PhD Program in Neuroscience

Prosocial behaviour in rodents: neural and behavioural substrates

Doctoral Thesis presented by

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"We can see brains but cannot see minds.
Yet, we can see the workings of minds in the logic of behaviours"

Carl Safina

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Acknowledgments

This is the clearest and yet the most challenging section of the thesis that I have faced while writing this manuscript. Clear because, in spite of all the extraordinary people I have met during this time, there are certain specific people who deserve this acknowledgement. But also challenging, because it is difficult to describe in a few lines everything I would like to transmit, that feeling of gratitude and affection, of remembering the moments we have spent together during this long road of learning and of animal adventures. A feeling that is sweet but sour, by remembering the beginnings when approaching the end.

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And to you mum, long gone, but always with me.

List of Figures and Tables

List of Abbreviations

AAV: Adeno Associated Virus

ACC: Anterior Cingulate Cortex

AI: Anterior Insula

Amy: Amygdala

AON: Anterior Olfactory Nucleus

ASD: Autism Spectrum Disorders

BDNF: Brain Derived Neurotrophic Factor

BL: Baseline

BLA: Basolateral Amygdala

CaMKIIa: Calcium/Calmodulin Dependent Protein Kinase II Alpha

CeA: Central Amygdala

dmPFC: Dorsomedial Prefrontal Cortex

D2: Dopamine 2 receptor

DA: Dopamine

DLC: DeepLabCut

DR: Dorsal Raphe Nucleus

fMRI: functional magnetic resonance imaging

FR: Fixed-Ratio

Hip: Hippocampus

IC: Insular Cortex

kHz: kilohertz

IR: Infrared

LE: Long Evans rats

LS: Lateral Septum

MDL: Mediodorsal Thalamus

MeA: Medial Amygdala

MPOA: Medial Preoptic Area

NAc: Nucleus Accumbens

OAMY: Olfactory Amygdala

OFC: Orbitofrontal Cortex

OT: Oxytocin

PAM: Perception Action Mechanism

PETH: Peri-event time histograms

PCI: Prosocial Choice Index

PCT: Prosocial Choice Task

PFC: Prefrontal Cortex

PrL: Prelimbic Cortex

PVN: Paraventricular Nucleus of Hypothalamus

PVT: Paraventricular Thalamic Nucleus

ROI: Regio Of Interest

SD: Sprague-Dawley rats

St: Striatum

Tac1: Tachykinin

TTL: Transistor-Transistor Logic

Vgat: Vesicular GABA transporter

VR: Variable Ratio

VS: Ventral Striatum

VTA: Ventral Tegmental Area

5-HT: Serotonin

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Abstract

Prosocial behaviors, actions that benefit others, are an essential part of the social life of humans and other animals, by promoting bonding and cohesion among individuals and groups. To study prosociality in rodents, scientists have developed behavioral paradigms where animals can display actions that improve the welfare of conspecifics in distress or need. Studies using these paradigms have provided insights into the role of social interactions and transfer of emotional states in the expression of prosociality, and into its neural bases. Rodents are thus powerful models to study these process as demonstrated by the intense research in the last years which is rapidly advancing our knowledge. Yet, despite the importance of positively valenced interactions in social cognition, most of our understanding on the neural circuits of prosocial actions and shared emotions is based in the study of negative affective states (stress, fear or pain). Here we developed different behavioural paradigms to assess prosociality and emotional transfer in reward-based contexts with mice and rats. Our work revealed that prosociality by reward provision is not a widespread phenomenon in mice as compared to rats, highlighting the very distinct social dynamics these two species display. Nevertheless, when prosociality was observed in mice, it was rooted in the same behavioural mechanisms that we have previously described in rats. Furthermore, we demonstrate that rats are capable of perceiving rewarding states from conspecifics, which is encoded as increases of ventral tegmental area (VTA) activity while witnessing a partner rat being rewarded. Finally, we found that these vicarious reward signals in the VTA are incorporated to guide social decisions that affect conspecifics. Optogenetic inhibition of the VTA neurons of decision-maker rats time-locked to the reward delivered to the partner in prosocial trials blocked the emergence of prosocial tendencies. We thus demonstrate that vicarious reward signals in rats are necessary for the emergence of prosociality, and that perceiving the rewarding states of others is the motivation underlying this interesting process. The results presented in this thesis provide an arising understanding of the behavioural and neural basis complementing our knowledge about the motivations of rodents to help others in positive contexts.

Resumen

Los comportamientos prosociales, acciones que benefician a los demás, son una parte esencial de la vida social de los seres humanos y otros animales, ya que fomentan los lazos afectivos y la cohesión entre individuos y grupos. Para estudiar la prosocialidad en roedores, los científicos han desarrollado paradigmas conductuales en los que los animales pueden mostrar acciones que mejoran el bienestar de congéneres en apuros o con necesidades. Los estudios realizados con estos paradigmas han permitido comprender mejor el papel de las interacciones sociales y la transferencia de estados emocionales en la expresión de la prosocialidad, y para conocer mejor sus bases neuronales. Los roedores son, por tanto, potentes modelos para estudiar estos procesos, como ha demostrado la intensa investigación en los últimos años, que está haciendo avanzar rápidamente nuestra comprensión. Sin embargo, a pesar de la importancia de las interacciones de valencia positiva en la cognición social, la mayoría de nuestros conocimientos sobre los circuitos neuronales de las acciones prosociales y las emociones compartidas se basan en el estudio de estados afectivos negativos (estrés, miedo o dolor). En este trabajo desarrollamos diferentes paradigmas conductuales para evaluar la prosocialidad y la transferencia emocional en contextos de recompensa con ratones y ratas. Nuestros resultados han revelado que la prosocialidad mediante la provisión de recompensas no es un fenómeno tan generalizado en los ratones en comparación con las ratas, lo que pone de manifiesto las dinámicas sociales tan distintas que muestran estas dos especies. No obstante, cuando se observó prosocialidad en ratones, ésta se basaba en los mismos mecanismos de comportamiento que hemos descrito anteriormente en ratas. Además, demostramos que las ratas son capaces de percibir estados de recompensa en congéneres, y esto es codificado como incrementos de la actividad del área tegmental ventral (VTA), al presenciar cómo se recompensa a una rata compañera. Finalmente, encontramos que estas señales vicarias de recompensa en el VTA se incorporan para guiar decisiones sociales que afectan a congéneres. Así, mediante inhibición optogenética de las neuronas del VTA de las ratas focales durante la recompensa entregada al compañero en la tarea prosocial, conseguimos bloquear la aparición de tendencias prosociales. De tal manera demostramos que las señales de recompensa vicaria son necesarias para la emergencia de la prosocialidad y que la percepción de estados positivos de otros es la motivación subyacente de este interesante proceso. Los resultados presentados en esta tesis proporcionan una comprensión emergente de las bases conductuales y neurales que complementan nuestro conocimiento sobre las motivaciones de los roedores para ayudar a los demás en contextos positivos.

1 | Introduction

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6 | Conclusions

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1 | Introduction

It should be noted that the following subsections of the Introduction; namely **1.1. Prosocial behavior in human and other animals**, **1.2.1. Russian Doll Model of Empathy**, **1.2.2. Three factors combination model of empathy**, and **1.3. Paradigms to study prosociality in rodents**, correspond to the original manuscript (Gachomba, Esteve-Agraz and Márquez, 2024).

1.1. Prosocial behavior in human and other animals

Helping someone in need, caregiving, comforting, donating money or volunteering are examples of prosocial actions largely common in human society. How prosociality is conserved across species and how the brain computes these types of actions are intense areas of research in neuroscience. For the purpose of introducing the topic of this thesis, I will start by providing a review about research performed with rodents and how recent findings using these species have been advancing our knowledge of the mechanisms underlying prosociality. I will begin giving a theoretical introduction of the main concepts and contextualization of the current views of the origins of prosociality, pioneered by research in humans and non-human primates, and enriched with research across different taxa. Then, I will focus on how research performed in rodents is helping advance the field.

At the most generic level, ***prosocial behavior****,* or *prosociality,* has been broadly defined as any behavior that benefits another, thus improving their condition (Dovidio et al., 2017). It is typically distinguished from *altruism* when considering motivations and costs associated with the behavior. Prosociality may or may not entail a cost for the actor and can be driven by several motivations. In contrast, altruistic behaviors are generally costly for the actor and other-regarding, implying no expectation of self-benefit (M. Lewis, 2018). Altruistic behaviors can thus be considered a subset of prosociality: all altruistic behaviors are prosocial but not all prosocial behaviors are altruistic. The terms prosocial behavior and altruism are generally distinguished from *cooperation*, which occurs when two or more individuals work together achieving common or mutual benefits (Marshall-Pescini et al., 2016). Prosociality, altruism and cooperation have been examined across scientific disciplines, resulting in similar terminology being used with different meanings (Kopp et al., 2024; Pfattheicher et al., 2022). The

above definitions for altruistic and cooperative behaviors reflect research in social and comparative psychology and differ from those developed in the field of evolutionary biology, where behaviors are defined as cooperative or altruistic based on costs and benefits for individuals' direct fitness (i.e., their reproductive success) (West et al., 2007). In evolutionary terms, cooperation is helping behavior that increases recipient's direct fitness and can result in mutual benefit (when also actor's direct fitness increases) or altruism (when actor's direct fitness decreases).

Several mechanisms have been proposed for explaining the evolution and maintenance of cooperative behaviors (Clutton-Brock, 2009; Nowak, 2006), including kin selection (Hamilton, 1964) direct reciprocity (Trivers, 1971) and generalized reciprocity (Hamilton & Taborsky, 2005; Pfeiffer et al., 2005). Evidence for reciprocity involving different commodities and services has been reported across several taxa *(for an issue in this topic see* (Schweinfurth, 2024)). Rats, for instance, reciprocate help for food sharing according to both direct and generalized reciprocity (Engelhardt & Taborsky, 2024; Rutte & Taborsky, 2007, 2008; Schneeberger et al., 2012; Schweinfurth & Taborsky, 2018; Wood et al., 2016). Since an overview of reciprocity and cooperation in rodents is beyond the scope of this review, we will focus here on studies using tasks where only one animal of the pair acts as helper. In addition, we will focus on the proximate mechanisms of prosociality, regardless of lifetime fitness consequences for the individuals involved, and thus we will use the term "*prosocial*" in relation to a behavior providing immediate benefit (e.g., food reward, stress reduction) to an individual in need. Furthermore, as intentionality of an action is difficult to assess in laboratory rodents, we will consider a decision as prosocial if it is learned, flexible and goal directed, which are parameters that can be evaluated experimentally.

1.2. Empathy at the base of prosociality

At the proximate level, it has been suggested that the type of prosociality shown by humans depends on socio-cognitive abilities well developed in our species, as well as on ethical and social attitudes appropriate to the culture (Penner. et al., 2005). For instance, *empathy,* broadly defined as the ability to sense and resonate with another's feeling, knowing that the shared feeling originates from the other (Decety & Jackson, 2004; Keysers et al., 2022). In humans, empathizing with others' distress, pain or needs can lead to personal distress as well as concern for others. As such, empathy allows us to quickly relate to another's state and can function as a major trigger for prosocial actions. Scientists have defined empathy in a variety of ways and long debated about its nature and evolution (Batson, 2009). Some authors distinguish empathy from perspective-taking, mentalizing and theory of mind, while others label these latter functions as cognitive components of empathy (cognitive empathy) as opposed to emotional/affective components (affective empathy). Some others consider empathy generally as an umbrella term aggregating various phenomena, including prosociality.

1.2.1. Russian Doll Model of Empathy

In this respect, a prevailing evolutionary model among the empathy literature is the Russian doll model, proposed by Frans de Waal, where empathy includes multiple affective, cognitive, and behavioral components organized into sequential layers (de Waal & Preston, 2017) (Figure 1a). Here, the inner and phylogenetically older layer is the perception-action mechanism (PAM), through which perceiving another's state activates one's own neural and mental representation of that state. PAM enables state matching between individuals, with its more basic expressions being motor mimicry and emotional contagion. New layers gradually evolved in some species, with each new layer being built on top of, and dependent on, older ones. These outer layers correspond to empathic phenomena requiring increased self-other distinction, emotion regulation and cognition, such as empathic concern, consolation, targeted helping and perspective-taking. Therefore, the model posits phylogenetic continuity in empathic abilities, which are supported by homologous neural and hormonal substrates. Advanced forms of empathy gradually developed from a simple, spontaneous mechanism, shared across a variety of species, with parental care and social attachment likely promoting this evolution. This model appears simple and has

inspired many researchers to investigate empathy in animals. However, the linear structure of the layers comes with some constraints for the expression of certain phenomena, by assuming that some of the processes are prerequisites for other ones. The model also implies that perspective taking and helping are built upon an emotional state-matching between the subjects, but not all prosocial processes have an emotional component, there can be understanding of others' needs and targeted helping without any emotional transfer.

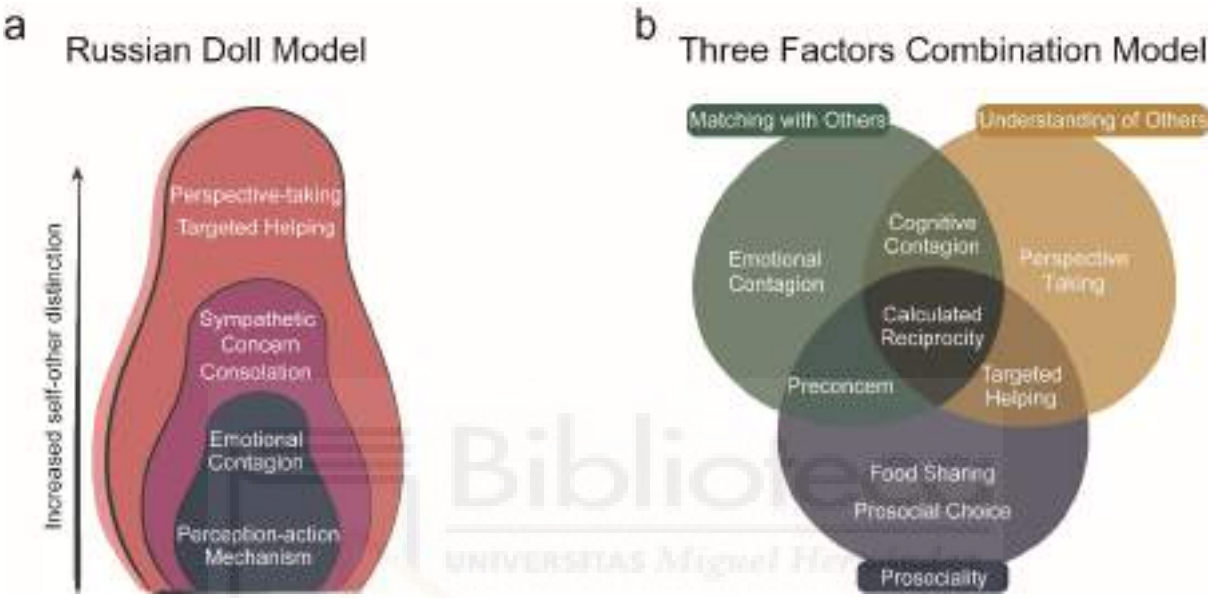

Figure 1. **Theoretical models of empathy**. **a.** Russian Doll Model of Empathy from de Waal and Preston. It reflects a conceptual framework where various affective, cognitive, and behavioral components of empathy are built into sequential layers developed during evolution. The inner and older layer corresponds to the perception-action mechanism, which induces emotional contagion in the observer. Outer layers are built upon increased self-other distinction, emotion regulation and cognition, such as empathic concern, consolation, targeted helping and perspective-taking**. b**. Three-Factor Combination Model from Yamamoto. This model posits that empathy is built upon three organizing factors: matching with others, understanding of others and prosociality. Most empathy-related phenomena can be categorized and mapped into appropriate contexts with these three factors and their combinations.

1.2.2. Three factors combination model of empathy

Yamamoto proposed a combination model as an alternative to the Russian doll model, observing that the related phenomena under the umbrella of empathy do not necessarily depend on each other sequentially but may have evolved independently, through convergent evolution, and can subsist separately (Yamamoto, 2017) (Figure 1b). This model suggests three independent but interacting factors: "*matching with others*"

(e.g., emotional contagion, mimicry), "*understanding of others*" (e.g., perspective-taking) and *prosociality.* Different empathic processes are mapped onto one of the three factors or onto their combination. Consequently, they are not strictly organized according to an increase in cognitive or emotional complexity, except for those mapped onto the overlaps between factors. Under this framework, prosociality and other empathic phenomena can be studied without assuming their dependence on other affective or cognitive capacities, with the potential to embrace a larger variety of prosocial behaviors across the entire animal kingdom. Indeed, the model lists food sharing and food-based prosocial choice in non-human primates as examples of behaviors which do not require assumptions of emotional state matching, or an understanding of others, in order to occur. Nevertheless, in both models, targeted helping would require perspective-taking, a mechanism less likely to be ascribed to cases of helping in social insects, such as the highly controlled rescue behavior shown by ants towards nestmates in danger (Hollis & Nowbahari, 2013; Nowbahari et al., 2009). While there is no a priori reason to fully rule out such abilities in insects, or that a mechanism similar to the PAM may be in place (de Waal & Preston, 2017), the matter awaits empirical evidence.

1.2.3. Empathically motivated prosocial behaviours

Discussions about the role of empathy in prosocial behaviour is plagued by disagreement and misunderstanding. Whether this affective experience can be a causal factor in eliciting a behavioural response to benefit another will likely depend on the measured components of empathy and prosocial actions. The aforementioned models highlight that empathy is a complex construct composed by the combination of multiple cognitive, affective and behavioural components.

As pointed out previously, some theorists have further distinguished distinct levels of empathy according to the nature of its components that interact in various ways (Decety & Jackson, 2004; De Waal & Preston, 2017). **Affective empathy** refers to bottom-up mechanisms involving low level and basic processing of vicarious sensory inputs that give rise to the automatic transfer and mimicking of other's emotional response as one's own. Thus, affective elements compose the bottom-up process of empathy, and have been linked to affective arousal, emotional contagion and shared representations between self and other (Preston & De Waal, 2002). Emotional mimicry is thought to be the

most basic reaction one can have to the affective state of another, coming from the idea that the autonomic nervous system of one species is genetically programmed to respond to an affective expression of another member of the same species, by generating a mirrored or similar response (Basch, 1983). On the other hand, **cognitive empathy** refers to top-down mechanisms involving higher level cognitive processing to understand the target's mental state, imagining how they feel, recognizing other's emotions and understanding their view. Thus, cognitive empathy is related to perspective taking and theory of mind (Batson, 2009; De Waal, 2008). Mentalizing abilities related to cognitive empathy are highly likely to vary depending on the situation in which the social interaction occurs, and they seem to only be found in more phylogenetically advanced mammals (Preston & De Waal, 2002). While there is a general agreement that empathy involves both affective and cognitive components, which rely on different brain regions (Decety & Jackson, 2004; Shamay-Tsoory et al., 2004; Bernhardt & Singer, 2012; De Waal & Preston, 2017), literature becomes mixed when determining how to understand **prosocial or empathic concern** (i.e. feeling of sorrow or concern for another, sympathy or compassion) (Stevens & Taber, 2021). There is disagreement in how this third component of empathy is conceptualized. However, whether prosocial concern is considered a proxy for affective empathy, a product of affective and/or cognitive empathy or a distinct phenomenon, it relates specifically to the caring for another's state rather than the sharing or understanding of it (Decety et al., 2015).

Empathy is thus thought to induce individuals to have a cognitive representation and emotional experience of another, which eases the perception of other's needs for help, promoting individuals' prosocial behaviour towards them (Van Der Graaff et al., 2018). Social psychologist C.D. Batson posited a model for linking empathy with prosocial behaviour known as the empathy-altruism hypothesis (Batson, 1987; Batson et al., 1991). It suggests that when a person finds another in distress will trigger empathy processes such as sympathy and compassion for that person, eliciting the motivation and behavioural response. For the author, terminating the observed distress of others leads to a relief from tension, thus the main motivation behind prosocial actions is to return to homeostasis (Batson, 1987). Moreover, the affective experience of personal distress by perceiving another in need might prompt prosocial behaviours to be decreased (Cameron & Payne, 2011; Lockwood et al., 2017). Experimental variations testing the empathy-altruism hypothesis have demonstrated a pluralism of prosocial motives (Batson & Shaw,

1991; Dovidio, 1991), which invite us to reconsider the overlap between egoistic and altruistic explanations found in humans.

The differentiation and conceptualization of the different elements composing empathy will ultimately help to understand the contributions of how each and combinations of these components can favour actions that help others. Research on humans has provided **conflicting evidence** regarding whether different elements of **empathy** positively **predicts prosocial behaviour** (Eisenberg & Miller, 1987; Eisenberg et al., 2010). Studies indicate that affective empathy does not necessarily translates into prosocial actions (Eisenberg & Miller, 1987; Jordan et al., 2016), and it could even have some opposite effects (Batson, 1987; Decety & Yoder, 2016). Some argue that an optimal level of **affective** empathy may be essential for promoting prosocial actions (Stevens & Taber, 2021). Too little might not lead to an empathetic concern, while too much might cause the individual to disregard another's suffering to cope with their own emotions (Lockwood et al., 2017). **Cognitive** empathy has also been associated with increased prosocial behaviour (Waytz et al., 2012), but it can be cognitively exhausting, hence deriving into decreased response to others in distress, even when a reward is offered (Cameron et al., 2019). Empathic **concern** has been found to be a factor increasing prosocial behaviours (FeldmanHall et al., 2015; Williams et al., 2014). Empathic concern is directly related to emotion regulation, which is the process of modulating/managing one's emotions to promote an optimal functioning and well-being (Gross, 1999). This process has also been studied in mediating the process for turning the affective empathy into subsequent prosocial action (Eisenberg, 2000). (Lockwood et al., 2014) studied this mediating relationship and found that individuals better at regulating emotions were more likely to engage in actions to benefit others. Alternatively, (Cameron & Payne, 2011) found opposing evidence, finding that individuals stronger at proactively regulating their emotions mitigated their affective responses, which could turned into a decrease in prosocial behaviours. Thus, different emotional regulation strategies might have distinct results (Lockwood et al., 2014). While empathic concern/compassion may be an emotional response, it is also shown to be adaptable through emotion regulation training (Stevens & Taber, 2021).

(Decety et al., 2016) proposed a rather complete model for empathetically motivated prosocial behaviours (Figure 2a), that recapitulates work from social psychology and neuroscience, which applies to humans and non-human animals. In their view, the

main motivation leading from the perception of someone in distress to performing a prosocial action towards them is empathy. In this model, empathetically motivated prosocial behaviour starts with the perception of another conspecific's cues of distress or need, increased attention to it can lead to an aversive affective arousal combined with a physiological response. The other's distress is experienced as aversive, recruiting neural circuits related to aversion, and a systemic stress response is initiated. Then, modulating factors such as resilience, emotional or stress reactivity will determine if an avoidance response is triggered instead of a prosocial action. Activation of decision-making and approach neural circuits are engaged when there is a prosocial drive. However, this is again modulated by assessments of perceived ability to help successfully, which can reduce the motivation for helping. Finally, successful helping is followed by a reduction of distress, returning to homeostasis in the victim, and in the helper by contagion. This social response is experienced as rewarding, causing an activation of reward circuits, which reinforce the behaviour, increasing the likelihood to be repeated in the future. For the authors of the model, helping and caring are inherently rewarding and become the main driver for helping others in distress (Decety et al., 2016), in contrast with tension-reduction models that posit a return to homeostasis (reducing one's own aversive empathic arousal) as the main motivation (Batson et al., 1987).

While there is a general agreement that certain empathic processes promote prosocial behaviours in the context of distress, some authors pointed out that the experience of prosociality and empathy-related phenomena is widespread and can also appear in situations of no distress (Schoenrade et al., 1986; Batson et al., 1991; Morelli et al., 2015; Telle & Pfister, 2016). We all experience positive events in our daily life, and they are thought to be associated with increased positive affect or emotion (Gable et al., 2000). Authors have described constructs related to positive empathy that help to give an explanation about the situations where empathy benefits others in non-distress. For instance, when people experience a negative or stressful event, they often turn to others for emotional support and comfort. On the other side of the coin, *capitalization* has been defined as the process of sharing/informing another person about the occurrence of a positive event and thereby deriving additional benefit from it (i.e. positive affect and well-being) (Langston, 1994; Gable et al., 2004). Capitalization normally occurs towards close relationships in humans and has been associated with increased positive affect (Gable et al., 2006; Gable

& Reis, 2010). (Fredrickson, 2001) proposed the "broaden-and-build" theory by which the function of positive emotions is to *broaden* individuals' scope of cognition, attention and action and to *build* by enhancing the individuals' physical, intellectual and social resources. The process of sharing positive emotions among individuals has been suggested to be central in the process of *building* resources (Gable et al., 2004, 2006).

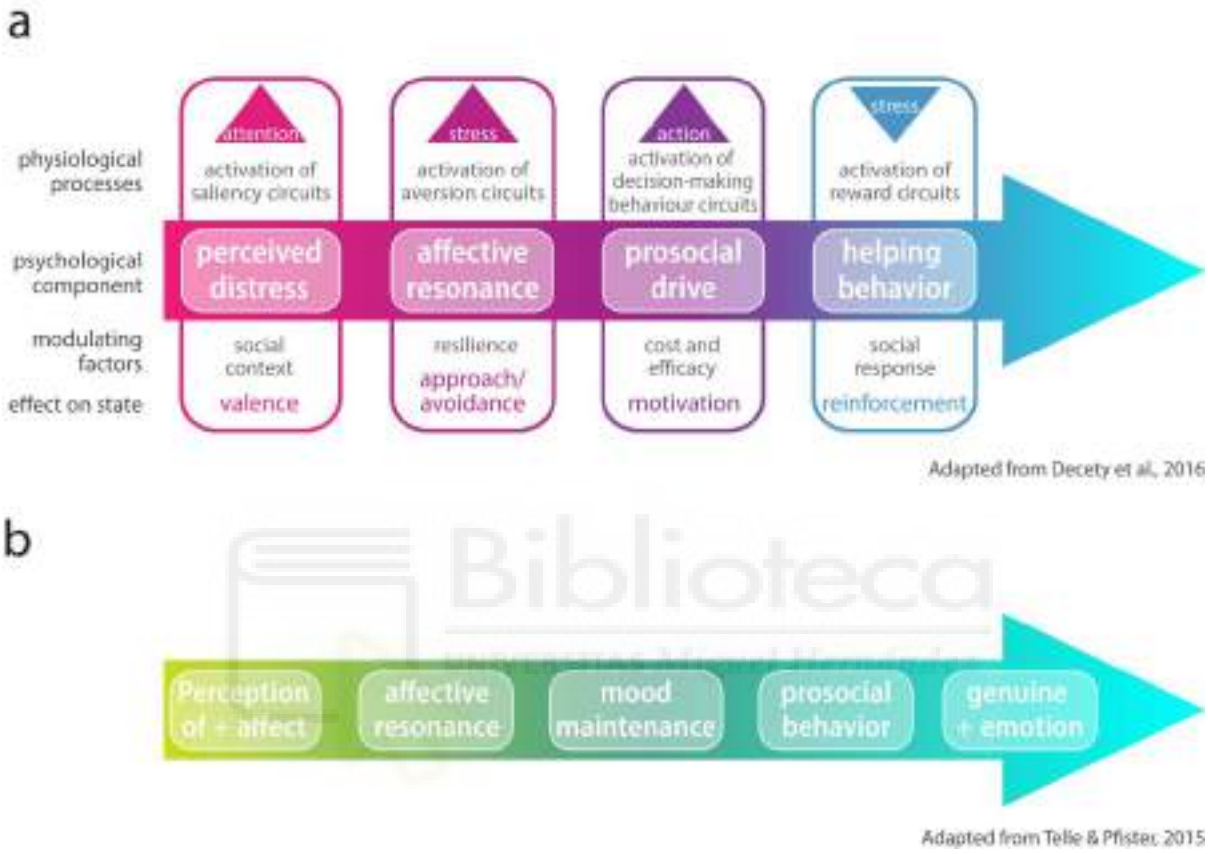

Figure 2. Models for empathetically motivated prosocial behaviours. a. Model from Decety et al., 2016. This model proposes that prosocial behaviour motivated by empathy starts by witnessing another's distress, which can lead to aversive affective arousal combined with a physiological stress response. Then, if appropriate, a prosocial drive is triggered, and depending on the context, can lead to prosocial behaviour. **b**. Model from Telle & Pfister, 2015. This model proposes that experiencing positive empathy can promote prosocial behaviour, mediated by mood-maintenance. It starts with the perception of a positive state of another, which causes an affective resonance by sharing the pleasant affect. While maintaining perspective of the source of the affect, the feelings experienced facilitate positive cognitions prompting prosocial behaviours, which are likely to be felt as rewarding and therefore reinforced. Hence, when an opportunity to help arises or is formulated, its likely to be seized, as it will serve to maintain or genuinely add to the empathic positive affect experienced previously.

These works reflect the responsiveness to others' positive emotional disclosures, and are tightly related to the experience of empathy for positive emotions, yet they do not provide an explanation about the situations where empathy benefits others in non-

distress. The *warm glow* effect is understood as the positive rewarding experience from the act of giving or helping others (Andreoni, 1989). Thus, promoting others' well-being and acting prosocial towards them in order to have a rewarding experience is thought to be a viable mean to explain this process. In this direction, early work pointed out to *anticipatory positive feedback/empathy* (Schoenrade et al., 1986) as another plausible way to promote prosocial behaviours in positive contexts. Additionally, the *mood-maintenance* hypothesis is a simple, yet widely supported, explanation as to why positive mood can drive increased prosocial actions (Telle & Pfister, 2016). By these means, negative mood often instigates people to actively engage in positive behaviours to mitigate its effects (Langston, 1994; Hess et al., 2006), whereas people in positive mood can prolong it by engaging in behaviours likely to yield a positive response, as for instance, prosocial actions. Positive empathy and positive affect show a significant amount of conceptual overlap (Morelli, Lieberman, et al., 2015), correlate with each other in child and adult humans (Light et al., 2009) and further show neural overlap (i.e. ventromedial prefrontal cortex) (Morelli, Lieberman, et al., 2015). Despite their connection, positive empathy occurs only when individuals learn of others' positive outcomes and vicariously share their affective state (Morelli, Lieberman, et al., 2015). This distinction is important as there is debate regarding whether the underlying motivations behind prosocial actions in positive contexts are rather explained by an egoistic motivation (i.e. to ultimately increase one's own positive affect).

As it will be discussed later, there is a bias on the fields studying prosociality and empathy-based processes in the context of negative affective states, perhaps because positive emotions do not fit existing models of emotion and emotion regulation (Fredrickson, 2001). Still, there is some scarce research on the link between positive emotional states and empathy-based prosociality. For example, (Telle & Pfister, 2016) proposed a model to explain why positive empathy (empathy felt for positive emotions) can trigger and mediate prosocial behaviour in humans (Figure 2b). In their view, witnessing a person displaying a pleasant affective state can trigger an affective resonance response in the observer, creating a positive affective arousal. While still knowing that the source of the experience of positive affect is the other person, the empathic experience facilitates positive cognitions and prompts positive behaviour that is likely to be felt as rewarding. Then, by means of mood-maintenance, a motivation to further increase and prolong the pleasant affect arises. Therefore, when

an opportunity to provide prosocial behaviour to another occurs, the likelihood that such opportunity is seized increases, as it serves to maintain or even genuinely add to the empathic positive affect previously experienced. This model, while simple, constitutes a plausible order of steps by which positive empathy may be elicited and translated into prosocial behaviour (Telle & Pfister, 2016). However, it is restricted to humans and based on the assumption that people want to maintain and prolong the empathically experienced positive affect, and each step is assumed to be necessary for this relationship to occur. While mood maintenance is a viable explanation to why positive mood leads to prosocial behaviour (Aknin et al., 2012), it should be acknowledged that alternative mechanisms could promote helping behaviour once positive empathy has been experienced (e.g. warm-glow effect, reciprocity or anticipatory positive affect). Others have suggested an even simpler view of this phenomenon, proposing a positive feedback loop between positive affect and prosocial behaviour, in which these two tend to reinforce one another within individuals in a daily basis (Aknin et al., 2018; Snippe et al., 2018). These authors reviewed evidence which suggest that **(1)** positive feelings and emotions serve as a valuable source of information for the actor and predict engagement in prosocial actions. And on the other hand, **(2)** evidence pointing out that acting prosocially can generate positive emotions in the actor. Thus, they proposed that if positive emotions cue and promote prosocial actions, then the emotional experience from engaging in prosocial behaviours should reward and predict subsequent prosocial actions (Snippe et al., 2018). However, is worth mentioning that, according to the authors, only tentative evidence with adults supports such possibilities (Aknin et al., 2018), and directionality of a causal relationship is hard to identify.

Most of the empathy related literature in humans is based on self and other report surveys, performing regression and correlational analysis in situations in which people simulate or imagine certain social scenarios rather than studying them on real contexts. Hence, much of the empirical data is grounded to indirect measures related to theoretical frameworks. Despite the diversity of instruments available to assess empathy and other psychometric measures, there is no *gold standard* to use as there exist important limitations regarding structural validity, standardization and response bias (Lima & Osório, 2021), likely due to the existing conceptual inconsistencies of empathy and related phenomena. Yet, thanks to advances in neuroimaging techniques (PET, fMRI, EEG), research in humans is providing useful insights with higher psychometric

quality and adequacy into the neural underpinnings of empathy in the general population, which are much required to have a unifying picture.

Lastly, while empathy is been studied as a powerful motivator for caring and helping behaviours (Decety et al., 2016), it is also assumed that not all prosocial behaviours are necessarily motivated by empathy (e.g. sharing and reciprocity or cooperation). Because psychological processes are hard to study in the field, testing animals in experimental tasks, within controlled laboratory or semi-natural settings, is useful to explore whether a species displays specific behavioural tendencies, as well as to identify shared cognitive or neural processes underlying those tendencies. Certainly, over the last few years, the field has grown tremendously in the study of emotional contagion in rodents, especially thanks to important contributions in fear and pain related paradigms (*see* (Keysers et al., 2022; Keysers & Gazzola, 2023)), that will be reviewed in the following section. During experiments with animals, if observing helping behaviour among them, researchers might be induced to conclude that empathic processes are involved and could represent the motivation for helping. However, it is necessary to verify the presence of emotional responses in order to conclude such claims, as (Vasconcelos et al., 2012) stated, human-like complex abilities found in animals may arise from simpler mechanisms than previously hypothesized. In this line, other authors recommend a multi-component approach to assess behavioural, physiological and valence related measures for better accessing the affective and cognitive basis of empathy-related phenomena in animals (Adriaense et al., 2020), although measuring such multiple readouts is often experimentally challenging and not always logistically feasible.

1.3. Paradigms to study prosociality in rodents

Research investigating the expression of empathic phenomena has provided evidence that animals can perceive, learn from and respond to the emotional states of conspecifics. For instance, findings of emotional contagion and affiliative response to distressed conspecifics are robust (*for reviews see* (J. Chen, 2017; Meyza et al., 2017; Pérez-Manrique & Gomila, 2018; Pérez-Manrique & Gomila, 2022). Moreover, a growing number of experimental studies has investigated whether animals display choices or actions that benefit others. This research has yielded evidence for the emergence of prosocial behaviours in a variety of species, (Nowbahari et al., 2009; Duque & Stevens, 2016; Horn et al., 2024; Nakahara et al., 2017; Satoh et al., 2021; Lalot, Liévin-Bazin, et al., 2021; Lalot et al., 2023; Jensen, 2016; Marshall-Pescini et al., 2016; Rault, 2019), further suggesting that convergent selective pressures may have driven the evolution of prosociality in distant taxa. Notably, studies with laboratory rodents, mainly rats, mice and voles, have started to map brain regions and neural circuits to specific types of prosocial behaviours that involve relieving the distress of, or providing reward to, a conspecific. For this, neuroscientists are testing rodents in innovative prosocial paradigms (Figure 3), and using tools to measure or manipulate neuronal activity, advancing our knowledge of the neural bases of prosocial actions.

It is timely to synthesize these findings, to help create a big picture of the puzzle of prosociality and identify the gaps in the field. To this aim, the presented thesis will continue with a review of results focused on four experimental paradigms that have been often employed to measure different types of prosocial behaviours in rodents, namely the ***consolation*** paradigm, which assesses animals' tendency to display affiliative social touch (e.g., allogrooming) towards a distressed conspecific; the ***rescue*** paradigm, where animals can perform an action that enables conspecifics to escape a situation of stress; the ***harm aversion*** paradigm, which measures animals' propensity to prevent others' distress; and the ***prosocial choice task***, where animals can choose to provide or not food to a partner. For this section of the introduction, I give an overview of the studies using these paradigms and summarize the results. It is mainly focused on behavioural outcomes, highlighting differences in task design and conditions tested, and reporting findings relative to neural mechanisms of prosociality when available (Table 1).

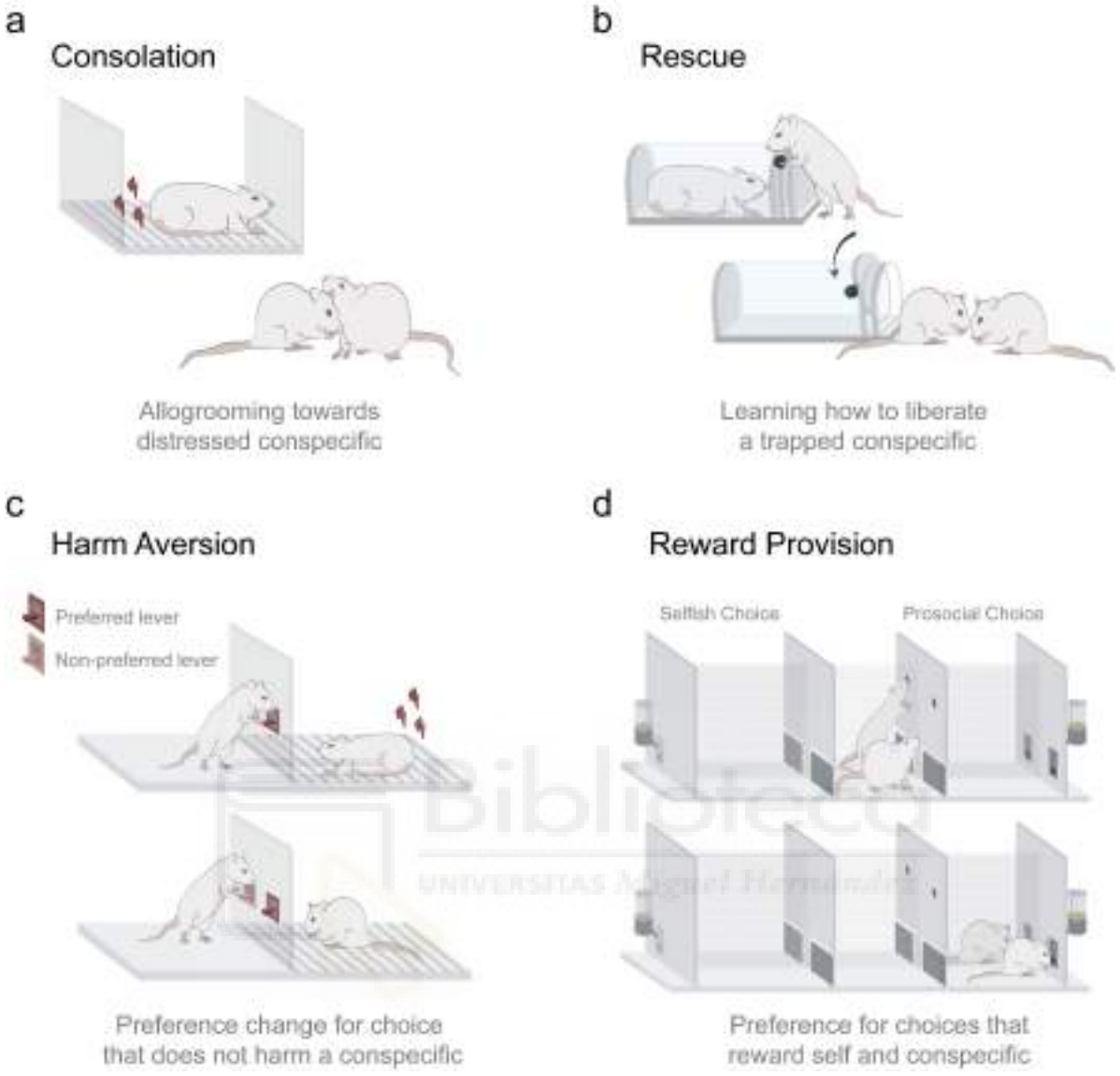

Figure 3. Experimental paradigms for assessing prosocial behaviors in rodents. a. Consolation paradigm. Focal rodents show increased allogrooming and/or allolicking towards a conspecific that experienced a distressful event (e.g. pain, fear, social defeat). **b**. Rescue paradigm. Rodents learn to free a trapped conspecific from a restrainer or water pool**. c**. Harm aversion paradigm. Rats are first tested for developing a preference between two options, then the preferred option is associated with shocks to a conspecific. Prosocial rats switch their previous preference and avoid the option that now shocks a conspecific**. d**. Reward provision paradigm. Decision-maker rodents can choose between two options: one option delivers reward to them and a conspecific (prosocial choice) and the other option only to them (selfish option). Over sessions, decision-makers develop a preference for the option that rewards both themselves and the conspecific.

1.3.1. Consolation paradigm

We tend to comfort familiar others who are experiencing pain, anxiety or fear, through reassuring words that have a calming effect. Depending on the context, culture, and our relationship with those others, we may comfort by means of physical gentle contact and affective gestures, such as patting or hugging. Such contacts communicate sympathetic concern, provide stress buffering and can strengthen social bonds (Jakubiak & Feeney, 2017; Morrison, 2016).

Several non-human animal species engage in affiliative contacts, such as allogrooming in mammals or allopreening in birds, which are likely to be maintained by mechanisms of reciprocity and mutual care (Lim & Hong, 2023; Schino & Aureli, 2010). Beyond improving hygiene, such interactions serve a social function, being crucial for the formation of relationships and for preserving group cohesion in multiple social species (Dunbar, 1991; Radford & Du Plessis, 2006). Affiliative contacts mediates post-conflict reconciliation in non-human primates (Jablonski, 2021; McFarland & Majolo, 2011) and provides social comfort for the recipient, buffering against stress and thus resembling the effects of consolation among humans (Clay & Waal, 2013; Fraser et al., 2008; Lim & Hong, 2023).

In rodents, as well as other animal species (Fraser & Bugnyar, 2010; Palagi & Cordoni, 2009; Plotnik & Waal, 2014) "consolation" is typically measured by quantifying affiliative interactions (e.g., duration, frequency, and latency of allogrooming in the case of rodents), towards distressed conspecifics, relative to non-stressed ones (Figure 3a). Burkett and colleagues were the first to provide experimental evidence of prosocial allogrooming in a rodent species, the prairie voles (*Microtus ochrogaster*) which engage in monogamous mating and biparental care (Burkett et al., 2016). The study aimed at showing that consolation behavior possesses characteristics consistent with an empathy mechanism: state matching, emotional contagion, familiarity bias, and self-other differentiation. Unstressed prairie voles (observers), both males and females, increased allogrooming towards a conspecific demonstrator after a separation during which the demonstrator was fear-conditioned, but not after a control separation without stressor, and the increase in allogrooming was selective towards a familiar partner (either mate, same-sex sibling, or unrelated same-sex cagemate). Stressed demonstrators that were kept alone for a short period of time after the stressor subsequently displayed increased anxiety-related behavior relative to unstressed controls, whereas those that were reunited with the observer for the same period

showed normalized responses. Therefore, social contact with a conspecific after the stressor had a buffering effect. Consistent with an empathy mechanism, prairie vole observers and stressed partners showed physiological state matching (correlated levels of plasma corticosterone between the observer and demonstrator), even if the association between state matching and prosocial allogrooming was not specifically assessed. The authors also tested meadow voles (*Microtus pennsylvanicus*), characterized by promiscuous breeding and uniparental care, and reported no increase in prosocial allogrooming in male observers tested with stressed female mates. At the neurobiological level, the anterior cingulate cortex (ACC), but not the prelimbic cortex (PrL) or nucleus accumbens (NAc) shell, showed increased expression of c-FOS, a marker of neuronal activation, in male prairie voles exposed to a stressed mate compared with those exposed to the unstressed partner. In addition, injection of an oxytocin receptor antagonist (OTA) either into the cerebral ventricles or into the ACC of male prairie voles, before the consolation test, abolished the subsequent increase in allogrooming towards the stressed female mate, indicating that oxytocin (OT) signaling in the ACC modulates consolation behaviours. This seminal work paved the way for other studies investigating consolation behaviours in response to different stressors, and its neural correlates.

Evidence for prosocial allogrooming has been reported for other rodent species. Monogamous mandarin voles (*Microtus mandarinus*), both males and females, showed higher frequency of, and more time spent on, allogrooming a mate that experienced stress via social defeat compared to mates that only experienced separation (L.-F. Li et al., 2019). Administration of either OTA, $GABA_A$ receptor antagonist, serotonin 5-HT1AR antagonist, or dopamine D2R antagonist, but not vasopressin V1a receptor antagonist, into the ACC of male observers significantly reduced the consolation response (L.-F. Li et al., 2019, 2020). In addition, dorsal raphe (DR) serotonergic neurons projecting to the ACC (DR-ACC 5HT neurons) were found to play a crucial role for consolation and sociability in both males and female mandarin voles. Activity of DR-ACC 5HT neurons and endogenous release of 5HT in the ACC increased during allogrooming bouts, social approaching, and sniffing directed towards the distressed partner, and optogenetic inhibition of DR-ACC 5HT neurons or their terminals in the ACC decreased consolation behavior (L.-F. Li et al., 2021). Since the same inhibitory manipulations also decreased sociability in a three-chamber test, the reduced

allogrooming towards stressed conspecifics may be due to an overall reduction in observers' sociability, as the authors pointed out. In contrast, activation of the DR-ACC 5-HT neurons did not elicit corresponding increases in allogrooming and sociability; thus, the effects of activation of this circuit on prosocial behavior may require further investigation.

Other studies reported consolation behavior in laboratory rats. Rats' allogrooming towards a same-sex conspecific experiencing physical pain, or stress induced by fear conditioning, was increased compared to that of rats interacting with an unstressed conspecific (C.-L. Li et al., 2018; Lu et al., 2018; Kiyokawa et al., 2019; Du et al., 2020), and no sex difference was found when comparing male and female cagemate dyads (Du et al., 2020). Differently from the familiarity selective response observed for prairie voles (Burkett et al., 2016), rats' prosocial allogrooming extends towards distressed unfamiliar partners (Lu et al., 2018; Kiyokawa et al., 2019; Luo et al., 2020), but at lower levels than that directed towards familiar ones (Lu et al., 2018; Luo et al., 2020), adding more evidence for familiarity as a factor promoting consolation behavior. Moreover, similar past experience with pain by observer rats and the display of visually-identifiable pain expressions by demonstrators are factors that enhance social transfer of pain and the consolation response (C.-L. Li et al., 2018; Luo et al., 2020). Furthermore, the paraventricular nucleus of the hypothalamus (PVN) and central amygdala (CeA), showed increased c-FOS expression in male rats that interacted freely with a fear-conditioned than a non-conditioned partner, suggesting that social cues from the fear-conditioned rat activated these brain regions in the observers (Kiyokawa et al., 2019).

Similar to rats, mice express consolation behavior towards both familiar and unfamiliar, same-sex conspecifics (Zeng et al., 2021; Matsumoto et al., 2021; Y. E. Wu et al., 2021; Carneiro de Oliveira et al., 2022; Phillips et al., 2023; Lee et al., 2021; Zhang et al., 2024), with no substantial difference between male and female dyads (Y. E. Wu et al., 2021; Phillips et al., 2023), although a study reported increased prosocial allogrooming duration and frequency in males compared to female mice (Du et al., 2020). Free social interactions with an unstressed cagemate reduce subsequent anxiety-like behavior in stressed mice (Zeng et al., 2021; Y. E. Wu et al., 2021; Phillips et al., 2023), while limited interactions through a transparent perforated barrier prevent stress relief (Y. E. Wu et al., 2021). This indicates that free

physical interactions between the animals provide stress buffering benefits that go beyond mere social proximity.

Recent research is providing insights into the brain areas and neural circuits mediating consolation behavior in mice. Here, the brain regions involved are diverse, maybe reflecting some differences in the neural pathways recruited depending on the type of stressor that the demonstrator mice have been subjected to. Zeng et al. identified several brain areas activated when mice would interact with a conspecific that underwent surgery, and functionally demonstrated that neurons in the paraventricular thalamic nucleus (PVT) containing orexin receptors have a role (Zeng et al., 2021). Phillips and colleagues linked consolation behavior to changes in PFC subregions, specifically cingulate area 1 (Cg1) and prelimbic cortex, reinforcing the idea that prefrontal cortex, especially the cingulate, has a role in prosocial behaviors (Phillips et al., 2023). Recent works have also elegantly reinforced the importance of the ACC in consolation paradigms where allogrooming was selectively targeted to a conspecific in pain suffering from bee venom injection (Zhang et al., 2024). Although the relevance of these cortical areas seems to gain momentum, important contributions have pointed to the critical role of non-cortical structures too, such as the medial amygdala (MeA) and its projections to the hypothalamus (Y. E. Wu et al., 2021)

Summary

The findings discussed in the section above show how different species of rodents have evolved instinctive behavioral strategies (e.g. allogrooming, allolicking) to address specific states and needs of others. Specifically, when interacting with conspecifics in negative emotional states (e.g. distress, pain or fear), rodents exhibit a form of prosocial behavior, consolation, in a context-appropriate manner. This behavior co-occurs with emotional contagion and has a stress buffering effect on the recipient. Affiliation of the pair tends to promote allogrooming towards distressed conspecifics in all tested species, although for voles it seems essential. Sex differences are not found in most of the studies, highlighting the relevance of consolation behaviors as a form of social bonding irrespective of sex. Dominance structures naturally shape the dynamics of the social interactions among individuals; however, we found that none of the works reviewed assess how social hierarchy influences the exhibition of this type of behavior. Finally, some of the reviewed work is

pinpointing the neural correlates underlying consolation behavior in rodents. Prosocial allogrooming depends on the activity of neurons in the Medial Amygdala, Paraventricular Thalamic Nucleus and Prefrontal Cortex (ACC, PrL) and involves the signaling of multiple neuropeptides and neurotransmitters (OT, orexin, 5HT and DA). We will benefit from understanding how these brain regions and neuromodulatory molecules act in concert to regulate the expression of consolation behavior.

1.3.2. Rescue paradigm

Instrumental paradigms have been developed to assess if rodents rescue trapped conspecifics (Figure 3b). Early work by Rice and Gainer showed that rats would press a lever to lower a distressed partner that was suspended from the floor, which was interpreted as altruistic behavior leading to relief of the distress (Rice & Gainer, 1962). This study met with some criticism, as a later work doubted that these actions were goal directed (Lavery & Foley, 1963). More recently, (Bartal et al., 2011) developed a door-opening paradigm where free rats are tested for their tendency to liberate a conspecific trapped in a restrainer. Over days of testing, the proportion of rats that opened the door increased and the latency to door opening decreased only when the free rats were tested with a trapped cagemate, but not in control conditions where the tube was empty or contained a toy. Rats opened the door even when the partner was released in a separate adjacent compartment, suggesting that expectation of full social contact is not required for eliciting rescue. When rats could free the partner or open another tube with chocolate, they opened both tubes and ate the chocolate together in half of the trials, suggesting that rats attributed value to releasing the trapped conspecific and tolerated the presence of the trapped animal while consuming high rewards. All female rats became door-openers in contrast to two thirds of male rats, suggesting that females are more likely to engage in rescue behavior; however, there is to consider that the size of the male sample in the study was four times larger. For the authors, rats freed their cagemates in order to end either their own distress or that of the trapped animal, thus their prosocial behavior possibly being empathy motivated. Indeed, corticosterone levels of the helper animal correlated negatively with the propensity to liberate the trapped animal, and pharmacologically manipulating the arousal/anxiety levels of the animals does have an impact on the levels of prosociality (Ben-Ami Bartal et al., 2016). On this line, male mice showing consistent helping were

characterized by a lower corticosterone increase compared to non-helpers, and their corticosterone response was positively correlated with that of the trapped cagemate (Pozo et al., 2023). This suggests that rescue behavior may entail some degree of physiological state matching between helper and trapped animals, and that a high stress response hinders helping.

(Sato et al., 2015) developed a task using a pool of water where a distressed rat was trying to escape from, and showed that unconfined rats, both males and females, learned to open a door over testing days, allowing the soaked cagemate to escape. Door-opening occurred mainly towards soaked rats and not towards those that were in a dry area, suggesting that rescue behavior depended on the partner's distress. Additionally, door-opening emerged more rapidly and with shorter latencies when the roles of the rats were switched, indicating that observational learning or prior experience with the stressor enhanced prosociality in this task. Prairie voles were also found to rescue littermates when tested in this task, regardless of the sex composition of dyad, and showing more prompt and stable door-opening when the partner was soaked (Kitano et al., 2022).

Following the works of (Bartal et al., 2011), and (Sato et al., 2015), other studies adapted the original protocols or implemented changes aimed at investigating the motivations driving the opening response as well as the contextual, neurobiological and physiological factors modulating it. An important modulator is the familiarity of the free rat with the strain of the trapped partner. Male rats were found to release both familiar and unfamiliar conspecifics of the same strain as well as conspecifics from a different strain they were cohoused with, but not conspecifics of a strain they never met, including their own genetic strain (Ben-Ami Bartal et al., 2014, 2021). The findings indicate an ingroup bias, which parallels effects observed for human empathy and helping. The ingroup bias for rescue has been proposed to emerge during development, since male adolescent rats, in contrast to adults, did release trapped conspecifics of an unfamiliar strain (Breton et al., 2022). Further studies will be useful to assess if a brief exposure to outgroup members during adolescence would reduce the ingroup bias later in life. Whether the unselective rescue displayed by adolescent rats is primarily driven by differences in affective arousal when facing the distressed partner or by an increased interest for novel social stimuli compared to adults, as suggested in the (Breton et al., 2022)

study, remains to be further investigated. (Havlik et al., 2020) reported that strain familiarity modulates the effect of bystander(s) on opening performance, by comparing male helper rats performing alone to helper rats performing in the presence of one or two other rats that were unable to help because slightly sedated with midazolam (passive bystanders). Compared to the alone condition, helper rats in the presence of passive bystanders opened the restrainer less often, but only when they were familiar with the bystander's strain. In contrast, rats were more likely to engage in door-opening when in the presence of one or two non-sedated rats that also engaged in the task (active bystanders), suggesting that releasing performance may be enhanced through social learning. This suggests that the performance of helper rats is influenced by the presence of conspecifics and their own capacity to perform.

To assess rats' motivation to engage in helping, (Kalamari et al., 2021) designed an operant version of the task where required lever pressing to open the restrainer was progressively increased. The authors studied how early life experiences, including short maternal deprivation during the postnatal period, and living in enriched environments from juveniles, affected male rats' helping behavior in adulthood. Compared to rats housed in pair and standard cages, rats housed in bigger cages, with physical and social enrichment, were less motivated to press a lever for releasing the restrained cagemate as well for gaining access to a free cagemate. Early-life stress (ELS) induced by a full day of maternal separation during the early postnatal period did not affect motivation to behave prosocially when adults. However, a study reported that adult male rats that received increased maternal care when pups (measured as frequency of grooming, licking, and nursing by the dam) were more likely to rescue a cagemate from water, and did it at shorter latency, compared to rats that received less maternal care, although this effect was restricted to a late stage of the task (Asadi et al., 2021). This parallels the observation that repeated periods of maternal separation during infancy reduced rats' prosociality for food reward later in adolescence (cf. section 2.4). Given the impact of ELS on different aspects of cognition and emotions later in life, research addressing the long-term effects of different kinds and degrees of ELS on helping behavior certainly deserves future attention.

Other physiological conditions associated with negative energy status, including hunger state and diabetes, have been found to prevent rescue behavior in male mice.

(Pozo et al., 2023) showed that mice with streptozotocin-induced diabetes, characterized by hyperglycemia, did not show any opening responses. In parallel, food-restricted mice did not release the partner on any day but did start releasing it once fed *ad libitum*, or when they had the simultaneous option to open a tube with palatable food. Notably, inducing a hunger-like state via chemogenetic activation of hypothalamic neurons expressing the agouti-related protein (AgRP) had a similar effect, as mice started releasing the partner only when AgRP neurons were no longer activated. In contrast, door-opening latencies of fed mice that had previously released the partner were not substantially affected by either food-deprivation or activation of AgRP neurons, indicating that these manipulations affected the learning phase rather than the maintenance of the behavior. These findings thus point to the role of the actor's internal state in learning prosocial behaviors. Energetic needs, under the influence of AgRP neuronal activity, compete with prosocial motivations, in accordance with observations that AgRP neurons influence other motivated behaviors.

Regarding how the brain engages in prosocial behavior during the rescue paradigm, the Bartal lab has been pioneer performing c-Fos whole brain analysis after this task and identifying network central hubs which were modulated by the familiarity levels of the trapped rat (Ben-Ami Bartal et al., 2021). Interestingly a shared network of frontal and insular cortices was active during the task, regardless of strain familiarity of the trapped rat; however, the NAc was selectively active in helper rats facing the familiar strain (where higher helping behavior was found). Further analyses, combining c-Fos labeling with retrograde tracing to identify active projections from the frontal cortex to the NAc, revealed that c-Fos+ ACC cells projecting to the NAc correlated with the percent of door-opening towards ingroup members. Further studies implementing loss and gain of function manipulations targeting NAc-projecting ACC neurons will help to elucidate the role of this circuit in rescue behavior, as this projection has been implicated in the social transfer of pain and analgesia in mice (Smith et al., 2021). Furthermore, Bartal laboratory found that male adolescent and adult rats showed different patterns of neural activity while freeing restrained ingroup or outgroup members, which may underlie the differences in rescue selectivity between the two age groups (Breton et al., 2022).

