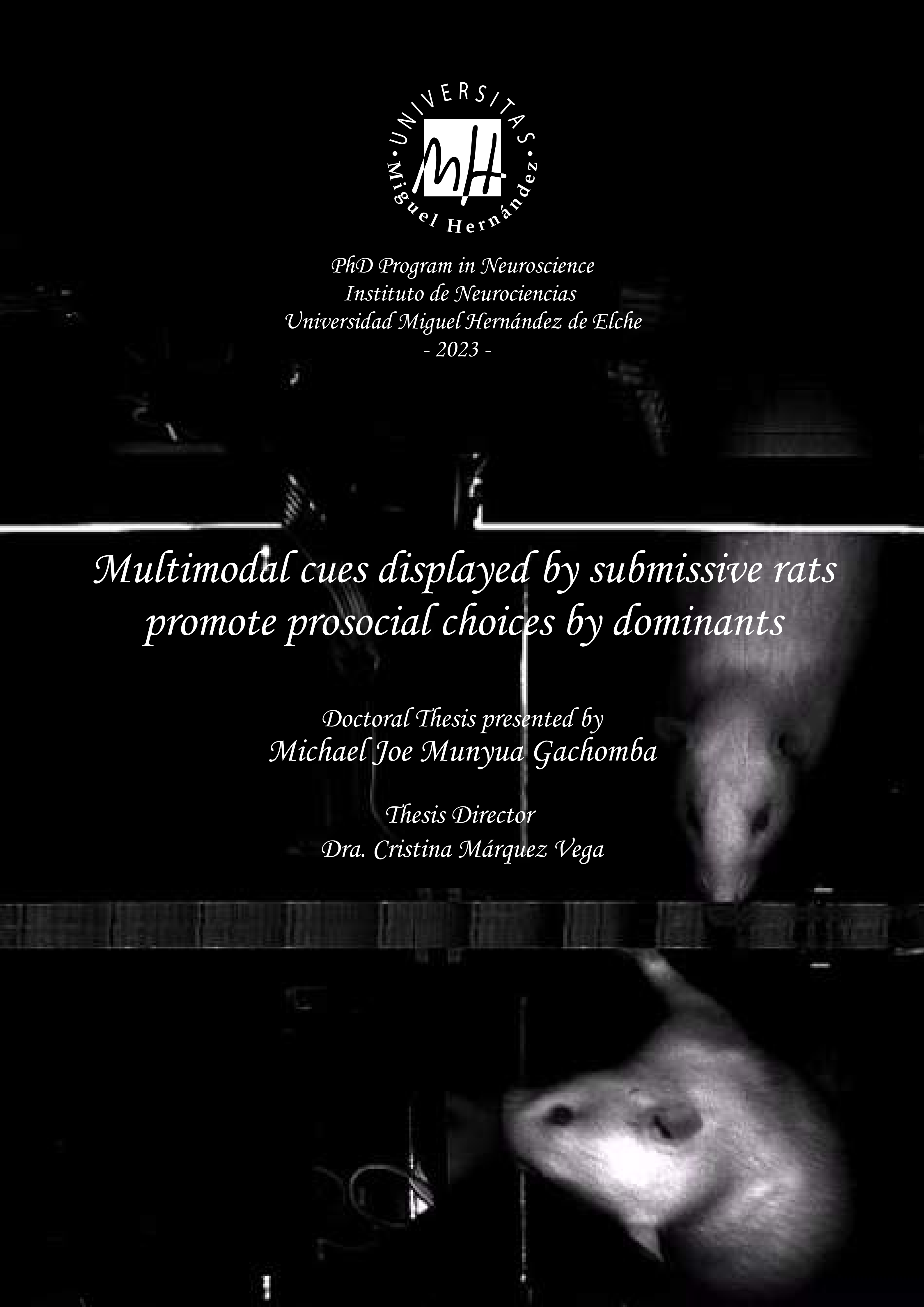

PhD Program in Neuroscience
Instituto de Neurociencias
Universidad Miguel Hernández de Elche
- 2023 -

Multimodal cues displayed by submissive rats promote prosocial choices by dominants

Doctoral Thesis presented by
Michael Joe Munyua Gachomba

Thesis Director
Dra. Cristina Márquez Vega

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Sant Joan d'Alacant, 2023

The doctoral thesis entitled: *“Multimodal cues displayed by submissive rats promote prosocial choices by dominants”* is presented as a compendium of publications and includes the following publication in which I am the first author:

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Dra. *Cristina Márquez Vega,* Director of the doctoral thesis entitled *"Multimodal cues displayed by submissive rats promote prosocial choices by dominants"*

INFORMS:

That Mr. *Michael Joe Munyua Gachomba* has carried out under our supervision the work entitled *"Multimodal cues displayed by submissive rats promote prosocial choices by dominants*" in accordance with the terms and conditions defined in his Research Plan and in accordance with the Code of Good Practice of the University Miguel Hernández of Elche, satisfactorily fulfilling the objectives foreseen for its public defense as a doctoral thesis.

I sign for appropriate purposes,

at Sant Joan d'Alacant, ... of .... of 2023

Thesis director

Dra. *Cristina Márquez Vega*

Sant Joan d'Alacant, 2023

Ms. Elvira de la Peña García, Coordinator of the Neuroscience PhD program at the Institute of Neuroscience in Alicante, a joint centre of the Miguel Hernández University (UMH) and the Spanish National Research Council (CSIC),

INFORMS:

That Mr. Michael Joe Munyua Gachomba has carried out under the supervision of our PhD Program the work entitled *“Multimodal cues displayed by submissive rats promote prosocial choices by dominants"* in accordance with the terms and conditions defined in his Research Plan and in accordance with the Code of Good Practice of the University Miguel Hernández de Elche, fulfilling the objectives satisfactorily for its public defence as a doctoral thesis.

Which I sign for the appropriate purposes, in Sant Joan d’Alacant, ………2023

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“Grandi sono le soddisfazioni di una vita laboriosa, agiata e tranquilla,

ma ancora più grande è l’attrazione dell’abisso”.

— Dino Buzzati, Il Colombre.

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Nomenclature

ACC - Anterior Cingulate Cortex

CBH - Cooperative Breeding Hypothesis

DA - Dopamine

DI - Dominant Index

DLC - DeepLabCut

Dom - Dominant

F - Focal

GLM - Geneleralized Linear Model

GSP - Group Service Paradigm

iPDC - Information Partial Directed Coherence

mFC - Modified Food Competition Test

PAM - Perception Action Mechanism

PCI - Prosocial Choice Index

PCT - Prosocial Choice Task

R - recipient

ROI - Region of Interest

Sub - Submissive

USV - Ultrasonic Vocalization

VAR - Vector Autoregression

VTA - Ventral Tegmental Area

List of figures and tables

Abstract

Prosocial behaviors, behaviors that benefit others, are an integral part of the life of humans and other animals, promoting social bonding and cooperation among individuals and groups. Field and laboratory research has shown evidence that, in several species, animals perform actions that improve the welfare of conspecifics and that factors of the social context, including sex, familiarity between the individuals, and their dominance relationship, lead to variation in the expression of prosociality. However, less effort has been devoted to investigating the behavioral correlates underlying such variation, which would improve our understanding of how animals integrate behavioral cues from those in need to make prosocial decisions. Here we used a two-choice task where rats can provide rewards to a conspecific in the absence of self-benefit and investigated which conditions promote prosociality by manipulating the social context of the interacting animals. Although sex or degree of familiarity did not affect prosocial choices in rats, social hierarchy revealed to be a potent modulator, with dominant decision-makers showing faster emergence and higher levels of prosocial choices toward their submissive cage mates. Leveraging quantitative analysis of multimodal social dynamics prior to choice, we identified that pairs with dominant decision-makers exhibited more proximal interactions. Interestingly, these closer interactions were driven by submissive animals, which were better at communicating their need for help, by modulating their position and movement towards their dominants and whose 50-kHz vocalization rate correlated with dominants' prosociality. Moreover, Granger causality revealed stronger bidirectional influences in pairs with dominant focals and submissive recipients, indicating increased behavioral coordination. Finally, multivariate analysis highlighted body language as the main information dominants use on a trial-by-trial basis to guide prosocial choices. Our results provide a refined understanding of the behavioral dynamics that rats use for action-selection upon perception of socially relevant cues and navigate social decision-making.

Resumen

Los comportamientos prosociales, comportamientos que benefician a los demás, son parte integral de la vida de los humanos y otros animales y favorecen los vínculos sociales y la cooperación entre individuos y grupos. Las investigaciones de campo y de laboratorio han mostrado evidencia de que, en varias especies, los animales realizan acciones que mejoran el bienestar de sus congéneres y que los factores del contexto social, como el sexo, la familiaridad entre los individuos y sus relaciones de dominancia, conducen a variabilidad en la expresión de prosocialidad. Sin embargo, se ha dedicado menos esfuerzo a investigar los correlatos de comportamiento que subyacen a esta variabilidad, lo que mejoraría nuestra comprensión de cómo los animales integran las señales de comportamiento de otros para tomar decisiones prosociales. Aquí usamos una tarea de dos opciones en la que las ratas pueden proporcionar recompensas a un congénere en ausencia de beneficio propio e investigamos qué condiciones promueven la prosocialidad mediante la manipulación del contexto social de los animales que interactúan. Aunque el sexo o el grado de familiaridad no afectaron las elecciones prosociales en las ratas, la jerarquía social se reveló como un potente modulador, donde las ratas dominantes mostraron una aparición más rápida y niveles más altos de elecciones prosociales hacia sus congéneres sumisos. Basándonos en el análisis cuantitativo de las dinámicas sociales multimodales antes de la toma de decisiones, identificamos que los pares en los que la rata que toma la decisión es la dominante, exhibieron interacciones más próximas. Curiosamente, estas interacciones más cercanas fueron impulsadas por los animales sumisos, que comunican mejor su necesidad de recibir ayuda, al modular su posición y movimiento hacia sus dominantes y cuya tasa de vocalización de 50-kHz correlaciona con la prosocialidad de los dominantes. Además, el análisis de causalidad de Granger reveló influencias bidireccionales más fuertes en parejas con focales dominantes y recipientes sumisos, lo que indica una mayor coordinación. Finalmente, el análisis multivariante destacó el lenguaje corporal como la principal información que los dominantes utilizan, trial por trial, para guiar las elecciones prosociales. Nuestros resultados proporcionan una comprensión refinada de las dinámicas de comportamiento que utilizan las ratas para la selección de acciones al percibir señales socialmente relevantes y navegar en la toma de decisiones sociales.

1 | Introduction

Chapter 1 | Introduction

1.1 Prosocial behavior in humans and other animals: concept and definitions

Social behaviors, which we can define as any behaviors directed to or influenced by others, permeate our existence. We devote a relevant part of our day interacting with others, by spending time with family members, romantic partners, gathering with friends and working with colleagues. Besides humans, many other animal species form groups and engage in social behaviors, involving social interactions of different nature and complexity[1]. In group-living societies, individuals experience a balance between risks, such as spread of diseases and competition for resources, and advantages, such as foraging efficiency, increased defense against threats, and formation of social bonds[2]. Animals interact with members of their group through behaviors which can be mainly affiliative (e.g., allogroming, huddling, food sharing, play), or agonistic (e.g., competing for space, food or potential mates). Both agonistic and affiliative behaviors are important for shaping animal social life, by making individuals interact preferentially with some while avoiding others and possibly defining social roles within the group. Therefore, they contribute to the formation, organization and maintenance of stable social relationships which favor group cohesion and survival.

Some affiliative behaviors are prosocial in nature. *Prosocial behaviors* have been broadly defined as any behaviors that benefit others, thus improving their welfare[3]. This comprises a huge variety of behaviors, which can differ from one another in terms of their function and mechanisms. Helping others in need, caregiving, donating goods and consolation are examples of prosocial acts largely common in our society. Acting prosocially may require a cost for the actor, in which case the behavior is defined as *altruistic*. Thus, all altruistic behaviors are prosocial but not all prosocial behaviors are altruistic[4]. Prosocial behavior between two or more individuals may lead to *cooperation*, which occurs when partners work together to achieve mutual benefits[5].

We often provide help even in situations in which we derive no personal gain and towards strangers. Some researchers argued that these kinds of unselfish behaviors may be peculiar of humans[6] in that they may require high socio-cognitive abilities which characterize our sociality, such as the understanding of others' goals or needs coupled with other-regarding preferences driving the motivation to help. Although rarely, basic forms of prosocial behaviors have been observed in the wild and captivity in other species[7], indicating that prosociality do occur outside humans. This has led researchers to take an interest in the study of the evolution, function and mechanisms underlying prosocial behaviors in animals. Such interest can be due in part to the fact that these behaviors have appeared difficult to explain under the theory of natural selection which emphasizes the role of competition in fitness and survival and predicts that individuals should behave to maximize their own benefit[8]. Prosocial acts where the actor does not receive any direct and immediate benefit are not fully predicted by the theory. However, researchers investigating prosocial behaviors in the field and the laboratory have obtained findings and advanced theories which can be integrated in the evolutionary framework. Currently, controlled experiments have been providing more evidence for the occurrence of prosocial behaviors in multiple taxa, including some species among non-human primates, birds, rodents, cetaceans, insects, and fishes[7,9–12], suggesting that prosociality may have deep evolutionary origins. This has triggered even more interest for elucidating how prosocial behaviors evolved, what are the motivations and the cognitive requirements to act prosocially and what factors lead to variation in the expression of prosociality within and between species, including the role of individual differences and that of the social context.

Despite the aforementioned definition being somewhat straightforward, multiple definitions have been applied to prosociality[13], reflecting the diversity of a natural phenomenon that is being studied across scientific domains, spanning the biological and psychological science. Whereas some definitions emphasize motives and intentionality, that is, the behavior is intended and thus require an intentional agent, others focus exclusively on the consequence and functional aspect, regardless of the behavior being intended or not, while other definitions combine both. It’s not surprising that some disagreement and debate has risen in the study of prosociality, especially when interpreting the potential causes and mechanisms of behaviors that appear similar in distant taxa, like the case of rescue behavior in ants and rats[11,14,15]. For the purpose of this thesis, I consider prosocial any behavior that benefit others, regardless of intentionality and whether it is altruistically or selfishly motivated. Considering the breadth of perspectives and levels of analysis with which researchers have approached this topic, together with the different forms and expressions that prosociality assumes, it seems reasonable to look at prosocial behaviors as a multidimensional phenomenon, associated with different proximate and ultimate explanations.

1.2 Ultimate and proximate explanations of prosociality

In biology, a distinction is often made between ultimate and proximate explanations for a behavior. Proximate explanations consider the physiological, structural, cognitive and contextual aspects of a behavior, aiming to elucidate how a particular behavior arises, in terms of its immediate causes. Ultimate explanations are concerned with the evolutionary history and function of a behavior, aiming to understand how it evolved and what it is for, focusing on its adaptive value in terms of reproduction and survival. These two levels of explanations are not alternative, but complementary and have been further extended by the ethologist Nikolaas Tinbergen, who pointed to four fundamentally types of questions faced in biology, which he expressed as 'causation', 'survival value', 'evolution,' and 'ontogeny', and that allow to gain an integrative understanding of animal behaviors[16].

Prosociality and cooperation are strictly linked, thus share models for their evolution and mechanisms. Researchers proposed different paths to the emergence and maintenance of prosociality and cooperation in animal societies, among which kin selection, reciprocal altruism, mutualism, and manipulation.[17]

1.2.1. Ultimate explanations

Kin selection

Kin selection is considered a leading theory for explaining how social behavior has evolved, offering a solution to the 'paradox' of altruism from a gene-centered view. It posits that altruism occurs more frequently between individuals who are genetically related. Animals that forego reproduction, and instead help others to breed, promote the reproduction and survival of their closed conspecifics and the transmission of shared genetic material to subsequent generations. By consequence, prosocial individuals will gain indirect fitness benefits. According to Hamilton's *inclusive fitness theory*[18], cost, benefit and degree of genetic relatedness can be connected to determine the likelihood of aid-giving another. Specifically, an altruistic behavior would be selected as a trait if the fitness cost for the benefactor were less than their recipient's benefit multiplied by their degree of kinship. Kin selection has been productive for explaining cooperation and altruistic behaviors in different taxa[19]. However, despite the large empirical evidence of the predictive power of relatedness in prosociality, individuals often behave prosocially towards non-kin when no apparent fitness benefit can be obtained, which remains a problem for the theory. That's where *reciprocal altruism* complements kin selection to account for the occurrence of prosocial behaviors among unrelated individuals.

Reciprocal altruism

Trivers proposed *reciprocal altruism*[20] to explain altruistic behaviors and maintenance of cooperation between unrelated conspecifics and cross-species individuals who assist each other repeatedly. Here, the cost of a prosocial act that temporally reduce a donors' fitness will be outweighed by the benefit given by recipients reciprocating in the future. An altruistic action would not ultimately be costly and would be driven by genetic self-interest. Thus, under certain conditions natural selection favors altruistic behaviors because in the long run they benefit the organism performing them. Even if Trivers named it reciprocal altruism, this is not altruistic in the pure sense but mutually beneficial, as it provides a direct fitness benefit. Therefore *direct reciprocity* has been considered a more appropriate term by some authors[21]. Alternating bouts of allogroming in social primates are one of the best documented cases of direct reciprocity[17]. Two other types of reciprocity have been proposed: one called *indirect reciprocity*, developed by Alexander[22], which predicts that individuals are more likely to help those who helped others; another one called *generalized reciprocity*[23,24], developed by Hamilton and Taborsky[23], which posits that individuals who have been recipient of an altruistic behavior are more likely to behave prosocially towards any individual, including the former benefactor. Support for the three types of reciprocity across various taxa has been growing[25–29], but remain rare compared to the levels of reciprocal, time-delayed exchanges between non-kins in humans, findings that some authors attributed to certain cognitive skills that would highly enhance the possibility for reciprocity[17]. Moreover, some authors pointed out that cases reported as examples of reciprocity among non-kins in animals may be explained better as cases of mutualism or manipulative strategies[17].

Mutualism and manipulation

Within or between-species mutualism occurs when cooperation produces immediate shared benefits that exceed the costs of aiding for the cooperators. In several animal societies, non-kins cooperate for foraging and hunting, defending mates and territories, building nests and shelters, achieving shared mutualistic benefits. Alternatively, prosociality and cooperation may arise from manipulative tactics through which individuals maximize their own fitness. Here, costs and benefits of the interaction tend to differ in magnitude between partners. For example, individuals may use coercion, by harassment or punishment, in order to force others to give them services or resources. In parallel, individuals may manipulate their own behavior acting prosocially toward conspecifics, with the consequence of obtaining protection, finding allies or mates, therefore promoting the establishment and maintenance of social relationships (see[17] for review).

In summary, prosociality in animals is more often observed between kins. Reciprocity among non-kins outside humans has been reported to a lesser extent, while mutualistic interactions and manipulative strategies, also observed more often in stable groups composed predominantly by kins, may explain some prosocial behaviors in animal societies[17].

1.2.2. Proximate explanations

In addition to evolutionary explanations, proximate mechanisms have been proposed to underlie prosociality, among which *empathy* and *social reinforcement*.

Empathy

Empathy has been considered a main motivator of prosociality. The German philosopher Robert Vischer invented the term *Einfühlung* ("feeling into") which was adopted and popularized by another German philosopher, Theodor Lipps, and translated into the English language with the word "empathy" by the psychologist Edward B. Titchener over 100 years ago[30]. Although it is difficult to find a common definition of empathy among researchers, there is substantial agreement that it can be defined, broadly, as the ability to perceive, share and understand the state of others. 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). Affective empathy tends to be associated to, and co-occur with, emotional contagion, when animals "shift, upon perceiving animals in an emotional state, their own affective state in the same direction"[31]. Unlike affective empathy however, the latter does not require the recipient of the emotion to be aware that the emotion originates in the other individual (social attribution), nor either individual to be aware of their emotional state[32]. Evidence of emotional contagion and basic empathic responses has been found in different taxa, indicating that animals can perceive, learn from and respond to the emotional states of conspecifics[32–36]. Findings of social transfer of emotional states in rodents are robust, at least when considering fear responses and negative emotions[37–47]. Frans de Wall proposed a Russian doll model, where empathy is considered as an umbrella term for vertical, sequential layers of increased emotional regulation and cognitive complexity (**Figure 1A**)**.** Here, doll's outer layers corresponding to cognitive levels of empathy and targeted helping build upon a simpler, older layer that he describes as the perception-action mechanism (PAM), according to which perception and actions share representations[48]. PAM directly links empathy with the nervous system suggesting that when subjects attend to the others' state, subjects' neural representations of similar states are automatically and unconsciously activated. Thus, de Wall argued that a basic form of empathy, based on emotional contagion, may be widespread across the animal kingdom and becomes more complex when combined with cognitive empathy (e.g., sympathetic concern, perspective-taking) in some species, allowing targeted helping. Though the doll's model appears elegant and simple and had inspired other researchers to investigate empathy in animals, its linear structure poses some constrains for the expression of certain phenomena, by assuming that some processes are prerequisites for other ones. For example, targeted helping would require perspective-taking, but studies reported helping behavior without evidence of perspective-taking, as discussed in[49]. Furthermore, the model implies that both perspective-taking and helping are built upon an emotional state-matching, but not all the phenomena under prosociality are affect-based and there can be understanding of other's needs without any emotional reaction. For instance, helping behaviors such as transferring to the partner out-of-reach tools are likely to involve some understanding of others' needs but less likely to entail an emotional aspect. Therefore, the Russian doll structure may hamper the study of each distinct phenomena under the umbrella of empathy. As an alternative to de Waal's model, Yamamoto proposed a combination model of empathy[49] (**Figure 1B**), made of three distinct but interacting concepts: "matching with others" (e.g., emotional contagion), "understanding of others" (e.g., perspective-taking) and "prosociality". Here, different

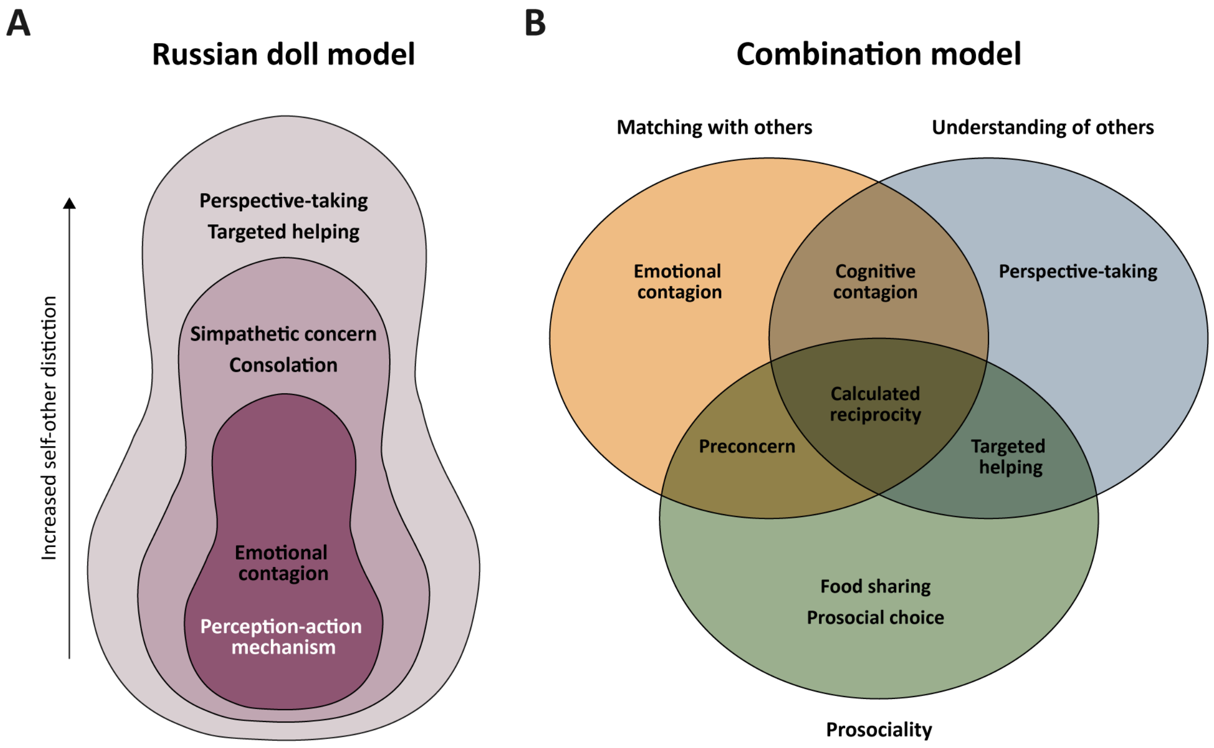

Figure 1. Models of empathy. (A) Russian doll model. The inner core is the Perception-Action Mechanism (PAM), which enables emotional contagion, i.e., a similar emotional state in the subject and the object. Outer layers of the doll build upon this hard-wired basis and reflect empathic phenomena marked by increased self-other distinction. Adapted from de Waal, 2008[48]. **(B) Combination model**. Empathy is organized around three major categorical concepts, namely 'Matching with others', 'Understanding of others' and 'Prosociality', which interact with each other. Empathic phenomena can be mapped onto one of three categories or their combinations. Adapted from Yamamoto, 2017[49].

phenomena can be mapped onto one of the three concepts or onto their combination. As a consequence, the absence of a sequential dependency allows to study the mechanisms of prosociality, emotional contagion and perspective-taking separately one from the other as well as how they interact between each other. Moreover, the model does not organize the different phenomena according to a strict increase of cognitive complexity, except for those mapped onto the overlaps between concepts. This allows to investigate certain phenomena in a given species focusing more on the functional relevance of that phenomena for the species ecology rather than its presumed degree of cognitive complexity. Therefore, some prosocial behaviors, may not require sophisticated cognitive capacities if they are part of the species' behavioral repertoire and the context is salient. Nevertheless, both models assume that targeted helping, for example, is accompanied by perspective-taking, possibly inducing researchers to conclude that empathic processes are involved and drive the motivation to help whenever helping behavior is observed in the experiments. However, demonstrating that animals share others' affective state and that there is causal link between the others' state and prosocial behavior would require stringent experimental controls that are rarely implemented, as discussed in Vasconcelos et al[50]. Moreover, some authors point out that inferring empathy-induced prosociality based only on behavioral measures remains problematic and recommend to also assess the physiological correlates of behavior and valence related measures for a better access to the affective basis of empathy-related phenomena in animals[51].

In summary, not all prosocial behaviors require or are motivated by empathy. However, empathy may have evolved in some species where it acts as a facilitator. Indeed, human studies have shown that empathy positively predicts prosocial behavior[52,53], sustaining the hypothesis that prosociality does depend on the ability to match the state of the potential recipients. This ability is thought to be affected by biological, social and contextual factors, such as sex/gender, the degree of social closeness, and the level of distress/need of the potential recipients. In nonhuman animals, empirical evidence of a positive association between empathy and prosociality is still scarce, due in part to the challenge of measuring empathic motivations. Nevertheless, studies on consolation in animals point to empathic processes[54,55] and there is evidence that some factors may similarly modulate emotional contagion and prosocial tendencies in some species. For example, familiarity seems to modulate emotional contagion in mice[45,46] and an in-group bias has been reported for rescue behavior in rats[56] (see also **1.4**).

Social reinforcement

While higher empathy may promote prosocial behavior, facilitating perception and sharing of others' state, the rewarding effect contingent on a prosocial act may be a parallel proximate mechanism that, by itself, sustains and reinforces prosocial behaviors in the long term. This may also explain why, especially in humans, prosociality often occurs spontaneously, without the potential recipients necessarily displaying need or distress. In reinforcement learning, action-outcome contingencies are learned through reinforcements. A positive reinforcement (e.g., reward) increases the likelihood that an operant behavior is repeated in the future, while a negative reinforcement (e.g., punishment) decreases the likelihood. Some authors suggested that prosocial preferences (or other-regarding preferences) can be studied under the framework of *social (*or *vicarious) reinforcement learning*[57,58], according to which social stimuli contingent on outcomes to others act as reinforcers on subjects' behavior. Behaviors leading to positive social outcomes will be reinforced through appetitive/rewarding stimuli from the partner, while those leading to negative social outcomes will be less likely to be repeated, being associated with putative aversive feedback. Positive and negative reinforcers are not mutually exclusive and can act together to influence behavior. Along this line, research with adults and children indicate that different types of prosocial behaviors are experienced as rewarding[59] and induce activation of brain areas commonly associated with reward processing[60], suggesting shared neural substrates for social and non-social reward (but see[61]). In monkeys, allogroming influences the release of ß-endorphin both in the recipient and the actor, accompanying a hygienic activity with a hedonic experience[62]. In rats, dopamine release in the nucleus accumbens, is modulated when observing a conspecific receiving reward[63], a possible neural correlate of vicarious reinforcement, and in response to playback of 'affiliative' 50-kHz ultrasonic vocalizations (USVs)[64], suggesting that positive outcomes to others and certain social signals may indeed have a self-rewarding effect. Studies using instrumental learning paradigms in different species have shown that animals, even if not consistently, display prosocial preferences, by learning actions and making choices that prevent damage or provide positive outcomes to conspecifics (see **1.3**). This suggests that benefitting others have a reinforcing value which may depend on brain structures involved in emotional contagion (see[32] for review). However, less details are known about which and how different kinds of social cues (visual, auditory, olfactory, tactile or their interaction) promote prosocial decisions in these paradigms.

1.3 Paradigms to study prosocial behavior in animals

Different paradigms have been developed to assess specific prosocial behaviors (see[32,65,66] for review), where the variety of task designs and experimental contexts has led to a large variation in reported levels of prosociality within and between species. For the purpose of this thesis, I will describe here some paradigms for measuring animals' prosocial tendencies towards a conspecific, involving reward provision and relief of others' distress (**Figure 2**) and outline some of the findings as examples.

1.3.1. Reward provision

Reward provision paradigms have been initially implemented for non-human primates to investigate the phylogeny of human prosociality and successively extended to some other taxa in recent years.

Instrumental helping

In instrumental helping experiments, animals are usually tested in pairs. One subject, the "actor" or "focal", is tested for its propensity to help a recipient partner in a problem it cannot solve alone. In some versions of this paradigm (*object transfer*), the actor can hand the recipient an out-of-reach tool that it uses for getting a reward (**Figure 2A**). In other versions (*obstacle removal*), the actor can perform an action to remove an obstacle (e.g., opening a door or releasing a peg), which prevented the recipient to access the reward. Actor's behavior is considered altruistic as being low-cost and not rewarded.

It is assumed that this kind of paradigm is cognitively demanding, requiring some degree of perspective-taking, because the actor has to infer the need or goals of the recipient in order to help. Among primates, most of the work of instrumental helping has been conducted with our closest

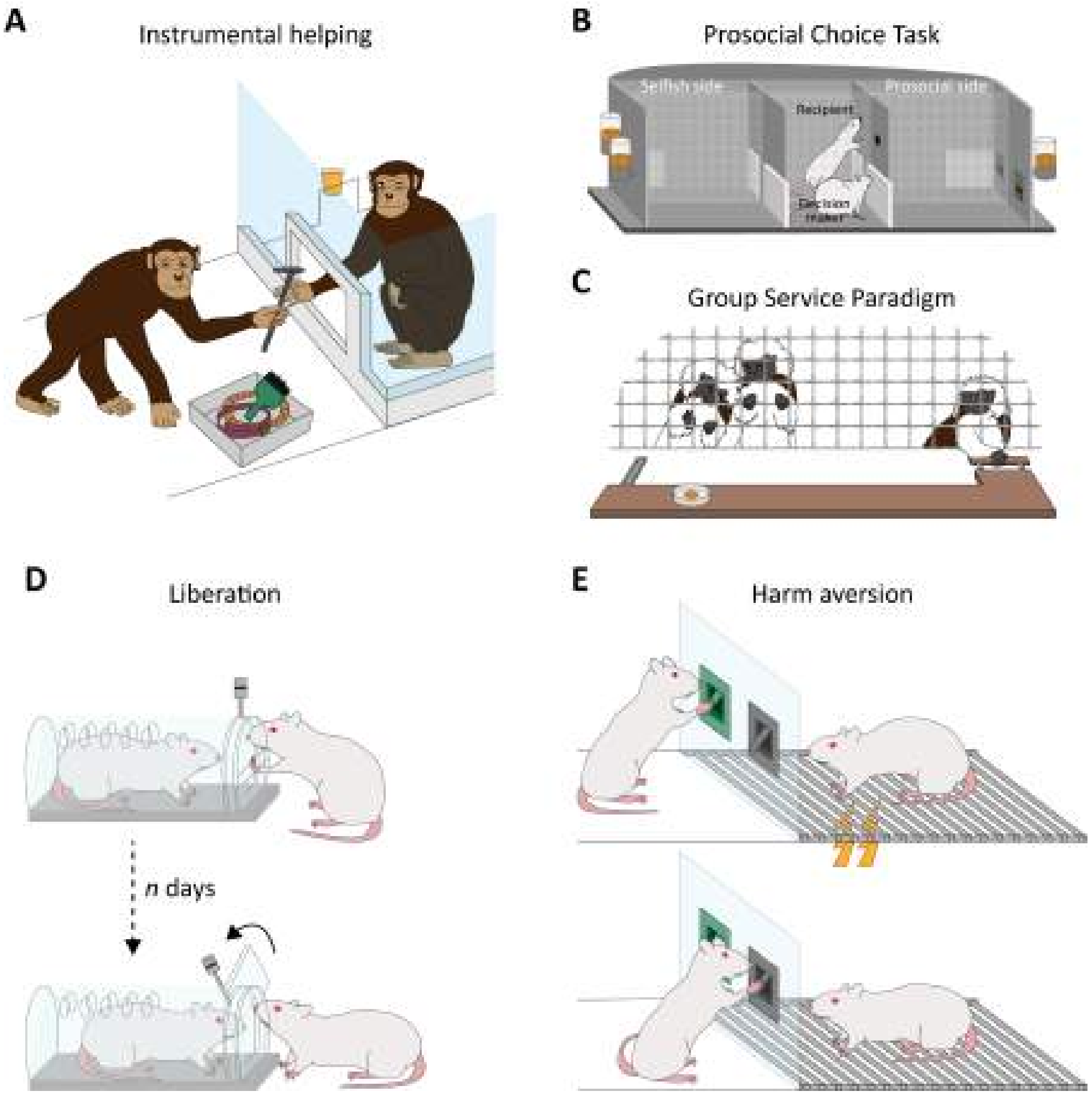

Figure 2. Example of paradigms to study prosocial behavior. (A) Chimpanzees help a partner to obtain a reward by transferring to it an appropriate tool selected from a set of objects. *Adapted from Yamamoto et al. 2012*. **(B)** Decision-maker rats prefer to enter the side of the maze providing a reward for themselves and a recipient rat than the side providing a reward for themselves only. **(C)** Cotton-top tamarins pull and hold a handle, allowing a group member to take a reward from a baited tray. *Adapted from Burkart et al. 2014*. **(D)** Across days, rats learn to open a door allowing a conspecific to escape from a restrainer. **(E)** During individual training, rats develop a preference for either one of two levers delivering reward. If that lever (green) is later made to deliver self-reward as well as footshocks to a conspecific (top panel), rats shift to press the alternative non-shock lever (grey, bottom panel). *Adapted from Keysers et al. 2022.*

relatives the great apes, providing evidence that under some circumstances they help others achieve their goal. For example, chimpanzees are able to hand a recipient an appropriate tool among a set of objects, according to the partner's need[67]. Both chimpanzees and bonobos are capable of opening a door allowing the recipient to enter a room with reward[68,69] and to release a hanging food that their partner tried to reach[70–72]. This is usually taken as evidence of the higher socio-cognitive abilities of great apes and as support for the hypothesis that they understood others' goals, which would allow them to engage in targeted helping. At the same time, chimpanzees help mostly when recipients display behavioral displays of intentions (e.g., "request"), suggesting a *reactive* prosociality, based on clear indications of others' needs, rather than a *proactive*, unsolicited prosociality. Along this line, some authors have advanced a "*signalling hypothesis*", according to which recipients' communicative signals in the form of attention-getter or request are salient information that allow animals to be more responsive to others' needs[70].

Studies of instrumental helping in other species outside great apes are still scarce. Negative findings have been reported for Capuchin monkeys (*Cebus apella*)[73]. In dolphins findings are mixed[74,75], and among birds there is positive evidence for helping based on tool/token transfer in African grey parrots (*Psittacus erithacus*)[76], Goffin's cockatoos (*Cacatua goffiniana*)[77], but not for Crows and Azure-Winged Magpies[78]. Rodent studies with similar task designs have been applied mostly when assessing reciprocity[25,26]. However, there is evidence from our lab that the recipient's attempts to reach the food are necessary for the emergence of prosociality in reward-based task[79] (see below). More studies are certainly needed to reach firm conclusions in these species and negative findings should be taken with caution as they may be due to animals' failure in understanding the task.

Prosocial Choice Task

Another widely used paradigm to study prosociality is the *prosocial choice task* (PCT)[80], which measures other-regarding preference for reward distribution. In this task, subjects are usually tested in pairs and are placed in adjacent compartments. The focal animal is the decision-maker, who can make a choice between two available options presented in each trial, determining the reward payoff for itself and a recipient partner. Choosing the *prosocial* (or mutualistic) option makes each animal gain a single reward, while choosing the *selfish* option provides a single reward for the decision-maker only. The prosocial choice is also referred to as 1/1 (one reward for the focal and one recipient), while the selfish as 1/0 (one reward for the focal and none for the recipient). Thus, the decision-making does not imply a cost or added-self benefit for the focal that always receives the same reward with the same effort, while affecting the outcome of the recipient. Generally, the proportion of trials on which animals make a prosocial choice when the recipient is present (test condition) is compared to that shown in a control condition, when the recipient is absent (nonsocial control) and/or a control condition where the recipient is present but unable to access the food (social facilitation). If animals choose the prosocial option significantly more often in the test than in the control condition, they are said to have a *prosocial preference*, which is taken as demonstration of their sensitivity to others' welfare. Variations of this task include a token version where subjects can choose between tokens that are exchanged with food items[81], designs using low and high-quality food[82], and a 0/0 vs 0/1 design[83] where the focal can choose between an action in which no one benefit versus one that gives reward to the recipient only (null versus altruistic choice). Subjects' roles remained fixed or can be reversed over sessions (the focal becomes the recipient and vice versa) to assess the emergence of reciprocity[75]. It is assumed that the PCT does not necessarily require an understanding of others' goals and needs[49] but does imply a certain degree of cognitive load because subjects have to track multiple reward outcomes simultaneously.

Again, most of the PCT studies have been conducted with primates. In contrast to positive findings from instrumental helping, chimpanzees and bonobos were often found to be indifferent in the PCT[80,84–89]. It has been suggested that food visibility would hamper prosociality in these animals due to competitive tendencies for food resources when these are limited. Indeed, in a token version chimpanzees behaved prosocially, with recipient's attention-getting behavior associated with increased prosociality by focals[81] (but see[90] for a critical review of how simpler mechanisms, such as associative learning, may explain the same results). Although this can be possibly the case, experiments reporting negative results could be alternatively explained by the animals not completely understanding the contingencies of the task. Unfortunately, not all studies implement a knowledge control where the focal experiences the outcomes of the recipient for example by accessing food trays in the recipient's position[66], or is asked to modulate its choices depending on reward contingencies, which would be necessary to demonstrate that this was indeed the case.

In other primates, positive findings come from long-tailed macaques[91,92], rhesus macaques[93], capuchin monkeys[82,94,95] (but see also[86,87] for negative results), common marmosets[83,96] and cotton-top tamarins[97]. In conclusion, although there is still controversy on how widespread prosociality is in different species of non-human primates, and whether negative results reflect differences on behavioral setups or differences on the ecology of each species, the consensus is that non-human primates display tendencies for prosocial behavior in this task.

