Multimodal cues displayed by submissive rats promote prosocial choices by dominants

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SUMMARY

Animals often display prosocial behaviours, performing actions that benefit others. Although prosociality is essential for social bonding and cooperation, we still know very little about how animals integrate behavioural cues from those in need to make decisions that increase their wellbeing. 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 interrogated which conditions promote prosociality by manipulating the social context of the interacting animals. While 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 towards 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 in social distance. Interestingly, these more coordinated interactions were driven by submissive animals that modulated their position and movement towards their dominants and increased 50kHz vocalisation rate when their partners were going to behave selfishly. This display of multimodal cues by submissive animals while signalling need promoted social saliency and a faster emergence of prosocial choices from dominant rats. Multivariate analysis highlighted non-canonical 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 behavioural dynamics that rats use for action-selection upon perception of socially relevant cues and navigate social decision-making.

INTRODUCTION

Prosocial actions support the development of positive social interactions, like cooperation, which sustain individual and group well-being[1–3]. Recent work has demonstrated that prosocial behaviours are not exclusive of humans but conserved across different species[4–23]. Several factors have been proposed to modulate prosocial behaviours, such as familiarity[2,19,24], sex[9,25,26], and social status[27,28]. 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. In this direction, enhanced prosocial behaviour by dominant individuals has been observed in some non-human primates. Massen et al. 2010, found that high-ranking long-tailed macaques, *Macaca fascicularis*, made more prosocial choices than low-ranking macaques, hypothesising that prosocial behaviour is not used by subordinates to obtain benefits from dominants, but by dominants to emphasise their dominant position[12]. In a later study, the same authors also found that the relative dominance position mattered more than the familiarity between the partners in modulating prosocial behaviour[28]. Other studies in macaques[24,29,30] and capuchin monkeys[31] also reported prosociality directed down the hierarchy, while in chimpanzees the direction of prosociality remains less clear[9,10,32]. Less effort has been devoted to the identification of the behavioural mechanisms that lead such directionalities, 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 to flexibility in the action-selection process of adequate behavioural strategies upon perception of these social cues, is still far from being understood. Mechanistic studies at the level of behaviour are needed to identify which are the behavioural 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 in order to identify the behavioural mechanisms 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 added self-benefit[17]. 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) (**Figure 1A-C** and **Movie S1**). We previously demonstrated that rats acquire a

prosocial choice preference which depends on the food-seeking behaviour displayed by recipients[17]. Here we hypothesise that social interactions during these attempts to reach the food might be crucial for increasing the social salience of recipients' behaviours 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 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, dominant rats are more prosocial with a faster emergence of prosocial biases. Beyond the description of this effect, we unravel the behavioural mechanism by which this effect is observed, based on the analysis of social interactions using DeepLabCut for unmarked pose estimation with subsecond resolution, entropy-based algorithms to reliably perform automatic assignment of ultrasonic vocalisation's agency, and behavioural modelling to establish causality of behaviour predictive of choice on a trial-by-trial basis. Strikingly, dominants' higher levels of prosociality are a consequence of the observation that their submissive partners are better at communicating need and capable of modifying the dominant's behaviour. 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 prosocial choice task (PCT), where a decision-maker rat (focal) can choose in each trial to provide food rewards to itself only (selfish choice) or to itself and a recipient rat (prosocial choice) (**Figure 1A-C** and **Movie S1**). First, focal and recipient rats of each pair were trained individually with separate protocols to learn to navigate in the maze in order to retrieve food (see methods). After individual training, 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 cagemates or unfamiliar conspecifics (repeated-measures ANOVA with "session" as within-subjects factor and "familiarity" as a between-subjects factor revealed a significant effect of "session" ($F_{(4, 100)}$=13.799, p =

5.366E-9), no significant interaction between "familiarity" and "session" ($F_{(4,100)}$= 0.268, p = 0.898) and no significant effect of "familiarity" ($F_{(1, 25)}$= 0.370, p = 0.548)) (**Figure 1D**). Moreover, we did not observe sex differences, with females being equally as prosocial as males (repeated-measures ANOVA with "session" as within-subjects factor and "sex" as a between-subjects factor revealed a significant effect of "session" ($F_{(4, 68)}$= 9.802, p = 0.000003), no significant interaction between "sex and "session" ($F_{(4, 68)}$= 0.439, p = 0.780) and no significant effect of "sex" ($F_{(1, 17)}$= 0.291, p = 0.597)) (**Figure 1E**). Our results support recent evidence that these factors do not modulate rats' prosocial tendencies towards animals under stress or in need[33] (but see[19,34]) and extend them to appetitive reward-related contexts.

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[35], 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 which could influence later prosocial tendencies. After identification of social status of the animals in the modified Food Competition Test (**Figure S1**), we tested for prosocial tendencies two parallel independent groups, where the focal rat, the decision-maker in our task, 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, providing access to food to the recipient, 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 with "session" as within-subjects factor and "hierarchy" as a between-subjects factor revealed a significant effect of "session": $F_{(4, 80)}$=8.545, p=0.000009, a significant interaction between "hierarchy" and "session": $F_{(4,80)}$=2.523, p=0.047 and a significant effect of "hierarchy": $F_{(1, 20)}$=9.070, p=0.007). Dominant and submissive decision-makers displayed similar choices on the first session of the prosocial choice task, 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: $t_{(20)}$ = 0.796, p = 0.435 for day 1). However, marked differences appeared from the second day of testing, where dominant animals displayed strong prosocial preferences while submissive focals were still at chance levels (independent sample *t* test comparing dominant/submissive focal groups $t_{(20)}$ = 4.184, p = 0.000457 for day 2; paired sample *t* test of proportion of prosocial choices in session 2 against baseline for dominant focals $t_{(8)}$ = 5.730, p = 0.000439; for

submissive focals $t_{(12)} = 0.840$, $p = 0.417$). These 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 onwards (*t* test comparing dominant/submissive focal groups $t_{(20)} = 1.934$, $p=0.067$ for day 3, $t_{(20)} = 1.847$, $p = 0.080$ for day 4, $t_{(20)} = 1.445$, $p = 0.164$ for day 5).