As regards to OT, evidence indicates that an intact OT system is important for prosocial behavior in the rescue paradigm with a soaked conspecific. Male and female rats receiving bilateral injection of an OTA into the ACC showed higher door-opening latency compared to controls when rescuing the soaked partner, suggesting that OT signaling in the ACC sustains prosocial learning (Yamagishi et al., 2020). On this line, prairie voles that were homozygous for the knocked out oxytocin receptor gene (*Oxtr–/–*) showed reduced rescue behavior as well as social interest (e.g., decreased social proximity and huddling) towards the soaked cagemate, compared to those that were wild-type (*Oxtr*+/+) (Kitano et al., 2022). Whether the effects of OTA into the ACC and Oxtr KO on rescue are specific for a partner in distress is yet not clear, since data from the no-distress condition are lacking.

Adding to the central role of OT in the ACC, the anterior insula (AI) has also been related to the propensity to help soaked partners. Pharmacological or chemogenetic inhibition of the AI on days 9-10 of the helping task increased door-opening latencies compared to days 7-8, when door-opening behavior was learnt (Cox et al., 2022). Furthermore, chemogenetic inhibition of the AI did not affect rats' preference for an unfamiliar animal over a novel object, when helper rats were further tested in a social reward place conditioning task, suggesting that AI activity may contribute to rescue behavior through mechanisms other than social interest. The authors suggest that inhibition of helper rats' AI activity likely reduces the emotional salience or valence of the distress of the trapped animals, increasing the latency to release them from the water. Indeed, previous reports have described AI to be important for mediating approach and avoidance responses to distressed conspecific rats (Rogers-Carter et al., 2018).

Finally, dynamic recordings of brain activity during the rescue paradigm are still scarce, but (W.-Y. Wu et al., 2023) described that ACC and insular cortex (IC) neuronal ensembles of helper rats increased activity around the time of door-opening when the restrainer contained a conspecific, but not when it was empty or contained a toy. These findings further suggest that these brain areas may encode aspects of the releasing response. Yet, it remains to be determined how this activity is specific for rescuing a distressed partner compared to gaining access to a non-distressed one.

The literature has generated substantial discussion on whether door-opening is primarily driven by empathic processes (Vasconcelos et al., 2012b), based on findings that other factors, such as seeking social contacts or interest in features of the apparatus (Ueno et al., 2019) may function as motivators and thus offer an alternative explanation. This has typically been evaluated by assessing order-effects and reinforcing aspects of the behavior. When having the opportunity to liberate a restrained conspecific or interacting with a free partner, some studies describe that rats showed no overall preference for rescue (Heslin & Brown, 2021), and were less motivated to engage in door-opening under a progressive ratio operant schedule, when the behavior did not result in social interaction compared with a condition that did result in interaction (Cox & Reichel, 2020). Seeking social contact is thus an important factor, with rewarding properties that can impact on social decisions in some contexts, and can facilitate helping in the rescue paradigm, but there is now robust evidence that is not necessary for prosocial behavior to occur.

Summary

The rescue paradigm, based on a tube or a water pool, has offered a novel, elegant and relatively simple instrumental learning paradigm to study prosocial behavior in rodents, being the most prolific tool according to the literature. It has been found that individual familiarity and sex do not seem to affect the exhibition of rescue behavior in rodents. Moreover, releasing performance is biased towards ingroup conspecifics in adult but not adolescent rats. Data about social dominance asymmetries is lacking in the literature and might result necessary to complement our knowledge on the topic. A moderate level of stress may facilitate prosocial learning, while higher stress levels and deficiency in energy status hinders helping. Furthermore, a few studies point to the modulatory effects of early life conditions on helping later in life. Exposure to the trapped rats likely recruits neuronal activity in the ACC and AI, consistent with the role of these brain regions in processing self and vicarious experience of fear and pain (Carrillo et al., 2019; Gu et al., 2012), and the activity of OT, since disrupting OT signaling impacts latency to door-opening.

1.3.3. Harm aversion

To date, very few studies have assessed the tendency of laboratory rodents to avoid actions that harm a conspecific. The firsts are the classic studies by (Church, 1959) and (Greene, 1969), where rats could instrumentally induce or relieve distress in a conspecific (Figure 3c), and that were echoed by studies in monkeys (Masserman et al., 1964). In Greene's study, actor rats were first trained in an operant box where they could obtain food by pressing either one of the two levers, with both levers delivering equal reward but one requiring twice the force as the other, so that most of the rats developed a stable preference for the easier lever. During testing, a second rat ("victim"), placed in an adjacent compartment, received foot shock whenever the actor rat pressed the preferred lever to feed itself. In this social condition, actors were considered to be prosocial or, in the own words of the author, to show "operationally defined altruism", if they changed their preference for lever pressing. To examine the role of prior experience with the stressor, two groups of actor rats were tested that were either naïve or had experienced foot shocks before training. According to the study, only in this second group the majority of rats changed their preference for the lever delivering food when the initially preferred lever delivered concurrent foot shock to the partner. This change in preference occurred even if, for most of the rats, pressing the nonpreferred lever required twice the effort. Thus, Greene's early work suggests that prior experience with the victim's stressor may increase rats' sensitivity to other's pain and, as a consequence, promote harm aversion. Indeed, in a more recent adaptation of these tasks, (Schaich Borg et al., 2017) reported that animals would avoid exploring spaces that would induced foot-shocks to conspecifics, avoidance that was enhanced by prior shock experience, and found that c-Fos activation in the ACC, OFC and Olfactory Amygdala and oscillations between and within these brain regions, correlated with individual differences in harm avoidance.

(Hernandez-Lallement et al., 2020) adopted, refined and expanded Greene's paradigm and results, investigating individual differences as well as the effects of sex, familiarity and reward cost, and demonstrating the necessity of the ACC in this type of prosociality. In line with the findings in (Greene, 1969), emergence of harm aversion at the group level was found to be dependent on prior experience with foot shock. In addition, actor rats pre-exposed to the stressor exhibited marked individual variability in harm aversion, with less than half of the animals showing switching (a significant reduction for the

initially preferred lever, then paired with foot shock to the partner) whereas the rest of the animals showed preference changes within chance level. Some animals stopped pressing levers, thereby also preventing shocks to the partner. Male and female rats displayed comparable levels of harm aversion towards a same-sex conspecific, and no effects of familiarity with the victim was found in males. Moreover, male rats significantly reduced their usage of the shock lever if it delivered twice, but not thrice, the number of sucrose pellets of the non-shock lever, suggesting that harm aversion is subject to cost-benefit evaluation. Furthermore, for prosociality to appear in this paradigm, animals should not be habitual in the individual pressing lever task, as overtraining in the individual part of the task would interfere with goal-directed switch when social contingencies change - i.e rats that were trained longer to keep a strong and stable preference over days did not switch their preference to the non-shock lever. Inactivation of the ACC (area 24a and 24b), via bilateral injections of muscimol, reduced harm aversion in male rats, an effect possibly mediated by cingulate deactivation also reducing rat's own distress when witnessing another receive a shock (Carrillo et al., 2019). In this work, the authors refrain from interpreting rats' behavior as truly altruistic in the sense of acting with the intention to benefit another and suggest that an account based on selfish motivations could offer a sufficient explanation. When delivering shocks to the partner, some rats experience distress or fear, via emotional contagion, accentuated by association with their prior exposure to the shock. Those rats would then avoid this negative state by switching to the non-shock lever. This account would be supported by the data showing that animals that switched more behaved more alertly to the shocks of the victim, delaying their entrance to the food magazine, shortening food consumption, and taking longer to perform trials. Thus, according to the author's view, rats showing harm aversion in this paradigm could be primarily motivated by the goal of reducing their own distress or fear.

Harm aversion has also been shown in mice (Song et al., 2023) and, as described for rats, is independent of sex and affiliation, but dependent on previous experience with foot shocks. (Song et al., 2023) further showed a crucial role of the ACC, and its connection to the Mediodorsal Thalamus (MDL), employing chemogenetic and optogenetic manipulations. These observations expand a previous study that found this projection to be important in modulating vicarious freezing behavior in rats (Zheng et al., 2020).

Recently, (E. M. Hess et al., 2023) developed a modified version of the task where, on each trial, actor rats could press a single lever that delivered a sucrose reward to them and a foot shock to a partner rat. By omitting lever pressing on a trial, actor rats could prevent harm to the partner at the cost of losing the reward. In agreement with previous studies, lever response decreased at the group level from baseline (no shock delivery) to test sessions, indicating harm aversion. However, this study did report sex differences, with male rats showing higher and more consistent harm aversion than females across seven days of testing. Notably, the intensity of the shock stimuli used in (Hernandez-Lallement et al., 2020) likely induced stronger behavioral and emotional reaction in the victim since it was higher than that used in (Hess et al., 2023), (1.5 and 0.8 mA, respectively). It is possible that shock intensity impacts female and male dyads differently, by modulating distress signals emitted by the victim as well as the aversive state triggered in the actor by those signals. It will be important to continue assessing behavioral and distress responses of both actor and victim rats during the task, including freezing and vocal emissions (i.e., squeaks, ultrasonic vocalizations). This assessment can be integrated with dyadic analysis methods that measure bidirectional transfer of information, as it has been performed to quantify mutual influences in freezing behavior (Han et al., 2019, 2020) or multimodal interactions (Gachomba et al., 2022). Such an approach would allow for a better understanding of the association between emotional contagion and harm aversion.

Summary

From the reviewed studies that assess prosocial behavior in response to negative emotions, the harm aversion paradigm is the least explored. Nonetheless, results show that rats and mice tend to avoid actions that produce distress on conspecifics, with marked individual differences. Data regarding whether other species also choose to avoid actions that hurt conspecifics is lacking. Harm avoidance, as for other types of decision-making, is subject to cost-benefit evaluation, and is not influenced by individual familiarity. Sex differences may emerge depending on task design and behavioral metrics and could be explored in further studies. Regarding the neural correlates associated with harm avoidance, ACC activity and its connections to downstream areas has been proved as necessary for this behavior, consolidating the role of ACC as a hub implicated in very different types of prosociality. Interestingly, ACC has been demonstrated to have a role in the processing of emotional responses

to vicarious fear in rodents (Carrillo et al., 2019; Zheng et al., 2020). The role of other brain areas deserves further study, as for example, witnessing a conspecific receiving shock also modulates DA release in the nucleus accumbens in rats (Lichtenberg et al., 2018), which may point to a possible role of DA in harm aversion.

1.3.4. Reward provision

Prosocial behaviors in the context of positive affective states of others is the least studied face of prosociality in rodents. Although the field has tremendously advanced in the last years as we have reviewed in the previous sections, it suffers from a strong bias towards the study of negatively valenced emotions. Helping others in distress (pain, fear, stress) is very relevant; however, adapting social decisions based on positively-valenced information from others is equally important, but has been much less studied. Several rodent species display affiliative behaviors in food-related contexts. For instance, food sharing among rats occurs naturally since the presence of shared feeding sites in the colony, where they allow conspecifics to eat in close proximity and even tolerate food stealing (Barnett, 1963; Galef et al., 2001). Consistently, wild and laboratory rats have been found to tolerate the presence of others in food locations, even if they could eat the food alone (Bartal et al., 2011; Colin & Desor, 1986; Grasmuck & Desor, 2002; Krafft et al., 2010). Thus, laboratory rodents could represent a valuable model to map reward-based prosocial choices to the mammalian brain.

An established paradigm for reward-based prosociality is the *prosocial choice task* (PCT) (Silk et al., 2005), which measures other-regarding preference for reward distribution. It was initially implemented for non-human primates to investigate the phylogeny of human prosociality and successively extended to other taxa (*for review see* (Cronin, 2012; Jensen, 2016; Marshall-Pescini et al., 2016)). In this task (Figure 3d), subjects are typically tested in pairs and often placed in adjacent compartments. The focal (decision-maker) can choose between two options presented in each trial, determining the reward payoff for itself and a recipient partner. Choosing the *prosocial* (or mutual rewards) option makes each animal gain a single reward, while choosing the *selfish* option provides a single reward for the focal only, and none to the recipient. Thus, the choice does not imply a cost or additional benefit for the actor in terms of reward number. To control for preference biases induced by reinforcing effects of food delivery,

the proportion of trials on which animals make a prosocial choice when the recipient is present (test condition) is generally compared to that shown in a control where the recipient is absent, or with a present recipient that is unable to access the food. If animals choose the prosocial option significantly more often in the test than in the control conditions, they are said to have a *prosocial preference*, which is taken as demonstration of their sensitivity to others' welfare. Variations of this task used across animal species have included a token version where subjects can choose between tokens that are exchanged with food items (Horner et al., 2011), designs using low and high-quality food (Lakshminarayanan & Santos, 2008), and designs where the focal can choose between an action in which no one benefits versus one that gives reward to the recipient only (null versus altruistic choice) (Burkart et al., 2007). Subjects' roles remain fixed or can be reversed over sessions (the focal becomes the recipient and vice versa) to assess the emergence of reciprocity (Lalot, Delfour, et al., 2021).

(Hernandez-Lallement et al., 2015) and (Márquez et al., 2015) were the first to adapt the PCT for laboratory rodents, showing evidence that rats display prosocial preferences in food-foraging contexts. These studies adapted this two-alternative forced choice task into different behavioral setups for rats, (Hernandez-Lallement et al., 2015) using a double box and (Márquez et al., 2015) in the form of an automated double T-maze. For both paradigms, in each trial over multiple day sessions, the focal rat could choose between the prosocial and selfish choice by entering either one of the two maze's arms, where food was delivered. (Hernandez-Lallement et al., 2015) tested non-cagemate male rats, food-deprived, that developed an overall prosocial choice bias of 55%, significantly higher compared to chance, and to a control where the recipient rat was replaced by a toy. Focal rats always entered the chosen rewarded arm before the recipient, reporting in this manner their choices, and having information of the recipient preferences and reward outcomes once the decision was made. (Márquez et al., 2015) tested male cagemates, non-food deprived, and reported an average prosocial choice bias of around 70%. In this task, instrumental helping, understood as prosocial actions performed to help others achieving a tangible resource (Warneken et al., 2007; Yamamoto et al., 2009) could be assessed, as recipients could display their attempts to access the rewarded arm before the choice of the decision maker, by repeatedly poking into a nose-port. Indeed, this work demonstrated that these displays of food seeking behavior by the recipients were necessary for the appearance of prosocial biases, but not sufficient, as information of

the reward contingencies of the recipient were also important for prosocial biases to emerge (i.e. choices of the focal animal flexibly adapted to changes in the provision of rewards to recipients). Furthermore, this work demonstrated that prosocial actions were goal-directed, being flexible upon changes of the reward contingencies for the recipient, and that local enhancement was not driving the emergence of prosocial choices. These findings indicate that prosocial choice for food provision in rats is enhanced by recipients' attempts to reach the reward, which may thus function as a social cue.

There are several differences in these two original paradigms that could explain the differences in the overall prosocial preferences, such as the different layouts of the setup itself, the strain of rats used, the use of familiar vs unfamiliar partners, using food deprived or non-food deprived animals, or the use of previous individual training or not before the social task. A highly likely explanation in this regard is the opportunity, or lack thereof, that the decision-maker animal has to gain social information of the recipient's preferences before and after the choice, which is in accordance with what it was shown before in chimpanzees (Warneken et al., 2007; Yamamoto et al., 2009).

In this direction, and further reinforcing the relevance of the actions of the recipient of help, Márquez's laboratory has recently studied how social dynamics prior to choice could explain the individual differences seen in prosocial preferences. (Gachomba et al., 2022) investigated the role of dominance relationship, sex of the pair, and familiarity of the recipient in the propensity to help others. Female dyads developed similar levels of prosocial choices compared to male dyads, and familiarity did not affect prosocial preference in males. Whether strain familiarity or affiliation, an important modulator in the rescue paradigm (Ben-Ami Bartal et al., 2014, 2021; Breton et al., 2022), affects prosocial choices for rewards is still unknown. To examine the effects of dominance in male rats, prosocial choices where compared between two groups of animals where the focal rat was either the dominant or the subordinate of the pair (and therefore its recipient was the subordinate or dominant, respectively), after pair social hierarchies were identified on the base of competition for food reward (Costa et al., 2021). Prosocial choices emerged faster and reached higher levels when the decision-maker was the dominant in the pair, with prosociality being positively correlated with dominance asymmetry. Prosocial choice directed "down the hierarchy" (i.e., more often from dominants to subordinates)

was accompanied by dyads in the two groups exhibiting different patterns of social interactions before the choice. Despite dominant and subordinate recipients displaying similar food-seeking behavior, the latter stayed closer to, and were more oriented towards, their dominant focal, especially before selfish choices, suggesting increased social attention. Consistently, rats from dyads with dominant decision-maker and subordinate recipient influenced more each other's movement relative to the decision ports, indicating increased attunement or coordinated behavior. Moreover, dominant focal's prosocial choice was found to positively correlate with the ultrasonic vocalization rate of subordinate recipients, while no such relationship was observed for the other group. These data associate the increased prosocial preference in dominant male rats with the dynamics of social interactions prior to choice. However, further research is needed to determine whether dominance status similarly modulates prosocial choices in female rat dyads, for which identification of stable social hierarchies has been elusive.

(Kentrop et al., 2020) developed an operant version of the PCT comprising one chamber divided into two compartments, one for the focal and one for the recipient rat, where the decision-maker was asked to report its choice by performing an instrumental action under different effort ratios. The focal could choose to either press a lever delivering mutual reward, a lever delivering reward to itself only, or an inactive lever. The location of reward delivery was the same for the prosocial or selfish choice, with feeder dispensers positioned at the center of the divider between the two compartments. The authors assessed the effects of early-life environmental enrichment on male rats' prosociality, by comparing adult rats that were pair-housed in standard cages to adult rats that were housed in more complex cages from juvenility, providing physical and social enrichment. Standard-housed males interacting with a same-sex familiar recipient were found to have on average 60% prosocial preference (significant against chance), under both a Fix ratio 3 (F3) and F5 lever press schedule. In contrast, complex-housed males did not show an overall prosocial bias. Interestingly, when the cost for prosociality was higher (by increasing the time for rewarding focal animals after the prosocial choice with respect to the selfish choice) no prosocial bias was observed, suggesting that rats do not necessarily show altruistic behavior in this task. In contrast to standard-housed males, no overall prosocial preference was observed in pairs of female rats (standard-housed), irrespective of the phase of the estrous

cycle. However, these results should be interpreted with caution as training and testing schedules were different between males and females.

How early life rearing can program prosocial choices in food-foraging contexts later in life has also been addressed in the context of stress. Male rats that experienced repeated periods of maternal separation during infancy showed reduced prosocial choice bias during juvenility compared to control animals (Joushi et al., 2022). Interestingly, this reduction in prosociality was prevented when rats experiencing maternal separation were either exposed to environmental enrichment or received intranasal OT administration for a short period after weaning. Given that both focals and recipients in the same group underwent maternal separation, it remains to be established whether maternal separation leads to decreased prosociality by affecting the social behavior of either the decision-maker or the recipient. Nevertheless, these findings highlight environmental enrichment and OT administration as potential interventions for preventing prosocial behavior impairments associated with conditions of early-life adversity. Future research would benefit from investigating whether these beneficial effects would extend to other types of distress that would negatively affect prosocial choices.

Recent works have started to evaluate prosocial tendencies for reward provision in laboratory mice. (Scheggia et al., 2022) expanded a standard operant cage hosting the focal mouse, with an adjacent compartment hosting the recipient. Naïve decision-maker mice learned to nosepoke on two ports, one delivering reward to themselves only, the other delivering mutual reward. Over testing days, focal mice paired with a recipient developed a bias for the prosocial option at the group level, whereas those trained without the recipient showed no overall preference. The former also performed higher amount of responses, suggesting that the presence of a conspecific increased learning performance or motivation to act. Focal mice classified as prosocial spent more time close to the divider separating the two animals, suggesting increased interest in the recipient. The authors trained animals under different conditions to identify factors modulating the preference. These included sex (only males developed an overall prosocial preference towards same-sex conspecifics whereas females did not, with half of the them preferring the prosocial choice and half preferring the selfish choice); effort (males previously classified as prosocial maintained a prosocial bias when the

effort for the prosocial option increased, while prosocial females switched faster to the easier selfish option); social contacts (impeding tactile contacts between mice prevented the emergence of the prosocial bias); familiarity (actors paired with non-cagemates acted more selfishly than actors paired with cagemates); recipient's hunger state (actors trained with food-restricted recipients had a higher prosocial preference compared to actors trained with sated recipients); and dominance (actors made more prosocial choices when they were dominant compared to their recipient, as assessed in the tube test, as also seen in rats (Gachomba et al., 2022)). Furthermore, this work linked prosocial biases with individual differences in social transfer of fearful emotional states. Interestingly, freezing duration of actor mice was positively correlated with their dominance rank, and it was higher in those categorized as prosocial than selfish. This suggests that prosocial and dominant mice show increased sensitivity to the negative affect state of a conspecific, which may facilitate prosociality. (Misiołek et al., 2023) also investigated food-based prosociality in adult mice, using a model partly based on the prosocial choice task for rats developed by (Hernandez-Lallement et al., 2015), where focal mice could choose to enter either one of two compartments associated with the different reward outcomes. Focals first underwent a pretest phase to determine that, on average, they had no preference for either compartment in the absence of the recipient. In contrast to (Scheggia et al., 2022), during testing with a same-sex partner, females, but not males, increased their prosocial choice preference relative to pretest. Further experiments showed that female and male mice showed comparable rewarding effects of social interactions in a social conditioned place preference test as well as similar affect state discrimination when interacting with a "neutral" vs food-deprived demonstrator, suggesting that these factors were not responsible for the sex differences observed in choice behavior.

The contradicting results between these two studies reflect the need of developing and implementing further paradigms to assess prosociality in food foraging contexts for mice, in order to expand our current knowledge on which factors promote and hinder prosociality in this species, and that allow for interspecies comparisons. The five studies assessing prosociality by reward provision in rats included in this review find that, even with marked individual differences between studies, overall rats are prosocial. However, the scarce data about mice shows contradicting results, which are hard to interpret altogether due to differences in protocols and paradigms used.

Providing future data in this direction is necessary to help enriching our knowledge about the basis of prosociality in food-related context. This will also improve our understanding of the natural differences about the social dynamics between rats and mice, helping researchers to better choose the appropriate animal model for addressing their questions, which in many cases are interchangeably used in neuroscience.

Little is still known about the neural bases of reward-based prosociality in rodents. Using the task they previously developed, (Hernandez-Lallement et al., 2016) investigated the effects of bilateral lesions to the basolateral amygdala (BLA) in adult male rats. Compared to control animals (sham operated), BLA-lesioned actor rats showed similar levels of prosocial choices in the non-social condition (toy as recipient) but made less prosocial choices in the social condition (recipient present) compared to controls (53%). Consistently with the involvement of BLA in rat prosociality, (Scheggia et al., 2022) showed that BLA neuronal activity of male mice increased at the onset of choice responses, with prosocial mice having higher BLA activity than selfish mice after prosocial choices. Chemogenetic inhibition of BLA glutamatergic neurons before daily test sessions prevented mice from developing a prosocial choice bias, and inhibiting BLA activity only during task learning had long-lasting effects, by reducing prosocial choices in the following testing days. Interestingly, BLA silencing also reduced social exploration, freezing during observational fear conditioning, and dominance rank in the tube test. It could be then hypothesized that BLA silencing, by modulating emotional contagion and dominance relationship, would affect prosocial choice in male mice. To provide insights into the role of cortico-amygdala projections, the authors targeted the prelimbic cortex. Chemogenetic inhibition of reciprocal BLA-PrL connections had different effects on choice preference. Inhibiting BLA→PrL projections slowed down the emergence of prosocial choices, whereas inhibiting PrL→BLA projections induced an overall shift towards the selfish choice. These findings reinforce the role of the amygdala in regulating distinct aspects of social behavior, including social decision-making, social transfer of fear, and dominance status and highlight cortico-amygdala connections as an important neural substrate coordinating prosocial and selfish decisions.

Interestingly, human fMRI studies showed that the sub region of ACC in the gyrus (ACCg) codes prediction error signals specifically when subjects learn to benefit others (Lockwood et al., 2016), and single-neuron recordings in rhesus macaques, *Macaca mulatta*, revealed that a high proportion of neurons in the ACCg exclusively responded to reward delivered to a conspecific (Chang et al., 2013). These findings highlight the role of the ACC in prosocial learning; however, if the ACC is required for developing prosocial choices in a PCT in rodents is yet to be assessed.

Summary

The prosocial choice task offers the possibility to study prosociality in rodents in a reward-based context. Variations of this instrumental learning paradigm have been useful to demonstrate that rodents often choose those choices that come with benefits for other conspecifics. Prosocial choice preference shows substantial variability, both intra and interspecies, it is modulated by different factors, and although results in reward-based prosociality are still scarce, some general principles are starting to be drawn. Food-seeking behavior is important in guiding rats' choice. Social dominance is a modulator (i.e., prosociality occurs more frequently from dominants to subordinates) both in mice and rats, while familiarity of the recipient affects prosocial choices in mice but not rats. In both rodent species, results regarding sex effects remain mixed, probably due to the lack of standardization between protocols. It has been shown that early life stress is associated with a reduction in prosociality later in life, which can be prevented by OT administration or environmental enrichment in the homecage; however environmental enrichment per se in non-stressed animals, seems to have a negative effect on adult prosociality. Regarding the neural correlates of prosocial choice, the evidences are still scarce, but prefrontal-amygdala circuits, which have been associated with social interest and social decisions in rodents and primates (Gangopadhyay et al., 2021; Huang et al., 2020), have been convincingly involved with the expression of reward-based prosociality. We are still lacking knowledge in key aspects of reward-based prosociality, most likely due to the bias that the field of social neuroscience has been suffering from favoring the study of negatively valenced emotions.

Table 1. Experimental studies revealing prosocial behaviors in rodents.

Experimental works revealing prosocial behaviour in rodents, along with the paradigm type, species and sex tested and main behavioural and neural substrates found.

PROSOCIAL BEHAVIOR IN **NEGATIVE** AFFECTIVE STATES

Species	Sex	Behavioral Readouts	Neural Substrates	References
		CONSOLATION		
Prairie voles	♂ → ♂ ♀ → ♀	↑ allogrooming towards stressed familiar conspecific.	c-FOS in ACC OT receptors in ACC	Burkett, et al., 2016
SD rats	♂ → ♂	↑ mechanical hypersensitivity, allogrooming and allolicking towards familiar conspecifics expressing pain.		C.-L. Li et al., 2018
SD rats	♂ → ♂	↑ mechanical hypersensitivity and allogrooming towards familiar conspecifics expressing pain.		Lu et al., 2018
Wistar rats	♂ → ♂	↑ allogrooming towards unfamiliar conspecific stressed by fear-conditioning.	c-FOS in PVN & CeA	Kiyokawa et al., 2019
Mandarin voles	♂ → ♂ ♀ → ♀	↑ allogrooming towards socially distressed conspecific. Chronic social defeat decreases consolation behaviour.	OT, GABAa, 5-HT1A and D2 receptors in the ACC DR→ACC 5HTergic	L.-T. Li et al., 2019, 2020, 2021
SD rats	♂ → ♂	Past pain experience increases allogrooming and observational contagious pain towards pain-experiencing unfamiliar conspecifics.		Luo et al., 2020
SD rats C57BL/6 mice	♂ → ♂ ♀ → ♀	↑ allogrooming and contagious pain towards familiar same-sex conspecifics experiencing pain. Modulated by sex in mice (↑ allogrooming by males than females) but not in rats.		Du et al., 2020
CD-1 mice	♂ → ♂	↑ allogrooming and allolicking towards conspecifics undergone surgery. Enhanced by familiarity.	PVT neurons containing orexin receptors	Zeng et al., 2021
C57BL/6 mice CD-1 mice	♂ → ♂ ♀ → ♀	↑ allogrooming towards socially defeated conspecifics. Enhanced by familiarity.	c-FOS OTR cells in AON, ACC, IC, LS, MeA (♀)	Matsumoto et al., 2021
C57BL/6J mice	♂ → ♂ ♀ → ♀	↑ allogrooming towards familiar conspecifics experiencing different types of stressors. Independent of sex.	Tac1⁺Vgat⁺ neurons in MeA →MPOA	Y. E. Wu et al., 2021
Swiss mice	♂ → ♂	↑ emotional contagion from and allogrooming directed towards chronically stressed familiar conspecifics.		Carneiro de Oliveira et al., 2022
C57BL/6J mice	♂ → ♂ ♀ → ♀	↑ allogrooming and body contacts towards fear conditioned conspecifics, independent on familiarity, sex, or observation of the partner's conditioning.	dmPFC	Philips et al., 2023
C57BL/6J mice	♂ → ♂ ♀ → ♀	↑ targeted allolicking and allogrooming towards familiar conspecifics experiencing pain via bee venom.	ACC	Zhang et al., 2024
		RESCUE		
Wistar rats	♂ → ♂ ♀ → ♀	Lever pressing to lower a distressed conspecific that was suspended from the floor.		Rice & Gainer, 1962
SD rats	♂ → ♂ ♀ → ♀	↓ door-opening latency to liberate a trapped conspecific from a restrainer. Modulated by familiarity with the conspecific's strain, and impaired by anxiolytic treatments.	c-FOS ACC →NAc	Bartal et al., 2011; 2014; 2016; 2021
SD rats	♂ → ♂ ♀ → ♀	↓ door-opening latency for liberating a soaked conspecific. Enhanced by prior experience.		Sato et al., 2015
SD rats	♂ → ♂	↓ door-opening latency for releasing a conspecific from a pool of water, independent of social interaction, and modulated by previous experience and conspecific familiarity.		Cox & Reichel, 2020
SD rats	♂ → ♂ ♀ → ♀	↓ door-opening latency for releasing a soaked conspecific from a pool.	OT receptors in ACC	Yamagishi et al., 2020
SD rats LE rats	♂ → ♂	Door-opening to liberate a trapped conspecific is hastened by the presence of potential helpers and hindered by incompetent bystanders, only when bystanders were from a familiar strain.		Havlik et al., 2020
SD rats	♂ → ♂	No overall preference for liberating a trapped conspecific over interacting with a free one.		Heslin & Brown, 2021
Wistar rats	♂ → ♂	Lever-pressing to release a trapped conspecific. Prosocial motivation modulated by the distress state of the trapped conspecific and housing conditions.		Kalamari et al., 2021
Wistar rats	♂ → ♂	High maternal care ↑ door-opening for liberating a soaked conspecific with ↓ latency in adulthood.	BDNF in Amy, Hip, IC, PFC, St	Asadi et al., 2021
SD rats LE rats	♂ → ♂	Adolescent rats, in contrast to adults, release restrained conspecifics of an unfamiliar strain.	Hip CA2	Breton et al., 2022
SD rats	♂ → ♂	↓ door-opening latency for releasing a conspecific from a pool of water. Distress USVs from trapped rat associated with attenuated rescue.	AI	Cox et al., 2022
Prairie voles	All combinations	↓ door-opening latency for liberating a soaked conspecific, more consistent for same- than opposite sex pairs. Reduced rescue behaviour, social proximity and huddling in Oxtr KO helper voles.	OT receptor	Kitano et al., 2022
LE rats	♀ → ♀	Door-opening to liberate a trapped conspecific from a restrainer.	mirror and anti-mirror neurons in IC and ACC	W.-Y. Wu et al., 2023
C57BL/6 mice AgRPcre/+	♂ → ♂	Door-opening to free a trapped conspecific from restrainer. Hindered by energy-deficit states (hunger, diabetes).	OT neurons in the PVN. AgRP neurons	Poza et al., 2023
		HARM AVERSION		
SD rats	♂ → ♂ ♀ → ♀	Forgo lever pressing associated with rewards to self and electric shocks to conspecific. Modulated by prior shock experience.		Church, 1956
SD rats	♂ → ♂	Change in preferred lever pressing to avoid electric shock to a conspecific. Modulated by prior shock experience.		Greene, 1969
Wistar rats	♂ → ♂	↓preference for time spent in naturally preferred dark chamber when associated with shocks to conspecific. Modulated by prior experience with shock.	c-Fos and specific oscillations in ACC, OFC and OAMY	Schaich Borg et al., 2017
SD rats	♂ → ♂ ♀ → ♀	Change in preferred lever pressing to avoid electric shock to a conspecific. Independent of sex and familiarity, modulated by reward benefit and prior shock experience.	ACC (24a, 24b)	Hernandez-Lallement et al., 2020
SD rats	♂ → ♂ ♀ → ♀	Forgo lever pressing associated with rewards to self and electric shocks to conspecific. Modulated by sex (♀ less prosocial).		Hess et al., 2023
C57BL/6 mice	♂ → ♂ ♀ → ♀	Switching preferred lever pressing to avoid shock to conspecific. Independent of sex and familiarity, modulated by self-experience with shock, and visual and social contacts (↓ when prevented).	ACC →MDL	Song and Wang et al., 2023

PROSOCIAL BEHAVIOR IN **POSITIVE** AFFECTIVE STATES

Species	Sex	Behavioral Readouts	Neural Substrates	References
		REWARD PROVISION		
SD rats	♂ → ♂	Decision-makers prefer choices that reward oneself and the recipient cagemate. Enhanced by recipients' food-seeking behaviour, goal directed. Non-food deprived.		Márquez et al., 2015
LE rats	♂ → ♂	Choice preference for mutual rewards in non-cagemates, food-restricted dyads.	BLA	Hernandez-Lallement et al., 2015, 2016
Wistar rats	♂ → ♂ ♀ → ♀	Preference for lever-pressing choices that reward oneself and recipient, in food-restricted dyads. Modulated by sex (females not prosocial), housing (complex housed rats not prosocial) and cost.		Kentrop et al., 2020
SD rats	♂ → ♂ ♀ → ♀	Focals prefer choices that reward oneself and recipient. Non-food deprived. Enhanced by social dominance (dominants ↑prosocial) which induces social attunement. Independent of familiarity or sex.		Gachomba et al., 2022
Wistar rats	♂ → ♂	Choice preference for mutual rewards in non-cagemates. Non-food deprived. ↓ by early maternal separation, ↑ by environmental enrichment.	OT	Joushi, et al., 2022
C57BL/6 mice	♂ → ♂ ♀ → ♀	Focals prefer choices that reward oneself and recipient, in food-restricted dyads. Dependent on sex (females ↓), effort, visual, olfactory and contact information (↓ when prevented), familiarity (↑ towards familiars), social hierarchy (dominants ↑) and recipients' hunger state (↑ towards food-deprived).	BLA - PLC	Schweggia et al., 2022
C57BL/6 mice	♂ → ♂ ♀ → ♀	Preference for choices that reward oneself and recipient, in food-restricted dyads. Dependent on sex (♂ not prosocial).		Misiolek et al., 2023

1.4. Vicarious reward as possible neural correlate of prosociality

Sharing emotions is a fundamental aspect of social animals living in groups. Early work started pointing out the relevance of studying emotion and its link to motivation in the context of prosociality and empathy. Vicarious emotions are thus an important part of our emotional repertoire, and despite the clear relevance for them in our social relationships, research attention to emotions of this kind has gained importance just recently (Batson & Shaw, 1991). Nowadays it is starting to be recognized that rodents are able to detect and react to negative affective states of others (Keysers et al., 2022; Gachomba, Esteve-Agraz et al., 2024) or the cessation of them (Scheggia et al., 2020). However, evidence about if and how rodents perceive positive states from conspecifics, and if those can promote the emergence of prosocial actions, has been scarce and far from conclusive. There is clearly a need for further studies on how positive emotional states are computed by the brain (*for review see* (Michon et al., 2023; Gachomba, Esteve-Agraz et al., 2024; Brosnan & Knapska, 2024)).

Vicarious reward processing is a form of emotional contagion based on the experience of conspecifics being rewarded. Recent studies have started examining the neural correlates of vicarious rewards in humans and found a notable involvement of different subregions of the prefrontal cortex (*for review see* (Morelli et al., 2015)). For instance, the ACC has been linked to the representation of the reward of others (Mobbs et al., 2009; Apps & Ramnani, 2014; Lockwood et al., 2015; Inomata & Shimada, 2020), and has been positively associated with prosociality (Contreras-Huerta et al., 2023). In resting state, (Inomata & Shimada, 2020) found an increased functional connectivity between regions of the default mode network (DMN) (i.e. ACC and precuneus) associated with an increased sensitivity to vicarious rewards. Interestingly, functional connectivity among other DMN brain regions have been previously studied in relation to empathy traits (Kim et al., 2017; Bilevicius et al., 2018). Some authors pointed out that the observer should internally simulate the other's action, intention and feelings when observing that person's action and consequent reward, in order to maximally appreciate another's reward (Shimada et al., 2016). Their results demonstrate that vicarious rewards by observing another person's successful actions are processed within the functionally coordinated activation of the reward system (vmPFC) with the premotor area, a principal component of the action

observation network (AON) (e.g. similar to the mirror neuron system but not requiring activation when performing an action by oneself) (Shimada et al., 2016). Brain regions pertaining to these two systems have been previously involved in observational learning (Monfardini et al., 2013), suggesting that components of the AON are sensitive to the outcome of another's action likely inducing vicarious rewards. In regard to the reward system, it has further been found that the Ventral Striatum (VS) activity increases during vicarious rewards, and it is modulated by the perceived similarity between the observer person and contestant of a game (Mobbs et al., 2009). In individuals with autism spectrum disorder (ASD), diminished activation of the NAc was found during vicarious, but not self-rewards (Greene et al., 2020).

In rodents, vicarious rewards have been hypothesised to be at the base of prosociality (Hernandez-Lallement et al., 2016), nevertheless experimental results on the neural circuits by which this perception might guide prosociality in foraging contexts are still scarce. There are evidences showing that mutual reward delivery drives associative learning about novel cues in a Pavlovian discrimination task in rats (van Gurp et al., 2020), and that mice can adapt their behaviours depending on the reward delivered to others (Choe et al., 2017), at least after strong rewards (electrical brain stimulation), as this was not observed when food rewards were used. Moreover, dopamine release in the NAc, a possible neural correlate of vicarious reinforcement, is initially increased in response to playback of affiliative 50-kHz ultrasonic vocalizations (Willuhn et al., 2014), and when observing a conspecific receiving reward (Kashtelyan et al., 2014). In this latter study, (Kashtelyan et al., 2014) described an increased release of DA in the NAc during the first trial of observation of a conspecific receiving a reward, however, this dynamic was followed by decreases in DA release in the following trials. Whether these complex DA dynamics might be explained by the conflicting information on reward outcomes, where a light-cue first predicts self-reward and then predicts reward for others (and lack of self-reward) needs to be clarified. Still, there is much to be learnt about the neuronal circuits supporting vicarious reward and reward-based prosocial choices. For example, if prosociality recruits mirror-like neurons (i.e., neurons that would respond when experiencing a rewarding state and witnessing another's rewarding state), in a similar fashion to what has been observed for attending another's pain experience (Carrillo et al., 2019; W.-Y. Wu et al., 2023), remains unexplored.

1.4.1. The ventral tegmental area and the reward system

In light of the above, it can be hypothesised that perceiving others being rewarded could involve neural activity of the reward system in a similar fashion as when receiving self-rewards (*see* (Morelli et al., 2015)). Rewards can be understood as natural processes during which the brain associates diverse stimuli with positive or desirable outcomes (Lewis et al., 2021). The mesolimbic system is the main dopaminergic reward pathway in the brain, but not the only one (*see* Figure 4), and it is composed by different structures responsible for the cognitive and physiological processing of rewards. Dopamine constitutes the main brain substrate in mediating the reward value for many different stimuli (e.g. food, sex, social interactions, and substances of abuse) (Robbins & Everitt, 1996). Upon recognition of a rewarding stimulus, the ventral tegmental area (VTA) stimulates the production of dopamine, and depending on the nature of rewards this mechanism can vary. Dopamine released from the VTA travels through the mesolimbic pathway towards the NAc neurons. DA binding to NAc neurons changes their activity, which is translated into feelings of pleasure and reward. VTA DA also projects through different pathways to other structures (Figure 4) like prefrontal cortex, amygdala or hippocampus that are also involved in limbic functions (Lewis et al., 2021). Altogether, the dopaminergic mesolimbic circuit gets interlinked to specific decision-making, memory and behavioural circuitries, giving rise to functionally relevant processes for refining and adjusting behaviours to optimize reward-seeking actions over time.

Although still uncertain, the VTA could possibly play a key role in the processing of positive emotional states of others through the release of DA into other brain regions. VTA DA release has been suggested as a neural substrate for social learning signals that drive motivated behaviours (Solié et al., 2022). Moreover, as stated before, DA release to the NAc seems to be involved in the processing of social rewards (Kashtelyan et al., 2014; Willuhn et al., 2014). Although most of the neurons in the VTA are dopaminergic, it is a heterogeneous region with a local architecture consisting of a variety of neurons (65% DA, 30% GABA, but also glutamate, acetylcholine) (Bouarab et al., 2019). Further evidence has pointed to it to play a crucial role in reward learning and to influence motivated behaviors through specific cell type functioning. For example, VTA GABA neurons are strongly modulated by drugs of abuse and stress (Bouarab et al., 2019). Glutamate in the VTA seems to regulate reinforcing behavior and aversive conditioning, in some cases independent from DA (Zell et al., 2020). Metabolic hormones

signalling in the VTA (ghrelin, amylin, leptin and insulin) have effects on synaptic and DA cell signalling function regulating motivated behaviours such as food-seeking (Geisler & Hayes, 2023). Whether and how this molecular heterogeneity is driving vicarious reward responses and prosocial choices still remains to be explored.

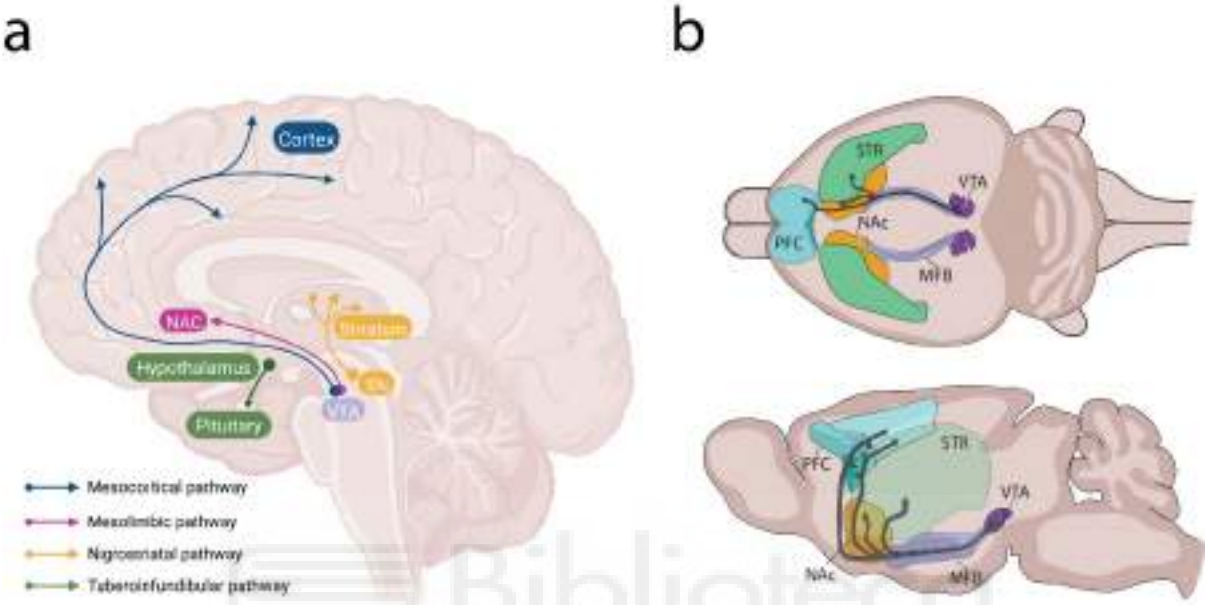

Figure 4. Dopaminergic pathways in human and mouse brain. a. Mesocortical dopaminergic pathway (blue) from VTA to the cortex, mesolimbic pathway from VTA to NAc (violet), nigrostriatal pathway (yellow) from substantia nigra to striatum and the tuberoinfundibular pathway (green) from hypothalamic nuclei to the pituitary (Fig adapted from Xu & Yang, 2022). **b.** Top and sagittal view of the mesocorticolimbic dopamine pathway in the mouse brain. DA projects from VTA to other limbic regions such NAc, striatum and prefrontal cortex. (Fig adapted from Reynolds & Flores, 2021).

Considering all these evidences, the ventral tegmental area could represent a major hub for processing self and other rewarding states, probably mediated by DA and/or other neurotransmitters release to other brain regions. Future work thus, should address if activity of specific VTA neurons process self and other rewards similarly, and if the experience of vicarious rewards could ease the emergence of prosocial choices.

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2 | Objectives

Nascent evidence based on works in rodent models is offering new foundational insights about the development of a sensitivity to the emotions of others. Rodents show a robust emotional contagion for the negative emotions of others and display prosocial behaviours relieving their distress, which ease the study of the behavioural and neural correlates of prosociality and empathy-related phenomena. However, despite the importance of sharing positive emotions, most of our knowledge about emotional transfer and prosocial behaviour is based in the context of distress.

My PhD work is aimed to address whether emotional contagion of positive emotional states can be studied in rodents, and further how they might be motivating prosocial behaviours, to advance in the knowledge of the underlying neural circuits. Although we considered advantages in continuing our studies with rats, the use of laboratory mice offers different benefits when aiming to circuit-level manipulations.

Thus, the current thesis establishes the following objectives and sub aims to be carried out:

2.1. Study prosocial choices in mice with reward-based paradigms:

- Development of a new custom-made behavioral paradigm for studying prosociality in mice;
- Detailed quantification and analysis of social and individual behaviours during prosocial choices;
- Assess how individual differences might modulate prosocial choices.

2.2. Identify the neural circuit underlying the motivation to help others:

- Assess whether vicarious reward responses are present in rats and can be measured at the neural level;
- Assess whether vicarious responses are necessary for the emergence of prosocial decisions in rats.

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3 | Materials and Methods

3.1. Animal subjects

Mice: 60 adult male C57BL6/J mice (632C57BL/6J, Charles River, France) were used, aged between 49-55 days at arrival to our facilities, with a body weight of 25±2 gr. Upon arrival from the commercial vendor, mice were group-housed (4 mice per cage) and maintained with *ad libitum* access to food and water in a reversed light cycle (12 hours dark/light; lights off 8 AM), in controlled temperature conditions. Paperboard and transparent acrylic cylinders were used as environmental enrichment in the home-cage. Mice were left undisturbed in their home-cages for the first two weeks at our Animal Facility to allow them to reverse their circadian rhythm and acclimate to the new environment and routines. Body weight was controlled weekly.

Rats: 40 adult male Sprague-Dawley rats (Charles River, France) were used in the experiments. Subjects were 8 weeks old and weighed between 226-250 g upon arrival to our facilities. Animals were pair-housed and maintained with *ad libitum* access to food and water under a reversed light cycle (12 hours dark/light cycle; lights off at 8:30 am) in controlled temperature conditions, and with a transparent red tunnel as environmental enrichment (8 cm diameter, Bio-Serv, # K3325). Rats were left undisturbed in their home-cages for two weeks, except for maintenance routines, allowing them to acclimatise to our Vivarium Facility and to reverse their circadian rhythm for the experiments. Experiments were performed during the dark cycle, waiting at least 1 hour and 30 minutes after the lights were off to start with behavioural procedures.

3.2. Experimental procedures to investigate prosocial choices in mice

We developed two different setups to evaluate prosocial tendencies in food foraging contexts in mice, one with a T-maze configuration (Akam et al., 2022), and a second one with a double chamber configuration. Both apparatuses were fully automated, in order to minimise interference by the experimenter while at the same time allowing for a precise control and detailed monitoring of the behaviour of the interacting individuals. In both tasks, the choices of an animal (***focal***, the decision-maker in the task) determined reward delivery for the ***recipient*** partner, allowing preference for 'prosocial' vs 'selfish' choices to be examined over sessions. Focal animals reported their choices by nose-poking between two available nose-pokes: one that provided food for itself and the recipient animal (prosocial choice) and another one that only rewarded itself (selfish choice). Recipient animals displayed attempts to obtain the reward by nose-poking repeatedly into a single nose-port. Mice worked for palatable pellets (20mg Dustless Precision Pellets, Bioserv #F0071) that were automatically delivered by a custom-made pellet dispenser into a food-receptacle. Before running any procedure with the animals, mice were habituated during a week to the experimenter by handling them for 5-10 minutes per day and to the food pellets used in the experiments to avoid stress related to neophobia.

The setups were derived and adapted from the Prosocial Choice Task (PCT) developed for rats by (Márquez et al., 2015), where two different processes were identified as crucial for the emergence of prosocial decision-making: **(1)** the food seeking behaviour displayed by the recipients of help while trying to obtain the food prior to the focal's choice, and **(2)** different reward contingencies in the reward areas of each choice and putative social information exchanged during these moments. The tasks developed and presented in this thesis were designed to include these two processes for the study of prosocial choices in mice. The differences in the structure and configuration of the two setups are explained below.

3.2.1. Maze-based configuration for prosocial decision-making task

The behavioural setup consisted of a fully automated double T-maze (Figure 5a-b). Each T-maze consisted of a central corridor (choice area) with nose-poke ports on

each side and two side arms (reward areas) each with a food receptacle connected to a pellet dispenser at the end. Access from the central choice area to the side arms was controlled by custom-made automated pneumatic sliding doors. Each individual maze (15x22x15cm) was built with laser cut white acrylic (3mm) and was connected to the other by a transparent and perforated acrylic (3mm). This transparent partition in the middle of the apparatus divided the maze in two, one for the decision maker and another for the recipient of help. For each individual maze, the central choice area was separated from the lateral reward areas with transparent acrylic walls, which allowed visibility of the animal in the side arms of the maze enabling tracking in the entire maze with one camera placed above the setup. These walls (12 mm) contained the mechanisms for the sliding doors (made from 3 mm transparent acrylic), animal's position IR detectors and 3D printed nose-pokes with sensors. All inner walls from the maze were gently scuffed with a fine sandpaper to avoid reflections of the mice in the walls that could interfere with automated pose estimation of the animals.

The task comprised two separate stages: **(1)** Individual training; in which animals learnt to navigate in the maze individually, opened doors by poking the ports in the central arms and retrieved pellets in the side arms. **(2)** Social task; where the decisions of the focal animal controlled the doors in both mazes, and determined rewards for both itself and the recipient animal.

3.2.1.1. Individual training protocols

During individual training, all animals were first habituated to the individual T-mazes in two sessions of 15 minutes, in which free exploration of the arena was allowed (i.e., all doors were open and nose-pokes and infrared beam detectors were inactive). Several food pellets were available for consumption in the food-magazines and floor of the maze in order to habituate animals to them. During the second session, mice were habituated to the gating of the automated doors, by opening and closing them non contingently of the animals' behaviour.

Then, mice were trained for three days to poke in the nose ports under a fixed ratio 1 (FR1) schedule (i.e. one nose-poke into the cued port required for obtaining a reward) in order to open the door that gave access to the food magazine where a food pellet was delivered for consumption. Both pokes were active during this stage and both sides were rewarded. Mild food restriction was performed during this early training, by

removing the home-cage food 2 h prior behavioural testing. Once training sessions finished, mice were allowed to eat *ad libitum* for the rest of the day.

Mice were randomly assigned to be the decision-maker (focal) or recipient of the help and tested in the social task with one of its cage-mates. From this moment onwards, focal and recipient mice were trained differently, and their roles were fixed throughout the entire experiment.

3.2.1.1.1. Focals' standard individual training

Focal animals continued individual training under a FR1 schedule during ten days for 20-30 minutes, where side biases were evaluated (Figure 6a). Briefly, a single poke in either of the two available LED-cued ports in the choice area triggered the opening of that same side door, allowing access to the lateral arm from where the animal could retrieve a pellet in the food magazine. An IR detector allowed to identify the moment when animals had reached the feeder area at the end of the lateral arm, moment in which the door safely closed in their back. Animals were allowed to retrieve and consume the pellets for a period of 10 seconds. Then, the door of the reward area opened again, allowing mice to go back to the choice area to start a new trial. Focal mice were allowed to freely choose any of the two pokes and hence get rewarded in the corresponding reward area.

3.2.1.1.2. Focals' reduced individual training

A shorter individual training was designed to assess whether the standard one was making animals to be less goal directed and inflexible in their choices once tested in the social task (*see results section* Assessing prosociality with low-trained decision-makers, Figure 12). In brief, focal animals in this experiment went through the two initial sessions of maze habituation. Then, they only performed a single session under fix ratio 1 schedule at the beginning of the training protocol, and a short training session just prior to social testing. This last session was limited to 20 mins or 6 trials, whatever was reached first. These two sessions were considered as the baseline for the side preference used to compare the prosocial preference during the PCT.

3.2.1.1.3. Recipients' individual training

During individual training for the recipient animals (Figure 6d), only one of the nose-poke ports in the central area was active (randomly assigned to be the right or left poke) cued with a LED, and the number of pokes required to access the reward arm increased over the training sessions. The rationale of this protocol was for recipients **(i)** to show a clear preference for only the rewarded side of the maze in the social task and **(ii)** to actively display food-seeking behaviour (nose-poking repeatedly). The first training sessions started with a FR1 schedule (i.e., only one nose-poke was necessary to open the door giving access to the reward). The quantity of pokes necessary to access the reward increased up to FR6 according to individual performance of the animals, thus ratio increased automatically when a given animal did five successful trials in a given FR schedule within a session (i.e., poking the required number of times, with a delay of less than 2 secs between pokes). Then, recipients were further trained under a variable ratio five schedule (VR5: pseudorandom list of pokes frequency needed to open the door, with an average of 5 pokes). In the last two sessions, recipient mice performing under this VR5 were forced to visit the unrewarded arm in 10% and 20% of the trials, in order to habituate them to enter and exit from the unrewarded area to initiate another trial. Finally, from the second day of PCT, a brief individual training was performed to the recipients before social testing to avoid extinction of food-seeking behaviour, as during the PCT they were not in control of their own reward delivery anymore.

3.2.1.2. Prosocial Choice Task protocol

During social testing, a pair of animals (focal and recipient) from the same home-cage were placed in the double T-maze, one in each side of the maze, separated by a transparent perforated partition. Animals were left to feed *ad libitum* during the entire period of social testing. In the social task, although both mice had access to the nose ports of their corresponding mazes, only those of the focal were active, and these controlled the automated doors of both mazes (i.e., a single poke to either port made by the focal animal opened the corresponding side doors in both mazes). The trial started when both mice were in the choice area. Recipient animals displayed food-seeking behaviour (poking into the port on the side where it would receive reward: prosocial side) while the focal animal controlled the recipient's access to the food-

baited arms. Importantly, the focal animal was rewarded for accessing either side, while the recipient animal was rewarded only on one side. The choice made by the focal animal therefore, determined whether the recipient animal received reward or not. Prosocial choices referred to choosing the side of the maze that provided access to food for both animals, whereas selfish choices referred to choosing the side of the maze that only provided food to the focal and not to the recipient. In this way, both choices provided the same amount of reward to focal mice. The reward of the focal animal was available immediately in the food receptacle after the choice was made, however, the recipient mouse only received its pellet once both animals were in the reward area, ensuring that information about the recipient receiving or not the food was available for the focal animal in each trial. Ten seconds after both animals entered the reward area, the doors of the recipient animal opened allowing it to return to the choice area and, once detected there by the corresponding infrared beam input, the focal's door opened allowing it to go back to the choice area to initiate a new trial. Seven sessions of 30 minutes were performed for each pair of animals.

3.2.2. Two-chamber configuration for prosocial choice task

This behavioural setup consisted of a custom-made white acrylic arena (16 cm long x 10 cm wide x 15cm high) which was divided in two individual chambers, one for each animal of a pair (8 cm long x 10 cm wide x 15 cm high). The two chambers were separated by a perforated and transparent partition that allowed the exchange of multimodal sensory information (Figure 13a-b). In each chamber there was a food-magasin connected by a tube to a custom-made pellet dispenser. In the decision-maker chamber there was a vertical double nose-port that animals used to display their choices, placed above the food receptacle. A start trial port was placed on the opposite wall. In the chamber of the recipient of help animal, there was a single nose-port above the food-magasin.

3.2.2.1. Individual training protocols in the two-chamber prosocial task

Before undergoing social testing, mice went through individual training according to their future role in the PCT. To this purpose we used a single chamber arena (8x10x15cm white acrylic box), which mimicked one chamber of the social setup, with a food receptacle and a dismountable poke-wall that allowed different training for focals and recipients. During the first two sessions, all mice were placed alone in the

chamber to allow free exploration and habituation to the arena for 10 minutes. Habituation to food-pellets was obtained by allowing animals to freely consume the available pellets placed on the floor of the chamber and in the food-receptacle. Then, all mice followed an individual training session with FR1 schedule for obtaining rewards, during three sessions, after which we randomly assigned the roles for the future social task (2 focals – 2 recipients per homecage). Individual training from this point diverged for focals and recipients. Short food restrictions (2h before the behavioural testing) were performed in all stages of the individual training to increase motivation for food-seeking behaviours.

3.2.2.1.1. Focals' individual training

Decision-maker mice were trained to obtain rewards under a FR1 throughout all the individual training (Figure 14a). Because of the configuration of this setup, focal mice reported their willingness to start a trial in a self-paced manner, by performing a poke in the start trial poke, after the 10th individual training session. During the early sessions of individual training, some of the focal mice showed an increased preference for the bottom poke (the one closer to the food magasin and that did not require to rear). When strong biases were observed, the preferred poke was blocked for some trials (i.e. pokes were not followed by reward delivery) forcing animals to explore the non-preferred option. Baseline sessions before the social task did not contain forced trials and thus reflected the individual preferences of each animal.

3.2.2.1.2. Recipients' individual training

Recipient animals were trained to poke in order to collect food rewards in the food magasin, by displaying a strong food-seeking behaviour (nose-poking repeatedly) (Figure 14c-d). The individual training comprised different sessions with an increasing nose-poke ratio, starting from FR1 until FR5 nose-pokes in order to obtain a reward. Recipient animals were re-trained in this individual protocol during the social testing days (except for the first session), in order to avoid extinction of food-seeking behaviour, as during the PCT they were not in control of their own reward delivery anymore.