Hernandez et al. 2015[98] and Márquez at al. 2015[79] were the first to adapt the PCT for laboratory rats, showing evidence that they behave prosocially, providing food to conspecifics without added-self benefit. Both studies made use of double-T maze design, one maze for the focal and one for the recipient rat. In each trial, the focal rat could choose to enter either the "prosocial" side of the double maze where both animals are rewarded, or the "selfish" side where it gets reward for itself only. In the Hernandez et al. study, the focal made its decision in a starting area where the recipient is not present and then enters either one of the two reward areas where it experiences the outcome of its partner. In this condition, rats developed a slight preference for the prosocial choice at group level (55%). In the Marquez et al. study, the focal made its decision in a choice area where it witnesses the recipient performing an action indicative of food-seeking behavior (i.e., repeatedly nose-poking on one side of the maze in an attempt to open an automatic door and reach the food, **Figure 2B**). Focals could choose to be prosocial, by poking on the same side of the recipient, or selfish, by poking on the opposite side. Focals' choice caused the opening of the automatic doors in the same side of both mazes, allowing both rats to enter either one of two reward areas where they experience each other's reward outcome. Thus, Márquez's study combines the standard PCT with elements of instrumental helping (obstacle removal). Results showed that rats acquired a prosocial preference close to 70% at group level, while they were indifferent in a control experiment where recipients were prevented from displaying food-seeking and could reach the food only after focals' choice (**Figure 3A-B**). This indicates that cues merely related to reward delivery, such as chewing by the recipient, were not sufficient to drive prosocial choices. One can argue that recipients' display of preference may function as local enhancement, attracting focals' attention to the prosocial side and thus increasing prosocial choices as a by-product of "going where the other goes". However, the study provided an additional control experiment showing that if recipients displayed food-seeking on one side but received reward on both sides, focals did not just follow their recipients (**Figure 3A-B**). This indicates that recipients' food-seeking was necessary but not sufficient for developing prosociality, with rats being also sensitive to the reward contingencies. Moreover, it excludes local enhancement as driver of prosociality in the study. An alternative explanation is that recipients' food-seeking behavior translates into signalling need, motivating focals to behave prosocially. This fits with the finding that, in the absence of recipients' attempts to reach the food, rats develop no prosocial bias or only a slight one which takes longer times to emerge, as in Hernandez et al. 2015. Thus, food provisioning in rats may resemble more a reactive than proactive prosociality. The specific mechanism(s) - e.g., sensory cues, social interaction - through which the recipients' food-seeking modulated focals' decisions remained to be addressed. Using a PCT with a different design (lever-pressing double operant box), a recent study with rats by Kentrop et al., also reported evidence of an overall prosocial preference, although large variability was present[99].

Recent works have started to evaluate prosocial tendencies in laboratory mice, which would suppose a great advantage for the dissection of neural circuits, and would expand our knowledge on how conserved this trait in different rodent species is. Scheggia et al.[100] reported that animals developed a prosocial bias compared to a nonsocial control group, with 11 out of 16 mice (69%) of the test group classified as prosocial[101]. However, unpublished work from our laboratory does not replicate this observation, being the proportion of prosocial animals much lower (17%) (Esteve-Agraz & Márquez, to be submitted). Whether these differences rely on differences on behavioral procedures or setup remain to be established.

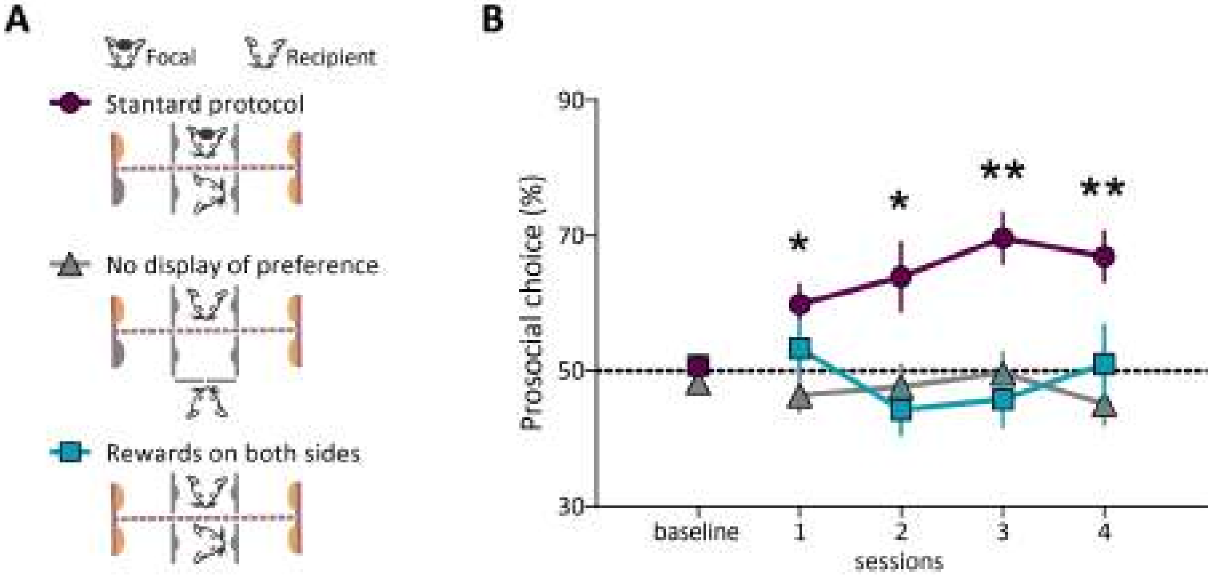

Figure 3. Prosocial choices in rats depend on food-seeking behavior displayed by recipients. (A) Schematic views of the different protocols used by Márquez et al. to disentangle the factors driving prosocial behavior for reward provision in rats (see '*Prosocial Choice Task*' in 1.3.1). In each view, orange semicircles represents food delivery. In the 'standard' protocol, the focal makes the choice while the recipient displays food-seeking behavior by nose-poking on its preferred side. In the 'no display of preference', the focal makes the choice while the recipient is prevented from displaying side-preference. In the 'rewards on both side', the recipient displays its side preference, but it is equally rewarded on each side. **(B)** Results from the three protocols show that the recipients' food-seeking behavior is necessary but not sufficient for the emergence of a prosocial preference over four consecutive daily sessions. *Adapted from Márquez et al. 2015.*

The PCT has been used in few other taxa. Jackdaws, *Corvus monedula*, don't show a prosocial preference *per se* but as a result of local or stimulus enhancement, since they benefit recipients only when the latter first showed interest at the location where food is available[102]. Dolphins have been proposed to show spontaneous prosocial tendencies[75,103], but strong conclusions are premature given the variability and the limited sample size. Among fishes, positive evidence has been shown by a study on *Amatitlania nigrofasciata*, a monogamous cichlid with biparental care, whose male individuals display prosocial choices providing food to their female mate[12]. In a 0/1 vs 0/0 design, pet dogs behave prosocially with a bar-pull apparatus[104], but not in a touch screen task, where wolves outperformed their domestic counterpart[105].

Group Service Paradigm

The *Group Service Paradigm* (GSP) was designed to measure proactive prosociality and social tolerance in group contexts across species[106]. Subjects can deliver reward to a member of their group but not to themselves, by performing a low-cost action, or not deliver reward at all. Therefore, the GSP has also been referred to as single-choice (go) task[66]. It was first presented in the study by Burkart & van Schaik[106]. Here, non-human primates of a social group are in their homecage and can pull the handle of a food-baited board outside of their enclosure to make food within reach of other group members (**Figure 2C**). The individual pulling the board cannot retrieve the food for itself and must hold the handle in order for a partner to retrieve the food. It is thought that this paradigm improves the socio-ecological validity of the experiments because it can be applied in both laboratory and zoo environments, and it allows subjects to freely choose who benefits. Moreover, it is considered less cognitive demanding than the standard PCT because animals have to track one reward item only. On the other hand, it has less statistical power since data points are represented by species or groups rather than dyads.

A comparative study testing fifteen primate species on the GSP, including humans, showed that cooperative breeders, such as marmosets and tamarins, behaved more prosocially than independent breeders, and the level of allomaternal care was the best predictor of prosociality across the species[107]. Cooperative breeding is a social system where the care of offspring is provided not only by the mother, but also by other members of the group, e.g., the father, siblings, uncles, aunts, and sometimes unrelated individuals. On this line, some authors sustain the *cooperative breeding hypothesis* (CBH)[108], according to which extensive allomaternal care is accompanied by socio-cognitive changes, such as increased social attention towards conspecifics and propensity to share resources which, by side-effect, would increase performance in prosocial tasks. Cooperative breeding is present in species among mammals, birds, fishes and insects. It occurs in less than 1% of mammals, primarily in primates, rodents and carnivores[109]. In birds, a study comparing eight corvid species on the GSP also reported a positive association between cooperative breeding and prosocial behavior[110], providing additional support for the CBH, while no studies have been conducted in rodents. However, debate remains about the role of cooperative breeding in prosociality and social learning across the animal kingdom, what precisely the cognitive consequences of cooperative breeding are, and whether humans can be considered cooperative breeders[111–114].

1.3.2. Relief of others' distress

In another class of paradigms, animals are tested for their propensity to relief others' distress, by liberating trapped conspecifics or avoiding harming them.

Liberation

In laboratory rodents, paradigms have been developed to assess if animals help conspecifics to escape a situation of stress. 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[115]. More recently, Bartal et al. developed a door-opening paradigm where a free rat in an arena is tested for its tendency to liberate a conspecific trapped in a restrainer tube[14] (**Figure 2D**). Results showed that, over several days of testing, rats learned to open the door of the restrainer and free their cagemates, even when they could not interact with them, but did not open an empty or toy-containing restrainer. When rats could free the partner or open another restrainer with chocolate, they opened both restrainers and ate the chocolate together in half of the trials, suggesting that rats attributed value to freeing the trapped conspecific. Moreover, a follow up study found that rats receiving anxiolytic treatment showed reduced rescue behavior but continued to open the restrainer with chocolate, suggesting that helping the partner requires some degree of arousal[116]. For the authors, the most parsimonious explanation is that rats free their cagemates in order to end either their own distress or that of the trapped animal. Thus, rats' helping behavior would be motivated by empathy (emotional contagion). In the same direction, but using a different paradigm where animals are soaked in water, Sato et al. showed that free rats learned to open a door, allowing the soaked partner to reach a safe area. Door-opening occurred mainly towards distressed partners and not towards rats that were out of the water, suggesting that the behavior is based on empathic processes[117]. Moreover, a following study showed that liberation decreased after local injection of oxytocin antagonist in the anterior cingulate cortex[118], a brain area involved in emotional contagion[43,119]. Studies using these paradigms in rodents continue to grow[120,121], showing that animals learn actions to liberate their partners but often reaching different conclusions and proposing alternative motivators for the rescue behavior, such as a desire for social contacts or interest in the apparatus[122–131]. Thus, debate remains about the motivations behind this form of helping and more investigation is needed to ascertain its empathic nature in rodents[50]. Interestingly, helping behavior has been studied in detail in some species of ants, which use precise rescue behavior exclusively directed towards nestmates, i.e. genetically closer, but not towards conspecifics of a different colony[11] and which is known to be based on pheromone release as eliciting stimulus.

<u>Harm aversion</u>

In harm aversion paradigms, animals are tested for their propensity to prevent others' distress. For example, in a study where laboratory rats[132] can choose to press either one of two levers which provide equal amount of food for themselves, but one lever additionally induces a footshock to a conspecific (**Figure 2E**), they decreased their usage of the preferred lever when it delivers shocks, even when this one provides the double of food or requires half of the pressing effort, compared to the non-shock lever. Interestingly, the behavior reflected large individual differences, with less than half of the rats showing consistent harm aversion. In addition, rats continued to press the shock lever when it was accompanied by the triple of food than the alternative lever, indicating that prosociality has a limit contingent to reward allocation. This suggests that for some rats causing harm to others acts as negative reinforcer and is subject to a cost-benefit evaluation when coupled with reward or effort. Moreover, inhibition of neural activity in area 24 of the anterior cingulate cortex abolished harm aversion in rats, indicating that the avoidance behavior is supported by brain structures involved in emotional contagion.

In summary, paradigms measuring prosocial behavior show evidence that animals, in some circumstances, prefer choices and perform actions that benefit others, by providing them with reward or relieving their distress, suggesting that prosocial acts might be reinforcing. It is clear that there is between- and -within species variation in the expression of prosociality: not all the tested animals are prosocial and the levels of prosociality show variability. Understanding which factors determine such variability and how they shape the behaviors of the subjects is therefore important to advance the field of animal prosociality, and social cognition more generally. While part of the variation derives from aspects mentioned above, i.e., - methodological differences, visibility of the food, presence or absence of signaling from the recipient, cooperative breeding - other biological and social factors have been proposed to enter into the equation.

1.4 Modulators of prosocial behavior: the role of familiarity, sex and social dominance

Several factors have been proposed to modulate prosocial behavior, including the sex of the individuals, the familiarity of the recipient(s) and the dominance status. However, empirical work incorporating systematic comparisons to determine how these factors affect prosociality, especially in reward-provisioning contexts, is still limited in the literature. Here, I outline some of the effects that have been observed.

<u>Sex of the individuals</u>

It is commonly assumed that females are more empathic and prosocial than males. This reflects the hypothesis that empathy in mammals has evolved as direct consequence of offspring care[48], which is primarily provided by the mother in most mammalian species. The idea is that evolutionary forces shaped females' biology and psychology promoting sensitivity to infants' affective state, nurturance behavior and social attention, which may have been cooped and extended to other conspecifics. From this point of view, sex difference in prosocial behavior and empathy should be traced back to an evolutionary history of maternal care[133]. In humans, experimental economic games and naturalistic data of charitable giving and volunteering reveal a tendency for female individuals to be more altruistic and score higher in self-report measures of empathy compared to males[133], but the literature emphasizes that the effects of sex on empathy are a complex phenomenon to analyze and sometimes different results have been found to depend on the methods used to measure human empathy (for instance, self-report versus measures based on physiology, face expressions, gestures and alike)[134]. Moreover, evolutionary theory is not the only one advancing explanations for sex differences in human social behavior. For instance, social role theory adopts a biosocial perspective which describes how gender-differentiated behavior are produced primarily by the distribution of men and women into social roles within their society[135].

In nonhuman animals, sex-differences in prosociality were more often considered in situation involving distress. For instance, from a database of spontaneous consolation in chimpanzees it was observed that females were more likely to offer consolation to recent victims of aggression compared to males[55], a trend also found among captive western gorillas[136], suggesting higher responsiveness to distressed others. In the liberation paradigm where rats can open the door of a restrainer to free a trapped cagemate, all female rats became door-openers in contrast to two thirds of male rats[14], which again would suggest that females are more likely to engage in prosociality towards distressed others; however, there is to consider that the size of the male sample in the study was four time larger. In a work investigating emotional contagion in rats, males and females in the role of observers reacted with the same amount of freezing to a given degree of freezing of a same-sex demonstrator[42], indicating that social transfer of distress is similar across the two sexes; that is, male and female rats express similar levels of emotional contagion. This is consistent with the finding that sex does not modulate harm aversion in rats, where females and males are equally prosocial in preventing others' distress[132]. When using food provision paradigms, despite extensive work with nonhuman primates, little investigation has been devoted to sex differences in these animal group when prosociality was found. One reason can be simply the small sample size which makes the comparison between groups more difficult. In the PCT study by Kentrop et al., male but not female rats developed a prosocial preference compared to chance, when facing a familiar, same-sex conspecific[99]. However, the study does not directly compare the level of prosociality between male and female pairs (by including sex of the pair as between-subjects factor in the statistical analysis), which would complement the results. Therefore, it remains unclear if prosocial choices for reward provision in rats emerge in equal manner in male and female dyads. In mice, the PCT study by Scheggia et al.[101] shows that male but not female pairs of familiar mice display a prosocial preference at the group level. They also show that prosociality in male pairs is significantly higher than that in female pairs across the testing sessions.

In conclusion, the role of sex differences in the emergence of prosociality evaluated in the different paradigms is not yet providing a clear picture, being further studies necessary to evaluate whether sex determines prosociality and if so, in which specific contexts.

Familiarity of the recipient

Some species of animals form social relationships with different degrees of selectivity and stability, by displaying preferential proximity and affiliation within their groups. Therefore, familiarity is thought to influence the expression of prosocial behavior, which would be more frequent among those individuals who are socially close and share a bond. However, the behavioral and physiological mechanisms underlying this influence are not completely understood. Moreover, familiarity may or may not have effects depending on the type of prosocial behavior under study.

Among children, prosocial responses are generally facilitated when the recipient is a friend compared to when the recipient is a known individual but not a friend[137–140], underlying the role of relationship quality. The effect of familiarity seems robust on consolation behavior directed towards distressed others, which was found to be expressed more often among close social partners, including mates and siblings, when assessed experimentally or by observation in different taxa[35,54,55,141,142]. In experiments with laboratory rodents, the effect of familiarity is often examined by considering as familiar animals those living in the same cage (cage-mates) and as unfamiliar those living in different cages (non cage-mates). When tested on the liberation and harm aversion paradigms[14,132], rats were found to behave prosocially towards conspecifics of the same strain, weather they were familiar or unfamiliar. This suggests that familiarity of the recipient does not influence rescue behavior and harm aversion in rats. However, the liberation paradigm also revealed that rats' early experience with others' strain was important for helping, since they released rats of strains with whom they were reared but not rats of strains they never met, even if it was their own genetic strain[56]. This indicates an in-group bias, where strain familiarity rather than individual familiarity modulates rats' rescue behavior of distressed conspecifics. The findings are interesting because an in-group bias for higher empathy, prosocial behavior and egalitarianism has been reported also in studies with human adults and children[138,143].

Less is known about the effects of individual familiarity on prosociality in reward-related contexts. Some studies of food provision with non-human primates, including, capuchin monkeys, cottontop tamarins and rhesus macaques, suggest a positive association between prosociality and social closeness (see Cronin, 2012[144] for review), but the limited sample size precludes strong conclusions. Mice tested on the PCT display more selfish choices when facing an unfamiliar than a familiar recipient[101], but the behavioral correlates underlying this effect remain unknown. Among rats, only familiar male dyads have been tested on the prosocial choice task so far[79,98,99]. Thus, whether rats develop a preference for mutual reward towards an unfamiliar conspecific remains to be investigated. According to the primate and mice literature on food provision tasks, we hypothesized that rats would display prosocial choices more frequently towards a familiar partner.

Dominance status

Dominance is a structuring feature of many animal societies, including humans, becoming a fundamental concept in social ecology and psychology. The origin of this concept can be traced back to the observations of domestic hens' pecking behavior that the Norwegian zoologist Thorleif Schjelderup-Ebbe made during his childhood. By collecting data on the patterns of aggressive interactions, he observed that hens were pecking each other during feeding competition, but not in an equal manner: some hens were "despots" pecking all the others, whereas other hens were pecked by all the others. Years later, in 1922, he published his observations[145] and described a "Hacklist", translated into English as "pecking order"[146], which explains how the hens could be ordered by their ability to peck other members of their group. The pecking order is now known as the first formally described example of *dominance hierarchy (*or *social dominance)*. Since then, an increasing number of studies have revealed how many species are structured by some types of dominance hierarchy[147,148] and shown how this influences important aspects of animal life, including survival, reproductive opportunities, access to resources, health and social behavior[149–153]. Although the early work by Schjelderup-Ebbe implies that dominance hierarchy arises from asymmetries in agonistic interactions, the concept of dominance has been defined in various ways since then, which is reflected in the different approaches used to study it. According to some definitions, dominance is an absolute attribute of the individuals, such as aggressiveness or size, whereas according to others it is an attribute of the relationship between individuals, which involves agonism but not necessarily aggression. In a review of the definitions, Drews[154] discusses dominance and offers a synthesis which serves as a basis for our current understanding of the concept: "dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterized by a consistent outcome in favor of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate" (p.283). This description views dominance as a relative measure rather than an absolute feature. A dominance hierarchy emerges when the same two individuals meet on several occasions and resolve conflicts in asymmetric pattern. In addition, Drews clarifies interrelated concepts. *Dominance status* refers to the status of an individual in a given dyad, being either dominant or subordinate according to the outcomes of several contests. *Dominance rank* refers to the position of an individual in the hierarchy. In large groups, certain individuals will be dominant to some and subordinate to others, therefore the terms "high-ranking" and "low-ranking" are more appropriate in a group context than dominant and subordinate. Finally, dominance does not necessarily imply leadership or control. The subordinate may control or influence aspects of the dominant's behavior, such as the direction of movement, without altering the dominance relationship[154]. The strength of the dominance may vary across dyads of the group, with some dyads expressing strong, stable asymmetry in their competitive interactions, while others having weak, unstable asymmetry[155]. Nevertheless, although dominance is primarily concerned with dyadic contests, ascending or descending in rank is associated with phenotypic changes and altered gene expression, leading high- and low-ranking individuals to show differences in behaviors, physiology and neural functioning, which in turn influence cognitive processes[151,152,156].

Several studies have shown that dominance hierarchies are largely stable and organized linearly in animal groups[157]. Maintaining a stable dominance hierarchy is advantageous especially in small groups with consistent membership, in that it allows animals to avoid new conflicts, minimizing risks and costs of aggression and saving energy. It emerges that, dominance relationships allow group-living animals of different taxa to successfully navigate social interactions over the long period. For instance, stable social relationships in flocks of zebra finches improves the coordination and synchronization of their group foraging[158]. Hierarchies are maintained by social dynamics given by different behaviors expressed by dominants and subordinates over their encounters as well as by signals of individual identity and dominance[157]. More broadly, *social hierarchies* may not be based exclusively on dominance. In humans for example, prestige-based status, which arises from access to information in the form of knowledge or skills, contributes along with dominance to the attainment of social rank in children and adults, impacting social influence and fitness[159].

Given the role of dominance in organizing the social environment, several studies have often examined the effects of dominance relationships on social behaviors, including prosociality. Some types of prosocial behaviors, such as allogroming, may be more often directed “up the hierarchy”, meaning more likely to occur from subordinate to dominant individuals, which may allow the former to increase social tolerance or gain access to resources[10]. Interestingly, it has been shown that dominance status modulates prosocial behavior in some non-human primate species tested on food-provision paradigms, where prosociality is more often directed “down the hierarchy”, meaning more likely to occur from dominants to submissives[58,91,92,95,160]. A similar trend has also been reported in male pinyon jays tested for food sharing[161]. However, little is known about the behavioral correlates underlying such directional effects[144]. For instance, is not clear if dominance status affects recipients’ interest in the reward or their attention towards the helper, or if it modulates helpers’ responsiveness to their recipient, which could be addressed by studying in more detail the behaviors of the interacting subjects in the tasks.

1.5 The Norway rat

Rattus norvegicus, also called “brown” rat or the Norway rat, is one of over 60 species in the mammalian genus *Rattus*. The wild Norway rats now inhabit many terrestrial environments on Earth and can be found living near most large human communities. They jointly dig underground burrows with shared tunnels and chambers, where they sleep, breed, build nest sites and storage food, away from potential predators. If handled from an early age, wild rats can be tamed[162], reducing aggressive and defensive behaviors towards humans. Their domestication, which started in the 19th century, has produced many inbred and outbred lines which differ from their wild counterparts in some aspects of behavior, as a result of selective breeding and adaptations to artificial environments. Nevertheless, domesticated rats, which thrive in captivity, share similarities with the wild ones and have been shown to survive and reproduce under semi-natural conditions[163–165]; they cannot be considered a subspecies. All laboratory rats, including the Albino rat, derive from *Rattus norvegicus* after selective breeding. Together with another member of the Muridae family, the house mouse (*Mus musculus*), laboratory rats have been used widely in research and have become an essential model in different scientific fields, including behavioral neuroscience.

The Norway rats (hereafter referred to as *rats*) are primarily nocturnal, displaying high activity mostly in the first hours after sunset (or laboratory equivalent). Compared to diurnal mammals, they do not count with a high developed eyesight, which is based on dichromatic color vision; in contrast, touch via facial whiskers (or vibrissae) and smell constitute main sensory inputs. In fact, olfaction plays an important role in their sensory world, guiding foraging, exploration and social recognition[162]. In addition, they are influenced by high pitch sounds and emit ultrasonic vocalizations (USVs) which are part of their communication system. This has been shown mainly in laboratory animals, while less information is available for wild ones. Adult rats' USVs have been classified into two main distinct classes, based on their frequency range: the 22 kHz or ''alarm calls'' (18-32 kHz, 300-3000 ms) and the 50 kHz calls (35-80 kHz, 10-150 ms)[166]. The former have been found to be emitted in situations of danger and stress, for instance when detecting a predator in presence of cospecifics[167], during fear learning[168], and in intermale defeat, probably as display of submission[169,170]. They are thought to reflect a state of anxiety or defense and may have different communicative functions depending on the situation. In contrast, the latter have been linked to several features of rats' behavior, including locomotor activity[171–173], reward anticipation[174], mating[175,176], play[177], and affiliative social contacts[178]. They are thought to reflect appetitive situations and a positive state. While the 22-kHz USVs are monotonous, the 50-kHz USVs can be highly modulated in their frequency and have been categorized often into four main classes: flat, trills, step-trills or other frequency calls[44].

Rats are explorative, a trait that has been framed into the dichotomy between exploration/exploitation in foraging contexts. Exploratory behavior of laboratory rats is easily displayed as spontaneous alternation in T- or Y- maze, where they tend to alternate the side visited at each run, especially when the time between runs decreases. Experiments shown that, in such exploratory behavior, animals are alternating on successive choices as a tendency to vary the sensory stimulus received, which underlies the importance of novelty[179,180].

Social relationships among wild rats are variable. They live in large colonies which may count more than 150 individuals, consisting of groups of pairs, harems with or without offspring, unisexual groups and/or single males and females, occupying different territories. Groups may comprise overlapping generations, allowing frequent interactions with differently familiar and related individuals. There is no evidence that adult wild rats form stable pair bonds. Females tend to be philopatric, that is, to remain and breed in their natal group, while male disperse more, although dispersal rates are relatively low. Males patrol and defend their territory from male intruders of other colonies, displaying aggression or signals of fights which in some cases would lead the intruder to retreat. Such resident-intruder aggression indicative of territoriality is also manifested by laboratory rats, but attacks are more immature given their reduced aggressive tendencies. Therefore, laboratory rats are less likely to harm unfamiliar conspecifics and more likely to tolerate them in their groups, compared to the wild ones (see [162,181,182] for review).

Perhaps more important than bonding is a social organization based on dominance hierarchy, which is maintained by the exchange of various social behaviors. Behavioral observations on a colony of twenty wild rats kept in an outdoor enclosure showed that males form a stable, near-linear dominance hierarchy, where challenges by subordinates and overt aggression by dominants in dyadic interactions were rare and primarily occurred between animals of similar rank. In contrast, wild female rats formed looser hierarchies under the same conditions, as interactions happened infrequently[183]. Also colonies of laboratory rats, Sprague-Dawley[164] and Long-Evans[165] organize in hierarchy when kept in semi-natural conditions. When studying dominance relationships in small groups, such as dyads, most of the experimental work has used tasks to evaluate the establishment of dominance between unfamiliar rats, usually isolated and under deprivation states before being tested in a neutral arena where they compete for food or water, engaging in strong agonistic interactions. However, once established, dominance is maintained in stable pairs where aggressive behaviors (biting, boxing, keep down, lateral threat) are reduced. Therefore, identification of status in stable dyads may depend on evaluating interactions that are more subtle. On this line, recent work revealed that stable dominance status can be identified in pairs of adult male rats, based on priority access to appetitive reinforcers. Costa et al.[155] implemented the modified Food Competition test (mFC), a novel trial-based dominance assay where a small conflict for access to a discrete number of palatable pellets is introduced in the homecage of nonfood deprived cage mate rats. In this test, only one animal of the pair can have access to the food at a time, which leads to subtle competitive interactions translating into increased food consumption by the dominant rat. Food consumption was found to reliably predict differences in other behaviors observed during testing, such as dominants being more efficient at displacing subordinates to gain access to the food.

Despite competitive interactions, rats engage in affiliative behaviors in several situations, including allogroming, huddling, play behavior and food sharing. This latter is expected to occur naturally since the presence of shared feeding sites in the colony, where rats allow conspecifics to eat in close proximity and even tolerate food steeling[162,184]. Consistently, wild and laboratory rats have been found to share food with others even if they could eat the food alone[185–187].

In addition, studies have shown rats' capacities for social learning, cooperation, reciprocity, and prosocial behaviors (reviewed in Schweinfurth, 2020[181]). For instance, where novel food is found rats leave urine markings, which are followed by other rats as olfactory cues to locate feeding sites[188]. They can coordinate their behavior with conspecifics, by turn-taking in locations[189], back-and-forth shuttling[190], synchronizing actions[191–193] in order to cooperate for reward outcomes in instrumental tasks. They reciprocate help for food sharing according to both direct[26,194,195] and generalized[25,26] reciprocity and possess the cognitive capacities to cooperate in the prisoner dilemma game[195,196]. Moreover, rats can select when they want to engage in competition for food with their partners[197]. Finally, as described in **1.3**, male rats display prosocial choices, providing reward to a familiar partner without added-self benefit[79,98,99].

Powered by the continuous development of new tasks for studying complex social behaviors in laboratory or semi-natural environments, this body of research emphasizes how rats display a rich repertoire of behavioral and socio-cognitive skills that enable them to perceive, react and learn from social cues, to coordinate their actions with conspecifics, to cooperate or compete for certain goals, and to benefit others. Given the availability of tools for recording and manipulating neuronal activity in a genetically and temporally precise manner while freely moving, rats represent a very good model for investigating the role of specific neural circuits of the mammalian brain in socio-cognitive processes, such as social decision-making, in both healthy and pathological conditions

2 | Objectives

Chapter 2 | Objectives

Animals often display prosocial behaviors, actions that benefit others, which are essential for social bonding and cooperation. Most studies on prosocial preference for reward provision come from the Primate Order and have highlighted how features of the social context, including familiarity, sex, and dominance status, may influence the expression of prosocial behavior. However, less effort has been devoted to the identification of the behavioral correlates that lead to such effects, which is needed to improve our understanding of the proximate mechanisms underlying prosocial choices, across species. With this work, we want to address this point in laboratory rats by using a previously developed Prosocial Choice Task in order to (i) assess the effects of the social context on rats' prosocial choices and (ii) identify their behavioral correlates, by performing a detailed behavioral analysis of the interacting subjects during decision-making. To this aim, the current thesis establishes the following four objectives:

1. Assess the effect of familiarity of the recipient on prosocial choices in male rats
2. Assess the effect of sex on rats' prosocial choices, by comparing male and female dyads
3. Assess the effect of the dominance relationship between decision-maker and recipient on prosocial choices in male rats
4. Analyze similarities and differences between dyads in the dynamics of multimodal social interactions when an effect is found.

3 | Materials and Methods

Chapter 3 | Materials and Methods

Materials and Methods section corresponding to the original manuscript[198]

3.1 Animal subjects

86 adult Sprague-Dawley rats, 74 males and 12 females (OFA, Charles-River, France) were used in this experiment, being 8 weeks old and weighing between 226-250 g upon arrival to our facilities. Rats 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 acclimatize to our Vivarium Facility, to reverse their circadian rhythm and start establishing their social hierarchy. After this period, animals were handled six times for two weeks, allowing them to habituate to the experimenter and to eat the new pellets, which were delivered inside the shavings or from a feeder magazine placed inside the homecage. Rats were 3-3.5 months old when starting the prosocial choice task. Experiments were performed during the dark cycle, waiting at least 1 hour and 30 minutes after the lights were off to start with behavioral procedures. Animals were provided by a commercial company, thus previous social experience, social status and degree of relatedness between the animals was not known. Animal husbandry and all experimental procedures were performed following Spanish Guidelines under the code 2016/VSC/PEA/00193 approved by the Dirección General de Agricultura, Ganadería y Pesca of the Generalitat Valenciana, which are in strict compliance with the European Directive 86/609/EEC of the European Council.

3.2 Experimental procedures

3.2.1. Prosocial Choice Task (PCT)

The propensity to perform actions that benefit others was evaluated in the prosocial choice task (PCT), where 43 pairs of non-food deprived rats were tested in a double T-maze, one per animal, as previously described[79]. The two individual mazes (one for the decision maker and the other for the recipient of help rat) are separated by a transparent perforated wall, thus allowing rats to see, hear, smell and partially touch each other. In each maze, a central arm gives access to a choice area and two reward areas where food is delivered in food magazines (**Figure 4A**). Access to the reward area is prevented by automated doors, controlled by nose ports placed above them. Rats had to poke on a nose port for the door underneath to open, thus allowing them to enter the reward area, reach the food magazine and run around the maze back to the choice area, initiating a new trial. 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, during which they could perform trials *ad libitum*. A trial would start when both animals were present in the central corridor, giving simultaneous access to the choice area. There, recipient animals could display food-seeking behavior by performing nose pokes on the side where they would expect the reward. Then, focals could choose between poking on the same side of the recipient, providing access to the lateral arm where both animals would receive one pellet (prosocial choice) or poking on the opposite side, entering the lateral arm where the focal would receive one pellet and the recipient none (selfish choice) (**Figure 4B**). In both choices, focal and recipient rats went to the same side of the maze, and returned to the central corridor to reinitiate a new trial.

Different types of pairs were tested in the PCT. To study the role of familiarity as a possible modulator of prosocial choices, two independent groups of male rats were tested: one where decision-maker and recipient were familiar animals (n=13), defined as cagemates living as a stable dyad for at least 1 month before behavioral testing; and another group where decision-maker and recipient were unfamiliar (n=14), defined as rats from the same strain that were not cagemates, that met for the first time in the PCT, and were maintained over the rest of sessions while not being cage mates. Furthermore, the role of the sex of the interacting dyads was studied by comparing the prosocial levels of males and females, both of the groups composed of familiar dyads (i.e., male cagemates (n=13) were tested together and compared to dyads of female cagemates (n=6)). Finally, the role of social hierarchy was evaluated by comparing two independent groups of male cagemates dyads, which lived together for at least one month before behavioral testing. In one group the dominant animal was the decision-maker of the pair and would decide whether to provide food to its submissive cage-mate (n=9), while in the other group the submissive animal would decide whether to be prosocial or not to its dominant partner (n=13).

3.2.2. Individual training for the PCT

All animals were habituated to the maze environment for 4 daily sessions of 15-20 min each. Rats were allowed to explore the maze and retrieve the pellets that the experimenter previously placed over the floor of the maze and in the food magazines. In addition, the doors of the maze were manually activated so that the animals could habituate to the noise produced by their opening/closing. After habituation, individual training started. On the first day, all animals were shaped to rear to poke in the nose port for opening the door that gave access to the food magazine. Rats could enter both arms that were rewarded with one pellet per trial. After this first day, each rat of a pair was randomly assigned to be the decision-maker (focal) or the recipient. From this moment, focal and recipient rats received distinct kinds of individual training, for a maximum of 12 daily sessions of 20-30 minutes each.

Focals learned to perform one poke on any side of the choice area, to access the lateral arms in order to retrieve the pellet and go back to the central arm to start a new trial, 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). For recipients, only the nose port on the rewarded side was active. Thus, recipients learned to poke only to one side, and the number of nose pokes required to open the automated door gradually increased over training, to ensure food-seeking behavior and clear side preference (for further details on nose poke training, see Márquez et al. 2015[79]). In the last 4 sessions, after nosepoking on the preferred side, the opposite door would open and recipients were forced to visit the unrewarded arm in 10 and 20% of the trials. In this manner, recipients would learn that even if no pellet was delivered in the unrewarded side, they would have to enter that lateral arm and go back to the central corridor to start a new trial. Finally, recipients were briefly re-trained immediately before each session of the PCT, to prevent extinction of food-seeking behavior. Focal and recipient role were fixed throughout the entire experiment.

3.2.3. Behavioral apparatus for the PCT

The setup consists of two identical, fully automated double T-mazes (Gravaplot, Sintra, Portugal), that are automatically controlled using Graphic State 3.03 software and the Habitest interface (Coulborun Instruments, Allentown, PA, USA). Custom-made automatic doors (WGT-Elektronic, Kolsass, Austria, and Mobiara R&D, Lisbon, Portugal) triggered by infrared beams control the positions of the rats in the mazes, such that when the rats activate the beam a specific door would open, allowing the animals to move to a different area of the maze. Each T-maze has a central corridor as starting point, which gives access to a choice area through an automated door. The choice area is flanked by two lateral reward arms, at the end of which there is a food magazine. To enter the lateral arms, rats had to poke in a light-cued nose port to activate the infrared beam controlling the door underneath. The moment when the focal animal pokes in one of these nose ports, thus opening the doors of the corresponding side that give access to the reward area, is defined as the moment of the decision, i.e., when the focal animal reports its choice. Once in the lateral arm, rats could retrieve the food (one pellet per trial), triggering the opening of the door that gives access to a small runway leading to the starting point at the central corridor, thus initiating a new trial. The roof of each maze consisted in transparent, 2 mm-thick acrylic walls, being perforated to facilitate the detection of ultrasonic vocalizations by the microphones above them. In addition, a transparent, 2 mm-thick acrylic wall was positioned on top of the central wall separating the two mazes and between the microphones to facilitate call assignment. 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 and the PCT started.

3.2.4. Modified Food Competition test (mFC)

After acclimation to the vivarium and handling by the experimenter, 23 pairs of male cage-mates rats were tested in the Food Competition Test (mFC)[155], to identify their social hierarchy. This tests reliably measures already established social hierarchies by introducing a subtle conflict for the access to palatable pellets in the homecage of nonfood-deprived pairs of animals. Briefly, for this test the homecage lid was replaced by a modified laser-cut acrylic one incorporating a fully transparent feeder for hosting the pellets (Dustless Precision Pellets, 45mg, Rodent Purified Diet). The feeder was designed so only one animal could access the palatable pellets at a time, leading to subtle conflict and competition for the reward. Moreover, the feeder counted with a sliding door to prevent the access to the pellets during inter-trial intervals and an opening on the top where the experimenter could deliver the pellets in each trial. Before testing, rats were habituated for three consecutive days to wait for the sliding door to open and to eat the pellets individually, while the partner rat was kept in a separate cage. In each day of habituation, the fur of the animal was marked to facilitate identification from video and the rat was placed alone in the homecage, with the new lid hosting 10 pellets per trial. In the habituation sessions, the structure of the trial was the following: the rat was allowed to explore in his home cage for 2 minutes with the sliding door of the modified lid closed, thus preventing access to the 10 pellets. Then the feeder was open and the rat had access to the 10 pellets for a period of 2 minutes, after which the sliding door would close for an inter-trial interval of 2 minutes. The number of trials in the habituation ranged from 2 to a maximum of 4 trials per daily session. After habituation, the pairs of cage-mates were re-marked and tested for 2 consecutive days in a social context, inducing now the competition for the positive reinforcers. In the test, a trial started with 1 minute of exploration, with the sliding door of the modified lid closed and hosting 10 pellets. Then the feeder was open and the rats had access to the 10 pellets for a period of 2 minutes, after which the sliding door would close for an inter-trial interval of 1 minute. In each day, rats performed 5 trials, having access to a total 50 pellets in a session of 15 minutes. To control for a possible mere effect of performing the modified Food Competition test on prosociality, the animals were divided into two groups: one group was tested twice for social hierarchy (n = 10 pairs), with two sessions of the modified Food Competition test performed before the start of individual training for the Prosocial Choice Task (PCT), followed by two sessions performed after the PCT, whereas the second group (n = 13 pairs) completed two sessions of the modified Food Competition test only after being tested for the PCT (**Figure S1K**). Consumption was quantified by video annotation and the total number of pellets eaten by each animal over the days indicated the social status between them. Statistics evaluating differences between dominant and submissive animals were performed in the average consumption of all days. To have a quantitative measure of the strength of the differences in hierarchy across dyads a Dominance Index (DI) was computed, as previously proposed[155]. Briefly, the pellets eaten by the recipient are subtracted from those of the focal and normalized by the total number of pellets eaten:

$$DI = \frac{Number\ of\ pellets\ of\ Focal - Number\ of\ pellets\ of\ Recipient}{Total\ number\ of\ pellets} * 100$$

The sign of the DI indicates whether the focal is dominant (positive values) or subordinate (negative values). One pair of animals displayed differences in pellet consumption between the interacting animals smaller than 5%. In this pair social hierarchy was not reliable and categorization of dominant and submissive was not possible, thus this pair of rats was excluded from the study.

3.3 Data acquisition and processing

3.3.1. Video and sound acquisition

All the experiments were performed during the dark phase of the animal's light cycle and video recordings were captured at 30 frames per second and 1280 x 960 pixel resolution under infra-red illumination (PointGrey Flea3-U3-13S2M CS, Canada, FlyCapture). We used two cameras, each positioned above one double maze, and centered on top of the choice area. Ultrasound was recorded at a sampling rate of 214285 Hz with two externally polarized condenser microphones (CM16/CMPA) connected to an UltraSoundGate 416H (Avisoft Bioacustics). We positioned two microphones on top of the choice area of each double maze, one microphone per T-maze (44 cm from the floor of the maze and 15 cm from the acrylic wall between them). For each session, video and audio acquisition start was simultaneously triggered through a common TTL delivered from visual reactive programming software Bonsai[32] through an Arduino Uno (ARDUINO). With Bonsai, we also sliced the entire session videos into video chunks, corresponding to the choice period of each trial. For the synchronization of the video with the data obtained from the interface controlling the mazes, we extracted the timestamps from the Coulbourn interface, and tracked the blinking of an infra-red LEDs placed in the visual field of the camera which was triggered at the time of each start trial and focal's choice. Sound recordings were synchronized to video data aligning each start trial with the recorded sound of the opening of the door that gave access to the choice area.

3.3.2. Pose estimation

A custom workflow of the Bonsai-DLC interface[199] (Python 3, DLC, version 2.2) was used to track unmarked body parts of the animals on both sides of the double maze simultaneously. Tracking was performed offline for all single trials' videos from the first two sessions of the PCT, with a confidence threshold set to 0.7. Video analysis' temporal resolution was determined by the camera's acquisition frame rate (33 ms), whereas spatial resolution was calculated by measuring a reference known distance in pixel values at the height where animals move in the maze (spatial resolution 0.59 mm). A cropped image corresponding to the choice area of one T-maze was used as region of interest (ROI) to train the model, applying an offset for each of the choice areas to maintain the original frame coordinates. DLC was trained on videos with one animal in the T-maze (26 videos from different animals). 25 frames per video (650 frames in total) were annotated and used to train a ResNet-50 neural network for 600,000 iterations.