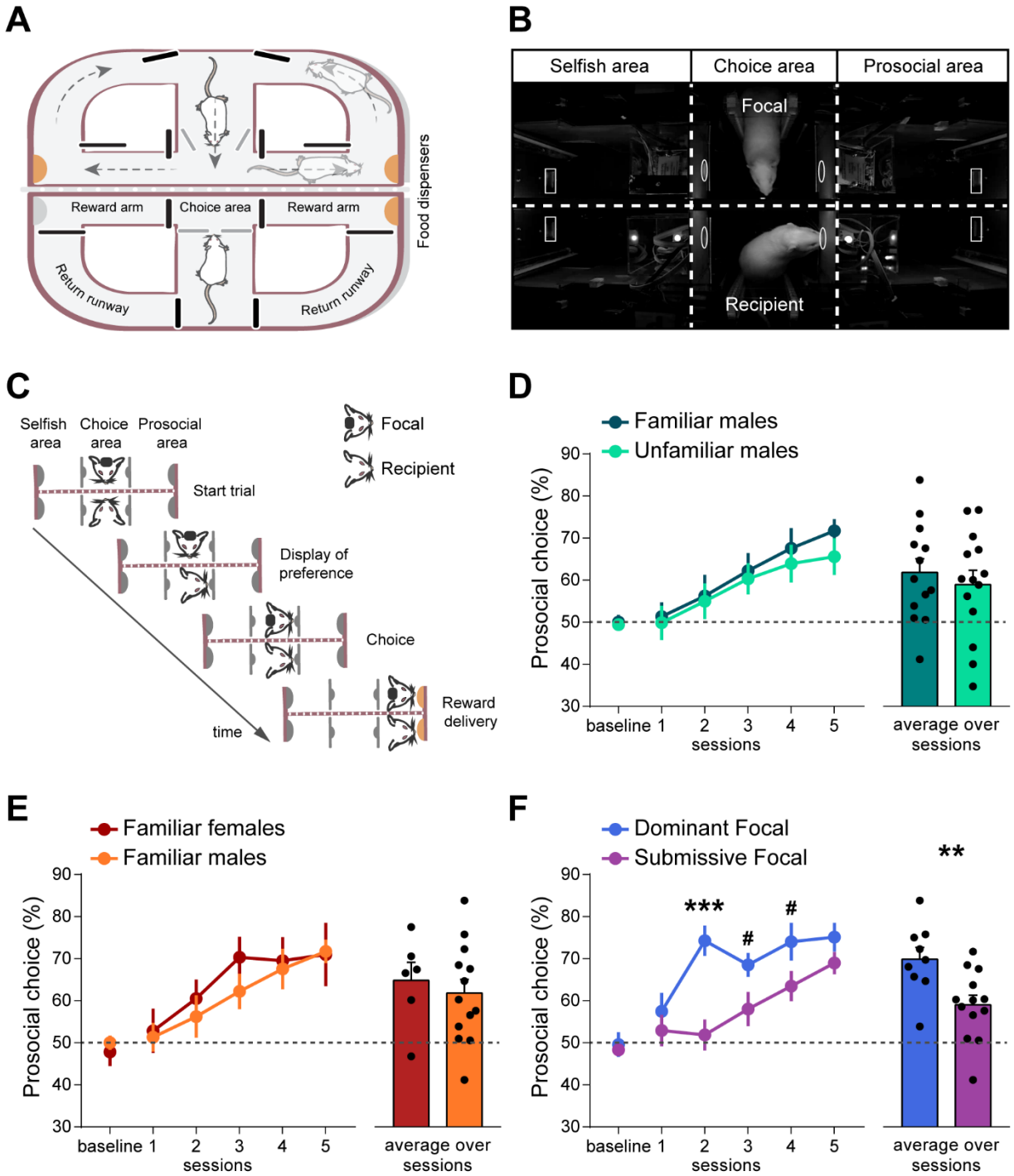

Figure1. Prosociality emerges faster when decision-makers are dominant, and is not affected by familiarity or sex

(**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. Access to the choice area is controlled by automated doors placed in the central arm (grey lines). Arrows in the upper maze represent the flow movements of the rats in the maze. Orange semi circles indicate reward delivery for both animals in prosocial (right arm) or selfish (left arm) trials.

(**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. Grey 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 (grey 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 behaviour.

(**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 (see 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 behaviour (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 of the recipient's preferred side (prosocial choice), will lead to both rats receiving one pellet in the reward area, while a nose poke on the opposite side (selfish choice) will lead to only the focal receiving one pellet and the recipient none. After food consumption, rats can pass through the return runway and go back to the central arm to start a new trial. See also Movie S1.

(**D**) Familiarity of the interacting animals doesn't 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 and focal animals that performed with a stranger recipient (non-cage mate). 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 to male rats. To study sex differences in the development of prosocial choices, we tested two independent groups where the focal animal was either male or female, 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. Dominant and submissive focals displayed a preference for the prosocial option but dominant focals showed faster emergence and higher proportion of prosocial choices compared to submissive focals. See also Figure S1. For D, E, 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 two days of individual training. Data represented as mean ± SEM. #p<0.1, *p<0.05, **p<0.01, **p<0.001.

These results indicate social dominance is a potent regulator of prosocial choices in rats, with prosociality being directed 'down the hierarchy': that is, prosociality is more likely to occur to benefit submissive rather than dominant recipients. While previous studies on non-human primates also show 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 endeavoured to identify the proximate mechanisms at the base of the enhanced prosociality in dominant animals. To this end, we performed a fine-grained analysis of rats' behaviour during the choice period (time from start

trial to focal's choice) in order to describe the potential mechanisms through which dominance status influences prosocial choices. We focused our analyses on the first two days of the task, when prosocial bias emerges, in order to identify the behavioural dynamics that promote integration of actions from others into decision making processes.