3.2.2.2. Prosocial Choice Task protocol in the two-chamber arena

After individual training, mice were tested with the prosocial choice task in the two-chamber setup. Trials started when the focal nose-poked into the start-trial poke. The recipient could display food-seeking behaviour by nose-poking repeatedly into its nose-port, while the decision-maker could choose the top or bottom poke, which were counterbalanced between the pairs of animals to be prosocial and selfish choices. Choosing any of the options would always deliver a rewarding pellet for the decision-makers; however, the rewards for the recipient would only be available after a prosocial choice. Twelve animals were tested in the social task, in eleven – 40 minutes sessions. In four of these animals data was only available for the first 4 sessions due to experimental problems. Mice underwent food-restriction 2h prior social testing.

3.2.3. Hardware and peripherals

Both behavioural setups (double T-maze and double chamber) were custom-made built using laser cuter (Epilog Laser – 60W) and 3D printing (MakerBot Replicator 2 and Ultimaker 3), and progression of the task structure was controlled by pyControl (Akam et al., 2022). State-machine peripherals used from pyControl were adapted to fit our configurational needs. The latest modifications used are explained below.

- Nose-ports (both setups)

Four pyControl nose-poke devices were used for controlling both decisions and food-seeking behaviour, two per individual maze. The IR-beam arms and LED were desoldered from the nose-poke boards and extended with wires so we could keep the components inside the setup and the boards outside the sound-attenuation box. These components were attached to a custom-made 3D printed nose-port piece which was attached inside the 1.2 cm inner walls that divided the different areas (Figure 5a-b)

- IR detectors for detecting animal position to control behavioural state (maze setup)

Three pyControl nose-poke devices were used to detect the animal position inside the mazes to drive the behavioural state machine. In order to use them, the devices were modified. We desoldered the IR-beam arms, extended with wires to keep the components inside the mazes and the boards outside the sound-isolation box, and we

also changed the IR emitter (TSUS5202, Vishay) to allow for a longer detection range given by the width of the corridors (6 cm for choice area and 7 cm for reward areas).

- Sound attenuation box (both setups)

Each double T-maze was located inside a custom-made sound attenuation box. They were built on a 40x40 cm wooden cabinet with a single door. Sound isolation material (Regular Panel 60.2 Premiere, EliAcoustic) was placed in all inner walls, which provided 20 dBs sound attenuation. The inside of the isolation boxes was illuminated with dim white light (4 lux) and infra-red stripes located on the ceiling of the box. There was a 5x5cm ventilator placed on the middle of the back wall to regulate the inner temperature, and a 3cm diameter hole below it to pull all hardware wires out of the boxes. In this way we organised all wiring and electronic boards outside the boxes, to decrease possible audible and temperature interferences.

- Custom-made pellet dispenser (both setups)

Food pellet rewards were delivered using custom-made pellet dispensers, which were built of a mix of 3D printed and laser cut parts, and actuated by stepper motors (NEMA 42HB34F08AB, e-ika electrónica y robótica, Spain) controlled by a stepper motor driver board. All of these were placed outside the sound attenuation box, to minimise the impact of the possible sound cues during the experiments. The palatable food pellets were dispensed to 3D printed food receptacles attached to the walls of the maze with magnets through a silicon tube that crossed the isolation box. Design files for the pellet dispenser and food receptacles can be found **here**.

- Custom-made pneumatic doors (maze setup)

The sliding doors that control access to the different areas were made from 3mm transparent acrylic and built on top of a 3D printed piece containing a ball-bearing to allow smooth sliding of the door. These were actuated by pneumatic cylinders (Cilindro ISO 6432, Vestonn Pneumatic, Spain) placed below the base of the maze, providing silent and smooth horizontal movement of the doors. These were in turn controlled via solenoid valves (8112005201, Vestonn Pneumatic, Spain) interfaced with pyControl by using an optocoupled relay board (Cebek- T1, Fadisel, Spain), to prevent from possible electrical interferences coming from the solenoid valves coils. The speed of

the opening/closing of the doors could be independently regulated by adjusting the pressure of the compressed air to the solenoid valves.

3.2.4. Data acquisition systems and data analysis

3.2.4.1. Video data

Individual training and experimental sessions were recorded with a high resolution infra-red sensitive camera (PointGrey Flea3-U3-13S2M CS, Canada) under infra-red illumination, capturing at 30fps with 1280x960 pixels resolution. Cameras were positioned centred above the setups to enable fine tracking of the animals' position and pose estimation. Visual reactive programming software *Bonsai* (Lopes et al., 2015) was used to trigger the recording of the cameras and to start the behavioural task. It was also used to temporarily crop the experimental session videos into single trial videos during post-processing, using the IR light of the nose-ports that indicated the start of each trial and the choice moment.

3.2.4.2. State machine and behavioural data

Behavioural data and general position of the animals in the automated mazes were extracted from pyControl software (Akam et al., 2022), and parsed with Python 3.0. For each session and animal, we extracted: number of trials, performance (trials/min), trial duration, prosocial preference (prosocial choices/total trials), frequency of nose-pokes, poke specificity, latency to decide. Moreover, for the experiments performed with the maze-like configuration, we extracted the latency to enter and to exit the reward areas after the automated doors opened.

3.2.4.3. Pose estimation of mice and behavioural quantification

For tracking and pose estimation of the animals, we used the Bonsai-DeepLabCut interface (Python 3, DLC 2.2) (Kane et al., 2020). We first trained a ResNet-50 neural network for pose-estimation on labels of single animals taken from videos of a single individual maze (cropped image). Specifically, we labelled 426 frames from 5 videos/animals (95% was used for training with a 0.6 p-cutoff) for 600,000 iterations keeping the default network parameters. Then, a custom workflow of the Bonsai-DLC interface was used to batch-track the body pose of the interacting mice in all single trial videos from all PCT sessions. The workflow was able to simultaneously track the

mice on any of the sides of the double maze, by applying a ROI for each side with an offset of the maze to maintain the original frame coordinates.

Finally, Python 3.0 scripts were written to analyse the social interactions happening during the experimental sessions. Tracking data from trials was split between prior and after choice period. We then extracted the coordinates position of body parts, which were used to compute different parameters to study the social dynamics during the social task. We extracted the location of the snouts of the mice and computed the euclidean distance between the animals and the distance in the X coordinate of the snouts from the central partition. Relative head-orientation of the two interacting animals was calculated by computing the angle-line from the label in between the ears of **animal A** and the label of the nose of **animal A**, minus the angle-line in between the ears of **animal A** to the nose label of the **animal B**. Furthermore, these variables were extracted in specific regions of interest such as the two nose-pokes in the choice area, the area around the wall dividing the two mazes in the choice area, and around both food-receptacles in the selfish and prosocial reward areas. Also, time spent in these ROIs was calculated.

3.2.5. Statistical analysis

Data extracted from the state-machine pyControl and the pose-estimation from DLC was parsed and processed with Python 3. We then used IBM SPSS Statistics 26 and JASP 0.16.2 to perform probabilistic and bayesian analysis on statistical differences between the extracted and studied variables.

Prosocial preference: repeated measures (RM) ANOVA with ‘session’ as within subjects factor was performed to assess the prosocial choice preference over the course of testing sessions (Figure 5d). In the 2-chambers setup, we further evaluated preference for the bottom nose-poke (Figure 13e), where proportion of choices towards the bottom choice was calculated over sessions using RM ANOVA the same way.

Prosocial Choice Index: we computed a prosocial choice index (PCI) to quantify individual differences on choice preference against chance over testing sessions,

$$PCI = \frac{Pref_{test} - Chance}{Pref_{test} + Chance}$$

where $Pref_{test}$ corresponds to the proportion of prosocial choices during social testing sessions, and $Chance$ is understood as the proportion of choices equal to 50%. The PCI values show the strength of change in prosocial preference from 50% preference for each mouse; [+] PCI show an increase on prosocial preference on social testing sessions compared to chance, [-] PCI show a decrease on prosocial preference from 50%, while the values close to 0 show no change. We performed a one-sample t-test to assess if the distribution of PCIs was different from chance (0) (Figure 5e). For the case of the bottom preference (Figure 13f), we computed the PCI for the opposite preference of those mice that had the prosocial option on the higher poke.

Permutation test to classify mice as prosocial, selfish or unbiased according to their preferences: to address individual variability on prosocial preference, we performed a permutation test to identify those mice that showed significant change on choice preference against chance. For each animal separately, we generated a distribution of 10.000 permuted PCIs by shuffling the sequences of all choices during social testing with same-length sequences of choices with prosocial preference equal to 50%. Mice then were assigned to three different categories by comparing their actual PCI to the 95% confidence interval (CI) of the distribution of randomised indexes (mouse with actual PCI in 2,5% upper bound was considered as prosocial, mouse with PCI in 2,5% lower bound was considered selfish, and those mice with PCI falling inside the 95% were considered as unbiased). Lower and upper bound for each individual's distribution of each experiment can be found in (Table 2).

Side preference (individual training): we used one sample t-test to check for differences in the side preference against chance (50) on the last two days of the individual training of mice for the sets of experiments. (Figure 6c)

Latency to decide: we compared the latency, from trial onset to nose-poke (choice) at either the prosocial or selfish pokes, using paired samples t-test. (Figure 8b)

Time exploring nose-pokes: for focal and recipient animals we computed the time spent exploring the area around the prosocial and selfish nose-pokes per trial. For this, we computed the time that the DLC labels of the head and nose of both animals was detected in the two different ROIs around the two nose-ports, to ensure that we were measuring exploratory behaviour. This included the moments where animals were nose-poking, but also sniffing and investigating around the port, and was considered as a more global measure of investigation of the options. We used paired

samples t-test to assess for differences between the conditions (i.e. prosocial or selfish ROIs). (Figure 7d and Figure 8c)

Latency to visit reward areas: we used paired samples t-test to assess differences in the averaged latency to enter into the prosocial and selfish reward areas after the choice moment, for focals and recipients. (Figure 7e and Figure 8d)

Time exploring feeders: we calculated the time that the head of focals and recipients was detected inside a ROI around the prosocial and selfish feeder (similar to that for the time exploring the nose-pokes) as a general measure for feeder investigation. We used paired samples t-test to assess for differences according to trial type. (Figure 7f and Figure 8e)

Latency to leave reward areas: we used related samples t-test to assess for differences in the latency to exit from the prosocial or from the selfish reward areas to start a new trial for both focals and recipients. (Figure 7g and Figure 8f)

Pokes frequency: we used paired samples t-test to assess differences between the frequency of pokes that recipients did to each type of nose-port per trial. (Figure 7b)

Pokes specificity: we calculated the specificity of the nose-pokes (nº prosocial pokes/ total nº pokes *100) done by recipients and used related samples t-test to assess differences according to trial type. (Figure 7c)

Time in close distance: using data from pose-estimation, we calculated the time that both animals spent in a distance less than 60 pixels (equivalent to nose-to-nose investigation). Paired samples t-test was used to evaluate differences according to trial type. (Figure 9b)

Interaction time in partition: we calculated the time that both animals spent together in a ROI around the partition in the choice area prior to decision. We used paired samples t-test to evaluate differences according to trial type. (Figure 9c)

Social distance during interactions in partition: we calculated the Euclidean distance between the nose label of focals and recipients during the interactions prior to choice in the partition ROI. Paired-samples t-test was used to assess for differences in this measure according to the trial type. (Figure 9d)

Distance to wall during interactions in partition: we calculated the distance of both focals and recipients towards the partition that separated both animals during the

interactions prior to choice in the partition ROI. RM ANOVA was used to assess for differences in the distance to the partition of focals and recipients according to the trial type. (Figure 9e)

Head orientation during partition interactions prior to choice: we calculated the head orientation towards the other animal for both focals and recipients during the interaction in the partition of the choice area. We used independent samples t-test to assess for differences between them. RM ANOVA was used to assess for differences in the head orientation of focals and recipients according to the trial type.(Figure 9f-g)

Time interacting in feeders: we checked the time that both animals spent interacting in the reward areas while being next to the feeders (ROI that comprised the feeders and the part of the corridor adjacent to the other animal) and used related samples t-test to check for differences according to the trial type. (Figure 10b)

Head orientation during interaction in feeders: we calculated the head orientation of both focals and recipients during the interaction time in each of the reward areas. We used independent samples t-test to check for differences between the angles of the animals according to their role. RM ANOVA was used to assess for differences in the head orientation of focals and recipients according to the trial type. (Figure 10c-d)

Distance to wall during interaction in feeders: we calculated the distance of both focals and recipients towards the partition that separated both animals during the interactions in the reward areas. We used independent samples t-test to check for differences between focals and recipients. RM ANOVA was used to assess for differences in the distance to the wall of focals and recipients according to the trial type. (Figure 10e-f)

Individual differences between prosocial and selfish pairs: we quantified (1) the total nose-pokes performed by recipient mice during the first session of PCT, (2) the latency of recipients from choice to until entering into the reward zones, (3) the distance to the wall of focals during the interactions in the reward areas, and (4) focals' latency to leave the prosocial reward area during the first session of the PCT. We then used one independent samples t-test to assess for differences in each of these variables comparing the extreme groups of the prosocial category (prosocial vs selfish animals). (Figure 11)

3.3. Experimental procedures in rats for identifying neural circuits of prosocial choices

3.3.1. Vicarious Reward Task

To assess whether rats react to positive affective states of conspecifics we designed a novel custom-made behavioral task (Figure 15a) where two animals are placed in contiguous compartments in a sound attenuation box, and where one animal (the focal) can witness a conspecific (the recipient) receiving rewards (45 mg Dustless Precision Pellets, Bioserv #F0021). During this task, we record calcium transients in VTA with fiber photometry (*see* Calcium imaging with fiber photometry *section*). The structure of the task was designed and run with pyControl and consisted in the alternate delivery of pellets to both focal and recipient animals.

Trial structure: a pellet was delivered first to the focal rat (self-reward) and after a pseudo-random time interval (between 3 to 5 seconds), a pellet was delivered to the recipient animal in the adjacent chamber (other-reward). Latency for delivery of the pellet to the recipient after self-reward, and inter-trial intervals (between 20 to 39 seconds) were pseudo-random to prevent predictability of the rewarding events (Figure 15b). 3 pairs of rats were tested in this task and underwent two consecutive daily sessions that lasted 30 minutes. One focal animal lost the fiber implant during the second day of testing thus data from this second session was not included in the analysis.

3.3.1.1. Vicarious reward task setup

The behavioural apparatus consisted of a custom-made 50 cm x 30 cm arena, made out of 5 mm black acrylic that was separated into two chambers (25 cm x 30 cm per animal) by a transparent and perforated partition to allow the interchange of multiple sensory information. Swinging doors placed in one of the walls of each chamber allowed to carefully place each animal into the setup. On the opposite wall, a protruding custom-made food receptacle allowed animals to safely retrieve the pellets without endangering the fiber implants. This food receptacle contained an IR detector which indicated when the pellet was consumed and was connected to a custom-made pellet dispenser located outside the setup. The arena was placed inside a custom-

made sound attenuation box, to minimize outer interferences during the experiments. A high-resolution infra-red camera was placed and held on top of the attenuation box which was illuminated by infrared LED stripes

3.3.1.2. Handling and habituation prior to social task

Before the social task, animals were handled for 5 sessions; the first 3 sessions occurred in consecutive days for 5 minutes each subject.

In the last two sessions, focal rats were habituated to the handling process plus to the attachment of the patch-cord to the implant in their heads. In the last session before the social test, focal rats were individually habituated to the arena, where they received pellets in the receptacle following a pseudo-random time interval (ranging between 5 to 60 seconds) for 30 minutes.

In the case of recipient rats, for the last two sessions before the social task, they were individually habituated to the arena, where they received pellets in the receptacle following a pseudo-random time interval (ranging between 5 to 60 seconds) for 30 minutes.

3.3.2. Prosocial Choice Task (PCT)

To assess how VTA activity linked to the perception of others being rewarded contributes to prosocial decision-making process, we tested 13 pairs of non-food deprived rats in the Prosocial Choice Task (PCT) (Figure 17a). During the task we performed closed-loop optogenetic loss of function of VTA activity of decision-makers, specifically during the moments of the reward to the recipient (i.e. only during prosocial trials).

Trial structure: in the prosocial choice task, the choices of a decision-maker animal (focal rat) determined the availability of rewards for a recipient animal, allowing for the assessment of prosocial preferences, which occurs naturally in rats (Márquez et al., 2015). For each pair, one rat was assigned to be the focal (decision-maker) and the other the recipient. Rats learned individually to move around the maze and retrieve pellets before the social task. After individual training, rats were tested in the PCT for five consecutive daily sessions of 40 minutes. A trial would start when both animals were present in the central corridor, giving simultaneous access to the choice area. There,

recipient animals would display food-seeking behaviour by performing nose pokes on the side where they were previously trained to receive the reward. Focals could choose between poking on the same side of the recipient, providing access to reward for both animals (prosocial choice) or poking on the opposite side, where only the focal received one pellet and the recipient none (selfish choice). In both choices, focals' doors for reward would open first, allowing them to access the pellet, and 2 seconds after the doors for recipients opened. This temporal delay in reward delivery for the recipients was set in order to enable manipulation of VTA neural activity during moments of the other-reward (i.e. reward to the recipient), without interfering with VTA natural dynamics during moments of self-reward (reward to the decision-maker). Wireless optogenetic inhibition of the VTA of decision-makers started from the moment the recipient approached the reward magasin and only after a prosocial choice.

3.3.2.1. Prosocial choice task setup

The behavioural setup consisted of a fully automated double T-maze (Gravaplot, Sintra, Portugal), controlled by Graphic State 3.03 software and the Habitest interface (Coulbourn Instruments, Allentown, PA, USA). The double T-maze was divided with a transparent and perforated partition into two fully identical individual mazes, one for the decision-maker and another for the recipient of help. Each T-maze comprised a central corridor, a choice area and two lateral arms for reward. The central corridor gave access to the choice area through an automated door. In the choice area there were two nose-ports, one in each side wall, which animals needed to poke in order to activate the infrared beam controlling the doors underneath. Once in the lateral arm, rats were able to retrieve the food (one pellet per trial), triggering the gating of the door that gives access to a runway leading to the starting point at the central corridor, thus initiating a new trial. The roof of each maze consisted in transparent and perforated, 2 mm-thick acrylic walls. In addition, a transparent, 2 mm-thick acrylic wall was positioned on top of the central wall separating the two mazes. These acrylics served to prevent the animals from jumping outside their own mazes. During individual training opaque acrylic walls were placed in each T-maze, thus isolating them, covering the communicating holes and preventing the rat in one maze from seeing the other maze. After the individual training, the opaque acrylic walls were removed for the PCT.

3.3.2.2. Individual training protocols

After two weeks of habituation to the new facilities and before individual training, rats were handled for a week allowing them to habituate to the experimenter. During handling days rats were also habituated to the palatable pellets used in the behavioural tasks (45 mg Dustless Precision Pellets, Bioserv #F0021), by placing them on the bedding of the home cage or from a feeder magazine placed inside he homecage.

Then for the individual training, each rat of a pair was randomly assigned to be the decision-maker or the recipient, and their roles were fixed throughout the entire experiment. Prior to individual training, decision maker rats underwent stereotaxic injection of viral vectors for optogenetic manipulation of the VTA during the PCT (*for further details see* AAV injections *section*). All animals were habituated to the individual T-maze for 4 daily sessions of 15-20 min each, during which rats were allowed to explore the full maze and retrieve the pellets that the experimenter previously placed over the floor and in the food receptacles. In the last two sessions, the doors of the maze were manually activated so that the animals could habituate to the noise produced by their gating.

After habituation, during the first session of individual training, all animals were shaped to rear in order to poke in the nose port for opening the door that gave access to the food receptacle. Rats could enter either side arm and were rewarded with one pellet per trial. From this moment, focal and recipient rats received distinct kinds of individual training, for a maximum of 12 sessions of 20-30 minutes each.

Focals learned to access both reward sides of the maze by performing choices under a fixed-ratio 1 (one poke into the nose-port to open the door that gave access to the reward arm) in the choice area. After the choice, they had access to the food receptacle on the corresponding reward arm, after eating they could go back to enter the central corridor through a runway for running another trial. Decision-makers ran up to 10 sessions, until they reached a performance of at least 1.5 trials/minute. Rats tend to alternate, and no side preference was observed at the end of the training (baseline). Before ending the individual training, decision-makers went through stereotaxic surgery for optic fiber implantation (*for further details, see* Optic fiber implantation for optogenetic loss of function experiments in the PCT). After at least 4 days of recovery,

focal rats were habituated to the attachment of the wireless device into the chronic implant with a dummy replica of the sensor and ran the last three sessions of individual training before the Prosocial Choice Task (baseline).

For recipients, only one reward arm and the corresponding nose-port in the choice area were active through the entire training sessions (counterbalanced between the pairs of rats). Thus, recipients learned to poke only to one side and the number of pokes required to access the reward arm increased over the training sessions, to ensure clear side preference and salient food-seeking behaviour (*for further details, see* (Márquez et al., 2015)). In the last 2 sessions, recipient rats were forced to visit the opposite unrewarded arm in 10 and 20% of the trials, respectively. The rationale was for recipients to learn that even if no pellet was delivered on the unrewarded side, they had to visit it to complete the trial and to start a new one. Finally, after the first day of the PCT, recipients underwent a brief individual training immediately before each session of the PCT to prevent extinction of food-seeking behavior.

3.3.3. Alone preference test

In order to account for a possible aversive effect of the VTA inhibition that could be influencing the preference of the focal rats during the social decision-making task, we ran an experiment to assess how the optogenetic inhibition of the VTA affected the choices of the focals being alone in the maze used for the PCT (Figure 17g).

The experiment comprised two different sessions:

- **Session 1**: **Alone baseline preference**. The day after the last session of the PCT, wireless optogenetic sensor was connected to the implant of focal rats. Then, focal animals were tested alone in the same side of the maze where they ran the PCT. During this 20-minute session, decision-makers could freely choose between the two sides of the maze under a fixed-ratio 1. Side preference was calculated to be used for the following session. No light was delivered at any time of the session.

- **Session 2**: **VTA inhibition after self-reward in preferred side**. The day after the baseline preference session, decision-makers underwent another session of 20 minutes alone in the maze with the optogenetic sensor attached to their implants. During this session, focals could freely choose between the two rewarded sides. However, two seconds after a reward delivery on the preferred side, the optogenetic

sensor turned the light ON for inhibition of the VTA activity, mimicking the temporal dynamics when recipients would be rewarded. In this manner, we could assess the preference change from session one to session two in a non-social setting, ascribing any possible change to the optogenetic manipulation effect.

3.3.4. Real-time place preference test

To further control for possible aversive effects of the inhibition in the VTA, we assessed the effects of the optogenetic loss of function of the focal rats on a non-social context preference test. During this experiment, animals were placed in the arena, allowing free exploration of the chambers for 5 minutes (Figure 17h). One of the two chambers was systematically associated with optogenetic inhibition while the other was not associated with any optical stimulation, in a counterbalanced manner across animals. Animals' position was recorded in real time with Bonsai; thus, the sensor turned the light ON for constant optogenetic inhibition if and while the animals were visiting the chamber that was associated with optical stimulation. With this closed-loop manipulation, we could assess if the preference for the time spent in any of the chambers was altered by the effect of the light inhibiting the VTA.

3.3.4.1. Real-time place preference test setup

The behavioural paradigm consisted in a rectangular arena (60 cm x 40 cm), divided into two separated chambers with different contextual patterns (circles and stripes) on the walls of each chamber. In between the two, there was a divisor wall separating the two chambers, and a small zone where animals were placed into the arena, and from where they could access any of the sides at any time of the session. An infra-red camera was placed on top of the arena to record the position of the animals within the session.

3.3.5. Stereotaxic procedures

3.3.5.1. AAV injections

For injections of the following viral vectors:

- **AAV-Syn-Flex-GCaMP6s** (Addgene plasmid # 100845; http://n2t.net/addgene:100845 ; RRID:Addgene_100845)

- **AAV-hSyn-EGFP** (Addgene plasmid # 50465; http://n2t.net/addgene:50465; RRID:Addgene_50465)

- **AAV-CKIIa-stGtACR2** (Addgene plasmid # 105669; http://n2t.net/addgene:105669; RRID:Addgene_105669), 1/100 dilution from original titer.

- **AAV-CAG-tdTomato** (Addgene plasmid # 59462; http://n2t.net/addgene:59462; RRID:Addgene_59462)

rats were anesthetized with a mixture of oxygen (1.5L/min) and isoflurane (4% for induction, 1-2% for maintenance), weighed and placed in the stereotaxic frame (KOPF, Germany). Rats were injected intraperitoneally with an analgesic compound buprenorphine (0,05mg/kg). Then, the skin of their heads was shaved and disinfected. A midline incision was performed with a scalpel and the area was cleaned. Bregma and Lambda points were identified in the skull and a craniotomy was performed for unilateral injection into the VTA (AP: -5.52, ML: +- 0.6, DV: -7.6 mm from Bregma). Viral injection was performed using a 1 µL Hamilton syringe filled with mineral oil, connected to an injector cannula, and using a micro syringe pump to control for volume and speed. 10 minutes after injection finished, the injector cannula was slowly extracted from the brain, and the skin was sutured with stitches. A dose of buprenorphine was administered intraperitoneally, and rats were kept under surveillance until they woken from the anaesthesia and then were put back to the homecage with their partner.

3.3.5.2. Fiber implantation for calcium imaging photometry

For the calcium imaging experiments (Figure 15), rats underwent infusion of AAVs, as explained above, and were not sutured but instead implanted with an optic fiber above the VTA after viral injection, during the same surgery. For this, three other small craniotomies were performed around the virus craniotomy, and ~0.5 mm diameter stainless steel screws were bolted into the holes in the skull to secure the implant. Then, an optic fiber (ø 400µm) was implanted above the VTA. For extra fixation of the implant to the skull, a thin layer of Super-Bond was applied onto the screws and the skull and finally, everything was covered with dental cement (Contemporary Ortho-Jet, LangDental #1530BLK), creating a long-lasting implant.

After the cement was dry, animals were removed from the stereotaxic frame, administered with buprenorphine and let to recover in a clean cage with food and water, placed on a heating pad until awakened, then moved to their homecage with their cagemate. Animals were checked every day but left undisturbed at least for one week to allow recovery before any additional procedure.

3.3.5.3. Optic fiber implantation for optogenetic loss of function experiments in the PCT

After 3-4 weeks of transfection of the viral vectors (AAV-stGtACR or tdTomato (*for controls*)) with stereotaxic surgery, rats were implanted with an optic fiber melded to a 465 nm LED above the VTA.

For this, animals were anesthetized with isoflurane, placed in a stereotactic frame, the skin was shaved, and a unilateral craniotomy was performed above the VTA. The optic fiber (ø 200 µm) was implanted at the following coordinates: (AP: -5.52, ML: +- 0.6, DV: -7.6 mm from Bregma). Three other craniotomies were performed around the fiber, and stainless-steel screws (ø ~0.5 mm) were bolted into the holes in the skull to secure the implant. The LED-melded fiber was attached to a 4-pin connector used to join the wireless sensor during the experiments. A thin layer of Super-Bond was applied onto the screws and every component was covered and fixed with dental cement creating a long-lasting implant, yet leaving intact the connector where the optogenetic device was connected in the testing sessions of the PCT, the Alone Preference Test and the Real-time place preference.

After the cement of the implant was dry, animals were removed from the stereotaxic frame, administered with buprenorphine and let to recover in a clean cage with food and water, placed on a heating pad until awakened, then moved to their homecage. Animals were checked every day but left undisturbed at least for three or four days to recover before any additional procedure. The connector on the implant was covered with a plastic cover to protect it from possible damage.

3.3.5.4. Opto-electrophysiological recordings

To perform electrophysiological recordings while optogenetically manipulating neurons activity (Figure 18), a different cohort of 2 rats were injected with the viral vector AAV-CKIIa-stGtACR2 and left for transduction for 3–4 weeks.

Rats were then weighed and placed in the stereotaxic frame (Kopf) and anesthetized with a mixture of oxygen (1L/min) and isoflurane (4% for induction, 1% for maintenance). Rats were injected intraperitoneally with an analgesic compound buprenorphine (0,05mg/kg). Then, the skin of their heads was shaved and disinfected. A midline incision was performed with a scalpel and the area was cleaned. Bregma and Lambda points were identified in the skull and a craniotomy window was performed into the VTA (AP: -5.52, ML: +- 0.6, DV: -7.6 mm from Bregma). A second craniotomy was performed lateral to the initial one at a distance corresponding to the insertion of the optic fiber into the VTA at a 20° angle with respect to the coronal plane.

An optic fiber (Ø 200 µm; Thorlabs Inc, Newton, NJ, USA) was connected to a 473 nm laser light source (Cobolt 06-MLD, 473nm) via a patch cord, which in turn was connected to a computer to control light parameters. A multichannel recording electrode (Neuronexus) was slowly lowered to VTA, and the optic fiber was closely inserted at a 20° angle after a delay of 30 minutes to allow the brain tissue to equilibrate to the insertion of the electrode.

3.3.6. Data acquisition systems and data analysis

3.3.6.1. Calcium imaging with fiber photometry

For the experiments regarding vicarious reward signals in the VTA with fiber photometry, rats were implanted with an optic fiber connected to a Doric system that allowed the measurement of Ca^{2+} dependent signal. Light from the LED (465nm) was emitted through a fluorescence minicube (Doric) composed of two dichroic mirrors fixed inside the main unit, allowing for 465nm light delivery and GCaMP6s and GFP fluorescence detection. The fluorescence emitted by GCaMP6s/GFP expressing neurons was then collected by the optic fiber and directed back to the photodetector integrated in the minicube. Data was recorded at 1kHz sampling rate and converted using a digital acquisition board (National Instruments, inc.), and a custom Bonsai workflow was used to control and synchronize the experimental session (recording

from fiber photometry, PointGrey video camera and pyControl behavioral state machine).

- Fiber photometry data analysis

Fiber photometry data were analysed using custom Python scripts. Raw data signals were extracted and processed to align the starting of each session with the corresponding behavioural data from pyControl. For each experiment, the fluorescence change was determined by ΔF/F which was calculated as $(F-F_0)/F_0$, where F is the fluorescence at each time point, and F_0 corresponds to the mean fluorescence of the entire session. We then applied a box-car filter sliding window of 500 time units to smooth the data. Next, we split the ΔF/F of the full session into time windows of 4s (hereafter “event window”), which were aligned to the behavioural events of interest (from pyControl). In order to normalize ΔF/F across experimental sessions and subjects we computed the z-scores, considering for each event window a baseline period defined as -3s to -1,5s prior to the event of interest (time 0s). We calculated for each event window the z-scores as $z = (x– \mu_{(BL)}) / \sigma_{(BL)}$, where x corresponds to the ΔF/F of a single time point, $\mu_{(BL)}$ corresponds to the averaged ΔF/F of the baseline period and $\sigma_{(BL)}$ corresponds to the standard deviation of the baseline period. In order to create the Peri-event time histograms (PTEH), we first determined if the z-scores dynamics of each event showed an increase at the time of the event (0s). Thus, we found the peak z-score in a time window of 1.5s, centred to the event (± 0.75s), and compared this value to the averaged z-score of the corresponding baseline period. If the peak was bigger than the mean baseline, the event was considered to be an increase. Otherwise, the event was considered as a decrease or no change. Finally, for plotting the PTEH we grouped all the event windows of the same category and calculated the mean and SEM for each time point (line and shadow on Figure 15c-d).

3.3.6.2. Wireless optogenetic inhibition

For the experimental sessions of the PCT and the Alone Preference Test, rats had implanted an optic fiber melded to a 465 nm LED in the VTA with a connector attached to the implant. During experimental sessions, the WEAR - Wireless motion sensor device from Champalimaud Foundation Hardware Platform (Tang et al., 2024), was

attached to the connector of the rats' implant for delivery of optical stimulation. During optogenetic behavioral experiments, light intensity at the tip of the fiber was 8mW.

The WEAR device was controlled via radiofrequency by the WEAR Basestation, which was connected to the PC and the Habitest Link controlling the mazes via a custom-made interface that converted the -28V outputs from the Habitest link through an optical relay into +5V TTL into the PC and the Basestation. A Bonsai workflow was created to control the stimulation protocol in the following manner:

For the Prosocial Choice Task during other-reward moments: **(1)** a signal from the state-machine Habitest was sent and converted from -28V to a TTL (+5V) into Bonsai, occurring when both animals entered into the choice area at the beginning of the trial. Then an image-based ROI **(2)** detected the entrance of the recipients into the prosocial reward area just before the food receptacle. This detection sent an output signal to the WEAR-basestation to start the stimulation protocol with a squared-pulse inhibition of 60 mA. Once the recipient rat would reach the food receptacle **(3)** it was detected by the state-machine Habitest and sent to Bonsai to change the stimulation protocol emitted by the optical sensor (4 seconds at 60mA and then gradually decrease in steps of -5mA over 1.2 seconds, to avoid rebound activity after optoinhibition). The workflow prevented another stimulation to occur if recipient rats would go back to the prosocial reward area in the same trial, as the initial detection of both animals at the beginning of the trial was a precondition for the stimulation protocol to happen.

For the Alone Preference Test after self-reward: the same system described above was used, but while rats were foraging for self-rewards alone in the maze. Mimicking the optogeneic inhibition performed during the social task, 2 seconds after pellet retrieval in the preferred side (compared from the baseline session), the device current was set to 60mA for 4 seconds and then gradually decreased in steps of -5mA over 1.2 seconds.

- Optogenetics data analysis

Regarding the optogenetic inhibition experiments, data from the WEAR system about the optical stimulation events was parsed and analysed with Python scripts. Data was aligned to the starting of each session and the files were split into the different trials performed by each pair of subjects across experimental sessions and synchronized

with behavioural data from the state-machine Coulbourn. From here, we extracted the total time of stimulation in each trial.

3.3.6.3. Opto-electrophysiological recordings in anaesthetized rats

Prior to each experiment, fiber optic cannulas were tested for adequate light delivery using the following procedure. The cannula was connected to the 473-nm laser via a patch cord, and the laser was connected via its analog control port (allowing for laser intensity to be controlled by voltage modulation) to a pulse generator (Multichannel Systems, Reutlingen, Germany) and also via its USB cable to a computer running Cobolt Monitor software. The pulse generator was in turn connected to a computer running MC_Stimulus II (Multichannel Systems), which allows for the creation of pulse trains of varying voltages and patterns. The voltage required to produce a light intensity of 8 mW, as measured by a light power meter (Thorlabs), was calibrated for each individual cannula. The light intensity of 8 mW was chosen to match the maximum intensity produced by the LEDs used during the wireless optogenetic behavioral experiments.

The stimulation protocol consisted of 10 trains of 4 s of continuous light stimulation followed by 30 s of no light. At the end of each 4-s stimulation, light power was linearly ramped down to 0 mW over a duration of either 0 ms (no ramp), 350 ms, 700 ms, 1000 ms, or 1500 ms.

For the acquisition of neural signals, a 16-channel linear electrode (100 µm spacing between electrode sites; NeuroNexus, Ann Arbor, MI, USA) was connected to a pre-amplification headstage, which was in turn connected to an amplifier (Multichannel Systems), where the signal was amplified. Signals were monitored online and recorded at a sampling rate of 25,000 Hz using MC_Rack (Multichannel Systems) and were band pass filtered [300-3000Hz]. Once the electrode and fiber were in place in their target locations in the anesthetized rat brain, electrophysiological signals from the six channels nearest to the target location (i.e., the channels closest to the electrode tip) were monitored for obvious spontaneous spiking activity. In the event that no spontaneous spiking activity was initially observed, the electrode was driven 100 µm deeper, and at least 10 minutes were allowed to elapse before initiating the recording and stimulation protocol. This process was repeated until spontaneous activity was observed.

- Electrophysiological recordings data analysis

To analyze the data, single units were isolated using the Python-based spike sorting utility NeuroSorter (developed by Javier Alegre, Instituto de Neurociencias, Alicante, Spain: https://github.com/Alegre-Cortes/NeuroSorter-Interface), which uses machine learning approaches to remove noise and identify units. Units were considered valid if <0.5% of their interspike intervals were >2 ms. Timestamps were then exported as .csv files to the statistical computing platform R (www.r-project.org) where they were further analyzed and plotted using custom scripts.

3.3.7. Statistical analysis

Data extracted from the state-machine pyControl (for vicarious reward experiments) and Coulbourn (for PCT experiments) was parsed and processed with Python 3. We then used IBM SPSS Statistics 26 and JASP 0.16.2 to perform probabilistic and bayesian analysis on statistical differences between the extracted and studied variables.

Peak response for self and other rewards: paired samples t-test was used to assess differences between the averaged peak response from all the self and other reward events from each animal (Figure 15e).

Prosocial preference: repeated measures (RM) ANOVA with ‘session’ as within subjects factor and “inhibition” as between subjects factor, was performed to assess the prosocial choice preference over the course of testing sessions (Figure 17e). After averaging each animal from each group, one sample t-test against chance (50) was used to assess a general effect from the experimental “inhibition” group. Same tests were used to assess the prosocial preference of the first session of the PCT in blocks of 10 mins (Figure 19a).

Preference change in alone preference control experiment: we calculated the preference from session one (where no inhibition occurred) and then the change of preference in session two (where inhibition happened) for both groups. Then used independent samples t-test to assess for differences according to the group. Figure 17e,g.

Place preference test: we calculated the time spent in the light-associated chamber for both groups. Then we used independent samples t-test to assess differences between both groups (Figure 17h).

Light duration comparison for opsin group between the different behavioural tests: we computed the averaged total time in seconds that the opsin group had inhibition through light stimulation. We then used one way ANOVA to check for differences between the measures (Figure 17i).

Light duration for alone preference test and real time place preference: total light stimulation in seconds was calculated for both groups (control and opsin), then independent samples t-test was used to compared between them (Figure 19c-d).

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4 | Results

4.1. Study of reward-based prosocial choices in mice

The scarce and contradictory evidence found for prosociality in mice in reward-based contexts makes it hard to find common behavioural and neural mechanisms of such processes. Testing with different paradigms to address similar questions might result in contradictory evidence but is also highly relevant and beneficial for the advancement of our knowledge. Due to the limited studies to test reward-based prosociality in mice, in the present work we introduce a new behavioural paradigm that we developed for mice inspired by the Prosocial Choice Task (PCT) for rats developed by (Márquez et al., 2015), which is proven to provide a good control and flexibility of the contingencies and quantitative studies of behaviour.

Statistical analysis shown in this section will include the standard and widely used frequentist approach besides the Bayesian approach on the presented data, being the latter a convenient tool to discern those results showing evidence of absence of an effect from absence of evidence (Keysers et al., 2020).

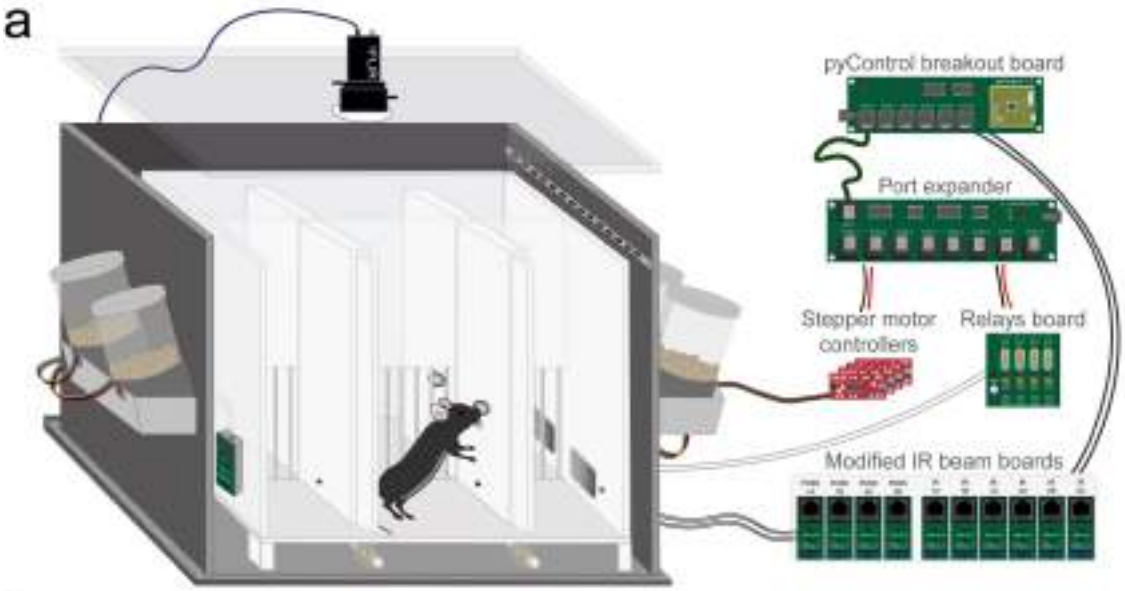
4.1.1. Prosocial choice task with double T-maze configuration

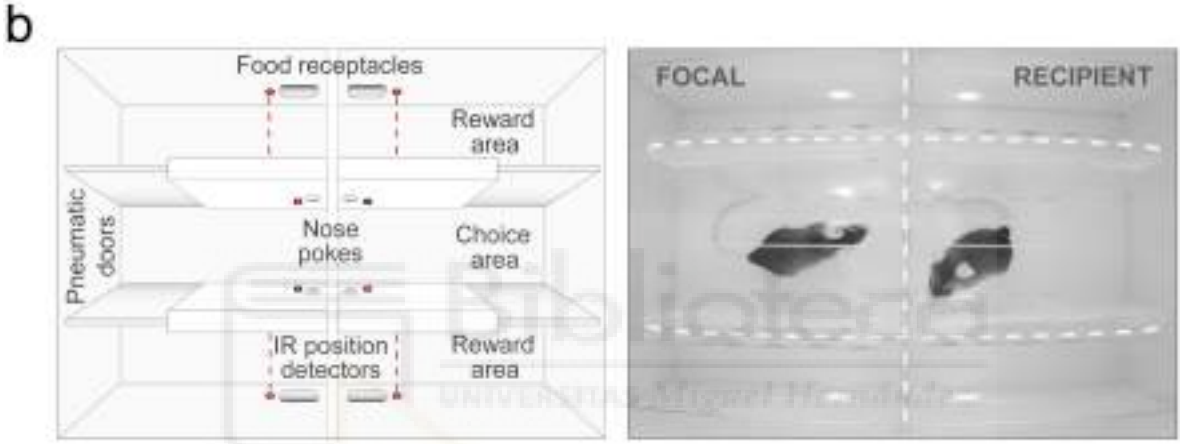
In previous work with rats, our group demonstrated that **(1)** decision-maker rats are sensitive to the food-seeking behavior displayed by the recipient animals prior to choice (Márquez et al., 2015). This is necessary for the emergence of a prosocial preference, but not sufficient, as **(2)** information about the reward contingencies of the recipient was also relevant for prosocial choices to emerge. Keeping these two important mechanisms in mind, we developed a fully automated double T-maze (Figure 5a), which decreased the possible interferences by the experimenter and also provided a precise and controlled monitoring of the behaviour of the interacting mice. The configuration of this maze separated spatially and temporally the moments of decision from those of reward delivery (Figure 5a-c). Each of the individual T-mazes (one per animal) contained a central ‘choice area’, where two nose ports for each animal were located, used for displaying food-seeking behaviour and decisions (i.e. by nose-poking into the IR ports). The central zone gave access to two lateral areas, gated by automatic doors, where mice retrieved the rewards from a food receptacle

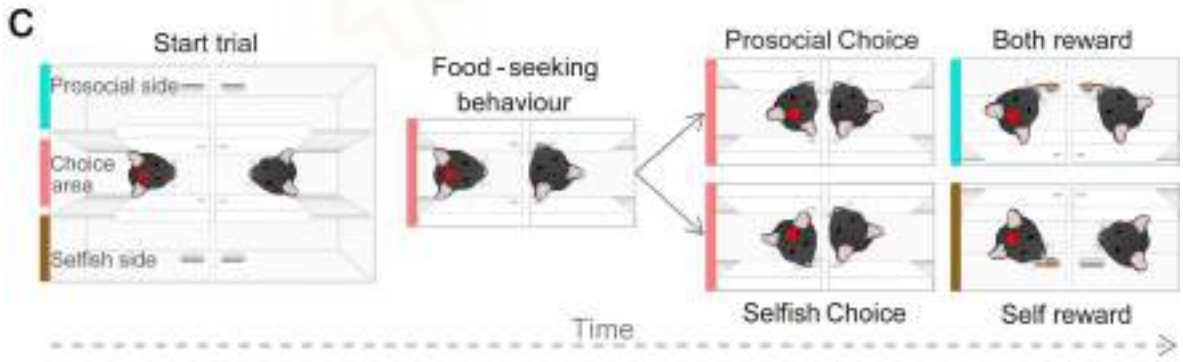
according to the contingencies of the task. After reward retrieval, the doors opened allowing the animals to go back to the choice area to start a new trial. The two individual T-mazes were connected by a perforated and transparent partition, which allowed mice to exchange different sensory information in the choice area as well as in the reward areas. To ensure that focals had the opportunity to perceive the displays of food-seeking behaviour and the reward retrieval by the recipient, the nose-ports of the focal mouse were active only after the recipient poked at least once in any of the IR ports, and the food pellet of the recipient was delivered only after the focal mouse entered the reward area.

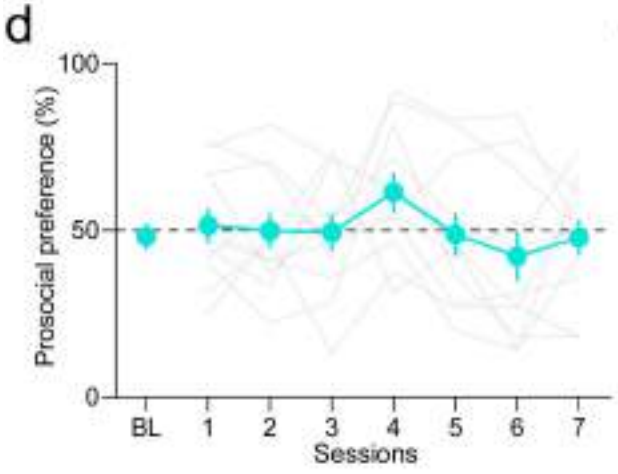
We tested pairs of mice in our PCT, where a decision-maker mouse (focal) could choose to provide food reward to itself (*selfish option*) or to itself and the recipient mouse (*prosocial option*). Before social testing, mice were individually trained for instrumental learning and maze navigation (Figure 6). By the end of the individual training, no general side bias was found (one sample t-test against chance (50), $t_{(11)}$=-0.489, p=0.634, BF_{10}=0.319) (Figure 6c). Then, focal and recipient mice were tested together in the PCT, where reward delivery for the two animals depended on the focals' choices. For this set of experiments, 12 pairs of male mice (C57BL/6) underwent 7 sessions of 30 minutes of the PCT. Importantly, mice only went through food-restriction during some early phases of the individual training, but in none of the PCT testing sessions to avoid possible stress-related behavioural effects.

During the social task, decision-makers did not develop a preference for prosocial or selfish options (repeated measures ANOVA with 'session' as within subjects: $F_{(6)}$ = 1.857, p=0.102, BF_{incl}= 0.637) (Figure 5d). These results suggest that mice did not have a preference for choosing the option that delivered food to their conspecifics, in absence of self-benefit, against what we observed in rats. However, most of the animals changed substantially their preference over sessions (increase and decrease), showing high individual variability. In order to account for the differences in prosociality between individuals, we computed a Prosocial Choice Index (PCI) (*see* Statistical analysis *for more details*). Positive PCIs reflect a change towards a prosocial preference from chance (PCI=0). We found moderate evidence supporting the lack of preference for any of the choices (one sample t-test: $t_{(11)}$=-0.251, p=0.59, BF_{+0}=0.242). A permutation test on the PCI of the individuals revealed that out of 12 mice, 2 were considered prosocial, 7 unbiased and 3 selfish (Figure 5e, Table 2).


a
pyControl breakout board
Port expander
Stepper motor controllers
Relays board
Modified IR beam boards


b
Food receptacles
Reward area
Pneumatic doors
Nose pokes
Choice area
IR position detectors
Reward area
FOCAL
RECIPIENT


c
Start trial
Prosocial side
Choice area
Selfish side
Food - seeking behaviour
Prosocial Choice
Both reward
Selfish Choice
Self reward
Time


d
Prosocial preference (%)
100
50
0
BL
1
2
3
4
5
6
7
Sessions

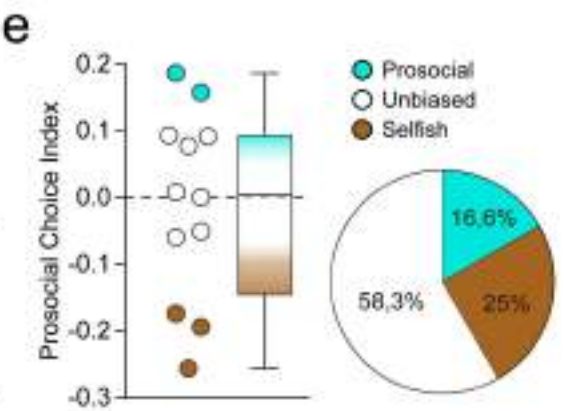

e
Prosocial Choice Index
0.2
0.1
0.0
-0.1
-0.2
-0.3
Prosocial
Unbiased
Selfish
16,6%
25%
58,3%

Figure 5. Mice prosocial choices in double T-maze

a. Hardware and peripherals used for the assessment of prosocial decision-making with a double-T maze arena. The arena is located inside a sound attenuation box and illuminated with IR and dim white light to enable high quality video recordings. The setup is made of laser-cut white acrylic treated to avoid reflections from the IR camera placed on the box centre-top. Custom-made pellet-dispensers hold outside the sound attenuation box to reduce head and noise. Pneumatic cylinders are below the base of the arena providing smooth gating of the doors. **b**. Hardware position schematic (left) and real top image (right) of the maze-based setup configuration. The T-mazes are joined by a perforated and transparent partition. For each side there is a central choice area with two nose-ports located in each wall (for decisions and displays of food-seeking behaviour), and modified IRs to detect the mice position. There are acrylic doors connected to pneumatic cylinders at the end of the corridor that give access to the reward areas. In these zones food pellets are delivered by automated food-dispensers located outside the arena. **c**. Timeline structure of prosocial choice task. Trials start with both animals in the choice area, the recipient will display food-seeking behaviour by nose-poking into any of the ports which will activate the decision ports of the focal mouse (red triangle in the head). The focal then will decide to go either side of the maze by nose-poking in any of the ports. Poking into the prosocial port will deliver a food-pellet to both animals while choosing the selfish port will only deliver a pellet for the focal and none to the recipient. The different separated areas are colour-coded (choice area: pink, prosocial side: blue, selfish side: brown). **d**. Prosocial preference of mice in maze-based arena over the seven testing sessions. BL refers to baseline, used to evaluate individuals' preference in the last two sessions of individual training. Blue thick line corresponds to mean±SEM, grey lines correspond to each individual. At population level, animals did not display any preference for prosocial or selfish choices. **e**. Distribution of Prosocial Choice Indexes to study individual differences in prosociality. Positive values show a preference for the prosocial option, negative values indicate preference for the selfish option, and values close to 0 indicate chance preference. Blue dots correspond to prosocial mice, grey dots are unbiased and brown selfish. On the right, pie chart: distribution of mice after permutation test of Prosocial Choice Indexes.

Table 2. Chance interval bounds generated by permutation test for each pair. Related to Figure 5, 12 and 13.
Low and high bounds show the 95% confidence interval for each focal mouse.

Standard PCT in double T-Maze						
Pair #	**1**	**2**	**3**	**4**	**5**	**6**
Lower bound	-0,096	-0,164	-0,179	-0,152	-0,128	-0,152
Upper bound	0,096	0,194	0,179	0,131	0,128	0,152
Pair #	**7**	**8**	**9**	**10**	**11**	**12**
Lower bound	-0,115	-0,131	-0,136	-0,115	-0,140	-0,140
Upper bound	0,115	0,131	0,136	0,115	0,140	0,140
Low training PCT in double T-Maze						
Pair #	**1**	**2**	**3**	**4**	**5**	**6**
Lower bound	-0,200	-0,175	-0,192	-0,176	-0,165	-0,186
Upper bound	0,200	0,175	0,192	0,176	0,165	0,186
Pair #	**7**	**8**	**9**	**10**	**11**	**12**
Lower bound	-0,263	-0,273	-0,278	-0,185	-0,176	-0,257
Upper bound	0,263	0,273	0,278	0,185	0,176	0,257
Standard PCT in double chamber						
Pair #	**1**	**2**	**3**	**4**	**5**	**6**
Lower bound	-0,063	-0,067	-0,099	-0,067	-0,078	-0,099
Upper bound	0,063	0,072	0,099	0,067	0,078	0,099
Bottom preference in double chamber						
Pair #	**1**	**2**	**3**	**4**	**5**	**6**
Lower bound	-0,063	-0,065	-0,063	-0,067	-0,078	-0,081
Upper bound	0,063	0,065	0,063	0,067	0,078	0,081

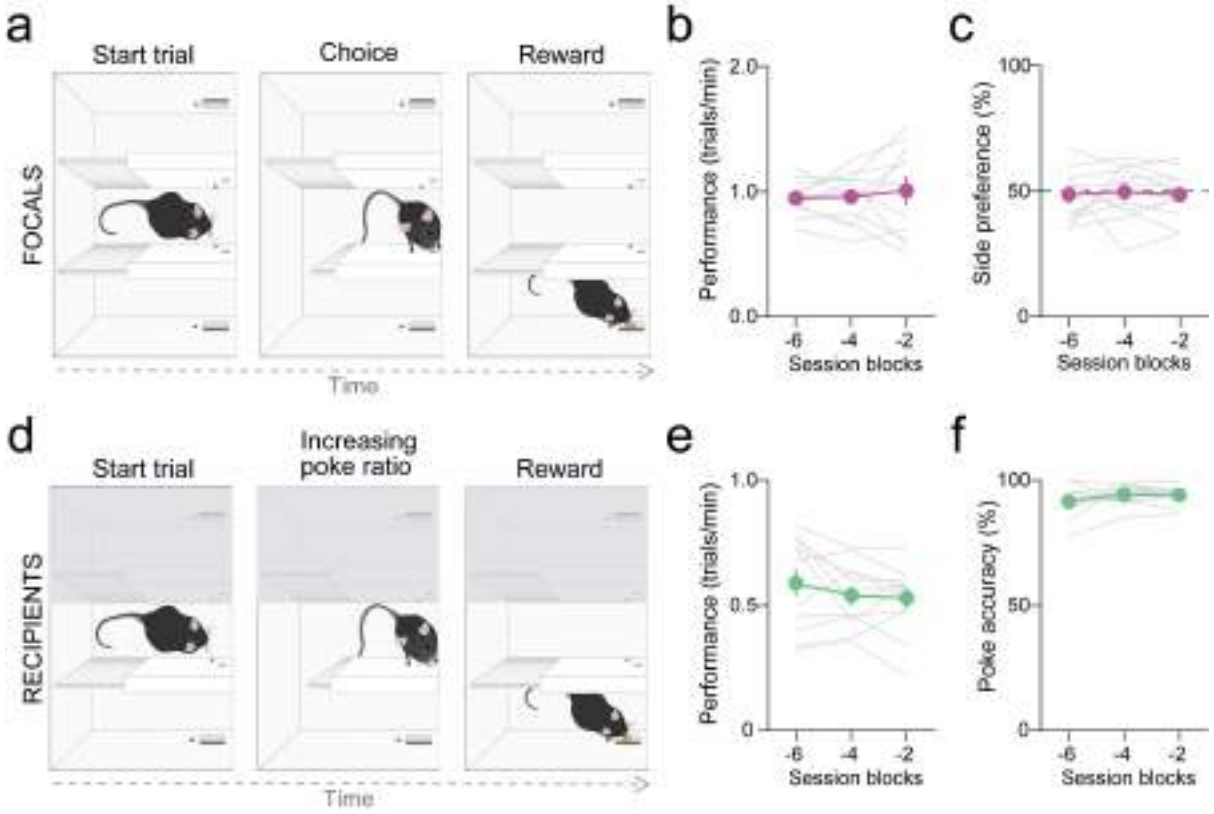


Figure 6. Individual training in the double T-maze before the PCT

a. Trial structure for focals' individual training in the double T-maze, where mice choose between two pokes in the choice area to gain access to the corresponding reward area to obtain a pellet (both sides rewarded). **b**. Performance (number of trials divided by the session duration in minutes) of focal mice during last 6 sessions of individual training before the social task, averaged in blocks of 2 sessions. **c.** Side preference during last phases of individual training. Proportion of choices during last sessions of individual training to the side that will be prosocial in the PCT. Animals perform at chance. **d**. Trial structure for recipients' individual training, where mice increase the poke ratio to gain access to reward only on one side of the maze. **e**. Performance of recipient mice during last sessions of individual training. Same as **b** for recipients. **f**. Nose-poke accuracy. Proportion of pokes towards the active nose-port over last sessions of individual training. Note that most of recipients pokes almost exclusively into the port which leads to reward, which corresponds to the prosocial port during the social task.

To improve our understanding of why focal mice did not prefer to choose the prosocial option, we analysed the behaviour of the animals according to their role during the social task. For this purpose, we performed a fine-grained analysis on the tracking data obtained by the animal pose estimation software DeepLabCut (DLC) and behavioural events extracted from pyControl (the platform used to control these experiments).

4.1.1.1. Recipient mice behaviour during the prosocial choice task

We first focused our analysis on understanding if the behaviour of recipient mice could explain the lack of choice preference found in focal mice. It has already been shown that the displays of food-seeking behaviour performed by the recipient rats are necessary for the emergence of a preference for the prosocial choices; therefore, we assessed if recipient mice performed clear food-seeking cues in the choice area (Figure 7a). To this end, we first quantified the number of pokes recipient mice did in each nose-port (prosocial and selfish ports) per trial (Figure 7b), and we found that the frequency of pokes into the prosocial port was much higher than those in the selfish nose-port (paired samples t-test: $t_{(11)}$=7.723, p=9.120e^{-6}, BF_{10}=2341.38). We next calculated the poke specificity towards the prosocial port in both prosocial and selfish trials (Figure 7c), and observed that recipient mice poked almost exclusively towards the prosocial port independently on whether the focal would decide to be prosocial or selfish (paired samples t-test: $t_{(11)}$=0.654, p=0.527, BF_{10}=0.345). With tracking data obtained with DLC, we performed a ROI analysis and measured the amount of time that the head of recipient mice was detected inside the ROI around each of the nose-ports (Figure 7d). We found that recipients spent a significantly higher amount of time near the nose port that gave access to reward in comparison to the 'selfish' port (paired samples t-test: $t_{(11)}$=4.654, p=7.005e-4, BF_{10}=54.187).