For each video frame in the recordings, we obtained the location of the noses used to compute the Euclidean distance between the rats in the choice area, and the y coordinate of the nose to retrieve the distance of each animal from the central wall. Moreover, we calculated the position of the nose of each animal in the maze, where movements in the x coordinate would indicate movement towards the selfish or prosocial port (being coordinate 0 cm the position of the selfish side and 17 cm the position of the prosocial side). In our task, focal and recipient animals of different pairs are counterbalanced when assigned to a side of the double maze, such that in some pairs the focal rat would appear in the upper part of the video and the recipient rat on the bottom part (as illustrated in **Figure 4B**), while the opposite occurs in the rest of the pairs. Furthermore, the prosocial side is also counterbalanced, such that it would be to the right side for some focals and to the left side for the remaining ones. Thus, we moved and scaled tracking data from different recordings to a common reference space. For this, we used the coordinates of the central wall of the double maze as space scale factor for pixel to meter conversion.

Orientation of one rat towards the other was computed as the angle between the vector from the middle of its head (halfway between the ears) to its own nose and the vector from the middle of its head to the other rat's nose. We obtained the nose instantaneous speed from the rate of change in its position. For this, we smoothed the nose position time series by independently convolving its two coordinates with a Gaussian window of 0.25 s (full width at half maximum). For each time point, we obtained the velocity vector as the derivative of each smoothed coordinate and computed instantaneous speed as its norm.

3.3.3. Detection, assignment and classification of USVs

We automatically detected and assigned USVs as thoroughly described in Sirotin et al. 2014[172] . Briefly, USVs were detected from the raw sound recordings with custom built MATLAB routines (The Mathworks). We first obtained the sonograms for each microphone, with a 0.25 ms time step and detected times with low entropy (<6.5 bits) of the frequency spectrum in the 18-100 kHz range. We then defined as USVs segments of low entropy, those lasting at least 3 ms and bounded by silence of >20 ms. USVs were then curated by automatically discarding as noise those with high power in the sonic range (5-18 kHz) and visually inspecting the sonograms, removing any noises detected as USVs by mistake. Next, each vocalization was assigned to either the focal or the recipient rat, by comparing the signal from both microphones. USVs that crossed the entropy threshold in only one microphone were assigned to the rat on the T-maze below it. If the same USV was picked up by both microphones, we assigned it to the rat under the microphone with lowest entropy values. Rats vocalizing at the same time will typically produce USVs with non-overlapping fundamental frequencies. When simultaneous signals from both microphones were found to differ by at least 1 kHz during >3 ms, we concluded that both rats vocalized simultaneously and assigned to each one the USV detected by the microphone on its side. As in Sirotin et al. 2014[172] ,we used recordings with only one rat in the double maze to validate the USV assignment, yielding an accuracy of 94% (**Figures S4A–B**).

We performed automated classification of the USVs that were already detected and assigned to the emitter rat from the dominance groups (n=45.898) into three different classes of 50-kHz USVs that correspond to different vocal programs: “flat”, “frequency-modulated” and “step”. We extracted a grayscale image of the sonogram for each vocalization (sonogram duration 200 ms, frequencies 25-100 kHz) and used a convolutional neural network for supervised image-based classification following the pipeline in VocalMat[200]. We manually selected and labelled flat (n=1002), frequency-modulated (n=1003) and step (n=921) calls, equally distributed across animals, randomly assigned 90% of each class as training set and trained the network using the original weights from VocalMat as starting point. Classification accuracy measured on the test set was 98%.

3.3.4. Multimodal analysis of USVs and tacking data

We temporally aligned audio and video of each recording session. This allowed to retrieve the video time and frame when a USV was emitted, tagging each USV with relevant behavioral information, i.e.- in which trial the USV was emitted and the location of the noses at the time a USV was emitted during the choice period (**Figure 8B**). We were then able to selectively quantify the USV number and rate during the interaction time (noses of both rats simultaneously detected in the choice area), which we used for **Figures 8**, **S4C**, and **S5**. After moving and scaling tracking data to a common reference, we were able to map the location of the noses at the time of USV emission from all recording sessions (**Figure 8C**).

3.4 Data analysis and statistics

3.4.1 Analysis of social interactions

All the analysis of social interactions prior to choice was restricted to the video frames where both focal's and recipient's noses were tracked in the choice area (the ROI for the training of DLC). We called this portion of the total choice time ''interaction time''.

Duration of mutual direct investigation

The duration of mutual investigation in a trial was calculated from the frames in which the nose-to-nose distance was shorter than 2 cm. Absolute thickness of central wall of the double maze was 1 cm, however, after manual observation of the nose coordinates, we decided to expand the distance to 2 cm in order to include in this measure all the mutual investigations that would happen in a diagonal, mostly across two separate holes in the perforated wall.

Duration of wall investigation

As a proxy of time sniffing through the wall, each rat's wall investigation was calculated from the frames in which the distance between the rat's nose and the central wall was equal to zero (**Figures 6M**, **S3N**, and **S3O**).

Distances, speed and head orientation

For each trial, we extracted the median of the time series of the different variables (the nose-to-nose distance, nose distance from the central wall, nose speed and orientation towards the partner). We then averaged the medians from all trials of a pair/rat to obtain a value for each subject, that we used for statistical analysis.

For the radar charts in **Figures 6I**, **6K**, **6L**, and **S3M** we retrieved in each trial all the frames with orientation value, which ranged from 0° (rat oriented to the nose of the partner) to 180° (rat oriented to the opposite direction of the nose of the partner). We then calculated the percentages of frames belonging to each of three ranges (0°-60° indicative of more direct gazing, 60°-120° and finally 120°-180° indicative of positions where one animal oriented opposite to the partner). For ''overlapping orientations'', we calculated the percentages of frames in which the orientations of both rats fell within the same range.

Visualization of early dynamics of social interactions

We selected, for each trial, the frames (time points) where both noses were tracked (interaction time). Next, we aligned the new time series, so that the first frame in each series was set as time 0. We then obtained an average time series for each hierarchy-trial type condition, by averaging the time series of the different trials at each interaction time point, up to the median duration of the interaction per trial (3.3 sec, line graphs of behavioral dynamics in **Figures 6** and **S3**). Finally, for the statistical comparisons we calculated the median of each new time series corresponding to a trial and averaged the medians of all the trials belonging to the same hierarchy-trial type category (dot graphs with Student-Newman-Keuls test in **Figures 6** and **S3**).

3.4.2 Granger causality via Partial Directed Coherence

To assess whether the behavior of one rat influences that of the other within trials (Figures 4 and 5), we applied partial directed coherence (PDC), a frequency decomposition of Granger causality[201], using routines from the AsympPDC implementation[202]. Briefly, for each condition (e.g., "focal animal is dominant") we fitted a single vector autoregressive (VAR) model to the time series of interest from focal and recipient rats and computed from it the information PDC (iPDC)[203] spectra from the focal to the recipient and vice versa. We integrated each iPDC spectra to obtain information flow (I_{flow}), scalar value representing the causality from one rat group (focal or recipient) to the other in units of information transfer (bits). We used trial-shuffle surrogates and resampling statistics to test for significance of each I_{flow} and of I_{flow} differences across conditions.

<u>Granger causality between focal and recipient positions</u>

In detail, to quantify within-trial causality between the positions of the rats along the x axis (running parallel to the wall separating the rats, from one nose port to the other, scaled such that the prosocial nose port is always represented at the "right", **Figure 7A**), we began by extracting from each trial the longest uninterrupted interaction between the rats from the early interaction time (the first 3.3 seconds selected for the analysis of behavioral dynamics). Trials not containing an uninterrupted interaction of at least 1 second were discarded from the analysis (93 out of 1998 total trials discarded). We subsampled the data by averaging every 3 time points, resulting in a sampling rate of 10 samples per second. At this point, each trial is represented by a vectorial time series of two dimensions $\left(x_{focal}(t)\,, x_{recipient}(t)\right)$ and 10 to 33 time points (1 to 3.3 seconds in duration). We sorted the trials into groups representing each condition and normalized x_{focal} and $x_{\mathrm{recipient}}$ by subtracting the mean of each variable in the whole condition and dividing by its standard deviation (note we did not normalize the data trial by trial). Next, we fitted a VAR model of order 2 to each individual trial vectorial time series. Our method requires a fixed order for the VAR models and 2 was the median optimal model order for individual trials as per Akaike's information criterion. We then computed the mean of all VAR models, thus producing a mean autoregressive model for each condition from which we calculated the iPDC spectra from focal to recipient and recipient to focal and we integrated each iPDC across all frequencies into I_{flow} as (adapted from equation 8 in Takahashi et al.[203]):

$$I_{\text{flow}} = -\frac{1}{fs} \cdot \int_{0}^{fs/2} \log_2\big(1 - \text{iPDC}(f)\big)\, df$$

Where fs is the sampling rate.

We implemented surrogates and resampling statistics to test for significance of I_{flow} and I_{flow} differences between conditions. We began by performing trial-shuffle surrogates within each condition. To construct each surrogate, we paired the data from the focal in each trial with data from the recipient in a random trial from the same condition. Since trials were of variable duration, we randomly matched each trial only with others having at least its duration and kept only data up to their common duration (481 of 1832 trials were of maximum duration, ensuring well-varied surrogates for all). In this way, surrogate datasets represent the null hypothesis whereby there is no interaction between the two rats within each trial. We obtained the iPDC and I_{flow} from each of 1000 surrogates and calculated a one-sided p value with finite-bias correction[204] as:

$$p = \frac{(\#\text{ of surrogate } I_{\text{flow}} > \text{ real } I_{\text{flow}}) + 1}{\#\text{ of surrogates} + 1}$$

To account for the positive bias in PDC, we subtracted the median surrogate iPDC from the real iPDC before calculating each final reported I_{flow} value.

We tested for significant differences between two given I_{flow} values by obtaining bootstrap distributions of their differences. For a condition with *n* trials, we get each single bootstrap estimate by selecting *n* random trials with replacement and obtaining iPDC, subtracting the median surrogate iPDC from it and calculating I_{flow} as before. We do 1000 subtractions of bootstrap estimates from each I_{flow} and compute a 2-sided finite-bias-corrected p value against the null hypothesis of there being no difference as:

$$p = 2 \cdot \min\left\{\frac{\big(\#\text{ of bootstrap } I_{flow} \text{ differences} > 0\big) + 1}{\#\text{ of bootstrap} + 1}, \frac{\big(\#\text{of bootstrap } I_{flow} \text{ differences} < 0\big) + 1}{\#\text{ of bootstrap} + 1}\right\}$$

Granger causality between recipient USVs and focal orientation towards the emitter

For analyzing causality from emission of USVs to orientation of the listener rat towards the emitter (**Figure 7F**) we followed the pipeline described above for rat positions, with adaptations as follows. We first constructed a binary time series with one sample per video frame valued 1 if the rat emitted a USV with onset in the time interval between that and the next frame and 0 otherwise. We then convolved this with a gaussian kernel of full width at half maximum of 0.25 s to obtain a continuous representation of vocal production and added to these time series gaussian noise with a sigma of 10% of their standard deviation as a necessary stochastic component as suggested in Sameshima and

Baccalá[202]. We then extracted the recipient USV and focal orientation time series from the rats, keeping the longest uninterrupted interaction between the rats for each trial up to 10 seconds of interaction time (1 second minimum duration), downsampled to 10 Hz and proceeded to obtain iPDC and I_{flow} from $\text{USV}_{\text{recipient}}$ to $\text{orientation}_{\text{focal}}$.

3.4.3 Generalized Linear Model (GLM) analysis

To examine the trial-by-trial contribution of a multitude of parameters to the focal's choice, we employed a multi-step GLM approach. First, we fitted a binomial GLM with 14 behavioral and categorical parameters (see **Figure 9** for regressor details) using the formula:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n$$

where p is the probability of a prosocial choice, $\beta_{0\ldots n}$ are the regressor weights, and $X_{1\ldots n}$ are the regressor values. Because Wald tests for statistical significance provide an incomplete interpretation of regressor contributions, we instead employed an alternative approach similar to that previously described by Musall and colleagues[205], in which we computed the proportion of deviance (D^2) explained individually by each regressor. To achieve this, for each regressor in the model, we shuffled the values of every *other* regressor's values, resulting in a dataset in which one regressor contained the actual values on each trial, but all the other regressors' values were shuffled. Then, we fitted a GLM to the shuffled trial-by-trial data and computed the D^2. To provide robustness against potential random imbalances in any single shuffling, we repeated this procedure 1,000 times per regressor and took the mean D^2 value for each one. Thus, we were able to determine the maximum explained deviance for each regressor. Because each regressor's D^2 value is computed independently, it does not describe the amount of unique information each regressor contributes to the model. Two regressors could have a similar D^2, but if they are related or dependent on each other, then their unique contributions to the predictive power of the model will be limited. Therefore, we also computed the ΔD^2 for each regressor: the proportion of the deviance that is *uniquely* explained by each regressor. To achieve this, for each regressor, we shuffled the values of only that regressor, leaving all others intact. We then fitted a GLM to this dataset and computed its D^2. Next, we subtracted this value from the D^2 of the full GLM with all regressors intact to obtain the ΔD^2. To provide robustness against any single shuffling of the data, we repeated this shuffling procedure 1,000 times for each regressor and took the mean D^2 for each one. In this manner, ΔD^2 is essentially a measure of how much predictive power the model loses when each variable is shuffled, thus revealing its unique contribution.

Next, to more deeply examine the regressors that explained most of the deviance as well as how they might interact with hierarchy and trial progression, we fitted a reduced GLM using only those regressors that explained more than 1% of the deviance: trial number, hierarchy, nose-to-nose distance, focal orientation angle, and recipient orientation angle. This time, we also included interaction terms between trial and the remaining variables, as well as for hierarchy and the remaining variables, since our previous observations strongly suggested that other variables may interact with those two.

Finally, to tease apart the resulting triple interaction involving the behavioral variables plus both trial and hierarchy, we fitted separate reduced GLMs on dominant focals and submissive focals, this time using 4 variables: trial, nose-to-nose distance, focal orientation angle, and recipient orientation angle, as well as an interaction term for trial. By fitting separate GLMs to each hierarchical group, we were able to remove hierarchy from the model, thus facilitating a more direct interpretation of the interactions between trial number and the behavioral variables.

3.4.4 Statistics

Choice preference

Repeated measure (RM) ANOVA with one between-subjects factor and "session" as within-subjects factor was performed to compare prosocial choices between the groups under study (dominant vs submissive focals, familiar males vs unfamiliar males, familiar females vs familiar males) over the course of the testing sessions. Independent sample *t*-test was performed to assess differences between the groups when examining prosocial choices in each testing day and the average prosocial preference over the 5 days. Paired-sample *t*-test was used for each focal to compare the prosocial choice in each testing day against rat's baseline preference in the last two days of individual training. One-sample t test was computed for each focal to compare its baseline preference against chance level (50% preference). Bayesian statistics complemented these analyses (Bayesian repeated measures and *t*-test analyses) in order to provide estimates of the strength of the effects. BF_{incl}, BF_{+0} (one-tailed) or BF_{10} (two-tailed) were provided accordingly[206].

Prosocial choice index

We computed a prosocial choice index (PCI) to quantify individual differences on choice preference against chance over testing sessions, using the formula:

$$\mathrm{PCI} = \frac{\mathrm{Pref}_{\mathrm{test}} - \mathrm{Chance}}{\mathrm{Pref}_{\mathrm{test}} + \mathrm{Chance}}$$

where $\mathrm{Pref}_{\mathrm{test}}$ 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 rat; [+] PCI show an increase on prosocial preference on social testing sessions compared to chance, [-] PCI show a decrease on prosocial preference from 50%. Distribution of PCIs for each group can be seen in **Figure S1**.

Permutation test analysis

To address individual variability on prosocial preference, we performed a permutation test to identify those rats 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%. Rats then were assigned to three different categories by comparing their actual PCI to the 95% confidence interval (CI) of the distribution of randomized indexes (rat with actual PCI in 2,5% upper bound was considered as *prosocial*, rat with PCI in 2,5% lower bound was considered *selfish*, and those rats with PCI falling inside the 95% were considered as *unbiased*). Lower and upper bound for each individual's distribution can be found in **Table S1**.

Proportions of prosocial, unbiased, and selfish rats

We used χ^2 test to analyse differences in the proportions of animals classified as prosocial, unbiased, or selfish, for every session of the PCT of the different tested groups (familiar males, unfamiliar males, familiar females, dominant focal males, and submissive focal males).

Social interactions

We performed the independent sample *t*-test or the Mann-Whitney *U*-test to assess differences between dominant and submissive focal groups, when examining tracking data extracted and averaged from all the trials of each session. When grouping data by prosocial and selfish trials, A RM-ANOVA with "hierarchy" as between-subjects factor and "choice" as within-subjects factor was used to test for differences between the two groups across trial type. Finally, a one-way ANOVA followed by Student-Newman-Keuls (SNK) post-hoc test was used to evaluate differences among dominance-trial type categories, when examining the early dynamics of the interaction time.

Social dominance

Dominance index as a measure of the strength of the social hierarchy was correlated with prosociality in the second day using Pearson correlation.

Task performance

A RM-ANOVA with "hierarchy" as between-subjects factor and "session" as within-subjects factor was used to compare dominant focal and submissive focal groups in number of trials performed over the 5 days of testing of prosocial choice task.

Nosepokes and choice time

Difference between hierarchy groups in the average number of recipient's nosepokes per trial was assessed with the independent sample *t*-test. For the recipient's latency to nosepoke, recipient's nosepoke duration (computed by subtracting the time of the rat's snout entering the nose port (activating the infrared beam) to the time the rat's snout exited the nose port (inactivating the infrared beam)) and focal's choice time, the non-parametric Mann-Witney *U*-test evaluated differences in the distributions.

Proportion of USV classes

We used RM-ANOVA to assess differences in the proportions of USVs classified as flat, frequency-modulated, and step, across days of testing and according to the emitter agent (focal, recipient) and their hierarchy (dominant, submissive).

Relationship between USV rate and speed

We divided for each rat the number of USVs emitted while its nose was moving within each of four instantaneous speed bins by the total time in each bin (**Figure S4D**). Then, differences in USV rate between focals and recipients was assessed across the different speed bins using RM-ANOVA. For cross-correlations between USV emission and nose speed (**Figure S4E**) we obtained the USV time series as explained for Partial Directed Coherence (without subsampling nor adding noise) together with instantaneous nose speed for each trial. For each rat, we concatenated all its trials leaving gaps of 1 second with missing values between them and run the cross-correlation with a maximum lag of +/- 1 second. For each lag, we normalized the cross-correlation value by the total number of non-missing samples used for its computation to obtain the unbiased cross-correlation estimate. We subtracted from each cross correlation the mean of 1000 within-rat trial-shuffle surrogates.

Analysis of partial correlations

We performed partial correlations with nose-speed of the animals as covariate, to study the correlation between USV rate of the different animals and prosociality.

4 | Results

Chapter 4 | Results

Results section corresponding to the original manuscript[198]

4.1 Prosociality emerges faster when decision-makers are dominant and is not affected by familiarity or sex

We tested pairs of rats in our PCT, where a decision-maker rat (focal) can choose in each trial to provide food reward to itself only (selfish choice) or to itself and a recipient rat (prosocial choice) (**Figure 4A–C**). After individual training for maze navigation, focal and recipient animals were tested together in PCT and learned the new reward contingencies where food delivery to the recipient depended on focal's choices. Each pair performed five daily consecutive sessions of 40 minutes each, over which focals' choice preference was assessed.

Rats' prosocial preferences in food-foraging contexts emerged over the testing sessions independently of familiarity or sex. Male rats displayed similar levels of prosociality when interacting with their cage mates or unfamiliar conspecifics (repeated-measures ANOVA with "session" as within-subjects factor and "familiarity" as a between-subjects factor; "session" [$F_{(4,100)} = 13.86$, $p = 5e{-}9$, $\eta^2 = 0.164$, $BF_{incl} = 2.961e{+}6$], "familiarity" by "session" [$F_{(4,100)} = 0.29$, $p = 0.882$, $\eta^2 = 0.003$, $BF_{incl} = 0.107$], and "familiarity" [$F_{(1,25)} = 0.36$, $p = 0.555$, $\eta^2 = 0.008$, $BF_{incl} = 0.328$]) (**Figure 4D**). Moreover, we did not observe sex differences, with females being equally as prosocial as males (repeated-measures ANOVA; "session" [$F_{(4,68)} = 9.83$, $p = 2e{-}6$, $\eta^2 = 0.181$, $BF_{incl} = 71{,}466$], "sex and "session" [$F_{(4,68)} = 0.44$, $p = 0.783$, $\eta^2 = 0.008$, $BF_{incl} = 0.223$], and "sex" [$F_{(1,17)} = 0.29$, $p = 0.596$, $\eta^2 = 0.008$, $BF_{incl} = 0.391$]) (**Figure 4E**). Although we did not find evidence for an effect of familiarity or sex in prosocial tendencies, it could be that the proportion of prosocial individuals would differently emerge over the testing sessions in each group. For this, we computed a prosocial choice index (PCI) that reflected the strength of the prosocial (or selfish) bias compared with chance. Using a permutation test, we categorized the animals as either prosocial, unbiased, or selfish over the days. The emergence of prosociality was comparable across groups (**Figure S1A-F**; **Table S1**).

To understand how social dominance may modulate prosocial choice, we first identified the social status within pairs of cage mate rats. For this, we used the modified food competition test[155], a novel trial-based dominance assay, where established social hierarchies can be identified in the home cage of non-food-deprived pairs of male rats. It has the added advantage of not inducing aggressive interactions during testing that could influence later prosocial tendencies. After identification of social status of the animals (**Figure S1J**), we tested for prosocial tendencies two parallel independent groups, where the decision-maker rat was either the dominant (and thus its recipient was submissive) or the submissive (and the recipient was the dominant). Thus, in both groups, a dominant animal would

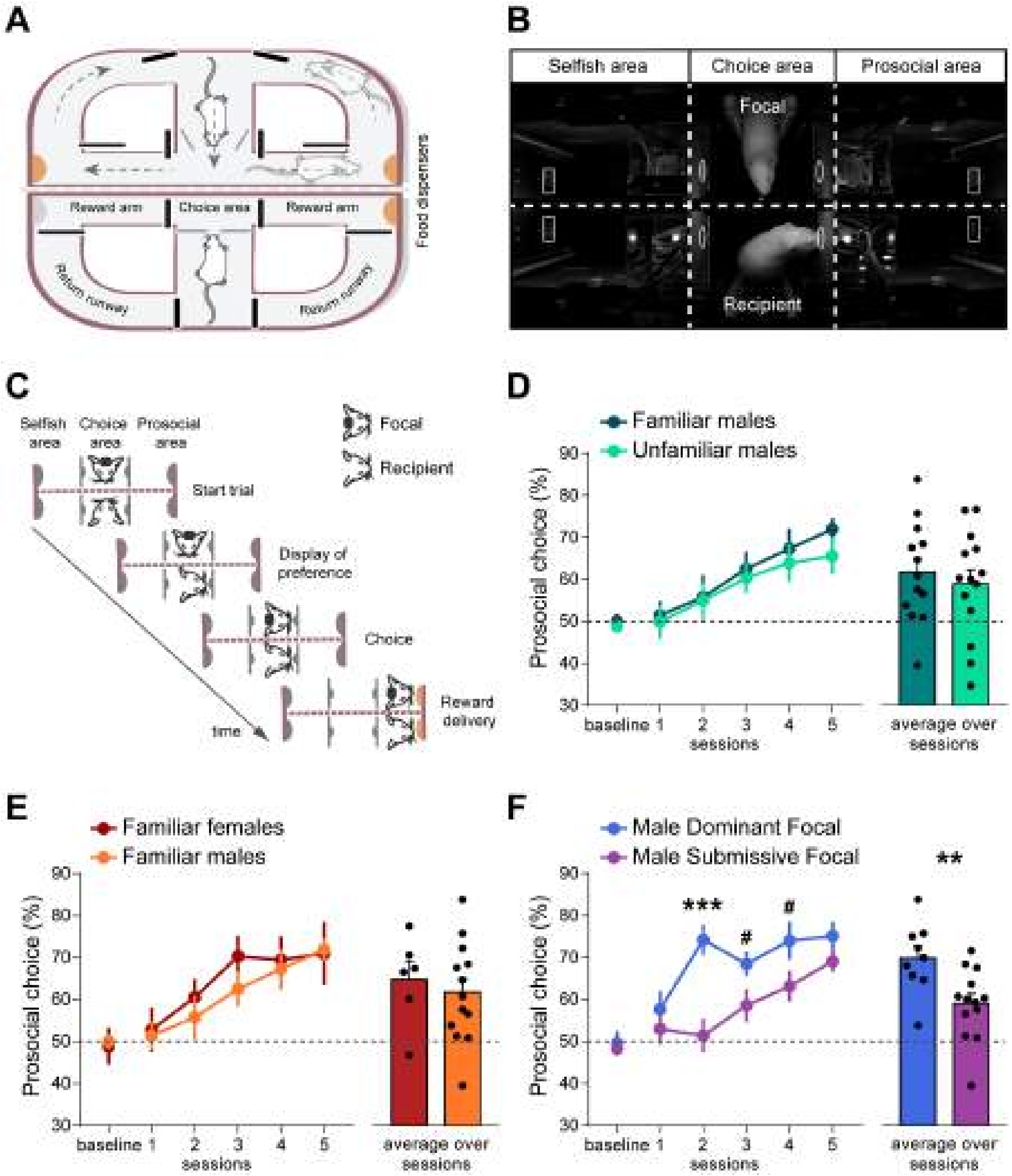

Figure 4. Prosociality emerges faster when decision-makers are dominant and is not affected by familiarity or sex. **(A)** Schematic overview of the double T-maze used for the prosocial choice task (PCT). Each T-maze (one per rat) consists of a central arm that gives access to a choice area and two reward areas gated by automated doors (black lines) at the end of which food is delivered (orange semi circles). Access to the choice area is controlled by automated doors placed in the central arm (gray lines). Arrows in the upper maze represent the flow movements of the rats in the maze. **(B)** Example image from a video recording, showing a top view of the central area of the double T-maze during one session. The horizontal dashed line marks the transparent and perforated wall that separates the two single mazes, which allows rats to see, hear, smell, and partially touch each other. Vertical dashed lines mark the separation between different areas of the maze: the choice area, where social decision-making occurs, and prosocial and selfish areas, where food is delivered depending on the contingencies of the task. White ellipses in the choice area mark the position of nose ports, which control the opening of doors located under them. When the decision-maker pokes one of its nose ports, the door underneath it and the door on the same side for the recipient animal opens, allowing them

to reach feeder magazines (white rectangles in the reward areas). In this example, the focal rat (decision-maker) is in the top of the image, whereas the recipient appears in the bottom, while displaying food-seeking behavior. **(C)** Schematic view of a trial: before the PCT focal and recipient rats are trained individually to navigate in the maze and learn to retrieve their own rewards. In the PCT, a trial starts when both rats are in the central arm, after opening of the central doors that give access to the choice area. There, the recipient rat will display food-seeking behavior (repeatedly poking in the side where it was previously trained to find food during individual training), and the focal animal can choose to nose poke on either side of its own maze. Focal animal will always be rewarded; however, recipient's reward will depend on focal's choice. A focal's nose poke on the same side where recipient is displaying food-seeking behavior (prosocial choice) will lead to both rats receiving one pellet in the reward area, whereas a nose poke on the opposite side (selfish choice) will lead to only the focal receiving one pellet and the recipient none. Prosocial and selfish sides remain fixed throughout all days, so that the focal animal does not need to read out the behavior of the recipient on each trial but can develop a preference over time. After food consumption, rats can pass through the return runway and go back to the central arm to start a new trial. **(D)** Familiarity of the interacting animals does not affect prosocial choices in male rats. To understand whether the familiarity of the recipient modulates the proportion of prosocial choices, we compared in the PCT two independent groups: focal animals that performed in the maze with their cage mate ("familiar males," n = 13 pairs) and focal animals that performed with a stranger, non-cage mate, recipient ("unfamiliar males," n = 14 pairs). Unfamiliar animals interacted for the first time in the first session of the PCT and were maintained over the rest of sessions while not being cage mates. We found that focals of the two groups developed similar proportions of prosocial choices along sessions, indicating that the degree of familiarity of the recipient does not affect prosociality in male rats. **(E)** Female rats show similar proportions of prosocial choices compared with male rats. To study sex differences in the development of prosocial choices, we tested two independent groups where the focal animal was either male ("familiar males," n = 13 pairs) or female ("familiar females," n = 6 pairs), interacting with a cage mate of the same sex. We found no difference in the proportions of prosocial choices along the five testing sessions, indicating that female and male rats are equally prosocial when interacting with a familiar conspecific of the same sex. **(F)** Social hierarchy modulates prosocial choices in male rats. Dominant (n = 9 pairs) and submissive (n = 13 pairs) focals displayed a preference for the prosocial option but dominant focals showed faster emergence and higher proportion of prosocial choices compared with submissive focals. See also **Figure S1**. For (**D**)–(**F**), baseline and five daily test sessions are shown. Baseline corresponds to the percentage of focal's choices for the side that would later correspond to the prosocial side during testing, averaged across the last 2 days of individual training. Data represented as mean ± SEM. #$p < 0.1$, ∗∗$p < 0.01$, ∗∗∗$p < 0.001$. See also **Figure S1** and **Table S1**.

interact with a submissive, but their roles in the decision process would differ. We found that both groups acquired a preference for the prosocial option over the days but that social hierarchy drastically modulated the emergence of this choice (**Figure 4F**). Specifically, dominant animals acquired faster prosocial tendencies and reached higher prosociality levels than submissive decision-makers (repeated-measures ANOVA; "session" [$F_{(4,80)} = 8.42$, $p = 1e{-5}$, $\eta^2 = 0.15$, $BF_{incl} = 3,445$], "hierarchy" by "session" [$F_{(4,80)} = 2.67$, $p = 0.038$, $\eta^2 = 0.048$, $BF_{incl} = 5.8$], and "hierarchy" [$F_{(1,20)} = 8.75$, $p = 0.008$, $\eta^2 = 0.136$, $BF_{incl} = 11.2$]). Dominant and submissive decision-makers displayed similar choices on the first session of the PCT, where animals are exposed to the social task for the first time after individual training and had not yet learned that their actions have consequences on the reward contingencies of the recipient (t test of the proportion of prosocial choices of dominant focals against submissive focals', $t_{(20)} = 0.81$, $p = 0.428$, $BF_{10} = 0.491$ for day 1). However, marked differences appeared from the second day of testing, where dominant animals displayed strong prosocial preferences, whereas submissive focals were still at chance levels (independent sample t test, $t_{(20)} =$

4.03, $p = 6.5e{-4}$, $BF_{10} = 42$, for day 2; paired sample t test of proportion of prosocial choices in session 2 against baseline for dominant focals, $t_{(8)} = 5.72$, $p = 4.4e{-4}$, $BF_{10} = 80$; for submissive focals, $t_{(12)} = 0.681$, $p = 0.509$, $BF_{10} = 0.34$). Interestingly, prosociality on this day positively correlated with the strength of the social hierarchy (**Figure S1L**), suggesting a parametric relationship between dominance and prosociality (Pearson correlation between prosocial choice in day 2 and dominance index, $r = 0.71$, $p < 0.001$). The differences between dominant and submissive focals were maintained over the sessions but progressively faded once submissive focals started to show prosocial biases from day 3 onward (t test, $t_{(20)} = 1.87$, $p = 0.077$, $BF_{10} = 1.28$ for day 3; $t_{(20)} = 1.88$, $p = 0.074$, $BF_{10} = 1.3$ for day 4; $t_{(20)} = 1.42$, $p = 0.171$, $BF_{10} = 0.79$ for day 5). We then assessed whether the proportion of prosocial, unbiased, and selfish animals would be different depending on their hierarchical status and observed a higher proportion of prosocial animals in pairs with dominant focals in the second day of testing (**Figures S1G–I**).

Although previous non-human primate studies also showed evidence of prosociality occurring down the hierarchy, the factors leading to such directionality are not known. Leveraging the controlled environment that experiments in laboratory rats provide, we endeavored to identify the behavioral correlates at the base of this enhanced prosociality in dominant animals. To this end, we performed a fine-grained analysis of rats' behavior during the choice period (time from trial start to focal's choice), focusing our analyses on the first 2 days of the task, when prosocial bias emerges, to identify the behavioral dynamics that promote integration of actions from others into decision-making processes.

4.2 Social dominance does not affect recipients' food-seeking behavior or focals' latency to decide

We have previously demonstrated that the recipient's display of food-seeking behavior, i.e., poking in the nose port that gives access to the food-baited arm, is necessary for the emergence of prosocial choices by focal rats[79]. Thus, one possibility was that submissive recipients were better at displaying food-seeking behavior, facilitating the learning of the contingencies of the task by dominant decision-makers. However, we did not find hierarchy differences in the number of nosepokes performed or in the vigor with which they were displayed (**Figure S2A**). Dominant humans are faster in making (non-social) decisions in stressful situations, without compromising their accuracy[207]. However, we did not observe differences in the latency to choose in rats performing our task (**Figure S2B**). It could still be possible that dominant focals developed faster prosocial preferences in the first days of testing because of increased task performance, thus accelerating the learning rate of the new contingencies in the social task. However, this was not the case either (**Figure S2C**). We then hypothesized that the social interactions displayed prior to the choice might be at the core of the faster learning of contingencies for dominant focals and that those pairs with dominant decision-makers would display richer social interactions.

4.3 Social dominance modulates the quality but not the quantity of social interactions prior to choice

We analyzed trial-by-trial social interactions in off-line video recordings using Bonsai[208] and DeepLabCut[209] that enabled us to precisely extract the position of unmarked body parts of the interacting animals with high spatial and temporal resolution. The time animals spent directly investigating each other was equivalent, regardless of the pronounced differences in prosociality (**Figure 5A**; independent sample t test for “mutual direct investigation,” $t_{(20)} = 0.411$, $p = 0.685$, $BF_{10} = 0.413$). Although direct contact is the standard measure of social interaction, we hypothesized that significant social interactions might still happen at a distance and not only through direct sniffing of the partner. Thus, we quantified the time that animals spent simultaneously in the choice area, regardless of the distance between them. Again, no differences were observed on the duration of these distant social interactions according to social status (**Figure 5B**; $t_{(20)} = 0.047$, $p = 0.96$, $BF_{10} = 0.39$).

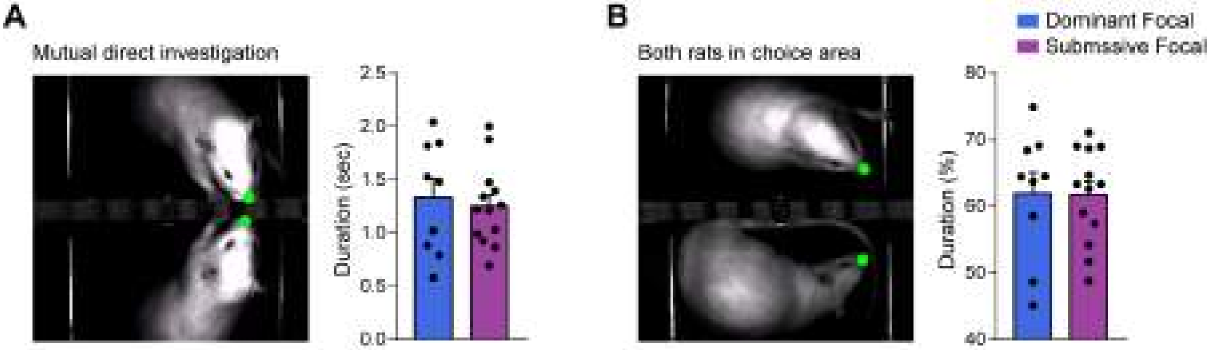

Figure 5. Social dominance does not affect the quantity of social interactions prior to choice. (A) Social dominance does not affect the duration of social investigation prior to the choice or **(B)** the percentage of choice time per trial that both rats are present in the choice area, as an index of social interactions in the distance. Data are shown as mean ± SEM; individual dots show the averaged trial value for each pair across the first two sessions. See also **Figure S2.**

Although the duration of mutual direct investigation and interaction time during choice was similar in the two groups, it was still possible that dominance status could account for differences in the quality of the social dynamics when animals were at a social distance. To this end, we quantified on each trial the median value of the distance between the focal and the recipient rat, whereas in the choice area, referred to as nose-to-nose distance, as a measure of social proximity. Indeed, pairs with a dominant focal maintained a closer distance prior to choice (independent sample t test, $t_{(20)} = -2.53$, $p = 0.020$,

BF_{10} = 3.15; **Figure 6A**). Interestingly, this effect was already present in the first testing session, where no differences in prosocial choice were yet observed (**Figures S3A**). Pairs with dominant decision-makers displayed closer interactions in a higher proportion of trials (**Figure 6B**), and these differences emerged early during the interaction time, where pairs with a dominant decision-maker would be closer to each other than those with a submissive focal, when the focal was going to choose the selfish option (**Figure 6C**, left panel; two-way ANOVA, “hierarchy” by “choice” [$F_{(1,1986)}$ = 4.77, p = 0.029], “choice” [$F_{(1,1986)}$ = 294.5, p = 1e−61], and “hierarchy” [$F_{(1,1986)}$ = 13.89, p = 0.0002]; further decomposition of the interaction followed by Student-Newman-Keuls [SNK] post hoc test revealed a significant difference across dominance categories in selfish but not prosocial trials [$F_{(3,1986)}$ = 120.3, p = 1e−71]; **Figure 6C**, right panel).

Social interactions are by definition bidirectional and highly dynamic[210], and although classical studies on decision-making have focused on the analysis of the decision-maker, it could well be possible that focals were influenced by the behavior of the recipient animal. To ascertain which animal (focal or recipient) was responsible for these more proximal interactions, we quantified the median distance between each rat’s nose and the central wall that divided the two mazes, as a proxy for social interest (**Figures 6D** and **S3D–F**). No significant differences were found between dominant and submissive decision-makers (Figure 3D, middle panel; repeated-measure ANOVA, “choice” [$F_{(1,20)}$ = 7.78, p = 0.011, BF_{incl} = 6.49], “choice” by “hierarchy” [$F_{(1,20)}$ = 4.32, p = 0.051, BF_{incl} = 1.76], and “hierarchy” [$F_{(1,20)}$ = 0.05, p = 0.820, BF_{incl} = 0.58]). However, submissive recipients were closer to the wall on selfish trials compared with dominant recipients (**Figure 6D**, right panel; “choice” [$F_{(1,20)}$ = 2.027, p = 0.170, BF_{incl} = 0.37], “choice” by “hierarchy” [$F_{(1,20)}$ = 10.86, p = 0.004, BF_{incl} = 8.44], and “hierarchy” [$F_{(1,20)}$ = 1.76, p = 0.200, BF_{incl} = 0.87]; independent sample t test for recipient rats in selfish trials, $t_{(20)}$ = −1.859, p = 0.088). Further analysis showed that this tendency for submissive recipients to stay closer to the wall occurred during the early phase of interaction when decision-makers were going to choose to be selfish (**Figure 6E**, left panel; two-way ANOVA; “hierarchy” by “choice” [$F_{(1,1986)}$ = 5.38, p = 0.020], “choice” [$F_{(1,1986)}$ = 11.17, p = 0.001], and “hierarchy” [$F_{(1,1986)}$ = 14.26, p = 0.0001]; further decomposition of the interaction [$F_{(3,1986)}$ = 7.388, p = 6e−5] followed by SNK post hoc test revealed that the distance from the wall of submissive recipients in selfish trials was different from the other three categories; **Figure 6E**, right panel).

The above results indicate that dominance status affects the recipient’s behavior: submissive recipients stay closer to the wall during selfish trials, thus decreasing the distance from the focal rat.