Social dominance does not affect recipient's food-seeking behaviour nor focal's latency to decide

Previously, we demonstrated that the recipient's display of food-seeking behaviour-poking in the nose port that gives access to the food-baited arm-is necessary for the emergence of prosocial choices by focal rats, indicating that the recipient's behaviour prior to choice influences the focal's decisions[17]. Thus, one possibility was that submissive recipients were better at displaying food seeking behaviour, facilitating the learning of the contingencies of the task by dominant decision makers. However, we did not find differences in the number of nosepokes performed, nor on the vigour with which they were displayed (**Figure S2A**). Dominant humans are faster in making (non-social) decisions in stressful situations, without compromising their accuracy[36]. However, we did not observe differences in the latency to choose in rats performing our social reward-based 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 hypothesised 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 implemented a workflow to systematically analyse trial-by-trial social interactions in off-line video recordings using Bonsai[37] and DeepLabCut[38] which 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 similar (independent sample *t* test for mutual social investigation: $t_{(20)} = 0.411$, $p = 0.685$) regardless of the pronounced differences in prosociality (**Figure 2A**). Although direct contact is the standard measure of social interaction, we hypothesised that significant social interactions might still happen at a social distance, and not only through direct sniffing of the partner. Thus, we quantified the time that both animals, the focal and the recipient, would spend 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 (independent sample *t* test for interaction time percentage: $t_{(20)} = 0.047$, $p = 0.963$) (**Figure 2B**).

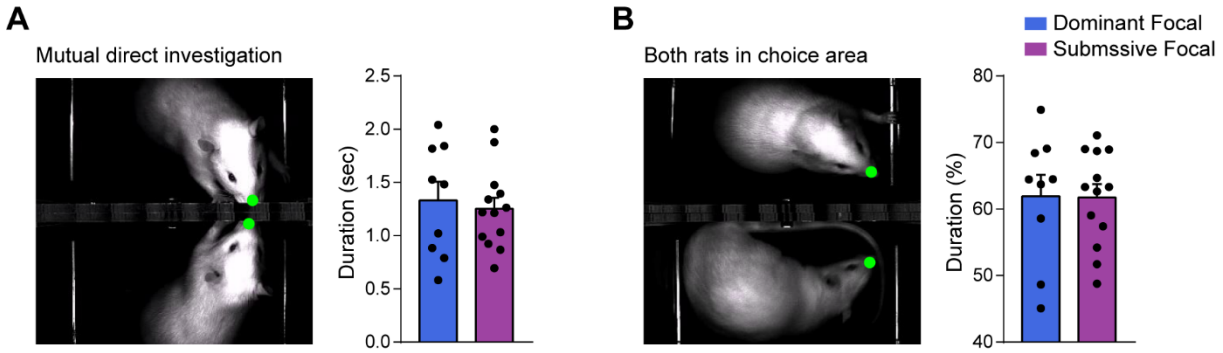

Figure2. Social dominance does not affect the quantity of social interactions prior to choice.
(**A**) Social dominance does not affect the time focal and recipient rats investigate each other prior to choice, measured as direct investigation where noses are in close proximity, nor (**B**) the percentage of choice time per trial that both rats are present in the choice area, as an index of social interactions in the distance. Data is shown as mean ± SEM, individual dots show the averaged trial value for each pair across the first two sessions. #p<0.1, *p<0.05, **p<0.01, **p<0.001.

Even though 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 in each trial the median value of the distance between the focal and the recipient rat while in the choice area, referred to as nose-to-nose distance, as a measure of social proximity. Indeed, pairs with a dominant focal maintained closer distances prior to choice (independent sample *t* test: $t_{(20)} = -2.534$, $p = 0.020$, **Figure 3A**). Interestingly, this effect was already present in the first testing session, where no differences in prosocial choice were yet observed (**Figure S2D**). Pairs with a dominant decision maker 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,** two-way ANOVA with "hierarchy" and "choice" as between-subjects factors revealed a significant interaction between the effects of hierarchy and choice $F_{(1, 1986)} = 4.765$, $p = 0.029$, a significant effect of "choice" $F_{(1,1986)} = 294.465$, $p = 1.181E-61$ and a significant effect of "hierarchy" $F_{(1,1986)} = 13.885$, $p = 0.0002$; further decomposition of the interaction revealed a significant difference across dominance categories in selfish but not prosocial trials $F_{(3, 1986)} = 120.304$, $p = 1.366E-71$ followed by SNK post hoc test, **Figure 3C** right panel).