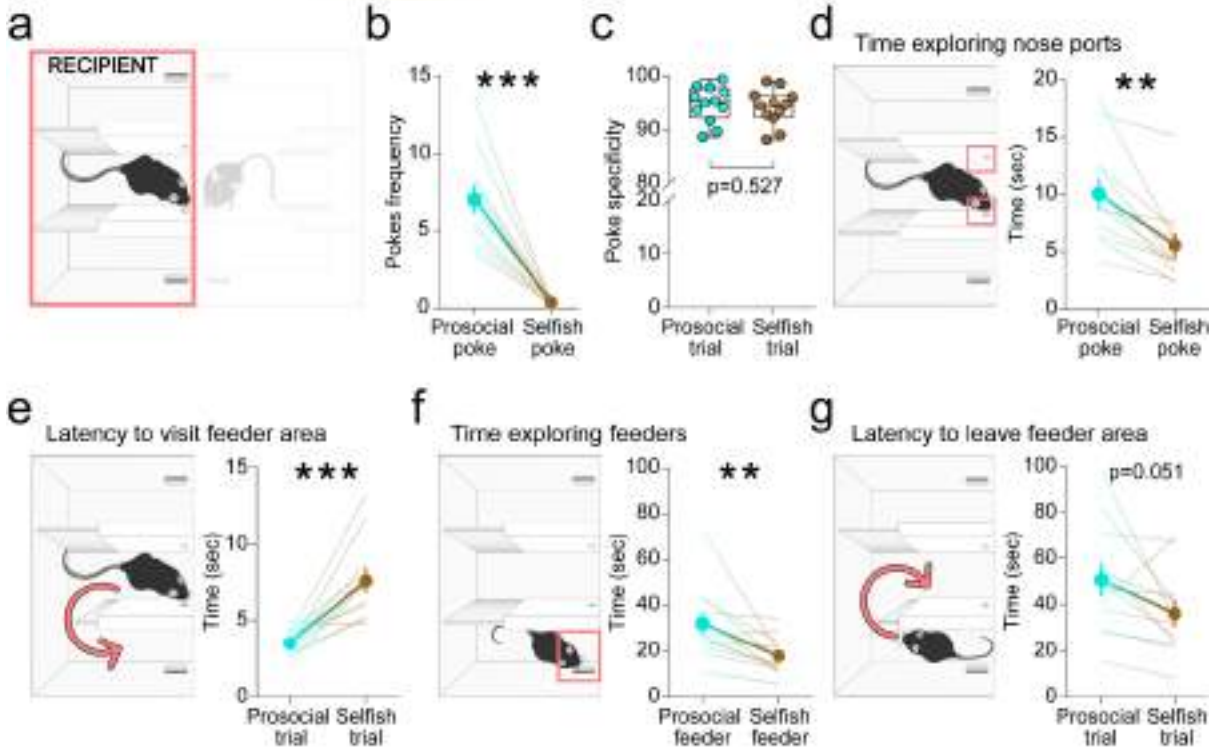


Figure 7. Recipient mice display food-seeking behaviour and react to reward contingencies

a. Illustration of the arena used for the PCT. The pink rectangle indicates that the following results are focused on recipients' behavioural data. **b**. Nose-pokes frequency per trial. Quantification of pokes per trial done in the prosocial and selfish ports. Recipients do a significantly higher number of pokes in the prosocial nose-port compared to the selfish. **c**. Nose-poke specificity. For each recipient mouse, we calculated the proportion of pokes towards the prosocial port, both in prosocial trials (blue dots) and selfish trials (brown dots). Specificity is similar for prosocial and selfish trials, being around the 95% of pokes towards the prosocial port. **d**. Time exploring nose-ports. With recipients pose data, we performed a ROI analysis for the nose-ports (pink squares). We measured the nose label spent inside each of the two ROIs and found out that recipients spend almost double of the time exploring the prosocial port compared to the selfish**. e**. Latency to visit feeder areas. Time in seconds from choice to detection of the recipient mouse in the reward areas was significantly different. Recipient mice enter faster in the area where they get rewarded. **f**. Time spent exploring the feeders. Same as **d** for the area around the food receptacles (pink square). We also considered the detection of the head label to avoid data loss by occlusions from the walls separating the different areas. Recipients stayed significantly longer around the feeder where they are rewarded compared to the selfish. **g**. Latency to leave the reward areas. Ten seconds after reward delivery, automated doors opened to allow going back into the choice area to start a new trial. We found a tendency for recipients to leave the selfish reward area faster than the prosocial. For all graphs: degraded thicker line shows mean±SEM, thinner lines represent data from each individual. Blue = prosocial, brown = selfish.

Taken together, these results show that recipient mice actually displayed clear attempts to reach the food and access the rewarded arm. We then extended our analysis to the moments of the testing sessions that occur after the decision is made (and before another trial starts) to elucidate if recipient mice altered their behaviour after being rewarded or not by their partners. We thus quantified the latency to go from the choice area to the reward area from the moment of the decision, as a proxy for reward anticipation (Figure 7e). Latency to access the reward area where recipients received a food pellet was significantly lower than when going to the 'reward area' where recipients did not eat (paired samples t-test: $t_{(11)}$=-5.306, p=2.500e^{-4}, BF_{10}=130.755). With the same strategy as before, we calculated how much time per trial the animals spent exploring the areas close to the food receptacles (Figure 7f). We found that recipient mice spent longer time near the feeder area where they retrieve a food pellet compared to the selfish area, where they did not receive any pellet (paired samples t-test: $t_{(11)}$=3.217, p=0.008, BF_{10}=6.957). Finally, we measured the latency to return to the choice area to start a new trial from the moment the automated doors opened after reward delivery (Figure 7g). We found that recipients tended to take longer to exit from the reward area after a prosocial choice (paired samples t-test: $t_{(11)}$=2.184, p=0.051, BF_{10}=1.621).

Together, these results indicate that recipient mice displayed food-seeking behaviour by nose-poking repeatedly and almost exclusively towards the 'prosocial' side, and

that their behaviour after a prosocial or selfish decision was very different too, being these distinct social cues that focal animals could base their decisions upon. Therefore, we next explored the behaviour of focal mice to disambiguate if they took the recipients' actions into account for modulating their decisions.

4.1.1.2. Focal mice behaviour during the prosocial choice task

Beyond the average lack of preference found in the PCT, we assessed whether the behaviour of focal mice was different before and after a prosocial and a selfish choice (Figure 8a). We first measured the latency, from trial onset to choose between prosocial and selfish options (Figure 8b), where no statistically significant differences were observed (related samples Wilcoxon signed rank tests: Z=-0.471, p=0.638, BF_{10}=0.334). We performed a ROI analysis to measure the time spent investigating both choice ports (Figure 8c), and focals spent similar amount of time exploring the prosocial and the selfish pokes per trial (related samples Wilcoxon signed rank tests: Z=-0.392, p=0.695, BF_{10}=0.338). We then quantified the latency to enter the reward area after performing prosocial and selfish choices, where focal animals were always rewarded but their partners were only after a prosocial choice (Figure 8d). No differences were observed (paired samples t-test: $t_{(11)}$=-0.512, p=0.619, BF_{10}=0.291). Once inside the reward areas, we measured how much time the animals spent exploring the feeder area per trial (Figure 8e). We found that the amount of time that focal mice spent exploring the two reward areas was no different (paired samples t-test: $t_{(11)}$=1.712, p=0.115, BF_{10}=0.894). Finally, we calculated the latency to leave the reward areas after the doors opened to start a new trial (Figure 8f), where no differences were observed depending on choice type (related samples Wilcoxon signed rank tests: Z=1.255, p=0.209, BF_{10}=0.604). Together these results show that decision-maker mice did not change their behaviour when deciding to provide food or not to another conspecific, not prior to making the decision nor during the reward periods, despite the differences reported in the behavior of the recipients. These observations suggest that focal mice did not perceive the food-seeking cues, nor the different behaviours that recipient mice displayed, or did not make use of this information in order to guide their decision. To further investigate this, we performed an analysis on the social dynamics happening prior and after decision to determine whether decision-makers were socially attentive and interacting with recipients, and whether they could have perceived these social cues.

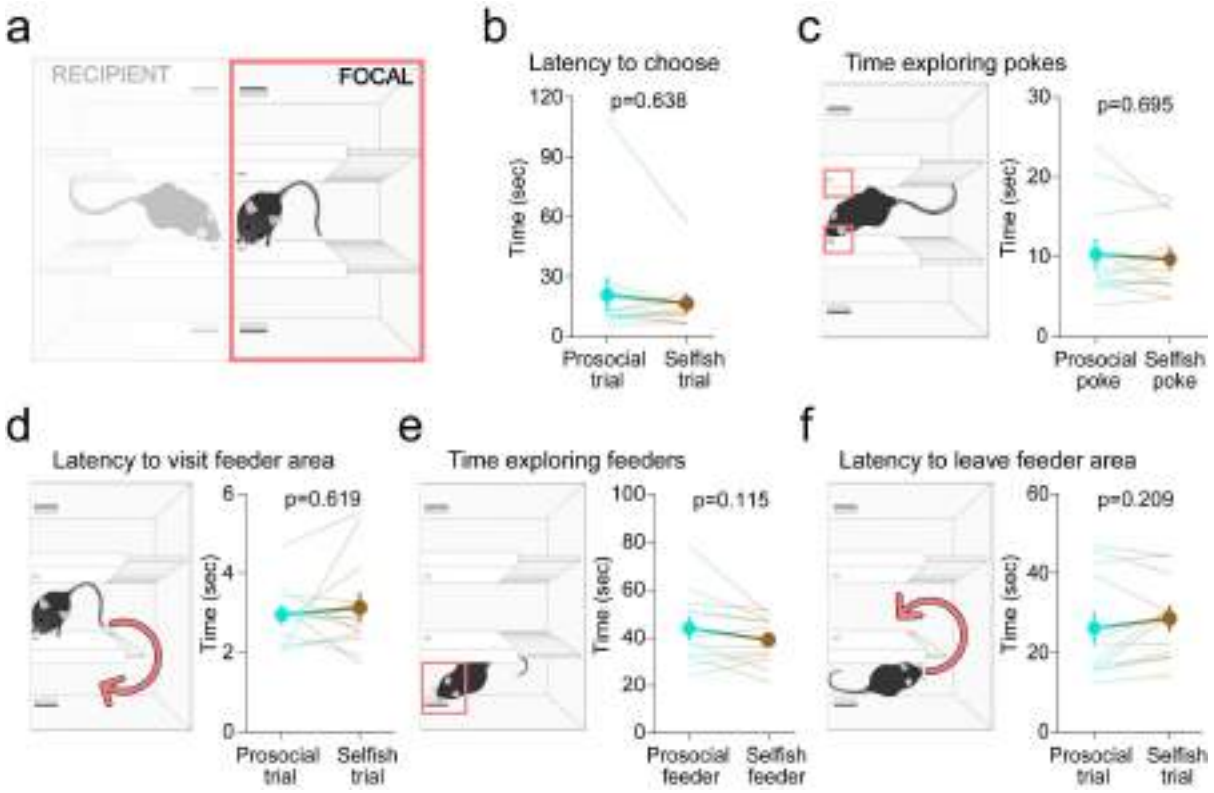


Figure 8. Focals do not change their behaviour according to choice type

a. Illustration of the arena used for the PCT. The pink rectangle indicates that the following results are focused on focals' behavioural data. **b**. Line graph showing the latency from trial onset to choose prosocial or selfish, in seconds, where no differences were found. **c**. Time spent exploring the choice ports, measured by quantifying the frames in which the snout of the focal mice was detected in a ROI around the nose-pokes (pink squares). The time, in seconds, spent exploring both ports is similar. **d**. Latency to enter reward areas. Time in seconds from choice to detection of the focal mouse in the reward areas was not different when choosing a prosocial or a selfish choice. **e**. Time spent exploring the feeder area. Same as C for the feeder areas (pink square), in this case we also considered the detection of the head label to avoid data loss by occlusions from the walls separating the different areas. Focals do not differ on the time spent on both feeder areas. **f**. Latency to leave reward areas. The time in seconds since the automated door opens after reward, until the focal goes back into the choice area to start a new trial, is not different in prosocial or selfish trials. For all graphs: degraded thicker line shows mean±SEM, thinner lines represent data from each individual. Blue = prosocial, brown = selfish.

4.1.1.3. Social interactions prior to choice during the prosocial choice task

With pose-estimation data obtained from DLC we first analysed the social interactions happening from trial start to the moment of the decision to examine whether focal mice were attentive to the displays of preference performed by their recipient conspecifics

(Figure 9a). We extracted the *X,Y* coordinates of different selected body parts across the frames of the experimental videos and calculated different quantitative parameters that would allow the study of social dynamics of the two interacting animals. We first calculated the social (*Euclidean*) distance between the snouts of the two mice. Then, we set a threshold of 60 pixels (1,4 cm) distance to be considered a close interaction (i.e. nose-to-nose direct investigation through the diving perforated wall), and measured the time prior to choice that animals spent closely interacting in both prosocial and selfish trials (Figure 9b). No differences were observed in the duration of close social interactions prior to choice (related samples Wilcoxon signed rank tests: $Z=0.863$, $p=0.388$, $BF_{10}=0.511$). However, relevant social interactions might have occurred at a distance. Thus, we calculated the time that both animals spent together in a defined ROI along the perforated and transparent partition that divides the arena (Figure 9c), which could provide more information about diverse social behaviours that might have happened before the decision was made. We found no significant differences in the time spent interacting near the division wall according to trial type (related samples Wilcoxon signed rank tests: $Z=0.863$, $p=0.388$, $BF_{10}=0.515$). We then checked if they interacted in the partition at a similar distance in prosocial and in selfish trials (Figure 9d), and found that they were closer to each other prior to prosocial choices (related samples t-test: $t_{(11)}=-2.918$, $p=0.014$, $BF_{10}=4.510$). Next, we measured the distance of each animal to the wall when they were interacting inside the partition ROI (Figure 9e). We use this variable as measure to know which animal is driving a close interaction by proximity to the partition, and hence to the other animal, that is not possible to know from the Euclidean distance. We found that during the interactions near the partition, focals and recipients maintained a similar distance to the wall both in prosocial and selfish trials (RM ANOVA with trial type as within subjects factor and role as between subjects factor: $F_{(1,22)}=0.174$, $p=0.680$ for trial type; $F_{(1,22)}=1.808e\text{-}4$, $p=0.989$ for interaction; $F_{(1,22)}=0.078$, $p=0.783$ for role. Simple main effects comparing trial type for focals: $F_{(1)}=0.166$, $p=0.692$, and for recipients: $F_{(1)}=0.062$, $p=0.809$. Simple main effects comparing according to the role for prosocial trials: $F_{(1)}=0.108$, $p=0.745$, and for selfish trials $F_{(1)}=0.051$, $p=0.824$). Yet, that both animals spent time together at a distance in the same space does not necessarily mean that they are paying attention to each other. Therefore, we measured the relative head orientation of both animals to get a proxy of visual interest during the moments prior to decision.

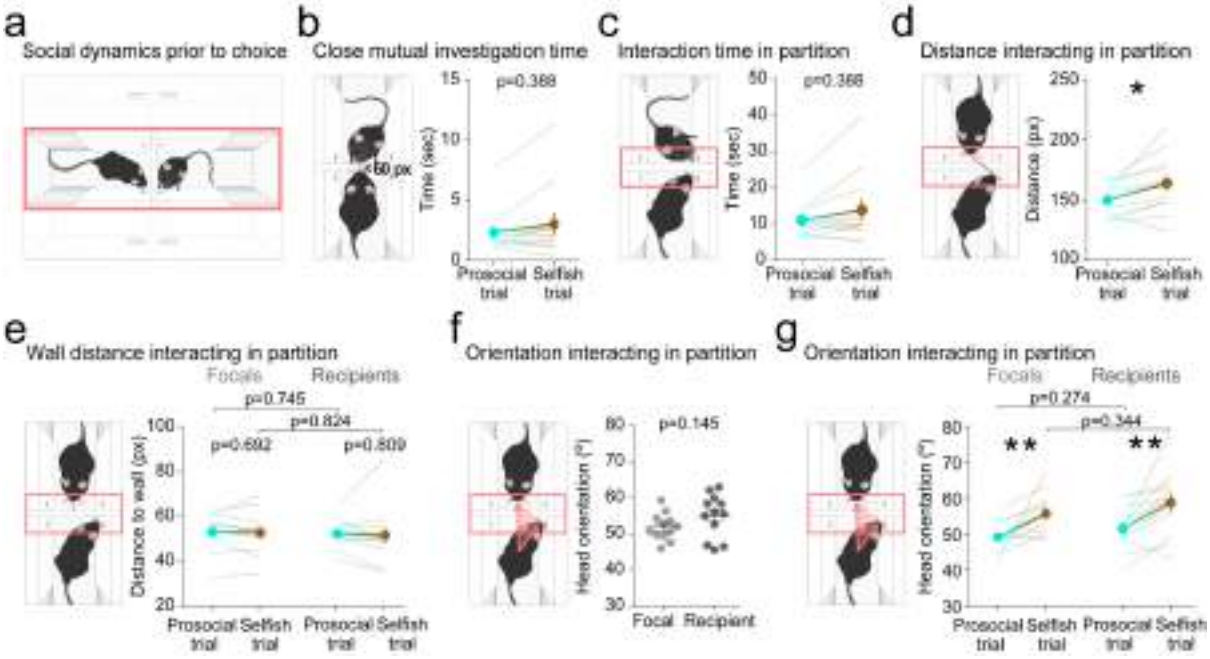


Figure 9. Social dynamics prior to choice

a. Illustration of the arena used for the PCT. The pink rectangle indicates that the following results are focused on the analysis of social behaviours in the choice area happening during the period from trial start to choice. **b**. Time in close distance. We calculated the amount of time mice interacted with a nose-nose distance lower than 60px (1,4 cm), which we considered to be a proximal interaction. We found that mice spend similar time interacting close to each other before a prosocial or a selfish decision. **c**. Interaction time in partition. Measurement of time spent by both mice detected together in a ROI around the divisor wall. Results show no differences on the time spent by both animals close to the partition before prosocial or selfish choices. **d**. Euclidean distance between mice during interactions in near the wall. Mice were closer during interactions prior to prosocial choices. **e.** Distance to the wall during interactions in the partition ROI. We measured the distance from each animal's nose x coordinate to the partition. We found that both focals and recipients kept a similar distance to the wall both in prosocial and selfish trials, also when compared between them. **f**. Head-orientation during interactions in the partition for focals and recipients independent on the trial type, similar between them. **g.** Orientation in the partition according to the trial type. We found that both focals and recipients, interacted more oriented to their partner in prosocial trials, but they did not differ according to the role. For all graphs: degraded thicker line shows mean±SEM, thinner lines represent data from each individual. Blue = prosocial, brown = selfish.

This parameter represents how straight the body-head angle is with respect to the other animal's head; values closer to 0 indicate an oriented position towards the other animal's face, values closer to 180 indicate a head orientation opposite to the other mouse. We focused the analysis when the animals were interacting in partition ROI (Figure 9f). We found that independently of the trial type, focal and recipient mice were interacting with a similar orientation to each other (independent samples t-test, $t_{(22)}$=-1.512, p=0.145), that was in the range of (50-60º), enough so they could be gazing each other. We then explored whether their orientations differed according to the trial type (Figure 9g), and found that in prosocial trials both focals and recipients were more

oriented to their partner (RM ANOVA with trial type as within subjects factor and role as between subjects factor: $F_{(1,22)}$=22.004, p=1.117e-4 for trial type, $F_{(1,22)}$=0.057, p=0.813 for interaction, and $F_{(1,22)}$=1.446, p=0.242 for role. Simple main effects comparing trial type for focals: $F_{(1)}$=11.587, p=0.006, and for recipients: $F_{(1)}$=10.616, p=0.008. Simple main effects comparing according to the role for prosocial trials: $F_{(1)}$=1.259, p=0.274, and for selfish trials: $F_{(1)}$=0.934, p=0.344).

Withall, we found that social interactions prior to the choice differed depending on the focals' decisions. In prosocial trials animals interacted at a closer distance and more oriented to each other, which should have enabled focal mice to perceive the food-seeking behaviour displayed by their partners.

4.1.1.4. Social interactions during reward periods in the prosocial choice task

With the previous analysis on the interactions prior to choice we observed that although focal mice were close and oriented towards the recipients during social interactions, these social interest proxies were not enough to drive prosocial choices. We next examined the social dynamics that happened during the reward period (Figure 10a). To start, we measured the time that both animals spent socially interacting close to the feeder areas by calculating the number of frames that any of the head labels was detected in determined ROIs around the food-magasins (Figure 10b). Mice interacted for a longer time in the area where the recipient receives reward compared to the selfish area (related samples Wilcoxon signed rank tests: Z=2.981, p=9.766e^{-4}, BF_{10}=59.45). During these interacting periods we calculated parameters such as the relative head orientation of the animals, and found that both focals and recipients were fairly oriented to each other (Figure 10c), being focals significantly more directed towards the recipients (independent samples t-test: $t_{(22)}$=-4.910, p=6.550e^{-5}, BF_{10}=290.919). We then explored how the head orientation of each mouse towards its partner was modulated by the type of trial during the interaction in the feeder areas (Figure 10d). Focals' orientation didn't differ according to the trial type but were more oriented than recipients both in prosocial and selfish trials. Recipients were more oriented to their focals in prosocial trials. (RM ANOVA with trial type as within subjects factor and role as between subjects factor: $F_{(1,22)}$=8.291, p=0.009 for trial type, $F_{(1,22)}$=0.359, p=0.555 for interaction, and $F_{(1,22)}$=22.497, p=9.830e-5 for role.

Simple main effects comparing trial type for focals: $F_{(1)}$=2.761, p=0.125, and for recipients: $F_{(1)}$=5.717, p=0.036. Simple main effects comparing according to the role for prosocial trials: $F_{(1)}$=10.947, p=0.003, and for selfish trials: $F_{(1)}$=17.561, p=3.788e-4). Finally, we examined the individuals' distance to the division wall while they were interacting in the feeder areas (Figure 10e). We found that both focals and recipients kept a similar distance towards the partition independently of the trial type (independent samples t-test: $t_{(22)}$=0.106, p=0.917, BF_{10}=0.375). This distance was not modulated by the trial type in the case of focals but recipients approached more to their focals in trials where they were not rewarded (i.e. selfish trials) (Figure 10f) (RM ANOVA with trial type as within subjects factor and role as between subjects factor: $F_{(1,22)}$=8.774, p=0.007 for trial type, $F_{(1,22)}$=14.080, p=0.001 for interaction, and $F_{(1,22)}$=0.087, p=0.770 for role. Simple main effects comparing trial type for focals: $F_{(1)}$=0.512, p=0.489, and for recipients: $F_{(1)}$=16.213, p=0.002. Simple main effects comparing according to the role for prosocial trials: $F_{(1)}$=2.114, p=0.160, and for selfish trials: $F_{(1)}$=4.921, p=0.037).

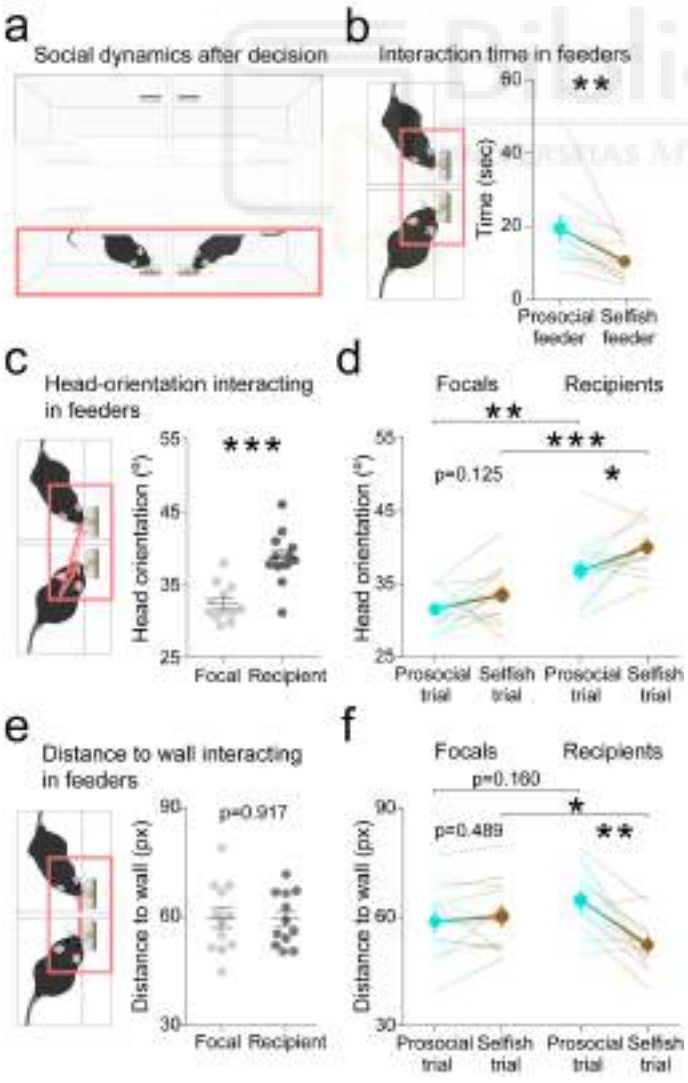


Figure 10. Social dynamics after decision.

a. Illustration of the arena used for the PCT. Results are focused on the social behaviours in the reward areas during the period from choice to reward. **b**. Interaction time in prosocial and selfish reward areas (pink squares). Mice interacted significantly longer in the prosocial area. **c**. Head-orientation of focals and recipients per trial during interaction in feeder areas. Focals are more oriented towards their recipients. **d**. Head-orientation of focals and recipients during interactions in prosocial and selfish trials. Focals keep the same orientation while recipients are more oriented towards their focals in the prosocial area. **e.** Distance to partition for focals and recipients. We measured the distance to check which animal is closer to the partition, hence to the other mouse. We found no differences between focals and recipients. **f**. Distance to partition for focals and recipients in prosocial and selfish trials. Focals keep the same distance, whereas recipients are closer in selfish trials. For all graphs: degraded thicker line shows mean±SEM, thinner lines represent data from each individual. Blue = prosocial, brown = selfish.

Taken together, these results show that focal mice were oriented towards the recipient during interaction periods both prior and after the decision was made, but this social interest was not modulated depending on the choices focal animals made. Recipient animals, however, did show differences on how they interact towards their partner (i.e. orientation and distance) when focal animals decided to act prosocial or selfishly, yet none of these behaviours seemed to affect the decisions of the focals.

4.1.1.5. Individual differences between prosocial and selfish mice

After observing marked individual differences in focals in terms of prosocial biases (Figure 5e), we investigated whether these biases would reflect differences in the behaviour of the focals, the recipients or their social interactions.

Indeed, we found that those recipients that were paired with a prosocial focal would initially display stronger food-seeking behaviours, having a higher frequency of prosocial pokes during the first session of the PCT (Figure 11a) (Independent samples t-test: $t_{(3)}$=3.962, p=0.029, BF_{10}=2.721). Furthermore, we found that recipients from prosocial focals tended to enter faster after all partner's choices (Figure 11b) (Independent samples t-test: $t_{(3)}$=-3.063, p=0.055, BF_{10}=1.939). Moreover, prosocial focals were closer to their recipients during interactions in both reward areas (Figure 11c) (Independent samples t-test: $t_{(3)}$=-3.893, p=0.030, BF_{10}=2.656). Finally, we also found that prosocial focals took longer to return to the choice area to start a new trial after a prosocial choice during the first session (Independent samples t-test: $t_{(3)}$=20.462, p=2.552e-4, BF_{10}=45.308) (Figure 11d).

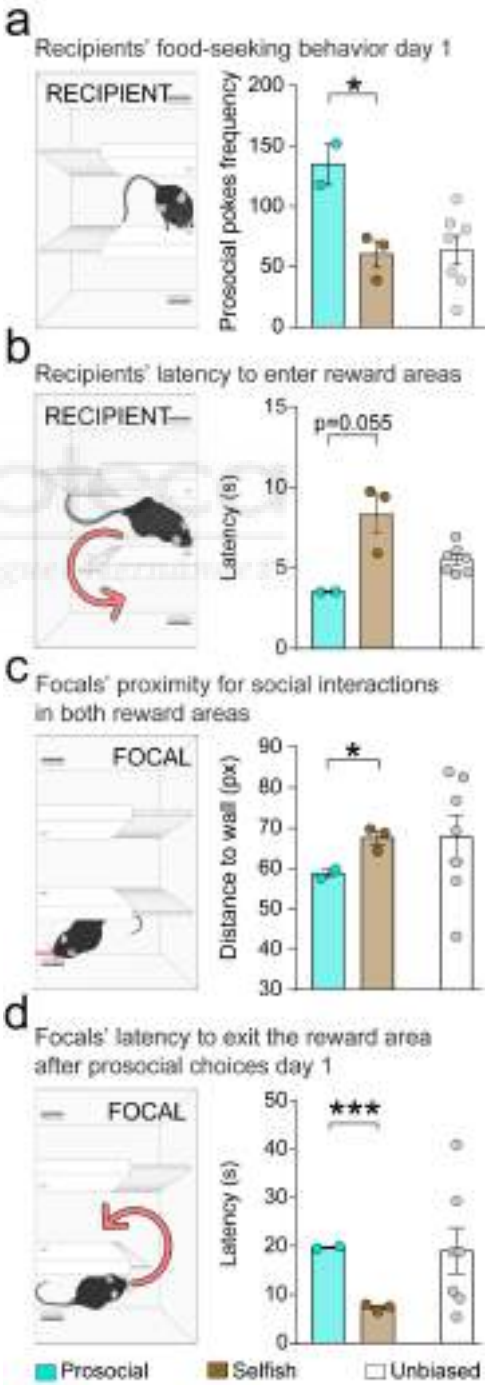


Figure 11. Individual differences between prosocial and selfish mice.

a. Recipients' prosocial nose-pokes frequency on day 1 of the PCT. We compared the averaged frequency of prosocial nose-pokes that recipient mice did during the first session of the PCT, according to the classification of the pairs as prosocial or selfish. We observed that those recipients from prosocial pairs do significantly more prosocial pokes compared to selfish. **b**. Recipients latency to enter reward after focals' choices. Recipients from selfish partners took longer to enter than prosocial after any type of choice. **c**. Distance to wall for focals during reward period. We found that prosocial focals were closer to the wall than selfish focals. **d.** Focals latency to exit the reward area after prosocial choices on day 1. Prosocial focals took longer than selfish focals to return to the choice area to start a new trial after prosocial choices on the first session of the PCT.

4.1.1.6. Assessing prosociality with low-trained decision-makers

To further investigate the lack of prosociality observed in our experiments performed with mice, we considered that the individual training protocols used could be influencing the decision-making process during the social task. It has been suggested that prolonged training of an instrumental action like nose-poking, can make such behaviour become habitual and thus, less goal dependent (Thrailkill & Daniels, 2024), and further demonstrated to have an effect in prosocial actions to avoid harm to others in rats (Hernandez-Lallement et al., 2020). Recent evidence shows that most male mice are prosocial in a reward-based operant paradigm (Scheggia et al., 2022), in which decision-maker mice learn the task contingencies during social testing, suggesting that individual training might not be required for this type of prosociality to emerge in mice. Yet, in this last study, authors found that when individually trained, most focal mice switched their preferences to that rewarding the recipients, but with a weaker magnitude than without individual training (Scheggia et al., 2022).

We thus evaluated whether overtraining of decision-makers was interfering with the emergence of prosocial tendencies in our hands. We performed an independent experiment (n=24), where decision-makers had a minimal individual training, consisting of two sessions of fixed-ratio 1 before social testing (Figure 12a-b). No food-restriction protocols were used in any session of this experiment. We assessed the decision-makers' preference (Figure 12c) and found that short training does not promote prosocial choices, as on average, focal mice had no preference for any of the options over days (repeated measures ANOVA with 'session' as within subjects: $F_{(6)}$=0.909, p=0.494, BF_{incl}=0.153). Interestingly, the preferences were very polarised (i.e. some focals were completely prosocial while others completely selfish) already in the first session. Thus, short training seemed to promote a foraging strategy for the single choice exploitation rather than both choices exploration. Categorization in

preference groups according to the Prosocial Choice Index revealed that most of the animals in this experiment (i.e. without training in the maze navigation) were unbiased, only one was selfish and none of them prosocial (Figure 12d, Table 2). These results indicate that, in our hands, shorter individual training did not increase the rate of prosocial choices in mice.

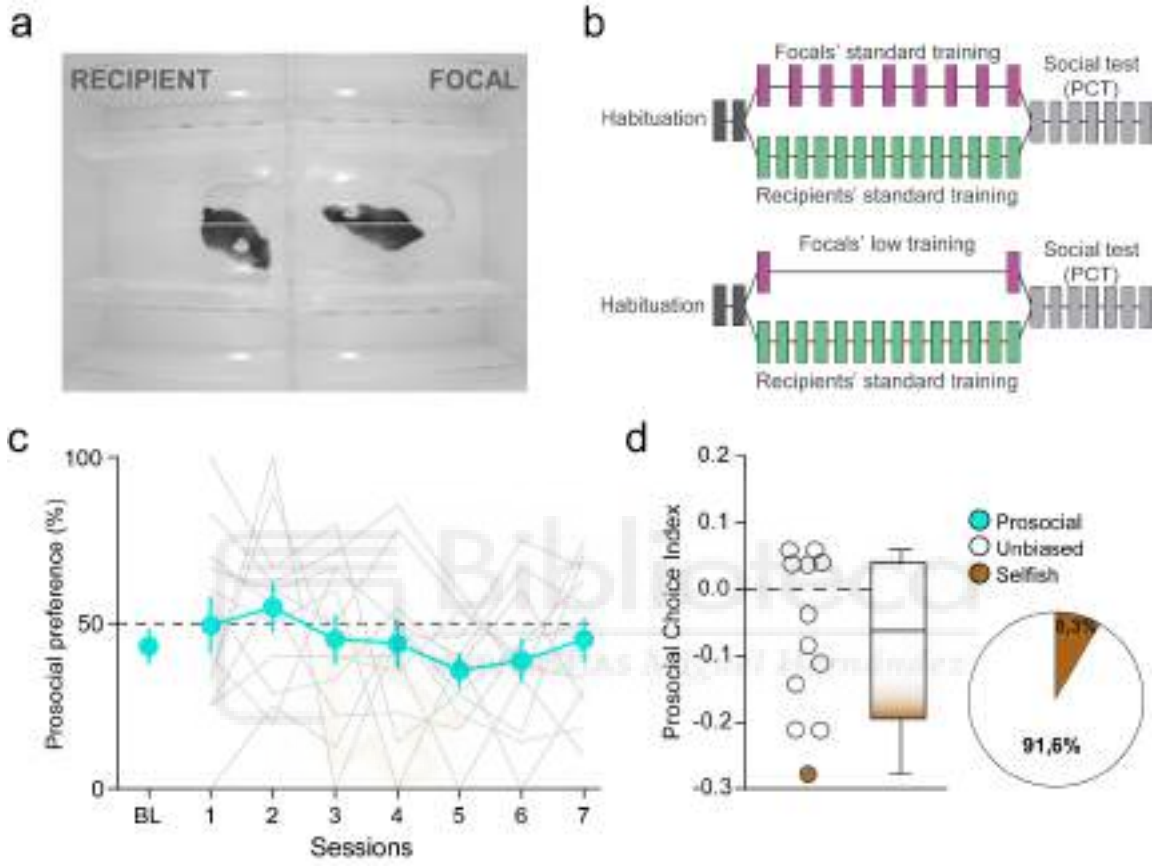


Figure 12. Choice preference of decision-makers with low training level

a. Real example image of mice in the choice area during a PCT session in the double T-maze setup configuration used for testing prosocial choices of focal mice with low-level training. **b**. Schema of standard individual training for recipients and low-level training for focals. Focals and recipients perform 2 sessions of habituation to the maze, then they all undergo a fixed-ratio 1 protocol session. Recipients continue their standard training protocol however, focals only perform an additional fixed-ratio 1 session just prior to social testing. **c**. Prosocial preference of low-trained focals running the Prosocial Choice Task. Percentage of prosocial choices (Y axis) over the seven testing sessions (X axis). BL refers to baseline, used to evaluate individuals' preference in the last two sessions of individual training. Blue thick line corresponds to mean±SEM, grey lines correspond to each individual. **d**. Distribution of Prosocial Choice Indexes. Positive values show a preference for the prosocial option, negative values indicate preference for the selfish option, and values close to 0 indicate chance preference. Blue dots correspond to prosocial mice, grey dots are unbiased and brown selfish. On the right, pie chart: distribution of mice after permutation test of Prosocial Choice Indexes.

4.1.2. Prosocial choice task with two-chamber setup configuration

An additional possible explanation about the lack of prosociality at the population level found in our previous experiments might be due to the paradigm we developed here inspired in our previous work in rats, which might be too demanding or complex for mice. The different compartments and temporally separated moments for choice and reward delivery in the double T-maze could interfere with the ability of mice to associate their choices with the food-preference and reward cues displayed by the recipients. We thus decided to develop another paradigm, closer to the design of (Scheggia et al., 2022) where, in contrast to our previous results, they found that most of the male mice tested developed a prosocial preference over days.

The design of this new setup consisted in an acrylic box, with a transparent and perforated partition in the middle which allows mice to see, hear, smell, and partially touch each other. Tthere are two contiguous areas, one for the decision-maker (focal mouse) and another for the recipient mouse (where delivery of reward depends on the focal's choices) (Figure 13a). In contrast to (Scheggia et al., 2022), the location of the choice ports (i.e. prosocial and selfish) where equidistant from the recipient (to avoid possible baseline preferences for the one closer to the partner or local enhancement effects in that poke) and the recipients had one nose-port to display food-seeking behaviour.

During individual training of the decision makers, mice were trained to poke into both options the same way, so by the end of the individual training they showed no strong preference for any of the options (Figure 14b) (one sample t-test against chance (50), $t_{(5)}$=-1.324, p=0.243, BF_{10}=0.698). Once the individual training was fulfilled mice underwent the prosocial choice task (PCT), following the protocol described in Prosocial Choice Task protocol in the two-chamber arena (Figure 13b). We tested six pairs of cage-mate male mice (C57BL/6) during 11 sessions of 40 minutes. As in (Scheggia et al., 2022), for this set of experiments, we mildly food restricted the animals before undergoing the PCT, to increase their motivation for obtaining the food pellets. Under this schedule, we evaluated if mice develop a preference towards the prosocial option and found that mice did not have any consistent preference over sessions in this double-chamber paradigm (repeated measures ANOVA with 'session' as within subjects factor: $F_{(10)}$=1.105, p=0.39, BF_{incl}=0.335) (Figure 13c). Yet, we observed marked individual differences over sessions, already present on the first session of the PCT (Figure 13c, grey lines).

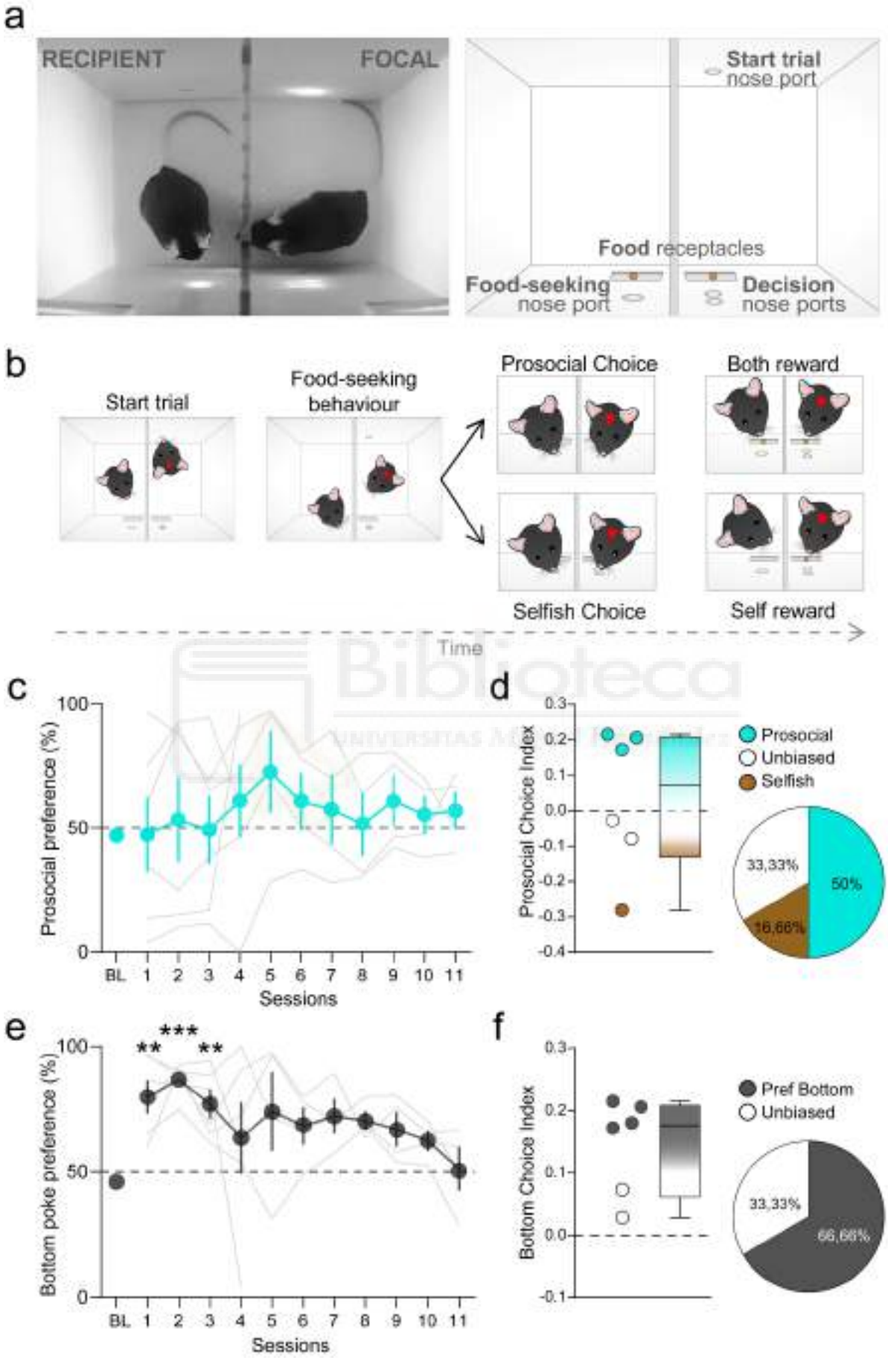


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Figure 13. Mice prosocial choices in the two-chamber PCT

a. Left, real top-view image of the two-chamber setup used for evaluating prosocial decision-making with mice. Focal (on the right) is checking the recipient mouse (on the left) through the perforated and transparent partition that divides the two chambers. Right, schematic of the peripherals used inside the setup. In the focals' side there is a nose-port to start the trials, on the opposite wall there are two other nose-ports placed vertically used for decision and below these there is a food magazine where food pellets are delivered. In the recipients' side there is a single nose-port to display food-seeking behaviour and below there is the food magazine for rewards. **b**. Timeline structure of the prosocial choice task. Trials begin when the decision-maker pokes into the nose-port to start, then the recipient mouse needs to poke into the nose-port to display food-seeking behaviour which activates the pokes for decision of the focal mouse. Poking into the prosocial port will deliver a food pellet to both animals however, choosing the selfish port will reward only the focal mouse. **c**. Prosocial preference of mice in two-chamber arena. Percentage of prosocial choices made by all focal mice (Y axis) during 11 experimental sessions on consecutive days (X axis). BL referrers to baseline, used to assess the preference of the focals on the last two days of individual training. Blue line corresponds to mean±SEM, grey lines correspond to data of each individual. At population level, prosocial preference was not different from chance (50%) in any experimental day. **d**. Distribution of Prosocial Choice Indexes. Positive values show a preference for the prosocial option, negative values indicate preference for the selfish option, and values close to 0 indicate chance preference. Blue dots correspond to prosocial mice, white dots are unbiased and brown selfish. On the right, pie chart: distribution of mice after permutation test of the Prosocial Choice Indexes. **e**. Preference for bottom choice. Percentage of choices towards the bottom option (Y axis) over the testing sessions (X axis). Black line corresponds to mean data from all individuals, grey lines correspond to each individual data. There is a significant preference for the bottom option already in the first 3 days, which slowly decays over sessions. **f**. Distribution of bottom choices. Positive values show a preference for the bottom option, negative values indicate preference for the upper option, and values close to 0 indicate chance preference. Grey dots represent mice with preference for the bottom option, and white represent unbiased. On the right, pie chart: distribution of mice after permutation test of the bottom choice index in percentages.

Indeed, with this double chamber paradigm, the categorization of focal animals according to their PCI scores after the permutation test revealed that 50% of the animals were prosocial, 2 focals were unbiased and only one was selfish (Figure 13d, Table 2), which represents a much higher proportion of animals compared to our doblue maze configuration paradigm. However, these preferences for the prosocial or selfish option were explained by a general preference for the poke that was placed closer to the food magasin, which did not required the animals to rear in order to activate, and thus was less costly (repeated measures ANOVA, with 'session' as within subjects factor: $F_{(10)}$=2.42, p=0.03, BF_{incl}=2.6) (Figure 13e), especially during the first three sessions of the PCT, when they are learning the new contingencies of the task: (one-sample t test against chance (50%): $t_{(5)}$ = 4.697, p=0.005, BF_{10}=11.069 for session 1; $t_{(5)}$=14.185, p=3.136e-5, BF_{10}=600 for session 2; $t_{(5)}$ =4.921, p=0.004, BF_{10}=12.86 for session 3). We also calculated the PCI for the bottom preference to assess individuals' differences in the preference, and found a significant increased preference from chance (one sample t-test against 0: $t_{(5)}$=4.65, p=0.003, BF_{+0}= 21.29) (Figure 13f, Table 2).

These results suggest that the marked individual differences we observed in the prosocial choice task were explained by a bias towards the option that would require less effort and was not explained by the recipient's reward contingencies.

In conclusion, in our hands, C57BL/6 mice do not show prosocial tendencies at the population level, or at least not as widespread as for rats. Interestingly, we find that there are marked individual differences in mice, and that those who develop a prosocial preference are those paying more attention to the recipients' behaviour during reward delivery, or those whose recipients more clearly display its food-seeking behaviours. We highlight here that these are factors that we previously demonstrated to promote prosociality in rats. It thus seems that there are some commonalities in the behavioural factors associated with reward-based prosociality for mice and rats, although this phenomenon is much more widespread in rats.

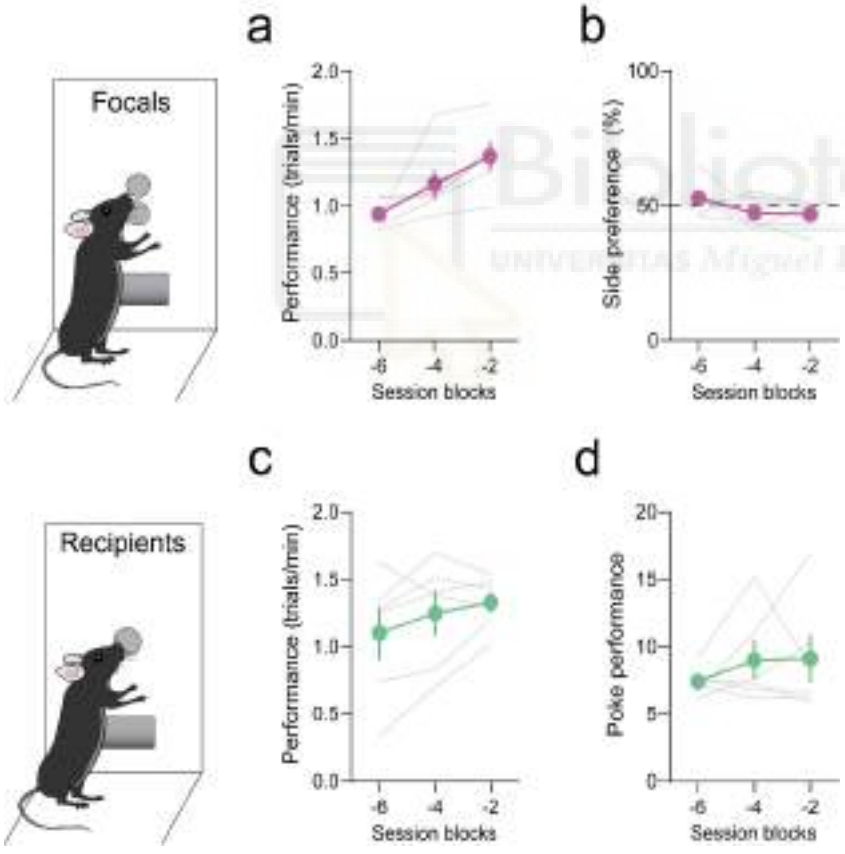

Figure 14. Individual training in the two-chamber before the PCT

a. Performance of focal mice during last phases of individual training, calculated as total trials/session duration in minutes. X-axis are the sessions before the PCT averaged in two (i.e., -2 is averaged data from the last two sessions of training before PCT). **b**. Side preference during the last phases of individual training. Proportion of choices during last sessions of individual training to the side that will be prosocial in the PCT. **c**. Performance of recipient mice during the last phases of individual training. Same as **a.** for recipient mice. **d**. Nose-poke performance during last sessions of individual training, calculated as total number of nose-pokes by number of trials. For all graphs: thicker line shows mean±SEM, thinner lines represent data from each individual.

As the second part of my PhD thesis objectives was to investigate the neural substrates of the motivation to help conspecifics, the following chapter is based on experiments performed with rats, where prosociality in reward-based contexts is more commonly found.

4.2. Identifying neural circuits underlying the motivation to help others

To study and identify the neural circuitry involved in the perception of positive affective states and underlying the motivation to help others, we performed a series of experiments in rats, where we recorded and manipulated activity of the VTA in different settings.

4.2.1. Male Sprague-Dawley rats display increases in VTA activity while observing conspecifics being rewarded.

To determine whether rats perceive positive affective states of other conspecifics and to study the neural activity underlying this process, we developed a new behavioral task where pairs of non-food deprived rats received palatable food pellets in a trial-based manner (Figure 15a) and calcium imaging transients (AAV2-Syn-GCaMP6s) were recorded in the VTA of one of the animals (the focal) using fiber photometry (*see* Figure 16 for controls of activity related signals of GCaMP vs GFP in our settings, where we can see that possible motion artifacts interfering with GCaMP signals are negligible). The behavioral paradigm consists in a custom-made acrylic arena (Figure 15a) which resembles, in a larger scale, one reward area from the double T-maze used for assessing prosocial decision-making in rats, developed by (Márquez et al., 2015). The arena is divided into two chambers separated by a perforated and transparent acrylic partition that allows the exchange of multisensory information between the pair of animals. In each chamber there is a food-receptacle where the pellets are delivered via a custom-made pellet dispenser.

The task follows a simple structure (Figure 15b) which consists in the delivery of alternate rewards to both animals. First, a pellet is delivered to the focal animal after a pseudo-random intertrial interval (20-39 seconds), followed by a pellet delivered to the partner rat after a pseudo-random time interval (3-5 seconds). Rewards are delivered in separated time points to be able to study independently the temporal dynamics of the VTA responses to the self and other-reward moments. As expected, delivery of unpredictable rewards to the focal rat (self-reward) induces increased calcium activity in the VTA, which peaks at the moment of pellet retrieval from the food

magasin and is observed in most of the trials (91%) (Figure 15c). Interestingly, increases in VTA activity of the focal rat were also present when the recipient rat was the one being rewarded, in a considerable amount of trials (64%) (Figure 15d).

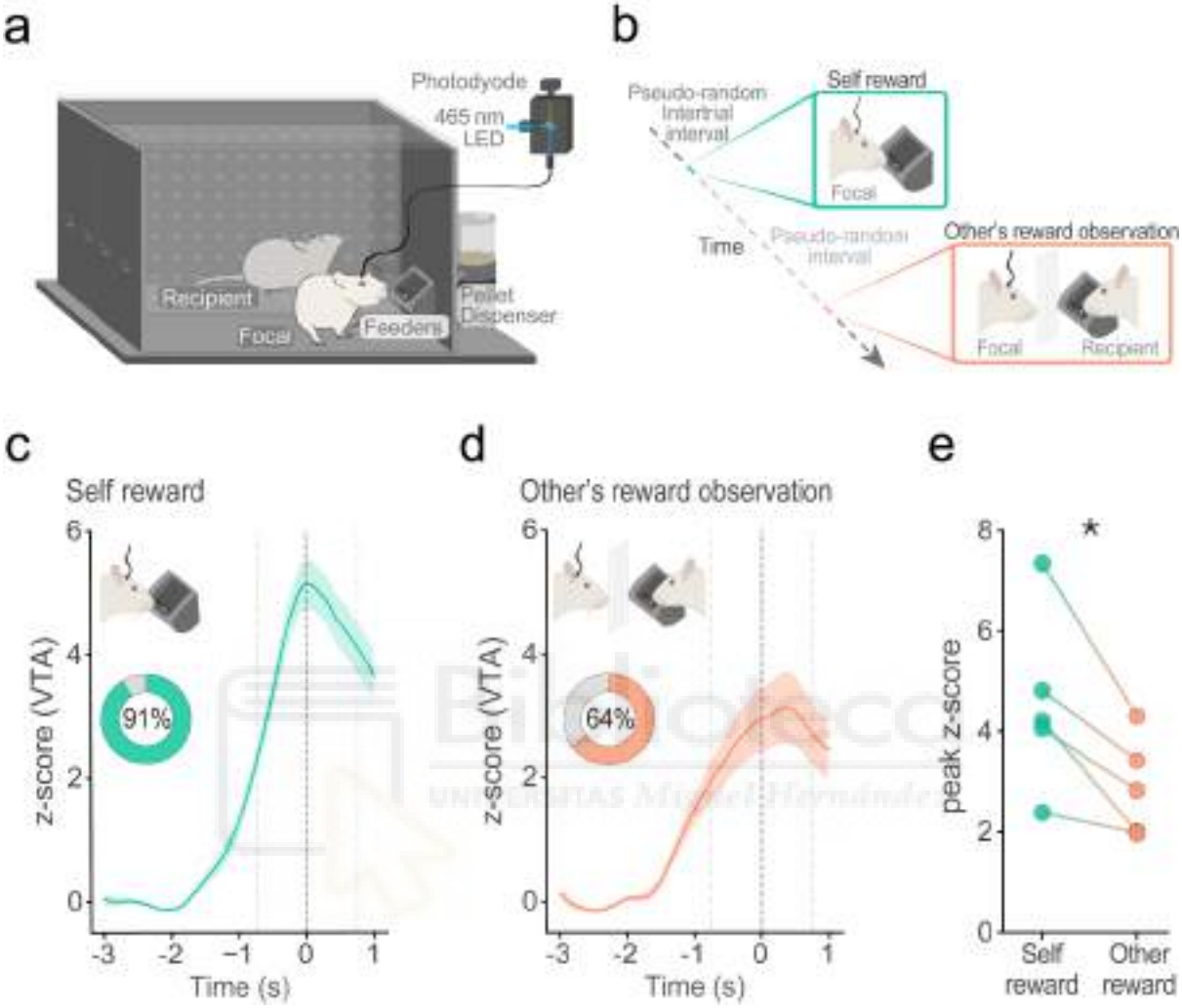

Figure 15. Self and vicarious rewards are encoded in VTA neurons activity

a. Behavioural setup used for recording calcium activity of neurons in the VTA during delivery of food rewards for focal (animal being recorded) and recipient animal. Rats are separated by a perforated and transparent partition. On each side, there is a food receptacle where food reward pellets are delivered via custom-made pellet dispensers. Calcium imaging system (Doric) is placed above the behavioural arena and a patch cable is connected to the fiber optic attached to the VTA of the focal rat**. b**. Trial structure of the task. Each trial starts by delivering a pellet first to the focal rat after a pseudo-random trial interval (20-39 seconds). Once the pellet is retrieved a reward pellet is then delivered to the recipient rat in the adjacent chamber after a pseudo-random time interval. **c.** Peri-event time histogram (PETH) of normalized ΔF/F (z-score) for VTA neurons during self-reward. Traces are aligned to the moment of pellet retrieval from the food receptacle (dashed line at time 0s). **d**. Peri-event time histogram (PETH) of normalized ΔF/F for VTA neurons during perception of the reward of another conspecific. Traces are aligned to the moment of pellet retrieval from the food receptacle (dashed line at time 0s). **e**. Mean normalized ΔF/F (z-Score) peaks around the time of pellet retrieval (dashed lines, ± 0.75s on C and D) for self-reward (green) and perception of the reward for others (orange). *p<0.05. Lines and shadow represent mean±sem. Data from 3 pairs of rats, 212 trials across 2 testing sessions.

These VTA increases during other-reward observation were not related to secondary order cues, such as the sound of the motor rotation for the pellet delivery acting as a reward predicting cue for the focal (Figure 16b-c). When we aligned the calcium traces of focals' activity to the starting of the rotation for the self (Figure 16b) and other reward (Figure 16c), we only found slow and subtle increases of fluorescence aligned to the rotation of the motor for the self-reward, but not for the other-reward, which if something, seemed to slightly decrease (Figure 16c). Therefore, we excluded the motor rotation noise as a reward-predicting cue to account for an explanation of the increases of VTA activity associated with the perception of the reward of others. Overall, these results demonstrate that rats display a vicarious experience of conspecifics being rewarded that involves activity of VTA neurons. These VTA increases during other rewards observation were smaller in magnitude than rewards to the self (paired samples t test, $t_{(4)}=3.683$, $p=0.021$) (Figure 15e). To investigate whether vicarious reward responses followed any specific temporal pattern, we explored the distribution of trials where increases of VTA activity were observed, but we did not find any specific temporal dynamic in the distribution within or across sessions (Figure 16d-e), which suggests that vicarious rewards encoded in VTA activity do no need learning to be observed and do not habituate over time, at least during the temporal window studied.