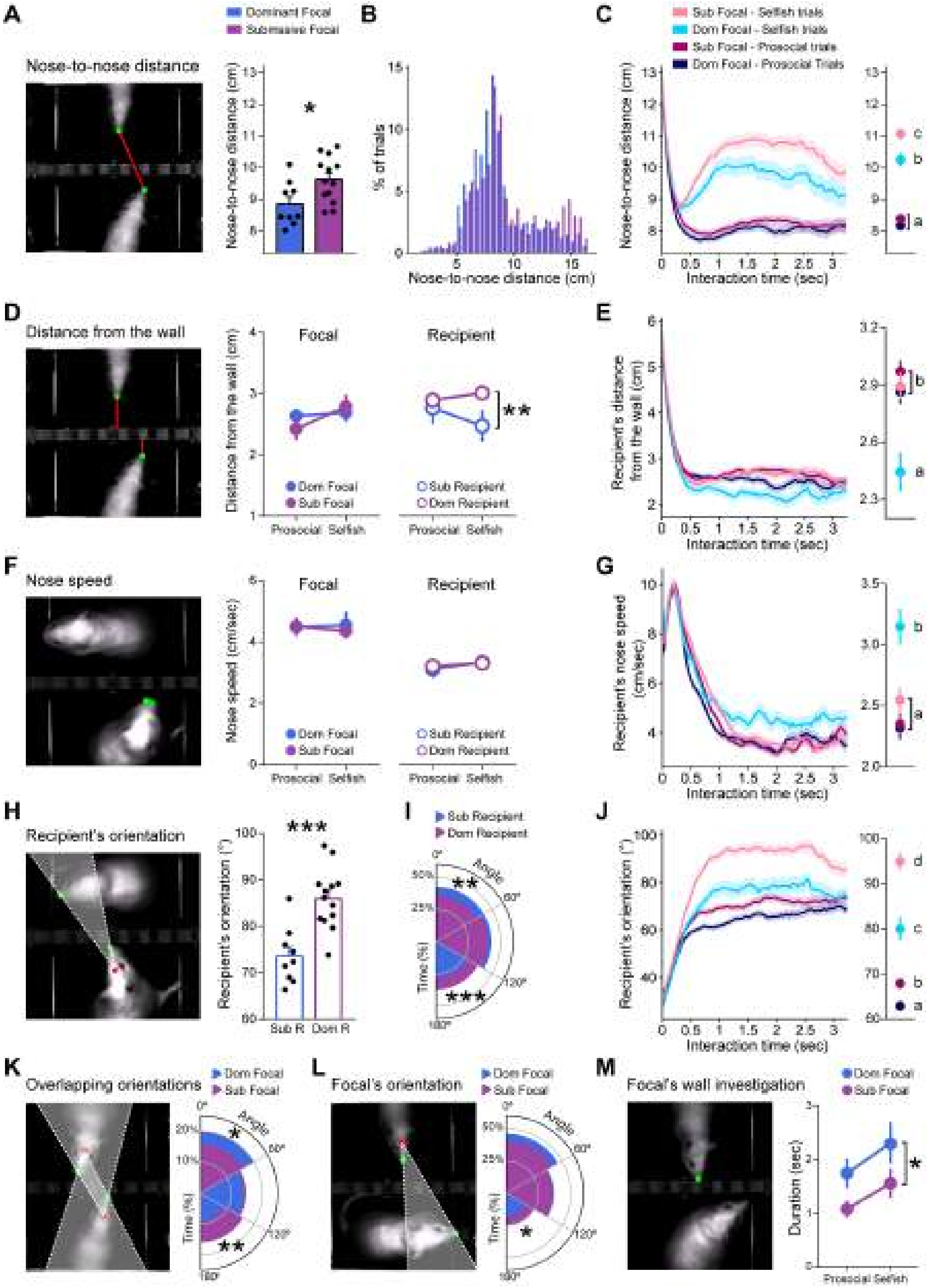

Figure 6. Social dominance modulates the dynamics of social interactions prior to choice. (A–C) Pairs with dominant rat as focal display more proximal interactions prior to choice. **(A)** The distance between focal and recipient noses, as a proxy for social interest of the pair, was measured during the interaction time, defined as the time that the two rats were simultaneously present in the choice area. The median nose-to-nose distance per trial was lower in pairs with

dominant focals. Moreover, **(B)** the proportion of trials with closer interactions was higher when dominants were decision-makers; **(C)** these more proximal interactions were already evident in the first seconds of interaction and only observed in selfish trials (see left panel showing temporal dynamics; right panel showing the average, SEM, and statistics of this time window). **(D–G)** Submissive recipients follow their dominant decision-makers. **(D)** To identify if one of the interacting animals was driving these more proximal social interactions, we measured the distance between the nose of each rat and the dividing wall that separated the animals in the choice area. The median distance from the wall per trial was similar for focal rats across dominance categories and trial type, although a tendency was found for submissive recipients to stay closer to the wall in selfish trials, suggesting an increased social interest toward their dominant focals when they were going to choose not to reward them. **(E)** This tendency was present during the early phase of interaction. **(F–G)** Movement dynamics indicated a similar pattern, where **(F)** median nose speed in the whole choice period did not reveal differences, but **(G)** submissive recipients showed higher nose speeds during the first seconds of interaction in selfish trials, suggesting again that they were following their dominant when it was going to poke in the selfish side. **(H–L)** Pairs with dominant focal and submissive recipient display more coordinated gazing. Orientation of each animal toward the partner was calculated as the angle between the vector from the center of its head (red dot) to its own nose (green dot), and the vector from the red dot and to the partners' nose. Lower values indicate more directed gazing. **(H–I)** Submissive recipients **(H)** were more oriented toward their focal when considering median head orientation toward the nose of the partner per trial, regardless of trial type, and **(I)** spent a higher proportion of time directly oriented in angles smaller than 60°. **(J)** These differences in head orientation were evident in the first seconds of interaction: submissive recipients were more oriented toward their dominant focal both in prosocial and selfish trials. **(K)** Pairs with dominant focal spent a higher proportion of time orienting to each other, whereas pairs with submissive focal spent a higher proportion of time orienting away from each other. **(L)** The same tendency was observed in the head orientation of the focals, although it was only significant in the case of submissive focals, which spent a higher proportion of time orienting away from their recipient, compared with dominant focals. **(M)** Dominant focals spent more time investigating the wall when their submissive recipient was in the choice area, compared with submissive focals, both in prosocial and in selfish trials, indicating a higher attention toward their recipients' behavior and suggesting increasing sniffing through the wall. Mean ± SEM is shown. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. In (C), (E), (G), and (J), right panels, letters denote statistically significant differences between conditions with significant level set to 0.05. Sub, subordinate; Dom, dominant; R, recipient. See also **Figures S2** and **S3**.

Since, in principle, the act of nosepoking would lead rats to show similar nose movements and trajectories, the increased proximity of submissive recipients to the wall may suggest that these animals move and/or orient themselves toward the focal when it is going to choose the selfish poke. Indeed, analysis of animals' movement (**Figures 6F and S3G–I**) showed that recipient rats continued moving the snout when the trial was going to be selfish. Similar values of nose speed were found across dominance categories for both focal and recipient rats (**Figure 6F**; no significant effects were found for focal or recipient rats, repeated-measure ANOVA; "choice" [focals, $F_{(1,20)} = 0.07$, $p = 0.798$, $BF_{incl} = 0.31$; recipients, $F_{(1,20)} = 2.526$, $p = 0.128$, $BF_{incl} = 0.71$], "choice" by "hierarchy" [focals, $F_{(1,20)} = 0.34$, $p = 0.568$, $BF_{incl} = 0.44$; recipients, $F_{(1,20)} = 0.354$, $p = 0.559$, $BF_{incl} = 0.43$], and "hierarchy" [focals, $F_{(1,20)} = 0.06$, $p = 0.814$, $BF_{incl} = 0.51$; recipients, $F_{(1,20)} = 0.017$, $p = 0.898$, $BF_{incl} = 0.55$]). Nevertheless, the dynamics of nose speed in the early phase of interaction (**Figure 6G**, left panel) showed that nose speed of submissive recipients was higher on selfish trials, especially after the first second of interaction (**Figure 6G**, right panel; two-way ANOVA; "hierarchy" by "choice" [$F_{(1,1925)} = 8.76$, $p = 0.003$], "choice" [$F_{(1,1925)} = 22.93$, $p = 2e{-}6$], and "hierarchy" [$F_{(1, 1925)} = 6.96$, $p = 0.008$]; one-way

ANOVA dissecting the interaction [$F_{(3,\ 1925)} = 9.609$, $p = 0.000003$] followed by SNK post hoc test revealed that nose speed of submissive recipients in selfish trials was higher compared with the other three categories).

We further asked whether dominance status affected the degree to which recipient rats were orienting toward their focal, as indication of increased social attention. To this end, we measured the orientation angle of the recipient's head toward the focal nose (**Figure 6H**, left panel) and found that submissive recipients were more oriented toward their focal compared with dominant recipients, with lower values indicative of a more directed orientation (**Figure 6H**, right panel; independent sample t test, $t_{(20)} = -4.52$, $p = 0.0002$, $BF_{10} = 106.48$). Further analyses revealed that over the interaction time prior to choice, submissive recipients spent a higher proportion of time orienting toward their focal, whereas dominant recipients spent a higher proportion of time orienting away from their focal (**Figure 6I**; independent sample t test for the proportion of time with orientation < 60°, $t_{(20)} = 3.80$, $p = 0.001$, $BF_{10} = 27.42$; time with orientation between 60° and 120°, $t_{(20)} = 1.18$, $p = 0.253$, $BF_{10} = 0.63$; time with orientation > 120°, $t_{(20)} = -4.32$, $p = 0.0003$, $BF_{10} = 72.84$). The same effect was observed in the dynamics of orientation during the early phase of interaction (**Figure 6J**, left panel), with submissive recipients more oriented to their dominant decision-maker both in prosocial and in selfish trials (**Figure 6J**, right panel; two-way ANOVA; "hierarchy" by "choice" [$F_{(1,1985)} = 9.20$, $p = 0.002$], "choice" [$F_{(1,1985)} = 183.43$, $p = 4e{-}40$], and "hierarchy" [$F_{(1,1985)} = 37.56$, $p = 1e{-}9$]; one-way ANOVA dissecting this interaction [$F_{(3,1985)} = 91.87$, $p = 1.136e{-}55$] followed by SNK post hoc test revealed a significant difference between all conditions). These results suggest that submissive recipients are more attentive to the behavior of the focal rat before the decision and change their orientation and position to maintain closer interactions with their dominant partner. Interestingly, this increased gazing from submissive recipients toward their dominant decision-maker was already observed in the first day of testing, whereas prosocial biases were not yet present (**Figures S3J–M**).

Next, we assessed whether this behavior of the recipient would lead to a more coordinated reciprocal interaction. We found that indeed, pairs with dominant focals spent a higher proportion of time orienting to each other, whereas pairs with submissive focals spent a higher proportion of time orienting away from each other (**Figure 6K**; independent sample t test for the proportion of time with both rats' orientations < 60°, $t_{(20)} = 2.36$, $p = 0.029$, $BF_{10} = 2.43$; both rats' orientations between 60° and 120°, $t_{(20)} = -0.33$, $p = 0.742$, $BF_{10} = 0.4$; time with both rats' orientations > 120°, $t_{(20)} = -3.37$, $p = 0.003$, $BF_{10} = 12.55$). In addition, submissive focals spent a higher proportion of time orienting away from their recipient, compared with dominant focals (**Figure 6L**; independent sample t test focal orientation < 60°, $t_{(20)} = 1.50$, $p = 0.148$, $BF_{10} = 0.85$; from 60° to 120°, $t_{(20)} = 0.34$, $p = 0.739$, $BF_{10} = 0.40$; orientation > 120°, $t_{(20)} = -2,42$, $p = 0.025$, $BF_{10} = 2.66$). Importantly, although orientation of focal animals was not as strongly modulated by hierarchy as observed for recipient's or mutual orientations, dominant decision-makers spent more time directly sniffing through the wall during the interaction period, suggesting enhanced social interest (**Figure 6M**; repeated-measure ANOVA; "choice" [$F_{(1,20)}$ = 5.70, p = 0.027, BF_{incl} = 3.29], "choice" by "hierarchy" [$F_{(1,20)}$ = 0.04, p = 0.844, BF_{incl} = 0.35], and "hierarchy" [$F_{(1,20)}$ = 4.95, p = 0.038, BF_{incl} = 2.16]). This effect was mainly driven by the behavior displayed in the second day of testing, when prosociality emerged (**Figure S3N**), and was not observed in recipient animals (**Figure S3O**).

4.4 Granger causality analysis of focal and recipient movements in the choice area reveals increased bidirectional influence in pairs with dominant focal

Overall, the results so far suggest that submissive recipients are more attentive to their dominants: they display more direct gazing prior to choice and increase proximity to their focals, specifically when decision-makers are going to be selfish (i.e., following them around the choice area). Dominant decision-makers might respond to these cues by showing increased social attention to their recipients which is reflected in increased sniffing time directed to the animal that needs help. In order to establish directionalities in the interactions between focals and recipients within trials, we implemented Granger causality from partial directed coherence, which evaluates whether the past of one time series contains exclusive information that helps predict the present value of another one. We computed the position of each rat's nose along the x axis (parallel to the dividing wall and ranging from the selfish port to the prosocial port) as a proxy for body movement between the two-choice options (**Figure 7A**), assessed whether the position of a rat would cause the other to follow (or move away), and whether this was dependent on hierarchy. Indeed, dominant focals strongly Granger-caused (g-caused) the position of their submissive recipient (**Figure 7B**; 0.018 bits, p = 0.002 against trial-shuffled surrogates; see **3.4.2**) and vice versa (**Figure 7C**; 0.006 bits, p = 0.001), indicating that both animals g-caused changes in the position of the other (**Figure 7D**). Considering that the positions of the animals are positively correlated (Pearson r = 0.277, $p < 0.00001$, n = 20,180), these results suggest that the movement of an animal causes movement of the other in the same direction, compatible with following behavior. The position of the rats was also positively correlated in dyads with submissive decision-makers (Pearson r = 0.146, $p < 0.00001$, n = 26,628); however, we found causality from focals to recipients (**Figure 7E**; 0.003 bits, p = 0.007) but not from recipients to focals (**Figure 7F**; 0.001 bits, p = 0.060), that is, unidirectional transfer of information (**Figure 7G**).

Interestingly, although both decision-makers g-caused changes in the position of their recipients, this influence was stronger when focals were the dominant of the pair (contrast focal dominant to recipient submissive larger than focal submissive to recipient dominant, p = 0.006). Importantly, the influence that the movement of the recipients caused in their decision-makers was stronger in the case of submissive recipients (contrast recipient submissive to dominant focal larger than dominant recipient to submissive focal, p = 0.032). Altogether, these results indicate that decision-maker and recipient become interdependent by influencing each other's movements, with dominance affecting the strength of such increased coordination.

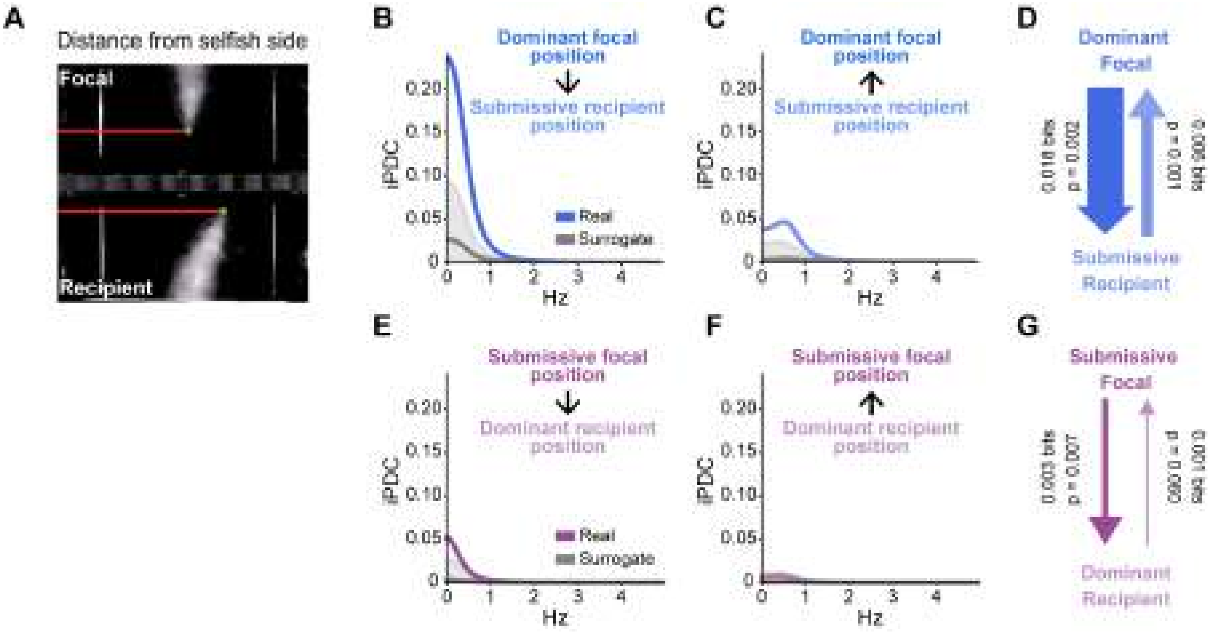

Figure 7. Granger causality analyses of animals' position reveal stronger bidirectional influences in dyads with dominant focals. (A) Rats' position was measured as horizontal distance from the selfish side of the choice area. **(B–C)** Information partial directed coherence (iPDC) **(B)** from dominant focal to submissive recipient and **(C)** from submissive recipient to dominant focal. **(D)** Information flow (I_{flow}) representing the causality from dominant focal to submissive recipient and vice versa in units of information transfer. **(E–F)** iPDC **(E)** from submissive focal to dominant recipient and **(F)** from dominant recipient to submissive focal. iPDC spectra from the real data are shown together with median and 95% confidence intervals from surrogate spectra distributions. **(G)** I_{flow} from submissive focal to dominant recipient and vice versa. Arrow widths are proportional to the I_{flow} values in each direction. p values account for significant differences between the real and surrogate iPDC.

4.5 Social dominance modulates recipient's call rate prior to choice, which correlates with the emergence of prosociality

In addition to body position, movement, and orientation, rats exchange social information through acoustic signals[166,211]. Adult rats emit vocalizations in ultrasonic frequencies of two distinct families: the 22-kHz or "alarm calls" and the 50-kHz calls[166]. The latter have been linked to different features of rat behavior, including mating[175,176], play[177], social contacts[178], reward anticipation[174], sniffing and locomotor activity[171–173]. However, the role of ultrasonic vocalizations (USVs) as communicative signals mediating animal prosocial decision-making has been largely unexplored. To address this, we recorded USVs during the first 2 days of the PCT, performed automated assignment of USVs agency based on the entropy of the signal (**Figures 8A** , **S4A**, and **S4B**), and combined this information with tracking and behavioral data in the maze (Figures **8B** and **8C**) to examine how this multimodal information may relate to dominance status and the emergence of prosocial choices.

All USVs recorded during the task were of the 50-kHz family, i.e., no alarm calls were observed, suggesting a positive emotional state of the interacting rats. Many vocalizations were emitted when the nose of the rats was close to the wall separating the two individual mazes and around the nose ports (**Figure 8C**). Nevertheless, normalizing the call rate by nose location revealed that rats vocalized with similar rates throughout the choice area, with no clear spatial preference (**Figure S4C**). Consistent with previous findings[173], rats in our task (both focal and recipient) partially synchronized the emission of calls with their own body movement, as evidenced by temporally precise correlations between nose speed and vocal production (**Figures S4D–E**). Interestingly, call rate was specifically modulated according to the role each animal had in the task, where focal animals vocalized at a higher rate than their recipients (**Figure S4D**).

To explore whether there were qualitative differences in the calls emitted by the animals, we classified their vocalizations into three different classes corresponding to different vocal programs (flat, frequency modulated, and step class). For this, we used VocalMat[200], a novel platform using convolutional neural networks for sonogram-based classification of rodent USVs. We did not find differences in the qualitative nature of USVs in focal/recipient or dominant/submissive animals (repeated-measures ANOVA with "USV class" as within-subjects factor and "hierarchy" as between-subjects factor; focal rats, "USV class" [F(1.36,27.26) = 100.57, p = 7e−12], "USV class" by "hierarchy" [F(1.36,27.26) = 0.05, p = 0.322], and "hierarchy" [$F_{(1,20)}$ = 0.01, p = 0.912]; recipients, "USV class" [F(1.07,21.35) = 80.38, p = 7e−9], "USV class" by "hierarchy" [F(1.07,21.35) = 0.36, p = 0.567], and "hierarchy" [$F_{(1,20)}$= 0.04, p = 0.847]) (**Figure 8D**) or in the evolution of this proportion across days and trial type (**Figures S5A–C**).

Then, we asked how focals' prosocial choices were related to the vocalization rates of the interacting animals. We included nose speed of the emitting rat as cofactor to isolate specific modulations of USVs rates from possible variations in movement. Prosocial choices were positively correlated with recipient's call rate but only when the recipient was the submissive of the pair (partial correlation between USV rate and prosocial choices, controlling for recipient speed; submissive recipients, r = 0.73, p = 0.037; dominant recipients, r = −0.56, p = 0.055) (**Figures 8E** and **S5D**). Since submissive recipients were also found to modulate their position and movement toward the focal, these results suggest that they may increase call rate to interact further with the focal, consistent with the proposed role of 50-kHz calls in promoting social contact[212].

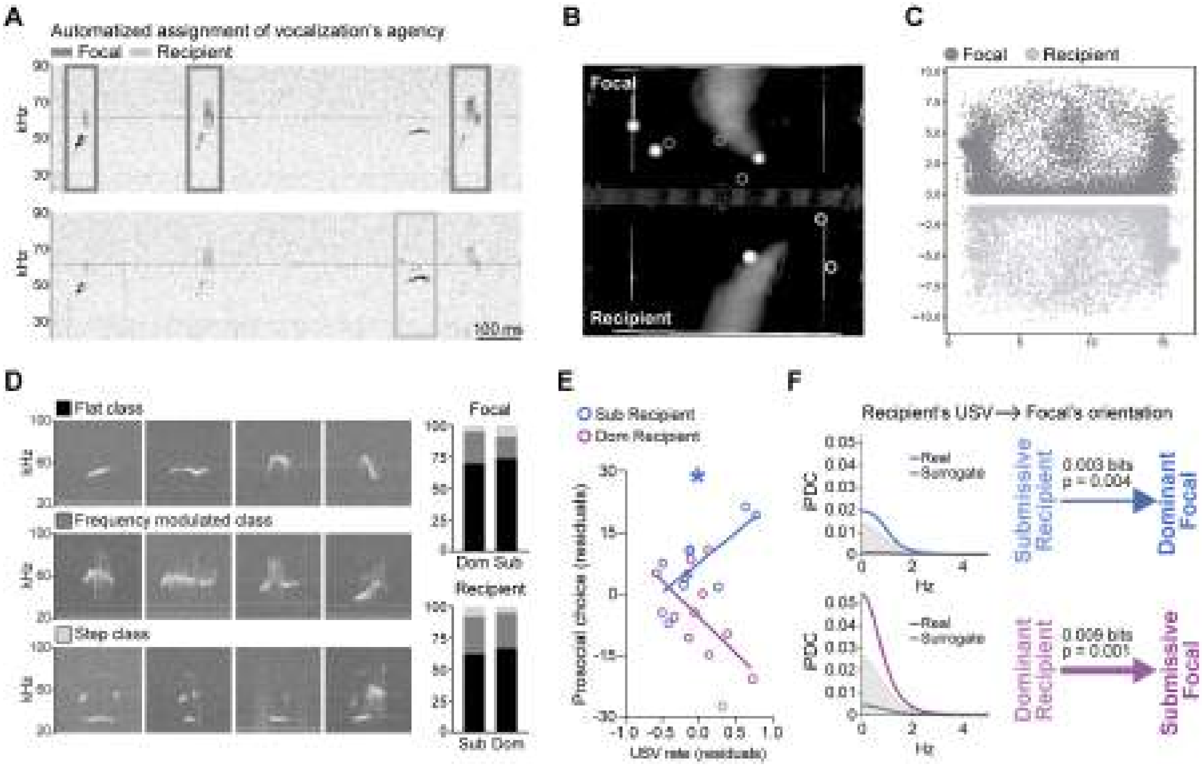

Figure 8. Social dominance modulates recipient's call rate prior to choice, which correlates with the emergence of prosociality. (A) Example USVs recording from a prosocial choice task session, showing sonograms for the two microphones, each one placed above the choice area of each maze. In this case, the top sonogram corresponds to the microphone placed above the focal rat and the bottom one to that on top of the recipient. Notice that USVs are detected from both microphones, but each USV is automatically assigned to either the focal (dark grey rectangles) or the recipient rat (light gray rectangles) according to the entropy levels (see 3.3.3). **(B)** Example image showing localization of agent-assigned USVs emitted during a trial by the focal and recipient rats while in the choice area. Circles indicate the position of the rats' nose at the time a USV was emitted. Filled circles correspond to the USVs shown in (A). **(C)** Nose location of focal (dark grey) and recipient (light grey) for all USVs detected, relative to the choice area, during the first 2 days of the prosocial choice task. USVs were emitted in the whole choice area; however, they were more frequent around nosepokes and in proximity to the wall that separated both mazes. See also **Figure S4**. **(D)** Four examples of spectrogram images are shown for each USV class: flat, frequency modulated, and step. Flat calls were the most frequent class observed, followed by frequency modulated, while step calls were rare. The proportions of calls (right panel) were similar in focals (top) and recipients (bottom), regardless of the hierarchy status. **(E)** Partial correlation between recipients' USVs rate and focals' prosocial choice preference in the first 2 days of the PCT, when recipients' speed was regressed out, indicating that the more the submissive recipient vocalizes, the more prosocial their dominant partner would be. This correlation was only marginally significant when recipients were the dominant in the pair. See also **Figure S5**. **(F)** Granger causality from recipients' USV to focals' orientation, showing information partial directed coherence (iPDC) from submissive recipient to dominant focal (top) and from dominant recipient to submissive focal (bottom). Independently of the hierarchy status of the animals, recipients' USVs would Granger-cause an orientation response from the focal rat. Real iPDC values, surrogate median, and 95% confidence intervals of the surrogates' distribution are shown. Arrow widths are proportional to the I_{flow} and p values account for significant differences between the real and surrogate iPDC. $^{*}p < 0.05$.

Interestingly, we found that social hierarchy modulated the direction of correlations between USV rate and prosociality where the more submissive rats would call the more prosociality, and conversely, the more dominants would call the less prosociality would be observed, especially on the second day of testing (**Figure S5D**). Although the sign of the correlations was independent of the role (focal/recipient) in the task, these correlations were mainly significant when considering the USV rate of recipients.

In light of this opposite correlation, we asked whether the effect that recipients' calls have on their decision-makers' behavior was different depending on their hierarchical status. We used partial directed coherence to test whether the emission of USVs by submissive and dominant recipients would affect gazing behavior of their focal differently (**Figure 8F**). We found that emission of calls from the recipient rat promoted more direct gazing from the focal, but this was independent of social hierarchy (I_{flow} from submissive recipients' USV to dominant focals' orientation, 0.003 bits, p = 0.004; Pearson r = −0.0732, p = 7e−32 [n = 25,641]; I_{flow} from dominant recipients' USV to submissive focals' orientation, 0.009 bits, p = 0.001; Pearson r = −0.0528, p = 5e−21 [n = 31,706]).

4.6 Identification of multimodal cues displayed by both animals as predictors of prosocial choices on a trial-by-trial basis

So far, we described that dominant animals are more prosocial, learning the contingencies of the PCT faster, and submissives, by following their dominants, have a stronger impact when communicating need. This is related to a more synchronized social interaction of both animals that builds upon multimodal cues displayed by submissive recipients, especially when decision-makers are going to behave selfishly. These different social dynamics are correlated with prosocial choice; however, it is still uncertain which cues animals utilize that predict prosocial choices on a trial-by-trial basis. To examine the contribution of a multitude of parameters to the focal's choice, we employed a multi-step generalized linear model (GLM). Given that nose-to-nose distance and the gazing angle of each animal of the pair were the regressors that explained most of the deviance (**Figures 9A–B** and **S6**), we asked how these parameters interact with social hierarchy and trial progression in the prediction of prosociality. Trial progression, often included in models of decision-making as a proxy for learning, was considered critical for our analyses as animals starting the social task learn about the new contingencies with respect to the individual training. Significant interactions between behavioral variables, learning, and social hierarchy were observed (**Table S2**). As our main objective was to disentangle the contribution of social hierarchy on the predictors of choice, we performed reduced GLM for data from each dominance category (**Figures 9C–D**). In both social hierarchy groups, trial progression positively influenced prosocial choice, i.e., both groups increased prosocial choice over time. However, only when dominant animals were the focals were there additional behavioral changes as sessions proceed. Specifically, when dominant animals were the decision-makers, orientation angles decreased (i.e., there was more direct gazing) as trials proceeded, indicating the

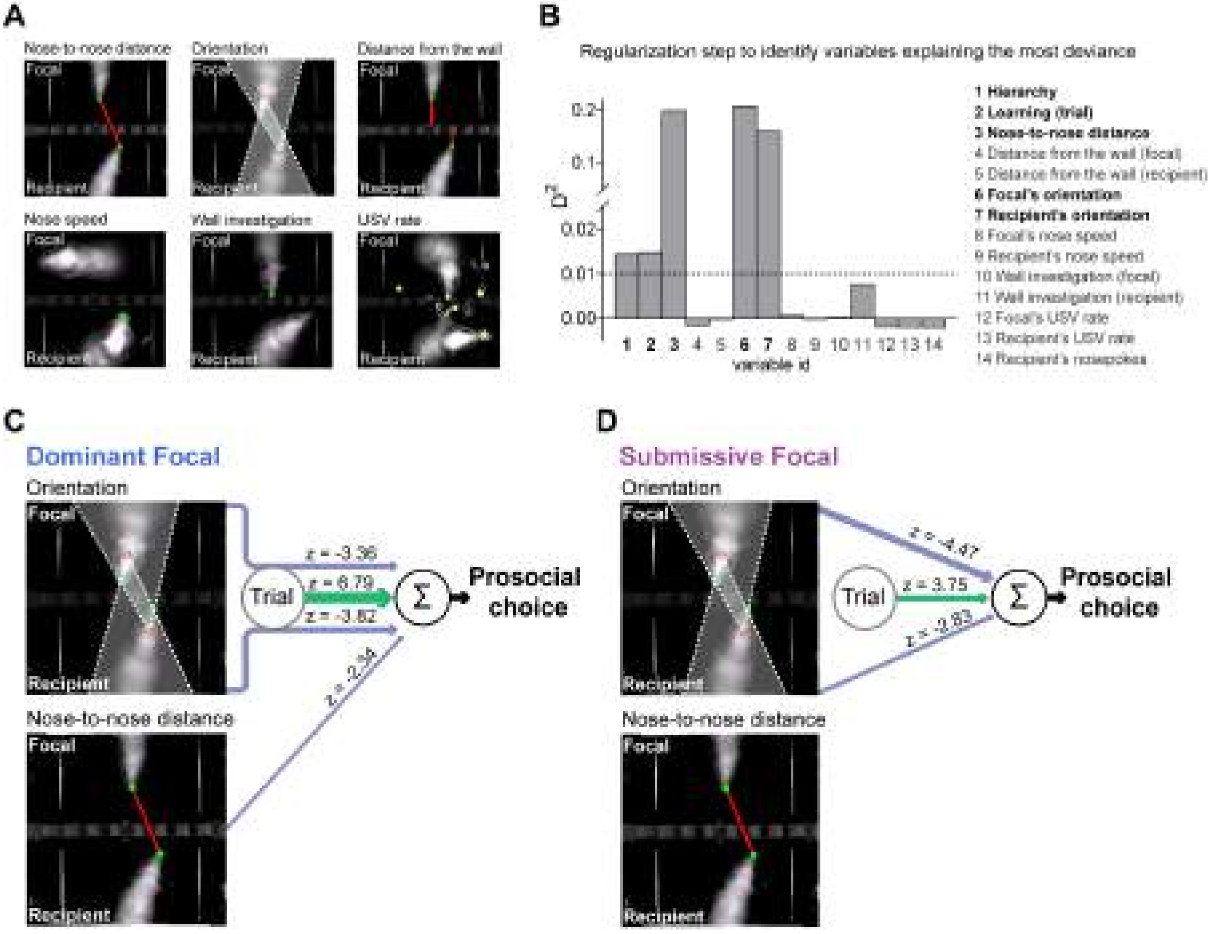

Figure 9. Behavioral predictors of prosocial choice on a trial-by-trial basis. (A) Images of the behavioral variables measured either in focal or recipient animals that were included in the analysis, together with the hierarchical status and trial number as a proxy for learning**. (B)** These 14 behavioral and categorical parameters were chosen as regressors and evaluated by their contribution to the explained deviance of the model. The graph shows the mean proportion of deviance (D^2) computed for each regressor. Trial number, hierarchy, nose-to-nose distance, focal orientation angle, and recipient orientation angle were selected as the regressors that explained more than 1% of the deviance (dashed line) and used to fit a reduced GLM (see **Figure S6** for unique contribution analysis of these variables). Because the latter 3 regressors were found to interact simultaneously with both trial number and hierarchy **(Table S2)**, we fitted separate GLMs for dominant and submissive animals, thus removing hierarchy from the models and facilitating the interpretation of interaction terms **(Tables S3** and **S4)**. **(C–D)** Representation of a trial-by-trial GLM analysis for pairs with **(C)** dominant and **(D)** submissive focal animals. Here, model terms are represented diagrammatically: the Σ symbol represents the summation of parameters that influence choice, green lines indicate regressors whose contributions correlate positively with prosocial choice, and blue lines indicate regressors whose contributions correlate negatively with prosocial choice. Line passing through "Trial" indicate that the interaction between that regressor and trial contributed significantly to choice and line thickness indicates the strength of those contributions as measured by the Z score of the regressor weight. The absence of a line from a behavioral parameter to the Σ symbol represents the absence of a statistical contribution to choice for that parameter.

occurrence of some form of learning on behalf of both animals that ultimately led to a higher proportion of prosocial choices (**Table S3**). However, this learning was not observed in pairs with submissive decision-makers (**Table S4**). Moreover, the nose-to-nose distance was negatively predictive of choice (the lower the distance between the animals, the more it predicted prosocial choice), a relationship that was not observed in submissive decision-makers. Interestingly, this relationship of social distance only in pairs with dominant focals was independent of trial progression, indicating that this regressor was a qualitative characteristic inherent to social status evident since the first interactions in the maze (**Table S3**).

Supplementary figures and tables

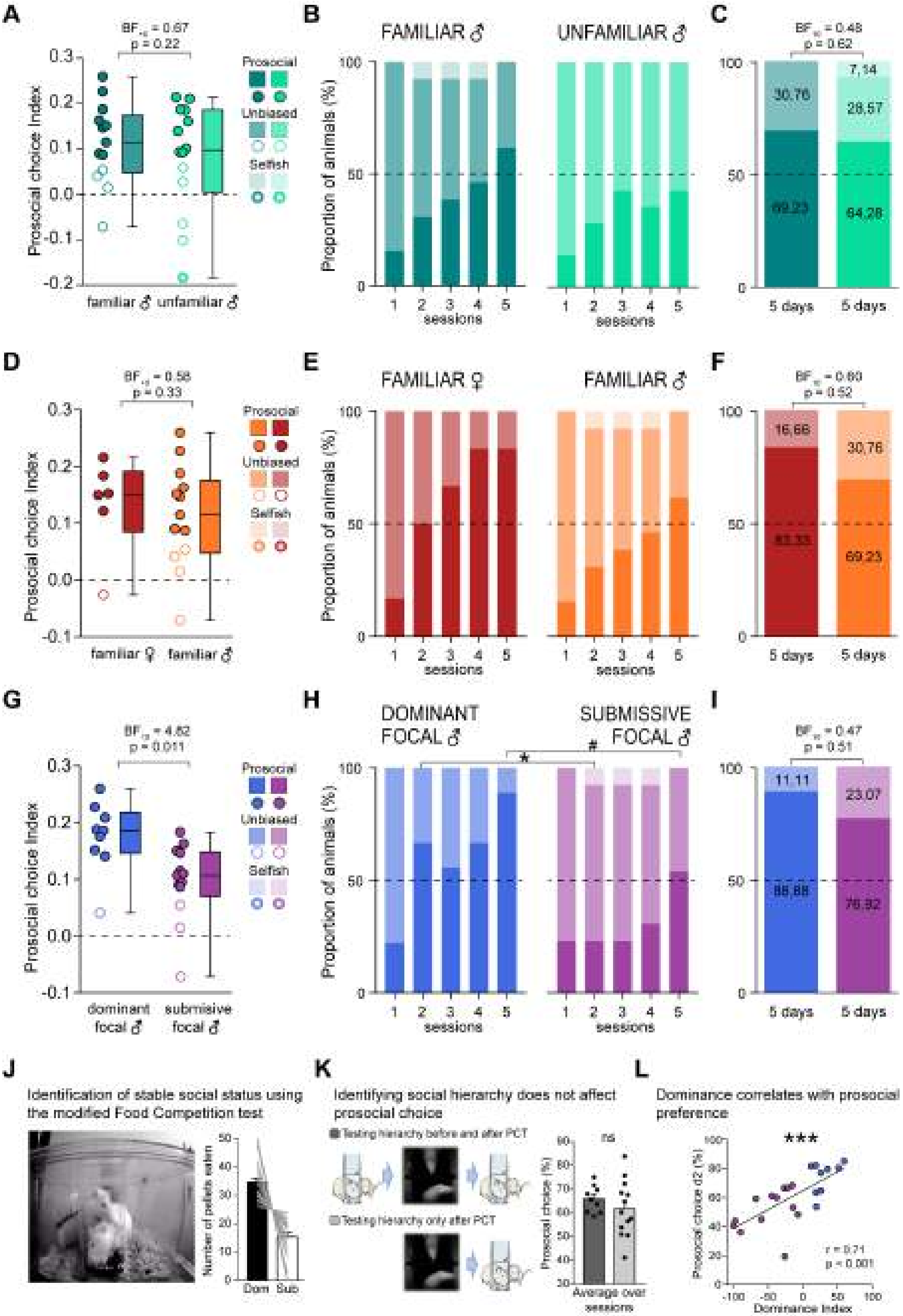

Figure S1. Individual differences in PCT and identification of social dominance. Related to Figure 4. (A) Distribution of Prosocial Choice Indexes (PCI, see 3.4.4) calculated for familiar and unfamiliar male groups. Each symbol represents the PCI for each rat over the five testing sessions; filled dots indicate prosocial rats, meaning a positive change on preference compared to chance (50%) after a permutation test; empty dots indicate unbiased rats, which preference is not different from chance; and degraded dots represent selfish animals, those having a negative change on preference. Boxplots show median and 1st and 3rd quartiles, whiskers 2.5% and 97.5% percentile values. Independent samples t-test showed no differences between the PCI of the groups $t_{(25)}$= 0.779, p= 0.222, BF_{+0}= 0.67. **(B)** We then analysed how the proportion of prosocial, unbiased and selfish animals emerged across testing days. No significant differences were observed between groups, with Bayesian statistics indicating absence of evidence (day1 X2 (1, 27) = 0.006, p = 0.936, BF_{10} =0.577; day2 X2 (2, 27) = 1.160, p = 0.560, BF_{10} =0.543; day3: X2 (2, 27) = 1.122, p = 0.571, BF_{10} =0.492; day4: X2 (2, 27) = 1.656, p = 0.437, BF_{10} =0.633; day5: X2 (1, 27) = 0.942, p = 0.332, BF_{10} =0.692). **(C)** In the same direction, the proportion of prosocial, unbiased, and selfish rats considering together the five sessions of PCT for familiar and unfamiliar male groups, was not significantly different (X2 (2, 27) = 0.964, p = 0.617, BF_{10} = 0.483). **(D)** Same as in A for the distribution of Prosocial Choice Indexes (PCI) for familiar females and males. Independent samples t-test showed no differences between males and females ($t_{(17)}$= 0.457, p= 0.327, BF_{+0}= 0.58). **(E)** Same as B for familiar females and males groups. Although a tendency for female animals to display faster emergence of prosociality could be observed, no significant differences were found across the days (day1 X2 (1, 19) = 0.005, p = 0.943, BF_{10} =0.60; day2 X2 (2, 19) = 1.644, p = 0.44, BF10 =0.67; day3: X2 (2, 19) = 1.516, p = 0.469, BF10 =0.59; day4: X2 (2, 19) = 2.411, p = 0.30, BF_{10} =0.87; day5: X2 (1, 19) = 0.903, p = 0.342, BF10 =0.71). **(F)** Same as C for familiar females and familiar males groups, where no significant differences between conditions were found (X2 (1, 19) = 0.421, p = 0.516, BF_{10} = 0.60). **(G)** Consistent with the percentage of prosocial choices results, PCI where higher for dominant focal males (Independent samples t-test: $t_{(20)}$= 0.457, p= 0.011, BF_{10}= 4.82). **(H)** Dominant focal groups showed significantly different distributions in the second day of testing, indicative of higher number of prosocial animals in early testing days. (day1 X2 (1, 22) = 0.002, p = 0.962, BF10 =0.54; day2 X2 (2, 22) = 6.249, p = 0.044, BF_{10} =5.67; day3: X2 (2, 22) = 2.788, p = 0.248, BF_{10} =1.13; day4: X2 (2, 22) = 3.046, p = 0.218, BF_{10} =1.25; day5: X2 (1, 22) = 3.010, p = 0.083, BF_{10} =1.98). **(I)** However, when taking into account all testing sessions, no significant differences on the proportions were observed anymore (X2 (1, 22) = 0.512, p = 0.474, BF_{10} = 0.68). **(J)** (Left) image showing two male cage-mate rats performing the modified Food Competition test for identification of stable social hierarchies in the homecage. In this task, only one of the two animals can gain access to palatable pellets in each trial, leading to a subtle conflict that results in higher consumption of food by one animal of the pair. (Right) Number of pellets eaten by the two rats within each pair averaged across the testing days. The rat eating more pellets over the testing days was categorized as the dominant ('Dom') and the rat eating less pellets as the submissive ('Sub') of the pair. **(K)** To control for any effect of testing for hierarchy in the modified Food Competition test on prosociality levels, we compared a group of pairs tested before and after the PCT (n = 9), with a group of pairs tested only after the PCT (n = 13). No substantial difference was found between the two groups in the proportion of prosocial choices over sessions, indicating that being tested for hierarchy does not affect prosocial tendencies (independent sample t test: $t_{(20)}$ = 0.989, p = 0.334, BF_{10} = 0.55). Data are represented as group MEAN ± SEM of the prosocial levels of the 5 testing sessions. **(L)** Dominance Index (DI) as a measure of social hierarchy strength positively correlates with prosocial preference displayed during day 2 of PCT testing (r= 0.71, p=0.0002). Blue dots indicate dominant focal rats, and purple dots submissive focal rats. #p<0.1, *p<0.05, ***p<0.001, ns = not significant.