Social interactions are by definition bidirectional and highly dynamic[39] and although classical studies on decision making and social interactions have focused on the analysis of behaviour of the decision-maker, it could well be possible that focals were influenced by the behaviour 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 (**Figure 3D**, left panel). No significant differences were found between dominant and submissive decision-makers (**Figure 3D** middle panel, repeated-measure ANOVA with "choice" as within-subjects factor and "hierarchy" as between-subjects factor revealed a significant effect of "choice" ($F_{(1,20)}$ = 7.781, p = 0.011), a marginally significant interaction between "choice" and "hierarchy" ($F_{(1,20)}$ = 4.324, p = 0.051) and no significant effect of "hierarchy" ($F_{(1,20)}$ = 0.053, p = 0.820)). However, submissive recipients were closer to the wall on selfish trials compared to dominant recipients (**Figure 3D** right panel, no significant effect of "choice" ($F_{(1,20)}$ = 2.027, p = 0.170, a significant interaction between "choice" and "hierarchy" ($F_{(1,20)}$ = 10.855, p = 0.004) and no significant effect of "hierarchy" ($F_{(1,20)}$ = 1.760, p = 0.200); further 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 was occurring during the early phase of interaction when decision makers were going to choose to be selfish (**Figure 3E**, two-way ANOVA with "hierarchy" and "choice" as between-subjects factors revealed a significant interaction between the effects of "hierarchy" and "choice" ($F_{(1, 1986)}$ = 5.379, p = 0.020), a significant effect of "choice" ($F_{(1, 1986)}$ = 11.171, p = 0.001) and a significant effect of "hierarchy" ($F_{(1, 1986)}$ = 14.262, p = 0.000164); further decomposition of the interaction : ($F_{(3, 1986)}$ = 7.388, p=0.000064) 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 behaviour: submissive recipients stay closer to the wall during selfish trials, thus decreasing the distance from 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 towards the focal when it is going to choose the selfish poke. Indeed, analysis of animals' movement (**Figure 3F** left panel) 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** right panel; for focal rats, a repeated-measure ANOVA with "choice" as within-subjects factor and "hierarchy" as between-subjects factor revealed no significant effect of "choice" ($F_{(1,20)}$ = 0.067, p = 0.798), no significant interaction between "choice" and "hierarchy" ($F_{(1,20)}$ = 0.338, p = 0.568) and no significant effect of "hierarchy" ($F_{(1,20)}$ = 0.057, p = 0.814); for recipient rats, the same analysis revealed no significant effect of "choice" ($F_{(1,20)}$ = 2.526, p = 0.128), no significant interaction between "choice" and "hierarchy" ($F_{(1,20)}$ = 0.354, p = 0.559) and no significant effect of "hierarchy" ($F_{(1,20)}$ = 0.017, p = 0.898)). 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; a two-way ANOVA with "hierarchy" and "choice" as between-subjects factors revealed a significant interaction between the effects of "hierarchy" and "choice" ($F_{(1,1925)} = 8.760$, $p = 0.003$,), a significant effect of "choice" ($F_{(1,1925)} = 22.929$, $p = 0.000002$) and a significant effect of "hierarchy" ($F_{(1, 1925)} = 6.957$, $p = 0.008$); one-way ANOVA dissecting the interaction ($F_{(3, 1925)} = 9.609$, $p = 0.000003$) followed by SNK post hoc test revealed that the nose speed of submissive recipients in selfish trials was higher compared to the other three categories).

We further asked whether dominance status affected the degree to which recipient rats were orienting toward their focal, as orientation towards the partner may be indicative 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), with lower values indicative of a more directed orientation. We found that submissive recipients were more oriented towards their focal compared to dominant recipients (**Figure 3H** right panel; independent sample *t* test $t_{(20)} = -4.516$, $p = 0.000211$). Further analyses revealed that over the interaction time prior to choice, 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 (**Figure 3I**; independent sample *t* test for the proportion of time with orientation <60°: $t_{(20)} = 3.801$, $p = 0.001$, time with orientation between 60° and 120°: $t_{(20)} = 1.177$, $p = 0.253$, time with orientation >120°: $t_{(20)} = -4.319$, $p = 0.000334$). 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 selfish trials (**Figure 3J**, right panel; a two-way ANOVA with "hierarchy" and "choice" as between-subjects factors revealed a significant interaction between "hierarchy" and "choice" ($F_{(1,1985)} = 9.196$, $p = 0.002$); a significant effect of "choice" ($F_{(1,1985)} = 183.428$, $p = 4.9034E-40$) and a significant effect of "hierarchy" ($F_{(1,1985)} = 37.560$, $p = 1.0662E-9$); one-way ANOVA dissecting this interaction ($F_{(3,1985)} = 91.867$, $p = 1.1363E-55$), followed by SNK post hoc test revealed a significant difference between all conditions). These results suggest that submissive recipients are more attentive to the behaviour of the focal rat before the decision, and change their orientation and position to maintain closer interactions with their dominant partner.

Next, we assessed whether this behaviour of the recipient would lead to a more coordinated reciprocal interaction. We found that indeed, over the interaction period, pairs with dominant focals spent a higher