These data indicate that VTA neurons do not respond exclusively to self-rewarding experiences but also to vicarious experiences, by perceiving a conspecific being rewarded. This fact opens new possibilities for understanding the biological mechanisms underlying the perception of emotional states of others and the emotional contagion of positive affective states, a largely understudied topic. It further poses pertinent questions to be answered regarding the influence of this phenomenon on different social behaviours. We have previously demonstrated that rats naturally perform prosocial behaviours (i.e., actions that benefit others) (Márquez et al., 2015; Gachomba et al., 2022) and that contingencies of the reward for the others are necessary for prosocial decisions (Márquez et al., 2015). One possibility is that the VTA increases we have examined upon observation of reward to a conspecific could be the neurobiological substrate mediating the motivation to help others. We thus setup to explore this possibility.

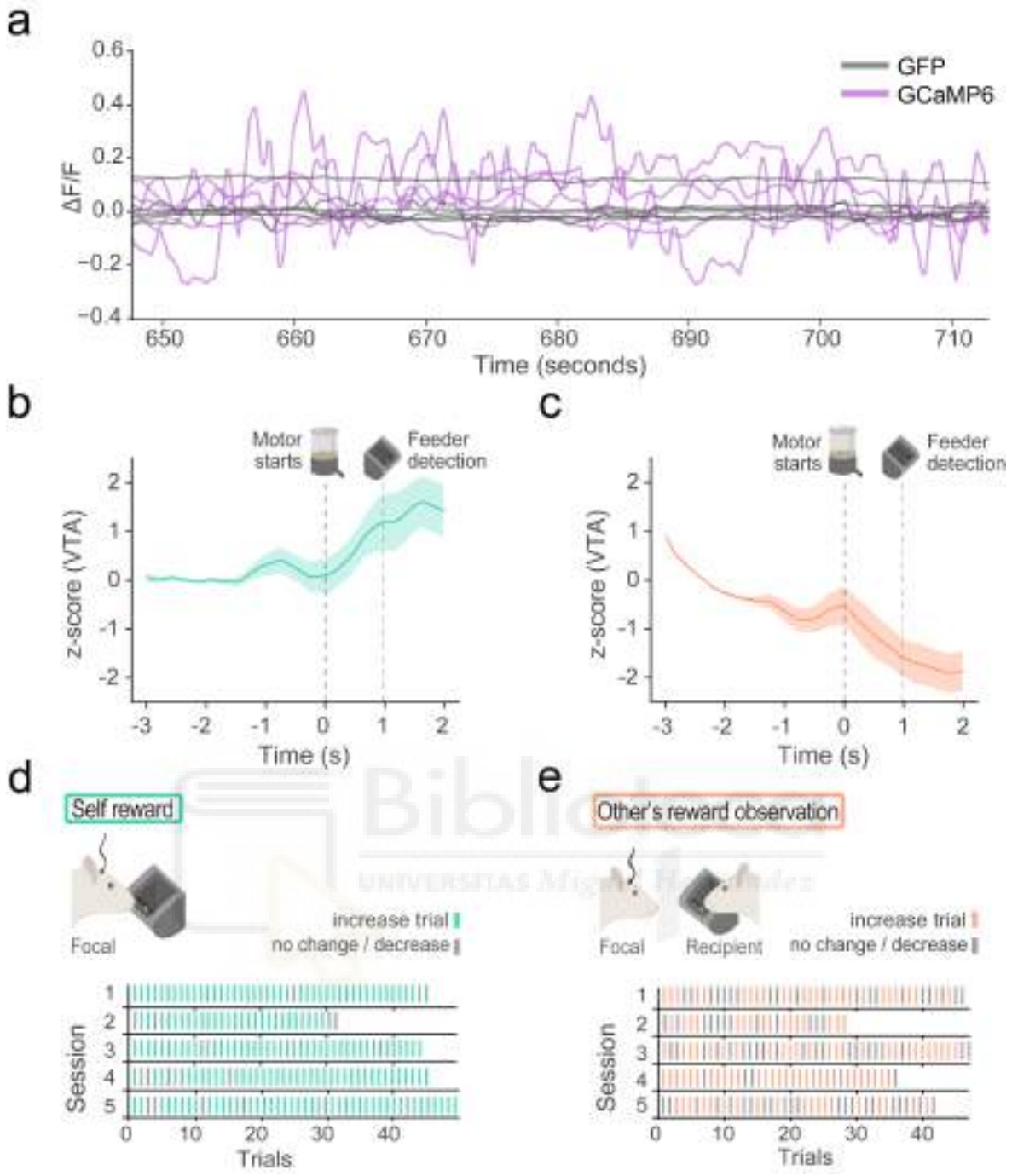

Figure 16. Calcium activity of VTA neurons during self-reward and during reward of other

a. Exemplary traces (60 seconds) of ΔF/F for GCaMP6 (activity-dependent, in purple) and GFP (non-activity dependent, in grey) fiber photometry recordings, to control for possible movement artifacts. Magnitude of changes in fluorescence in GFP are negligible compared to GCaMP fluorescence variations. **b.** Peri-event time histogram (PETH) of normalized ΔF/F for VTA neurons during start of motor rotation (dashed line at time 0s) for self-reward delivery. The other dashed line represents the time when the pellet arrives to the food receptacle. Green line and shadow represent mean ± SEM. **c.** Same as **b** for other-reward, in orange. **d.** Identification of all trials from all sessions that show an increase around the moment of self-consumption of the pellet compared to the baseline (91%, from Figure 15c). In green, increase trials. In grey, trials determined as no change/decrease. **e.** Identification of all trials from all sessions that show an increase around the moment of consumption of the pellet by the recipient rat compared to the baseline (64%, from Figure 15d). In orange, increase trials. In grey, trials determined as no change/decrease. Data from 3 pairs of rats, 212 trials across 2 testing sessions.

4.2.2. Closed-loop optogenetic inhibition of VTA neurons during perception of others' rewards is necessary for the emergence of prosocial preferences in the PCT.

To address if these vicarious reward responses are necessary for rats to act prosocially to one another, we performed an optogenetic closed-loop loss of function experiment, in which we inhibited VTA activity specifically during vicarious reward moments of decision-makers that were choosing to help their cagemates. We used our previously validated double T-maze (Márquez et al., 2015; Gachomba et al., 2022) to prove the role of VTA activity during the perception of the reward of others in the Prosocial Choice Task (Figure 17a) . Briefly, in this task pairs of animals are tested, but only the decision-maker (focal rat) controls the access to rewards for both animals. In each trial, the focal rat can choose between one side of the maze, which provides food only to itself (selfish choice) or the opposite side, where both animals are rewarded (prosocial choice). Thus, focal rats are always rewarded, but their decisions affect the reward of others.

For this experiment, decision-maker rats were injected in the VTA with either soma-targeted anion-conducting ChR2 (AAV1-CaMKIIa-stGtACR2 FusionRed, n=6) or AAV1-CAG-tdTomato (*for controls, n=7*), and implanted with an optic fiber over the VTA, creating a long-lasting implant to which a wireless optogenetic device (HARP WEAR motion sensor v2.1, developed by the Champalimaud Foundation Hardware Platform) (Tang et al., 2024) was attached at the beginning of the experiments (Figure 17b). Closed-loop optogenetic inhibition of transfected cells in the VTA was achieved by a custom-made interface that linked the information between the state-machine controller of the behavioural arena with real-time video recordings (with Bonsai) and the WEAR device (Figure 17c). A Bonsai workflow ran and controlled the optical stimulation protocol as follows: after a prosocial choice, the focal rat had access to its own reward in the prosocial area, and two seconds after pellet consumption, the recipient had access to its reward. Then, a vision-based ROI detected when the recipient approached the feeder into the prosocial area, triggering the starting of the light stimulation. Upon pellet consumption, a digital output from the state-machine continued the stimulation for 4 seconds followed by a ramp down (Figure 17d) to avoid possible rebound activity effects (Figure 18b) (*for more details see* Wireless

optogenetic inhibition). In this manner, we were able to specifically inhibit the VTA activity of decision-makers during the reward of the recipient leaving intact the self-reward responses. In the case of selfish choices, the structure of the trial and the gating of the doors remained as explained but no optical stimulation was delivered.

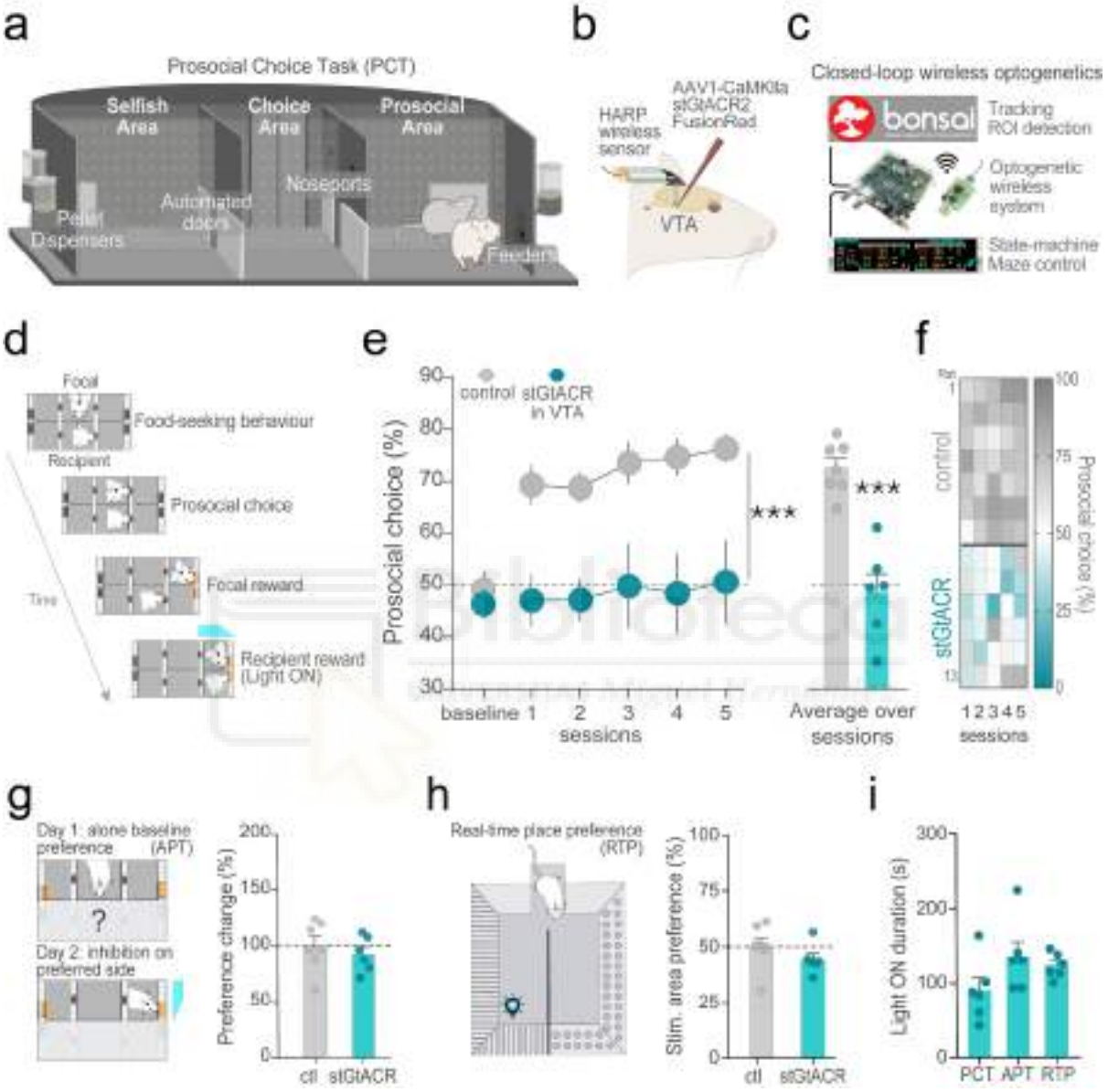

Figure 17. Closed-loop optogenetic inhibition of VTA neurons during perception of others' rewards blocks the emergence of prosocial preferences in the PCT.

a. Illustration of the behavioural arena used for the Prosocial Choice Task (PCT). The double T-maze is separated into two by a perforated and transparent partition that allows exchange of multisensory information between the animals. For each T-maze there is a central choice area with two nose-ports located in each wall (for decisions and displays of food-seeking behaviour), and infrared beams to detect the rats' position. There are automated doors below the nose-ports that give access to the reward areas (Prosocial and Selfish areas), where food pellets are delivered by motor dispensers into receptacles for the animals to retrieve. After food retrieval, rats can go back to the central area to run another trial. **b**. Schema of viral injection and wireless sensor device for optogenetics. AAV1-CaMKIIa stGtACR2 FusionRed or tdTomato was

injected on the VTA of decision-makers rats and a fiber optic was implanted on top of the area. The HARP wireless device for optogenetics was attached to the implant at the beginning of the experimental sessions to provide light to the transfected neurons in the VTA. **c**. Schema of the system used to perform closed-loop wireless optogenetics during the PCT. A custom-made interface linked the information from the state-machine controller with the camera recordings and the HARP device. The optical stimulation protocol was controlled by using a Bonsai workflow that: (1) detected the position of the recipient rat approaching the pellet receptacle in prosocial trials to trigger the light stimulation. Then (2) detection of the pellet being retrieved by the recipient triggered a ramp down of the light voltage. **d**. Timeline structure of a prosocial trial. Each trial started with both rats entering the choice area, where the recipient would display food-seeking behaviour into the preferred side of the maze, and the focal could choose to poke in either side of the maze. After a prosocial choice, the door opened for the focal to access the reward area and after pellet retrieval, the recipient's door opened. When the recipient entered the prosocial reward area the focal's device turned the light ON for inhibition of the VTA neurons, which continued for some seconds after the recipient retrieved the food pellet. **e**. Prosocial preference of rats was blocked by inhibition of the VTA neurons during vicarious reward moments. On the Y-axis, percentage of prosocial choices over testing sessions (X-axis). Baseline corresponds to the last two sessions of individual training. Grey dots and line, control group (injected with tdTomato in the VTA). Blue dots and line, group of animals injected with stGtACR2 in the VTA. Right bar graph: averaged proportion of prosocial choices from all sessions. Inhibiting the VTA specifically during the reward of the recipient after a prosocial choice significantly reduced the proportion of prosocial choices in the experimental group. **f.** Heatmap of individual prosocial preferences. Each row corresponds to a single subject (n=13) and each column corresponds to a single session of the PCT (n=5). Higher prosocial preference is marked in grey and lower in blue. **g**. Alone preference test. To control for possible aversive effects of unilateral VTA inhibition, decision-makers were tested alone in the maze in a different session after the PCT. On day 1, possible side preferences while performing alone in the maze were studied. The day after, focals' VTA activity was inhibited after reward retrieval on the preferred side. Bar graph represents the change in preference from session 1 (without any optical stimulation) to session 2 (with optical inhibition of the VTA), i.e., 100% indicates same preference as the previous session. No significant change was observed for any of the groups in their preference from session 1 to session 2. **h**. Real-time place preference. To further control for possible aversive effects of unilateral VTA inhibition, decision-makers were tested in a contextual place preference. The arena consisted in two separated chambers with contextual differences (stripes or dots on the walls) which the focal rats could freely explore. In a closed-loop system, one of the chambers was systematically associated with optical stimulation, inhibiting the VTA neurons, thus if and while the focal was visiting the corresponding area, light was provided by the optogenetics device. Bar graph: time (in percentage) that rats spent on the stimulating chamber. No difference was observed in either group. **i**. Duration of optogenetic inhibition for the stGtACR2 group in the different optogenetic experiments. We quantified the total duration in seconds of optical stimulation for the stGtACR2 group and compared between the different experiments. Light stimulation used in the PCT and control experiments was comparable.

To confirm that light power at the intensity produced by the LED/fiber optic cannulas used in the behavioural experiments (8 mW) was capable of inhibiting VTA neurons in vivo, we performed a series of optoelectrophysiological experiments in which a fiber optic cannula was placed in close proximity to a recording electrode targeting the VTA in anesthetized rats. We found that out of 11 total units isolated, 10 reduced their average firing rate across all trials upon light stimulation compared to a 20-s pre-stimulus baseline (Figure 18a-b). Because baseline firing rate for most units was low

(0.74 Hz on average), it was not possible to determine whether the decrease in firing was statistically significant. We found that 4 s long optogenetic inhibition of the VTA neurons cause rebound activity (Figure 18b), hence we tested different protocols to ramp down the light power to 0 mW (Figure 18c). Ramping the light power over 700 ms probed to decrease the possible rebound spikes that could cause unwanted behavioural effects. Based on these results, we decided to use a ramp of 1200 ms duration for the optogenetic inhibition during the PCT.

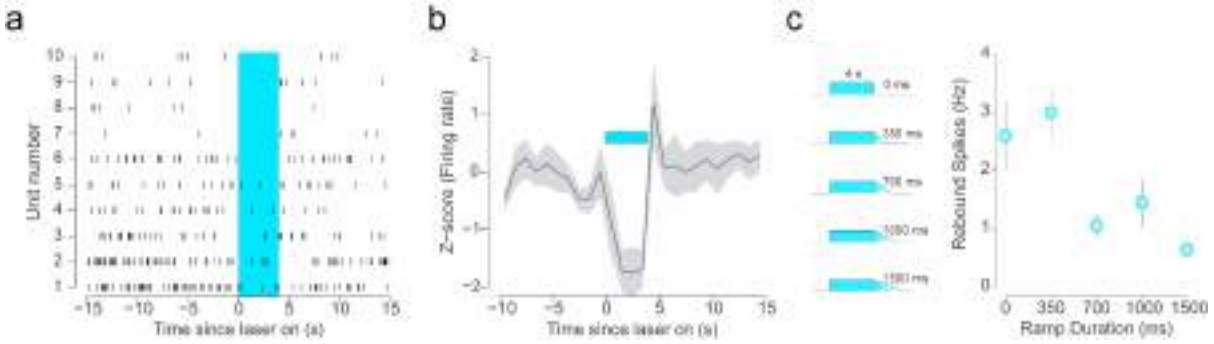

Figure 18. Electrophysiological recordings during light stimulation in the VTA of anesthetized rats

a. Raster plot showing firing for each of the 10 neurons from 15 s before to 15 s after laser onset during the trial with the highest number of baseline spikes. Units are ordered by the number of spikes occurring during light stimulation. The laser stimulation epoch is represented by the blue rectangle (4 s). **b**. A peri-event time histogram showing the average firing across all trials for all 10 units from 10 s before to 15 s after laser onset. Z-score was computed using a 20-s pre- stimulus baseline, and the bin width = 1000 ms. Data are represented as mean ± SEM. The laser stimulation epoch is represented by the blue rectangle (4 s). **c.** Left, light power was linearly ramped down to 0 mW over a duration of either 0 ms (no ramp), 350 ms, 700 ms, 1000 ms, or 1500 ms. Right, rebound spikes found after the different ramping protocols.

We found that, by doing this precise closed-loop optogenetic inhibition of the VTA activity, time-locked to the reward consumption of the recipient, we were able to block the prosocial preference in our experimental group, while the control group maintained a high prosocial preference over the testing sessions (Figure 17e). Specifically, the proportion of prosocial choices was significantly reduced in the stGtACR2 group (repeated-measures ANOVA with ‘‘session’’ as within-subjects factor and ‘‘inhibition’’ as a between-subjects factor; ‘‘session’’ [$F_{(4,44)}$=0.501, p=0.735], “inhibition” by “session” [$F_{(4,44)}$=0.089, p=0.985], and “inhibition” [$F_{(1,11)}$=37.66, p=7.342e-5]) compared to the control group at the population level, decrease evident also at the individual level (Figure 17f). This decrease did not reflect a switch to a selfish preference, as it was not different from chance (one sample t-test against chance (50), $t_{(5)}$=-0.383, p=0.717, BF_{10}=0.379). Interestingly, the control group showed a high

prosocial preference already on the first session compared to the stGtACR2 group, although when studying the emergence of prosociality in this session, both groups started at chance levels (RM ANOVA with “session 1 in 10 mins blocks” as within-subjects factor and “inhibition” as a between-subjects factor; “10 mins blocks” [$F_{(3,33)}$=1.936, p=0.143], “inhibition” by “10 mins blocks” [$F_{(3,33)}$=2.264, p=0.099], and “inhibition” [$F_{(1,11)}$=15.030, p=0.003]). Simple main effects tests indicated that preference was similar for the first 10’ and 20’ blocks, but significantly higher for the control group on the 30’ (p=0.006) and 40’ (p=0.003). Furthermore, this increased preference of the control group was emerging throughout the session, while stGtACR2 animals remained at chance (one sample t-test against chance (50), [in first 10’ $t_{(6)}$=1.465, p=0.193 for control, $t_{(5)}$=-0.932, p=0.394 for stGtACR2], [for the next 10’, $t_{(6)}$=3.645, p=0.011 for control, $t_{(5)}$=0.878, p=0.420 for stGtACR2], [for the third 10’ $t_{(6)}$=5.182, p=0.0021 for control, $t_{(5)}$=-1.177, p=0.292 for stGtACR2], [for the last 10 mins, and $t_{(6)}$=7.084, p=0.0004 for control, $t_{(5)}$=-1.089, p=0.326 for opsin]) (Figure 19a).

Previous reports have shown that optogenetic inhibition of DA in the VTA can evoke immediate aversive behavioral responses, mediated by D2 receptors in the NAc (Danjo et al., 2014). Although we performed a unilateral (side counterbalanced between subjects) inhibition of VTA neurons under CaMKIIa promoter, the decrease in proportion of prosocial choices found in the present study could have been explained by an aversive effect of the optogenetic inhibition, affecting DA D2 neurons projecting to the NAc or other cell types in the VTA. Thus, we carried out control experiments to rule out this possibility. The first control experiment we implemented happened after the PCT last session, in which we placed the implanted focal rats back to the maze, alone, to perform a baseline preference session. During this session we let the decision-makers to freely perform trials for 20 minutes and their side preferences were annotated. During the session on the following day, the structure of the experimental session remained the same, but optical inhibition was delivered into the VTA of focals two seconds after reward consumption in the preferred side from session 1. We measured the percentage change on side preference between the two sessions and found no difference between the experimental and control groups (independent t test, $t_{(11)}$=0.717, p=0.488) (Figure 17g), and the duration of the optical stimulation delivered to both groups was similar (independent samples t test, $t_{(11)}$=0.302, p=0.768) (Figure 19b). To further discard aversive effects of the VTA inhibition, we next performed a

real-time place preference test in a different context. During this experiment focal rats were able to freely explore two different chambers for 5 minutes. One of the chambers was systematically associated with sustained optical stimulation as long as the rats were visiting the area. We assessed the time rats spent in each area and we found that inhibition of VTA neurons activity was not sufficient to produce an effect on the place preference, for any of the groups (independent samples t test, $t_{(11)}$=1.175, p=0.265) (Figure 17h), being the duration of the light delivery similar for both groups (independent samples t test, $t_{(11)}$=1.211, p=0.251) (Figure 19c). We further quantified the duration of stimulation delivered in the different optogenetic experiments and found that the stGtACR2 group did not differ in the duration of stimulation delivered between the tests (one-way ANOVA, $F_{(2,15)}$=2.29, p=0.136) (Figure 17i).

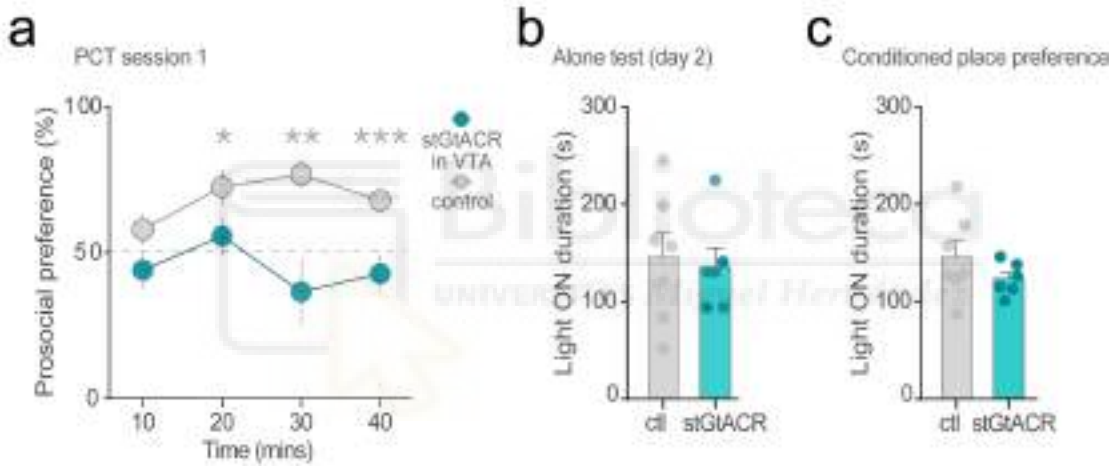

Figure 19. Optical stimulation time during VTA optogenetic inhibition experiments.

a. Emergence of prosocial preferences on the Prosocial Choice Task, during the first day of testing, evaluated in 10-minute blocks. While control animals (in grey) develop a preference significantly higher than chance in the last thirty minutes of the session, stGtACR2 group's preference (in blue) was not different from chance across the whole session. **b**. Duration of optogenetic inhibition during the second session of the Alone Preference Test. Quantification of total duration (in seconds) of optical stimulation on the second session for both groups, when VTA is inhibited after the reward on the preferred side from session one (grey: control, blue: stGtACR2). No significant difference is found between the groups. **c.** Duration of optogenetic inhibition during the Real-Time Place Preference test. Quantification of total duration in seconds of optical stimulation performed during the real-time place preference test for both groups, in which VTA activity of focal rats was inhibited while the animals visited one of the two chambers. No significant difference was observed on the stimulation duration between the two groups. For all graphs, mean ± SEM are reported. (*) report differences against chance.

Taken together, these results indicate that optogenetic inhibition of VTA activity during vicarious rewards blocks the emergence of prosocial choices in the PCT, which cannot be explained by an aversion induced by our VTA inhibition due to the light stimulation. With all, this data demonstrates that rats perceive positive states from conspecifics and use this information to guide social decisions, being the necessary motivation to help others.

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5 | Discussion

Prosocial behaviours are commonly found in the animal kingdom. Nascent evidence reports that also rodents display this type of actions, as reviewed in the introduction of this manuscript. However, there is a strong bias favouring the study of emotion transfer and prosocial behaviours in the context of negative affective states. Over the last years, rodents have become an emerging model ideal to study the neural correlates of such processes due to their robust emotional contagion found for the distress of conspecifics (Keysers et al., 2022; Hernandez-Lallement et al., 2022). However, this does not fully capture the complexity of emotional processing repertoire of rodents, and it might further bias our understanding of such. With the current thesis, we aimed to assess if rodents are capable of perceiving positive states from conspecifics, and the neural correlates behind this process. Furthermore, we aimed to study prosocial choices in reward provision paradigms in mice that, as reviewed in the literature (Gachomba, Esteve-Agraz, et al., 2024), is one of the least studied. Providing extra evidence in this direction is essential to unravel the conflicting results obtained by the few different studies about reward provision in mice. Overall, the results of the current thesis have revealed interesting insights into behavioural substrates of reward-based prosociality in mice, suggesting that prosocial tendencies are not overly prevalent in this species, but when they emerge seem to depend on behavioural elements similar as for rats. On the other hand, this thesis has provided evidence about rats perceiving rewarding states of conspecifics, and encoding these vicarious rewards in the VTA, being this a relevant neural process found to be necessary for making prosocial choices in food-foraging contexts.

5.1. Mice prosociality in reward provision paradigms

We developed a new custom-made behavioural paradigm for the study of prosocial choices in mice. The design and development of this setup was based on the one previously described in (Márquez et al., 2015) to assess prosociality by reward provision in rats. Three main reasons motivated the adaptation of this paradigm to mice: **(1)** the use of the larger genetic toolbox available nowadays for the manipulation and monitoring of neural circuits for mice in comparison to rats. **(2)** the current setup used for assessing prosocial choices in rats in our lab was incompatible with the use of

tethered animals due to the configuration of the automated doors on the corridors, which limited the tools available to study the underlying neural circuitry in rats. And **(3)** the possibility for direct interspecies comparison using the same paradigm. To date, different labs have developed and used their own paradigm to give answers to similar questions about prosociality. On the one hand, being able to develop a specific paradigm to flexibly address a question of interest is very relevant and beneficial to further expand our knowledge. However, on the other hand, this might be problematic when obtaining conflicting results between studies that use different training or testing procedures, which makes it difficult to interpret the results due to the variability in the different factors included among them. Thus, replication of results using the same paradigms and protocols would be useful and would allow for direct interspecies comparison.

Withall, there are diverse PCT paradigms used to assess reward provision in rats that, in general, find these to be prosocial by preferring the choices that reward a conspecific literature (Gachomba, Esteve-Agraz, et al., 2024). In contrast, the results found in mice studies give contradictory conclusions. Despite the clear differences in the paradigms and testing protocols used (*for more details see* Reward provision), a main difference found between the studies is concerning prosocial reward provision in mice to be sex-dependent in two opposing directions. While (Scheggia et al., 2022) found that most of tested male mice (75%) were prosocial, in comparison to only 47% of tested female mice. (Misiołek et al., 2023) found that female mice are more willing to choose the prosocial choice more often than males (75% prosocial female to 30% prosocial male mice). However, (Misiołek et al., 2023) did not find any sex-specific difference in emotional state discrimination that could account for their different proportions in prosocial preference. The results presented in this thesis about prosociality in mice using our double T-maze seem to favour this second study, as only 17% of the tested males were prosocial (in the standard training protocol). However, we limited our experiments to test only male mice, as we first aimed to validate the task and paradigm, which for rats provides evidence for no sex differences (Gachomba et al., 2022).

In an attempt to explain the generalized lack of prosociality found in our double-T maze experiments with mice, we performed a detailed quantification and analysis of social and individual behaviours occurring during this task. We first focused on the recipients' actions and found that they displayed robust food-seeking behaviour that was almost exclusive towards the prosocial nose-port. In previous work, we found these actions

to be important and necessary for the emergence of prosocial preferences in rats, as when prevented, decision-maker rats did not become prosocial. Additionally, we explored several behavioural measures from the recipient animals prior and after the decision-makers' choices. We found that recipients displayed very distinct behavioural patterns in prosocial and selfish trials, reflecting that they were aware of when they were going to be rewarded and showed clear behaviours that decision-makers could have used to guide their decisions. However, despite the clear behavioural cues from recipients, we found that focal animals behaved similarly whether they decided the prosocial or the selfish option. Because decision-makers did not seem to be affected by the different behaviors displayed by recipients depending on the trial type, we asked whether animals were actually socially interacting and attentive to the behavior of their partners. In broad terms, we did not find any predictive variable in their social interactions prior to choice, although the social distance and orientation they displayed should have enabled focals to perceive the food-seeking and reward-related behaviours their partners displayed. After choice, those differences found in their social dynamics arose from the behaviour of recipients according to the trial type, whereas focals remained consistent.

In a previous publication with rats, we found that the quality of social dynamics and not the quantity of social interactions prior to choice was predictive of the choice. When extracting more quantitative measures of the social and individual behaviours, they found that social hierarchies shaped the interactions between the pairs of animals. The recipients of the pairs were the ones driving the changes in the measured variables, according to their dominance status and the trial type, being submissive recipients better at communicating their need through multimodal cues, especially during those interactions before selfish choices (Gachomba et al., 2022). In light of the above, we can discuss that prosocial choices displayed by mice and rats using the reward provision paradigm developed by (Márquez et al., 2015) maintain certain behavioural similarities between the two species. Indeed, we found that recipients of those few mice ending up being prosocial were better a displaying food-seeking on the first session of the PCT, which as mentioned previously, is a necessary action for focal rats to develop a prosocial preference. We also noted that recipient mice from prosocial pairs entered more rapidly to the reward areas after their partners' choices. Furthermore, prosocial decision-maker mice interacted closer to the partition, and took longer to leave the prosocial reward area during the first session of the PCT.

Altogether, these insights suggest that behaviours and social interactions during the first session were relevant for the prosocial decision-maker mice to pay increased attention to their partners' behaviour which eased the understanding of the reward contingencies of their choices. We did not test for social hierarchy in any of the experiments performed with mice. Thus, we could not assess if the differences presented on the recipients' behaviour could be modulated by their social rank, as in the case for rats. In this direction, (Scheggia et al., 2022) assessed mice's dominance status after testing them in each session in their reward provision paradigm and found that prosocial choices are dependent on the social rank between the pairs of mice, in a similar fashion as for rats (Gachomba et al., 2022). These two studies provide evidence that reward provision in rodents is directed down the hierarchy, being dominant decision-makers more prosocial towards their submissive recipients than on the contrary. This is consistent with previous work with macaques, in which it was stated that dominant animals might be more likely to engage in such other-regarding behaviours to sustain their rank and promote group cohesion (Massen et al., 2010; Chang et al., 2011), suggesting that this social mechanism could be conserved across rodent and other species. However, whether prosociality levels modulated by dominance found in mice are mainly driven by the behaviour of the recipient, as it happens for rats, remains to be established, as social dynamics have not been deeply studied in paradigms where high prosocial preferences are observed (Scheggia et al., 2022).

To keep further investigating the general lack of prosociality observed in our experiments performed with mice, we considered the individual training protocols used as a possible factor interfering with the decision-making process during the social task. It has been suggested that prolonged training of an instrumental action like nose-poking, can make such behaviour become habitual and thus, less flexible and goal directed (Thrailkill & Daniels, 2024). Furthermore, prior individual training has been further demonstrated to have an effect in prosocial actions to avoid harm to others in rats (Hernandez-Lallement et al., 2020). Thus, the comparable individual behaviours displayed by focal mice in prosocial and selfish trials and their average lack of preference found in our experiments could reflect choosing both options similarly as a habitual action, thus becoming less dependent on the actions and behaviours displayed by their partners. In the work of Scheggia and colleagues, decision-maker mice learnt the task contingencies during social testing and most male mice ended up being prosocial. Additionally, they assessed whether sharing food with a recipient could motivate a

change in choice preference when already having a prior stable preference alone. They found in this case that most mice also switch their preferred option to favour the partner, although with a weaker effect. Withall, this suggests that mice tested in our paradigm could show prosocial tendencies with a different (lower) training schedule. Short training of instrumental actions prior to the PCT did not favour prosocial choices, and in any case, diluted the individuals' preference by promoting a foraging strategy for the single choice exploitation rather than promoting their natural tendency to alternate between choices (Lalonde, 2002). Overall, mice showed very polarized choices from one session to another, revealing that no decision-maker was prosocial over sessions and most remain unbiased (except for a selfish one). Moreover, it has been shown that male mice tend to change their choice strategies more frequently during learning than females (C. S. Chen et al., 2021), and are strongly influenced by immediate prior experience of reinforcement. Having tested female mice would have been beneficial for addressing such scenario, and this limitation should be overcome in future work to be able to draw clear conclusions.

An interesting observation comparing our results with Scheggia's work is regarding unbiased choices and familiarity. Our experiments were performed with non-food deprived cagemates and our results show most of the mice having no clear preference (unbiased). On the other hand, (Scheggia et al., 2022) tested food-deprived familiar and unfamiliar dyads, finding unbiased animals only in the unfamiliar condition. When testing with familiar recipients, most focal mice were prosocial, but 24% remained selfish, intriguingly no decision-makers were unbiased. In contrast, (Gachomba et al., 2022) found similar levels of prosocial choices when pairing focal rats with familiar or unfamiliar recipient rats. This observation prompts intriguing questions regarding why in our work most mice remain with no clear preferences to provide or not food to familiars, whereas in Scheggia's study, most their familiar animals have contrasting clear preferences away from chance.

All things considered, we could argue that our double-T paradigm for reward provision was either too cognitively demanding for mice or did not fully capture the ability of these animals to display prosocial choices in this context. Although mice prosociality in reward provision is not very widespread, these experiments served to compare those results with rats, who naturally display a prosocial preference in different settings. In a final attempt to probe mice prosociality in a reward-based context, we developed a two-chamber PCT paradigm, more similar to the one from Scheggia et al.,

2022, but with main differences. **(1)** the recipient mouse had an active role in the task, by displaying food-seeking behaviour in a single nose-poke to incentivize focals' decisions. **(2)** the choice nose-pokes of the focal mouse were placed vertically instead of horizontally and equidistant to the recipient in order to avoid a possible local enhancement effect towards the option being closer to the recipient. **(3)** all animals underwent an individual training prior to social testing. The results from this experiment, yet with a low sample, reflect that this setup configuration was not optimal for assessing prosocial choices in mice in our hands, as most of them had an overall preference for the nose-port placed below, requiring a lower effort. In this way, we argue that decision-makers could possibly no disentangle the different social cues that, in this case, happen in the same area and close in time, and which result important for the emergence of prosocial behaviours in rats.

Overall, prosocial tendencies in reward provision in mice are not as widespread as for rats and come with marked individual differences. In those few animals we found to be prosocial, the behavioural mechanisms promoting prosociality are similar to the ones in rats. These results provide a framework to perform direct interspecies comparisons, thus once we understand the neural circuits underlying these intricate behaviours in rats, would be interesting to perform gain of function experiments with mice.

5.2. Mice and rats are different in the wild and in the lab

Rats and mice are widely used models for studying mechanisms of mammalian social behaviour and cognition, and while some might think that a rat is just a “bigger” mouse, substantial differences in social cognition and behaviour have been described between these two species, likely linked to their distinct natural social structures (Ellenbroek & Youn, 2016). It has been described that both rats and mice live in hierarchical groups in the wild, yet rats are much less territorial and the hierarchy between males is not as absolute as for mice (Schweinfurth, 2020). Rats are commonly found to live in mixed-sex colonies, where all males mate with all females, and they rarely fight over resources, further they have been described to forage for food together (Weiss et al., 2017). In contrast, mice are commonly found to live in more territorial structures, which are founded by a single male that mates with all the females (Lipp & Wolfer, 2013). As a result, interactions between males are much rarer, and when they occur, are more aggressive and territorial in nature. These differences in the natural social dynamics in the wild also translate when studying them in laboratory settings. For instance, different studies have examined the motivation for seeking social contacts in both rats and mice. Using a socially-conditioned place preference test, (Kummer et al., 2014) described that most of the rats found the socially-paired compartment rewarding, spending more time, compared to half of the tested mice, even describing that a small proportion could find it aversive. Moreover, rats found social interaction as rewarding as 15mg/kg body weight cocaine, whereas mice preferred this dose much more than social interaction. In this line, others have described similar effects comparing the motivation for seeking food or social contacts between rats and mice. (Reppucci & Veenema, 2020) developed a social vs. food preference test, and found that in sated conditions, rats were generally more social-preferring whilst mice were more food-preferring (Reppucci et al., 2020) (Figure 20**)**. In food deprivation conditions, their preferences switched; rats had no bias whereas mice preferred the food stimulus. (Netser et al., 2020) replicated such observations and further examined the dynamics of their social investigation. The authors found distinct exploration strategies employed by rats and mice when choosing to interact with familiar or unfamiliar conspecifics. Their results demonstrate a stronger immediate motivation for interactions with same-sex social stimuli in male rats compared to mice. Moreover, species-specific characteristics also bring about other differences in addictive and impulsive behaviours and cognition (*for review see*

(Ellenbroek & Youn, 2016)), but also in emotional processing and transfer. Both rodent species can be affected by and respond to negative affective states of conspecifics at comparable levels, yet different factors such familiarity, strain or prior experience with emotional stimuli affect them differently *(for review see* (Hernandez-Lallement et al., 2022; Keysers et al., 2022)).

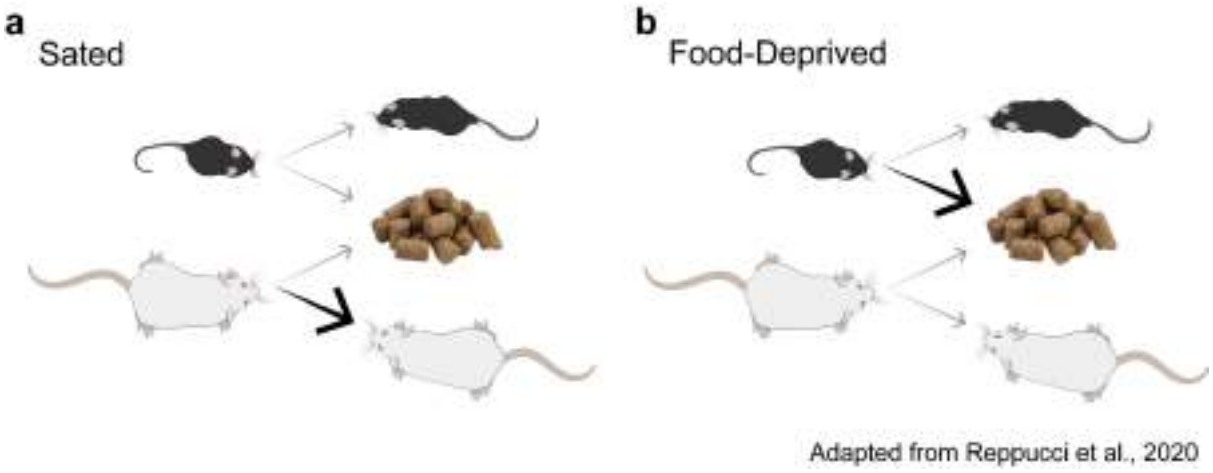

Figure 20. Social vs Food preference for mice and rats

a. In baseline sated conditions, mice have no preference between seeking for food or to interact with another conspecific. In contrast, rats have a preference for social interactions over food. **b.** In food deprived conditions, mice are more motivated to seek for food than social contacts. Rats on the other hand, have no preference for either the social or the food stimuli. Adapted from Reppucci et al., 2020.

Thus, one could argue that the conflicting results in reward provision paradigms found in mice could arise from any of these components of the distinct natural repertoires displayed by rats and mice. From an ecological standpoint, sharing food with other individuals always reduces the amount of potential food available to oneself (Chang et al., 2011). Considering the differences found in the natural social dynamics of mice and rats, it would not be surprising that prosociality measured in reward provision paradigms differed substantially between these rodent species, favouring the appearance of prosocial choices in rats, in contrast to the more solitary and competitive nature of male mice. Furthermore, different levels of sensitivity to the affective states of others could represent a causal factor for the differences observed in reward-provision. Both Misiolek and Scheggia studies tested for affective state discrimination, in negative or relieved conditions, the former finding similar levels between male and female mice, and the latter finding a link between emotional contagion and increased prosocial choices. However, evidence about processing of positive affective states of others in mice is scarce. As demonstrated in this work,

vicarious rewards are found in rats and are proposed as a causal mechanism driving prosocial choices in reward-provision. Considering again the natural differences between these two species, it would not be strange that a sensitivity to positive states of others is widely more conserved in rats. Thus, a generalized reduced (or lack thereof) sensitivity for vicarious reward in mice could account for the individual variability and opposing effects found between these studies, and surely deserves attention in future works.

Finally, mice often need substantially longer training and habituation sessions to perform certain tasks that rats do (Jaramillo & Zador, 2014), and additionally experience more stress and anxiety (Ellenbroek & Youn, 2016). All of these variations we have been discussing emphasise the importance of choosing an appropriate animal model for studies in behavioural neuroscience, especially those with a social scope. While checking the literature about prosocial behaviour in rodents (Gachomba, Esteve-Agraz et al., 2024), we found that mice and rats are differentially used according to the cognitive demand of the task. As shown in (Figure 21)**,** those tasks based on instrumental actions to measure prosocial behaviours use mostly rats (namely *rescue, reward provision and harm aversion* paradigms). In contrast, mice and rats are similarly used in consolation paradigms, which measure immediate natural responses that require no learning to be displayed. Yet, the proportions showed in (Figure 21) are merely illustrative of the different usage of rodent species for studying prosociality as reviewed in the literature (*see* Table 1), and should not be taken as an indication of differences in cognitive/affective components between mice and rats for choosing an animal model for addressing specific questions of interest.

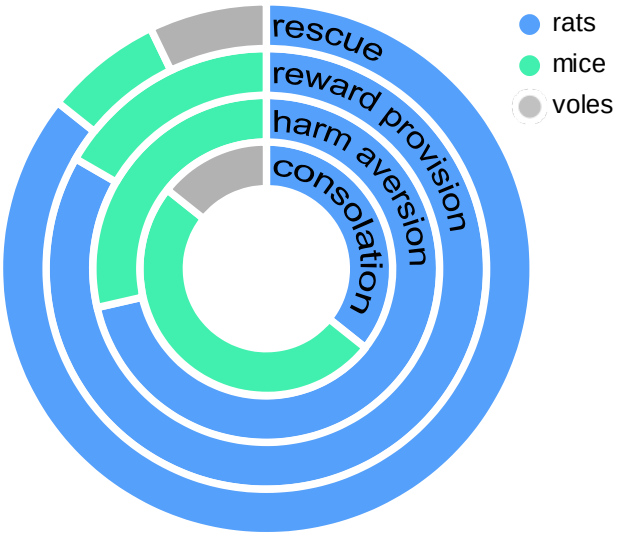

Figure 21. Proportion of rodent models in prosocial paradigms.

Doughnut chart reflecting the proportion of rodent models (rats:blue, mice:green or voles:grey) used for studying prosocial behaviours in each paradigm. Paradigms are organized according to the maximum proportion of studies using rats. Data extracted from the review about prosocial behaviour in rodents (Gachomba, Esteve-Agraz, et al., 2024).

5.3. Vicarious rewards in the VTA drive prosocial choices in rats

To assess whether rats are sensitive to the reward of others and to study the neural correlates underlying this process, we developed a task to measure activity of neurons in the VTA via calcium imaging with fiber photometry, while pairs of non-food deprived rats receive palatable food pellets in a structured manner. We then asked if rats use this socioemotional information to guide decisions in order to reward others.

We focused our study on the possible role of the VTA in positive emotional transfer, a heterogeneous region with a local architecture consisting of a variety of neurons, mainly known for its role in releasing dopamine into the reward system and which could represent a brain hub for processing self and other rewarding states (*see* The ventral tegmental area and the reward system)**.** VTA DA release has been suggested as a neural substrate for social learning signals that drive motivated behaviours (Solié et al., 2022), and its projections to the NAc (known as the main mesolimbic DA pathway) seem to be a possible neural correlate of vicarious reinforcement in rats (Kashtelyan et al., 2014; Willuhn et al., 2014). As mentioned in the introduction, previous reports have shown transients of DA release in the NAc of a rat when witnessing another getting a food reward (Kashtelyan et al., 2014). In this work, authors observed increased DA release during the first trial while witnessing a conspecific receiving a reward, being followed by decreases in DA release. Whether these complex DA dynamics might be explained by reward prediction error through conflicting information on cue-reward outcomes (a cue-light first predicts self-reward and then predicts reward for the other, and lack of self-reward) is uncertain. Other reports showed that DA is initially released in the NAc in response to playback of affiliative 50-kHz ultrasonic vocalisations (Willuhn et al., 2014), a response that also rapidly fades away over trials (only present in the first two presentations). Our VTA calcium recordings complement our knowledge about this region showing it to encode rewarding states of the self but also from conspecifics. Expanding these previous reports, our results show that the VTA of the focal rats respond with increased activity to the reward delivered to a conspecific. This response is present in 64% of the trials, not only in early trials but throughout the entire thirty-minute session and does not fade away following any temporal pattern. A main difference of our study is regarding the lack of reward-cues and the structure of our trial-based task, in which the focal animal always gets rewarded first, followed by

reward to the conspecific after a pseudorandom time interval. In contrast, in the work of Kashtelyan, a light-cue first signals the self-reward and later the same cue signals the reward for the other animal and lack of self-reward, resulting in conflicting reward-cue information. On the other hand, Willuhn et al, present a different scenario in which they playback ultrasonic vocalizations to rats. Besides DA release in the first trial, they also found an increased approach behaviour to the USV-emitting speaker which correlated with DA release, that faded along with DA transients after the second trial. Thus, the lack of increased DA release over time could arise from the lack of a real social agent. Another main difference of our study is the recording and manipulation of population activity of the VTA cells, in contrast to DA-specific assessments of these reports aforementioned. Consequently, we do not know if vicarious reward signals measured in this study are driven either by DA or a combination of other cell types in the VTA acting in concert, a question that remains to be addressed in future work. Furthermore, it will be important to disentangle whether the self and other reward signals found in the VTA recruit the same or different cell populations, which might account by a mirror neuron hypothesis.

Our results further suggest that vicarious reward signals in the VTA are incorporated in prosocial decision-making processing. This is shown by the abolished prosocial preference found in the experimental group when we optogenetically inhibited this signal in the precise moments of the reward for the recipient. It was not the case for the control group, which maintained its normal prosocial preference over sessions, and could not be explained by aversion due to the VTA inhibition. An interesting observation is that during the first PCT session, the control group already had an emerging prosocial preference that reached 70% on average. Rats in this group did not receive optogenetic inhibition of the VTA neurons, but show an abnormally increased preference for the prosocial option during the first day that resembles that of more advanced sessions in previous reports (*see* (Márquez et al., 2015), *and* (Gachomba et al., 2022)). For the experiments in this study, we modified the standard protocol of the PCT by delaying the opening of the doors for the recipient to access the reward areas after the decision-maker got their reward in each trial, which in previous works were simultaneously opened for both animals. The logic behind this modification was to not interfere (via optogenetic inhibition) with the self-reward increases that happen in the VTA when focals get their reward. Therefore, we decided to only open the recipients' doors after the neural activity for the focal rats would go back to baseline levels after

eating their own reward, to then be able to inhibit that specifically linked to the vicarious reward experience. We consider that this modification enabled decision-makers of the control group to have increased possibilities to perceive the reward of their partners, thus reinforcing the learning of the contingencies of this task faster than usual. This can be seen during the first 10 minutes of the first PCT session, when control group's preference is not different from chance, but it increases throughout the rest of the session (Figure 19a). Finally, as discussed previously, we found in previous work that rats' social dominance shaped the dynamics of social interactions prior to choice and the prosocial preferences. Such observation suggests that the social interactions during the reward period of the PCT could vary in similar ways; dominant decision-makers could be paying more attention to the reward of their submissive partners, thus easing the learning of the task contingencies. Future studies should address this hypothesis and whether social dominance modulates the activity of the VTA in response to vicarious rewards.

In the pursuit of the proximal causes for the emergence of prosocial behaviors, several works have proposed empathy-related processes as a core mechanism leading to prosociality in humans and non-human animals ((Batson et al., 1987); (Decety et al., 2016); (Lahvis, 2017)). In most of these models, empathetically motivated prosocial behaviours start with the perception of another in a distress state, that can lead to an aversive affective arousal combined with a physiological stress response in the observer. Then, if appropriate, prosocial drive is triggered and depending on the context, can lead to prosocial behaviour. These different processes can be influenced by social context affecting the valence and intensity of the affective response. Thus, these models emphasise the ability to share the negative affective state of others as a critical component eliciting prosocial behaviour. Under the scope of these models, the food-seeking behavior displayed by recipient rats prior to the focals' choices in the PCT, necessary for the prosocial preference to emerge (Márquez et al., 2015), could be interpreted as distress signals that lead to prosociality. However, rats are not food-deprived in any of our studies to avoid stress related-effects, and show no signs of aversive states measured by a lack of 22-kHz ultrasonic vocalizations and increased number of 55-kHz affiliative calls (Gachomba et al., 2022). Thus, the food-seeking behaviour displayed by recipients rats in the PCT is a social signalling behaviour that promotes instrumental helping (Warneken & Tomasello, 2006) in a non-distress context. Here we propose that rats are sensitive and respond to the rewarding states of others, an

affective element found to be critical for reward-based prosocial decision-making in rats. Moreover, (Morelli et al., 2018) provided insights about neural sensitivity to vicarious reward and its relation to prosociality and well-being in humans. Their results expand Batson's idea of empathetically motivated prosocial behaviours, demonstrating that individuals with greater sensitivity to vicarious rewards reported increases in everyday helping behaviours. This broadens the assumption that neural responses to vicarious reward may track the tendency to behave prosocially across various contexts. Additionally, neural sensitivity to self and other rewards correlated with individuals' reports of psychological well-being, which allowed them to conclude that vicarious rewards can serve as an affective engine that can drive prosocial behaviour and boost well-being for the self as well as for others. This supports the idea that emotional processing and contagion feeds into prosociality, but does it through a filter that allows vicarious rewards or distress to affect prosociality under specific conditions (Keysers et al., 2022). Components of social cognition such as subjective implicit or explicit attitudes towards the observed person (Braams et al., 2014), familiarity, closeness, perceived similarity or attractiveness, are factors modulating such relationship in humans (Singer et al., 2006). In this line, our results seem to expand this notion in rodents, suggesting that differences in the neural and behavioural sensitivity to vicarious rewards could account for differences in prosocial behavior by reward provision in rats, which comes from the interplay of multiple positive socioemotional cues which are integrated to guide decisions that impact others.

The nascent evidence of work with rodents is offering new insights about the development of a sensitivity to the emotions of others, perhaps under a more selfish perspective. Perceiving and sharing others' negative emotions allow animals to gather information in order to anticipate and prepare for what might happen to them (Keysers et al., 2022). On the other hand, perceiving and sharing positive emotions of others has undeniable benefits for social living (Michon et al., 2023) by aligning incentives to promote collaboration, fostering the perception of an environment as safe, allowing the exchange of information about food resources, and reducing conflict among individuals. Our results seem to contribute to this perspective, as by reinforcing prosocial choices, rats maximize the possibility of obtaining more rewards.

Finally, the presented results in this thesis expand our knowledge about how the brain integrates emotional cues used to guide social decisions, a complex set of processes

that is severely affected in certain psychopathologies, such as autism spectrum disorders. Furthermore, the interest in the neurobiology underlying vicarious reward responses goes beyond the study of prosociality. Research has found evidence of the reward system to be involved in the use of social media, a phenomenon acquiring much relevance lately, as there were over 5 billion social media users worldwide at the start of 2024 (62,3% of the population, according to the Digital Report 2024). Researchers found an activation of the reward system of people both when giving and receiving 'likes', especially in the VTA while receiving those (Sherman et al., 2018). In addition, another interesting phenomenon gaining importance over the last years is vicarious gaming. Watching others play video games has been around for as long as video games, but since the introduction of internet and more recently COVID-19, videogame live streaming audience has grown exponentially, having crossed the billion users in 2023 (Newzoo, Global Esports & Live Streaming Market Report, 2022). While many factors might play a role in the motivations behind vicarious gaming, such as cognitive (e.g. learning gameplay strategies), social (e.g. relatedness and integration by belonging to a community or parasocial interactions (i.e. unreciprocated feelings of connection from the audience to the broadcaster)); vicarious rewards could also represent an affective component at the base of this phenomenon. Video games are played for the experience of "fun" and short-term increase in subjective well-being (Przybylski et al., 2010). Indeed, it has been found that playing videogames is associated with endogenous DA release in the VS (Koepp et al., 1998), and higher structural grey matter volume and stronger functional activation during loss processing is found in frequent videogame players (Kühn et al., 2011). Additionally, videogaming has been associated with alterations of the neural reward processing in the VS (Lorenz et al., 2015). Together, this body of neuroimaging evidence points to videogaming to be experienced as rewarding, and thus vicarious rewards could represent a possible motivation for the vicarious gaming phenomenon. In this line, a study found NAc and putamen activity linked to self and vicarious gaming experiences, mostly during win events (Kätsyri et al., 2013). Altogether, this is of special relevance, as a growing body of literature is linking excessive and possibly addictive use of digital and social media with adverse psychological, physical and social consequences (Lissak, 2018). Thus, future studies should address how these vicarious reward signals are affected or conserved in different animal models of neurodevelopmental diseases and gaming/media abuse, paving the way for new interventions.

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6 | Conclusions

This work aimed to improve our knowledge about the basis of prosociality in rodents in reward-based contexts. First by assessing prosocial tendencies in laboratory mice to provide comparable measures to rats and then by studying the neural correlates of vicarious rewards as possible mediator of prosocial choices food-foraging contexts, in rats. The findings of this work lead to the following conclusions:

1. C57BL/6 mice show no preference for prosocial choices in any of the two reward provision paradigms tested. Training protocols do not influence their preference. However, individual differences were observed, with a relatively small percentage of animals having prosocial biases.

2. Recipient mice display clear food-seeking and distinct reward-related behaviours depending on the trial type (i.e. whether they are going to be rewarded or not), that focal mice do not seem to account for guiding decision-making in the prosocial choice task.

3. Behavioural correlates of prosociality found in rats are kept in those mice dyads found to be prosocial (namely, the food-seeking of recipients and reward-related behaviours and proximity by the focals during interactions in reward areas).

4. Social dynamics and interactions prior to choice are not predictive of prosocial decisions in mice, in contrast to rats, although social dominance (known to affect social interactions prior to choice in rats) was not tested in mice.

5. We demonstrated that rats are sensitive to rewarding states of conspecifics. These vicarious reward responses were measured at the neural level as increased activity in neurons of the ventral tegmental area (VTA) while witnessing a conspecific consuming rewards.

6. VTA encodes about self and other-rewards information, generating the former more occurring and bigger activity increases than the latter.

7. Vicarious rewards happening during conspecifics' food consumption reinforce prosocial choices in rat dyads. Optogenetic inhibition of vicarious rewards abolish the prosocial preference normally found in rats.

8. The reduction in prosocial choices found by optogenetic inhibition of the VTA concurrent to vicarious rewards could not be explained by an aversion experience of the optogenetic VTA inhibition.

6 | Conclusiones

Con este trabajo se pretende mejorar nuestros conocimientos sobre las bases de la prosocialidad en roedores, en contextos de recompensas. Primero mediante la evaluación de tendencias prosociales en ratones de laboratorio para obtener medidas comparables a las de las ratas, y luego estudiando las bases neurales de las recompensas vicarias como posible mediador de las conductas prosociales mediante búsqueda de comida en ratas. Los resultados de este trabajo llevan a las siguientes conclusiones:

1. Los ratones C57BL/6 no muestran preferencia por las elecciones prosociales en ninguno de los dos paradigmas de recompensa probados. Los protocolos de entrenamiento no influyen en su preferencia. Existen diferencias individuales, con un pequeño porcentaje de animales siendo prosociales.