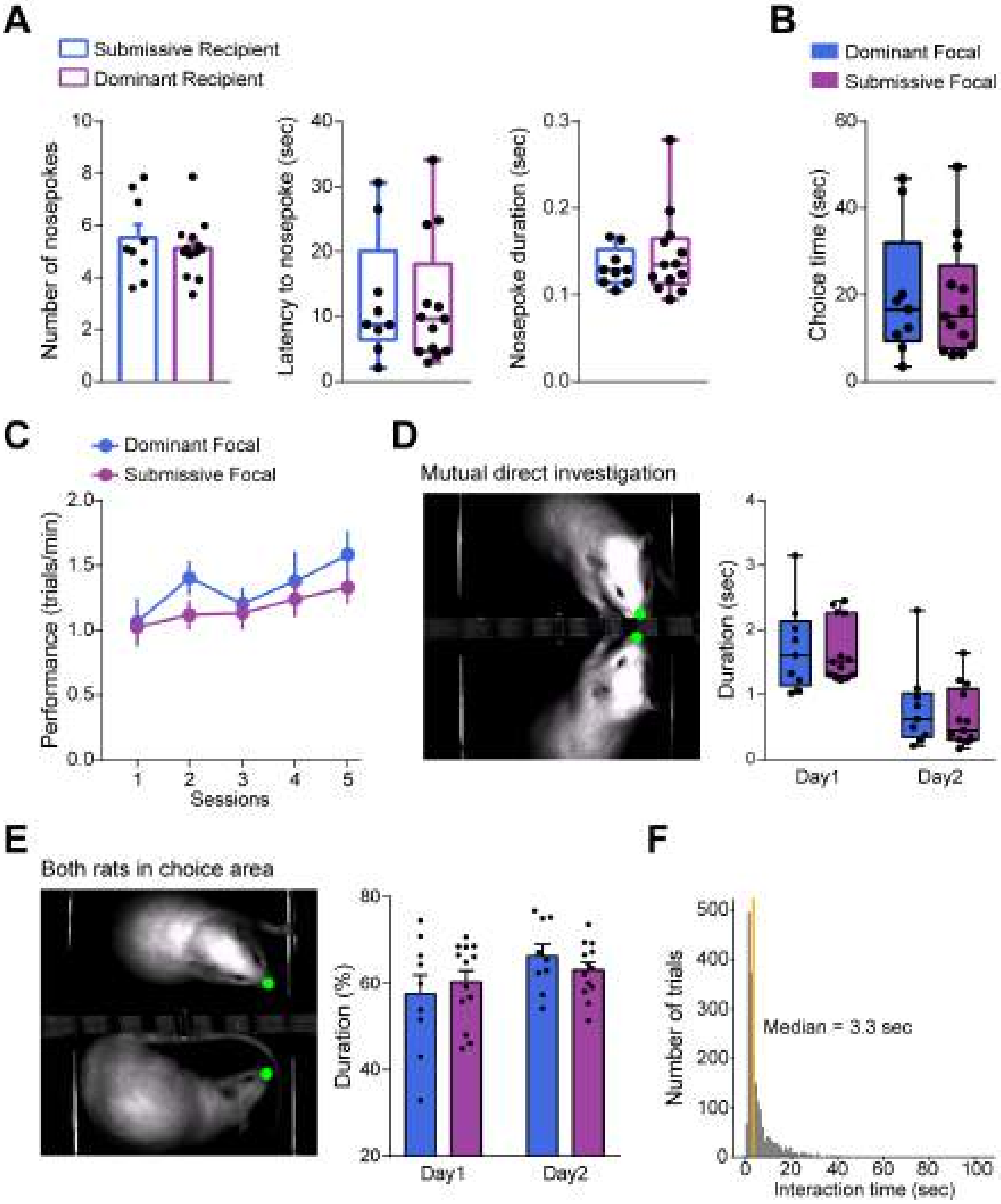

Figure S2. Effects of dominance status on recipient's nosepokes, choice time, task performance, mutual investigation and interaction time. Related to Figure 4, 5 and 6. (A) Social hierarchy of the recipients does not affect displays of food seeking behavior. Submissive and dominant recipients showed no difference in the number of nosepokes per trial (left), nor in the latency to perform the first nosepoke from start trial (middle), neither in the duration of nosepoke (right) prior to choice (independent sample *t* test for the number of nosepokes of submissive against dominant recipients: $t_{(20)} = 0.71$, $p = 0.488$, $BF_{10} = 0.465$; Mann-Whitney U Test for latency to nosepoke and nosepoke duration: $U = 63$, $p = 0.794$, $BF_{10} = 0.384$; $U = 54$, $p = 0.794$, $BF_{10} = 0.412$). **(B)** The duration of the choice period was similar between dominant and submissive focals (Mann-Whitney U Test: $U = 61$, $p = 0.896$, $BF_{10} = 0.372$). **(C)** Dominance status did not affect the performance in the PCT, measured as number of trials per minute (repeated-measure ANOVA with "session" as within-subjects factor and "hierarchy" as between-subjects: "session" ($F_{(4,80)}=5.577$, $p=0.004$, $BF_{incl} = 39.9$), "session" by "hierarchy" ($F_{(4,80)}$)$=0.695$, $p=0.535$, $BF_{incl} = 0.15$) and "hierarchy" ($F_{(1,20)}=0.821$, p=0.376, BF_{incl} = 0.61). **(D)** Despite the changes in prosociality observed in day 2, these were not accompanied by differences in the duration of social investigation across the testing days (Mann-Whitney U Test for “mutual direct investigation” on day 1: *U* = 55, p = 0.845, BF_{10}=0.415; on day 2: *U* = 65.5, p = 0.647, BF_{10}=0.433), nor by differences in **(E)** the percentage of choice time per trial that both rats are present in the choice area, as an index of duration of social interactions in the distance (independent sample t test for “both rats in choice area” on day 1: $t_{(20)}$ = -0.593, p = 0.560, BF_{10}=0.44; on day 2: $t_{(20)}$ = 1.049, p = 0.307, BF_{10}=0.57) **(F)** Histogram including all trials from the first two sessions of both dominance groups, showing the median time (orange line) of interaction (total time per trial in which the noses of both rats were simultaneously tracked in the choice area). This value was selected as upper limit for all the trials to visualise the early dynamics of nose-to-nose distance, recipient’s distance from the wall, nose speed and orientation, plotted in **Figure 6** and **Figure S3**. Bar graphs in (A) and (E) show MEAN ± SEM and individual values; line plot in (C) shows MEAN ± SEM; box plots in (A), (B) and (D) show median, first and third quartiles, with whiskers indicating maximum and minimum values. Individual values correspond to the mean over days for each animal.

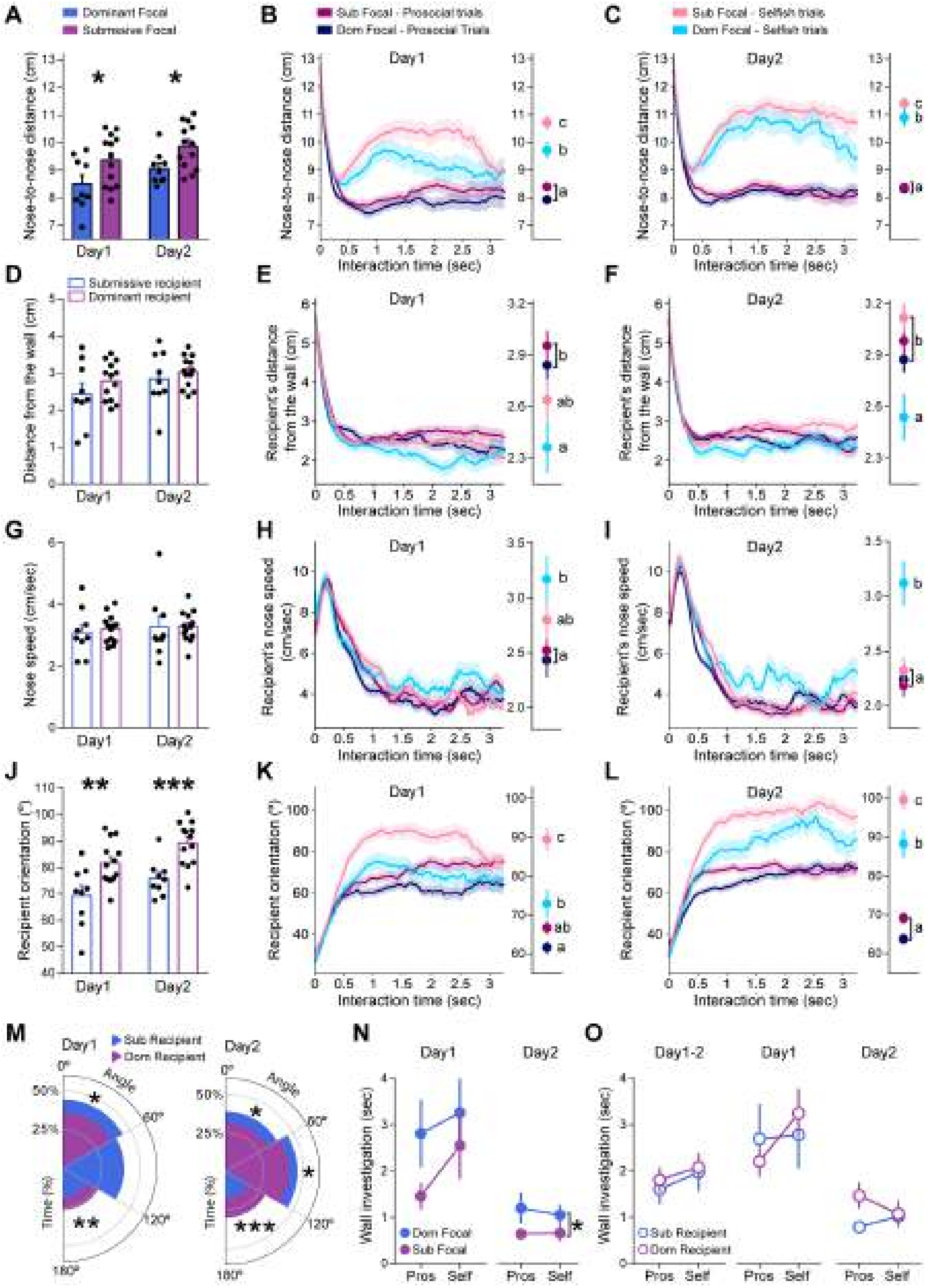

Figure S3. Social dominance modulates the dynamics of social interactions prior to choice across days. Related to Figure 6. (A) The median distance between focal and recipient noses per trial, as a proxy for social interest of the pair, was measured during the interaction time, defined as the time that the two rats were simultaneously present in the choice area. Pairs with dominant focals showed lower nose-to-nose distance on both the first and second day of the

PCT (independent sample t test for day 1: $t_{(20)}$ = -2.11, p = 0.048, BF_{10} = 1.73; for day 2: $t_{(20)}$ = -2.54, p = 0.02, BF_{10} = 3.18), suggesting that more proximal interactions preceded the emergence of prosocial choice (observed from day 2). Dynamics of nose-to-nose distance in the first seconds of interaction on **(B)** day 1 and **(C)** day 2. Pairs with dominant focal maintained shorter nose-to-nose distance in selfish trials on both days (one-way ANOVA comparing the four conditions for day 1: $F_{(3, 907)}$ = 38.72, p = 1.5e-23; for day 2: $F_{(3, 1075)}$ = 92.91 , p = 1.825e-53). **(D)** The median distance between recipients' nose and the central wall per trial was similar between submissive and dominant recipients on both the first and second day of the PCT when not taking into account trial type (independent sample t test for day 1: $t_{(20)}$ = -1.21, p = 0.242, BF_{10} = 0.65; for day 2: $t_{(20)}$ = -0.79, p = 0.442, BF_{10} =0.48). Nevertheless, the dynamics of the distance in the early phase of interaction on **(E)** day 1 and **(F)** day 2 showed that submissive recipients stayed closer to the wall than dominant recipients on day 2 in selfish trials, being this difference only marginal on day 1 (one-way ANOVA comparing the four conditions for day 1: $F_{(3,907)}$ = 4.61 , p = 0.003; for day 2: $F_{(3, 1075)}$ = 4.84, p = 0.002). **(G)** The median nose speed per trial was similar between submissive and dominant recipients on both days of testing (independent samples t-test for day 1: $t_{(20)}$ = 0.47, p = 0.64, BF_{10} =0.42; for day 2: $t_{(20)}$=0.05, p=0.96, BF_{10}=0.39. Nevertheless, the dynamics of nose speed on **(H)** day 1 and **(I)** day 2 showed that submissive recipients kept moving the snout faster than dominant recipients on day 2 in selfish trials, and tended to do so on day 1, after the first second of interaction (one-way ANOVA comparing the four conditions for day 1: $F_{(3,884)}$ = 3.29, p = 0.02; for day 2 $F_{(3,1037)}$ = 6.63, p = 0.0002). (**J**) On both days, submissive recipients were more oriented towards their focal compared to dominant recipients (independent samples t test for day 1: $t_{(20)}$ = -2.96 , p = 0.008, BF_{10} =6.21; for day 2: $t_{(20)}$ = -4.01, p = 0.0007, BF_{10}=40.76). The same effect was observed in the dynamics of orientation **(K-L)**, with submissive recipients being more oriented to their dominant decision-maker in selfish trials of both days. Interestingly on day 1, the orientation of submissive recipients in selfish trials was more similar to the orientation of dominant recipients in prosocial trials than that displayed in selfish trials. One-way ANOVA comparing the four conditions for day 1: $F_{(3,906)}$ = 26.58, p = 1.76E-16; for day 2: $F_{(3,1075)}$ = 76.92, p = 4.45E-45). **(M)** On both days, submissive recipients spent a higher proportion of time orienting towards their focal, while dominant recipients spent a higher proportion of time orienting away from their focal (for day 1, independent samples t test for the proportion of time with orientation <60°: $t_{(20)}$ = 2.69 , p = 0.015, BF_{10}=3.8, time with orientation between 60° and 120°: $t_{(20)}$ = -0.08, p = 0.939, BF_{10}=0.39, time with orientation >120°: $t_{(20)}$ = -3.08, p = 0.006, BF_{10}=7.64 ; for day 2, time with orientation <60°: $t_{(20)}$ = 2.49, p = 0.029, BF_{10}=4.39, time with orientation between 60° and 120°: $t_{(20)}$ = 2.26, p = 0.035, BF_{10}=2.11, time with orientation >120°: $t_{(20)}$ = -4.39 , p = 0.0003, BF_{10} =82.7). Regarding mutual orientation, this trend was already significant on day 1 (on day 1, independent sample t test for the proportion of time with both rats' orientations <60°: $t_{(20)}$ = 2.249, p = 0.036, BF_{10} = 2.1, with both orientations between 60° and 120°: $t_{(20)}$ = -1.335, p = 0.197, BF_{10} = 0.73, with both orientations >120°: $t_{(20)}$ = -2.654, p = 0.015, BF_{10} = 3.8); on day 2, time with both orientations <60°: $t_{(20)}$ = 1.8, p = 0.087, BF_{10} = 1.12, with both orientations between 60° and 120°: $t_{(20)}$ = 0.606, p = 0.551, BF_{10} = 0.44, with both orientations >120°: $t_{(20)}$ = -3.274, p = 0.004, BF_{10} = 10.61). **(N)** Dominant focals investigate the wall that separates them from their submissive partner for longer durations regardless of trial type. Although this trend was not significant on day 1 due to the high variability observed (repeated measures ANOVA, "choice" ($F_{(1,20)}$ = 2.29, p = 0.146, BF_{incl} = 0.96), "choice" by "hierarchy" ($F_{(1,20)}$ = 0.39, p = 0.541, BF_{incl} =0.49) and "hierarchy" ($F_{(1,20)}$ = 1.93, p=0.180, BF_{incl}=0.74), it reached significant levels on day 2 ((repeated measures ANOVA, "choice" $F_{(1,20)}$ = 0.12, p = 0.729, BF_{incl} =0.3, "choice" by "hierarchy" $F_{(1,20)}$ = 0.18, p = 0.672, BF_{incl}=0.42, "hierarchy" $F_{(1,20)}$ = 4.65, p = 0.043, BF_{incl} =1.5). **(O)** Submissive and dominant recipients spent a similar amount of time investigating the wall when the focal was in the choice area, both in prosocial and selfish trials (RM-ANOVA for d1-2: "choice" ($F_{(1,20)}$ = 2.14, p = 0.159, BF_{incl} =0.69), "choice" by "hierarchy" ($F_{(1,20)}$ = 0.05, p = 0.827, BF_{incl}=0.38) and "hierarchy" ($F_{(1,20)}$ = 0.17, p=0.687, BF_{incl}=0.47)). No effects were observed on the single days either (on day 1: "choice" $F_{(1,20)}$ = 1.82, p = 0.193, BF_{incl} =0.79, "choice" by "hierarchy" $F_{(1,20)}$ = 1.28, p = 0.272, BF_{incl}=0.63, "hierarchy" $F_{(1,20)}$ = 0.0002, p = 0.989, BF_{incl}=0.42; on day 2: "choice" $F_{(1,18)}$ = 0.12, p = 0.739, BF_{incl} =0.36, "choice" by "hierarchy" $F_{(1,18)}$ = 2.35, p = 0.143, BF_{incl}=1.05, "hierarchy" $F_{(1,18)}$ = 0.93, p = 0.347, BF_{incl} = 0.62. On **B,C,E,F,H,I,K,L** the left panel shows the temporal dynamics for each condition; the right panel shows the average, SEM and statistics of this time window. Letters show results of Student-Newman-Keuls (SNK) post-hoc test used to evaluate differences among dominance-trial type categories. Pros: prosocial, Self: selfish. *p<0.05, **p<0.01, ***p<0.001.

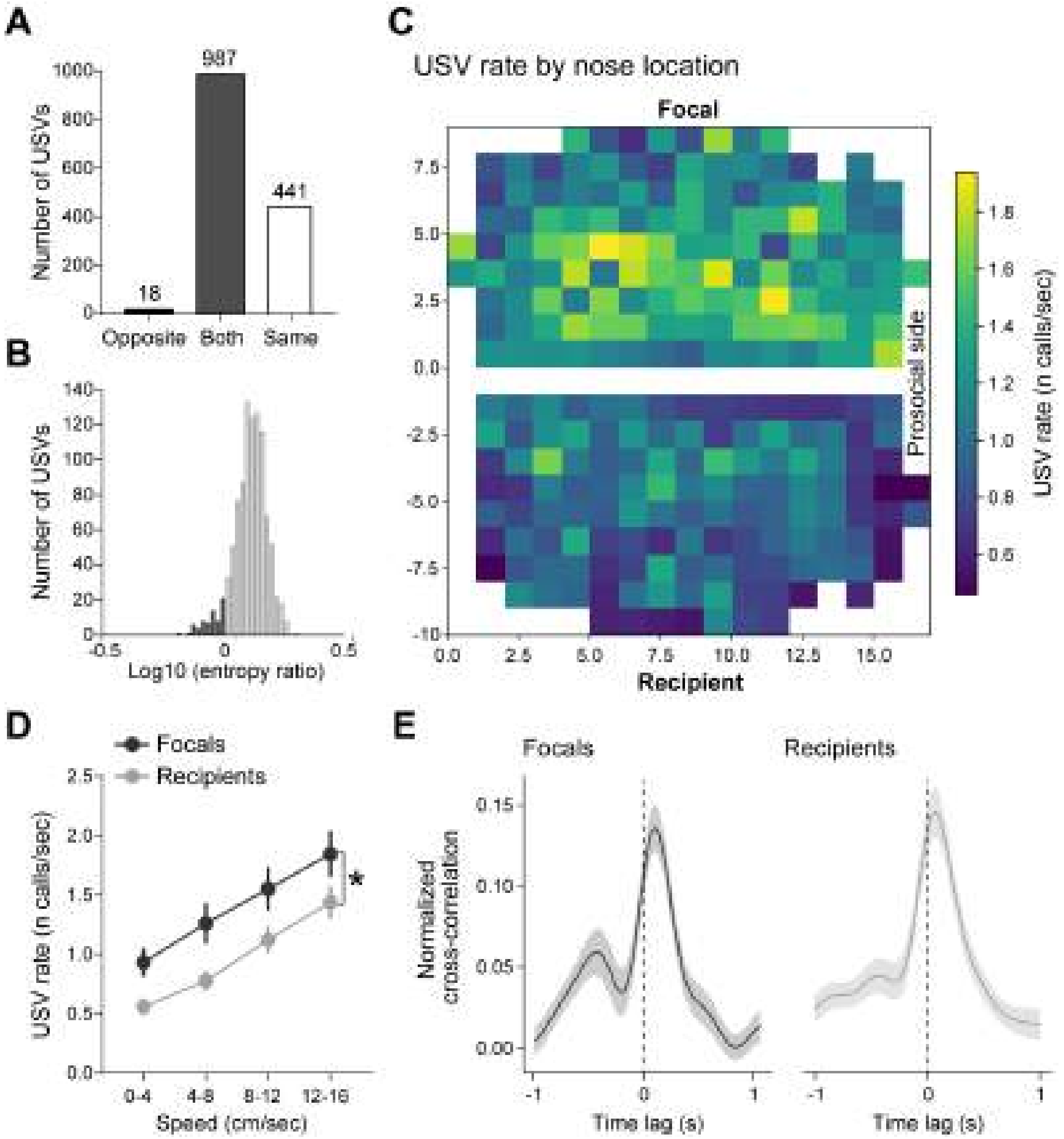

Figure S4. Validation of the USV assignment to individual animals within a pair, USVs rates by rat position and correlation between USV emission and snout speed. Related to Figure 8. (A) In recordings with only one rat placed alone in the double T-maze, all USVs should ideally be assigned to the side of the arena where the rat is**.** Of the 1446 USVs detected from 15 recordings obtained from 10 rats performing alone in one side of the double maze, 441 (31%) were only detected by the microphone over its side, 18 (1%) only by the opposite microphone and 987 (68%) were detected by both microphones. **(B)** Of those USVs detected by both microphones, 917 (93%) would have been correctly assigned to the one on the occupied side, as its signal had lower entropy (log10(entropy ratio occupied/empty) > 0, in grey). Overall, 94% of the detected calls were correctly assigned to the emitting rat (6% assignment error). **(C)** Heat map showing USVs rate of focal (top) and recipient rat (bottom) normalized by the time spent in each nose location of the choice area. Locations visited in less than 250 video frames were left out when normalising (white space). Prosocial side is on the right side of the image and dimensions are shown in centimetres. **(D)** USV rate vs instantaneous speed, showing that both focal and recipients increased call rate with speed. A RM-ANOVA indicated a significant effect of "speed bin" ($F_{(1.7,71.68)} = 130.26$, $p = 6e\text{-}23$, $\eta2 = 0.195$, $BF_{incl} = 2e{+}35$), no significant interaction between "speed bin" and "role" ($F_{(1.7,71.68)} = 0.47$, $p = 0.597$, $\eta2 = 7e\text{-}4$, $BF_{incl} = 0.107$) and a significant effect of "role" ($F_{(1,42)} = 4.87$, $p = 0.033$, $\eta2 = 0.077$, $BF_{incl} = 1.48$). *$p<0.05$. **(E)** Normalized cross-correlation of instantaneous speed and call rate for focals (left panel) and recipients (right panel). The peak of cross -correlation was consistently shifted from zero-time, revealing that vocal production preceded the speed increase by about 70ms. Mean across rats ± SEM are shown.

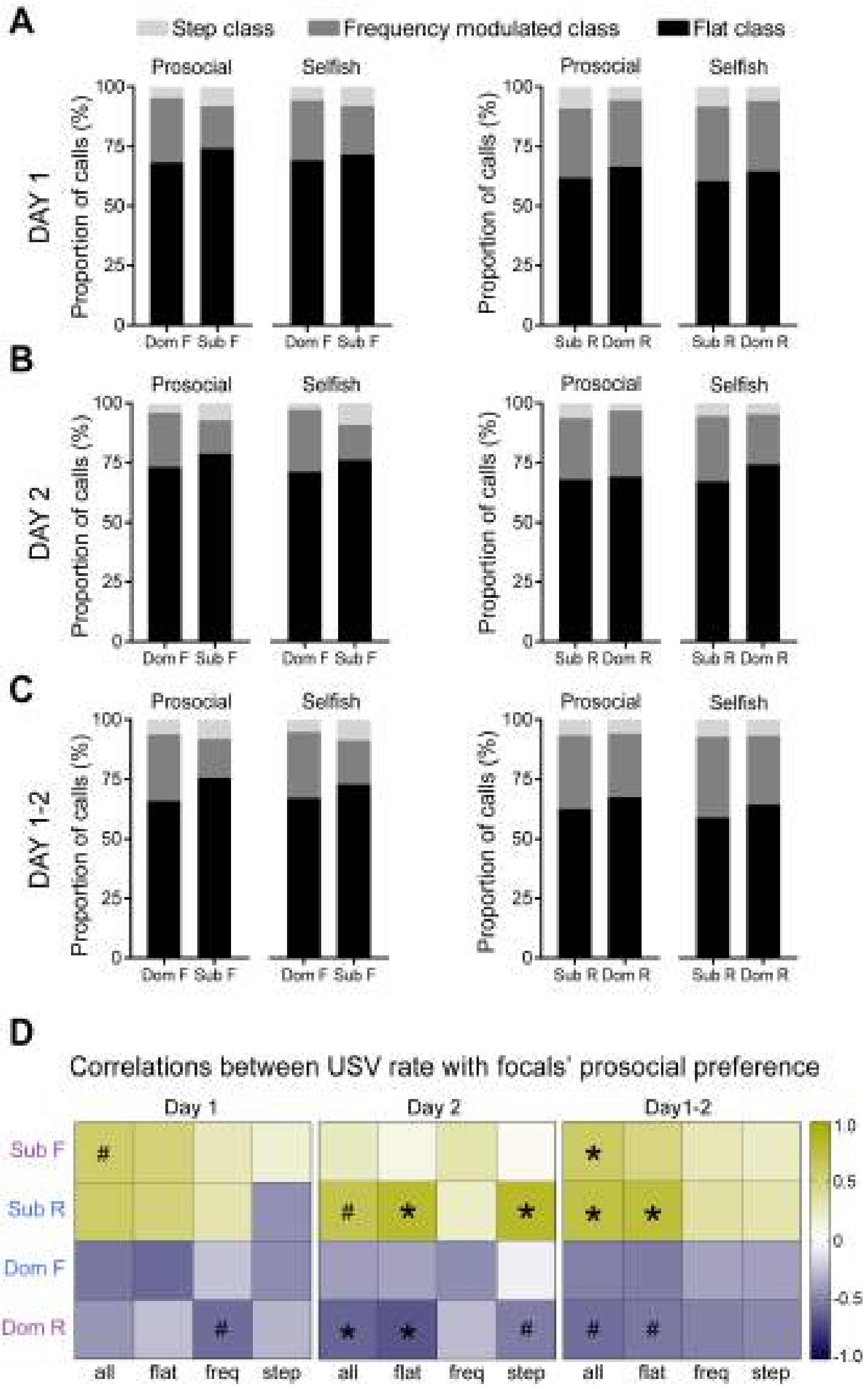

Figure S5. USV class proportions across days and correlation between USV rate and focal's prosociality. Related to Figure 8. Proportion of USVs by class (flat, frequency modulated and step) on **(A)** day 1, **(B)** day 2 and **(C)** day1-2 in prosocial and selfish trials for both focal (left) and recipient (right) rats, according to hierarchy. Flat calls were the most frequent, followed by frequency modulated and step. No effect of dominance was observed in any condition, regardless of the day of testing and the role of the animals (repeated measure ANOVA of proportion of USVs class and trial type as within subjects factors. For focal rats: Class by hierarchy on day 1 $F_{(2,36)}$=0.70 p = 0.501; day 2 $F_{(2,40)}$=1.63 p = 0.208; day1-2 $F_{(2,40)}$=1.157, p=0.325; Class by trial type and by hierarchy on day 1 $F_{(2,36)}$=0.70 p = 0.502; day 2 $F_{(2,40)}$=1.03 p = 0.365; day1-2 $F_{(2,40)}$=0.163, p=0.85. For recipient rats: Class by hierarchy on day 1 $F_{(2,36)}$=0.25 p =

0.78; day 2 $F_{(2,40)}$=0.293 p = 0.748; day1-2 $F_{(2,40)}$=0.355, p=0.703; Class by trial type and by hierarchy on day 1 $F_{(2,36)}$=0.025 p = 0.976; day 2 $F_{(2,40)}$=1.23 p = 0.304; day1-2 $F_{(2,40)}$=1.561, p=0.222). **(D)** Partial correlations between USV rate and focals' prosocial choice on day 1 (left panel), day 2 (middle panel) and day1-2 (right panel). Prosocial preference was positively correlated with recipient's call rate only when the recipient was submissive (on day2 and day 1-2). Interestingly, a positive correlation was found also between prosociality of submissive focals and their own USV rate, only marginal on day 1 and significant on day 1-2, suggesting that increased prosociality is associated with increased call rate by submissive animals, regardless of their role in the task. In contrast, the USV rate of dominant recipients correlated negatively with prosociality by their submissive focals. This negative correlation was significant on day 2 and only marginal on day 1 and day 1-2. Dom F = Dominant Focal, Sub F = Submissive Focal, Sub R = Submissive Recipient, Dom R = Dominant Recipient. # p<0.1, * p<0.05.

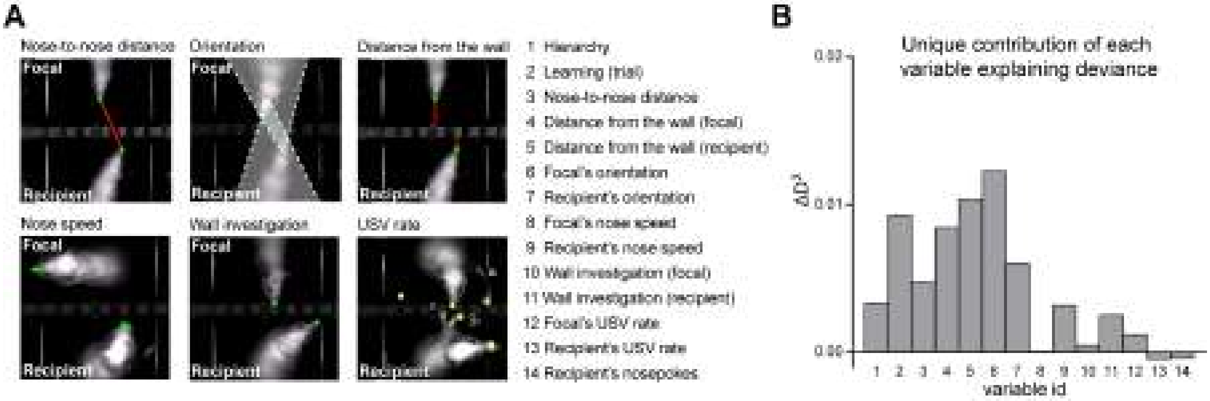

Figure S6. Unique contribution of each variable to the variability of the dataset. Related to Figure 9. (A) 14 behavioral and categorical parameters were chosen as regressors and evaluated by their contribution to the explained deviance of the model. Pictures represent the 14 behavioral variables measured either in focal or recipient animals, listed below. **(B)** The proportion of unique contribution (delta D^2) of each regressor was very low, indicating that all variables were partially dependent on each other.

Pair #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Low bound	-0,09	-0,09	-0,07	-0,09	-0,09	-0,07	-0,08	-0,10	-0,07	-0,06	-0,07	-0,08	-0,09	-0,06	-0,08
High bound	0,10	0,09	0,07	0,09	0,09	0,07	0,08	0,10	0,07	0,07	0,07	0,08	0,09	0,06	0,08
Pair #	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Low bound	-0,06	-0,10	-0,04	-0,06	-0,14	-0,07	-0,11	-0,09	-0,08	-0,07	-0,06	-0,06	-0,09	-0,13	-0,08
High bound	0,06	0,10	0,04	0,06	0,14	0,07	0,11	0,10	0,08	0,07	0,06	0,06	0,09	0,13	0,08
Pair #	31	41	42	43	44	45	46	47	48	49	50	51	52		
Low bound	-0,07	-0,10	-0,09	-0,13	-0,07	-0,07	-0,10	-0,07	-0,07	-0,06	-0,07	-0,06	-0,07		
High bound	0,07	0,10	0,09	0,13	0,07	0,07	0,10	0,07	0,07	0,06	0,07	0,06	0,07		

Table S1. Chance interval bounds generated by permutation test for each pair. Related to Figure 4 and S1. Low and high bounds show the 95% confidence interval for each focal animal. Dominant focals #: 3,7,8,9,10,12,15,19,46; Submissive focals #: 1,2,4,5,6,11,13,16,17,18,20,42,43; Familiar males #: 1,2,3,4,5,8,10,16,19,20,42,43,46; Unfamiliar males #: 21,22,23,24,25,26,27,28,29,30,31,41,44,45; Familiar females #: 47,48,49,50,51,52.

Prosocial choice ~ trial * hierarchy * (nose-to-nose distance + focal's orientation + recipient's orientation)

Variable	Estimate	z	p	
(Intercept)	2.771	6.234	<0.00001	***
Trial	0.033	3.748	0.00020	***
Hierarchy	-1.331	-1.988	0.04690	*
Nose-to-nose distance	0.147	1.326	0.18480	
Focal's orientation	-0.033	-4.469	<0.00001	***
Recipient's orientation	-0.020	-2.828	0.00470	**
Trial × Hierarchy	0.049	3.268	0.00110	**
Trial × nose-to-nose distance	-0.004	-1.572	0.11590	
Trial × focal's orientation	<0.001	1.013	0.31090	
Trial × recipient's orientation	<-0.001	-0.193	0.84670	
Hierarchy × nose-to-nose distance	-0.449	-2.639	0.00830	**
Hierarchy × focal's orientation	0.032	2.945	0.00320	**
Hierarchy × recipient's orientation	0.035	3.058	0.00220	**
Trial × hierarchy × nose-to-nose distance	0.009	2.424	0.01530	*
Trial × hierarchy × focal's orientation	-0.001	-3.250	0.00120	**
Trial × hierarchy × recipient's orientation	-0.001	-3.008	0.00260	**

Table S2. Generalized Linear Model with all behavioral variables. Related to Figure 9. Prosocial choice is a binary variable (1:prosocial choice, 0:selfish choice). Trial is an ordinal variable indicating cumulative trial number over the first two days of the PCT. The dataset includes 1995 observations from 22 pairs of animals. *p<0.05, **p<0.01, *** p<0.001.

Prosocial choice ~ trial * (nose-to-nose distance + focal's orientation + recipient's orientation)

Variable	Estimate	z	p	
(Intercept)	1.441	2.878	0.00400	**
Trial	0.082	6.788	<0.00001	***
Nose-to-nose distance	-0.302	-2.340	0.01930	*
Focal's orientation	-0.001	-0.123	0.90210	
Recipient's orientation	0.014	1.633	0.10250	
Trial × nose-to-nose distance	0.005	1.848	0.06460	#
Trial × focal's orientation	-0.001	-3.358	0.00080	***
Trial × recipient's orientation	-0.001	-3.817	0.00010	***

Table S3. Reduced GLM: pairs with dominant focal. Related to Figure 9. Prosocial choice is a binary variable (1:prosocial choice, 0:selfish choice). Trial is an ordinal variable indicating cumulative trial number over the first two days of the PCT. The dataset includes 885 observations from 9 pairs of animals. #p<0.1, *p<0.05, **p<0.01, *** p<0.001.

Prosocial choice ~ trial * (nose-to-nose distance + focal's orientation + recipient's orientation)

Variable	Estimate	z	p	
(Intercept)	2.771	6.234	<0.00001	***
Trial	0.033	3.749	0.00020	***
Nose-to-nose distance	0.147	1.326	0.18480	
Focal's orientation	-0.033	-4.469	<0.00001	***
Recipient's orientation	-0.020	-2.828	0.00470	**
Trial × nose-to-nose distance	-0.004	-1.572	0.11590	
Trial × focal's orientation	<0.001	1.013	0.31090	
Trial × recipient's orientation	<-0.001	-0.193	0.84660	

Table S4. Reduced GLM: pairs with submissive focal. Related to Figure 9. Prosocial choice is a binary variable (1:prosocial choice, 0:selfish choice). Trial is an ordinal variable indicating cumulative trial number over the first two days of the PCT. The dataset includes 1110 observations from 13 pairs of animals. **p<0.01, *** p<0.001.

5 | Discussion

Chapter 5 | Discussion

The main objective of the current thesis was to understand how familiarity, sex and dominance status modulate rats' decisions to benefit others in a PCT, where a decision-maker animal can choose between providing food only to itself (selfish choice) or to itself and a recipient conspecific (prosocial choice). These factors of the social context have been proposed to affect other-regarding preferences in some non-human primate species performing similar paradigms, but the behavioral correlates underlying such effects remained to be investigated. We addressed this point by modulating the social context of the interacting animals. We show that familiarity in males and sex of the pair did not affect rats' prosocial choices. In contrast, dominance status was found to be a potent modulator in males, with faster emergence and increased prosociality by dominant decision-makers towards submissive recipients. The dominance relationship between decision-maker and recipient affected the dynamics of multimodal interactions, which revealed how dominants' higher levels of prosociality are a consequence of their submissive partners being better at communicating need and capable of modifying the dominant's behavior.

5.1 Familiarity and sex

Similar levels of prosociality were found in pairs of familiar compared to unfamiliar male rats, and in pairs of familiar females compared to familiar males. The proportion of prosocial, unbiased, and selfish animals were also similar across the groups under comparison. These results thus show that familiarity in males and sex of the pair do not affect rat's prosocial choices for reward provision.

Rats generally present high levels of social tolerance for conspecifics in feeding or foraging contexts, which may explain their prosocial choices regardless of the familiarity of the recipients. Our results support previous work showing that familiarity do not influence harm aversion[132] and rescue behavior[14] towards distressed partners, and extend them to appetitive reward-related contexts, which have been less studied. However, since adult rats have been found to release trapped rats of a familiar but not unfamiliar strain in the liberation paradigm[56], indicating an in-group bias[213], it remains to be tested if strain familiarity would affect in a similar manner rats' prosocial choices for reward allocation. Our findings diverge from those reported by Kentrop et al.[99] where only male rats tested with same-sex conspecific in a PCT developed a prosocial preference, while females' preference was not different from chance. As the authors pointed out, this result might be attributed to the different testing conditions for the two groups, where females performed both as focal and recipient on separate occasions, whereas male animals performed only as focal or recipient. Alternatively, it is possible that the lack of food-seeking behavior displayed by the recipient prevented the emergence of robust prosocial behavior in females and only a modest prosocial bias in males. Again, our study parallels previous work on prosociality in stress-related contexts, which showed that male and female rats are equally prosocial when choosing not to harm a same-sex partner and highlights that both sexes are suitable to study prosocial choices in our reward task. Providing reward to a conspecific in need seems to be part of rats' social behavioral repertoire.