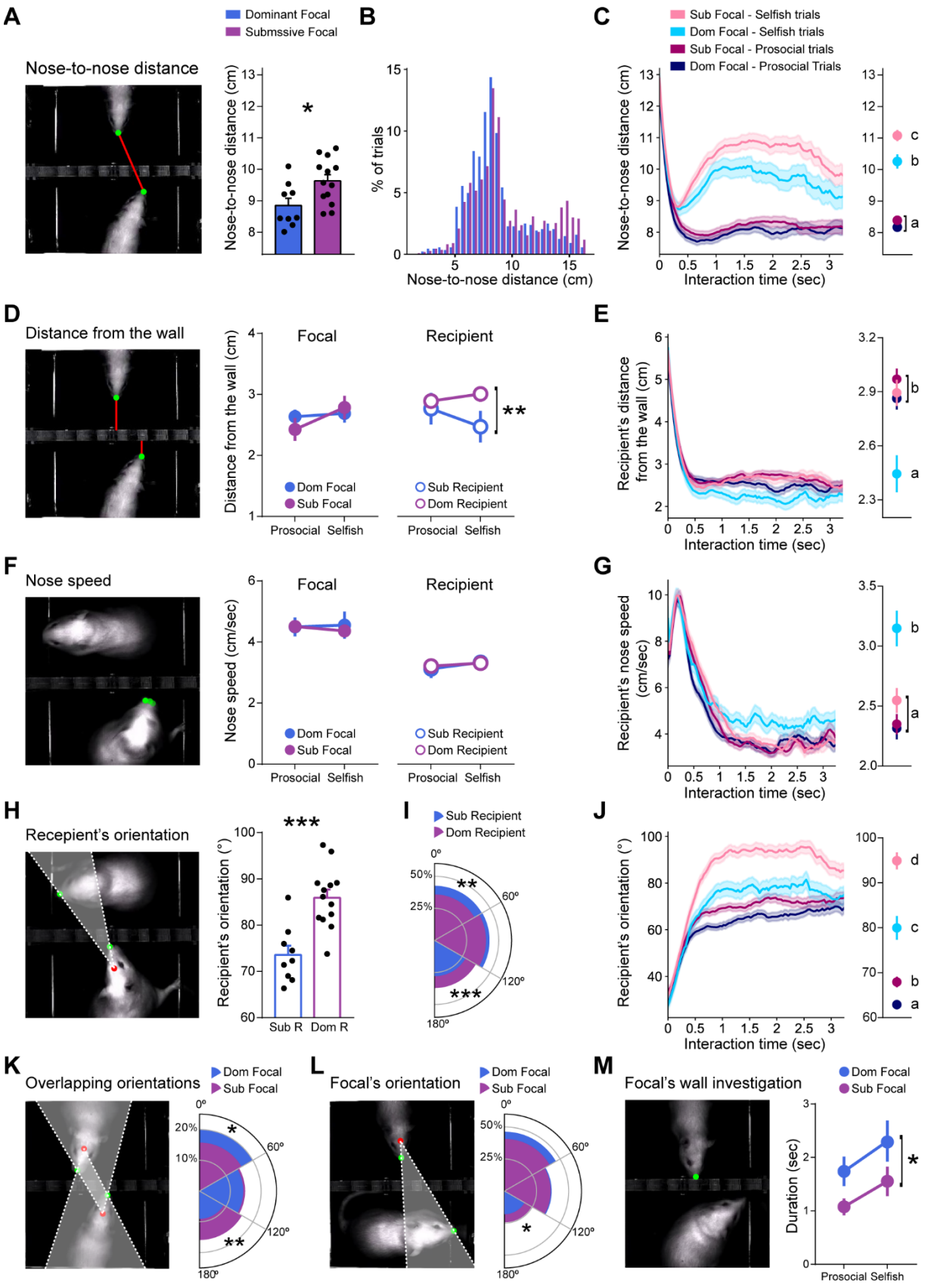
A
Dominant Focal
Submssive Focal
Nose-to-nose distance
Nose-to-nose distance (cm)
B
% of trials
Nose-to-nose distance (cm)
C
Sub Focal - Selfish trials
Dom Focal - Selfish trials
Sub Focal - Prosocial trials
Dom Focal - Prosocial Trials
Nose-to-nose distance (cm)
Interaction time (sec)
D
Distance from the wall
Distance from the wall (cm)
Focal
Recipient
Dom Focal
Sub Focal
Sub Recipient
Dom Recipient
Prosocial Selfish
E
Recipient's distance from the wall (cm)
Interaction time (sec)
F
Nose speed
Nose speed (cm/sec)
Focal
Recipient
Dom Focal
Sub Focal
Sub Recipient
Dom Recipient
Prosocial Selfish
G
Recipient's nose speed (cm/sec)
Interaction time (sec)
H
Recepient's orientation
Recipient's orientation (°)
Sub R
Dom R
I
Sub Recipient
Dom Recipient
J
Recipient's orientation (°)
Interaction time (sec)
K
Overlapping orientations
Dom Focal
Sub Focal
L
Focal's orientation
Dom Focal
Sub Focal
M
Focal's wall investigation
Dom Focal
Sub Focal
Duration (sec)
Prosocial Selfish

Figure3. 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, being (**C**) these more proximal interactions 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, while a tendency was found for submissive recipients to stay closer to the wall in selfish trials, suggesting an increased social interest towards 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**) Movement dynamics indicated a similar pattern, where median nose speed in the whole choice period did not revealed 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 (opposite to where recipients display their food seeking behaviours). (**H-L**) **Pairs with dominant focal and submissive recipient display more coordinated gazing.** (**H**) Submissive recipients were more oriented towards their focal when considering median head orientation towards 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 60º. (**J**) These differences in head orientation were evident in the first seconds of interaction, being submissive recipients more oriented towards their dominant focal both in prosocial and selfish trials. (**K**) We then calculated the proportion of time in which the recipient's orientation towards the focal and the focal's orientation towards the recipient were within the same defined range, as an index for coordinated gazing. Pairs with dominant focal spent a higher proportion of time orienting to each other, while 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 only significant in the case of submissive focals, which spent a higher proportion of time orienting away from their recipient, compared to dominant focals. (**M**) Dominant focals spent more time investigating the wall when their submissive recipient was in the choice area, compared to submissive focals, both in prosocial and selfish trials, indicating a higher attention towards their recipients' behaviour and suggesting increasing sniffing through the wall. MEAN ± SEM is shown. #p<0.1, *p<0.05, **p<0.01, **p<0.001. In C, E, G, J right panels: letters denote statistically significant differences between conditions with significant level set to 0.05. Sub= subordinate; Dom= dominant; R= recipient.

proportion of time orienting to each other, while pairs with submissive focals spent a higher proportion of time orienting away from each other (**Figure 3K** right panel; independent sample *t* test for the proportion of time with both rats orientations <60°: t(20) = 2.356, p = 0.029, time with both rats orientations between 60° and 120°: t(20) = -0.334, p = 0.742, time with both rats orientations >120°: t(20) = -3.368, p = 0.003). In addition, we saw that less prosocial (submissive) focals spent a higher proportion of time orienting away from their recipient, compared to dominant focals (**Figure 3L** right panel; independent sample *t* test for the proportion of time with focal orientation <60°: $t_{(20)}$ = 1.503, p = 0.148, with focal orientation between 60° and 120°: $t_{(20)}$ = 0.338, p = 0.739, with focal orientation >120°: $t_{(20)}$ = -2,417, p = 0.025). 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**; a repeated-measure ANOVA with "choice" as within-subject factors and "hierarchy" as between-

subject factors for focal rats revealed a significant effect of "choice" ($F_{(1,20)} = 5.698$, $p = 0.027$), no significant interaction ($F_{(1,20)} = 0.040$, $p = 0.844$) and a significant effect of "hierarchy" ($F_{(1,20)} = 4.945$, $p = 0.038$)). This difference was not observed in recipient animals (**Figure S2F**).