2. Los ratones recipientes realizan una conducta clara de búsqueda de comida y distintos comportamientos relacionados con las recompensas dependiendo del tipo de decisión. Los ratones focals, sin embargo, no parecen tener en cuenta para guiar la toma de decisiones en esta tarea prosocial.

3. Las variables conductuales relacionadas con la prosocialidad halladas en ratas se mantienen en aquellas parejas de ratones prosociales (acciones relacionadas con la búsqueda de comida de los recipientes, y la proximidad de los focales durante las interacciones en las áreas de recompensa).

4. La interacciones y dinámicas sociales previas a la elección no predicen las decisiones prosociales en ratones, en contraste con las ratas; aunque la dominancia social (conocida por afectar a las interacciones sociales previas a la elección en ratas) no se comprobó en ratones.

5. Demostramos que las ratas son sensibles a los estados de recompensa de congéneres. Estas recompensas vicarias son medidas a nivel neural como un aumento de la actividad en las neuronas del área tegmental ventral mientras presenciaban a un congénere consumiendo recompensas.

6. El VTA codifica información sobre recompensas propias y ajenas, generando las primeras mayores incrementos de actividad y más ocurrentes que las segundas.

7. Las recompensas vicarias que ocurren durante la recompensa de congéneres refuerzan las elecciones prosociales en parejas de ratas. La inhibición optogenética de las recompensas vicarias suprime la preferencia prosocial normalmente encontrada en ratas.

8. La reducción en las elecciones prosociales mediante la inhibición optogenética del VTA concurrente a las recompensas vicarias no puede ser explicada por una experiencia de aversión a la inhibición optogenética.

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References

1. Adriaense, J. E. C., Koski, S. E., Huber, L., & Lamm, C. (2020). Challenges in the comparative study of empathy and related phenomena in animals. Neuroscience & Biobehavioral Reviews, 112, 62–82. https://doi.org/10.1016/j.neubiorev.2020.01.021
2. Akam, T., Lustig, A., Rowland, J. M., Kapanaiah, S. K., Esteve-Agraz, J., Panniello, M., Márquez, C., Kohl, M. M., Kätzel, D., Costa, R. M., & Walton, M. E. (2022). Open-source, Python-based, hardware and software for controlling behavioural neuroscience experiments. eLife, 11, e67846. https://doi.org/10.7554/eLife.67846
3. Aknin, L. B., Dunn, E. W., & Norton, M. I. (2012). Happiness Runs in a Circular Motion: Evidence for a Positive Feedback Loop between Prosocial Spending and Happiness. Journal of Happiness Studies, 13(2), 347–355. https://doi.org/10.1007/s10902-011-9267-5
4. Aknin, L. B., Van De Vondervoort, J. W., & Hamlin, J. K. (2018). Positive feelings reward and promote prosocial behavior. Current Opinion in Psychology, 20, 55–59. https://doi.org/10.1016/j.copsyc.2017.08.017
5. Andreoni, J. (1989). Giving with Impure Altruism: Applications to Charity and Ricardian Equivalence. Journal of Political Economy, 97(6), 1447–1458.
6. Apps, M. A. J., & Ramnani, N. (2014). The Anterior Cingulate Gyrus Signals the Net Value of Others' Rewards. The Journal of Neuroscience, 34(18), 6190–6200. https://doi.org/10.1523/JNEUROSCI.2701-13.2014
7. Asadi, E., Khodagholi, F., Asadi, S., Mohammadi Kamsorkh, H., Kaveh, N., & Maleki, A. (2021). Quality of early-life maternal care predicts empathy-like behavior in adult male rats: Linking empathy to BDNF gene expression in associated brain regions. Brain Research, 1767, 147568. https://doi.org/10.1016/j.brainres.2021.147568
8. Barnett, S. A. (1963). The rat: A study in behaviour (pp. xvi, 248). Aldine.
9. Bartal, I. B.-A., Decety, J., & Mason, P. (2011). Empathy and Pro-Social Behavior in Rats. Science, 334(6061), 1427–1430. https://doi.org/10.1126/science.1210789
10. Batson, C. D. (1987). Prosocial Motivation: Is it ever Truly Altruistic? In Advances in Experimental Social Psychology (Vol. 20, pp. 65–122). Elsevier. https://doi.org/10.1016/S0065-2601(08)60412-8
11. Batson, C. D. (2009). These things called empathy: Eight related but distinct phenomena. In The social neuroscience of empathy (pp. 3–15). Boston Review. https://doi.org/10.7551/mitpress/9780262012973.003.0002

12. Batson, C. D., Batson, J. G., Slingsby, J. K., Harrell, K. L., Peekna, H. M., & Todd, R. M. (1991). Empathic joy and the empathy-altruism hypothesis. Journal of Personality and Social Psychology, 61(3), 413–426. https://doi.org/10.1037/0022-3514.61.3.413
13. Batson, C. D., Fultz, J., & Schoenrade, P. A. (1987). Distress and Empathy: Two Qualitatively Distinct Vicarious Emotions with Different Motivational Consequences. Journal of Personality, 55(1), 19–39. https://doi.org/10.1111/j.1467-6494.1987.tb00426.x
14. Batson, C. D., & Shaw, L. L. (1991). Evidence for Altruism: Toward a Pluralism of Prosocial Motives. Psychological Inquiry, 2(2), 107–122. https://doi.org/10.1207/s15327965pli0202_1
15. Ben-Ami Bartal, I., Breton, J. M., Sheng, H., Long, K. L., Chen, S., Halliday, A., Kenney, J. W., Wheeler, A. L., Frankland, P., Shilyansky, C., Deisseroth, K., Keltner, D., & Kaufer, D. (2021). Neural correlates of ingroup bias for prosociality in rats. eLife, 10, e65582. https://doi.org/10.7554/eLife.65582
16. Ben-Ami Bartal, I., Rodgers, D. A., Bernardez Sarria, M. S., Decety, J., & Mason, P. (2014). Pro-social behavior in rats is modulated by social experience. eLife, 3, e01385. https://doi.org/10.7554/eLife.01385
17. Ben-Ami Bartal, I., Shan, H., Molasky, N. M. R., Murray, T. M., Williams, J. Z., Decety, J., & Mason, P. (2016). Anxiolytic Treatment Impairs Helping Behavior in Rats. Front. Psychol., 7. https://doi.org/10.3389/fpsyg.2016.00850
18. Bernhardt, B. C., & Singer, T. (2012). The Neural Basis of Empathy. Annual Review of Neuroscience, 35(1), 1–23. https://doi.org/10.1146/annurev-neuro-062111-150536
19. Bilevicius, E., Kolesar, T. A., Smith, S. D., Trapnell, P. D., & Kornelsen, J. (2018). Trait Emotional Empathy and Resting State Functional Connectivity in Default Mode, Salience, and Central Executive Networks. Brain Sciences, 8(7), 128. https://doi.org/10.3390/brainsci8070128
20. Bouarab, C., Thompson, B., & Polter, A. M. (2019). VTA GABA Neurons at the Interface of Stress and Reward. Frontiers in Neural Circuits, 13, 78. https://doi.org/10.3389/fncir.2019.00078
21. Braams, B. R., Güroğlu, B., De Water, E., Meuwese, R., Koolschijn, P. C., Peper, J. S., & Crone, E. A. (2014). Reward-related neural responses are dependent on the beneficiary. Social Cognitive and Affective Neuroscience, 9(7), 1030–1037. https://doi.org/10.1093/scan/nst077
22. Breton, J. M., Eisner, J. S., Gandhi, V. S., Musick, N., Zhang, A., Long, K. L. P., Perloff, O. S., Hu, K. Y., Pham, C. M., Lalchandani, P., Barraza, M. K., Kantor, B., Kaufer, D., & Ben-Ami Bartal, I. (2022). Neural activation associated with outgroup helping in adolescent rats. iScience, 25(6), 104412. https://doi.org/10.1016/j.isci.2022.104412

23. Brosnan, A., & Knapska, E. (2024). Cheerful tails: Delving into positive emotional contagion. Neuroscience & Biobehavioral Reviews, 161, 105674. https://doi.org/10.1016/j.neubiorev.2024.105674
24. Burkart, J. M., Fehr, E., Efferson, C., & van Schaik, C. P. (2007). Other-regarding preferences in a non-human primate: Common marmosets provision food altruistically. Proceedings of the National Academy of Sciences, 104(50), 19762–19766. https://doi.org/10.1073/pnas.0710310104
25. Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B. M., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. Science, 351(6271), 375–378. https://doi.org/10.1126/science.aac4785
26. Cameron, C. D., Hutcherson, C. A., Ferguson, A. M., Scheffer, J. A., Hadjiandreou, E., & Inzlicht, M. (2019). Empathy is hard work: People choose to avoid empathy because of its cognitive costs. Journal of Experimental Psychology: General, 148(6), 962–976. https://doi.org/10.1037/xge0000595
27. Cameron, C. D., & Payne, B. K. (2011). Escaping affect: How motivated emotion regulation creates insensitivity to mass suffering. Journal of Personality and Social Psychology, 100(1), 1–15. https://doi.org/10.1037/a0021643
28. Carneiro de Oliveira, P. E., Carmona, I. M., Casarotto, M., Silveira, L. M., Oliveira, A. C. B., & Canto-de-Souza, A. (2022). Mice Cohabiting With Familiar Conspecific in Chronic Stress Condition Exhibit Methamphetamine-Induced Locomotor Sensitization and Augmented Consolation Behavior. Frontiers in Behavioral Neuroscience, 16. https://www.frontiersin.org/articles/10.3389/fnbeh.2022.835717
29. Carrillo, M., Han, Y., Migliorati, F., Liu, M., Gazzola, V., & Keysers, C. (2019). Emotional Mirror Neurons in the Rat's Anterior Cingulate Cortex. Current Biology, 29(8), 1301-1312.e6. https://doi.org/10.1016/j.cub.2019.03.024
30. Chang, S. W. C., Gariépy, J.-F., & Platt, M. L. (2013). Neuronal reference frames for social decisions in primate frontal cortex. Nat Neurosci, 16(2), 243–250. https://doi.org/10.1038/nn.3287
31. Chang, S. W. C., Winecoff, A. A., & Platt, M. L. (2011). Vicarious Reinforcement in Rhesus Macaques (Macaca Mulatta). Frontiers in Neuroscience, 5. https://doi.org/10.3389/fnins.2011.00027
32. Chen, C. S., Ebitz, R. B., Bindas, S. R., Redish, A. D., Hayden, B. Y., & Grissom, N. M. (2021). Divergent Strategies for Learning in Males and Females. Current Biology, 31(1), 39-50.e4. https://doi.org/10.1016/j.cub.2020.09.075

33. Chen, J. (2017). Empathy for Distress in Humans and Rodents. Neuroscience Bulletin, 34(1), 216–236. https://doi.org/10.1007/s12264-017-0135-0
34. Choe, I.-H., Byun, J., Kim, K. K., Park, S., Kim, I., Jeong, J., & Shin, H.-S. (2017). Mice in social conflict show rule-observance behavior enhancing long-term benefit. Nature Communications, 8(1), 1176. https://doi.org/10.1038/s41467-017-01091-5
35. Church, R. M. (1959). Emotional reactions of rats to the pain of others. Journal of Comparative and Physiological Psychology, 52(2), 132–134. https://doi.org/10.1037/h0043531
36. Clay, Z., & Waal, F. B. M. de. (2013). Bonobos Respond to Distress in Others: Consolation across the Age Spectrum. PLOS ONE, 8(1), e55206. https://doi.org/10.1371/journal.pone.0055206
37. Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. Nature, 462(7269), 51–57. https://doi.org/10.1038/nature08366
38. Colin, C., & Desor, D. (1986). Differenciations comportementales dans des groupes de rats soumis a une difficulte d'acces a la nourriture. Behavioural Processes, 13(1–2), 85–100. https://doi.org/10.1016/0376-6357(86)90019-7
39. Contreras-Huerta, L. S., Coll, M.-P., Bird, G., Yu, H., Prosser, A., Lockwood, P. L., Murphy, J., Crockett, M. J., & Apps, M. A. J. (2023). Neural representations of vicarious rewards are linked to interoception and prosocial behaviour. NeuroImage, 269, 119881. https://doi.org/10.1016/j.neuroimage.2023.119881
40. Costa, D. F., Moita, M. A., & Márquez, C. (2021). Novel competition test for food rewards reveals stable dominance status in adult male rats. Sci Rep, 11(1), 14599. https://doi.org/10.1038/s41598-021-93818-0
41. Cox, S. S., Kearns, A. M., Woods, S. K., Brown, B. J., Brown, S. J., & Reichel, C. M. (2022). The role of the anterior insula during targeted helping behavior in male rats. Scientific Reports, 12(1), Article 1. https://doi.org/10.1038/s41598-022-07365-3
42. Cox, S. S., & Reichel, C. M. (2020). Rats display empathic behavior independent of the opportunity for social interaction. Neuropsychopharmacology, 45(7), 1097–1104. https://doi.org/10.1038/s41386-019-0572-8
43. Cronin, K. A. (2012). Prosocial behaviour in animals: The influence of social relationships, communication and rewards. Animal Behaviour, 84(5), 1085–1093. https://doi.org/10.1016/j.anbehav.2012.08.009
44. Danjo, T., Yoshimi, K., Funabiki, K., Yawata, S., & Nakanishi, S. (2014). Aversive behavior induced by optogenetic inactivation of ventral tegmental area dopamine neurons is mediated by dopamine D2 receptors in the nucleus accumbens. Proceedings of the National Academy of Sciences, 111(17), 6455–6460. https://doi.org/10.1073/pnas.1404323111

45. De Waal, F. B. M. (2008). Putting the Altruism Back into Altruism: The Evolution of Empathy. Annual Review of Psychology, 59(1), 279–300. https://doi.org/10.1146/annurev.psych.59.103006.093625
46. De Waal, F. B. M., & Preston, S. D. (2017). Mammalian empathy: Behavioural manifestations and neural basis. Nature Reviews Neuroscience, 18(8), 498–509. https://doi.org/10.1038/nrn.2017.72
47. Decety, J., Bartal, I. B.-A., Uzefovsky, F., & Knafo-Noam, A. (2016). Empathy as a driver of prosocial behaviour: Highly conserved neurobehavioural mechanisms across species. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1686), 20150077. https://doi.org/10.1098/rstb.2015.0077
48. Decety, J., & Jackson, P. (2004). The Functional Architecture of Human Empathy. Behavioral and Cognitive Neuroscience Reviews, 3, 71–100. https://doi.org/10.1177/1534582304267187
49. Decety, J., Lewis, K. L., & Cowell, J. M. (2015). Specific electrophysiological components disentangle affective sharing and empathic concern in psychopathy. Journal of Neurophysiology, 114(1), 493–504. https://doi.org/10.1152/jn.00253.2015
50. Decety, J., & Yoder, K. J. (2016). Empathy and motivation for justice: Cognitive empathy and concern, but not emotional empathy, predict sensitivity to injustice for others. Social Neuroscience, 11(1), 1–14. https://doi.org/10.1080/17470919.2015.1029593
51. Dovidio, J. F. (1991). The Empathy- Altruism Hypothesis: Paradigm and Promise. Psychological Inquiry, 2(2), 126–128. https://doi.org/10.1207/s15327965pli0202_4
52. Dovidio, J. F., Piliavin, J. A., Schroeder, D. A., & Penner, L. A. (2017). The Social Psychology of Prosocial Behavior. Psychology Press. https://doi.org/10.4324/9781315085241
53. Du, R., Luo, W.-J., Geng, K.-W., Li, C.-L., Yu, Y., Wei, N., & Chen, J. (2020). Empathic Contagious Pain and Consolation in Laboratory Rodents: Species and Sex Comparisons. Neuroscience Bulletin, 36(6), 649–653. https://doi.org/10.1007/s12264-020-00465-y
54. Dunbar, R. (1991). Functional Significance of Social Grooming in Primates. Folia Primatologica, 57, 121–131. https://doi.org/10.1159/000156574
55. Duque, J. F., & Stevens, J. R. (2016). Voluntary food sharing in pinyon jays: The role of reciprocity and dominance. Animal Behaviour, 122, 135–144. https://doi.org/10.1016/j.anbehav.2016.09.020
56. Eisenberg, N. (2000). Emotion, Regulation, and Moral Development. Annual Review of Psychology, 51(1), 665–697. https://doi.org/10.1146/annurev.psych.51.1.665
57. Eisenberg, N., Eggum, N. D., & Di Giunta, L. (2010). Empathy-Related Responding: Associations with Prosocial Behavior, Aggression, and Intergroup Relations: Empathy-Related Responding.

Social Issues and Policy Review, 4(1), 143–180. https://doi.org/10.1111/j.1751-2409.2010.01020.x

58. Eisenberg, N., & Miller, P. A. (n.d.). The Relation of Empathy to Prosocial and Related Behaviors.
59. Ellenbroek, B., & Youn, J. (2016). Rodent models in neuroscience research: Is it a rat race? Disease Models & Mechanisms, 9(10), 1079–1087. https://doi.org/10.1242/dmm.026120
60. Engelhardt, S. C., & Taborsky, M. (2024). Reciprocal altruism in Norway rats. Ethology, 130(4), e13418. https://doi.org/10.1111/eth.13418
61. FeldmanHall, O., Dalgleish, T., Evans, D., & Mobbs, D. (2015). Empathic concern drives costly altruism. NeuroImage, 105, 347–356. https://doi.org/10.1016/j.neuroimage.2014.10.043
62. Fraser, O. N., & Bugnyar, T. (2010). Do Ravens Show Consolation? Responses to Distressed Others. PLoS ONE, 5(5), e10605. https://doi.org/10.1371/journal.pone.0010605
63. Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. Proc. Natl. Acad. Sci. U.S.A., 105(25), 8557–8562. https://doi.org/10.1073/pnas.0804141105
64. Fredrickson, B. L. (2001). The Role of Positive Emotions in Positive Psychology. American Psychologist.
65. Gable, S. L., Gonzaga, G. C., & Strachman, A. (2006). Will you be there for me when things go right? Supportive responses to positive event disclosures. Journal of Personality and Social Psychology, 91(5), 904–917. https://doi.org/10.1037/0022-3514.91.5.904
66. Gable, S. L., & Reis, H. T. (2010). Good News! Capitalizing on Positive Events in an Interpersonal Context. In Advances in Experimental Social Psychology (Vol. 42, pp. 195–257). Elsevier. https://doi.org/10.1016/S0065-2601(10)42004-3
67. Gable, S. L., Reis, H. T., & Elliot, A. J. (2000). Behavioral activation and inhibition in everyday life. Journal of Personality and Social Psychology, 78(6), 1135–1149. https://doi.org/10.1037/0022-3514.78.6.1135
68. Gable, S. L., Reis, H. T., Impett, E. A., & Asher, E. R. (2004). What Do You Do When Things Go Right? The Intrapersonal and Interpersonal Benefits of Sharing Positive Events. Journal of Personality and Social Psychology, 87(2), 228–245. https://doi.org/10.1037/0022-3514.87.2.228
69. Gachomba, M. J. M., Esteve-Agraz, J., Caref, K., Maroto, A. S., Bortolozzo-Gleich, M. H., Laplagne, D. A., & Márquez, C. (2022). Multimodal cues displayed by submissive rats promote prosocial choices by dominants. Current Biology, S096098222200985X. https://doi.org/10.1016/j.cub.2022.06.026
70. Gachomba, M. J. M., Esteve-Agraz, J., & Márquez, C. (2024). Prosocial behaviors in rodents. Neuroscience & Biobehavioral Reviews, 163, 105776. https://doi.org/10.1016/j.neubiorev.2024.105776

71. Galef, B. G., Marczinski, C. A., Murray, K. A., & Whiskin, E. E. (2001). Food stealing by young Norway rats (Rattus norvegicus). Journal of Comparative Psychology (Washington, D.C.: 1983), 115(1), 16–21. https://doi.org/10.1037/0735-7036.115.1.16
72. Gangopadhyay, P., Chawla, M., Dal Monte, O., & Chang, S. W. C. (2021). Prefrontal–amygdala circuits in social decision-making. Nature Neuroscience, 24(1), 5–18. https://doi.org/10.1038/s41593-020-00738-9
73. Geisler, C. E., & Hayes, M. R. (2023). Metabolic hormone action in the VTA: Reward-directed behavior and mechanistic insights. Physiology & Behavior, 268, 114236. https://doi.org/10.1016/j.physbeh.2023.114236
74. Grasmuck, V., & Desor, D. (2002). Behavioural differentiation of rats confronted to a complex diving-for-food situation. Behavioural Processes, 58(1–2), 67–77. https://doi.org/10.1016/S0376-6357(01)00209-1
75. Greene, J. T. (1969). Altruistic behavior in the albino rat. Psychon Sci, 14(1), 47–48. https://doi.org/10.3758/BF03336420
76. Greene, R. K., Damiano-Goodwin, C. R., Walsh, E., Bizzell, J., & Dichter, G. S. (2020). Neural Mechanisms of Vicarious Reward Processing in Adults with Autism Spectrum Disorder. Autism Research and Treatment, 2020, 1–12. https://doi.org/10.1155/2020/8014248
77. Gross, J. J. (1999). Emotion Regulation: Past, Present, Future. Cognition & Emotion, 13(5), 551–573. https://doi.org/10.1080/026999399379186
78. Gu, X., Gao, Z., Wang, X., Liu, X., Knight, R. T., Hof, P. R., & Fan, J. (2012). Anterior insular cortex is necessary for empathetic pain perception. Brain, 135(9), 2726–2735. https://doi.org/10.1093/brain/aws199
79. Hamilton, I. M., & Taborsky, M. (2005). Contingent movement and cooperation evolve under generalized reciprocity. Proc. R. Soc. B., 272(1578), 2259–2267. https://doi.org/10.1098/rspb.2005.3248
80. Hamilton, W. D. (1964). The genetical evolution of social behaviour. I. Journal of Theoretical Biology, 7(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4
81. Han, Y., Bruls, R., Soyman, E., Thomas, R. M., Pentaraki, V., Jelinek, N., Heinemans, M., Bassez, I., Verschooren, S., Pruis, I., Van Lierde, T., Carrillo, N., Gazzola, V., Carrillo, M., & Keysers, C. (2019). Bidirectional cingulate-dependent danger information transfer across rats. PLoS Biol, 17(12), e3000524. https://doi.org/10.1371/journal.pbio.3000524
82. Han, Y., Sichterman, B., Carrillo, M., Gazzola, V., & Keysers, C. (2020). Similar levels of emotional contagion in male and female rats. Sci Rep, 10(1), 2763. https://doi.org/10.1038/s41598-020-59680-2

83. Havlik, J. L., Vieira Sugano, Y. Y., Jacobi, M. C., Kukreja, R. R., Jacobi, J. H. C., & Mason, P. (2020). The bystander effect in rats. Sci. Adv., 6(28), eabb4205. https://doi.org/10.1126/sciadv.abb4205

84. Hernandez-Lallement, J., Attah, A. T., Soyman, E., Pinhal, C. M., Gazzola, V., & Keysers, C. (2020). Harm to Others Acts as a Negative Reinforcer in Rats. Current Biology, 30(6), 949-961.e7. https://doi.org/10.1016/j.cub.2020.01.017

85. Hernandez-Lallement, J., Gómez-Sotres, P., & Carrillo, M. (2022). Towards a unified theory of emotional contagion in rodents—A meta-analysis. Neuroscience & Biobehavioral Reviews, 132, 1229–1248. https://doi.org/10.1016/j.neubiorev.2020.09.010

86. Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., & Kalenscher, T. (2015). Rats prefer mutual rewards in a prosocial choice task. Frontiers in Neuroscience, 8. https://doi.org/10.3389/fnins.2014.00443

87. Hernandez-Lallement, J., van Wingerden, M., Schäble, S., & Kalenscher, T. (2016a). A Social Reinforcement Learning Hypothesis of Mutual Reward Preferences in Rats. In M. Wöhr & S. Krach (Eds.), Social Behavior from Rodents to Humans (Vol. 30, pp. 159–176). Springer International Publishing. https://doi.org/10.1007/7854_2016_436

88. Hernandez-Lallement, J., van Wingerden, M., Schäble, S., & Kalenscher, T. (2016b). Basolateral amygdala lesions abolish mutual reward preferences in rats. Neurobiology of Learning and Memory, 127, 1–9. https://doi.org/10.1016/j.nlm.2015.11.004

89. Heslin, K. A., & Brown, M. F. (2021). No preference for prosocial helping behavior in rats with concurrent social interaction opportunities. Learn Behav, 49(4), 397–404. https://doi.org/10.3758/s13420-021-00471-8

90. Hess, E. M., Venniro, M., & Gould, T. D. (2023). Relative to females, male rats are more willing to forego obtaining sucrose reward in order to prevent harm to their cage mate. Psychopharmacology. https://doi.org/10.1007/s00213-023-06435-2

91. Hess, J. D., Kacen, J. J., & Kim, J. (2006). Mood-management dynamics: The interrelationship between moods and behaviours. British Journal of Mathematical and Statistical Psychology, 59(2), 347–378. https://doi.org/10.1348/000711005X81133

92. Hollis, K. L., & Nowbahari, E. (2013). Toward a Behavioral Ecology of Rescue Behavior. Evolutionary Psychology, 11(3). https://doi.org/10.1177/147470491301100311

93. Horn, L., Bugnyar, T., & Massen, J. J. M. (2024). Testing the effects of kinship, reciprocity and dominance on prosocial food provisioning in azure-winged magpies (Cyanopica cyana), carrion crows (Corvus corone) and common ravens (C. corax). Ethology, 130(4), e13420. https://doi.org/10.1111/eth.13420

94. Horner, V., Carter, J. D., Suchak, M., & de Waal, F. B. M. (2011). Spontaneous prosocial choice by chimpanzees. Proceedings of the National Academy of Sciences, 108(33), 13847–13851. https://doi.org/10.1073/pnas.1111088108

95. Huang, W.-C., Zucca, A., Levy, J., & Page, D. T. (2020). Social Behavior Is Modulated by Valence-Encoding mPFC-Amygdala Sub-circuitry. Cell Reports, 32(2), 107899. https://doi.org/10.1016/j.celrep.2020.107899

96. Inomata, T., & Shimada, S. (2020). Vicarious Reward Response Is Positively Correlated with Functional Connectivity in the Resting State Default Mode Network. Psychology, 11(10), 1574–1591. https://doi.org/10.4236/psych.2020.1110100

97. Jablonski, N. G. (2021). Social and affective touch in primates and its role in the evolution of social cohesion. Neuroscience, 464, 117–125. https://doi.org/10.1016/j.neuroscience.2020.11.024

98. Jakubiak, B. K., & Feeney, B. C. (2017). Affectionate Touch to Promote Relational, Psychological, and Physical Well-Being in Adulthood: A Theoretical Model and Review of the Research. Personality and Social Psychology Review, 21(3), 228–252. https://doi.org/10.1177/1088868316650307

99. Jaramillo, S., & Zador, A. M. (2014). Mice and rats achieve similar levels of performance in an adaptive decision-making task. Frontiers in Systems Neuroscience, 8. https://doi.org/10.3389/fnsys.2014.00173

100. Jensen, K. (2016). The Prosocial Primate—A Critical Review. In Advances in the Study of Behavior (Vol. 48, pp. 387–441). Elsevier. https://doi.org/10.1016/bs.asb.2016.03.001

101. Jordan, M. R., Amir, D., & Bloom, P. (2016). Are empathy and concern psychologically distinct? Emotion, 16(8), 1107–1116. https://doi.org/10.1037/emo0000228

102. Joushi, S., Taherizadeh, Z., Esmaeilpour, K., & Sheibani, V. (2022). Environmental enrichment and intranasal oxytocin administration reverse maternal separation-induced impairments of prosocial choice behavior. Pharmacology Biochemistry and Behavior, 213, 173318. https://doi.org/10.1016/j.pbb.2021.173318

103. Kalamari, A., Kentrop, J., Hinna Danesi, C., Graat, E. A. M., van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Joëls, M., & van der Veen, R. (2021). Complex Housing, but Not Maternal Deprivation Affects Motivation to Liberate a Trapped Cage-Mate in an Operant Rat Task. Front. Behav. Neurosci., 15, 698501. https://doi.org/10.3389/fnbeh.2021.698501

104. Kane, G. A., Lopes, G., Saunders, J. L., Mathis, A., & Mathis, M. W. (2020). Real-time, low-latency closed-loop feedback using markerless posture tracking. eLife, 9, e61909. https://doi.org/10.7554/eLife.61909

105. Kashtelyan, V., Lichtenberg, N. T., Chen, M. L., Cheer, J. F., & Roesch, M. R. (2014). Observation of Reward Delivery to a Conspecific Modulates Dopamine Release in Ventral Striatum. Current Biology, 24(21), 2564–2568. https://doi.org/10.1016/j.cub.2014.09.016

106. Kätsyri, J., Hari, R., Ravaja, N., & Nummenmaa, L. (2013). Just watching the game ain't enough: Striatal fMRI reward responses to successes and failures in a video game during active and vicarious playing. Frontiers in Human Neuroscience, 7. https://doi.org/10.3389/fnhum.2013.00278

107. Kentrop, J., Kalamari, A., Danesi, C. H., Kentrop, J. J., van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Joëls, M., & van der Veen, R. (2020). Pro-social preference in an automated operant two-choice reward task under different housing conditions: Exploratory studies on pro-social decision making. Developmental Cognitive Neuroscience, 45, 100827. https://doi.org/10.1016/j.dcn.2020.100827

108. Keysers, C., & Gazzola, V. (2023). Vicarious Emotions of Fear and Pain in Rodents. Affective Science, 4(4), 662–671. https://doi.org/10.1007/s42761-023-00198-x

109. Keysers, C., Gazzola, V., & Wagenmakers, E.-J. (2020). Using Bayes factor hypothesis testing in neuroscience to establish evidence of absence. Nature Neuroscience, 23(7), 788–799. https://doi.org/10.1038/s41593-020-0660-4

110. Keysers, C., Knapska, E., Moita, M. A., & Gazzola, V. (2022). Emotional contagion and prosocial behavior in rodents. Trends in Cognitive Sciences, S1364661322001115. https://doi.org/10.1016/j.tics.2022.05.005

111. Kim, S. J., Kim, S.-E., Kim, H. E., Han, K., Jeong, B., Kim, J.-J., Namkoong, K., & Kim, J.-W. (2017). Altered Functional Connectivity of the Default Mode Network in Low-Empathy Subjects. Yonsei Medical Journal, 58(5), 1061. https://doi.org/10.3349/ymj.2017.58.5.1061

112. Kitano, K., Yamagishi, A., Horie, K., Nishimori, K., & Sato, N. (2022). Helping behavior in prairie voles: A model of empathy and the importance of oxytocin. iScience, 25(4), 103991. https://doi.org/10.1016/j.isci.2022.103991

113. Kiyokawa, Y., Li, Y., & Takeuchi, Y. (2019). A dyad shows mutual changes during social buffering of conditioned fear responses in male rats. Behavioural Brain Research, 366, 45–55. https://doi.org/10.1016/j.bbr.2019.03.024

114. Koepp, M. J., Gunn, R. N., Lawrence, A. D., Cunningham, V. J., Dagher, A., Jones, T., Brooks, D. J., Bench, C. J., & Grasby, P. M. (1998). Evidence for striatal dopamine release during a video game. Nature, 393(6682), 266–268. https://doi.org/10.1038/30498

115. Kopp, K. S., Kanngiesser, P., Brügger, R. K., Daum, M. M., Gampe, A., Köster, M., van Schaik, C. P., Liebal, K., & Burkart, J. M. (2024). The proximate regulation of prosocial behaviour:

Towards a conceptual framework for comparative research. Animal Cognition, 27(1), 5. https://doi.org/10.1007/s10071-024-01846-w

116. Krafft, B., Colin, C., & Peignot, P. (2010). Diving-for-Food: A New Model to Assess Social Roles in a Group of Laboratory Rats. Ethology, 96(1), 11–23. https://doi.org/10.1111/j.1439-0310.1994.tb00877.x

117. Kühn, S., Romanowski, A., Schilling, C., Lorenz, R., Mörsen, C., Seiferth, N., Banaschewski, T., Barbot, A., Barker, G. J., Büchel, C., Conrod, P. J., Dalley, J. W., Flor, H., Garavan, H., Ittermann, B., Mann, K., Martinot, J.-L., Paus, T., Rietschel, M., … The IMAGEN Consortium. (2011). The neural basis of video gaming. Translational Psychiatry, 1(11), e53–e53. https://doi.org/10.1038/tp.2011.53

118. Kummer, K. K., Hofhansel, L., Barwitz, C. M., Schardl, A., Prast, J. M., Salti, A., El Rawas, R., & Zernig, G. (2014). Differences in social interaction- vs. Cocaine reward in mouse vs. Rat. Frontiers in Behavioral Neuroscience, 8. https://doi.org/10.3389/fnbeh.2014.00363

119. Lahvis, G. P. (2017). Social Reward and Empathy as Proximal Contributions to Altruism: The Camaraderie Effect. Current Topics in Behavioral Neurosciences, 30, 127–157. https://doi.org/10.1007/7854_2016_449

120. Lakshminarayanan, V. R., & Santos, L. R. (2008). Capuchin monkeys are sensitive to others' welfare. Current Biology, 18(21), R999–R1000. https://doi.org/10.1016/j.cub.2008.08.057

121. Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. Neuroscience & Biobehavioral Reviews, 26(1), 91–104. https://doi.org/10.1016/S0149-7634(01)00041-0

122. Lalot, M., Bourgeois, A., Jalme, M. S., & Bovet, D. (2023). Family first! Influence of parental investment in Guinea pigs (Cavia porcellus) prosocial choices. Animal Cognition, 26(5), 1713–1732. https://doi.org/10.1007/s10071-023-01813-x

123. Lalot, M., Delfour, F., Mercera, B., & Bovet, D. (2021). Prosociality and reciprocity in bottlenose dolphins (Tursiops truncatus). Animal Cognition, 24(5), 1075–1086. https://doi.org/10.1007/s10071-021-01499-z

124. Lalot, M., Liévin-Bazin, A., Bourgeois, A., Saint Jalme, M., & Bovet, D. (2021). Prosociality and reciprocity in capybaras (Hydrochoerus hydrochaeris) in a non-reproductive context. Behavioural Processes, 188, 104407. https://doi.org/10.1016/j.beproc.2021.104407

125. Langston, C. A. (n.d.). Capitalizing On and Coping With Daily-Life Events: Expressive Responses to Positive Events.

126. Lavery, J. J., & Foley, P. J. (1963). Altruism or Arousal in the Rat? Science, 140(3563), 172–173. https://doi.org/10.1126/science.140.3563.172

127. Lee, I.-C., Yu, T.-H., Liu, W.-H., & Hsu, K.-S. (2021). Social Transmission and Buffering of Hippocampal Metaplasticity after Stress in Mice. Journal of Neuroscience, 41(6), 1317–1330. https://doi.org/10.1523/JNEUROSCI.1751-20.2020

128. Lewis, M. (2018). Prosocial Behavior. In T. K. Shackelford & V. A. Weekes-Shackelford (Eds.), Encyclopedia of Evolutionary Psychological Science (pp. 1–6). Springer International Publishing. https://doi.org/10.1007/978-3-319-16999-6_158-1

129. Lewis, R. G., Florio, E., Punzo, D., & Borrelli, E. (2021). The Brain's Reward System in Health and Disease. In O. Engmann & M. Brancaccio (Eds.), Circadian Clock in Brain Health and Disease (Vol. 1344, pp. 57–69). Springer International Publishing. https://doi.org/10.1007/978-3-030-81147-1_4

130. Li, C.-L., Yu, Y., He, T., Wang, R.-R., Geng, K.-W., Du, R., Luo, W.-J., Wei, N., Wang, X.-L., Wang, Y., Yang, Y., Yu, Y.-Q., & Chen, J. (2018). Validating Rat Model of Empathy for Pain: Effects of Pain Expressions in Social Partners. Frontiers in Behavioral Neuroscience, 12. https://www.frontiersin.org/articles/10.3389/fnbeh.2018.00242

131. Li, L.-F., Yuan, W., He, Z.-X., Ma, H., Xun, Y.-F., Meng, L.-R., Zhu, S.-J., Wang, L.-M., Zhang, J., Cai, W.-Q., Zhang, X.-N., Guo, Q.-Q., Lian, Z.-M., Jia, R., & Tai, F.-D. (2020). Reduced Consolation Behaviors in Physically Stressed Mandarin Voles: Involvement of Oxytocin, Dopamine D2, and Serotonin 1A Receptors Within the Anterior Cingulate Cortex. International Journal of Neuropsychopharmacology, 23(8), 511–523. https://doi.org/10.1093/ijnp/pyz060

132. Li, L.-F., Yuan, W., He, Z.-X., Wang, L.-M., Jing, X.-Y., Zhang, J., Yang, Y., Guo, Q.-Q., Zhang, X.-N., Cai, W.-Q., Hou, W.-J., Jia, R., & Tai, F.-D. (2019). Involvement of oxytocin and GABA in consolation behavior elicited by socially defeated individuals in mandarin voles. Psychoneuroendocrinology, 103, 14–24. https://doi.org/10.1016/j.psyneuen.2018.12.238

133. Li, L.-F., Zhang, L.-Z., He, Z.-X., Ma, H., Zhang, Y.-T., Xun, Y.-F., Yuan, W., Hou, W.-J., Li, Y.-T., Lv, Z.-J., Jia, R., & Tai, F.-D. (2021). Dorsal raphe nucleus to anterior cingulate cortex 5-HTergic neural circuit modulates consolation and sociability. eLife, 10, e67638. https://doi.org/10.7554/eLife.67638

134. Lichtenberg, N. T., Lee, B., Kashtelyan, V., Chappa, B. S., Girma, H. T., Green, E. A., Kantor, S., Lagowala, D. A., Myers, M. A., Potemri, D., Pecukonis, M. G., Tesfay, R. T., Walters, M. S., Zhao, A. C., Blair, R. J. R., Cheer, J. F., & Roesch, M. R. (2018). Rat behavior and dopamine release are modulated by conspecific distress. eLife, 7, e38090. https://doi.org/10.7554/eLife.38090

135. Light, S. N., Coan, J. A., Zahn-Waxler, C., Frye, C., Goldsmith, H. H., & Davidson, R. J. (2009). Empathy Is Associated With Dynamic Change in Prefrontal Brain Electrical Activity During

Positive Emotion in Children. Child Development, 80(4), 1210–1231. https://doi.org/10.1111/j.1467-8624.2009.01326.x

136. Lim, K. Y., & Hong, W. (2023). Neural mechanisms of comforting: Prosocial touch and stress buffering. Hormones and Behavior, 153, 105391. https://doi.org/10.1016/j.yhbeh.2023.105391

137. Lima, F. F. D., & Osório, F. D. L. (2021). Empathy: Assessment Instruments and Psychometric Quality – A Systematic Literature Review With a Meta-Analysis of the Past Ten Years. Frontiers in Psychology, 12, 781346. https://doi.org/10.3389/fpsyg.2021.781346

138. Lipp, H.-P., & Wolfer, D. P. (2013). Natural neurobiology and behavior of the mouse: Relevance for behavioral studies in the laboratory. In W. E. Crusio, F. Sluyter, R. T. Gerlai, & S. Pietropaolo (Eds.), Behavioral Genetics of the Mouse (1st ed., pp. 5–16). Cambridge University Press. https://doi.org/10.1017/CBO9781139541022.003

139. Lissak, G. (2018). Adverse physiological and psychological effects of screen time on children and adolescents: Literature review and case study. Environmental Research, 164, 149–157. https://doi.org/10.1016/j.envres.2018.01.015

140. Lockwood, P. L., Apps, M. A. J., Valton, V., Viding, E., & Roiser, J. P. (2016). Neurocomputational mechanisms of prosocial learning and links to empathy. Proc. Natl. Acad. Sci. U.S.A., 113(35), 9763–9768. https://doi.org/10.1073/pnas.1603198113

141. Lockwood, P. L., Hamonet, M., Zhang, S. H., Ratnavel, A., Salmony, F. U., Husain, M., & Apps, M. A. J. (2017). Prosocial apathy for helping others when effort is required. Nature Human Behaviour, 1(7), 0131. https://doi.org/10.1038/s41562-017-0131

142. Lockwood, P. L., Seara-Cardoso, A., & Viding, E. (2014). Emotion Regulation Moderates the Association between Empathy and Prosocial Behavior. PLoS ONE, 9(5), e96555. https://doi.org/10.1371/journal.pone.0096555

143. Lockwood, Patricia. L., Apps, M. A. J., Roiser, J. P., & Viding, E. (2015). Encoding of Vicarious Reward Prediction in Anterior Cingulate Cortex and Relationship with Trait Empathy. The Journal of Neuroscience, 35(40), 13720–13727. https://doi.org/10.1523/JNEUROSCI.1703-15.2015

144. Lopes, G., Bonacchi, N., FrazÃ£o, J., Neto, J. P., Atallah, B. V., Soares, S., Moreira, L., Matias, S., Itskov, P. M., Correia, P. A., Medina, R. E., Calcaterra, L., Dreosti, E., Paton, J. J., & Kampff, A. R. (2015). Bonsai: An event-based framework for processing and controlling data streams. Frontiers in Neuroinformatics, 9. https://doi.org/10.3389/fninf.2015.00007

145. Lorenz, R. C., Gleich, T., Gallinat, J., & KÃ¼hn, S. (2015). Video game training and the reward system. Frontiers in Human Neuroscience, 9. https://doi.org/10.3389/fnhum.2015.00040

146. Lu, Y.-F., Ren, B., Ling, B.-F., Zhang, J., Xu, C., & Li, Z. (2018). Social interaction with a cagemate in pain increases allogrooming and induces pain hypersensitivity in the observer rats. Neuroscience Letters, 662, 385–388. https://doi.org/10.1016/j.neulet.2017.10.063

147. Luo, W.-J., Li, C.-L., Geng, K.-W., Wang, X.-L., Du, R., Yu, Y., Wei, N., He, T., Wang, Y., Yu, Y.-Q., & Chen, J. (2020). The similar past pain experience evokes both observational contagious pain and consolation in stranger rat observers. Neuroscience Letters, 722, 134840. https://doi.org/10.1016/j.neulet.2020.134840

148. Márquez, C., Rennie, S. M., Costa, D. F., & Moita, M. A. (2015). Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients. Current Biology, 25(13), 1736–1745. https://doi.org/10.1016/j.cub.2015.05.018

149. Marshall-Pescini, S., Dale, R., Quervel-Chaumette, M., & Range, F. (2016). Critical issues in experimental studies of prosociality in non-human species. Anim Cogn, 19(4), 679–705. https://doi.org/10.1007/s10071-016-0973-6

150. Massen, J. J. M., Van Den Berg, L. M., Spruijt, B. M., & Sterck, E. H. M. (2010). Generous Leaders and Selfish Underdogs: Pro-Sociality in Despotic Macaques. PLoS ONE, 5(3), e9734. https://doi.org/10.1371/journal.pone.0009734

151. Masserman, J. H., Wechkin, S., & Terris, W. (1964). 'ALTRUISTIC' BEHAVIOR IN RHESUS MONKEYS. American Journal of Psychiatry, 121(6), 584–585. https://doi.org/10.1176/ajp.121.6.584

152. Matsumoto, M., Yoshida, M., Jayathilake, B. W., Inutsuka, A., Nishimori, K., Takayanagi, Y., & Onaka, T. (2021). Indispensable role of the oxytocin receptor for allogrooming toward socially distressed cage mates in female mice. Journal of Neuroendocrinology, 33(6), e12980. https://doi.org/10.1111/jne.12980

153. McFarland, R., & Majolo, B. (2011). Grooming Coercion and the Post-Conflict Trading of Social Services in Wild Barbary Macaques. PLOS ONE, 6(10), e26893. https://doi.org/10.1371/journal.pone.0026893

154. Meyza, K. Z., Bartal, I. B.-A., Monfils, M. H., Panksepp, J. B., & Knapska, E. (2017). The roots of empathy: Through the lens of rodent models. Neuroscience & Biobehavioral Reviews, 76, 216–234. https://doi.org/10.1016/j.neubiorev.2016.10.028

155. Michon, F., Packheiser, J., Gazzola, V., & Keysers, C. (2023). Sharing Positive Affective States Amongst Rodents. Affective Science, 4(3), 475–479. https://doi.org/10.1007/s42761-023-00201-5

156. Misiołek, K., Klimczak, M., Chrószcz, M., Szumiec, Ł., Bryksa, A., Przyborowicz, K., Rodriguez Parkitna, J., & Harda, Z. (2023). Prosocial behavior, social reward and affective state

discrimination in adult male and female mice. Scientific Reports, 13(1), Article 1. https://doi.org/10.1038/s41598-023-32682-6

157. Monfardini, E., Gazzola, V., Boussaoud, D., Brovelli, A., Keysers, C., & Wicker, B. (2013). Vicarious Neural Processing of Outcomes during Observational Learning. PLoS ONE, 8(9), e73879. https://doi.org/10.1371/journal.pone.0073879

158. Morelli, S. A., Knutson, B., & Zaki, J. (2018). Neural sensitivity to personal and vicarious reward differentially relate to prosociality and well-being. Social Cognitive and Affective Neuroscience, 13(8), 831–839. https://doi.org/10.1093/scan/nsy056

159. Morelli, S. A., Lieberman, M. D., & Zaki, J. (2015). The Emerging Study of Positive Empathy. Social and Personality Psychology Compass, 9(2), 57–68. https://doi.org/10.1111/spc3.12157

160. Morelli, S. A., Sacchet, M. D., & Zaki, J. (2015). Common and distinct neural correlates of personal and vicarious reward: A quantitative meta-analysis. NeuroImage, 112, 244–253. https://doi.org/10.1016/j.neuroimage.2014.12.056

161. Morrison, I. (2016). Keep Calm and Cuddle on: Social Touch as a Stress Buffer. Adaptive Human Behavior and Physiology, 2(4), 344–362. https://doi.org/10.1007/s40750-016-0052-x

162. Nakahara, F., Komaba, M., Sato, R., Ikeda, H., Komaba, K., & Kawakubo, A. (2017). Spontaneous prosocial choice by captive bottlenose dolphins, Tursiops truncatus. Behavioural Processes, 135, 8–11. https://doi.org/10.1016/j.beproc.2016.11.009

163. Netser, S., Meyer, A., Magalnik, H., Zylbertal, A., De La Zerda, S. H., Briller, M., Bizer, A., Grinevich, V., & Wagner, S. (2020). Distinct dynamics of social motivation drive differential social behavior in laboratory rat and mouse strains. Nature Communications, 11(1), 5908. https://doi.org/10.1038/s41467-020-19569-0

164. Nowak, M. A. (2006). Five Rules for the Evolution of Cooperation. Science, 314(5805), 1560–1563. https://doi.org/10.1126/science.1133755

165. Nowbahari, E., Scohier, A., Durand, J.-L., & Hollis, K. L. (2009). Ants, Cataglyphis cursor, Use Precisely Directed Rescue Behavior to Free Entrapped Relatives. PLoS ONE, 4(8), e6573. https://doi.org/10.1371/journal.pone.0006573

166. Palagi, E., & Cordoni, G. (2009). Postconflict third-party affiliation in Canis lupus: Do wolves share similarities with the great apes? Animal Behaviour, 78(4), 979–986. https://doi.org/10.1016/j.anbehav.2009.07.017

167. Penner., L. A., Dovidio., J. F., Piliavin., J. A., & Schroeder., D. A. (2005). Prosocial Behavior: Multilevel Perspectives. Annu. Rev. Psychol., 56(1), 365–392. https://doi.org/10.1146/annurev.psych.56.091103.070141

168. Pérez-Manrique, A., & Gomila, A. (2018). The comparative study of empathy: Sympathetic concern and empathic perspective-taking in non-human animals: Comparative study of empathy. Biol Rev, 93(1), 248–269. https://doi.org/10.1111/brv.12342

169. Pérez-Manrique, A., & Gomila, A. (2022). Emotional contagion in nonhuman animals: A review. WIRES Cognitive Science, 13(1). https://doi.org/10.1002/wcs.1560

170. Pfattheicher, S., Nielsen, Y. A., & Thielmann, I. (2022). Prosocial behavior and altruism: A review of concepts and definitions. Current Opinion in Psychology, 44, 124–129. https://doi.org/10.1016/j.copsyc.2021.08.021

171. Pfeiffer, T., Rutte, C., Killingback, T., Taborsky, M., & Bonhoeffer, S. (2005). Evolution of cooperation by generalized reciprocity. Proc. R. Soc. B., 272(1568), 1115–1120. https://doi.org/10.1098/rspb.2004.2988

172. Phillips, H. L., Dai, H., Choi, S. Y., Jansen-West, K., Zajicek, A. S., Daly, L., Petrucelli, L., Gao, F.-B., & Yao, W.-D. (2023). Dorsomedial prefrontal hypoexcitability underlies lost empathy in frontotemporal dementia. Neuron, 111(6), 797-806.e6. https://doi.org/10.1016/j.neuron.2022.12.027

173. Plotnik, J. M., & Waal, F. B. M. de. (2014). Asian elephants (Elephas maximus) reassure others in distress. PeerJ, 2, e278. https://doi.org/10.7717/peerj.278

174. Pozo, M., Milà-Guasch, M., Haddad-Tóvolli, R., Boudjadja, M. B., Chivite, I., Toledo, M., Gómez-Valadés, A. G., Eyre, E., Ramírez, S., Obri, A., Ben-Ami Bartal, I., D'Agostino, G., Costa-Font, J., & Claret, M. (2023). Negative energy balance hinders prosocial helping behavior. Proceedings of the National Academy of Sciences, 120(15), e2218142120. https://doi.org/10.1073/pnas.2218142120

175. Preston, S. D., & De Waal, F. B. M. (2002). Empathy: Its ultimate and proximate bases. Behavioral and Brain Sciences, 25(1), 1–20. https://doi.org/10.1017/S0140525X02000018

176. Przybylski, A. K., Rigby, C. S., & Ryan, R. M. (2010). A Motivational Model of Video Game Engagement. Review of General Psychology, 14(2), 154–166. https://doi.org/10.1037/a0019440

177. Radford, A. N., & Du Plessis, M. A. (2006). Dual function of allopreening in the cooperatively breeding green woodhoopoe, Phoeniculus purpureus. Behavioral Ecology and Sociobiology, 61(2), 221–230. https://doi.org/10.1007/s00265-006-0253-6

178. Rault, J.-L. (2019). Be kind to others: Prosocial behaviours and their implications for animal welfare. Applied Animal Behaviour Science, 210, 113–123. https://doi.org/10.1016/j.applanim.2018.10.015

179. Reppucci, C. J., Brown, L. A., Chambers, A. Q., & Veenema, A. H. (2020). Wistar rats and C57BL/6 mice differ in their motivation to seek social interaction versus food in the Social versus

Food Preference Test. Physiology & Behavior, 227, 113162. https://doi.org/10.1016/j.physbeh.2020.113162

180. Reppucci, C. J., & Veenema, A. H. (2020). The social versus food preference test: A behavioral paradigm for studying competing motivated behaviors in rodents. MethodsX, 7, 101119. https://doi.org/10.1016/j.mex.2020.101119

181. Rice, G. E., & Gainer, P. (1962). 'Altruism' in the albino rat. Journal of Comparative and Physiological Psychology, 55(1), 123–125. https://doi.org/10.1037/h0042276

182. Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. Current Opinion in Neurobiology,6(2),228–236. https://doi.org/10.1016/S0959-4388(96)80077-8

183. Rogers-Carter, M. M., Varela, J. A., Gribbons, K. B., Pierce, A. F., McGoey, M. T., Ritchey, M., & Christianson, J. P. (2018). Insular cortex mediates approach and avoidance responses to social affective stimuli. Nature Neuroscience, 21(3), 404–414. https://doi.org/10.1038/s41593-018-0071-y

184. Rutte, C., & Taborsky, M. (2007). Generalized Reciprocity in Rats. PLoS Biol, 5(7), e196. https://doi.org/10.1371/journal.pbio.0050196

185. Rutte, C., & Taborsky, M. (2008). The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): Direct vs generalised reciprocity. Behav Ecol Sociobiol, 62(4), 499–505. https://doi.org/10.1007/s00265-007-0474-3

186. Sato, N., Tan, L., Tate, K., & Okada, M. (2015). Rats demonstrate helping behavior toward a soaked conspecific. Animal Cognition, 18(5), 1039–1047. https://doi.org/10.1007/s10071-015-0872-2

187. Satoh, S., Bshary, R., Shibasaki, M., Inaba, S., Sogawa, S., Hotta, T., Awata, S., & Kohda, M. (2021). Prosocial and antisocial choices in a monogamous cichlid with biparental care. Nat Commun, 12(1), 1775. https://doi.org/10.1038/s41467-021-22075-6

188. Schaich Borg, J., Srivastava, S., Lin, L., Heffner, J., Dunson, D., Dzirasa, K., & de Lecea, L. (2017). Rat intersubjective decisions are encoded by frequency-specific oscillatory contexts. Brain and Behavior, 7(6), e00710. https://doi.org/10.1002/brb3.710

189. Scheggia, D., La Greca, F., Maltese, F., Chiacchierini, G., Italia, M., Molent, C., Bernardi, F., Coccia, G., Carrano, N., Zianni, E., Gardoni, F., Di Luca, M., & Papaleo, F. (2022). Reciprocal cortico-amygdala connections regulate prosocial and selfish choices in mice. Nature Neuroscience. https://doi.org/10.1038/s41593-022-01179-2

190. Scheggia, D., Managò, F., Maltese, F., Bruni, S., Nigro, M., Dautan, D., Latuske, P., Contarini, G., Gomez-Gonzalo, M., Requie, L. M., Ferretti, V., Castellani, G., Mauro, D., Bonavia, A., Carmignoto, G., Yizhar, O., & Papaleo, F. (2020). Somatostatin interneurons in the prefrontal

cortex control affective state discrimination in mice. Nature Neuroscience, 23(1), 47–60. https://doi.org/10.1038/s41593-019-0551-8

191. Schino, G., & Aureli, F. (2010). The relative roles of kinship and reciprocity in explaining primate altruism. Ecology Letters, 13(1), 45–50. https://doi.org/10.1111/j.1461-0248.2009.01396.x

192. Schneeberger, K., Dietz, M., & Taborsky, M. (2012). Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evol Biol, 12(1), 41. https://doi.org/10.1186/1471-2148-12-41

193. Schoenrade, P. A., Batson, C. D., Brandt, J. R., & Loud, R. E. (n.d.). Attachment, Accountability, and Motivation to Benefit Another Not in Distress.