5.2 Dominance status

The effect of dominance on rats' prosocial choices was assessed by comparing dyads with dominant focal and submissive recipient against dyads with submissive focal and dominant recipient, tested on the PCT for five consecutive days. Both groups developed prosocial choices over testing, but dominant focals were faster, acquiring a prosocial preference from the second day, when submissive focals were still at chance level. Overall, dominant focals showed higher rates of prosocial choices than their submissive counterparts in most testing days and dominance asymmetry was positively correlated with prosociality. Therefore, this study extends previous work with non-human primates by providing evidence that also in rats prosocial behavior for reward provision occurs more often "down the hierarchy". This finding thus suggests that the effect of dominance status on prosociality may be conserved across species. Why dominant individuals behave more prosocially? Massen et al.[92], described that high-ranking long-tailed macaques, *Macaca fascicularis*, are more prosocial than low-ranking macaques, hypothesizing that prosocial behavior is not used by subordinates to obtain benefits from dominants, but by dominants to emphasize their dominant position. According to this view dominant animals, who are those with greater access to, and in control of, resources, would engage in more prosocial acts, providing resources to others, as a strategy to enhance or maintain status. This resembles what is expected by the handicap principle, proposed by Zahavi[214], according to which many forms of altruism are a handicap, an honest signal which advertises some quality of the helper, such as rank, and allows animals to attract potential collaborators or mates. Zahavi developed this hypothesis by observing that allofeeding in adult Arabian babblers (*Argya squamiceps*) occurs mostly unidirectionally from an individual to another of lower rank, and that high-ranking babblers are more likely to refuse the food offered[215]. However, the handicap principle concerns costly behaviors, which is not the case of food provision in our prosocial choice task. Whether rats would behave prosocially when facing a cost in foraging contexts has to be tested.

The hypothesis of prosocial behavior as a strategy for status signaling or maintenance offers a possible explanation of dominants' higher prosociality from a functional perspective, but it is difficult to prove experimentally. With this work, beyond assessing the impact of dominance on choice behavior, we approached the question of how the phenomenon occurs from the perspective of social interactions, in order to identify the behavioral correlates that would help explaining the enhanced prosociality by dominant animals at more proximate levels. To this aim, we examined how dominance status affected different behaviors of both rats during decision-making. Dominants' higher prosociality cannot be explained by recipients' motivation or interest in accessing the reward, focals' latency to choose or number of trials performed by the pair, since these factors were similar across the two dominance groups. Neither by the quantity of social interaction, since rats in both groups spent similar amount of time co-occupying the choice area and engaging in mutual investigation through the holes of the separating wall. This latter result also suggests that olfactory cues potentially exchanged through mutual sniffing prior to the choice are unlikely to contribute to the differences observed in prosociality. However, compared to submissive focals, dominant focals spent more time investigating the separating wall when their recipient was in the choice area, suggesting increased interest in their conspecific, a behavior that may be more pronounced in dominant rats in general.

More than quantity, dominance affected the quality of social interactions in the distance. Pairs with dominant focals stayed closer to each other before the choice. Interestingly, this increased social proximity was driven by submissive recipients who stayed closer to the central wall, increased their movement and were more oriented towards their decision-maker, especially when it was going to choose the selfish option. This indicates that, while dominant recipients mainly directed their attention towards the access of the reward, submissive recipients directed increased attention towards the focal animal. Importantly, some of these behavioral patterns would not have been observed by analyzing the short period of the interactions (seconds) as a whole but were detected only when examining their dynamics at higher temporal resolution (subseconds). This highlights that looking at how social interactions unfold over time can provide insights into how animals may differently modulate their behaviors. Moreover, social dynamics were only observed when social interactions were studied over distance and included into the equation the behaviors of both the decision-maker and the recipient, two aspects often neglected in studies of social decision-making. Our results showing increased proximity in the more prosocial pairs are consistent with a study in humans demonstrating that cooperation in a prisoner's dilemma game decreased with the physical distance between the players[216]. Moreover, experiment of gaze monitoring have found that both humans and monkeys shape social attention according to their relative social status, with individuals preferentially allocating attentional resources to high-status conspecifics[217–219]. This mechanism is likely to be particularly relevant for low-status individuals, allowing them to monitor and attend more closely the behavior of high-status individuals, on which they depend. In the context of prosocial decision-making, this helps to explain why recipient rats direct more attention to a conspecific controlling and deciding on their reward when this conspecific is dominant.

The different dynamics of social proximity and orientation according to dominance may have affected rats’ behavior as a dyadic system, measured as bidirectional influences in nose position. Pairs with dominant focal exhibited stronger bidirectional influences, revealing that rats were following more each other’s movements compared to dyads of the other group. Importantly, submissive but not dominant recipients significantly influenced the nose position of their decision-maker, suggesting that they had a heavier influence on guiding focals’ choice. These results are consistent with the mechanism proposed by Michael et al.,[220] where the ability of an individual to adapt its behavior to a partner can trigger prosociality. Overall, these findings strongly indicate that dyads with dominant focals and submissive recipients displayed higher body movement coordination, which likely favored increased prosocial choices. Evidence for coordination positively associated with prosociality has been reported in humans, where subjects increased helping behavior after engaging in coordination compared to a condition without coordination[221], with these effects emerging in early childhood[222]. In our study, we do not manipulate coordination or test its effect on prosocial choices. However, we show that it can be a behavioral correlate in social decision-making tasks where freely moving subjects engage in social interactions. We found that the influence in movement was slightly higher from focals to recipients than vice versa; that is, decision-makers tend to lead, and recipient tend to follow, the movement. This may be specific of our task or a general trend in dyadic prosocial tasks involving coordination. Importantly, we show that dominance status modulated this pattern, by affecting the strength of the influences. Thus, further research would benefit from investigating if body or other forms of coordination emerge in prosocial tasks where subjects socially interact and how they are affected by the relationship between the partners. More generally, it would be interesting to assess if dominance affects performance in tasks testing for coordination.

Despite large inter-individual variability, rats emitted a high amount of 50-kHz USVs during the task, suggesting an appetitive behavioral state, likely driven by an environment involving social interactions and reward seeking. 50-kHz USVs have been showed to induce approach in playback experiments[212,223] and have been proposed to serve a prosocial communication function as contact calls, important for rats to maintain social proximity and coordinating their behavior[224]. Here, we highlight their potential role in prosocial decision-making. Interestingly, we found that social hierarchy modulated how USV rate correlated with prosociality. Prosocial choices tended to correlate positively with the USV rate of submissive rats and negatively with the USV rate of dominant rats, regardless of their role in the task, which underlies a rather complex relationship between dominance status, prosociality and vocalizations. However, these correlations were mainly significant when considering the call rate of recipients, that is the more a submissive recipient would call the more its decision maker would help. One hypothesis to explain this relationship would be that dominant and submissive focals behaved differently in response to recipients' calls, with only dominant focals being responsive and increasing help. However, we found that recipients' calls promoted more direct gazing from focals independently of social hierarchy. Another hypothesis would be that submissive and dominant recipients may react differently when receiving help for food. Only submissive recipients increased call rate with more help received, suggesting that receiving help may affect their emotional state positively or induce them to seek social contacts. In contrast, dominant recipients may not react in this manner.

Going beyond the analysis of USV rate, we explored whether qualitative differences in these calls would increase our understanding on the cues animals use to behave prosocially. By classifying rats' 50-kHz USVs into three main categories, we found that most calls were flat, followed by frequency modulated, while step calls were infrequent. This result suggests that rats' vocal production in our task reflected primarily a social coordination function, as flat 50-kHz calls have been mainly associated with social approach or contacts[224]. At the same time, it likely reflected reward and positive affective state, which are particularly associated with the frequency-modulated calls[225]. The proportion of the different classes were not affected by dominance, role in the task, choice type or day of testing.

Finally, by assessing the predictive power of different behavioral and task variables to prosocial choices in a generalized linear model, we found that nose-to-nose distance and social orientation of both rats were the predictors that explained most of the deviance (~20%), followed by dominance status and trial number as a proxy for learning over time (~1.4%). Trial number positively predicted prosocial choice, indicating that rats did more prosocial choices as trials were advancing, consistent with a learning curve. Since both hierarchy and trial number interacted with the three behavioral variables and our main goal was to study how the two dominance groups differed, we performed the analysis for each dominance category. Only pairs with dominant decision-maker became more oriented to each other over trials, suggesting behavioral adaptation. Moreover, social proximity between rats positively predicted prosociality only in these pairs, indicating that dominant animals use this information to guide decisions. These results point to a modulation by dominance status of how rats use body language as learning signal for prosocial choices on a trial-by-trial basis and concur with findings that socially dominant individuals show an enhanced reliance, relative to subordinate individuals, on social learning when in a complex decision-making situation[226].

Altogether, our study shows that dominance status modulates the emergence prosocial biases in rats by affecting the social dynamics established within the dyads. We propose a model (**Figure 10**) where submissive animals, when in need of help, are influenced by the behavior of their dominant decision-makers, follow them more and display multisensory cues that accelerate learning about choice impact on others by dominant rats. In contrast, when dominant animals are those in need of help and not in control of the situation, they try to obtain their own food displaying clear food-seeking behavior, but showing reduced body-following, approach, and orientation towards their submissive focal animals, which are the ones making the decisions. Therefore, submissive recipients may be more effective at using body-language to signal need and soliciting attention when the probability to receive help is low, enhancing social salience and facilitating the emergence of prosocial choices by dominant rats over testing. Our findings are consonant with studies of helping in chimpanzees, showing that recipients' attention-grabbing behaviors towards the donor increased the success of receiving help and thus function as social signals[70].

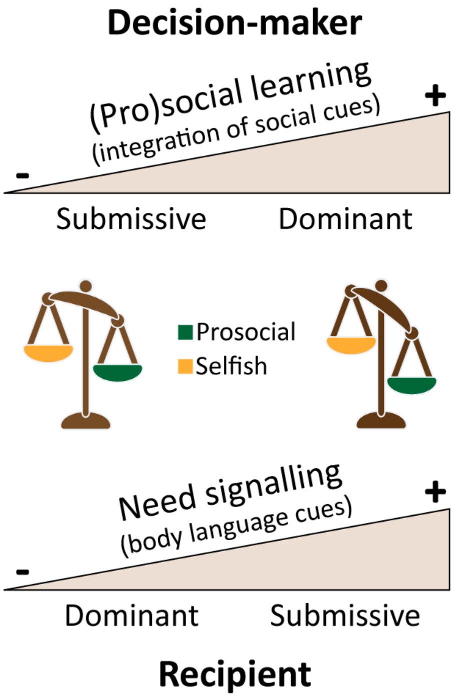

Figure 10. Model for the proximate mechanisms underlying prosociality directed down the hierarchy in rats.
Dominance modulates how rats use body language to signal need for reward and how they integrate social signals into prosocial learning to guide choices. Signals of need, mediated by body language cues, are enhanced when recipients are submissive to their decision-makers. Concurrently, (pro)social learning, based on the integration of social cues, is enhanced when decision-makers are dominant to their recipient. These patterns lead prosociality in male rats to be directed down the hierarchy, that is, more often from dominant to submissive animals.

Through detailed quantification of social interactions, this work describes the importance of social dynamics and information flow as factors underlying the effects of dominance on social decision-making, highlighting the role of the recipient, and pinpointing multimodal cues in social distance as behavioral salient correlates motivating prosocial behavior. Dominance turns to be an important factor in modulating rats' ability to communicate need for reward to conspecifics and their propensity to help for this need.

5.3 Limitations and future directions.

This work shows how dominance status modulates prosocial choices in stable rat dyads. An interesting question is how this translates to larger groups of individuals, since wild rats are common to form colonies organized in a dominance hierarchy. That is, to understand if the effect of prosociality occurring more often "down the hierarchy" is preserved in a group context, with individuals providing more rewards to others of lower rank, or if more complex patterns exist. Testing animals in a group service paradigm, in semi-natural conditions, such as the novel setups proposed for mice[227,228], and identifying their dominance rank would be ideal to start addressing this question. Moreover, it would be interesting to study whether submissive animals are better at displaying need in contexts other than reward seeking, for instance when in need of help to avoid stress or a danger, which could be examined by incorporating the study of dominance into the liberation and harm aversion paradigms.

Assessing how familiarity and dominance modulate female rats' prosociality would have increased the complexity of our study but provided a more complete view of the effects of these factors in both sexes and if any interaction exists. Even though the identification of stable social hierarchies in female rat dyads remains largely unexplored, future research should determine whether dominance status modulates female rats' prosocial choices in a similar manner. In addition, although we considered only dyads where rats interacted with a same-sex conspecific, an interesting unanswered question is about rats' prosociality in mixed-sex dyads, where the decision-maker is either a male or a female interacting with a recipient of the opposite sex. This would reveal, for instance, whether prosocial choices in these animals occurred at equal levels from one sex to the other. Furthermore, among other factors which possibly modulate rats' prosociality, age may be an important candidate to investigate. In fact, age has been found to modulate the effect of strain familiarity on rats' rescue behavior in the liberation paradigm, where adult rats selectively released trapped conspecifics of a familiar strain, while adolescent rats helped conspecifics of both familiar and unfamiliar strain, suggesting that in-group bias for this kind of helping emerges in adulthood[229]. Moreover, dyadic agonistic interactions in a colony of wild adult males were more likely to be won by the older rat in the dyads, and this likelihood increased with the age difference between the animals, revealing that age, which in stable groups is equivalent with time spent in the colony, was a good predictor of dominance rank[183]. Considering that all rats in our study had the same age when tested, we can rule out that this factor contributed to mediate the effects of dominance status on prosocial choices. Given the positive association between age and dominance, it could be expected that prosocial choices in our task would occur more often when decision-maker rats are older than their recipient. More interesting would be to examine whether rats' prosociality changes with the age of the dyad, i.e., if dyads of different ages are equally prosocial. Furthermore, age, sex, and social status may interact in social decision-making processes, as a study with humans reported a combinatorial effect of these factors in modulating gaze following behavior and prosocial attitudes[230].

Our study restricted the analysis of dominant and submissive rats' behavior to the choice area, where recipients show food-seeking displays and focals make their choice. However, rats are able to see, smell, hear and partially touch each other also in the reward area, where they witness each other's reward outcome. Social interactions occurring during the reward period (the time from focal rat's choice to the start of the following trial) seem important, as in the previous study by Marquez et al.[79] rats showed increased social investigation after selfish than prosocial choices. Thus, rats are likely to integrate information from both the choice and the reward period to guide their decisions. Future studies could therefore expand the analysis of the effects of dominance status on multimodal interactions after the choice in order to evaluate if similar behavioral patterns emerge.

Shedding light onto the brain mechanisms underlying rats' prosocial choices in our task and how they may be differently modulated according to dominance is also a target of future research. While it would be very challenging to perform electrophysiological recordings for monitoring neuronal activity in our paradigm, experiments of neuronal activity manipulation provide an approach to identify brain areas and neural circuits controlling prosocial behavior. This is relevant considering that ongoing research has been discovering neural correlates of prosocial learning and preference, but loss and gain of function experiments to prove if specific neural circuits control prosocial choices have been rarely implemented. The connection between the ventral tegmental area and the anterior cingulate cortex (VTA-ACC) is a candidate neural circuit. The VTA, which is involved in motivation and reward processing, represents a major source of dopamine (DA) for the central nervous system. About 65% of cells in the VTA are dopaminergic neurons which project to several brain areas, through the mesolimbic and mesocortical pathways[231]. Unpublished data from *in-vivo* calcium imaging experiments in our lab indicate that VTA neural activity increases when rats witness a neighboring partner receiving reward, which adds to the finding that observation of reward delivery to a conspecific modulates DA release in the ventral striatum[63]. Since the activity of VTA dopaminergic neurons is considered to play a crucial role in reward learning and to influence action selection by DA release[232,233], it can be hypothesized that normal VTA or DA functioning supports prosocial learning. In accordance with this, other unpublished data from our lab indicate that if focal rats undergo optogenetic inhibition of neural activity in the VTA while witnessing reward delivery to the recipient, they do not develop a prosocial preference at the group level. It would be interesting to understand the relationship between the activity of dopaminergic neurons and prosociality, and if it is differently modulated in dominant and submissive decision-makers, given that dominance status has been found to impact DA D_2 receptor binding potential in cynomolgus monkeys, *Macaca fascicularis*[234].

The ACC has been associated with a variety of behavioral, cognitive, and affective processes. For instance, the rat ACC (area 24) contains mirror-like neurons responding to pain experienced by self and others[119] and its deactivation abolishes harm aversion. In the monogamous prairie voles (*Microtus ochrogaster*), injection of oxytocin antagonist (OTA) into the ACC prevents the consolation response towards a distressed conspecific[54]. When considering reward-based tasks, 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, highlighting its role in prosocial learning[235]. In rhesus macaques, *Macaca mulatta*, single-neuron recordings revealed that a high proportion of neurons in the ACCg exclusively responded to reward delivered to a conspecific[236], and excitotoxic lesions to the ACC decreased subjects' acceptance that rewards offered would be delivered to a partner compared to no one in a vicarious reinforcement task[237]. However, if the ACC is required for developing prosocial choices in a PCT remains to be assessed. In rats performing a cost-benefit decision-making task with a reversal component, 4-Hz ACC-to-VTA signal increased when animals initiated and sustained choices towards a new preferred default after the reversal, suggesting that ACC-to-VTA signal may influence motivational state so as to deviate from habitual behavior when the environment changes[238]. This can resemble the conditions of our task, where prosocial rats deviate from the unbiased behavior in individual sessions towards a prosocial bias in social sessions. It would then be interesting to understand how ACC-to-VTA signal relates to preference in the PCT and if it is shaped by dominance status.

6 | Conclusions

Chapter 6 | Conclusions

This work aimed to understand how different factors of the social context modulate rats' prosocial choices when providing reward to a conspecific in need. Our findings lead to the following conclusions:

1. Familiarity does not affect prosocial choices for reward provision in male rats.
2. The sex of the dyads does not affect prosocial choices for reward provision in rats, as males and females show similar levels of prosociality when providing reward to a familiar, same-sex conspecific.
3. Social hierarchy is a strong modulator of prosocial choices for reward provision in male rats. Prosociality is directed down the hierarchy, that is, more frequently from dominant decision-makers to submissive recipients, and correlates positively with dominance asymmetry.
4. Recipients' attempts to access the reward, trial performance, decision-making time, and the amount of social interactions are unlikely to contribute to the higher prosociality of dominant rats, as these factors are independent of dominance status.
5. Dominance status modulates the dynamics of social interactions prior to the choice. Dominant decision-makers and submissive recipients stay closer to each other. The increased social proximity in these dyads is driven by submissive recipients, who move closer to and gaze more at their decision-makers. These behaviors may qualify as attention-grabbing and thus function as social signals.
6. Dominant decision-makers and submissive recipients influence more each other's body-movement, indicating that these dyads engage in increased coordination in the prosocial choice task.
7. Recipient rats' 50-kHz USVs influence decision-makers' movement and its correlation with prosociality depends on dominance. Submissive recipients' USVs may reinforce the prosocial behavior of their dominant helpers.
8. Dominance status modulates how rats signal need of reward and provide help for it. When recipients of help, rats may be more effective at using body language for signalling need to a conspecific of higher status. Concurrently, helper rats may be more effective at integrating social signals from conspecifics of lower status, accelerating and enhancing prosocial learning. Together, these dynamics direct prosociality in male rats down the hierarchy.

Conclusiones

El objetivo de este trabajo ha sido comprender cómo diferentes factores del contexto social modulan las elecciones prosociales de ratas que proporcionan recompensas a un conespecífico en necesidad. Nuestros hallazgos conducen a las siguientes conclusiones:

1. La familiaridad no afecta las elecciones prosociales para la provisión de recompensas en ratas macho.
2. El sexo de las díadas no afecta las elecciones prosociales para la provisión de recompensas en ratas, ya que machos y hembras muestran niveles similares de prosocialidad cuando proporcionan recompensas a una rata familiar del mismo sexo.
3. La jerarquía social es un fuerte modulador de elecciones prosociales para la provisión de recompensas en ratas macho. La prosocialidad se dirige hacia abajo en la jerarquía, es decir, con más frecuencia de dominantes a sumisos, y correlaciona positivamente con la asimetría de dominancia.
4. Es poco probable que los intentos de los recipientes de acceder a la recompensa, el número de trials, el tiempo de toma de decisiones y la cantidad de interacciones sociales contribuyan a la mayor prosocialidad de las ratas dominantes, ya que estos factores son independientes del estado de dominancia.
5. El estado de dominancia modula la dinámica de las interacciones sociales antes de la elección. Los tomadores de decisiones dominantes y los recipientes sumisos se mantienen más cerca unos de otros. La mayor proximidad social en estas díadas está impulsada por recipientes sumisos, que se acercan y miran más a quienes toman las decisiones. Estos comportamientos podrían calificarse como captación de atención y, por lo tanto, funcionan como señales sociales.
6. Los tomadores de decisiones dominantes y los recipientes sumisos influyen más en el movimiento corporal del otro, lo que indica que estas díadas manifiestan una mayor coordinación en la tarea de elección prosocial.
7. Las USVs de 50-kHz de las ratas recipientes influyen en el movimiento de los tomadores de decisiones y su correlación con la prosocialidad depende de la dominancia. Las USVs de los destinatarios sumisos podrían reforzar el comportamiento prosocial de sus ayudantes dominantes.

8. El estado de dominancia modula cómo las ratas señalan la necesidad de recompensa y proporcionan ayuda para ello. Cuando reciben ayuda, las ratas pueden ser más efectivas en el uso del lenguaje corporal para señalar sus necesidades a un congénere con mayor estatus. Al mismo tiempo, las ratas ayudantes pueden ser más efectivas para integrar señales sociales de congéneres con menor estatus, acelerando y mejorando el aprendizaje prosocial. Juntas, estas dinámicas dirigen la prosocialidad en ratas macho hacia abajo en la jerarquía.

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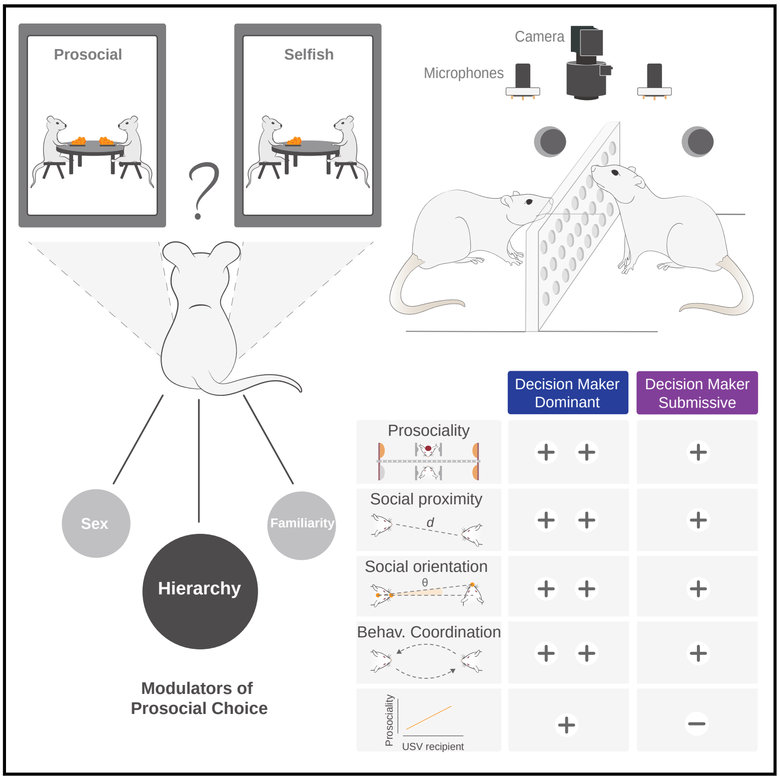
Annex

Article

Current Biology

Multimodal cues displayed by submissive rats promote prosocial choices by dominants

Graphical abstract



Highlights

- Social hierarchy, but not sex or familiarity, modulates prosocial choices
- Dominant male rats are more prosocial
- Granger causality reveals increased coordination when decision-makers are dominants
- Rats use partner's body language to guide social decisions on a trial-by-trial basis

Authors

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In brief

Gachomba et al. show that social status modulates prosocial choices. Dominant decision-makers are more prosocial, and submissive rats are better at communicating their need for help. Quantitative analysis of multimodal social dynamics reveals body language and increased behavioral coordination as factors promoting the emergence of prosociality.

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Current Biology

Article

Multimodal cues displayed by submissive rats promote prosocial choices by dominants

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SUMMARY

Animals often display prosocial behaviors, performing actions that benefit others. Although prosociality is essential for social bonding and cooperation, we still know little about how animals integrate behavioral cues from those in need to make decisions that increase their well-being. To address this question, we used a two-choice task where rats can provide rewards to a conspecific in the absence of self-benefit and investigated which conditions promote prosociality by manipulating the social context of the interacting animals. Although sex or degree of familiarity did not affect prosocial choices in rats, social hierarchy revealed to be a potent modulator, with dominant decision-makers showing faster emergence and higher levels of prosocial choices toward their submissive cage mates. Leveraging quantitative analysis of multimodal social dynamics prior to choice, we identified that pairs with dominant decision-makers exhibited more proximal interactions. Interestingly, these closer interactions were driven by submissive animals that modulated their position and movement following their dominants and whose 50-kHz vocalization rate correlated with dominants' prosociality. Moreover, Granger causality revealed stronger bidirectional influences in pairs with dominant focals and submissive recipients, indicating increased behavioral coordination. Finally, multivariate analysis highlighted body language as the main information dominants use on a trial-by-trial basis to learn that their actions have effects on others. Our results provide a refined understanding of the behavioral dynamics that rats use for action-selection upon perception of socially relevant cues and navigate social decision-making.

INTRODUCTION

Prosocial actions, those that benefit others, support the development of positive social interactions, like cooperation, which sustain individual and group well-being.[1–3] Recent work has demonstrated that prosocial behaviors are not exclusive to humans but conserved across different species.[4–24] Several factors have been proposed to modulate prosocial behaviors, such as familiarity,[2,13,25] sex,[24,26,27] and social status.[28,29] Related to the latter, flexibly adapting decision-making based on the social hierarchy of the interacting partner can be cost-effective and, in some cases, a crucial survival strategy. Less effort has been devoted to the identification of the behavioral correlates that lead to such effects, which is important and necessary to determine the proximate mechanisms underlying prosocial choices. Whether enhanced prosociality is due to an improvement in the perception and integration of socially relevant information, or it is due to flexibility in the action-selection process of adequate behavioral strategies upon perception of these social cues, is still far from being understood. Studies at the level of behavior are needed to identify which are the factors that inform individuals' social decisions to benefit others.

We evaluated how laboratory rats adapt their decision to help or not to help depending on social context to identify the behavioral correlates by which animals incorporate the actions of others into social decision-making. We previously showed that male rats behave prosocially in a two-alternative forced choice task, providing food to a familiar conspecific in the absence of self-benefit, being food-seeking behavior displayed by recipients necessary for prosociality to emerge.[11] Here, we used this task to ask about the factors that promote or hinder prosociality by modulating familiarity, sex, and social status of the interacting animals. Briefly, our prosocial choice task (PCT) is based on a double T-maze where only the focal animal (decision-maker) controls the access to the food-baited arms of its own and the recipient rat's maze. In each trial, the focal rat can choose between one side of the maze, providing food only to itself (selfish choice) or the opposite side, providing food to itself and the recipient rat (prosocial choice) (Figures 1A–1C; Video S1). We hypothesized that social interactions prior to choice might be crucial for increasing the social salience of recipients' attempts to reach the food and thus might impel decision-makers to learn that their choices have an impact on others. With this aim in mind, we first identified the social

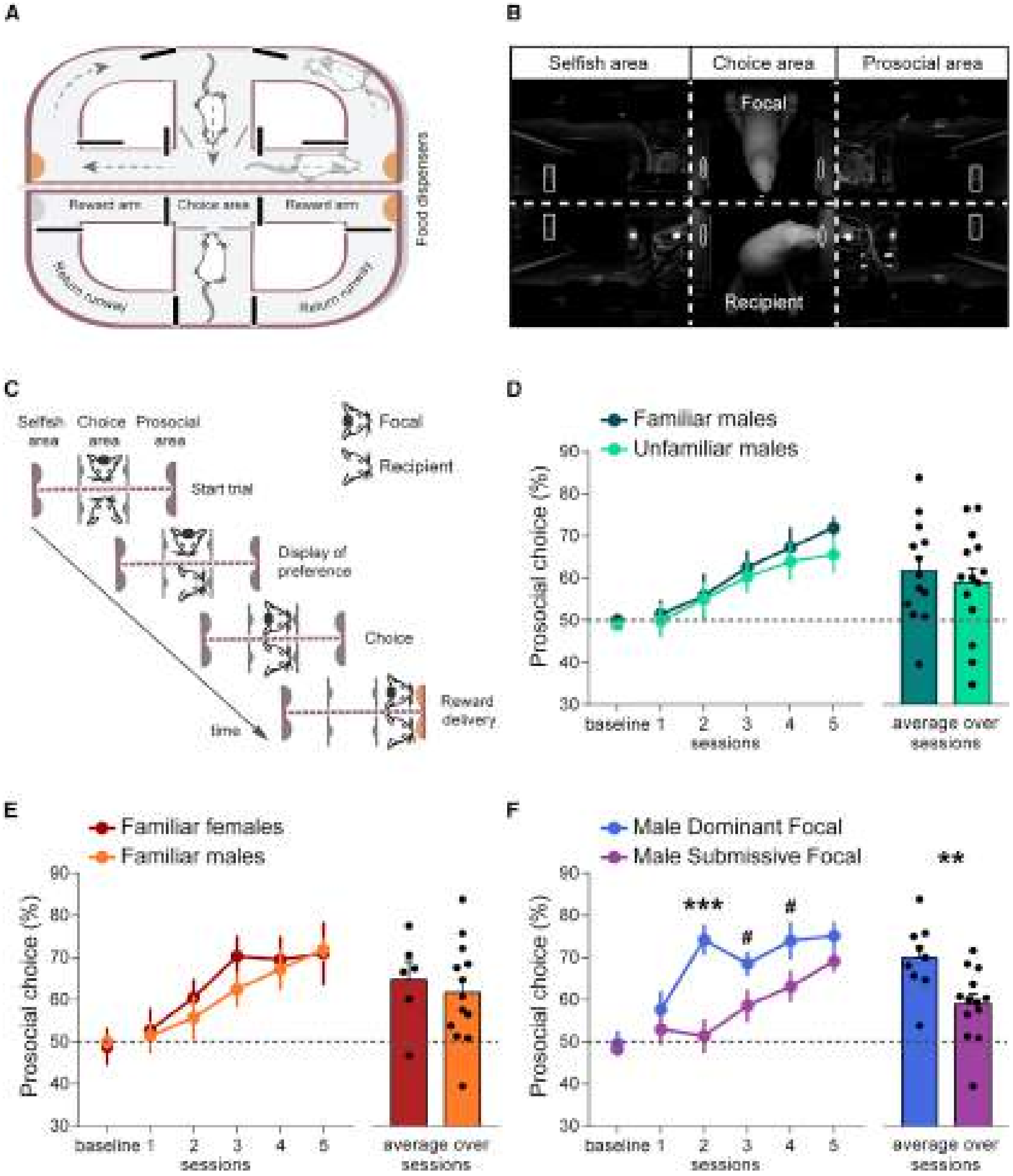

Figure 1. Prosociality emerges faster when decision-makers are dominant and is not affected by familiarity or sex

(A) Schematic overview of the double T-maze used for the prosocial choice task (PCT). Each T-maze (one per rat) consists of a central arm that gives access to a choice area and two reward areas gated by automated doors (black lines) at the end of which food is delivered (orange semi circles). Access to the choice area is controlled by automated doors placed in the central arm (gray lines). Arrows in the upper maze represent the flow movements of the rats in the maze.

(B) Example image from a video recording, showing a top view of the central area of the double T-maze during one session. The horizontal dashed line marks the transparent and perforated wall that separates the two single mazes, which allows rats to see, hear, smell, and partially touch each other. Vertical dashed lines mark the separation between different areas of the maze: the choice area, where social decision-making occurs, and prosocial and selfish areas, where food is delivered depending on the contingencies of the task. White ellipses in the choice area mark the position of nose ports, which control the opening of doors located under them. When the decision-maker pokes one of its nose ports, the door underneath it and the door on the same side for the recipient animal opens, allowing them to reach feeder magazines (white rectangles in the reward areas). In this example, the focal rat (decision-maker) is in the top of the image, whereas the recipient appears in the bottom, while displaying food-seeking behavior.

(C) Schematic view of a trial: before the PCT focal and recipient rats are trained individually to navigate in the maze and learn to retrieve their own rewards (STAR Methods). In the PCT, a trial starts when both rats are in the central arm, after opening of the central doors that give access to the choice area. There, the recipient rat will display food-seeking behavior (repeatedly poking in the side where it was previously trained to find food during individual training), and the focal animal can

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conditions where differences in prosociality can be detected and performed a refined analysis of the social interactions observed.

We show that as observed in non-human primates, male dominant rats are more prosocial with a faster emergence of prosocial biases. Beyond the description of this effect, we unravel the behavioral correlates through which this effect is observed, based on the analysis of social interactions using DeepLabCut for unmarked pose estimation with subsecond resolution, entropy-based algorithms for ultrasonic vocalization's agency, Granger causality to assess directionality in animal's interactions and behavioral modeling to identify behaviors predictive of choice on a trial-by-trial basis. Strikingly, dominants' higher levels of prosociality are a consequence of their submissive partners being better at communicating need and capable of modifying the dominant's behavior. This interesting effect emerges in the form of multimodal social dynamics and highlights the importance of embracing the bidirectionality of social interactions in decision-making.

RESULTS

Prosociality emerges faster when decision-makers are dominant and is not affected by familiarity or sex

We tested pairs of rats in our PCT, where a decision-maker rat (focal) can choose in each trial to provide food reward to itself only (selfish choice) or to itself and a recipient rat (prosocial choice) (Figures 1A–1C; Video S1). After individual training for maze navigation, focal and recipient animals were tested together in PCT and learned the new reward contingencies where food delivery to the recipient depended on focal's choices. Each pair performed five daily consecutive sessions of 40 min each, over which focals' choice preference was assessed.

Rats' prosocial preferences in food-foraging contexts emerged over the testing sessions independently of familiarity or sex. Male rats displayed similar levels of prosociality when interacting with their cage mates or unfamiliar conspecifics (repeated-measures ANOVA with "session" as within-subjects factor and "familiarity" as a between-subjects factor; "session" [$F_{(4,100)}$ = 13.86, $p = 5e{-}9$, $\eta^2 = 0.164$, BF_{incl} = 2.961e+6], "familiarity" by "session" [$F_{(4,100)}$ = 0.29, $p = 0.882$, $\eta^2 = 0.003$, $BF_{incl} = 0.107$], and "familiarity" [$F_{(1,25)}$ = 0.36, $p = 0.555$, $\eta^2 = 0.008$, $BF_{incl} = 0.328$]) (Figure 1D). Moreover, we did not observe sex differences, with females being equally as prosocial as males (repeated-measures ANOVA; "session" [$F_{(4,68)} = 9.83$, $p = 2e{-}6$, $\eta^2 = 0.181$, BF_{incl} = 71,466], "sex and "session" [$F_{(4,68)} = 0.44$, $p = 0.783$, $\eta^2 = 0.008$, $BF_{incl} = 0.223$], and "sex" [$F_{(1,17)} = 0.29$, $p = 0.596$, $\eta^2 = 0.008$, $BF_{incl} = 0.391$]) (Figure 1E). Although we did not find evidence for an effect of familiarity or sex in prosocial tendencies, it could be that the proportion of prosocial individuals would differently emerge over the testing sessions in each group. For this, we computed a prosocial choice index (PCI) that reflected the strength of the prosocial (or selfish) bias compared with chance. Using a permutation test, we categorized the animals as either prosocial, unbiased, or selfish over the days. The emergence of prosociality was comparable across groups (Figures S1A–S1F; Table S1).

To understand how social dominance may modulate prosocial choice, we first identified the social status within pairs of cage mate rats. For this, we used the modified food competition test,[30] a novel trial-based dominance assay, where established social hierarchies can be identified in the home cage of non-food-deprived pairs of male rats. It has the added advantage of not inducing aggressive interactions during testing that could influence later prosocial tendencies. After identification of social status of the animals (Figure S1J), we tested for prosocial tendencies two parallel independent groups, where the decision-maker rat was either the dominant (and thus its recipient was submissive) or the submissive (and the recipient was the dominant). Thus, in both groups, a dominant animal would interact with a submissive, but their roles in the decision process would differ. We found that both groups acquired a preference for the prosocial option over the days but that social hierarchy drastically modulated the emergence of this choice (Figure 1F). Specifically, dominant animals acquired faster prosocial tendencies and reached higher prosociality levels than submissive decision-makers (repeated-measures ANOVA; "session" [$F_{(4,80)}$ = 8.42, $p = 1e{-}5$, $\eta^2 = 0.15$, BF_{incl} = 3,445], "hierarchy" by "session" [$F_{(4,80)} = 2.67$, $p = 0.038$, $\eta^2 = 0.048$, $BF_{incl} = 5.8$], and "hierarchy"

choose to nose poke on either side of its own maze. Focal animal will always be rewarded; however, recipient's reward will depend on focal's choice. A focal's nose poke on the same side where recipient is displaying food-seeking behavior (prosocial choice) will lead to both rats receiving one pellet in the reward area, whereas a nose poke on the opposite side (selfish choice) will lead to only the focal receiving one pellet and the recipient none. Prosocial and selfish sides remain fixed throughout all days, so that the focal animal does not need to read out the behavior of the recipient on each trial but can develop a preference over time. After food consumption, rats can pass through the return runway and go back to the central arm to start a new trial. See also Video S1.

(D) Familiarity of the interacting animals does not affect prosocial choices in male rats. To understand whether the familiarity of the recipient modulates the proportion of prosocial choices, we compared in the PCT two independent groups: focal animals that performed in the maze with their cage mate ("familiar males," n = 13 pairs) and focal animals that performed with a stranger, non-cage mate, recipient ("unfamiliar males," n = 14 pairs). Unfamiliar animals interacted for the first time in the first session of the PCT and were maintained over the rest of sessions while not being cage mates. We found that focals of the two groups developed similar proportions of prosocial choices along sessions, indicating that the degree of familiarity of the recipient does not affect prosociality in male rats.

(E) Female rats show similar proportions of prosocial choices compared with male rats. To study sex differences in the development of prosocial choices, we tested two independent groups where the focal animal was either male ("familiar males," n = 13 pairs) or female ("familiar females," n = 6 pairs), interacting with a cage mate of the same sex. We found no difference in the proportions of prosocial choices along the five testing sessions, indicating that female and male rats are equally prosocial when interacting with a familiar conspecific of the same sex.

(F) Social hierarchy modulates prosocial choices in male rats. Dominant (n = 9 pairs) and submissive (n = 13 pairs) focals displayed a preference for the prosocial option but dominant focals showed faster emergence and higher proportion of prosocial choices compared with submissive focals. See also Figure S1.

For (D)–(F), baseline and five daily test sessions are shown. Baseline corresponds to the percentage of focal's choices for the side that would later correspond to the prosocial side during testing, averaged across the last 2 days of individual training. Data represented as mean ± SEM. #$p < 0.1$, **$p < 0.01$, ***$p < 0.001$. See also Figure S2 and Table S1.

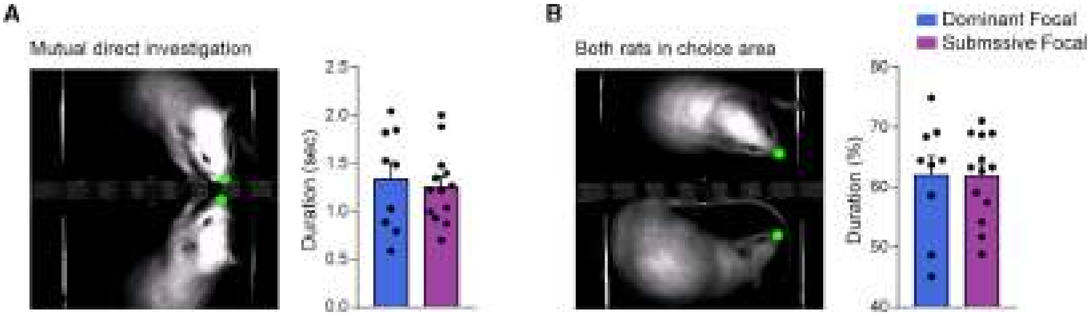


Figure 2. Social dominance does not affect the quantity of social interactions prior to choice
(A) Social dominance does not affect the duration of social investigation prior to the choice or (B) the percentage of choice time per trial that both rats are present in the choice area, as an index of social interactions in the distance. Data are shown as mean ± SEM; individual dots show the averaged trial value for each pair across the first two sessions. See also Figure S2.