Overall, these results indicate that submissive recipients are more attentive to their dominants: they display more direct gazing prior to choice and they exhibit very salient behaviours, specifically when decision makers are going to be selfish (e.g., following them around the choice area). Dominant decision makers respond to these cues by showing increased social attention to their recipients which is reflected in increased sniffing times directed to the animal that needs help.

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[40,41]. Adult rats emit vocalisations in ultrasonic frequencies of two distinct families: the 22-kHz or "alarm calls" and the 50-kHz calls[41]. The latter have been linked to different features of rat behaviour, including mating[42,43], play[44], social contacts[45], reward anticipation[46], sniffing and locomotor activity[47–49]. However, the role of USVs as communicative signals mediating animal prosocial decision-making has been largely unexplored. To address this, we recorded USVs during the first two days of the prosocial choice task, performed automated assignment of USVs agency based on the intensity and entropy of the signal (**Figure 4A, Figure S3A-B** and methods section) and combined this information with tracking and behavioural data in the maze (**Figure 4B-C and Movie S2**) in order to examine how this multimodal information may relate to dominance status and the emergence of prosocial choices.

All USVs recorded during the task were in the 50kHz range and no alarm calls were observed, suggesting a positive emotional state of the interacting rats. Many vocalisations were emitted when the nose of the rats was close to the wall separating the two individual mazes and around the nose-ports where focals perform their decisions, or for recipients, around the nose port where they display food seeking behaviour in the prosocial side (**Figure 4C**). Nevertheless, further analysis normalising the call rate by nose location revealed that rats vocalised throughout the choice area, with no clear space preference (**Figure S3C**).

Interestingly, call rate was modulated according to the role each animal had in the task, where focal animals vocalised at a higher rate than their recipients during the time of interaction prior to the choice (**Figure S3D**). Then, we asked how focal's prosocial choices were related to vocalisations rate of the interacting animals. Prosocial choices were positively correlated with recipient's call rate but only when the recipient was the submissive of the pair (Pearson correlation between vocalisations rate from

submissive recipients and prosocial choices: r = 0.776, p = 0.014; for dominant recipients: r = -0.436, p = 0.136) (**Figure 4D and Figure S3E**).