194. Schweinfurth, M. K. (2020). The social life of Norway rats (Rattus norvegicus). eLife, 9, e54020. https://doi.org/10.7554/eLife.54020

195. Schweinfurth, M. K. (2024). Interdisciplinary perspectives on the principle of reciprocation. Ethology, 130(4), e13445. https://doi.org/10.1111/eth.13445

196. Schweinfurth, M. K., & Taborsky, M. (2018). Relatedness decreases and reciprocity increases cooperation in Norway rats. Proceedings of the Royal Society B: Biological Sciences, 285(1874), 20180035. https://doi.org/10.1098/rspb.2018.0035

197. Shamay-Tsoory, S. G., Tomer, R., Goldsher, D., Berger, B. D., & Aharon-Peretz, J. (2004). Impairment in Cognitive and Affective Empathy in Patients with Brain Lesions: Anatomical and Cognitive Correlates. Journal of Clinical and Experimental Neuropsychology, 26(8), 1113–1127. https://doi.org/10.1080/13803390490515531

198. Sherman, L. E., Hernandez, L. M., Greenfield, P. M., & Dapretto, M. (2018). What the brain 'Likes': Neural correlates of providing feedback on social media. Social Cognitive and Affective Neuroscience, 13(7), 699–707. https://doi.org/10.1093/scan/nsy051

199. Shimada, S., Matsumoto, M., Takahashi, H., Yomogida, Y., & Matsumoto, K. (2016). Coordinated activation of premotor and ventromedial prefrontal cortices during vicarious reward. Social Cognitive and Affective Neuroscience, 11(3), 508–515. https://doi.org/10.1093/scan/nsv134

200. Silk, J. B., Brosnan, S. F., Vonk, J., Henrich, J., Povinelli, D. J., Richardson, A. S., Lambeth, S. P., Mascaro, J., & Schapiro, S. J. (2005). Chimpanzees are indifferent to the welfare of unrelated group members. Nature, 437(7063), 1357–1359. https://doi.org/10.1038/nature04243

201. Singer, T., Seymour, B., O'Doherty, J. P., Stephan, K. E., Dolan, R. J., & Frith, C. D. (2006). Empathic neural responses are modulated by the perceived fairness of others. Nature, 439(7075), 466–469. https://doi.org/10.1038/nature04271

202. Smith, M. L., Asada, N., & Malenka, R. C. (2021). Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia. Science, 371(6525), 153–159. https://doi.org/10.1126/science.abe3040

203. Snippe, E., Jeronimus, B. F., Aan Het Rot, M., Bos, E. H., De Jonge, P., & Wichers, M. (2018). The Reciprocity of Prosocial Behavior and Positive Affect in Daily Life. Journal of Personality, 86(2), 139–146. https://doi.org/10.1111/jopy.12299

204. Solié, C., Girard, B., Righetti, B., Tapparel, M., & Bellone, C. (2022). VTA dopamine neuron activity encodes social interaction and promotes reinforcement learning through social prediction error. Nature Neuroscience, 25(1), 86–97. https://doi.org/10.1038/s41593-021-00972-9

205. Song, D., Wang, C., Jin, Y., Deng, Y., Yan, Y., Wang, D., Zhu, Z., Ke, Z., Wang, Z., Wu, Y., Ni, J., Qing, H., & Quan, Z. (2023). Mediodorsal thalamus-projecting anterior cingulate cortex neurons modulate helping behavior in mice. Current Biology, 33(20), 4330-4342.e5. https://doi.org/10.1016/j.cub.2023.08.070

206. Stevens, F., & Taber, K. (2021). The neuroscience of empathy and compassion in pro-social behavior. Neuropsychologia, 159, 107925. https://doi.org/10.1016/j.neuropsychologia.2021.107925

207. Tang, J. C. Y., Paixao, V., Carvalho, F., Silva, A., Klaus, A., Da Silva, J. A., & Costa, R. M. (2024). Dynamic behaviour restructuring mediates dopamine-dependent credit assignment. Nature, 626(7999), 583–592. https://doi.org/10.1038/s41586-023-06941-5

208. Telle, N.-T., & Pfister, H.-R. (2016). Positive Empathy and Prosocial Behavior: A Neglected Link. Emotion Review, 8(2), 154–163. https://doi.org/10.1177/1754073915586817

209. Thrailkill, E. A., & Daniels, C. W. (2024). The temporal structure of goal-directed and habitual operant behavior. Journal of the Experimental Analysis of Behavior, 121(1), 38–51. https://doi.org/10.1002/jeab.896

210. Trivers, R. L. (1971). The Evolution of Reciprocal Altruism. The Quarterly Review of Biology, 46(1), 35–57. https://doi.org/10.1086/406755

211. Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Takahashi, Y., Matsumoto, Y., Okamoto, M., & Ishihara, T. (2019). Rescue-like Behaviour in Mice is Mediated by Their Interest in the Restraint Tool. Sci Rep, 9(1), 10648. https://doi.org/10.1038/s41598-019-46128-5

212. Van Der Graaff, J., Carlo, G., Crocetti, E., Koot, H. M., & Branje, S. (2018). Prosocial Behavior in Adolescence: Gender Differences in Development and Links with Empathy. Journal of Youth and Adolescence, 47(5), 1086–1099. https://doi.org/10.1007/s10964-017-0786-1

213. van Gurp, S., Hoog, J., Kalenscher, T., & van Wingerden, M. (2020). Vicarious reward unblocks associative learning about novel cues in male rats. eLife, 9, e60755. https://doi.org/10.7554/eLife.60755

214. Vasconcelos, M., Hollis, K., Nowbahari, E., & Kacelnik, A. (2012). Pro-sociality without empathy. Biology Letters, 8(6), 910–912. https://doi.org/10.1098/rsbl.2012.0554

215. Warneken, F., Hare, B., Melis, A. P., Hanus, D., & Tomasello, M. (2007). Spontaneous Altruism by Chimpanzees and Young Children. PLoS Biol, 5(7), e184. https://doi.org/10.1371/journal.pbio.0050184

216. Warneken, F., & Tomasello, M. (2006). Altruistic Helping in Human Infants and Young Chimpanzees. Science, 311(5765), 1301–1303. https://doi.org/10.1126/science.1121448

217. Waytz, A., Zaki, J., & Mitchell, J. P. (2012). Response of Dorsomedial Prefrontal Cortex Predicts Altruistic Behavior. The Journal of Neuroscience, 32(22), 7646–7650. https://doi.org/10.1523/JNEUROSCI.6193-11.2012

218. Weiss, O., Dorfman, A., Ram, T., Zadicario, P., & Eilam, D. (2017). Rats do not eat alone in public: Food-deprived rats socialize rather than competing for baits. PLOS ONE, 12(3), e0173302. https://doi.org/10.1371/journal.pone.0173302

219. West, S. A., Griffin, A. S., & Gardner, A. (2007). Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. J Evolution Biol, 20(2), 415–432. https://doi.org/10.1111/j.1420-9101.2006.01258.x

220. Williams, A., Oâ€TMDriscoll, K., & Moore, C. (2014). The influence of empathic concern on prosocial behavior in children. Frontiers in Psychology, 5. https://doi.org/10.3389/fpsyg.2014.00425

221. Willuhn, I., Tose, A., Wanat, M. J., Hart, A. S., Hollon, N. G., Phillips, P. E. M., Schwarting, R. K. W., & Wohr, M. (2014a). Phasic Dopamine Release in the Nucleus Accumbens in Response to Pro-Social 50 kHz Ultrasonic Vocalizations in Rats. Journal of Neuroscience, 34(32), 10616–10623. https://doi.org/10.1523/JNEUROSCI.1060-14.2014

222. Wood, R. I., Kim, J. Y., & Li, G. R. (2016). Cooperation in rats playing the iterated Prisoner's Dilemma game. Animal Behaviour, 114, 27–35. https://doi.org/10.1016/j.anbehav.2016.01.010

223. Wu, W.-Y., Cheng, Y., Liang, K.-C., Lee, R. X., & Yen, C.-T. (2023). Affective mirror and anti-mirror neurons relate to prosocial help in rats. iScience, 26(1), 105865. https://doi.org/10.1016/j.isci.2022.105865

224. Wu, Y. E., Dang, J., Kingsbury, L., Zhang, M., Sun, F., Hu, R. K., & Hong, W. (2021). Neural control of affiliative touch in prosocial interaction. Nature, 599(7884), Article 7884. https://doi.org/10.1038/s41586-021-03962-w

225. Yamagishi, A., Lee, J., & Sato, N. (2020). Oxytocin in the anterior cingulate cortex is involved in helping behaviour. Behavioural Brain Research, 393, 112790. https://doi.org/10.1016/j.bbr.2020.112790

226. Yamamoto, S. (2017). Primate empathy: Three factors and their combinations for empathy-related phenomena. WIREs Cogn Sci, 8(3). https://doi.org/10.1002/wcs.1431

227. Yamamoto, S., Humle, T., & Tanaka, M. (2009). Chimpanzees Help Each Other upon Request. PLoS ONE, 4(10), e7416. https://doi.org/10.1371/journal.pone.0007416

228. Zell, V., Steinkellner, T., Hollon, N. G., Warlow, S. M., Souter, E., Faget, L., Hunker, A. C., Jin, X., Zweifel, L. S., & Hnasko, T. S. (2020). VTA Glutamate Neuron Activity Drives Positive Reinforcement Absent Dopamine Co-release. Neuron, 107(5), 864-873.e4. https://doi.org/10.1016/j.neuron.2020.06.011

229. Zeng, Q., Shan, W., Zhang, H., Yang, J., & Zuo, Z. (2021). Paraventricular thalamic nucleus plays a critical role in consolation and anxious behaviors of familiar observers exposed to surgery mice. Theranostics, 11(8), 3813–3829. https://doi.org/10.7150/thno.45690

230. Zhang, M., Wu, Y. E., Jiang, M., & Hong, W. (2024). Cortical regulation of helping behaviour towards others in pain. Nature, 626(7997), Article 7997. https://doi.org/10.1038/s41586-023-06973-x

231. Zheng, C., Huang, Y., Bo, B., Wei, L., Liang, Z., & Wang, Z. (2020). Projection from the Anterior Cingulate Cortex to the Lateral Part of Mediodorsal Thalamus Modulates Vicarious Freezing Behavior. Neuroscience Bulletin, 36(3), 217–229. https://doi.org/10.1007/s12264-019-00427-z

Annex 1

Quality criteria used for this doctoral thesis, which is presented as a compendium of publications:

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Prosocial behaviors in rodents

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ABSTRACT

Prosocial behaviors (i.e., actions that benefit others) are central for social interactions in humans and other animals, by fostering social bonding and cohesion. To study prosociality in rodents, scientists have developed behavioral paradigms where animals can display actions that benefit conspecifics in distress or need. These paradigms have provided insights into the role of social interactions and transfer of emotional states in the expression of prosociality, and increased knowledge of its neural bases. However, prosociality levels are variable: not all tested animals are prosocial. Such variation has been linked to differences in animals' ability to process another's state as well as to contextual factors. Moreover, evidence suggests that prosocial behaviors involve the orchestrated activity of multiple brain regions and neuromodulators. This review aims to synthesize findings across paradigms both at the level of behavior and neural mechanisms. Growing evidence confirms that these processes can be studied in rodents, and intense research in the past years is rapidly advancing our knowledge. We discuss a strong bias in the field towards the study of these processes in negative valence contexts (e.g., pain, fear, stress), which should be taken as an opportunity to open new venues for future research.

1. Introduction

We would like to dedicate this review to the memory of Franz de Waal and Larry Young, for their influential careers in the field of social neuroscience, but especially, for inspiring us in our research and pioneering the field of the study of prosociality in laboratory settings.

Helping someone in need, caregiving, comforting, donating money or volunteering are examples of prosocial actions largely common in human society. How prosociality is conserved across species and how the brain computes these types of actions are intense areas of research in neuroscience. In the present review we will focus on research performed with rodents and how recent findings using these species have been advancing our knowledge of the mechanisms underlying prosociality. We will start by a theoretical introduction of the main concepts and contextualization of the current views of the origins of prosociality, pioneered by research in humans and non-human primates, and enriched with research across different taxa. We will then focus on how research performed in rodents is helping advance the field.

At the most generic level, *prosocial behavior,* or *prosociality,* has been broadly defined as any behavior that benefits another, thus improving their condition (Dovidio et al., 2017). It is typically distinguished from *altruism* when considering motivations and costs associated with the behavior. Prosociality may or may not entail a cost for the actor and can be driven by several motivations. In contrast, altruistic behaviors are generally costly for the actor and other-regarding, implying no expectation of self-benefit (Lewis, 2018). Altruistic behaviors can thus be considered a subset of prosociality: all altruistic behaviors are prosocial but not all prosocial behaviors are altruistic. The terms prosocial behavior and altruism are generally distinguished from *cooperation*, which occurs when two or more individuals work together achieving common or mutual benefits (Marshall-Pescini et al., 2016). Prosociality, altruism and cooperation have been examined across scientific disciplines, resulting in similar terminology being used with different meanings (Kopp et al., 2024; Pfattheicher et al., 2022). The above definitions for altruistic and cooperative behaviors reflect research in social and comparative psychology and differ from those developed in the field of evolutionary biology, where behaviors are defined as cooperative or altruistic based on costs and benefits for individuals' direct fitness (i.e., their reproductive success) (West et al., 2007). In evolutionary terms,

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cooperation is helping behavior that increases recipient's direct fitness, and can result in mutual benefit (when also actor's direct fitness increases) or altruism (when actor's direct fitness decreases).

Several mechanisms have been proposed for explaining the evolution and maintenance of cooperative behaviors (Clutton-Brock, 2009; Nowak, 2006), including kin selection (Hamilton, 1964), direct reciprocity (Trivers, 1971) and generalized reciprocity (Hamilton and Taborsky, 2005; Pfeiffer et al., 2005). Evidence for reciprocity involving different commodities and services has been reported across several taxa (Schweinfurth, 2024). Rats, for instance, reciprocate help for food sharing according to both direct and generalized reciprocity (Engelhardt and Taborsky, 2024; Rutte and Taborsky, 2007, 2008; Schneeberger et al., 2012; Schweinfurth and Taborsky, 2018; Wood et al., 2016). Since an overview of reciprocity and cooperation in rodents is beyond the scope of this review, we will focus here on studies using tasks where only one animal of the pair acts as helper. In addition, we will focus on the proximate mechanisms of prosociality, regardless of lifetime fitness consequences for the individuals involved, and thus we will use the term "*prosocial*" in relation to a behavior providing immediate benefit (e.g., food reward, stress reduction) to an individual in need. Furthermore, as intentionality of an action is difficult to assess in laboratory rodents, we will consider a decision as prosocial if it is learned, flexible and goal directed, which are parameters that can be evaluated experimentally.

At the proximate level, it has been suggested that the type of prosociality shown by humans depends on socio-cognitive abilities well developed in our species, as well as on ethical and social attitudes appropriate to the culture (Penner et al., 2005). For instance, *empathy,* broadly defined as the ability to sense and resonate with another's feeling, knowing that the shared feeling originates from the other (Decety and Jackson, 2004). In humans, empathizing with others' distress, pain or needs can lead to personal distress as well as concern for others. As such, empathy allows us to quickly relate to another's state and can function as a major trigger for prosocial actions.

Scientists have defined empathy in a variety of ways and long debated about its nature and evolution (Batson, 2009). Some consider empathy as an umbrella term aggregating various phenomena, including prosociality. In this respect, a prevailing evolutionary model among the empathy literature is the Russian doll model (de Waal and Preston, 2017), where empathy includes multiple affective, cognitive, and behavioral components organized into sequential layers (Fig. 1**A**). Here, the inner and phylogenetically older layer is the perception-action mechanism (PAM), through which perceiving another's state activates one's own neural and mental representation of that state. PAM enables state matching between individuals, with its more basic expressions being motor mimicry and emotional contagion. New layers gradually evolved in some species, with each new layer being built on top of, and dependent on, older ones. These outer layers correspond to empathic phenomena requiring increased self-other distinction, emotion regulation and cognition, such as empathic concern, consolation, targeted helping and perspective-taking. Therefore, the model posits phylogenetic continuity in empathic abilities, which are supported by homologous neural and hormonal substrates. Advanced forms of empathy gradually developed from a simple, spontaneous mechanism, shared across a variety of species, with parental care and social attachment likely promoting this evolution.

As an alternative to the Russian doll model, Yamamoto proposed a combination model, observing that the related phenomena under the umbrella of empathy do not necessarily depend on each other sequentially but may have evolved independently, through convergent evolution, and can subsist separately (Yamamoto, 2017) (Fig. 1**B**). The model suggests three independent but interacting factors: "matching with others" (e.g., emotional contagion, mimicry), "understanding of others" (e.g., perspective-taking) and prosociality. Different empathic phenomena are mapped onto one of the three factors or onto their combination. Consequently, they are not strictly organized according to an increase in cognitive or emotional complexity, except for those mapped onto the overlaps between factors. Under this framework, prosociality can be studied without assuming its dependence on other affective or cognitive capacities, with the potential to embrace a larger variety of prosocial behaviors across the entire animal kingdom. Indeed, the model lists food sharing and food-based prosocial choice in non-human primates as examples of behaviors which do not require assumptions of emotional state matching, or an understanding of others, in order to occur. Nevertheless, in both models, targeted helping would require perspective-taking, a mechanism less likely to be ascribed to cases of helping in social insects, such as the highly controlled rescue behavior

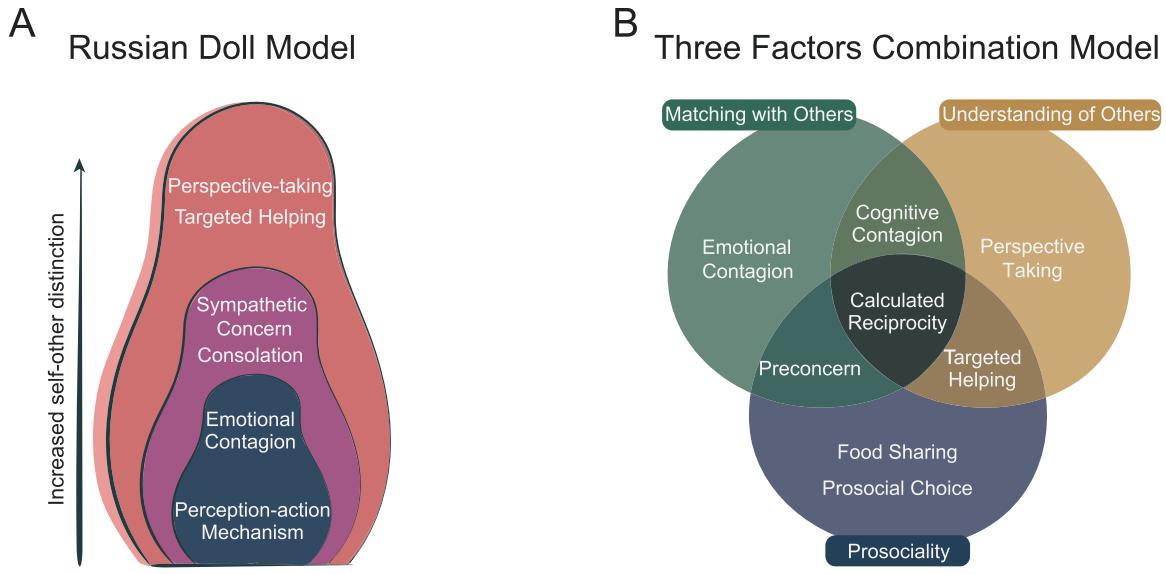

Fig. 1. Theoretical models of empathy. A. Russian Doll Model of Empathy from de Waal and Preston. It reflects a conceptual framework where various affective, cognitive, and behavioral components of empathy are built into sequential layers developed during evolution. The inner and older layer corresponds to the perception-action mechanism, which induces emotional contagion in the observer. Outer layers are built upon increased self-other distinction, emotion regulation and cognition, such as empathic concern, consolation, targeted helping and perspective-taking. B. Three-Factor Combination Model from Yamamoto. This model posits that empathy is built upon three organizing factors: matching with others, understanding of others and prosociality. Most empathy-related phenomena can be categorized and mapped into appropriate contexts with these three factors and their combinations.

shown by ants towards nestmates in danger (Hollis and Nowbahari, 2013; Nowbahari et al., 2009). While there is no a priori reason to fully rule out such abilities in insects, or that a mechanism similar to the PAM may be in place (de Waal and Preston, 2017), the matter awaits empirical evidence.

Because psychological processes are hard to study in the field, testing animals in experimental tasks, in controlled laboratory or semi-natural settings, is useful to explore whether a species displays specific behavioral tendencies as well as to identify shared cognitive or neural processes underlying those tendencies. On this line, research investigating the expression of empathic phenomena has provided evidence that animals can perceive, learn from and respond to the emotional states of conspecifics. For instance, findings of emotional contagion and affiliative response to distressed conspecifics are robust (for review Chen, 2017; Meyza et al., 2017; Pérez-Manrique and Gomila, 2018; Pérez-Manrique and Gomila, 2022; Keysers et al., 2022). Moreover, a growing number of experimental studies has investigated whether animals display choices or actions that benefit others. This research has yielded evidence for the emergence of prosocial behaviors in a variety of species, (Nowbahari et al., 2009; Duque and Stevens, 2016; Horn et al., 2024; Nakahara et al., 2017; Satoh et al., 2021; Lalot, Liévin-Bazin, et al., 2021; Lalot et al., 2023; for review see Jensen, 2016; Marshall-Pescini et al., 2016; Rault, 2019), further suggesting that convergent selective pressures may have driven the evolution of prosociality in distant taxa. Notably, studies with laboratory rodents, mainly rats, mice and voles, have started to map brain regions and neural circuits to specific types of prosocial behaviors that involve relieving the distress of, or providing reward to, a conspecific. For this, neuroscientists are testing rodents in innovative prosocial paradigms and using tools to measure or manipulate neuronal activity, advancing our knowledge of the neural bases of prosocial actions.

We feel it is timely to synthesize these findings, to help create a big picture of the puzzle of prosociality and identify the gaps in the field. To this aim, the current review focuses on four experimental paradigms that have been often employed to measure different types of prosocial behaviors (Fig. 2), namely the "*consolation*" paradigm, which assesses animals' tendency to display affiliative social touch (e.g., allogrooming) towards a distressed conspecific; the *rescue* paradigm, where animals can perform an action that enables conspecifics to escape a situation of stress; the *harm aversion* paradigm, which measures animals' propensity to prevent others' distress; and the *prosocial choice task*, where animals can choose to provide or not food to a partner. We give an overview of the studies using these paradigms and summarize the results, focusing on behavioral outcomes, highlighting differences in task design and conditions tested, and reporting findings relative to neural mechanisms of prosociality when available (Table 1).

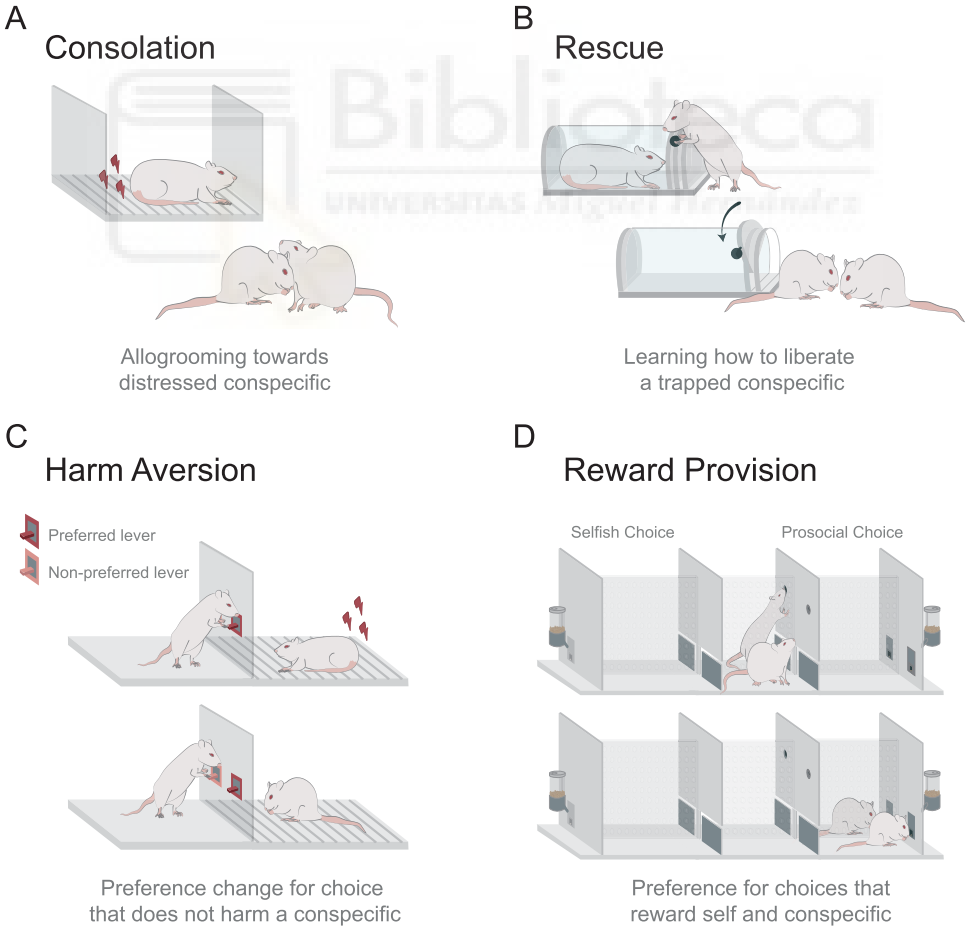

Fig. 2. Experimental paradigms revealing prosocial behavior in rodents. A. Consolation paradigm. Focal rodents show increased allogrooming and/or allolicking towards a conspecific that experienced a distressful event (e.g. pain, fear, social defeat). B. Rescue paradigm. Rodents learn to free a trapped conspecific from a restrainer or water pool. C. Harm aversion paradigm. Rats are first tested for developing a preference between two options, then the preferred option is associated with shocks to a conspecific. Prosocial rats switch their previous preference and avoid the option that now shocks a conspecific. D. Reward provision paradigm. Decision-maker rodents can choose between two options: one option delivers reward to them and a conspecific (prosocial choice) and the other option only to them (selfish option). Over sessions, decision-makers develop a preference for the option that rewards both themselves and the conspecific.

Table 1
Experimental studies revealing prosocial behavior in rodents. Experimental works revealing prosocial behavior in rodents, along with the paradigm type, species and sex tested, and main behavioral and neural substrates found.

PROSOCIAL BEHAVIOR IN NEGATIVE AFFECTIVE STATES				
Species	Sex	Behavioral Readouts	Neural Substrates	References
		CONSOLATION		
Prairie voles	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming towards stressed familiar conspecific.	c-FOS in ACC OT receptors in ACC	Burkett et al. (2016)
SD rats	♂ ➜ ♂	↑ mechanical hypersensitivity, allogrooming and allolicking towards familiar conspecifics expressing pain.		Li et al. (2018)
SD rats	♂ ➜ ♂	↑ mechanical hypersensitivity and allogrooming towards familiar conspecifics expressing pain.		Lu et al. (2018)
Wistar rats	♂ ➜ ♂	↑ allogrooming towards unfamiliar conspecific stressed by fear-conditioning.	c-FOS in PVN & CeA	Kiyokawa et al. (2019)
Mandarin voles	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming towards socially distressed conspecific. Chronic social defeat decreases consolation behaviour.	OT, GABAa, 5-HT1A and D2 receptors in the ACC. DR➜ACC 5HTergic	Li et al. (2019), (2020), (2021)
SD rats	♂ ➜ ♂	Past pain experience increases allogrooming and observational contagious pain towards pain-experiencing unfamiliar conspecifics.		Luo et al. (2020)
SD rats C57BL/6 mice	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming and contagious pain towards familiar same-sex conspecifics experiencing pain. Modulated by sex in mice (↑ allogrooming by males) but not in rats.		Du et al. (2020)
CD-1 mice	♂ ➜ ♂	↑ allogrooming and allolicking towards conspecifics undergone surgery. Enhanced by familiarity.	PVT neurons containing orexin receptors	Zeng et al. (2021)
C57BL/6 mice CD-1 mice	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming towards socially defeated conspecifics. Enhanced by familiarity.	c-FOS OTR cells in AON, ACC, IC, LS, MeA (♀)	Matsumoto et al. (2021)
C57BL/6 J mice	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming towards familiar conspecifics experiencing different types of stressors. Independent of sex.	$Tac1^{+}Vgat^{+}$ neurons in MeA ➜ MPOA	Wu et al. (2021)
Swiss mice	♂ ➜ ♂	↑ emotional contagion from and allogrooming directed towards chronically stressed familiar conspecifics.		Carneiro de Oliveira et al. (2022)
C57BL/6 J mice	♂ ➜ ♂ ♀ ➜ ♀	↑ allogrooming and body contacts towards fear conditioned conspecifics, independent on familiarity, sex, or observation of the partner's conditioning.	dmPFC	Phillips et al. (2023)
C57BL/6 J mice	♂ ➜ ♂ ♀ ➜ ♀	↑ targeted allolicking and allogrooming towards familiar conspecifics experiencing pain via bee venom.	ACC	Zhang et al. (2024)
		RESCUE		
Wistar rats	♂ ➜ ♂ ♀ ➜ ♀	Lever pressing to lower a distressed conspecific that was suspended from the floor.		Rice and Gainer (1962)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	↓ door-opening latency to liberate a trapped conspecific from a restrainer. Modulated by familiarity with the conspecific's strain, and impaired by anxiolytic treatments.	c-FOS ACC ➜ NAc	Bartal et al. (2011); Ben-Ami Bartal et al. (2014), (2016), (2021)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	↓ door-opening latency for liberating a soaked conspecific. Enhanced by prior experience.		Sato et al. (2015)
SD rats	♂ ➜ ♂	↓ door-opening latency for releasing a conspecific from a pool of water, independent of social interaction, and modulated by previous experience and conspecific familiarity.		Cox and Reichel (2020)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	↓ door-opening latency for releasing a soaked conspecific from a pool.	OT receptors in ACC	Yamagishi et al. (2020)
SD rats LE rats	♂ ➜ ♂	Door-opening to liberate a trapped conspecific is hastened by the presence of potential helpers and hindered by incompetent bystanders, only when bystanders were from a familiar strain.		Havlik et al. (2020)
SD rats	♂ ➜ ♂	No overall preference for liberating a trapped conspecific over interacting with a free one.		Heslin and Brown (2021)
Wistar rats	♂ ➜ ♂	Lever-pressing to release a trapped conspecific. Prosocial motivation modulated by the distress state of the trapped conspecific and housing conditions.		Kalamari et al. (2021)

(*continued on next page*)

Table 1 (*continued*)

PROSOCIAL BEHAVIOR IN NEGATIVE AFFECTIVE STATES				
Species	Sex	Behavioral Readouts	Neural Substrates	References
Wistar rats	♂ ➜ ♂	High maternal care associated with ↑ door-opening for liberating a soaked conspecific and ↓ latency in adulthood.	BDNF in Amy, Hip, IC, PFC, St	Asadi et al. (2021)
SD rats LE rats	♂ ➜ ♂	Adolescent rats, in contrast to adults, release restrained conspecifics of an unfamiliar strain.	Hip CA2	Breton et al. (2022)
SD rats	♂ ➜ ♂	↓ door-opening latency for releasing a conspecific from a pool of water. Distress USVs from trapped rat associated with attenuated rescue.	AI	Cox et al. (2022)
Prairie voles	All combinations	↓ door-opening latency for liberating a soaked conspecific, more consistent for same- than opposite sex pairs. Reduced rescue behaviour, social proximity and huddling in *Oxtr* KO helper voles.	OT receptor	Kitano et al. (2022)
LE rats	♀ ➜ ♀	Door-opening to liberate a trapped conspecific from a restrainer.	Mirror and anti-mirror neurons in IC and ACC	Wu et al. (2023)
C57BL/6 mice AgRPcre/+	♂ ➜ ♂	Door-opening to free a trapped conspecific from restrainer. Hindered by energy-deficit states (hunger, diabetes).	OT neurons in the PVN. AgRP neurons	Pozo et al. (2023)
		HARM AVERSION		
SD rats	♂ ➜ ♂ ♀ ➜ ♀	Forgo lever pressing associated with rewards to self and electric shocks to conspecific. Modulated by prior shock experience.		Church (1959)
SD rats	♂ ➜ ♂	Change in preferred lever pressing to avoid electric shock to a conspecific. Modulated by prior shock experience.		Greene (1969)
Wistar rats	♂ ➜ ♂	↓ preference for time spent in naturally preferred dark chamber when associated with shocks to conspecific. Modulated by prior experience with shock.	c-Fos and specific oscillations in ACC, OFC and OAMY	Schaich Borg et al. (2017)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	Change in preferred lever pressing to avoid electric shock to a conspecific. Independent of sex and familiarity, modulated by reward benefit and prior shock experience.	ACC (24a, 24b)	Hernandez-Lallement et al. (2020)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	Forgo lever pressing associated with rewards to self and electric shocks to conspecific. Modulated by sex (♀ less prosocial).		Hess et al. (2023)
C57BL/6 mice	♂ ➜ ♂ ♀ ➜ ♀	Switch preferred lever pressing to avoid shock to conspecific. Independent of sex and familiarity, modulated by self-experience with shock, and visual and social contacts (↓ when prevented).	ACC ➜ MDL	Song and Wang et al. (2023)
PROSOCIAL BEHAVIOR IN POSITIVE AFFECTIVE STATES				
		REWARD PROVISION		
SD rats	♂ ➜ ♂	Decision-makers prefer choices that reward oneself and the recipient cagemate. Enhanced by recipients' food-seeking behaviour, goal directed. Non-food deprived.		Márquez et al. (2015)
LE rats	♂ ➜ ♂	Choice preference for mutual rewards in non-cagemates, food-restricted dyads.	BLA	Hernandez-Lallement et al. (2015), (2016b)
Wistar rats	♂ ➜ ♂ ♀ ➜ ♀	Preference for lever-pressing choices that reward oneself and recipient, in food-restricted dyads. Modulated by sex (females not prosocial), housing (complex housed rats not prosocial) and cost.		Kentrop et al. (2020)
SD rats	♂ ➜ ♂ ♀ ➜ ♀	Focals prefer choices that reward oneself and recipient. Non-food deprived. Enhanced by social dominance (dominants ↑ prosocial) which induces social attunement. Independent of familiarity or sex.		Gachomba et al. (2022)
Wistar rats	♂ ➜ ♂	Choice preference for mutual rewards in non-cagemates. Non-food deprived. ↓ by early maternal separation, ↑ by environmental enrichment.	OT	Joushi et al. (2022)
C57BL/6 mice	♂ ➜ ♂ ♀ ➜ ♀	Focals prefer choices that reward oneself and recipient, in food-restricted dyads. Dependent on sex (females ↓), effort, visual, olfactory and contact information (↓ when prevented), familiarity (↑ towards familiars), social hierarchy (dominants ↑) and recipients' hunger state (↑ towards food-deprived).	BLA - PrL	Scheggia et al. (2022)
C57BL/6 mice	♂ ➜ ♂ ♀ ➜ ♀	Preference for choices that reward oneself and recipient, in food-restricted dyads. Dependent on sex (♂ not prosocial).		(Misiołek et al., 2023)

ACC: Anterior Cingulate Cortex, **AI**: Anterior Insula, **Amy**: Amygdala, **AON**: Anterior Olfactory Nucleus, **BDNF**: Brain Derived Neurotrophic Factor, **BLA**: Basolateral Amygdala, **CeA**: Central Amygdala, **dmPFC**: Dorsomedial Prefrontal Cortex, **DR**: Dorsal Raphe Nucleus, **D2**: Dopamine 2 receptor, **Hip**: Hippocampus, **IC**: Insular Cortex, **LE**: Long Evans rats, **LS**: Lateral Septum, **MDL**: Mediodorsal Thalamus, **MeA**: Medial Amygdala, **MPOA**: Medial Preoptic Area, **NAc**: Nucleus Accumbens, **OAMY**: Olfactory Amygdala, **OFC**: Orbitofrontal Cortex, **OT**: Oxytocin, **PFC**: Prefrontal Cortex, **PrL**: Prelimbic Cortex, **PVN**: Paraventricular Nucleus of Hypothalamus, **PVT**: Paraventricular Thalamic Nucleus, **SD**: Sprague-Dawley rats, **St:** Striatum, **Tac1**: Tachykinin, **Vgat**: Vesicular GABA transporter, **5-HT**: Serotonin.

2. Paradigms to study prosociality in rodents

2.1. Consolation paradigm

We tend to comfort familiar others who are experiencing pain, anxiety or fear, through reassuring words that have a calming effect. Depending on the context, culture, and our relationship with those others, we may comfort by means of physical gentle contact and affective gestures, such as patting or hugging. Such contacts communicate sympathetic concern, provide stress buffering and can strengthen social bonds (Jakubiak and Feeney, 2017; Morrison, 2016).

Several non-human animal species engage in affiliative contacts, such as allogrooming in mammals or allopreening in birds, which are likely to be maintained by mechanisms of reciprocity and mutual care (Lim and Hong, 2023; Schino and Aureli, 2010). Beyond improving hygiene, such interactions serve a social function, being crucial for the formation of relationships and for preserving group cohesion in multiple social species (Dunbar, 1991; Radford and Du Plessis, 2006). Affiliative contacts mediates post-conflict reconciliation in non-human primates (Jablonski, 2021; McFarland and Majolo, 2011) and provides social comfort for the recipient, buffering against stress and thus resembling the effects of consolation among humans (Clay and Waal, 2013; Fraser et al., 2008; Lim and Hong, 2023).

In rodents, as well as other animal species (Fraser and Bugnyar, 2010; Palagi and Cordoni, 2009; Plotnik and Waal, 2014) "consolation" is typically measured by quantifying affiliative interactions (e.g., duration, frequency, and latency of allogrooming in the case of rodents), towards distressed conspecifics, relative to non-stressed ones (Fig. 2**A**). Burkett and colleagues were the first to provide experimental evidence of prosocial allogrooming in a rodent species, the prairie voles (*Microtus ochrogaster*) which engage in monogamous mating and biparental care (Burkett et al., 2016). The study aimed at showing that consolation behavior possesses characteristics consistent with an empathy mechanism: state matching, emotional contagion, familiarity bias, and self-other differentiation. Unstressed prairie voles (observers), both males and females, increased allogrooming towards a conspecific demonstrator after a separation during which the demonstrator was fear-conditioned, but not after a control separation without stressor, and the increase in allogrooming was selective towards a familiar partner (either mate, same-sex sibling, or unrelated same-sex cagemate). Stressed demonstrators that were kept alone for a short period of time after the stressor subsequently displayed increased anxiety-related behavior relative to unstressed controls, whereas those that were reunited with the observer for the same period showed normalized responses. Therefore, social contact with a conspecific after the stressor had a buffering effect. Consistent with an empathy mechanism, prairie vole observers and stressed partners showed physiological state matching (correlated levels of plasma corticosterone between the observer and demonstrator), even if the association between state matching and prosocial allogrooming was not specifically assessed. The authors also tested meadow voles (*Microtus pennsylvanicus*), characterized by promiscuous breeding and uniparental care, and reported no increase in prosocial allogrooming in male observers tested with stressed female mates. At the neurobiological level, the anterior cingulate cortex (ACC), but not the prelimbic cortex (PrL) or nucleus accumbens (NAc) shell, showed increased expression of c-FOS, a marker of neuronal activation, in male prairie voles exposed to a stressed mate compared with those exposed to the unstressed partner. In addition, injection of an oxytocin receptor antagonist (OTA) either into the cerebral ventricles or into the ACC of male prairie voles, before the consolation test, abolished the subsequent increase in allogrooming towards the stressed female mate, indicating that oxytocin (OT) signaling in the ACC modulates consolation behavior. This seminal work paved the way for other studies investigating consolation behavior in response to different stressors, and its neural correlates.

Evidence for prosocial allogrooming has been reported for other rodent species. Monogamous mandarin voles (*Microtus mandarinus*), both males and females, showed higher frequency of, and more time spent on, allogrooming a mate that experienced stress via social defeat compared to mates that only experienced separation (Li et al., 2019). Administration of either OTA, $GABA_A$ receptor antagonist, serotonin 5-HT1AR antagonist, or dopamine D2R antagonist, but not vasopressin V1a receptor antagonist, into the ACC of male observers significantly reduced the consolation response (Li et al., 2019, 2020). In addition, dorsal raphe (DR) serotonergic neurons projecting to the ACC (DR-ACC 5HT neurons) were found to play a crucial role for consolation and sociability in both males and females mandarin voles. Activity of DR-ACC 5HT neurons and endogenous release of 5HT in the ACC increased during allogrooming bouts, social approaching, and sniffing directed towards the distressed partner, and optogenetic inhibition of DR-ACC 5HT neurons or their terminals in the ACC decreased consolation behavior (L.-F. Li et al., 2021). Since the same inhibitory manipulations also decreased sociability in a three-chamber test, the reduced allogrooming towards stressed conspecifics may be due to an overall reduction in observers' sociability, as the authors pointed out. In contrast, activation of the DR-ACC 5-HT neurons did not elicit corresponding increases in allogrooming and sociability; thus, the effects of activation of this circuit on prosocial behavior may require further investigation.

Other studies reported consolation behavior in laboratory rats. Rats' allogrooming towards a same-sex conspecific experiencing physical pain, aggressive encounters or stress induced by fear conditioning, was increased compared to that of rats interacting with an unstressed conspecific (Li et al., 2018; Lu et al., 2018; Kiyokawa et al., 2019; Du et al., 2020; Heinla et al., 2020). No sex difference was found when comparing male and female cagemate dyads (Du et al., 2020), although a study reported greater sensitivity for vicarious aggression in females (Heinla et al., 2020). Differently from the familiarity selective response observed for prairie voles (Burkett et al., 2016), rats' prosocial allogrooming extends towards distressed unfamiliar partners (Lu et al., 2018; Kiyokawa et al., 2019; Luo et al., 2020), but at lower levels than that directed towards familiar ones (Lu et al., 2018; Luo et al., 2020), adding more evidence for familiarity as a factor promoting consolation behavior. Moreover, similar past experience with pain by observer rats and the display of visually-identifiable pain expressions by demonstrators are factors that enhance social transfer of pain and the consolation response (Li et al., 2018; Luo et al., 2020). Furthermore, the paraventricular nucleus of the hypothalamus (PVN) and central amygdala (CeA), showed increased c-FOS expression in male rats that interacted freely with a fear-conditioned than a non-conditioned partner, suggesting that social cues from the fear-conditioned rat activated these brain regions in the observers Kiyokawa et al., 2019).

Similar to rats, mice express consolation behavior towards both familiar and unfamiliar, same-sex conspecifics (Zeng et al., 2021; Matsumoto et al., 2021; Wu et al., 2021; Carneiro de Oliveira et al., 2022; Phillips et al., 2023; Lee et al., 2021; Zhang et al., 2024), with no substantial difference between male and female dyads (Wu et al., 2021; Phillips et al., 2023), although a study reported increased prosocial allogrooming duration and frequency in males compared to female mice (Du et al., 2020). Free social interactions with an unstressed cagemate reduce subsequent anxiety-like behavior in stressed mice (Zeng et al., 2021; Wu et al., 2021; Phillips et al., 2023), while limited interactions through a transparent perforated barrier prevent stress relief (Wu et al., 2021). This indicates that free physical interactions between the animals provide stress buffering benefits that go beyond mere social proximity.

Recent research is providing insights into the brain areas and neural circuits mediating consolation behavior in mice. Here, the brain regions involved are diverse, maybe reflecting some differences in the neural pathways recruited depending on the type of stressor that the demonstrator mice have been subjected to. (Zeng et al., 2021) identified several brain areas activated when mice would interact with a conspecific that underwent surgery, and functionally demonstrated that neurons in the paraventricular thalamic nucleus (PVT) containing orexin receptors

have a role (Zeng et al., 2021). Phillips and colleagues linked consolation behavior to changes in activity of PFC subregions, specifically cingulate area 1 (Cg1) and prelimbic cortex (PL), reinforcing the idea that prefrontal cortex, especially the cingulate, has a role in prosocial behaviors (Phillips et al., 2023). Recent works have also elegantly reinforced the importance of the ACC in consolation paradigms where allogrooming was selectively targeted to a conspecific in pain suffering from bee venom injection (Zhang et al., 2024). Although the relevance of these cortical areas seems to gain momentum, important contributions have pointed to the critical role of non-cortical structures too, such as the medial amygdala (MeA) and its projections to the hypothalamus (Wu et al., 2021). Specifically, tachykinin (Tac1) positive neurons in the MeA projecting to the medial preoptic area (MPOA) were functionally demonstrated to guide allogrooming in mice in the context of consoling conspecifics after emotional stressors (Wu et al., 2021).

2.1.1. Summary

The findings discussed in the section above show how different species of rodents have evolved behavioral strategies (e.g. allogrooming, allolicking) to address specific states and needs of others. Specifically, when interacting with conspecifics in negative emotional states (e.g. distress, pain or fear), rodents exhibit a form of prosocial behavior, consolation, in a context-appropriate manner. This behavior co-occurs with emotional contagion and has a stress buffering effect on the recipient. Affiliation of the pair tends to promote allogrooming towards distressed conspecifics in all tested species, although for voles it seems essential. Sex differences are not found in most of the studies, highlighting the relevance of consolation behaviors as a form of social bonding irrespective of sex. Dominance structures naturally shape the dynamics of the social interactions among individuals; however, we found that none of the works reviewed assess how social hierarchy influences the exhibition of this type of behavior. Finally, some of the reviewed work is pinpointing the neural correlates underlying consolation behavior in rodents. Prosocial allogrooming depends on the activity of neurons in the Medial Amygdala, Paraventricular Thalamic Nucleus and Prefrontal Cortex (ACC and PrL) and involves the signaling of multiple neuropeptides and neurotransmitters (OT, orexin, 5HT and DA). We will benefit from understanding how these brain regions and neuromodulatory molecules act in concert to regulate the expression of consolation behavior.

2.2. Rescue paradigm

Instrumental paradigms have been developed to assess if rodents rescue trapped conspecifics (Fig. 2**B**). Early work by Rice and Gainer showed that rats would press a lever to lower a distressed partner that was suspended from the floor, which was interpreted as altruistic behavior leading to relief of the distress (Rice and Gainer, 1962). This study met with some criticism, as a later work doubted that these actions were goal directed (Lavery and Foley, 1963). More recently, (Bartal et al., 2011) developed a door-opening paradigm where free rats are tested for their tendency to liberate a conspecific trapped in a restrainer. Over days of testing, the proportion of rats that opened the door increased and the latency to door opening decreased only when the free rats were tested with a trapped cagemate, but not in control conditions where the tube was empty or contained a toy. Rats opened the door even when the partner was released in a separate adjacent compartment, suggesting that expectation of full social contact is not required for eliciting rescue. When rats could free the partner or open another tube with chocolate, they opened both tubes and ate the chocolate together in half of the trials, suggesting that rats attributed value to releasing the trapped conspecific and tolerated the presence of the trapped animal while consuming high rewards. All female rats became door-openers in contrast to two thirds of male rats, suggesting that females are more likely to engage in rescue behavior; however, there is to consider that the size of the male sample in the study was four times larger. For the authors, rats freed their cagemates in order to end either their own distress or that of the trapped animal, thus their prosocial behavior possibly being empathy-motivated. Indeed, corticosterone levels of the helper animal correlated negatively with the propensity to liberate the trapped animal, and pharmacologically manipulating the arousal/anxiety levels of the animals had an impact on the levels of prosociality (Ben-Ami Bartal et al., 2016). On this line, male mice showing consistent helping were characterized by a lower corticosterone increase compared to non-helpers, and their corticosterone response was positively correlated with that of the trapped cagemate (Pozo et al., 2023). This suggests that rescue behavior may entail some degree of physiological state matching between helper and trapped animals, and that a high stress response hinders helping.

(Sato et al., 2015) developed a task using a pool of water where a distressed rat was trying to escape from, and showed that unconfined rats, both males and females, learned to open a door over testing days, allowing the soaked cagemate to escape. Door-opening occurred mainly towards soaked rats and not towards those that were in a dry area, suggesting that rescue behavior depended on the partner's distress. Additionally, door-opening emerged more rapidly and with shorter latencies when the roles of the rats were switched, indicating that observational learning or prior experience with the stressor enhanced prosociality in this task. Prairie voles were also found to rescue littermates when tested in this task, regardless of the sex composition of dyad, and showed more prompt and stable door-opening when the partner was soaked (Kitano et al., 2022).

Following the works of (Bartal et al., 2011), and (Sato et al., 2015), other studies adapted the original protocols or implemented changes aimed at investigating the motivations driving the opening response as well as the contextual, neurobiological and physiological factors modulating it. An important modulator is the familiarity of the free rat with the strain of the trapped partner. Male rats were found to release both familiar and unfamiliar conspecifics of the same strain as well as conspecifics from a different strain they were cohoused with, but not conspecifics of a strain they never met, including their own genetic strain (Ben-Ami Bartal et al., 2014, 2021). The findings indicate an ingroup bias, which parallels effects observed for human empathy and helping. The ingroup bias for rescue has been proposed to emerge during development, since male adolescent rats, in contrast to adults, did release trapped conspecifics of an unfamiliar strain (Breton et al., 2022). Further studies will be useful to assess if a brief exposure to outgroup members during adolescence would reduce the ingroup bias later in life. Whether the unselective rescue displayed by adolescent rats is primarily driven by differences in affective arousal when facing the distressed partner or by an increased interest for novel social stimuli compared to adults, as suggested in the (Breton et al., 2022) study, remains to be further investigated. (Havlik et al., 2020) reported that strain familiarity modulates the effect of bystander(s) on opening performance, by comparing male helper rats performing alone to helper rats performing in the presence of one or two other rats that were unable to help because slightly sedated with midazolam (passive bystanders). Compared to the alone condition, helper rats in the presence of passive bystanders opened the restrainer less often, but only when they were familiar with the bystander's strain. In contrast, rats were more likely to engage in door-opening when in the presence of one or two non-sedated rats that also engaged in the task (active bystanders), suggesting that releasing performance may be enhanced through social learning. This suggests that the performance of helper rats is influenced by the presence of conspecifics and their own capacity to perform.

To assess rats' motivation to engage in helping, (Kalamari et al., 2021) designed an operant version of the task where required lever pressing to open the restrainer was progressively increased. The authors studied how early life experiences, including short maternal deprivation during the postnatal period, and living in enriched environments from juveniles, affected male rats' helping behavior in adulthood. Compared to rats housed in pairs and standard cages, rats housed in bigger cages,

with physical and social enrichment, were less motivated to press a lever for releasing the restrained cagemate as well for gaining access to a free cagemate. Early-life stress (ELS) induced by a full day of maternal separation during the early postnatal period did not affect motivation to behave prosocially when adults. However, a different study reported that adult male rats that received increased maternal care when pups (measured as frequency of grooming, licking, and nursing by the dam) were more likely to rescue a cagemate from water, and did it at shorter latency, compared to rats that received less maternal care, although this effect was restricted to a late stage of the task (Asadi et al., 2021). This parallels the observation that repeated periods of maternal separation during infancy reduced rats' prosociality for food reward later in adolescence (cf. Section 2.4). Given the impact of ELS on different aspects of cognition and emotions later in life, research addressing the long-term effects of different kinds and degrees of ELS on helping behavior certainly deserves future attention.

Other physiological conditions associated with negative energy status, including hunger state and diabetes, have been found to prevent rescue behavior in male mice. (Pozo et al., 2023) showed that mice with streptozotocin-induced diabetes, characterized by hyperglycemia, did not show any opening responses. In parallel, food-restricted mice did not release the partner on any day but did start releasing it once fed *ad libitum*, or when they had the simultaneous option to open a tube with palatable food. Notably, inducing a hunger-like state via chemogenetic activation of hypothalamic neurons expressing the agouti-related protein (AgRP) had a similar effect, as mice started releasing the partner only when AgRP neurons were no longer activated. In contrast, door-opening latencies of fed mice that had previously released the partner were not substantially affected by either food-deprivation or activation of AgRP neurons, indicating that these manipulations affected the learning phase rather than the maintenance of the behavior. These findings thus point to the role of the actor's internal state in learning prosocial behaviors. Energetic needs, under the influence of AgRP neuronal activity, compete with prosocial motivations, in accordance with observations that AgRP neurons influence other motivated behaviors.

Regarding how the brain engages in prosocial behavior during the rescue paradigm, the Bartal lab has been pioneer performing whole brain c-Fos analysis after this task and identifying network central hubs which were modulated by the familiarity levels of the trapped rat (Ben-Ami Bartal et al., 2021). Interestingly, a shared network of frontal and insular cortices was active during the task, regardless of strain familiarity of the trapped rat; however, the NAc was selectively active in helper rats facing the familiar strain (where higher helping behavior was found). Further analyses, combining c-Fos labeling with retrograde tracing to identify active projections from the frontal cortex to the NAc, revealed that c-Fos+ ACC cells projecting to the NAc correlated with the percent of door-opening towards ingroup members. Future studies implementing loss and gain of function manipulations targeting NAc-projecting ACC neurons will help to elucidate the role of this circuit in rescue behavior, as this projection has been implicated in the social transfer of pain and analgesia in mice (Smith et al., 2021). Furthermore, Bartal laboratory found that male adolescent and adult rats showed different patterns of neural activity while freeing restrained ingroup or outgroup members, which may underlie the differences in rescue selectivity between the two age groups (Breton et al., 2022).

As regards to OT, evidence indicates that an intact OT system is important for prosocial behavior in the rescue paradigm with a soaked conspecific. Male and female rats receiving bilateral injection of an OTA into the ACC showed higher door-opening latency compared to controls when rescuing the soaked partner, suggesting that OT signaling in the ACC sustains prosocial learning (Yamagishi et al., 2020). On this line, prairie voles that were homozygous for the knocked out oxytocin receptor gene (*Oxtr*–/–) showed reduced rescue behavior as well as social interest (e.g., decreased social proximity and huddling) towards the soaked cagemate, compared to those that were wild-type (*Oxtr*+/+) (Kitano et al., 2022). Whether the effects of OTA into the ACC and Oxtr KO on rescue are specific for a partner in distress is yet not clear, since data from the no-distress condition are lacking.

Adding to the central role of OT in the ACC, the anterior insula (AI) has also been related to the propensity to help soaked partners. Pharmacological or chemogenetic inhibition of the AI on days 9–10 of the helping task increased door-opening latencies compared to days 7–8, when door-opening behavior was learnt (Cox et al., 2022). Furthermore, chemogenetic inhibition of the AI did not affect rats' preference for an unfamiliar animal over a novel object, when helper rats were further tested in a social reward place conditioning task, suggesting that AI activity may contribute to rescue behavior through mechanisms other than social interest. The authors suggest that inhibition of helper rats' AI activity likely reduces the emotional salience or valence of the distress of the trapped animals, increasing the latency to release them from the water. Indeed, previous reports have described AI to be important for mediating approach and avoidance responses to distressed conspecific rats (Rogers-Carter et al., 2018).

Finally, dynamic recordings of brain activity during the rescue paradigm are still scarce, but (Wu et al., 2023) described that ACC and insular cortex (IC) neuronal ensembles of helper rats increased activity around the time of door-opening when the restrainer contained a conspecific, but not when it was empty or contained a toy. These findings further suggest that these brain areas may encode aspects of the releasing response. Yet, it remains to be determined how this activity is specific for rescuing a distressed partner compared to gaining access to a non-distressed one.

The literature has generated substantial discussion on whether door-opening is primarily driven by empathic processes (Vasconcelos et al., 2012), based on findings that other factors, such as seeking social contacts or interest in features of the apparatus (Ueno et al., 2019) may function as motivators and thus offer an alternative explanation. This has typically been evaluated by assessing order-effects and reinforcing aspects of the behavior. When having the opportunity to liberate a restrained conspecific or interacting with a free partner, some studies describe that rats showed no overall preference for rescue (Heslin and Brown, 2021), and were less motivated to engage in door-opening under a progressive ratio operant schedule, when the behavior did not result in social interaction compared with a condition that did result in interaction (Cox and Reichel, 2020). Seeking social contact is thus an important factor, with rewarding properties that can impact on social decisions in some contexts, and can facilitate helping in the rescue paradigm, but there is now robust evidence that is not necessary for prosocial behavior to occur.

2.2.1. Summary

The rescue paradigm, based on a tube or a water pool, has offered a novel, elegant and relatively simple instrumental learning paradigm to study prosocial behavior in rodents, being the most prolific tool according to the literature. It has been found that individual familiarity and sex do not seem to affect the exhibition of rescue behavior in rodents. Moreover, releasing performance is biased towards ingroup conspecifics in adult but not in adolescent rats. Data about social dominance asymmetries is lacking in the literature and might result necessary to complement our knowledge on the topic. A moderate level of stress may facilitate prosocial learning, while higher stress levels and deficiency in energy status hinders helping. Furthermore, a few studies point to the modulatory effects of early life conditions on helping later in life. Exposure to the trapped rats likely recruits neuronal activity in the ACC and AI, consistent with the role of these brain regions in processing self and vicarious experience of fear and pain (Carrillo et al., 2019; Gu et al., 2012), and activity of OT, since disrupting OT signaling impacts the latency to door-opening.

2.3. Harm aversion

To date, very few studies have assessed the tendency of laboratory rodents to avoid actions that harm a conspecific. The firsts are the classic studies by (Church, 1959) and (Greene, 1969), where rats could instrumentally induce or relieve distress in a conspecific (Fig. 2**C**), and that were echoed by studies in monkeys (Masserman et al., 1964). In Greene's study, actor rats were first trained in an operant box where they could obtain food by pressing either one of the two levers, with both levers delivering equal reward but one requiring twice the force as the other, so that most of the rats developed a stable preference for the easier lever. During testing, a second rat ("victim"), placed in an adjacent compartment, received foot shock whenever the actor rat pressed the preferred lever to feed itself. In this social condition, actors were considered to be prosocial or, in the own words of the author, to show "operationally defined altruism", if they changed their preference for lever pressing. To examine the role of prior experience with the stressor, two groups of actor rats were tested that were either naïve or had experienced foot shocks before training. According to the study, only in this second group the majority of rats changed their preference for the lever delivering food when the initially preferred lever delivered concurrent foot shock to the partner. This change in preference occurred even if, for most of the rats, pressing the nonpreferred lever required twice the effort. Thus, Greene's early work suggests that prior experience with the victim's stressor may increase rats' sensitivity to other's pain and, as a consequence, promote harm aversion. Indeed, in a more recent adaptation of these tasks, (Schaich Borg et al., 2017) reported that animals would avoid exploring spaces that would induced foot-shocks to conspecifics, avoidance that was enhanced by prior shock experience, and found that c-Fos activation in the ACC, OFC and Olfactory Amygdala and oscillations between and within these brain regions, correlated with individual differences in harm avoidance.

(Hernandez-Lallement et al., 2020) adopted, refined and expanded Greene's paradigm and results, investigating individual differences as well as the effects of sex, familiarity and reward cost, and demonstrating the necessity of the ACC in this type of prosociality. In line with the findings in (Greene, 1969), emergence of harm aversion at the group level was found to be dependent on prior experience with foot shock. In addition, actor rats pre-exposed to the stressor exhibited marked individual variability in harm aversion, with less than half of the animals showing switching (a significant reduction for the initially preferred lever, then paired with foot shock to the partner) whereas the rest of the animals showed preference changes within chance level. Some animals stopped pressing levers, thereby also preventing shocks to the partner. Male and female rats displayed comparable levels of harm aversion towards a same-sex conspecific, and no effects of familiarity with the victim was found in males. Moreover, male rats significantly reduced their usage of the shock lever if it delivered twice, but not thrice, the number of sucrose pellets of the non-shock lever, suggesting that harm aversion is subject to cost-benefit evaluation. Furthermore, for prosociality to appear in this paradigm, animals should not be habitual in the individual pressing lever task, as overtraining in the individual part of the task would interfere with goal-directed switching when social contingencies change - i.e rats that were trained longer to keep a strong and stable preference over days did not switch their preference to the non-shock lever. Inactivation of the ACC (area 24a and 24b), via bilateral injections of muscimol, reduced harm aversion in male rats, an effect possibly mediated by cingulate deactivation also reducing the rat's own distress when witnessing another receive a shock (Carrillo et al., 2019). In this work, the authors refrain from interpreting rats' behavior as truly altruistic in the sense of acting with the intention to benefit another and suggest that an account based on selfish motivations could offer a sufficient explanation. When delivering shocks to the partner, some rats experience distress or fear, via emotional contagion, accentuated by association with their prior exposure to the shock. Those rats would then avoid this negative state by switching to the non-shock lever. This account would be supported by the data showing that animals that switched more behaved more alertly to the shocks of the victim, delaying their entrance to the food magazine, shortening food consumption, and taking longer to perform trials. Thus, according to the author's view, rats showing harm aversion in this paradigm could be primarily motivated by the goal of reducing their own distress or fear.

Harm aversion has also been shown in mice (Song et al., 2023) and, as described for rats, is independent of sex and affiliation, but dependent on previous experience with foot shocks. (Song et al., 2023) further showed a crucial role of the ACC, and its connection to the Mediodorsal Thalamus (MDL), employing chemogenetic and optogenetic manipulations. These observations expand a previous study that found this projection to be important in modulating vicarious freezing behavior in rats (Zheng et al., 2020).