[$F_{(1,20)} = 8.75$, $p = 0.008$, $\eta^2 = 0.136$, $BF_{incl} = 11.2$]). Dominant and submissive decision-makers displayed similar choices on the first session of the PCT, where animals are exposed to the social task for the first time after individual training and had not yet learned that their actions have consequences on the reward contingencies of the recipient (t test of the proportion of prosocial choices of dominant focals against submissive focals' $t_{(20)} = 0.81$, $p = 0.428$, $BF_{10} = 0.491$ for day 1). However, marked differences appeared from the second day of testing, where dominant animals displayed strong prosocial preferences, whereas submissive focals were still at chance levels (independent sample t test, $t_{(20)} = 4.03$, $p = 6.5e-4$, $BF_{10} = 42$, for day 2; paired sample t test of proportion of prosocial choices in session 2 against baseline for dominant focals, $t_{(8)} = 5.72$, $p = 4.4e-4$, $BF_{10} = 80$; for submissive focals, $t_{(12)} = 0.681$, $p = 0.509$, $BF_{10} = 0.34$). Interestingly, prosociality on this day positively correlated with the strength of the social hierarchy (Figure S1L), suggesting a parametric relationship between dominance and prosociality (Pearson correlation between prosocial choice in day 2 and dominance index, $r = 0.71$, $p < 0.001$). The differences between dominant and submissive focals were maintained over the sessions but progressively faded once submissive focals started to show prosocial biases from day 3 onward (t test, $t_{(20)} = 1.87$, $p = 0.077$, $BF_{10} = 1.28$ for day 3; $t_{(20)} = 1.88$, $p = 0.074$, $BF_{10} = 1.3$ for day 4; $t_{(20)} = 1.42$, $p = 0.171$, $BF_{10} = 0.79$ for day 5). We then assessed whether the proportion of prosocial, unbiased, and selfish animals would be different depending on their hierarchical status and observed a higher proportion of prosocial animals in pairs with dominant focals in the second day of testing (Figures S1G–S1I).

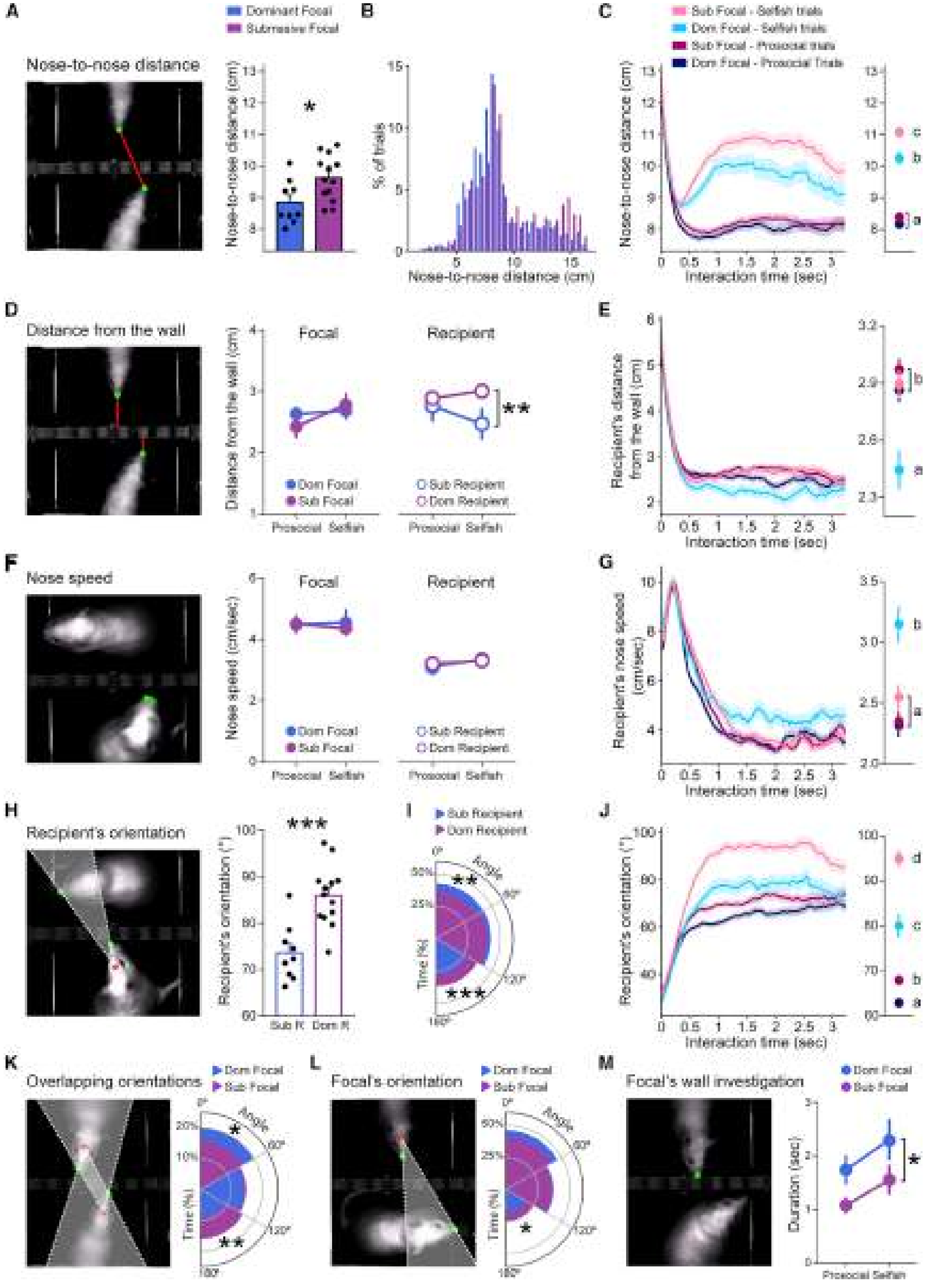
Although previous non-human primate studies also showed evidence of prosociality occurring down the hierarchy, the factors leading to such directionality are not known. Leveraging the controlled environment that experiments in laboratory rats provide, we endeavored to identify the behavioral correlates at the base of this enhanced prosociality in dominant animals. To this end, we performed a fine-grained analysis of rats' behavior during the choice period (time from trial start to focal's choice), focusing our analyses on the first 2 days of the task, when prosocial bias emerges, to identify the behavioral dynamics that promote integration of actions from others into decision-making processes.

Social dominance does not affect recipients' food-seeking behavior or focals' latency to decide

We have previously demonstrated that the recipient's display of food-seeking behavior—poking in the nose port that gives access to the food-baited arm—is necessary for the emergence of prosocial choices by focal rats.[11] Thus, one possibility was that submissive recipients were better at displaying food-seeking behavior, facilitating the learning of the contingencies of the task by dominant decision-makers. However, we did not find hierarchy differences in the number of nosepokes performed or in the vigor with which they were displayed (Figure S2A). Dominant humans are faster in making (non-social) decisions in stressful situations, without compromising their accuracy.[31] However, we did not observe differences in the latency to choose in rats performing our task (Figure S2B). It could still be possible that dominant focals developed faster prosocial preferences in the first days of testing because of increased task performance, thus accelerating the learning rate of the new contingencies in the social task. However, this was not the case either (Figure S2C). We then hypothesized that the social interactions displayed prior to the choice might be at the core of the faster learning of contingencies for dominant focals and that those pairs with dominant decision-makers would display richer social interactions.

Social dominance modulates the quality but not the quantity of social interactions prior to choice

We analyzed trial-by-trial social interactions in off-line video recordings using Bonsai[32] and DeepLabCut[33] that enabled us to precisely extract the position of unmarked body parts of the interacting animals with high spatial and temporal resolution. The time animals spent directly investigating each other was equivalent, regardless of the pronounced differences in prosociality (Figure 2A; independent sample t test for "mutual direct investigation," $t_{(20)} = 0.411$, $p = 0.685$, $BF_{10} = 0.413$). Although direct contact is the standard measure of social interaction, we hypothesized that significant social interactions might still happen at a distance and not only through direct sniffing of the partner. Thus, we quantified the time that animals spent simultaneously in the choice area, regardless of the distance between them. Again, no differences were observed on the duration of these distant social interactions according to social status (Figure 2B; $t_{(20)} = 0.047$, $p = 0.96$, $BF_{10} = 0.39$).



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Although the duration of mutual direct investigation and interaction time during choice was similar in the two groups, it was still possible that dominance status could account for differences in the quality of the social dynamics when animals were at a social distance. To this end, we quantified on each trial the median value of the distance between the focal and the recipient rat, whereas in the choice area, referred to as nose-to-nose distance, as a measure of social proximity. Indeed, pairs with a dominant focal maintained a closer distance prior to choice (independent sample t test, $t_{(20)} = -2.53$, $p = 0.020$, $BF_{10} = 3.15$; Figure 3A). Interestingly, this effect was already present in the first testing session, where no differences in prosocial choice were yet observed (Figures S3A). Pairs with dominant decision-makers displayed closer interactions in a higher proportion of trials (Figure 3B), and these differences emerged early during the interaction time, where pairs with a dominant decision-maker would be closer to each other than those with a submissive focal, when the focal was going to choose the selfish option (Figure 3C, left panel; two-way ANOVA, "hierarchy" by "choice" [$F_{(1,1986)} = 4.77$, $p = 0.029$], "choice" [$F_{(1,1986)} = 294.5$, $p = 1e-61$], and "hierarchy" [$F_{(1,1986)} = 13.89$, $p = 0.0002$]; further decomposition of the interaction followed by Student-Newman-Keuls [SNK] post hoc test revealed a significant difference across dominance categories in selfish but not prosocial trials [$F_{(3,1986)} = 120.3$, $p = 1e-71$]; Figure 3C, right panel).

Social interactions are by definition bidirectional and highly dynamic,[34] and although classical studies on decision-making have focused on the analysis of the decision-maker, it could well be possible that focals were influenced by the behavior of the recipient animal. To ascertain which animal (focal or recipient) was responsible for these more proximal interactions, we quantified the median distance between each rat's nose and the central wall that divided the two mazes, as a proxy for social interest (Figures 3D and S3D–S3F). No significant differences were found between dominant and submissive decision-makers (Figure 3D, middle panel; repeated-measure ANOVA, "choice" [$F_{(1,20)} = 7.78$, $p = 0.011$, $BF_{incl} = 6.49$], "choice" by "hierarchy" [$F_{(1,20)} = 4.32$, $p = 0.051$, $BF_{incl} = 1.76$], and "hierarchy" [$F_{(1,20)} = 0.05$, $p = 0.820$, $BF_{incl} = 0.58$]). However, submissive recipients were closer to the wall on selfish trials compared with dominant recipients (Figure 3D, right panel; "choice" [$F_{(1,20)} = 2.027$, $p = 0.170$, $BF_{incl} = 0.37$], "choice" by "hierarchy" [$F_{(1,20)} = 10.86$, $p = 0.004$, $BF_{incl} = 8.44$], and "hierarchy" [$F_{(1,20)} = 1.76$, $p = 0.200$, $BF_{incl} = 0.87$]; independent sample t test for recipient rats in selfish trials, $t_{(20)} = -1.859$, $p = 0.088$). Further analysis showed that this tendency for submissive recipients to stay closer to the wall occurred during the early phase of interaction when decision-makers were going to choose to be selfish (Figure 3E, left panel; two-way ANOVA; "hierarchy" by "choice" [$F_{(1,1986)} = 5.38$, $p = 0.020$], "choice" [$F_{(1,1986)} = 11.17$, $p = 0.001$], and "hierarchy" [$F_{(1,1986)} = 14.26$, $p = 0.0001$]; further decomposition of the interaction [$F_{(3,1986)} = 7.388$, $p = 6e-5$] followed by SNK post hoc test revealed that the distance from the wall of submissive recipients in selfish trials was different from the other three categories; Figure 3E, right panel).

The above results indicate that dominance status affects the recipient's behavior: submissive recipients stay closer to the wall during selfish trials, thus decreasing the distance from

Figure 3. Social dominance modulates the dynamics of social interactions prior to choice

(A–C) Pairs with dominant rat as focal display more proximal interactions prior to choice.

(A) The distance between focal and recipient noses, as a proxy for social interest of the pair, was measured during the interaction time, defined as the time that the two rats were simultaneously present in the choice area. The median nose-to-nose distance per trial was lower in pairs with dominant focals.

(B and C) Moreover, (B) the proportion of trials with closer interactions was higher when dominants were decision-makers; (C) these more proximal interactions were already evident in the first seconds of interaction and only observed in selfish trials (see left panel showing temporal dynamics; right panel showing the average, SEM, and statistics of this time window).

(D–G) Submissive recipients follow their dominant decision-makers.

(D) To identify if one of the interacting animals was driving these more proximal social interactions, we measured the distance between the nose of each rat and the dividing wall that separated the animals in the choice area. The median distance from the wall per trial was similar for focal rats across dominance categories and trial type, although a tendency was found for submissive recipients to stay closer to the wall in selfish trials, suggesting an increased social interest toward their dominant focals when they were going to choose not to reward them.

(E) This tendency was present during the early phase of interaction.

(F and G) Movement dynamics indicated a similar pattern, where (F) median nose speed in the whole choice period did not reveal differences, but (G) submissive recipients showed higher nose speeds during the first seconds of interaction in selfish trials, suggesting again that they were following their dominant when it was going to poke in the selfish side.

(H–L) Pairs with dominant focal and submissive recipient display more coordinated gazing. Orientation of each animal toward the partner was calculated as the angle between the vector from the center of its head (red dot) to its own nose (green dot), and the vector from the red dot and to the partners' nose. Lower values indicate more directed gazing.

(H and I) Submissive recipients (H) were more oriented toward their focal when considering median head orientation toward the nose of the partner per trial, regardless of trial type, and (I) spent a higher proportion of time directly oriented in angles smaller than 60°.

(J) These differences in head orientation were evident in the first seconds of interaction: submissive recipients were more oriented toward their dominant focal both in prosocial and selfish trials.

(K) Pairs with dominant focal spent a higher proportion of time orienting to each other, whereas pairs with submissive focal spent a higher proportion of time orienting away from each other.

(L) The same tendency was observed in the head orientation of the focals, although it was only significant in the case of submissive focals, which spent a higher proportion of time orienting away from their recipient, compared with dominant focals.

(M) Dominant focals spent more time investigating the wall when their submissive recipient was in the choice area, compared with submissive focals, both in prosocial and in selfish trials, indicating a higher attention toward their recipients' behavior and suggesting increasing sniffing through the wall.

Mean ± SEM is shown. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. In (C), (E), (G), and (J), right panels, letters denote statistically significant differences between conditions with significant level set to 0.05. Sub, subordinate; Dom, dominant; R, recipient.

See also Figures S2 and S3.

the focal rat. Since, in principle, the act of nosepoking would lead rats to show similar nose movements and trajectories, the increased proximity of submissive recipients to the wall may suggest that these animals move and/or orient themselves toward the focal when it is going to choose the selfish poke. Indeed, analysis of animals’ movement (Figures 3F and S3G–S3I) showed that recipient rats continued moving the snout when the trial was going to be selfish. Similar values of nose speed were found across dominance categories for both focal and recipient rats (Figure 3F; no significant effects were found for focal or recipient rats, repeated-measure ANOVA; “choice” [focals, $F_{(1,20)} = 0.07$, $p = 0.798$, $BF_{incl} = 0.31$; recipients, $F_{(1,20)} = 2.526$, $p = 0.128$, $BF_{incl} = 0.71$], “choice” by “hierarchy” [focals, $F_{(1,20)} = 0.34$, $p = 0.568$, $BF_{incl} = 0.44$; recipients, $F_{(1,20)} = 0.354$, $p = 0.559$, $BF_{incl} = 0.43$], and “hierarchy” [focals, $F_{(1,20)} = 0.06$, $p = 0.814$, $BF_{incl} = 0.51$; recipients, $F_{(1,20)} = 0.017$, $p = 0.898$, $BF_{incl} = 0.55$]). Nevertheless, the dynamics of nose speed in the early phase of interaction (Figure 3G, left panel) showed that nose speed of submissive recipients was higher on selfish trials, especially after the first second of interaction (Figure 3G, right panel; two-way ANOVA; “hierarchy” by “choice” [$F_{(1,1925)} = 8.76$, $p = 0.003$], “choice” [$F_{(1,1925)} = 22.93$, $p = 2e{-}6$], and “hierarchy” [$F_{(1,\ 1925)} = 6.96$, $p = 0.008$]; one-way ANOVA dissecting the interaction [$F_{(3,\ 1925)} = 9.609$, $p = 0.000003$] followed by SNK post hoc test revealed that nose speed of submissive recipients in selfish trials was higher compared with the other three categories).

We further asked whether dominance status affected the degree to which recipient rats were orienting toward their focal, as indication of increased social attention. To this end, we measured the orientation angle of the recipient’s head toward the focal nose (Figure 3H, left panel) and found that submissive recipients were more oriented toward their focal compared with dominant recipients, with lower values indicative of a more directed orientation (Figure 3H, right panel; independent sample t test, $t_{(20)} = -4.52$, $p = 0.0002$, $BF_{10} = 106.48$). Further analyses revealed that over the interaction time prior to choice, submissive recipients spent a higher proportion of time orienting toward their focal, whereas dominant recipients spent a higher proportion of time orienting away from their focal (Figure 3I; independent sample t test for the proportion of time with orientation $< 60^\circ$, $t_{(20)} = 3.80$, $p = 0.001$, $BF_{10} = 27.42$; time with orientation between 60° and 120°, $t_{(20)} = 1.18$, $p = 0.253$, $BF_{10} = 0.63$; time with orientation $> 120^\circ$, $t_{(20)} = -4.32$, $p = 0.0003$, $BF_{10} = 72.84$). The same effect was observed in the dynamics of orientation during the early phase of interaction (Figure 3J, left panel), with submissive recipients more oriented to their dominant decision-maker both in prosocial and in selfish trials (Figure 3J, right panel; two-way ANOVA; “hierarchy” by “choice” [$F_{(1,1985)} = 9.20$, $p = 0.002$], “choice” [$F_{(1,1985)} = 183.43$, $p = 4e{-}40$], and “hierarchy” [$F_{(1,1985)} = 37.56$, $p = 1e{-}9$]; one-way ANOVA dissecting this interaction [$F_{(3,1985)} = 91.87$, $p = 1.136e{-}55$] followed by SNK post hoc test revealed a significant difference between all conditions). These results suggest that submissive recipients are more attentive to the behavior of the focal rat before the decision and change their orientation and position to maintain closer interactions with their dominant partner. Interestingly, this increased gazing from submissive recipients toward their dominant decision-maker was already observed in the first day of testing, whereas prosocial biases were not yet present (Figures S3J–S3M).

Next, we assessed whether this behavior of the recipient would lead to a more coordinated reciprocal interaction. We found that indeed, pairs with dominant focals spent a higher proportion of time orienting to each other, whereas pairs with submissive focals spent a higher proportion of time orienting away from each other (Figure 3K; independent sample t test for the proportion of time with both rats’ orientations $< 60^\circ$, $t_{(20)} = 2.36$, $p = 0.029$, $BF_{10} = 2.43$; both rats’ orientations between 60° and 120°, $t_{(20)} = -0.33$, $p = 0.742$, $BF_{10} = 0.4$; time with both rats’ orientations $> 120^\circ$, $t_{(20)} = -3.37$, $p = 0.003$, $BF_{10} = 12.55$). In addition, submissive focals spent a higher proportion of time orienting away from their recipient, compared with dominant focals (Figure 3L; independent sample t test focal orientation $< 60^\circ$, $t_{(20)} = 1.50$, $p = 0.148$, $BF_{10} = 0.85$; from 60° to 120°, $t_{(20)} = 0.34$, $p = 0.739$, $BF_{10} = 0.40$; orientation $> 120^\circ$, $t_{(20)} = -2{,}42$, $p = 0.025$, $BF_{10} = 2.66$). Importantly, although orientation of focal animals was not as strongly modulated by hierarchy as observed for recipient’s or mutual orientations, dominant decision-makers spent more time directly sniffing through the wall during the interaction period, suggesting enhanced social interest (Figure 3M; repeated-measure ANOVA; “choice” [$F_{(1,20)} = 5.70$, $p = 0.027$, $BF_{incl} = 3.29$], “choice” by “hierarchy” [$F_{(1,20)} = 0.04$, $p = 0.844$, $BF_{incl} = 0.35$], and “hierarchy” [$F_{(1,20)} = 4.95$, $p = 0.038$, $BF_{incl} = 2.16$]). This effect was mainly driven by the behavior displayed in the second day of testing, when prosociality emerged (Figure S3N), and was not observed in recipient animals (Figure S3O).

Granger causality analysis of focal and recipient movements in the choice area reveals increased bidirectional influence in pairs with dominant focal

Overall, the results so far suggest that submissive recipients are more attentive to their dominants: they display more direct gazing prior to choice and increase proximity to their focals, specifically when decision-makers are going to be selfish (i.e., following them around the choice area). Dominant decision-makers might respond to these cues by showing increased social attention to their recipients which is reflected in increased sniffing time directed to the animal that needs help. In order to establish directionalities in the interactions between focals and recipients within trials, we implemented Granger causality from partial directed coherence, which evaluates whether the past of one time series contains exclusive information that helps predict the present value of another one. We computed the position of each rat’s nose along the x axis (parallel to the dividing wall and ranging from the selfish port to the prosocial port) as a proxy for body movement between the two-choice options (Figure 4A), assessed whether the position of a rat would cause the other to follow (or move away), and whether this was dependent on hierarchy. Indeed, dominant focals strongly Granger-caused (g-caused) the position of their submissive recipient (Figure 4B; 0.018 bits, $p = 0.002$ against trial-shuffled surrogates; STAR Methods) and vice versa (Figure 4C; 0.006 bits, $p = 0.001$), indicating that both animals g-caused changes in the position of the other (Figure 4D). Considering that the positions of the animals are positively correlated (Pearson $r = 0.277$, $p < 0.00001$, $n = 20{,}180$), these results suggest that the movement of an animal causes movement of

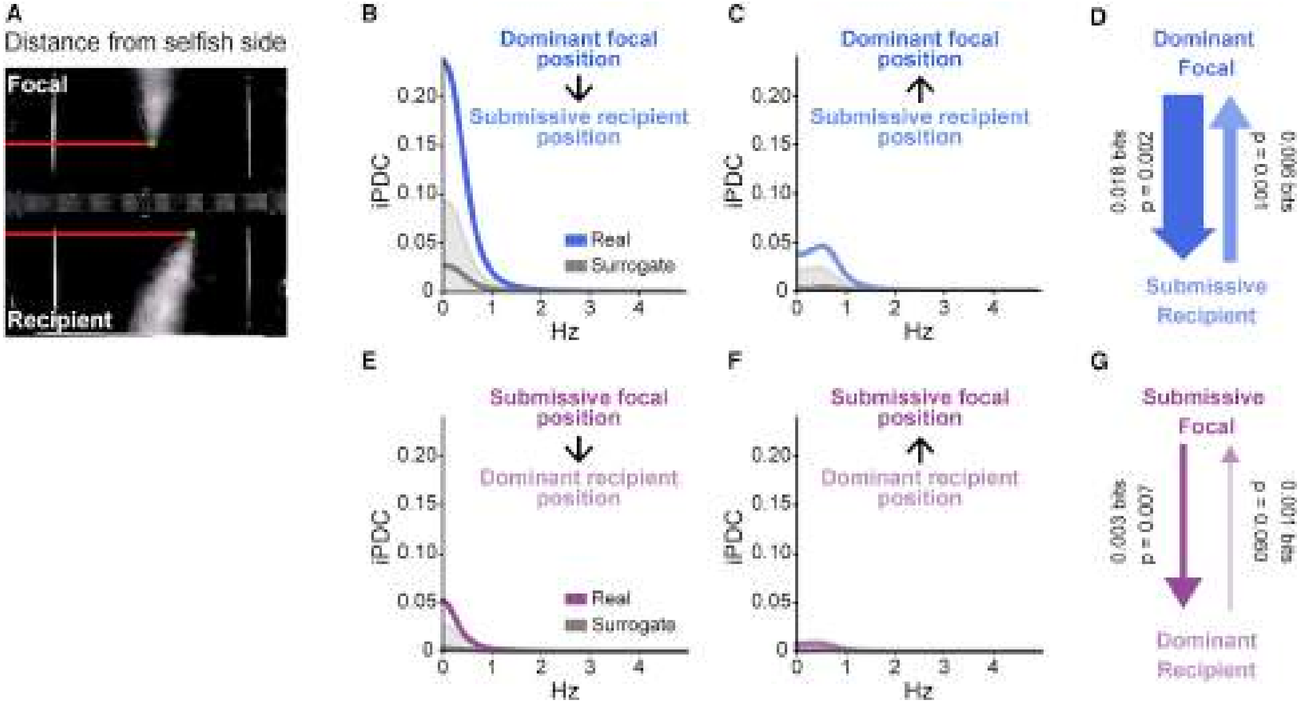

Figure 4. Granger causality analyses of animals' position reveal stronger bidirectional influences in dyads with dominant focals

(A) Rats' position was measured as horizontal distance from the selfish side of the choice area.

(B and C) Information partial directed coherence (iPDC) (B) from dominant focal to submissive recipient and (C) from submissive recipient to dominant focal.

(D) Information flow (I_{flow}) representing the causality from dominant focal to submissive recipient and vice versa in units of information transfer.

(E and F) iPDC (E) from submissive focal to dominant recipient and (F) from dominant recipient to submissive focal. iPDC spectra from the real data are shown together with median and 95% confidence intervals from surrogate spectra distributions.

(G) I_{flow} from submissive focal to dominant recipient and vice versa. Arrow widths are proportional to the I_{flow} values in each direction. p values account for significant differences between the real and surrogate iPDC.

the other in the same direction, compatible with following behavior. The position of the rats was also positively correlated in dyads with submissive decision-makers (Pearson r = 0.146, $p < 0.00001$, n = 26,628); however, we found causality from focals to recipients (Figure 4E; 0.003 bits, p = 0.007) but not from recipients to focals (Figure 4F; 0.001 bits, p = 0.060), that is, unidirectional transfer of information (Figure 4G).

Interestingly, although both decision-makers g-caused changes in the position of their recipients, this influence was stronger when focals were the dominant of the pair (contrast focal dominant to recipient submissive larger than focal submissive to recipient dominant, p = 0.006). Importantly, the influence that the movement of the recipients caused in their decision-makers was stronger in the case of submissive recipients (contrast recipient submissive to dominant focal larger than dominant recipient to submissive focal, p = 0.032). Altogether, these results indicate that decision-maker and recipient become interdependent by influencing each other's movements, with dominance affecting the strength of such increased coordination.

Social dominance modulates recipient's call rate prior to choice, which correlates with the emergence of prosociality

In addition to body position, movement, and orientation, rats exchange social information through acoustic signals.[35,36] Adult rats emit vocalizations in ultrasonic frequencies of two distinct families: the 22-kHz or "alarm calls" and the 50-kHz calls.[36] The latter have been linked to different features of rat behavior, including mating,[37,38] play,[39] social contacts,[40] reward anticipation,[41] sniffing, and locomotor activity.[42–44] However, the role of ultrasonic vocalizations (USVs) as communicative signals mediating animal prosocial decision-making has been largely unexplored. To address this, we recorded USVs during the first 2 days of the PCT, performed automated assignment of USVs agency based on the entropy of the signal (Figures 5A, S4A, and S4B), and combined this information with tracking and behavioral data in the maze (Figures 5B and 5C; Video S2) to examine how this multimodal information may relate to dominance status and the emergence of prosocial choices.

All USVs recorded during the task were of the 50-kHz family—i.e., no alarm calls were observed—suggesting a positive emotional state of the interacting rats. Many vocalizations were emitted when the nose of the rats was close to the wall separating the two individual mazes and around the nose ports (Figure 5C). Nevertheless, normalizing the call rate by nose location revealed that rats vocalized with similar rates throughout the choice area, with no clear spatial preference (Figure S4C). Consistent with previous findings,[44] rats in our task (both focal and recipient) partially synchronized the emission of calls with their own body movement, as evidenced by temporally precise correlations between nose speed and vocal production (Figures S4D and S4E). Interestingly, call rate was specifically modulated according to the role each animal had in the task, where focal animals vocalized at a higher rate than their recipients (Figure S4D).

To explore whether there were qualitative differences in the calls emitted by the animals, we classified their vocalizations into three different classes corresponding to different vocal programs (flat, frequency modulated, and step class). For this, we used VocalMat,[45] a novel platform using convolutional neural networks for sonogram-based classification of rodent USVs. We did not find differences in the qualitative nature of USVs in focal/recipient or dominant/submissive animals (repeated-measures ANOVA with "USV class" as within-subjects factor and "hierarchy" as between-subjects factor; focal rats, "USV class" [F(1.36,27.26) = 100.57, p = 7e−12], "USV class" by "hierarchy" [F(1.36,27.26) = 0.05, p = 0.322], and "hierarchy" [$F_{(1,20)}$ = 0.01, p = 0.912]; recipients, "USV class" [F(1.07,21.35) = 80.38, p = 7e−9], "USV class" by "hierarchy" [F(1.07,21.35) = 0.36, p = 0.567], and "hierarchy" [$F_{(1,20)}$= 0.04, p = 0.847]) (Figure 5D) or in the evolution of this proportion across days and trial type (Figures S5A–S5C).

Then, we asked how focals' prosocial choices were related to the vocalization rates of the interacting animals. We included nose speed of the emitting rat as cofactor to isolate specific modulations of USVs rates from possible variations in movement. Prosocial choices were positively correlated with recipient's call rate but only when the recipient was the submissive of the pair (partial correlation between USV rate and prosocial choices, controlling for recipient speed; submissive recipients, r = 0.73, p = 0.037; dominant recipients, r = −0.56, p = 0.055) (Figures 5E and S5D). Since submissive recipients were also found to modulate their position and movement toward the focal, these results suggest that they may increase call rate to interact further with the focal, consistent with the proposed role of 50-kHz calls in promoting social contact.[46]

Interestingly, we found that social hierarchy modulated the direction of correlations between USV rate and prosociality where the more submissive rats would call the more prosociality, and conversely, the more dominants would call the less prosociality would be observed, especially on the second day of testing (Figure S5D). Although the sign of the correlations was independent of the role (focal/recipient) in the task, these correlations were mainly significant when considering the USV rate of recipients.

In light of this opposite correlation, we asked whether the effect that recipients' calls have on their decision-makers' behavior was different depending on their hierarchical status. We used partial directed coherence to test whether the emission of USVs by submissive and dominant recipients would affect gazing behavior of their focal differently (Figure 5F). We found that emission of calls from the recipient rat promoted more direct gazing from the focal, but this was independent of social hierarchy (I_{flow} from submissive recipients' USV to dominant focals' orientation, 0.003 bits, p = 0.004; Pearson r = −0.0732, p = 7e−32 [n = 25,641]; I_{flow} from dominant recipients' USV to submissive focals' orientation, 0.009 bits, p = 0.001; Pearson r = −0.0528, p = 5e−21 [n = 31,706]).

Identification of multimodal cues displayed by both animals as predictors of prosocial choices on a trial-by-trial basis

So far, we described that dominant animals are more prosocial, learning the contingencies of the PCT faster, and submissives, by following their dominants, have a stronger impact when communicating need. This is related to a more synchronized social interaction of both animals that builds upon multimodal cues displayed by submissive recipients, especially when decision-makers are going to behave selfishly. These different social dynamics are correlated with prosocial choice; however, it is still uncertain which cues animals utilize that predict prosocial choices on a trial-by-trial basis. To examine the contribution of a multitude of parameters to the focal's choice, we employed a multi-step generalized linear model (GLM). Given that nose-to-nose distance and the gazing angle of each animal of the pair were the regressors that explained most of the deviance (STAR Methods; Figures 6A, 6B, and S6), we asked how these parameters interact with social hierarchy and trial progression in the prediction of prosociality. Trial progression, often included in models of decision-making as a proxy for learning, was considered critical for our analyses as animals starting the social task learn about the new contingencies with respect to the individual training. Significant interactions between behavioral variables, learning, and social hierarchy were observed (Table S2). As our main objective was to disentangle the contribution of social hierarchy on the predictors of choice, we performed reduced GLM for data from each dominance category (Figures 6C and 6D). In both social hierarchy groups, trial progression positively influenced prosocial choice, i.e., both groups increased prosocial choice over time. However, only when dominant animals were the focals were there additional behavioral changes as sessions proceed. Specifically, when dominant animals were the decision-makers, orientation angles decreased (i.e., there was more direct gazing) as trials proceeded, indicating the occurrence of some form of learning on behalf of both animals that ultimately led to a higher proportion of prosocial choices (Table S3).

However, this learning was not observed in pairs with submissive decision-makers (Table S4). Moreover, the nose-to-nose distance was negatively predictive of choice (the lower the distance between the animals, the more it predicted prosocial choice), a relationship that was not observed in submissive decision-makers. Interestingly, this relationship of social distance only in pairs with dominant focals was independent of trial progression, indicating that this regressor was a qualitative characteristic inherent to social status evident since the first interactions in the maze (Table S3).

DISCUSSION

In this study, we show that rats' prosocial tendencies are modulated by social hierarchy, with faster emergence and increased prosociality by dominant decision-makers toward submissive recipients. The emergence of prosocial biases depends on the social dynamics established within the dyads. Submissive animals, when in need of help, are influenced by the behavior of their dominant decision-makers, follow them more and display multimodal cues that facilitate social synchrony, and accelerate learning about choice impact on others by dominant rats. On

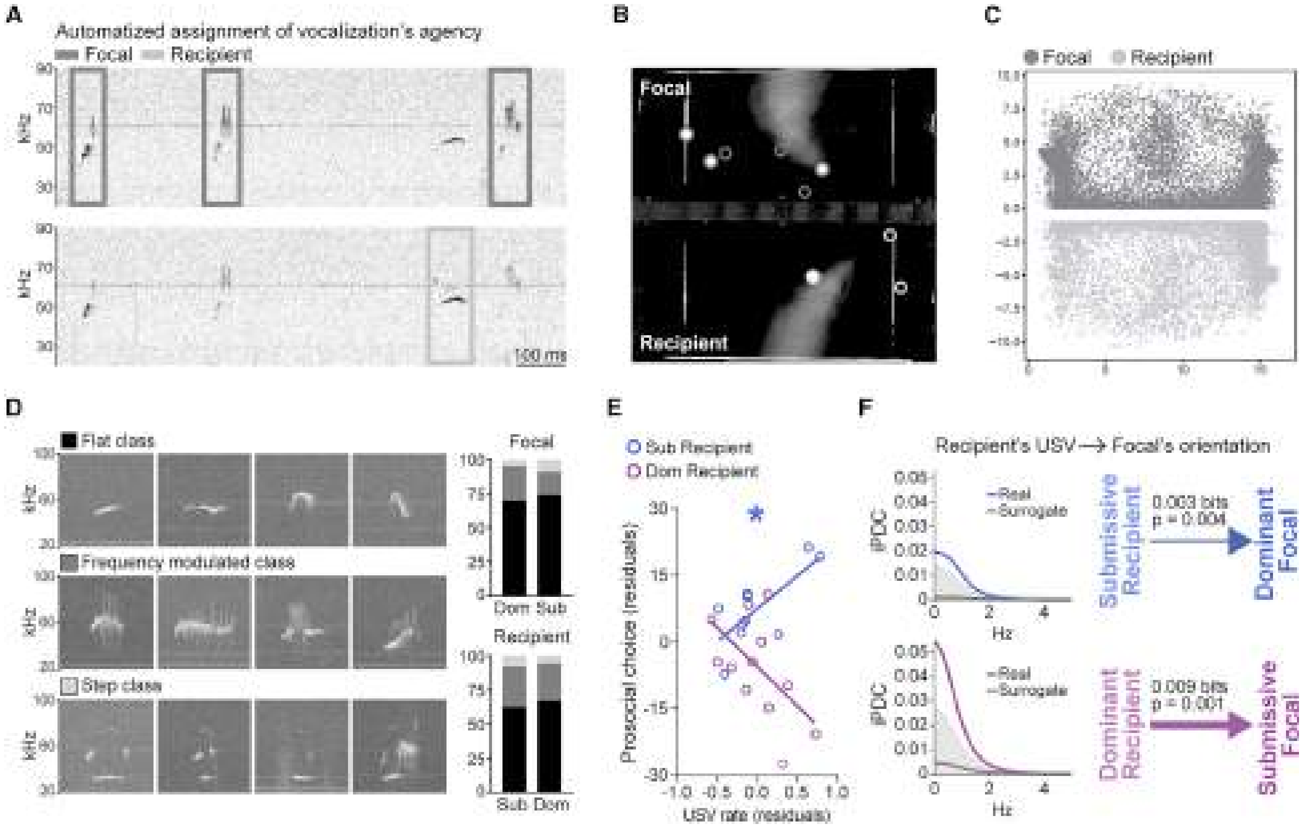

Figure 5. Social dominance modulates recipient's call rate prior to choice, which correlates with the emergence of prosociality

(A) Example USVs recording from a prosocial choice task session, showing sonograms for the two microphones, each one placed above the choice area of each maze. In this case, the top sonogram corresponds to the microphone placed above the focal rat and the bottom one to that on top of the recipient. Notice that USVs are detected from both microphones, but each USV is automatically assigned to either the focal (dark gray rectangles) or the recipient rat (light gray rectangles) according to the entropy levels (STAR Methods; Video S2).

(B) Example image showing localization of agent-assigned USVs emitted during a trial by the focal and recipient rats while in the choice area. Circles indicate the position of the rats' nose at the time a USV was emitted. Filled circles correspond to the USVs shown in (A).

(C) Nose location of focal (dark gray) and recipient (light gray) for all USVs detected, relative to the choice area, during the first 2 days of the prosocial choice task. USVs were emitted in the whole choice area; however, they were more frequent around nosepokes and in proximity to the wall that separated both mazes. See also Figure S4.

(D) Four examples of spectrogram images are shown for each USV class: flat, frequency modulated, and step. Flat calls were the most frequent class observed, followed by frequency modulated, while step calls were rare. The proportions of calls (right panel) were similar in focals (top) and recipients (bottom), regardless of the hierarchy status (Figure S5) .

(E) Partial correlation between recipients' USVs rate and focals' prosocial choice preference in the first 2 days of the PCT, when recipients' speed was regressed out, indicating that the more the submissive recipient vocalizes, the more prosocial their dominant partner would be. This correlation was only marginally significant when recipients were the dominant in the pair.

(F) Granger causality from recipients' USV to focals' orientation, showing information partial directed coherence (iPDC) from submissive recipient to dominant focal (top) and from dominant recipient to submissive focal (bottom). Independently of the hierarchy status of the animals, recipients' USVs would Granger-cause an orientation response from the focal rat. Real iPDC values, surrogate median, and 95% confidence intervals of the surrogates' distribution are shown. Arrow widths are proportional to the I_{flow} and p values account for significant differences between the real and surrogate iPDC. $^{*}p < 0.05$.

the other hand, when dominant animals are the ones in need of help and not in control of the situation, they try to obtain their own food displaying clear food-seeking behavior, but without approaching and orienting toward their submissive focal animals, which are the ones making the decisions.

In nature, social hierarchy has an important role in social organization, survival, reproductive success, and health of animals in a group.[47,48] Rats are very social animals that live in large communities in the wild and display rich social interactions.[47,49] Our findings show how dominance status modulates prosocial choice in a dyad with established social hierarchy. Prosocial modulation "down the hierarchy" has been also observed in some species of non-human primates[6,25,29,50,51] (for review, see Cronin[52]), indicating that the effect of dominance status on prosocial behavior may be conserved across species. Previous work described that high-ranking long-tailed macaques, *Macaca fascicularis*, are more prosocial than low-ranking macaques, hypothesizing that prosocial behavior is not used by subordinates to obtain benefits from dominants, but by dominants to emphasize their dominant position.[6] Furthermore, the relative dominance position mattered more than familiarity between the partners in modulating prosocial behavior in this species.[29] Other studies in

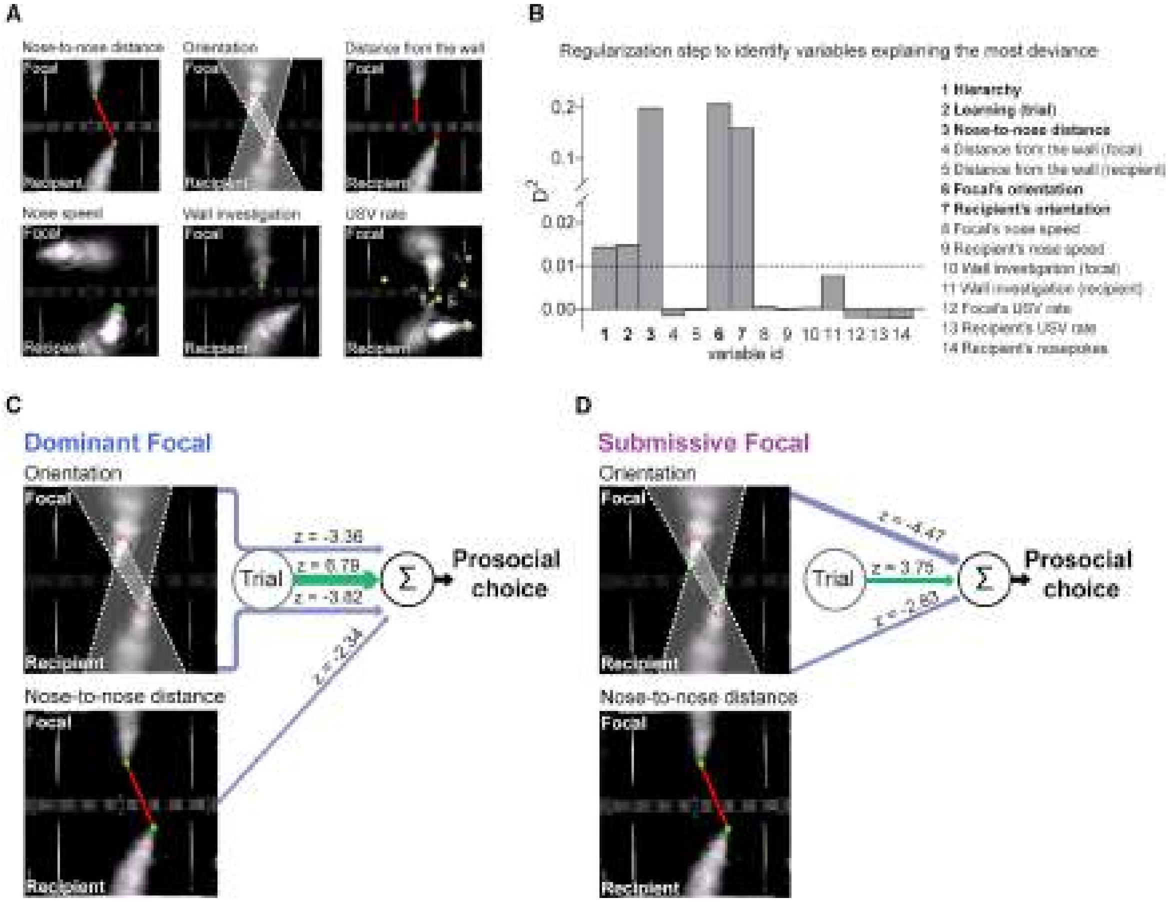

Figure 6. Behavioral predictors of prosocial choice on a trial-by-trial basis

(A) Images of the behavioral variables measured either in focal or recipient animals that were included in the analysis, together with the hierarchical status and trial number as a proxy for learning.