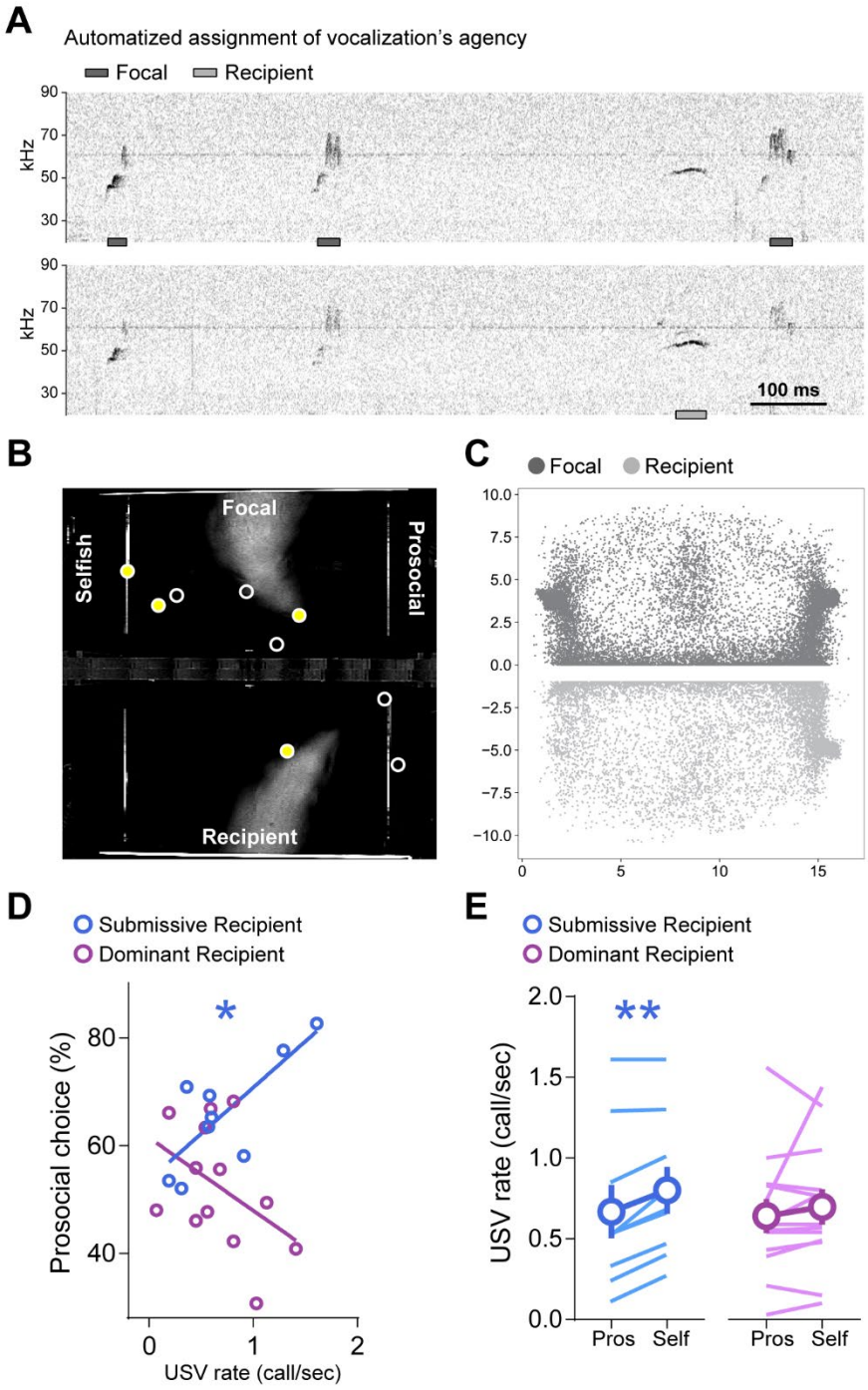

Figure4. **Social dominance modulates recipient's call rate prior to choice which correlates with the emergence of prosociality.** (**A**) Example USVs recording from a prosocial choice task session, showing sonograms for the two microphones, each one placed above the choice area of each maze. In this 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 (grey rectangles) or the recipient rat (light grey rectangles) according to the entropy levels (see methods). (**B**) Example image showing localization of agent-assigned USVs emitted during a trial by the focal (on the top) and recipient (on the bottom) rats while in the choice area. Circles indicate the position of the rats' nose at the time a USV was emitted. Circles filled in yellow correspond to the USVs shown in (A). (**C**) Nose location of focal (grey) and recipient (light grey) for all USVs detected, relative to the choice area, during the first two days of the prosocial choice task. USVs were emitted in the whole choice area, however they were more frequent around nose-pokes and in proximity to the wall that separated both mazes. (**D**) Correlation between recipient's USVs rate and focal's prosocial choice preference in the first two days of the PCT, indicating that the more the submissive recipient would vocalise, the more prosocial their dominant partner would be. This correlation was not observed when recipients were the dominant in the pair. (**E**) In accordance with what we observed with the tracking data, submissive recipients showed higher USVs rate in selfish than prosocial trials.

Strikingly, only submissive recipients modulated call rate depending on trial type, increasing the rate of their calls when dominant decision makers were going to behave selfishly (paired sample *t* test of prosocial vs selfish trials for submissive recipients: $t_{(8)} = -4.670$, $p = 0.002$; for dominant recipients $t_{(12)} = -0.990$, $p = 0.342$) (**Figure 4E** and **S3F**). Since submissive recipients were also found to modulate their position and movement toward the focal with trial type, 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[50].

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

So far, we have described that dominant animals are more prosocial, learning the contingencies of the prosocial choice task faster, and submissives are more efficient communicators while signalling need. This is related to a more synchronised 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 that emerge between dominant and submissive animals are correlated with prosocial choice, however it is still uncertain which cues animals utilise that causally 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 General 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 (see methods and **Figure S4**), 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 behavioural variables, learning and social hierarchy were observed (**Supplementary Table 1**). As our main objective was to disentangle the contribution of social hierarchy on the causal predictors of choice, we performed reduced GLM for data from each dominance category (**Figure 5**). 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 behavioural 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 (**Supplementary Table 2**).

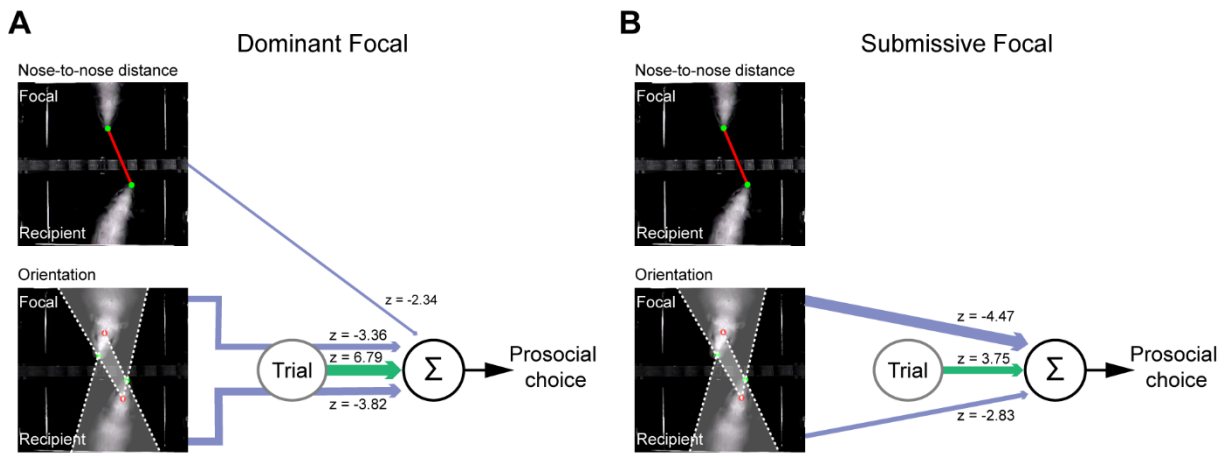

Figure5. Behavioural predictors of prosocial choice on a trial-by-trial basis.
Diagrammatic representation of a trial-by-trial Generalised Linear Model (GLM) analysis for pairs with Dominant (left) and Submissive (right) focal animals. To examine the contribution of a multitude of behavioural parameters to the focals' choices, we employed a multi-step GLM approach. A binomial GLM was fitted to trial-by-trial data from all rats for 14 parameters (see Methods for full details of these procedures). These regressors were further evaluated by their contribution to the explained deviance of the model, from which 5 were selected for further analysis: trial number, hierarchy, nose-to-nose distance, focal orientation angle, and recipient orientation angle (see **Figure S4**). Because the latter 3 regressors were found to interact simultaneously with both trial number and hierarchy (**Supplementary Table 1**), we fitted separate GLMs for dominant and submissive animals, thus removing hierarchy from the models and facilitating the interpretation of interaction terms (**Supplementary Tables 2 and 3**). 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, blue lines indicate regressors whose contributions correlate negatively with prosocial choice, and line thickness indicates the strength of those contributions as measured by the p value of the regressor weight. The absence of a line from a behavioural parameter to the Σ symbol represents the absence of a statistical contribution to choice for that parameter. For both groups of animals, trial number was found to positively influence prosocial choice (green arrows), i.e., both groups make more prosocial choices as sessions proceed. Strikingly, however, only when dominant animals are the focals are there additional behavioural changes as sessions proceed. Specifically, when dominant animals are the focals, orientation angles of both focal and recipient rats decrease (i.e., there is more direct gazing) as trials proceed (blue arrows passing through Trial), indicating the occurrence of some form of learning on behalf of both animals that ultimately leads to a higher proportion of prosocial choices. In contrast, for submissive focals, while orientation angles tend to be lower on trials in which the animal makes a prosocial choice (blue arrows) most probably explained by the architecture of the maze, these angles do not change as a function of trial number (i.e., blue arrows do not pass through Trial due to the absence of an interaction), indicating the lack of a learned 'concerted effort' by the pairs. Moreover, distance between the interacting animals was a further predictor of prosocial choice, but only in pairs where focals were dominant.