Recently, (Hess et al., 2023) developed a modified version of the task where, on each trial, actor rats could press a single lever that delivered a sucrose reward to them and a foot shock to a partner rat. By omitting lever pressing on a trial, actor rats could prevent harm to the partner at the cost of losing the reward. In agreement with previous studies, lever response decreased at the group level from baseline (no shock delivery) to test sessions, indicating harm aversion. However, this study did report sex differences, with male rats showing higher and more consistent harm aversion than females across seven days of testing. Notably, the intensity of the shock stimuli used in Hernandez-Lallement et al., (2020) likely induced stronger behavioral and emotional reaction in the victim since it was higher than that used in Hess et al., 2023, (1.5 and 0.8 mA, respectively). It is possible that shock intensity impacts female and male dyads differently, by modulating distress signals emitted by the victim as well as the aversive state triggered in the actor by those signals. It will be important to continue assessing behavioral and distress responses of both actor and victim rats during the task, including freezing and vocal emissions (i.e., squeaks, ultrasonic vocalizations). This assessment can be integrated with dyadic analysis methods that measure bidirectional transfer of information, as it has been performed to quantify mutual influences in freezing behavior (Han et al., 2019, 2020) or multimodal interactions (Gachomba et al., 2022). Such an approach would allow for a better understanding of the association between emotional contagion and harm aversion.

2.3.1. Summary

From the reviewed studies that assess prosocial behavior in response to negative emotions, the harm aversion paradigm is the least explored. Nonetheless, results show that rats and mice tend to avoid actions that produce distress on conspecifics, with marked individual differences. Data regarding whether other species also choose to avoid actions that hurt conspecifics is lacking. Harm avoidance, as for other types of decision-making, is subject to cost-benefit evaluation, and is not influenced by individual familiarity. Sex differences may emerge depending on task design and behavioral metrics, and could be explored in further studies. Regarding the neural correlates associated with harm avoidance, ACC activity and its connection to downstream areas has been proved as necessary for this behavior, consolidating the role of ACC as a hub implicated in very different types of prosociality. Interestingly, ACC has been demonstrated to have a role in the processing of emotional responses to vicarious fear in rodents (Carrillo et al., 2019; Zheng et al., 2020). The role of other brain areas deserves further study, as for example, witnessing a conspecific receiving shock also modulates DA release in the nucleus accumbens in rats (Lichtenberg et al., 2018), which points to a possible role of DA in harm aversion.

2.4. Reward provision

Prosocial behaviors in the context of positive affective states of others is the least studied face of prosociality in rodents. Although the field has tremendously advanced in the last years, as we have reviewed in the previous sections, it suffers from a strong bias towards the study of

negatively valenced emotions. Helping others in distress (pain, fear, stress) is very relevant; however, adapting social decisions based on positively-valenced information from others is equally important, but has been much less studied. Several rodent species display affiliative behaviors in food-related contexts. For instance, food sharing among rats occurs naturally since the presence of shared feeding sites in the colony, where they allow conspecifics to eat in close proximity and even tolerate food stealing (Barnett, 1963; Galef et al., 2001). Consistently, wild and laboratory rats have been found to tolerate the presence of others in food locations, even if they could eat the food alone (Bartal et al., 2011; Colin and Desor, 1986; Grasmuck and Desor, 2002; Krafft et al., 2010). Thus, laboratory rodents could represent a valuable model to map reward-based prosocial choices to the mammalian brain.

An established paradigm for reward-based prosociality is the *prosocial choice task* (PCT) (Silk et al., 2005), which measures other-regarding preference for reward distribution. It was initially implemented for non-human primates to investigate the phylogeny of human prosociality and successively extended to other taxa (for review see (Cronin, 2012; Jensen, 2016; Marshall-Pescini et al., 2016)). In this task (Fig. 2**D**), subjects are typically tested in pairs and often placed in adjacent compartments. The focal (decision-maker) can choose between two options presented in each trial, determining the reward payoff for itself and a recipient partner. Choosing the *prosocial* (or mutual rewards) option makes each animal gain a single reward, while choosing the *selfish* option provides a single reward for the focal only, and none to the recipient. Thus, the choice does not imply a cost or additional benefit for the actor in terms of reward number. To control for preference biases induced by reinforcing effects of food delivery, the proportion of trials on which animals make a prosocial choice when the recipient is present (test condition) is generally compared to that shown in a control where the recipient is absent, or with a present recipient that is unable to access the food. If animals choose the prosocial option significantly more often in the test than in the control conditions, they are said to have a *prosocial preference*, which is taken as demonstration of their sensitivity to others' welfare. Variations of this task used across animal species have included a token version where subjects can choose between tokens that are exchanged with food items (Horner et al., 2011), designs using low and high-quality food (Lakshminarayanan and Santos, 2008), and designs where the focal can choose between an action in which no one benefits versus one that gives reward to the recipient only (null versus altruistic choice) (Burkart et al., 2007). Subjects' roles remain fixed or can be reversed over sessions (the focal becomes the recipient and vice versa) to assess the emergence of reciprocity (Lalot, Delfour, et al., 2021).

(Hernandez-Lallement et al., 2015) and (Márquez et al., 2015) were the first to adapt the PCT for laboratory rodents, showing evidence that rats display prosocial preferences in food-foraging contexts. These studies adapted this two-alternative forced choice task into different behavioral setups for rats, (Hernandez-Lallement et al., 2015) using a double box and (Márquez et al., 2015) in the form of an automated double T-maze. For both paradigms, in each trial over multiple daily sessions, the focal rat could choose between the prosocial and selfish choice by entering either one of the two maze's arms, where food was delivered. (Hernandez-Lallement et al., 2015) tested non-cagemate male rats, food-deprived, that developed an overall prosocial choice bias of 55 %, significantly higher compared to chance, and to a control where the recipient rat was replaced by a toy. Focal rats always entered the chosen rewarded arm before the recipient, reporting in this manner their choices, and having information of the recipient preferences and reward outcomes once the decision was made. (Márquez et al., 2015) tested male cagemates, non-food deprived, and reported an average prosocial choice bias of around 70 %. In this task, instrumental helping, understood as prosocial actions performed to help others achieving a tangible resource (Warneken et al., 2007; Yamamoto et al., 2009) could be assessed, as recipients could display their attempts to access the rewarded arm before the choice of the decision maker, by repeatedly poking into a nose-port. Indeed, this work demonstrated that these displays of food seeking behavior by the recipients were necessary for the appearance of prosocial biases, but not sufficient, as information of the reward contingencies of the recipient were also important for prosocial biases to emerge (i.e. choices of the focal animal flexibly adapted to changes in the provision of rewards to recipients). Furthermore, this work demonstrated that prosocial actions were goal-directed, being flexible upon changes of the reward contingencies for the recipient, and that local enhancement was not driving the emergence of prosocial choices. These findings indicate that prosocial choice for food provision in rats is enhanced by recipients' attempts to reach the reward, which may thus function as a social cue.

There are several differences in these two original paradigms that could explain the differences in the overall prosocial preferences, such as the different layouts of the setup itself, the strain of rats used, the use of familiar vs unfamiliar partners, using food deprived or non-food deprived animals, or the use of previous individual training or not before the social task. A highly likely explanation in this regard is the opportunity, or lack thereof, that the decision-maker animal has to gain social information of the recipient's preferences before and after the choice, which is in accordance with what it was shown before in chimpanzees (Warneken et al., 2007; Yamamoto et al., 2009).

In this direction, and further reinforcing the relevance of the actions of the recipient of help, Márquez's laboratory has recently studied how social dynamics prior to choice explain the individual differences seen in prosocial preferences. (Gachomba et al., 2022) investigated the role of dominance relationship, sex of the pair, and familiarity of the recipient in the propensity to help others. Female dyads developed similar levels of prosocial choices compared to male dyads, and familiarity did not affect prosocial preference in males. Whether strain familiarity or affiliation, an important modulator in the rescue paradigm (Ben-Ami Bartal et al., 2014, 2021; Breton et al., 2022), affects prosocial choices for rewards is still unknown. To examine the effects of dominance in male rats, prosocial choices where compared between two groups of animals where the focal rat was either the dominant or the subordinate of the pair (and therefore its recipient was the subordinate or dominant, respectively), after social hierarchies were identified on the base of competition for food reward (Costa et al., 2021). Prosocial choices emerged faster and reached higher levels when the decision-maker was the dominant in the pair, with prosociality being positively correlated with dominance asymmetry. Prosocial choice directed "down the hierarchy" (i.e., more often from dominants to subordinates) was accompanied by dyads in the two groups exhibiting different patterns of social interactions before the choice. Despite dominant and subordinate recipients displaying similar food-seeking behavior, the latter stayed closer to, and were more oriented towards, their dominant focal, especially before selfish choices, suggesting increased social attention. Consistently, rats from dyads with dominant decision-maker and subordinate recipient influenced more each other's movement relative to the decision ports, indicating increased attunement or coordinated behavior. Moreover, dominant focals' prosocial choice was found to positively correlate with the ultrasonic vocalization rate of subordinate recipients, while no such relationship was observed for the other group. These data associate the increased prosocial preference in dominant male rats with the dynamics of social interactions prior to choice. Further research is needed to determine whether dominance status similarly modulates prosocial choices in female rat dyads, for which identification of stable social hierarchies has been elusive.

(Kentrop et al., 2020) developed an operant version of the PCT comprising one chamber divided into two compartments, one for the focal and one for the recipient rat, where the decision-maker was asked to report its choice by performing an instrumental action under different effort ratios. The focal could choose to either press a lever delivering mutual reward, a lever delivering reward to itself only, or an inactive lever. The location of reward delivery was the same for the prosocial or selfish choice, with feeder dispensers positioned at the center of the divider between the two compartments. The authors assessed the effects

of early-life environmental enrichment on male rats' prosociality, by comparing adult rats that were pair-housed in standard cages to adult rats that were housed in more complex cages from juvenility, providing physical and social enrichment. Standard-housed males interacting with a same-sex familiar recipient were found to have on average 60 % prosocial preference (significant against chance), under both a Fix ratio 3 (F3) and F5 lever press schedule. In contrast, complex-housed males did not show an overall prosocial bias. Interestingly, when the cost for prosociality was higher (by increasing the time for rewarding focal animals after the prosocial choice with respect to the selfish choice) no prosocial bias was observed, suggesting that rats do not necessarily show altruistic behavior in this task. In contrast to standard-housed males, no overall prosocial preference was observed in pairs of female rats (standard-housed), irrespective of the phase of the estrous cycle. However, these results should be interpreted with caution as training and testing schedules were different between males and females.

How early life rearing can program prosocial choices in food-foraging contexts later in life has also been addressed in the context of stress. Male rats that experienced repeated periods of maternal separation during infancy showed reduced prosocial choice bias during juvenility compared to control animals (Joushi et al., 2022). Interestingly, this reduction in prosociality was prevented when rats experiencing maternal separation were either exposed to environmental enrichment or received intranasal OT administration for a short period after weaning. Given that both focals and recipients in the same group underwent maternal separation, it remains to be established whether maternal separation leads to decreased prosociality by affecting the social behavior of either the decision-maker or the recipient. Nevertheless, these findings highlight environmental enrichment and OT administration as potential interventions for preventing prosocial behavior impairments associated with conditions of early-life adversity. Future research would benefit from investigating whether these beneficial effects would extend to other types of distress that would negatively affect prosocial choices.

Recent works have started to evaluate prosocial tendencies for reward provision in laboratory mice.

(Scheggia et al., 2022) expanded a standard operant cage hosting the focal mouse, with an adjacent compartment hosting the recipient. Naïve decision-maker mice learned to nosepoke on two ports, one delivering reward to themselves only, the other delivering mutual reward. Over testing days, focal mice paired with a recipient developed a bias for the prosocial option at the group level, whereas those trained without the recipient showed no overall preference. The former also performed a higher amount of responses, suggesting that the presence of a conspecific increased learning performance or motivation to act. Focal mice classified as prosocial spent more time close to the divider separating the two animals, suggesting increased interest in the recipient. The authors trained animals under different conditions to identify factors modulating the preference. These included sex (only males developed an overall prosocial preference towards same-sex conspecifics whereas females did not, with half of the them preferring the prosocial choice and half preferring the selfish choice); effort (males previously classified as prosocial maintained a prosocial bias when the effort for the prosocial option increased, while prosocial females switched faster to the easier selfish option); social contacts (impeding tactile contacts between mice prevented the emergence of the prosocial bias); familiarity (actors paired with non-cagemates acted more selfishly than actors paired with cagemates); recipient's hunger state (actors trained with food-restricted recipients had a higher prosocial preference compared to actors trained with sated recipients); and dominance (actors made more prosocial choices when they were dominant compared to their recipient, as assessed in the tube test, as also seen in rats (Gachomba et al., 2022)). Furthermore, this work linked prosocial biases with individual differences in social transfer of fearful emotional states. Interestingly, freezing duration of actor mice was positively correlated with their dominance rank, and it was higher in those categorized as prosocial than selfish. This suggests that prosocial and dominant mice show increased sensitivity to the negative affect state of a conspecific, which may facilitate prosociality. (Misiołek et al., 2023) also investigated food-based prosociality in adult mice, using a model partly based on the prosocial choice task for rats developed by (Hernandez-Lallement et al., 2015), where focal mice could choose to enter either one of two compartments associated with the different reward outcomes. Focals first underwent a pretest phase to determine that, on average, they had no preference for either compartment in the absence of the recipient. In contrast to (Scheggia et al., 2022), during testing with a same-sex partner, females, but not males, increased their prosocial choice preference relative to pretest. Further experiments showed that female and male mice showed comparable rewarding effects of social interactions in a social conditioned place preference test as well as similar affect state discrimination when interacting with a "neutral" vs food-deprived demonstrator, suggesting that these factors were not responsible for the sex differences observed in choice behavior.

Little is still known about the neural bases of reward-based prosociality in rodents. Using the task they previously developed, (Hernandez-Lallement et al., 2016b) investigated the effects of bilateral lesions to the basolateral amygdala (BLA) in adult male rats. Compared to control animals (sham operated), BLA-lesioned actor rats showed similar levels of prosocial choices in the nonsocial condition (toy as recipient) but made less prosocial choices in the social condition (recipient present) compared to controls (53 %). Consistently with the involvement of BLA in rat prosociality, (Scheggia et al., 2022) showed that BLA neuronal activity of male mice increased at the onset of choice responses, with prosocial mice having higher BLA activity than selfish mice after prosocial choices. Chemogenetic inhibition of BLA glutamatergic neurons before daily test sessions prevented mice from developing a prosocial choice bias, and inhibiting BLA activity only during task learning had long-lasting effects, by reducing prosocial choices in the following testing days. Interestingly, BLA silencing also reduced social exploration, freezing during observational fear conditioning, and dominance rank in the tube test. It could be then hypothesized that BLA silencing, by modulating emotional contagion and dominance relationship, would affect prosocial choice in male mice. To provide insights into the role of cortico-amygdala projections, the authors targeted the prelimbic cortex (PrL). Chemogenetic inhibition of reciprocal BLA-PrL connections had different effects on choice preference. Inhibiting BLA→PrL projections slowed down the emergence of prosocial choices, whereas inhibiting PrL→BLA projections induced an overall shift towards the selfish choice. These findings reinforce the role of the amygdala in regulating distinct aspects of social behavior, including social decision-making, social transfer of fear, and dominance status and highlight cortico-amygdala connections as an important neural substrate coordinating prosocial and selfish decisions.

Interestingly, human fMRI studies showed that the sub region of ACC in the gyrus (ACCg) codes prediction error signals specifically when subjects learn to benefit others (Lockwood et al., 2016), and single-neuron recordings in rhesus macaques, *Macaca mulatta*, revealed that a high proportion of neurons in the ACCg exclusively responded to reward delivered to a conspecific (Chang et al., 2013). These findings highlight the role of the ACC in prosocial learning; however if the ACC is required for developing prosocial choices in a PCT in rodents is yet to be assessed.

2.4.1. Summary

The prosocial choice task offers the possibility to study prosociality in rodents in a reward-based context. Variations of this instrumental learning paradigm have been useful to demonstrate that rodents often choose those choices that come with benefits for other conspecifics. Prosocial choice preference shows substantial variability, both intra and interspecies, it is modulated by different factors, and although results in reward-based prosociality are still scarce, some general principles are starting to be drawn. Food-seeking behavior is important in guiding rats'

choice. Social dominance is a modulator (i.e., prosociality occurs more frequently from dominants to subordinates) both in mice and rats, while familiarity of the recipient affects prosocial choices in mice but not rats. In both rodent species, results regarding sex effects remain mixed, probably due to the lack of standardization between protocols. It has been shown that early life stress is associated with a reduction in prosociality later in life, which can be prevented by OT administration or environmental enrichment in the homecage; however environmental enrichment per se in non-stressed animals, seems to have a negative effect on adult prosociality. Regarding the neural correlates of prosocial choice, the evidences are still scarce, but prefrontal-amygdala circuits, which have been associated with social interest and social decisions in rodents and primates (Gangopadhyay et al., 2021; Huang et al., 2020), have been convincingly involved with the expression of reward-based prosociality. We are still lacking knowledge in key aspects of reward-based prosociality, most likely due to the bias that the field of social neuroscience has been suffering from favoring the study of negatively-valenced emotions. That rodents are able to detect and react to negative affective states of others (Keysers et al., 2022) or the cessation of them (Scheggia et al., 2020), is starting to be recognized; however, there is a need for further studies on how positive emotional states are computed by the brain (see (Michon et al., 2023) for review and (Brosnan and Knapska, 2024) in this issue).

Vicarious reward processes have been proposed to be at the base of prosociality (Hernandez-Lallement et al., 2016a), nevertheless experimental results on the neural circuits by which this perception might guide prosociality in foraging contexts are still scarce. There are evidences showing that mutual reward delivery drives associative learning about novel cues in a Pavlovian discrimination task in rats (van Gurp et al., 2020), and that mice can adapt their behavior depending on the reward delivered to others (Choe et al., 2017), at least after strong rewards (electrical brain stimulation), as this was not observed when food rewards were used. Moreover, dopamine release in the nucleus accumbens, a possible neural correlate of vicarious reinforcement, is initially increased in response to playback of affiliative 50-kHz ultrasonic vocalizations (Willuhn et al., 2014), and when observing a conspecific receiving reward (Kashtelyan et al., 2014). In this latter study, (Kashtelyan et al., 2014) described that DA release was increased during the first trial of observation of a conspecific receiving a reward, being then followed by decreases in DA release. Whether these complex DA dynamics might be explained by the conflicting information on reward outcomes, where a light-cue first predicts self-reward and then predicts reward for others (and lack of self-reward) needs to be clarified.

There is still much to learn about the neuronal circuits supporting vicarious reward and reward-based prosocial choices. Moreover, if prosociality recruits mirror-like neurons (i.e., neurons that would respond when experiencing a rewarding state and witnessing another's rewarding state), in a similar fashion to what has been observed for attending another's pain experience (Carrillo et al., 2019; Wu et al., 2023), remains unexplored. We hope that the literature reviewed here will offer a solid base to inspire future research.

3. Discussion/Concluding remarks

Here, we reviewed four experimental paradigms broadly used in the literature to assess prosocial behaviors in rodents: the consolation paradigm, the rescue paradigm, the harm-aversion paradigm and the reward-provision paradigm. We briefly discussed the results, similarities and differences between studies, and pointed out the neural substrates important for the emergence of prosocial behaviors.

The vast majority of the work reviewed here has focused on the demonstration of prosociality in rodents at the behavioral level. We feel that this is a reflection of the endeavor of early works that needed to demonstrate that indeed, prosociality is not exclusive to highly complex brains, and devoted efforts to understand and characterize it in rodents. There is growing consensus that these types of socio-cognitive processes can be indeed studied in laboratory rodents. However, there is still a need for implementing standardized paradigms and protocols for providing a more comprehensive understanding of the results, which sometimes are opposite or lack clear interpretations. Nevertheless, the time is ripe now to further assess how these fascinating behaviors are computed at the brain level, and how different types of prosocial behaviors, reviewed here, map onto distinct or common neural circuits.

There is mounting evidence for a key role of the anterior cingulate in the emergence of prosociality in different paradigms, which has been mostly related to its role in empathy-related processes on negative affective states. Furthermore, several works point to the involvement of different sub areas of the amygdaloid complex and its projections in the regulation of this valenced motivated behaviors; or highlight the modulating role of oxytocin. We are, however, far from having a unifying picture that would help understand prosociality in the healthy brain, and how these processes are impaired in psychopathology and neurodevelopmental disorders.

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Competing interest

The authors have declared that no competing interests exist.

References

Asadi, E., Khodagholi, F., Asadi, S., Mohammadi Kamsorkh, H., Kaveh, N., Maleki, A., 2021. Quality of early-life maternal care predicts empathy-like behavior in adult male rats: Linking empathy to BDNF gene expression in associated brain regions. Brain Res. *1767*, 147568 https://doi.org/10.1016/j.brainres.2021.147568.

Barnett, S.A., 1963. The rat: A study in behaviour (pp. xvi, 248). Aldine.

Bartal, I.B.-A., Decety, J., Mason, P., 2011. Empathy and Pro-Social Behavior in Rats. Science *334* (6061), 1427–1430. https://doi.org/10.1126/science.1210789.

Batson, C.D., 2009. These things called empathy: Eight related but distinct phenomena (In The social neuroscience of empathy). Boston Rev. 3–15. https://doi.org/10.7551/mitpress/9780262012973.003.0002.

Ben-Ami Bartal, I., Breton, J.M., Sheng, H., Long, K.L., Chen, S., Halliday, A., Kenney, J. W., Wheeler, A.L., Frankland, P., Shilyansky, C., Deisseroth, K., Keltner, D., Kaufer, D., 2021. Neural correlates of ingroup bias for prosociality in rats. eLife *10*, e65582. https://doi.org/10.7554/eLife.65582.

Ben-Ami Bartal, I., Rodgers, D.A., Bernardez Sarria, M.S., Decety, J., Mason, P., 2014. Pro-social behavior in rats is modulated by social experience. eLife *3*, e01385. https://doi.org/10.7554/eLife.01385.

Ben-Ami Bartal, I., Shan, H., Molasky, N.M.R., Murray, T.M., Williams, J.Z., Decety, J., Mason, P., 2016. Anxiolytic Treatment Impairs Helping Behavior in Rats. Front. Psychol. *7* https://doi.org/10.3389/fpsyg.2016.00850.

Breton, J.M., Eisner, J.S., Gandhi, V.S., Musick, N., Zhang, A., Long, K.L.P., Perloff, O.S., Hu, K.Y., Pham, C.M., Lalchandani, P., Barraza, M.K., Kantor, B., Kaufer, D., Ben-Ami Bartal, I., 2022. Neural activation associated with outgroup helping in adolescent rats. iScience *25* (6), 104412. https://doi.org/10.1016/j.isci.2022.104412.

Brosnan, A., Knapska, E., 2024. Cheerful tails: Delving into positive emotional contagion. Neurosci. Biobehav. Rev. *161*, 105674 https://doi.org/10.1016/j.neubiorev.2024.105674.

Burkart, J.M., Fehr, E., Efferson, C., van Schaik, C.P., 2007. Other-regarding preferences in a non-human primate: Common marmosets provision food altruistically. Proc. Natl. Acad. Sci. *104* (50), 19762–19766. https://doi.org/10.1073/pnas.0710310104.

Burkett, J.P., Andari, E., Johnson, Z.V., Curry, D.C., de Waal, F.B.M., Young, L.J., 2016. Oxytocin-dependent consolation behavior in rodents. Science *351* (6271), 375–378. https://doi.org/10.1126/science.aac4785.

Carneiro de Oliveira, P.E., Carmona, I.M., Casarotto, M., Silveira, L.M., Oliveira, A.C.B., Canto-de-Souza, A., 2022. Mice Cohabiting With Familiar Conspecific in Chronic Stress Condition Exhibit Methamphetamine-Induced Locomotor Sensitization and

Augmented Consolation Behavior. Front. Behav. Neurosci. *16*. ⟨https://www.frontiersin.org/articles/10.3389/fnbeh.2022.835717⟩.
Carrillo, M., Han, Y., Migliorati, F., Liu, M., Gazzola, V., Keysers, C., 2019. Emotional Mirror Neurons in the Rat's Anterior Cingulate Cortex. Curr. Biol. *29* (8), 1301–1312.e6. https://doi.org/10.1016/j.cub.2019.03.024.
Chang, S.W.C., Gariépy, J.-F., Platt, M.L., 2013. Neuronal reference frames for social decisions in primate frontal cortex. Nat. Neurosci. *16* (2), 243–250. https://doi.org/10.1038/nn.3287.
Chen, J., 2017. Empathy for Distress in Humans and Rodents. Neurosci. Bull. *34* (1), 216–236. https://doi.org/10.1007/s12264-017-0135-0.
Choe, I.-H., Byun, J., Kim, K.K., Park, S., Kim, I., Jeong, J., Shin, H.-S., 2017. Mice in social conflict show rule-observance behavior enhancing long-term benefit. Nat. Commun. *8* (1), 1176. https://doi.org/10.1038/s41467-017-01091-5.
Church, R.M., 1959. Emotional reactions of rats to the pain of others. J. Comp. Physiol. Psychol. *52* (2), 132–134. https://doi.org/10.1037/h0043531.
Clay, Z., Waal, F.B.M. de, 2013. Bonobos Respond to Distress in Others: Consolation across the Age Spectrum. PLOS ONE *8* (1), e55206. https://doi.org/10.1371/journal.pone.0055206.
Clutton-Brock, T., 2009. Cooperation between non-kin in animal societies. Nature *462* (7269), 51–57. https://doi.org/10.1038/nature08366.
Colin, C., Desor, D., 1986. Differenciations comportementales dans des groupes de rats soumis a une difficulte d'acces a la nourriture. Behav. Process. *13* (1–2), 85–100. https://doi.org/10.1016/0376-6357(86)90019-7.
Costa, D.F., Moita, M.A., Márquez, C., 2021. Novel competition test for food rewards reveals stable dominance status in adult male rats. Sci. Rep. *11* (1), 14599 https://doi.org/10.1038/s41598-021-93818-0.
Cox, S.S., Kearns, A.M., Woods, S.K., Brown, B.J., Brown, S.J., Reichel, C.M., 2022. The role of the anterior insula during targeted helping behavior in male rats (Article). Sci. Rep. *12* (1), 1. https://doi.org/10.1038/s41598-022-07365-3.
Cox, S.S., Reichel, C.M., 2020. Rats display empathic behavior independent of the opportunity for social interaction. Neuropsychopharmacology *45* (7), 1097–1104. https://doi.org/10.1038/s41386-019-0572-8.
Cronin, K.A., 2012. Prosocial behaviour in animals: The influence of social relationships, communication and rewards. Anim. Behav. *84* (5), 1085–1093. https://doi.org/10.1016/j.anbehav.2012.08.009.
de Waal, F.B.M., Preston, S.D., 2017. Mammalian empathy: Behavioural manifestations and neural basis (Article). Nat. Rev. Neurosci. *18* (8), 8. https://doi.org/10.1038/nrn.2017.72.
Decety, J., Jackson, P., 2004. The Functional Architecture of Human Empathy. Behav. Cogn. Neurosci. Rev. *3*, 71–100. https://doi.org/10.1177/1534582304267187.
Dovidio, J.F., Piliavin, J.A., Schroeder, D.A., Penner, L.A., 2017. The Social Psychology of Prosocial Behavior. Psychology Press. https://doi.org/10.4324/9781315085241.
Du, R., Luo, W.-J., Geng, K.-W., Li, C.-L., Yu, Y., Wei, N., Chen, J., 2020. Empathic Contagious Pain and Consolation in Laboratory Rodents: Species and Sex Comparisons. Neurosci. Bull. *36* (6), 649–653. https://doi.org/10.1007/s12264-020-00465-y.
Dunbar, R., 1991. Functional Significance of Social Grooming in Primates. Folia Primatol. *57*, 121–131. https://doi.org/10.1159/000156574.
Duque, J.F., Stevens, J.R., 2016. Voluntary food sharing in pinyon jays: The role of reciprocity and dominance. Anim. Behav. *122*, 135–144. https://doi.org/10.1016/j.anbehav.2016.09.020.
Engelhardt, S.C., Taborsky, M., 2024. Reciprocal altruism in Norway rats. Ethology *130* (4), e13418. https://doi.org/10.1111/eth.13418.
Fraser, O.N., Bugnyar, T., 2010. Do Ravens Show Consolation? Responses to Distressed Others. PLoS ONE *5* (5), e10605. https://doi.org/10.1371/journal.pone.0010605.
Fraser, O.N., Stahl, D., Aureli, F., 2008. Stress reduction through consolation in chimpanzees. Proc. Natl. Acad. Sci. U. S. A. *105* (25), 8557–8562. https://doi.org/10.1073/pnas.0804141105.
Gachomba, M.J.M., Esteve-Agraz, J., Caref, K., Maroto, A.S., Bortolozzo-Gleich, M.H., Laplagne, D.A., Márquez, C., 2022. Multimodal cues displayed by submissive rats promote prosocial choices by dominants. S096098222200985X Curr. Biol.. https://doi.org/10.1016/j.cub.2022.06.026.
Galef, B.G., Marczinski, C.A., Murray, K.A., Whiskin, E.E., 2001. Food stealing by young Norway rats (Rattus norvegicus). J. Comp. Psychol. (Wash., D. C.: 1983) *115* (1), 16–21. https://doi.org/10.1037/0735-7036.115.1.16.
Gangopadhyay, P., Chawla, M., Dal Monte, O., Chang, S.W.C., 2021. Prefrontal–amygdala circuits in social decision-making. Nat. Neurosci. *24* (1), 5–18. https://doi.org/10.1038/s41593-020-00738-9.
Grasmuck, V., Desor, D., 2002. Behavioural differentiation of rats confronted to a complex diving-for-food situation. Behav. Process. *58* (1–2), 67–77. https://doi.org/10.1016/S0376-6357(01)00209-1.
Greene, J.T., 1969. Altruistic behavior in the albino rat. Psychon. Sci. *14* (1), 47–48. https://doi.org/10.3758/BF03336420.
Gu, X., Gao, Z., Wang, X., Liu, X., Knight, R.T., Hof, P.R., Fan, J., 2012. Anterior insular cortex is necessary for empathetic pain perception. Brain *135* (9), 2726–2735. https://doi.org/10.1093/brain/aws199.
Hamilton, W.D., 1964. The genetical evolution of social behaviour. I. Journal of Theoretical Biology *7* (1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4.
Hamilton, I.M., Taborsky, M., 2005. Contingent movement and cooperation evolve under generalized reciprocity. Proc. R. Soc. B. *272* (1578), 2259–2267. https://doi.org/10.1098/rspb.2005.3248.
Han, Y., Bruls, R., Soyman, E., Thomas, R.M., Pentaraki, V., Jelinek, N., Heinemans, M., Bassez, I., Verschooren, S., Pruis, I., Van Lierde, T., Carrillo, N., Gazzola, V., Carrillo, M., Keysers, C., 2019. Bidirectional cingulate-dependent danger information transfer across rats. PLoS Biol. *17* (12), e3000524 https://doi.org/10.1371/journal.pbio.3000524.
Han, Y., Sichterman, B., Carrillo, M., Gazzola, V., Keysers, C., 2020. Similar levels of emotional contagion in male and female rats. Sci. Rep. *10* (1), 2763. https://doi.org/10.1038/s41598-020-59680-2.
Havlik, J.L., Vieira Sugano, Y.Y., Jacobi, M.C., Kukreja, R.R., Jacobi, J.H.C., Mason, P., 2020. The bystander effect in rats. Sci. Adv. *6* (28), eabb4205 https://doi.org/10.1126/sciadv.abb4205.
Heinla, I., Heijkoop, R., Houwing, D.J., Olivier, J.D.A., Snoeren, E.M.S., 2020. Third-party prosocial behavior in adult female rats is impaired after perinatal fluoxetine exposure. Physiology & behavior 222, 112899. https://doi.org/10.1016/j.physbeh.2020.112899.
Hernandez-Lallement, J., Attah, A.T., Soyman, E., Pinhal, C.M., Gazzola, V., Keysers, C., 2020. Harm to Others Acts as a Negative Reinforcer in Rats. Curr. Biol. *30* (6), 949–961.e7. https://doi.org/10.1016/j.cub.2020.01.017.
Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., Kalenscher, T., 2015. Rats prefer mutual rewards in a prosocial choice task. Front. Neurosci. *8* https://doi.org/10.3389/fnins.2014.00443.
Hernandez-Lallement, J., van Wingerden, M., Schäble, S., Kalenscher, T., 2016a. A Social Reinforcement Learning Hypothesis of Mutual Reward Preferences in Rats. In: Wöhr, M., Krach, S. (Eds.), Social Behavior from Rodents to Humans, Vol. 30. Springer International Publishing, pp. 159–176. https://doi.org/10.1007/7854_2016_436.
Hernandez-Lallement, J., van Wingerden, M., Schäble, S., Kalenscher, T., 2016b. Basolateral amygdala lesions abolish mutual reward preferences in rats. Neurobiol. Learn. Mem. *127*, 1–9. https://doi.org/10.1016/j.nlm.2015.11.004.
Heslin, K.A., Brown, M.F., 2021. No preference for prosocial helping behavior in rats with concurrent social interaction opportunities. Learn Behav. *49* (4), 397–404. https://doi.org/10.3758/s13420-021-00471-8.
Hess, E.M., Venniro, M., Gould, T.D., 2023. Relative to females, male rats are more willing to forego obtaining sucrose reward in order to prevent harm to their cage mate. Psychopharmacology. https://doi.org/10.1007/s00213-023-06435-2.
Hollis, K.L., Nowbahari, E., 2013. Toward a Behavioral Ecology of Rescue Behavior. Evolut. Psychol. *11* (3) https://doi.org/10.1177/147470491301100311.
Horn, L., Bugnyar, T., Massen, J.J.M., 2024. Testing the effects of kinship, reciprocity and dominance on prosocial food provisioning in azure-winged magpies (Cyanopica cyana), carrion crows (Corvus corone) and common ravens (C. corax). Ethology *130* (4), e13420. https://doi.org/10.1111/eth.13420.
Horner, V., Carter, J.D., Suchak, M., de Waal, F.B.M., 2011. Spontaneous prosocial choice by chimpanzees. Proc. Natl. Acad. Sci. *108* (33), 13847–13851. https://doi.org/10.1073/pnas.1111088108.
Huang, W.-C., Zucca, A., Levy, J., Page, D.T., 2020. Social Behavior Is Modulated by Valence-Encoding mPFC-Amygdala Sub-circuitry. Cell Rep. *32* (2), 107899 https://doi.org/10.1016/j.celrep.2020.107899.
Jablonski, N.G., 2021. Social and affective touch in primates and its role in the evolution of social cohesion. Neuroscience *464*, 117–125. https://doi.org/10.1016/j.neuroscience.2020.11.024.
Jakubiak, B.K., Feeney, B.C., 2017. Affectionate Touch to Promote Relational, Psychological, and Physical Well-Being in Adulthood: A Theoretical Model and Review of the Research. Personal. Soc. Psychol. Rev. *21* (3), 228–252. https://doi.org/10.1177/1088868316650307.
Jensen, K., 2016. The Prosocial Primate—A Critical Review. In: Advances in the Study of Behavior, Vol. 48. Elsevier, pp. 387–441. https://doi.org/10.1016/bs.asb.2016.03.001.
Joushi, S., Taherizadeh, Z., Esmaeilpour, K., Sheibani, V., 2022. Environmental enrichment and intranasal oxytocin administration reverse maternal separation-induced impairments of prosocial choice behavior. Pharmacol. Biochem. Behav. *213*, 173318 https://doi.org/10.1016/j.pbb.2021.173318.
Kalamari, A., Kentrop, J., Hinna Danesi, C., Graat, E.A.M., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Joëls, M., van der Veen, R., 2021. Complex Housing, but Not Maternal Deprivation Affects Motivation to Liberate a Trapped Cage-Mate in an Operant Rat Task. Front. Behav. Neurosci. *15*, 698501 https://doi.org/10.3389/fnbeh.2021.698501.
Kashtelyan, V., Lichtenberg, N.T., Chen, M.L., Cheer, J.F., Roesch, M.R., 2014. Observation of Reward Delivery to a Conspecific Modulates Dopamine Release in Ventral Striatum. Curr. Biol. *24* (21), 2564–2568. https://doi.org/10.1016/j.cub.2014.09.016.
Kentrop, J., Kalamari, A., Danesi, C.H., Kentrop, J.J., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Joëls, M., van der Veen, R., 2020. Pro-social preference in an automated operant two-choice reward task under different housing conditions: Exploratory studies on pro-social decision making. Dev. Cogn. Neurosci. *45*, 100827 https://doi.org/10.1016/j.dcn.2020.100827.
Keysers, C., Knapska, E., Moita, M.A., Gazzola, V., 2022. Emotional contagion and prosocial behavior in rodents. S1364661322001115 Trends Cogn. Sci.. https://doi.org/10.1016/j.tics.2022.05.005.
Kitano, K., Yamagishi, A., Horie, K., Nishimori, K., Sato, N., 2022. Helping behavior in prairie voles: A model of empathy and the importance of oxytocin. iScience *25* (4), 103991. https://doi.org/10.1016/j.isci.2022.103991.
Kiyokawa, Y., Li, Y., Takeuchi, Y., 2019. A dyad shows mutual changes during social buffering of conditioned fear responses in male rats. Behav. Brain Res. *366*, 45–55. https://doi.org/10.1016/j.bbr.2019.03.024.
Kopp, K.S., Kanngiesser, P., Brügger, R.K., Daum, M.M., Gampe, A., Köster, M., van Schaik, C.P., Liebal, K., Burkart, J.M., 2024. The proximate regulation of prosocial behaviour: Towards a conceptual framework for comparative research. Anim. Cogn. *27* (1), 5. https://doi.org/10.1007/s10071-024-01846-w.
Krafft, B., Colin, C., Peignot, P., 2010. Diving-for-Food: A New Model to Assess Social Roles in a Group of Laboratory Rats. Ethology *96* (1), 11–23. https://doi.org/10.1111/j.1439-0310.1994.tb00877.x.

Lakshminarayanan, V.R., Santos, L.R., 2008. Capuchin monkeys are sensitive to others' welfare. Curr. Biol. *18* (21), R999–R1000. https://doi.org/10.1016/j.cub.2008.08.057.

Lalot, M., Bourgeois, A., Jalme, M.S., Bovet, D., 2023. Family first! Influence of parental investment in Guinea pigs (Cavia porcellus) prosocial choices. Anim. Cogn. *26* (5), 1713–1732. https://doi.org/10.1007/s10071-023-01813-x.

Lalot, M., Delfour, F., Mercera, B., Bovet, D., 2021. Prosociality and reciprocity in bottlenose dolphins (Tursiops truncatus). Anim. Cogn. *24* (5), 1075–1086. https://doi.org/10.1007/s10071-021-01499-z.

Lalot, M., Liévin-Bazin, A., Bourgeois, A., Saint Jalme, M., Bovet, D., 2021. Prosociality and reciprocity in capybaras (Hydrochoerus hydrochaeris) in a non-reproductive context. Behav. Process. *188*, 104407 https://doi.org/10.1016/j.beproc.2021.104407.

Lavery, J.J., Foley, P.J., 1963. Altruism or Arousal in the Rat? Science *140* (3563), 172–173. https://doi.org/10.1126/science.140.3563.172.

Lee, I.-C., Yu, T.-H., Liu, W.-H., Hsu, K.-S., 2021. Social Transmission and Buffering of Hippocampal Metaplasticity after Stress in Mice. J. Neurosci. *41* (6), 1317–1330. https://doi.org/10.1523/JNEUROSCI.1751-20.2020.

Lewis, M., 2018. Prosocial Behavior. In: Shackelford, T.K., Weekes-Shackelford, V.A. (Eds.), Encyclopedia of Evolutionary Psychological Science. Springer International Publishing, pp. 1–6. https://doi.org/10.1007/978-3-319-16999-6_158-1.

Li, C.-L., Yu, Y., He, T., Wang, R.-R., Geng, K.-W., Du, R., Luo, W.-J., Wei, N., Wang, X.-L., Wang, Y., Yang, Y., Yu, Y.-Q., Chen, J., 2018. Validating Rat Model of Empathy for Pain: Effects of Pain Expressions in Social Partners. Front. Behav. Neurosci. *12*. ⟨https://www.frontiersin.org/articles/10.3389/fnbeh.2018.00242⟩.

Li, L.-F., Yuan, W., He, Z.-X., Wang, L.-M., Jing, X.-Y., Zhang, J., Yang, Y., Guo, Q.-Q., Zhang, X.-N., Cai, W.-Q., Hou, W.-J., Jia, R., Tai, F.-D., 2019. Involvement of oxytocin and GABA in consolation behavior elicited by socially defeated individuals in mandarin voles. Psychoneuroendocrinology *103*, 14–24. https://doi.org/10.1016/j.psyneuen.2018.12.238.

Li, L.-F., Yuan, W., He, Z.-X., Ma, H., Xun, Y.-F., Meng, L.-R., Zhu, S.-J., Wang, L.-M., Zhang, J., Cai, W.-Q., Zhang, X.-N., Guo, Q.-Q., Lian, Z.-M., Jia, R., Tai, F.-D., 2020. Reduced Consolation Behaviors in Physically Stressed Mandarin Voles: Involvement of Oxytocin, Dopamine D2, and Serotonin 1A Receptors Within the Anterior Cingulate Cortex. Int. J. Neuropsychopharmacol. *23* (8), 511–523. https://doi.org/10.1093/ijnp/pyz060.

Li, L.-F., Zhang, L.-Z., He, Z.-X., Ma, H., Zhang, Y.-T., Xun, Y.-F., Yuan, W., Hou, W.-J., Li, Y.-T., Lv, Z.-J., Jia, R., Tai, F.-D., 2021. Dorsal raphe nucleus to anterior cingulate cortex 5-HTergic neural circuit modulates consolation and sociability. eLife *10*, e67638. https://doi.org/10.7554/eLife.67638.

Lichtenberg, N.T., Lee, B., Kashtelyan, V., Chappa, B.S., Girma, H.T., Green, E.A., Kantor, S., Lagowala, D.A., Myers, M.A., Potemri, D., Pecukonis, M.G., Tesfay, R.T., Walters, M.S., Zhao, A.C., Blair, R.J.R., Cheer, J.F., Roesch, M.R., 2018. Rat behavior and dopamine release are modulated by conspecific distress. eLife *7*, e38090. https://doi.org/10.7554/eLife.38090.

Lim, K.Y., Hong, W., 2023. Neural mechanisms of comforting: Prosocial touch and stress buffering. Horm. Behav. *153*, 105391 https://doi.org/10.1016/j.yhbeh.2023.105391.

Lockwood, P.L., Apps, M.A.J., Valton, V., Viding, E., Roiser, J.P., 2016. Neurocomputational mechanisms of prosocial learning and links to empathy. Proc. Natl. Acad. Sci. U. S. A. *113* (35), 9763–9768. https://doi.org/10.1073/pnas.1603198113.

Lu, Y.-F., Ren, B., Ling, B.-F., Zhang, J., Xu, C., Li, Z., 2018. Social interaction with a cagemate in pain increases allogrooming and induces pain hypersensitivity in the observer rats. Neurosci. Lett. *662*, 385–388. https://doi.org/10.1016/j.neulet.2017.10.063.

Luo, W.-J., Li, C.-L., Geng, K.-W., Wang, X.-L., Du, R., Yu, Y., Wei, N., He, T., Wang, Y., Yu, Y.-Q., Chen, J., 2020. The similar past pain experience evokes both observational contagious pain and consolation in stranger rat observers. Neurosci. Lett. *722*, 134840 https://doi.org/10.1016/j.neulet.2020.134840.

Márquez, C., Rennie, S.M., Costa, D.F., Moita, M.A., 2015. Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients. Curr. Biol. *25* (13), 1736–1745. https://doi.org/10.1016/j.cub.2015.05.018.

Marshall-Pescini, S., Dale, R., Quervel-Chaumette, M., Range, F., 2016. Critical issues in experimental studies of prosociality in non-human species. Anim. Cogn. *19* (4), 679–705. https://doi.org/10.1007/s10071-016-0973-6.

Masserman, J.H., Wechkin, S., Terris, W., 1964. ALTRUISTIC' BEHAVIOR IN RHESUS MONKEYS. Am. J. Psychiatry *121* (6), 584–585. https://doi.org/10.1176/ajp.121.6.584.

Matsumoto, M., Yoshida, M., Jayathilake, B.W., Inutsuka, A., Nishimori, K., Takayanagi, Y., Onaka, T., 2021. Indispensable role of the oxytocin receptor for allogrooming toward socially distressed cage mates in female mice. J. Neuroendocrinol. *33* (6), e12980 https://doi.org/10.1111/jne.12980.

McFarland, R., Majolo, B., 2011. Grooming Coercion and the Post-Conflict Trading of Social Services in Wild Barbary Macaques. PLOS ONE *6* (10), e26893. https://doi.org/10.1371/journal.pone.0026893.

Meyza, K.Z., Bartal, I.B.-A., Monfils, M.H., Panksepp, J.B., Knapska, E., 2017. The roots of empathy: Through the lens of rodent models. Neurosci. Biobehav. Rev. *76*, 216–234. https://doi.org/10.1016/j.neubiorev.2016.10.028.

Michon, F., Packheiser, J., Gazzola, V., Keysers, C., 2023. Sharing Positive Affective States Amongst Rodents. Affect. Sci. *4* (3), 475–479. https://doi.org/10.1007/s42761-023-00201-5.

Misiołek, K., Klimczak, M., Chrószcz, M., Szumiec, Ł., Bryksa, A., Przyborowicz, K., Rodriguez Parkitna, J., Harda, Z., 2023. Prosocial behavior, social reward and affective state discrimination in adult male and female mice (Article). Sci. Rep. 13 (1), 1. https://doi.org/10.1038/s41598-023-32682-6.

Morrison, I., 2016. Keep Calm and Cuddle on: Social Touch as a Stress Buffer. Adapt. Hum. Behav. Physiol. *2* (4), 344–362. https://doi.org/10.1007/s40750-016-0052-x.

Nakahara, F., Komaba, M., Sato, R., Ikeda, H., Komaba, K., Kawakubo, A., 2017. Spontaneous prosocial choice by captive bottlenose dolphins, Tursiops truncatus. Behav. Process. *135*, 8–11. https://doi.org/10.1016/j.beproc.2016.11.009.

Nowak, M.A., 2006. Five Rules for the Evolution of Cooperation. Science *314* (5805), 1560–1563. https://doi.org/10.1126/science.1133755.

Nowbahari, E., Scohier, A., Durand, J.-L., Hollis, K.L., 2009. Ants, Cataglyphis cursor, Use Precisely Directed Rescue Behavior to Free Entrapped Relatives. PLoS ONE *4* (8), e6573. https://doi.org/10.1371/journal.pone.0006573.

Palagi, E., Cordoni, G., 2009. Postconflict third-party affiliation in Canis lupus: Do wolves share similarities with the great apes? Anim. Behav. *78* (4), 979–986. https://doi.org/10.1016/j.anbehav.2009.07.017.

Penner, L.A., Dovidio, J.F., Piliavin, J.A., Schroeder, D.A., 2005. Prosocial Behavior: Multilevel Perspectives. Annu. Rev. Psychol. *56* (1), 365–392. https://doi.org/10.1146/annurev.psych.56.091103.070141.

Pérez-Manrique, A., Gomila, A., 2018. The comparative study of empathy: Sympathetic concern and empathic perspective-taking in non-human animals: Comparative study of empathy. Biol. Rev. *93* (1), 248–269. https://doi.org/10.1111/brv.12342.

Pérez-Manrique, A., Gomila, A., 2022. Emotional contagion in nonhuman animals: A review. WIRES Cogn. Sci. *13* (1) https://doi.org/10.1002/wcs.1560.

Pfattheicher, S., Nielsen, Y.A., Thielmann, I., 2022. Prosocial behavior and altruism: A review of concepts and definitions. Curr. Opin. Psychol. *44*, 124–129. https://doi.org/10.1016/j.copsyc.2021.08.021.

Pfeiffer, T., Rutte, C., Killingback, T., Taborsky, M., Bonhoeffer, S., 2005. Evolution of cooperation by generalized reciprocity. Proc. R. Soc. B. *272* (1568), 1115–1120. https://doi.org/10.1098/rspb.2004.2988.

Phillips, H.L., Dai, H., Choi, S.Y., Jansen-West, K., Zajicek, A.S., Daly, L., Petrucelli, L., Gao, F.-B., Yao, W.-D., 2023. Dorsomedial prefrontal hypoexcitability underlies lost empathy in frontotemporal dementia. Neuron *111* (6), 797–806.e6. https://doi.org/10.1016/j.neuron.2022.12.027.

Plotnik, J.M., Waal, F.B.M. de, 2014. Asian elephants (Elephas maximus) reassure others in distress. PeerJ *2*, e278. https://doi.org/10.7717/peerj.278.

Pozo, M., Milà-Guasch, M., Haddad-Tóvolli, R., Boudjadja, M.B., Chivite, I., Toledo, M., Gómez-Valadés, A.G., Eyre, E., Ramírez, S., Obri, A., Ben-Ami Bartal, I., D'Agostino, G., Costa-Font, J., Claret, M., 2023. Negative energy balance hinders prosocial helping behavior. Proc. Natl. Acad. Sci. *120* (15), e2218142120 https://doi.org/10.1073/pnas.2218142120.

Radford, A.N., Du Plessis, M.A., 2006. Dual function of allopreening in the cooperatively breeding green woodhoopoe, Phoeniculus purpureus. Behav. Ecol. Sociobiol. *61* (2), 221–230. https://doi.org/10.1007/s00265-006-0253-6.

Rault, J.-L., 2019. Be kind to others: Prosocial behaviours and their implications for animal welfare. Appl. Anim. Behav. Sci. *210*, 113–123. https://doi.org/10.1016/j.applanim.2018.10.015.

Rice, G.E., Gainer, P., 1962. Altruism' in the albino rat. J. Comp. Physiol. Psychol. *55* (1), 123–125. https://doi.org/10.1037/h0042276.

Rogers-Carter, M.M., Varela, J.A., Gribbons, K.B., Pierce, A.F., McGoey, M.T., Ritchey, M., Christianson, J.P., 2018. Insular cortex mediates approach and avoidance responses to social affective stimuli. Nature neuroscience 21 (3), 404–414. https://doi.org/10.1038/s41593-018-0071-y.

Rutte, C., Taborsky, M., 2007. Generalized Reciprocity in Rats. PLoS Biol. *5* (7), e196 https://doi.org/10.1371/journal.pbio.0050196.

Rutte, C., Taborsky, M., 2008. The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): Direct vs generalised reciprocity. Behav. Ecol. Socio *62* (4), 499–505. https://doi.org/10.1007/s00265-007-0474-3.

Sato, N., Tan, L., Tate, K., Okada, M., 2015. Rats demonstrate helping behavior toward a soaked conspecific. Anim. Cogn. *18* (5), 1039–1047. https://doi.org/10.1007/s10071-015-0872-2.

Satoh, S., Bshary, R., Shibasaki, M., Inaba, S., Sogawa, S., Hotta, T., Awata, S., Kohda, M., 2021. Prosocial and antisocial choices in a monogamous cichlid with biparental care. Nat. Commun. *12* (1), 1775. https://doi.org/10.1038/s41467-021-22075-6.

Schaich Borg, J., Srivastava, S., Lin, L., Heffner, J., Dunson, D., Dzirasa, K., de Lecea, L., 2017. Rat intersubjective decisions are encoded by frequency-specific oscillatory contexts. Brain Behav. *7* (6), e00710 https://doi.org/10.1002/brb3.710.

Scheggia, D., La Greca, F., Maltese, F., Chiacchierini, G., Italia, M., Molent, C., Bernardi, F., Coccia, G., Carrano, N., Zianni, E., Gardoni, F., Di Luca, M., Papaleo, F., 2022. Reciprocal cortico-amygdala connections regulate prosocial and selfish choices in mice. Nat. Neurosci. https://doi.org/10.1038/s41593-022-01179-2.

Scheggia, D., Managò, F., Maltese, F., Bruni, S., Nigro, M., Dautan, D., Latuske, P., Contarini, G., Gomez-Gonzalo, M., Requie, L.M., Ferretti, V., Castellani, G., Mauro, D., Bonavia, A., Carmignoto, G., Yizhar, O., Papaleo, F., 2020. Somatostatin interneurons in the prefrontal cortex control affective state discrimination in mice. Nat. Neurosci. *23* (1), 47–60. https://doi.org/10.1038/s41593-019-0551-8.

Schino, G., Aureli, F., 2010. The relative roles of kinship and reciprocity in explaining primate altruism. Ecol. Lett. *13* (1), 45–50. https://doi.org/10.1111/j.1461-0248.2009.01396.x.

Schneeberger, K., Dietz, M., Taborsky, M., 2012. Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evol. Biol. *12* (1), 41. https://doi.org/10.1186/1471-2148-12-41.

Schweinfurth, M.K., 2024. Interdisciplinary perspectives on the principle of reciprocation. Ethology *130* (4), e13445. https://doi.org/10.1111/eth.13445.

Schweinfurth, M.K., Taborsky, M., 2018. Relatedness decreases and reciprocity increases cooperation in Norway rats. Proc. R. Soc. B: Biol. Sci. *285* (1874), 20180035. https://doi.org/10.1098/rspb.2018.0035.

Silk, J.B., Brosnan, S.F., Vonk, J., Henrich, J., Povinelli, D.J., Richardson, A.S., Lambeth, S.P., Mascaro, J., Schapiro, S.J., 2005. Chimpanzees are indifferent to the

welfare of unrelated group members. Nature *437* (7063), 1357–1359. https://doi.org/10.1038/nature04243.

Smith, M.L., Asada, N., Malenka, R.C., 2021. Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia. Science *371* (6525), 153–159. https://doi.org/10.1126/science.abe3040.

Song, D., Wang, C., Jin, Y., Deng, Y., Yan, Y., Wang, D., Zhu, Z., Ke, Z., Wang, Z., Wu, Y., Ni, J., Qing, H., Quan, Z., 2023. Mediodorsal thalamus-projecting anterior cingulate cortex neurons modulate helping behavior in mice. Curr. Biol. *33* (20), 4330–4342. e5. https://doi.org/10.1016/j.cub.2023.08.070.

Trivers, R.L., 1971. The Evolution of Reciprocal Altruism. The Quarterly Review of Biology 46 (1), 35–57. http://www.jstor.org/stable/2822435.

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Takahashi, Y., Matsumoto, Y., Okamoto, M., Ishihara, T., 2019. Rescue-like Behaviour in Mice is Mediated by Their Interest in the Restraint Tool. Sci. Rep. *9* (1), 10648 https://doi.org/10.1038/s41598-019-46128-5.

van Gurp, S., Hoog, J., Kalenscher, T., van Wingerden, M., 2020. Vicarious reward unblocks associative learning about novel cues in male rats. eLife *9*, e60755. https://doi.org/10.7554/eLife.60755.

Vasconcelos, M., Hollis, K., Nowbahari, E., Kacelnik, A., 2012. Pro-sociality without empathy. Biol. Lett. *8* (6), 910–912. https://doi.org/10.1098/rsbl.2012.0554.

Warneken, F., Hare, B., Melis, A.P., Hanus, D., Tomasello, M., 2007. Spontaneous Altruism by Chimpanzees and Young Children. PLoS Biol. *5* (7), e184 https://doi.org/10.1371/journal.pbio.0050184.

West, S.A., Griffin, A.S., Gardner, A., 2007. Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. J. Evol. Biol. *20* (2), 415–432. https://doi.org/10.1111/j.1420-9101.2006.01258.x.

Willuhn, I., Tose, A., Wanat, M.J., Hart, A.S., Hollon, N.G., Phillips, P.E.M., Schwarting, R.K.W., Wohr, M., 2014. Phasic Dopamine Release in the Nucleus Accumbens in Response to Pro-Social 50 kHz Ultrasonic Vocalizations in Rats. J. Neurosci. *34* (32), 10616–10623. https://doi.org/10.1523/JNEUROSCI.1060-14.2014.

Wood, R.I., Kim, J.Y., Li, G.R., 2016. Cooperation in rats playing the iterated Prisoner's Dilemma game. Anim. Behav. *114*, 27–35. https://doi.org/10.1016/j.anbehav.2016.01.010.

Wu, W.-Y., Cheng, Y., Liang, K.-C., Lee, R.X., Yen, C.-T., 2023. Affective mirror and anti-mirror neurons relate to prosocial help in rats. iScience *26* (1), 105865. https://doi.org/10.1016/j.isci.2022.105865.

Wu, Y.E., Dang, J., Kingsbury, L., Zhang, M., Sun, F., Hu, R.K., Hong, W., 2021. Neural control of affiliative touch in prosocial interaction (Article). Nature *599* (7884), 7884. https://doi.org/10.1038/s41586-021-03962-w.

Yamagishi, A., Lee, J., Sato, N., 2020. Oxytocin in the anterior cingulate cortex is involved in helping behaviour. Behav. Brain Res. *393*, 112790 https://doi.org/10.1016/j.bbr.2020.112790.

Yamamoto, S., 2017. Primate empathy: Three factors and their combinations for empathy-related phenomena. WIREs Cogn. Sci. *8* (3) https://doi.org/10.1002/wcs.1431.

Yamamoto, S., Humle, T., Tanaka, M., 2009. Chimpanzees Help Each Other upon Request. PLoS ONE *4* (10), e7416. https://doi.org/10.1371/journal.pone.0007416.

Zeng, Q., Shan, W., Zhang, H., Yang, J., Zuo, Z., 2021. Paraventricular thalamic nucleus plays a critical role in consolation and anxious behaviors of familiar observers exposed to surgery mice. Theranostics *11* (8), 3813–3829. https://doi.org/10.7150/thno.45690.

Zhang, M., Wu, Y.E., Jiang, M., Hong, W., 2024. Cortical regulation of helping behaviour towards others in pain (Article). Nature *626* (7997), 7997. https://doi.org/10.1038/s41586-023-06973-x.

Zheng, C., Huang, Y., Bo, B., Wei, L., Liang, Z., Wang, Z., 2020. Projection from the Anterior Cingulate Cortex to the Lateral Part of Mediodorsal Thalamus Modulates Vicarious Freezing Behavior. Neurosci. Bull. *36* (3), 217–229. https://doi.org/10.1007/s12264-019-00427-z.

Biblioteca
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