(B) These 14 behavioral and categorical parameters were chosen as regressors and evaluated by their contribution to the explained deviance of the model. The graph shows the mean proportion of deviance (D^2) computed for each regressor. Trial number, hierarchy, nose-to-nose distance, focal orientation angle, and recipient orientation angle were selected as the regressors that explained more than 1% of the deviance (dashed line) and used to fit a reduced GLM (see Figure S6 for unique contribution analysis of these variables). Because the latter 3 regressors were found to interact simultaneously with both trial number and hierarchy (Table S2), we fitted separate GLMs for dominant and submissive animals, thus removing hierarchy from the models and facilitating the interpretation of interaction terms (Tables S3 and S4).

(C and D) Representation of a trial-by-trial GLM analysis for pairs with (C) dominant and (D) submissive focal animals. Here, model terms are represented diagrammatically: the Σ symbol represents the summation of parameters that influence choice, green lines indicate regressors whose contributions correlate positively with prosocial choice, and blue lines indicate regressors whose contributions correlate negatively with prosocial choice. Line passing through "Trial" indicate that the interaction between that regressor and trial contributed significantly to choice and line thickness indicates the strength of those contributions as measured by the *Z* score of the regressor weight. The absence of a line from a behavioral parameter to the Σ symbol represents the absence of a statistical contribution to choice for that parameter.

macaques[25,50] and capuchin monkeys[51] also reported prosociality directed down the hierarchy, whereas in chimpanzees, the direction of prosociality remains less clear[24,53] (but see Yamamoto et al.[4]). Less effort has been devoted to the detailed investigation of the behaviors explaining these effects. We addressed this point, analyzing how dominance status affected different behaviors of both rats during decision-making. Importantly, we found that submissive recipients were the ones that adapted the most their behavior toward the dominant focals before their choice; they showed closer proximity in social distance, more direct orientation toward the focal, and their 50-kHz call rate correlated with focals' prosociality. The cues displayed by recipients were multimodal, and analysis of the predictive value of this information to prosocial choices on a trial-by-trial basis pointed toward body language as the learning signal that animals used to drive their prosocial choices. Indeed, the position of submissive recipients affected the behavior of their dominants, whereas this influence was not observed in couples where the recipient was the dominant of the pair. We observed an increased bidirectional influence in couples with dominant decision-makers, compatible with increased body movement coordination. Dominant recipients mainly directed their attention to the access to the food, whereas submissive recipients also directed their attention to the focal animal when it was going to choose the selfish option. We propose that these multimodal cues displayed by submissive recipient rats may enhance the social salience of signaling need, when the probability to receive help is low, facilitating the emergence of prosocial choices by dominant rats over testing. Our findings are consonant with studies of helping behavior in chimpanzees, showing that recipients' communicative displays toward the donor (begging direct requests) increased the success of receiving help.[4,53]

Furthermore, our results showing increased proximity in the more prosocial pairs are consistent with a study in humans demonstrating that cooperation in a prisoner's dilemma game decreased with the physical distance between the players.[54] Moreover, both humans and monkeys have been found to shape social attention according to their relative social status, with individuals preferentially allocating attentional resources to high-status conspecifics.[55–57] This mechanism is likely to be particularly relevant for low-status individuals who more heavily depend on high-status individuals and may allow them to monitor and attend more closely the behavior of their leaders. In the context of prosocial decision-making, this helps to explain why recipient rats direct more attention to a conspecific controlling and deciding on their reward when this conspecific is dominant. Future studies should investigate whether these higher levels of prosociality displayed by dominant animals are a consistent trait that can be observed when interacting with novel submissive rats from other cages or whether it emerges through the interactions of an already established hierarchy with a cage mate. In the same direction, it would be interesting to study whether submissive animals are better at displaying need in different contexts, not only in reward-related tasks, i.e., when in need of help to avoid a danger. Moreover, although the identification of stable social hierarchies in females has been elusive until now, it would be interesting to investigate whether prosociality is modulated in a similar manner in female social hierarchies.

We did not find evidence of an effect of familiarity or sex of the interacting animals in the emergence of prosociality. Our results support recent reports that these factors do not modulate emotional contagion or rats' prosocial tendencies toward animals under stress[13,16,58,59] (but see Kentrop et al.[60]) and extend them to appetitive reward-related contexts, which have been less studied. Previous works have shown that different degrees of familiarity do have an impact in the levels of prosocial behavior under stress, where the effect of familiarity is not observed when comparing cage mates versus non-cage mates of the same strain, but it does when comparing familiar and unfamiliar strains.[13] Future studies should address whether strain familiarity might affect prosocial choices in our reward-based task.

Through detailed quantification of social interactions, we describe the importance of social dynamics and information flow as factors underlying the effects of hierarchy on social decision-making, highlighting the role of the recipient and pinpointing multimodal cues in social distance as behavioral salient correlates motivating prosocial behavior. Our work has identified complex behavioral dynamics that emerge during social decision-making, paving the way for the study of the neural circuits by which the brain monitors others' actions to guide social decisions, a complex process that is dramatically affected in several psychopathologies, such as autism spectrum disorders.[61,62] Importantly, these social dynamics were only observed when social interactions were studied over distance and included into the equation the behaviors not only of the decision-maker but also those of the recipient of help. Strikingly, standard measures of social interaction were blind to the rich social dynamics that emerged in this social decision-making task. This highlights the importance of embracing the complexity of social interactions by expanding their analysis to more quantitative and sophisticated venues and taking into account the contribution of each individual to the joint social interaction. This is still scarce in mechanistic studies regarding the neural circuits of social behavior, with some recent notable exceptions,[17,59,63–66] and invite us to adopt a more complex approach in the study of social behavior going beyond the standard measurement of direct social investigation of one of the interacting individuals.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.06.026.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.M. and M.J.M.G.; methodology, software, and formal analysis, M.J.M.G., J.E.-A., K.C., and D.A.L.; investigation, M.J.M.G., A.S.M., and M.H.B.-G.; resources: D.A.L., writing – original draft, M.J.M.G. and C.M.; writing – review & editing, M.J.M.G., C.M., D.A.L., K.C., and J.E.-A.; visualization, M.J.M.G., D.A.L., and J.E.-A.; supervision, C.M.; funding acquisition, C.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure sex balance in the selection of non-human subjects. One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. One or more of the authors of this paper self-identifies as a member of the LGBTQ+ community. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Behavioural data	This paper	https://doi.org/10.17632/nzjch5ydnf.1
Codes for Granger causality analysis and multi-step GLM	This paper	https://doi.org/10.17632/nzjch5ydnf.1
Experimental models: Organisms/strains		
Rattus norvegicus with name Crl:OFA(SD)	Charles River Laboratories	RRID: RGD_2312474
Software and algorithms		
Bonsai 2.4	Lopes et al.[32]	https://bonsai-rx.org/
DeepLabCut 2.2	Mathis et al.[33]	https://github.com/DeepLabCut/DeepLabCut
VocalMat	Fonseca et al.[45]	https://github.com/ahof1704/VocalMat
Anaconda Python 3	Anaconda	https://www.anaconda.com/
Matlab 2018a	The Mathworks	https://matlab.mathworks.com
R Project for Statistical Computing	R Project	https://www.r-project.org/
IBM SPSS Statistics v23	IBM	https://www.ibm.com/mx-es/products/spss-statistics
Prism 7	GraphPad	https://www.graphpad.com/scientific-software/prism/
Graphic State 3.03	Coulbourn Instruments	https://www.coulbourn.com/
Avisoft-RECORDER software	Avisoft Bioacustics	http://www.avisoft.com/recorder/
USV detection and assignment	Sirotin et al.[42]	Sinatrat
JASP 0.16.1.0	JASP Team	https://jasp-stats.org/
AsympPDC Package	Sameshima and Baccalá [67]	https://www.lcs.poli.usp.br/∼baccala/pdc/CRCBrainConnectivity/AsympPDC/
Other		
Fully automated double T-mazes	Márquez et al.[11]	Fully automated double T-mazes
Habitest Modular System	Coulbourn Instruments	https://www.coulbourn.com/
Custom-made automatic doors	WGT-Elektronic	https://www.wgt.at/
Custom-made vertical automatic doors	Mobiara Research & Development Design Portugal	Custom-made vertical automatic doors
Dustless Precision Pellets 45mg	Bio-Serv	F0021 https://www.bio-serv.com/product/DPP_RP.html
Condenser Ultrasound Microphones (CM16/CMPA part #40011)	Avisoft Bioacustics	http://www.avisoft.com/ultrasound-microphones/cm16-cmpa/
UltrasoundGate 416H	Avisoft Bioacustics	http://www.avisoft.com/ultrasoundgate/416h/
PointGrey FL3-U3-13S2M CS	TELEDYNE FLIR	https://www.flir.com/products/flea3-usb3/
Arduino Uno board	Arduino	https://arduino.cl/arduino-uno/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Cristina Márquez (cmarquez@umh.es).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper have been deposited at Mendeley Data and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- Original codes for Granger causality analysis and multi-step GLM have been deposited at Mendeley Data and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

86 adult Sprague-Dawley rats, 74 males and 12 females (OFA, Charles-River, France) were used in this experiment, being 8 weeks old and weighing between 226-250 g upon arrival to our facilities. Rats 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, to reverse their circadian rhythm and start establishing their social hierarchy. After this period, animals were handled six times during two weeks, allowing them to habituate to the experimenter and to eat the new pellets, which were delivered inside the shavings or from a feeder magazine placed inside the homecage. Rats were 3-3.5 months old when starting the prosocial choice task. 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. Animals were provided by a commercial company, thus previous social experience, social status and degree of relatedness between the animals was not known. Animal husbandry and all experimental procedures were performed following Spanish Guidelines under the code 2016/VSC/PEA/00193 approved by the Dirección General de Agricultura, Ganadería y Pesca of the Generalitat Valenciana, which are in strict compliance with the European Directive 86/609/EEC of the European Council.

METHOD DETAILS

Modified food competition test

After acclimation to the vivarium and handling by the experimenter, 23 pairs of male cage-mates rats were tested in the Food Competition Test (mFC),[30] to identify their social hierarchy. This tests reliably measures already established social hierarchies by introducing a subtle conflict for the access to palatable pellets in the homecage of non food-deprived pairs of animals. Briefly, for this test the homecage lid was replaced by a modified laser-cut acrylic one incorporating a fully transparent feeder for hosting the pellets (Dustless Precision Pellets, 45mg, Rodent Purified Diet). The feeder was designed so only one animal could access the palatable pellets at a time, leading to subtle conflict and competition for the reward. Moreover, the feeder counted with a sliding door to prevent the access to the pellets during inter-trial intervals and an opening on the top where the experimenter could deliver the pellets in each trial. Before testing, rats were habituated for three consecutive days to wait for the sliding door to open and to eat the pellets individually, while the partner rat was kept in a separate cage. In each day of habituation, the fur of the animal was marked to facilitate identification from video and the rat was placed alone in the homecage, with the new lid hosting 10 pellets per trial. In the habituation sessions, the structure of the trial was the following: the rat was allowed to explore in his home cage for 2 minutes with the sliding door of the modified lid closed, thus preventing access to the 10 pellets. Then the feeder was open and the rat had access to the 10 pellets for a period of 2 minutes, after which the sliding door would close for an inter-trial interval of 2 minutes. The number of trials in the habituation ranged from 2 to a maximum of 4 trials per daily session. After habituation, the pairs of cage-mates were re-marked and tested for 2 consecutive days in a social context, inducing now the competition for the positive reinforcers. In the test, a trial started with 1 minute of exploration, with the sliding door of the modified lid closed and hosting 10 pellets. Then the feeder was open and the rats had access to the 10 pellets for a period of 2 minutes, after which the sliding door would close for an inter-trial interval of 1 minute. In each day, rats performed 5 trials, having access to a total 50 pellets in a session of 15 minutes. To control for a possible mere effect of performing the modified Food Competition test on prosociality, we divided the animals into two groups: one group was tested twice for social hierarchy (n = 10 pairs), with two sessions of the modified Food Competition test performed before the start of individual training for the Prosocial Choice Task (PCT), followed by two sessions performed after the PCT, whereas the second group (n = 13 pairs) completed two sessions of the modified Food Competition test only after being tested for the PCT (Figure S1K). Consumption was quantified by video annotation and the total number of pellets eaten by each animal over the days indicated the social status between them. Statistics evaluating differences between dominant and submissive animals were performed in the average consumption of all days. To have a quantitative measure of the strength of the differences in hierarchy across dyads we computed a Dominance Index (DI), as previously proposed.[30] Briefly, the pellets eaten by the recipient are substracted from those of the focal and normalized by the total number of pellets eaten:

$$DI = \frac{Number\ of\ pellets\ of\ Focal\ -\ Number\ of\ pellets\ of\ Recipient}{Total\ number\ of\ pellets} * 100$$

The sign of the DI indicates whether the focal is dominant (positive values) or subordinate (negative values). One pair of animals displayed differences in pellet consumption between the interacting animals smaller than 5%. In this pair social hierarchy was not reliable and categorisation of dominant and submissive was not possible, thus this pair of rats was excluded from the study.

Prosocial choice task (PCT)

The propensity to perform actions that benefit others was evaluated in the prosocial choice task (PCT), where 43 pairs of non-food deprived rats were tested in a double T-maze, one per animal, as previously described.[11] The two individual mazes (one for the decision maker and the other for the recipient of help rat) are separated by a transparent perforated wall, thus allowing rats to see, hear, smell and partially touch each other. In each maze, a central arm gives access to a choice area and two reward areas where food is delivered in food magazines (Figure 1A). Access to the reward area is prevented by automated doors, controlled by nose ports placed above them. Rats had to poke on a nose port for the door underneath to open, thus allowing them to enter the reward area, reach the food magazine and run around the maze back to the choice area, initiating a new trial. 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, during which they could perform trials *ad libitum*. A trial would start when both animals were present in the central corridor, giving simultaneous access to the choice area. There, recipient animals could display food-seeking behaviour by performing nose pokes on the side where they would expect the reward. Then, focals could choose between poking on the same side of the recipient, providing access to the lateral arm where both animals would receive one pellet (prosocial choice) or poking on the opposite side, entering the lateral arm where the focal would receive one pellet and the recipient none (selfish choice) (Figure 1B). In both choices, focal and recipient rats went to the same side of the maze, and returned to the central corridor to reinitiate a new trial.

Different types of pairs were tested in the PCT. To study the role of familiarity as a possible modulator of prosocial choices, two independent groups of male rats were tested: one where decision-maker and recipient were familiar animals (n=13), defined as cagemates living as a stable dyad for at least 1 month before behavioral testing; and another group where decision-maker and recipient were unfamiliar (n=14), defined as rats from the same strain that were not cagemates, that met for the first time in the PCT, and were maintained over the rest of sessions while not being cage mates. Furthermore, we studied the role of the sex of the interacting dyads by comparing the prosocial levels of males and females, both of the groups composed of familiar dyads (i.e male cagemates (n=13) were tested together and compared to dyads of female cagemates (n=6)). Finally, the role of social hierarchy was evaluated by comparing two independent groups of male cagemates dyads, which lived together for at least one month before behavioural testing. In one group the dominant animal was the decision maker of the pair and would decide whether to provide food to its submissive cage-mate (n=9), while in the other group the submissive animal would decide whether to be prosocial or not to its dominant partner (n=13).

Behavioural apparatus for the Prosocial Choice Task

The setup consists of two identical, fully automated double T-mazes (Gravaplot, Sintra, Portugal), that are automatically controlled using Graphic State 3.03 software and the Habitest interface (Coulborun Instruments, Allentown, PA, USA). Custom-made automatic doors (WGT-Elektronic, Kolsass, Austria, and Mobiara R&D, Lisbon, Portugal) triggered by infrared beams control the positions of the rats in the mazes, such that when the rats activate the beam a specific door would open, allowing the animals to move to a different area of the maze. Each T-maze has a central corridor as starting point, which gives access to a choice area through an automated door. The choice area is flanked by two lateral reward arms, at the end of which there is a food magazine. To enter the lateral arms, rats had to poke in a light-cued nose port to activate the infrared beam controlling the door underneath. The moment when the focal animal pokes in one of these nose ports, thus opening the doors of the corresponding side that give access to the reward area, is defined as the moment of the decision, i.e when the focal animal reports its choice. Once in the lateral arm, rats could retrieve the food (one pellet per trial), triggering the opening of the door that gives access to a small runway leading to the starting point at the central corridor, thus initiating a new trial. Before being tested in the PCT, rats were trained individually. The roof of each maze consisted in transparent, 2 mm-thick acrylic walls, being perforated to facilitate the detection of ultrasonic vocalizations by the microphones above them. In addition, a transparent, 2 mm-thick acrylic wall was positioned on top of the central wall separating the two mazes and between the microphones to facilitate call assignment. 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 and the PCT started.

Individual training

All animals were habituated to the maze environment for 4 daily sessions of 15-20 min each. Rats were allowed to explore the maze and retrieve the pellets that the experimenter previously placed over the floor of the maze and in the food magazines. In addition, the doors of the maze were manually activated so that the animals could habituate to the noise produced by their opening/closing. After habituation, individual training started. On the first day, all animals were shaped to rear to poke in the nose port for opening the door that gave access to the food magazine. Rats could enter both arms that were rewarded with one pellet per trial. After this first day, each rat of a pair was randomly assigned to be the decision-maker (focal) or the recipient. From this moment, focal and recipient rats received distinct kinds of individual training, for a maximum of 12 daily sessions of 20-30 minutes each. Focals learned to perform one poke on any side of the choice area, to access the lateral arms in order to retrieve the pellet and go back to the central arm to start a new trial, 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). For recipients, only the nose port on the rewarded side was active. Thus, recipients learned to poke only to one side, and the number of nose pokes required to open the automated door gradually increased over training, to ensure food-seeking behaviour and clear side preference (for further details on nose poke training, see Márquez et al.[11]). In the last 4 sessions, after nosepoking on the preferred side, the opposite door would open and recipients were forced to visit the unrewarded arm in 10 and 20% of the trials. In this manner, recipients would learn that even if no pellet was delivered in the unrewarded side, they would have to enter that lateral arm and go back to the central corridor to start a new trial. Finally, recipients were briefly re-trained immediately before each session of the PCT, to prevent extinction of food-seeking behavior. Focal and recipient role were fixed throughout the entire experiment.

Video and Sound acquisition

All the experiments were performed during the dark phase of the animal's light cycle and video recordings were captured at 30 frames per second and 1280 x 960 pixel resolution under infra-red illumination (PointGrey Flea3-U3-13S2M CS, Canada, FlyCapture). We used two cameras, each positioned above one double maze, and centred on top of the choice area. Ultrasound was recorded at a sampling rate of 214285 Hz with two externally polarized condenser microphones (CM16/CMPA) connected to an UltraSoundGate 416H (Avisoft Bioacoustics). We positioned two microphones on top of the choice area of each double maze, one microphone per T-maze (44 cm from the floor of the maze and 15 cm from the acrylic wall between them). For each session, video and audio acquisition start was simultaneously triggered through a common TTL delivered from visual reactive programming software Bonsai[32] through an Arduino Uno (ARDUINO). With Bonsai, we also sliced the entire session videos into video chunks, corresponding to the choice period of each trial. For the synchronisation of the video with the data obtained from the interface controlling the mazes, we extracted the timestamps from the coulbourn interface, and tracked the blinking of an infra-red LEDs placed in the visual field of the camera which was triggered at the time of each start trial and focal's choice. Sound recordings were synchronised to video data aligning each start trial with the recorded sound of the opening of the door that gave access to the choice area.

Pose estimation of unmarked socially interacting animals via Bonsai-DeepLabCut

A custom workflow of the Bonsai-DLC interface[68] (Python 3, DLC, version 2.2) was used to track unmarked body parts of the animals on both sides of the double maze simultaneously. Tracking was performed offline for all single trials' videos from the first two sessions of the PCT, with a confidence threshold set to 0.7. Video analysis' temporal resolution was determined by the camera's acquisition frame rate (33 ms), whereas spatial resolution was calculated by measuring a reference known distance in pixel values at the height where animals move in the maze (spatial resolution 0.59 mm). A cropped image corresponding to the choice area of one T-maze was used as region of interest (ROI) to train the model, applying an offset for each of the choice areas to maintain the original frame coordinates. DLC was trained on videos with one animal in the T-maze (26 videos from different animals). 25 frames per video (650 frames in total) were annotated and used to train a ResNet-50 neural network for 600,000 iterations.

For each video frame in the recordings, we obtained the location of the noses used to compute the euclidean distance between the rats in the choice area, and the y coordinate of the nose to retrieve the distance of each animal from the central wall. Moreover, we calculated the position of the nose of each animal in the maze, where movements in the x coordinate would indicate movement towards the selfish or prosocial port (being coordinate 0 cm the position of the selfish side and 17 cm the position of the prosocial side). In our task, focal and recipient animals of different pairs are counterbalanced when assigned to a side of the double maze, such that in some pairs the focal rat would appear in the upper part of the video and the recipient rat on the bottom part (as illustrated in Figure 1B), while the opposite occurs in the rest of the pairs. Furthermore, the prosocial side is also counterbalanced, such that it would be to the right side for some focals and to the left side for the remaining ones. Thus, we moved and scaled tracking data from different recordings to a common reference space. For this, we used the coordinates of the central wall of the double maze as space scale factor for pixel to meter conversion.

Orientation of one rat towards the other was computed as the angle between the vector from the middle of its head (halfway between the ears) to its own nose and the vector from the middle of its head to the other rat's nose. We obtained the nose instantaneous speed from the rate of change in its position. For this, we smoothed the nose position time series by independently convolving its two coordinates with a Gaussian window of 0.25 s (full width at half maximum). For each time point, we obtained the velocity vector as the derivative of each smoothed coordinate and computed instantaneous speed as its norm.

Automatic detection and assignment of Ultrasonic Vocalisations (USVs) in the Prosocial Choice Task

We automatically detected and assigned USVs as thoroughly described in Sirotin et al.[42] Briefly, USVs were detected from the raw sound recordings with custom built MATLAB routines (The Mathworks). We first obtained the sonograms for each microphone, with a 0.25 ms time step and detected times with low entropy (<6.5 bits) of the frequency spectrum in the 18-100 kHz range. We then defined as USVs segments of low entropy, those lasting at least 3 ms and bounded by silence of >20 ms. USVs were then curated by automatically discarding as noise those with high power in the sonic range (5-18 kHz) and visually inspecting the sonograms, removing any noises detected as USVs by mistake. Next, each vocalisation was assigned to either the focal or the recipient rat, by comparing the signal from both microphones. USVs that crossed the entropy threshold in only one microphone were assigned to the rat on the T-maze below it. If the same USV was picked up by both microphones, we assigned it to the rat under the microphone with lowest entropy values. Rats vocalising at the same time will typically produce USVs with non-overlapping fundamental frequencies. When simultaneous signals from both microphones were found to differ by at least 1 kHz during >3 ms, we concluded that both rats vocalized simultaneously and assigned to each one the USV detected by the microphone on its side. As in Sirotin et al.,[42] we used recordings with only one rat in the double maze to validate the USV assignment, yielding an accuracy of 94% (Figures S4A and S4B).

Multimodal analysis of USVs and tracking data

We temporally aligned audio and video of each recording session. This allowed to retrieve the video time and frame when a USV was emitted, tagging each USV with relevant behavioral information, i.e.- in which trial the USV was emitted and the location of the noses at the time a USV was emitted during the choice period (Figure 5B). We were then able to selectively quantify the USV number and rate during the interaction time (noses of both rats simultaneously detected in the choice area), which we used for Figures 5, S4C, and S5. After moving and scaling tracking data to a common reference, we were able to map the location of the noses at the time of USV emission from all recording sessions (Figure 5C).

USVs Classification

We performed automated classification of the USVs that were already detected and assigned to the emitter rat from the dominance groups (n=45.898) into three different classes of 50-kHz USVs that correspond to different vocal programs: ‘‘flat’’, ‘‘frequency-modulated’’ and ‘‘step’’. We extracted a grayscale image of the sonogram for each vocalisation (sonogram duration 200 ms, frequencies 25-100 kHz) and used a convolutional neural network for supervised image-based classification following the pipeline in VocalMat.[45] We manually selected and labelled flat (n=1002), frequency-modulated (n=1003) and step (n=921) calls, equally distributed across animals, randomly assigned 90% of each class as training set and trained the network using the original weights from VocalMat as starting point (original script available here: https://github.com/ahof1704/VocalMat/blob/master/vocalmat_classifier/training/train_model.m). Classification accuracy measured on the test set was 98%.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data obtained from the interface controlling the mazes, video analysis and USVs recordings was parsed and processed with Python (Python Software Foundation, version 3, https://www.python.org/) and MATLAB (version 2018a, The Mathworks, https://matlab.mathworks.com/). Probabilistic statistical analyses were performed using IBM SPSS Statistics version 23 for Windows (https://www.ibm.com/analytics/spss-statistics-software) and Bayesian statistics with JASP version 0.16.1 (https://jasp-stats.org/). GLM analysis was performed using the package modEvA[69] from the R Project for Statistical Computing (https://www.R-project.org/). Normality of the data was tested with the Shapiro-Wilk method.

Analysis of social interactions prior to choice

All the analysis of social interactions prior to choice was restricted to the video frames where both focal’s and recipient’s noses were tracked in the choice area (the ROI for the training of DLC). We called this portion of the total choice time ‘‘interaction time’’.

Duration of mutual direct investigation

The duration of mutual investigation in a trial was calculated from the frames in which the nose-to-nose distance was shorter than 2 cm. Absolute thickness of central wall of the double maze was 1 cm, however, after manual observation of the nose coordinates we decided to expand the distance to 2 cm in order to include in this measure all the mutual investigations that would happen in a diagonal, mostly across two separate holes in the perforated wall.

Wall investigation of each animal

As a proxy of time sniffing through the wall, rat’s wall investigation was calculated from the frames in which the distance between the rat’s nose and the central wall was equal to zero (Figures 3M, S3N, and S3O).

Quantification of social interactions in social distance

For each trial, we extracted the median of the time series of the different variables (the nose-to-nose distance, nose distance from the central wall, nose speed and orientation towards the partner). We then averaged the medians from all trials of a pair/rat to obtain a value for each subject, that we used for statistical analysis.

Radar plots of head orientation towards the partner

For the radar charts in Figures 3I, 3K, 3L, and S3M we retrieved in each trial all the frames with orientation value, which ranged from 0 (rat oriented to the nose of the partner) to 180 degrees (rat oriented to the opposite direction of the nose of the partner). We then calculated the percentages of frames belonging to each of three ranges (0-60 indicative of more direct gazing, 60-120 and finally 120-180 indicative of positions where one animal oriented opposite to the partner). For ‘‘overlapping orientations’’, we calculated the percentages of frames in which the orientations of both rats fell within the same range.

Visualization of early dynamics of social interactions in social distance

We selected, for each trial, the frames (time points) where both noses were tracked (interaction time). Next, we aligned the new time series, so that the first frame in each series was set as time 0. We then obtained an average time series for each hierarchy-trial type condition, by averaging the time series of the different trials at each interaction time point, up to the median duration of the interaction per trial (3.3 sec, line graphs of behavioral dynamics in Figures 3 and S3). Finally, for the statistical comparisons we calculated the median of each new time series corresponding to a trial and averaged the medians of all the trials belonging to the same hierarchy-trial type category (dot graphs with SNK test in Figures 3 and S3).

Granger causality from Partial Directed Coherence

To assess whether the behaviour of one rat influences that of the other within trials (Figures 4 and 5), we applied partial directed coherence (PDC), a frequency decomposition of Granger causality,[70] using routines from the AsympPDC implementation[67] (package available at http://www.lcs.poli.usp.br/∼baccala/pdc/CRCBrainConnectivity). Briefly, for each condition (e.g. “focal animal is dominant”) we fitted a single vector autoregressive (VAR) model to the time series of interest from focal and recipient rats and computed from it the information PDC (iPDC)[71] spectra from the focal to the recipient and vice versa. We integrated each iPDC spectra to obtain information flow (I_{flow}), a scalar value representing the causality from one rat group (focal or recipient) to the other in units of information transfer (bits). We used trial-shuffle surrogates and resampling statistics to test for significance of each I_{flow} and of I_{flow} differences across conditions.

Granger causality between focal and recipient positions

In detail, to quantify within-trial causality between the positions of the rats along the x axis (running parallel to the wall separating the rats, from one nose port to the other, scaled such that the prosocial nose port is always represented at the “right”, Figure 4A), we began by extracting from each trial the longest uninterrupted interaction between the rats from the early interaction time (the first 3.3 seconds selected for the analysis of behavioural dynamics). Trials not containing an uninterrupted interaction of at least 1 second were discarded from the analysis (93 out of 1998 total trials discarded). We subsampled the data by averaging every 3 time points, resulting in a sampling rate of 10 samples per second. At this point, each trial is represented by a vectorial time series of two dimensions $(x_{focal}(t),\ x_{recipient}(t))$ and 10 to 33 time points (1 to 3.3 seconds in duration). We sorted the trials into groups representing each condition and normalized x_{focal} and $x_{\text{recipient}}$ by subtracting the mean of each variable in the whole condition and dividing by its standard deviation (note we did not normalize the data trial by trial). Next, we fitted a VAR model of order 2 to each individual trial vectorial time series. Our method requires a fixed order for the VAR models and 2 was the median optimal model order for individual trials as per Akaike’s information criterion. We then computed the mean of all VAR models, thus producing a mean autoregressive model for each condition from which we calculated the iPDC spectra from focal to recipient and recipient to focal and we integrated each iPDC across all frequencies into I_{flow} as (adapted from equation 8 in Takahashi et al.[71]):

$$I_{\text{flow}} = -\frac{1}{fs}\cdot\int_0^{fs/2} \log_2(1-\text{iPDC}(f))df,$$

where fs is the sampling rate.

We implemented surrogates and resampling statistics to test for significance of I_{flow} and I_{flow} differences between conditions. We began by performing trial-shuffle surrogates within each condition. To construct each surrogate, we paired the data from the focal in each trial with data from the recipient in a random trial from the same condition. Since trials were of variable duration, we randomly matched each trial only with others having at least its duration and kept only data up to their common duration (481 of 1832 trials were of maximum duration, ensuring well-varied surrogates for all). In this way, surrogate datasets represent the null hypothesis whereby there is no interaction between the two rats within each trial. We obtained the iPDC and I_{flow} from each of 1000 surrogates and calculated a one-sided p value with finite-bias correction[72] as:

$$p = \frac{(\#\ \text{of surrogate } I_{\text{flow}} > \text{ real } I_{\text{flow}}) + 1}{\#\ \text{of surrogates} + 1}$$

To account for the positive bias in PDC, we subtracted the median surrogate iPDC from the real iPDC before calculating each final reported I_{flow} value.

We tested for significant differences between two given I_{flow} values by obtaining bootstrap distributions of their differences. For a condition with n trials, we get each single bootstrap estimate by selecting n random trials with replacement and obtaining iPDC, subtracting the median surrogate iPDC from it and calculating I_{flow} as before. We do 1000 subtractions of bootstrap estimates from each I_{flow} and compute a 2-sided finite-bias-corrected p value against the null hypothesis of there being no difference as:

$$p = 2\cdot\min\left\{\frac{(\#\ \text{of bootstrap } I_{flow}\text{ differences} > 0) + 1}{\#\ \text{of bootstrap} + 1},\ \frac{(\#\ \text{of bootstrap } I_{flow}\text{ differences} < 0) + 1}{\#\ \text{of bootstrap} + 1}\right\}$$

Granger causality between recipient USVs and focal orientation towards the emitter

For analysing causality from emission of USVs to orientation of the listener rat towards the emitter (Figure 4F) we followed the pipeline described above for rat positions, with adaptations as follows. We first constructed a binary time series with one sample per video frame valued 1 if the rat emitted a USV with onset in the time interval between that and the next frame and 0 otherwise. We then convolved this with a gaussian kernel of full-width at half-maximum of 0.25 s to obtain a continuous representation of vocal production and added to these time series gaussian noise with a sigma of 10% of their standard deviation as a necessary stochastic component as suggested in Sameshima and Baccalá.[67] We then extracted the recipient USV and focal orientation time series from the rats, keeping the longest uninterrupted interaction between the rats for each trial up to 10 seconds of interaction time (1 second minimum duration), downsampled to 10 Hz and proceeded to obtain iPDC and I_{flow} from $USV_{recipient}$ to $orientation_{focal}$.

Generalised Linear Model (GLM) Analysis

To examine the trial-by-trial contribution of a multitude of parameters to the focal's choice, we employed a multi-step GLM approach. First, we fitted a binomial GLM with 14 behavioural and categorical parameters (see Figure 6) for regressor details) using the formula

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n,$$

where p is the probability of a prosocial choice, $\beta_{0\ldots n}$ are the regressor weights, and $X_{1\ldots n}$ are the regressor values. Because Wald tests for statistical significance provide an incomplete interpretation of regressor contributions, we instead employed an alternative approach similar to that previously described by Musall and colleagues,[73] in which we computed the proportion of deviance (D^2) explained individually by each regressor. To achieve this, for each regressor in the model, we shuffled the values of every *other* regressor's values, resulting in a dataset in which one regressor contained the actual values on each trial, but all the other regressors' values were shuffled. Then, we fitted a GLM to the shuffled trial-by-trial data and computed the D^2. To provide robustness against potential random imbalances in any single shuffling, we repeated this procedure 1,000 times per regressor and took the mean D^2 value for each one. Thus, we were able to determine the maximum explained deviance for each regressor. Because each regressor's D^2 value is computed independently, it does not describe the amount of unique information each regressor contributes to the model. Two regressors could have a similar D^2, but if they are related or dependent on each other, then their unique contributions to the predictive power of the model will be limited. Therefore, we also computed the ΔD^2 for each regressor: the proportion of the deviance that is *uniquely* explained by each regressor. To achieve this, for each regressor, we shuffled the values of only that regressor, leaving all others intact. We then fitted a GLM to this dataset and computed its D^2. Next, we subtracted this value from the D^2 of the full GLM with all regressors intact to obtain the ΔD.[2] To provide robustness against any single shuffling of the data, we repeated this shuffling procedure 1,000 times for each regressor and took the mean D^2 for each one. In this manner, ΔD^2 is essentially a measure of how much predictive power the model loses when each variable is shuffled, thus revealing its unique contribution.

Next, to more deeply examine the regressors that explained most of the deviance as well as how they might interact with hierarchy and trial progression, we fitted a reduced GLM using only those regressors that explained more than 1% of the deviance: trial number, hierarchy, nose-to-nose distance, focal orientation angle, and recipient orientation angle. This time, we also included interaction terms between trial and the remaining variables, as well as for hierarchy and the remaining variables, since our previous observations strongly suggested that other variables may interact with those two.

Finally, to tease apart the resulting triple interaction involving the behavioural variables plus both trial and hierarchy, we fitted separate reduced GLMs on dominant focals and submissive focals, this time using 4 variables: trial, nose-to-nose distance, focal orientation angle, and recipient orientation angle, as well as an interaction term for trial. By fitting separate GLMs to each hierarchical group, we were able to remove hierarchy from the model, thus facilitating a more direct interpretation of the interactions between trial number and the behavioural variables.

Statistics

Repeated measure (RM) ANOVA with one between-subjects factor and "session" as within-subjects factor was performed to compare prosocial choices between the groups under study (dominant vs submissive focals, familiar males vs unfamiliar males, familiar females vs familiar males) over the course of the testing sessions. Independent sample t test was performed to assess differences between the groups when examining prosocial choices in each testing day and the average prosocial preference over the 5 days. Paired-sample t test was used for each focal to compare the prosocial choice in each testing day against rat's baseline preference in the last two days of individual training. One-sample t test was computed for each focal to compare its baseline preference against chance level (50% preference). Bayesian statistics complemented these analyses (Bayesian repeated measures and t test analyses) in order to provide estimates of the strength of the effects. We provide the BF_{incl}, BF_{+0} (one-tailed) or BF_{10} (two-tailed) accordingly.[74]

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 rat; [+] PCI show an increase on prosocial preference on social testing sessions compared to chance, [-] PCI show a decrease on prosocial preference from 50%. Distribution of PCIs for each group can be seen in Figure S1.

Permutation test analysis

To address individual variability on prosocial preference, we performed a permutation test to identify those rats 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%. Rats then were assigned to three different categories by comparing their actual PCI to the 95% confidence interval (CI) of the distribution of randomized indexes (rat with actual PCI in 2,5% upper bound was considered as *prosocial*, rat with PCI in 2,5% lower bound was considered *selfish*, and those rats with PCI falling inside the 95% were considered as *unbiased*). Lower and upper bound for each individual's distribution can be found in (Table S1).

Proportions of prosocial, unbiased, and selfish rats

We used χ^2 test to analyse differences in the proportions of animals classified as prosocial, unbiased, or selfish, for every session of the PCT of the different tested groups (familiar males, unfamiliar males, familiar females, dominant focal males, and submissive focal males).

Social interactions

We performed the independent sample t test or the Mann-Whitney U test to assess differences between dominant and submissive focal groups, when examining tracking data extracted and averaged from all the trials of each session. When grouping data by prosocial and selfish trials, A RM-ANOVA with "hierarchy" as between-subjects factor and "choice" as within-subjects factor was used to test for differences between the two groups across trial type. Finally, a one-way ANOVA followed by Student-Newman-Keuls (SNK) post-hoc test was used to evaluate differences among dominance-trial type categories, when examining the early dynamics of the interaction time.

Social dominance

Dominance index as a measure of the strength of the social hierarchy was correlated with prosociality in the second day using Pearson correlation.

Task performance

A RM-ANOVA with "hierarchy" as between-subjects factor and "session" as within-subjects factor was used to compare dominant focal and submissive focal groups in number of trials performed over the 5 days of testing of prosocial choice task.

Nosepokes and choice time

Difference between hierarchy groups in the average number of recipient's nosepokes per trial was assessed with the independent sample t test. For the recipient's latency to nosepoke, recipient's nosepoke duration (computed by subtracting the time of the rat's snout entering the nose port (activating the infrared beam) to the time the rat's snout exited the nose port (inactivating the infrared beam)) and focal's choice time, the non-parametric Mann-Witney U test evaluated differences in the distributions.

Proportion of USV classes

We used RM-ANOVA to assess differences in the proportions of USVs classified as flat, frequency-modulated, and step, across days of testing and according to the emitter agent (focal, recipient) and their hierarchy (dominant, submissive).

Relationship between USV rate and speed

We divided for each rat the number of USVs emitted while its nose was moving within each of four instantaneous speed bins by the total time in each bin (Figure S4D). Then, differences in USV rate between focals and recipients was assessed across the different speed bins using RM-ANOVA. For cross-correlations between USV emission and nose speed (Figure S4E) we obtained the USV time series as explained for Partial Directed Coherence (without subsampling nor adding noise) together with instantaneous nose speed for each trial. For each rat, we concatenated all its trials leaving gaps of 1 second with missing values between them and run the cross-correlation with a maximum lag of +/- 1 second. For each lag, we normalized the cross-correlation value by the total number of non-missing samples used for its computation to obtain the unbiased cross-correlation estimate. We subtracted from each cross correlation the mean of 1000 within-rat trial-shuffle surrogates.

Analysis of partial correlations

We performed partial correlations with nose-speed of the animals as covariate, to study the correlation between USV rate of the different animals and prosociality.