However, this learning was not observed in pairs with submissive decision-makers (**Supplementary Table 3**). 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 (**Supplementary Table 2**).

DISCUSSION

In this study, we show that rats' prosocial tendencies are strongly modulated by social hierarchy, and the emergence of prosocial biases depend on the social dynamics established between dominant and submissive rats. Submissive animals, when in need of help, follow their dominant focals and display multimodal cues that enhance social saliency, which facilitates social synchrony and accelerates learning about choice impact on others by dominant decision-makers. On the other hand, when dominant animals are the ones in need of help and not in control of the situation, they tend to continue doing what they learned during individual training, trying to obtain their own food by displaying clear food seeking behaviour, but without orienting towards their submissive focal animals, which are the ones making the decisions.

In nature, social hierarchy has an important role in social organisation, survival, reproductive success and health of animals in a group[51,52]. Rats are very social animals that live in large communities in the wild and display rich social interactions[51,53]. Our findings show how dominance status modulates prosocial choice in a dyad with established social hierarchy, with faster emergence and increased prosociality by dominant decision-makers. While prosocial modulation down the hierarchy has been also observed in some species of non-human primates[12,24,28–31], indicating that the effect of dominance status on prosocial behaviour may be conserved across species, less effort has been devoted to the detailed investigation and characterisation of the behaviours of subjects, which is necessary to determine the proximate mechanisms leading to such directionality. We addressed this point in our study, analysing how dominance status affected different behaviours of both rats during decision-making. Importantly, we found that submissive recipients were the ones that adapted their behaviour towards the dominant focals before their choice; they showed closer proximity in social distance, more direct orientation towards the focal, and increased 50-kHz call rate, especially during selfish trials. 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 towards non-canonical body language as the learning signal that animals used to drive their

prosocial choices. 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, by approaching and vocalising. We propose that these multimodal cues displayed by submissive recipient rats may enhance the social salience of signalling 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 a study of helping behaviour in chimpanzees, showing that recipients' communicative displays towards the donor (begging direct requests) increased the success of receiving help[10].

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 behaviour 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 for their reward when this conspecific is dominant.

Through detailed quantification of social interactions, we describe the proximate factors determining these directional effects, highlighting the role of the recipient and pinpointing multimodal cues in social distance as behavioural salient mechanisms motivating prosocial behaviour. Our work has identified sophisticated behavioural 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 which is dramatically affected in several psychopathologies, such as autism spectrum disorders[58,59]. Importantly, these social dynamics were only observed when social interactions were studied over distance, and included into the equation the behaviours not only of the decision-maker but also the ones 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 and that were mainly driven by the recipient of help. 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. Unfortunately, this approach is still scarce in mechanistic studies regarding the neural circuits of social behaviour, with some recent notable

exceptions[23,60–63], and invites us to revisit previous negative results when studying social interactions based solely on the measurement of direct social investigation of one of the interacting individuals.

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AUTHORS CONTRIBUTION

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

DECLARATION OF INTERESTS

The authors declare no competing interests

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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)

Data and code availability

- All data reported in this paper will be shared by the lead contact upon reasonable request
- All original codes will be deposited at Github and made publicly available as of the date of publication
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODELS AND SUBJECT DETAILS

Subjects

86 adult Sprague-Dawley rats (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)[35], to identify the stable social hierarchies. 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 mFC 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 mFC 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 mFC only after the being tested PCT (**Figure S1B**). 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. 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[17]. 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 a minimum of two 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.

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. 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 vocalisations 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 see 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 Marquez et al., 2015). 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 behaviour. 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 polarised 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[37] 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[64] (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. 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.

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 metre conversion.

Orientation of one rat towards the other was computed as the angle between the segment from the middle of its head (halfway between the ear) to its own nose and the segment 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 0.1 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, 2014[47] (Methods and Figure S3). 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 1kHz during >3 ms, we concluded that both rats vocalised simultaneously and assigned to each one the USV detected by the microphone on its side. As in Sirotin et al., 2014[47], we used recordings with only one rat in the double maze to validate the USV assignment (total 15 recordings).

Multimodal analysis of USVs and tracking data

A time scale factor was computed to align 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 behavioural 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 4B**). We were then able to selectively quantify the USV rate during the interaction time (noses of both rats simultaneously detected in the choice area), which we used for **Figures 4D-E** and **S3D-F**. 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 4C**).

QUANTIFICATION OF BEHAVIOURAL DATA 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/). Statistical analyses were performed using IBM SPSS Statistics version 23 for Windows (https://www.ibm.com/analytics/spss-statistics-software). GLM analysis was performed using the package modEvA[65] 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 (**Figure 3M** and **S2F**).

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** and **3L** 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, 60-120, 120-180).

Visualisation of early dynamics of social interactions in social distance: We selected, for each trial, the frames 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 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). 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.

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, effect of testing for social hierarchy with the modified food Competition test on the prosocial choice task)) 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).

Social interactions: we performed the independent sample t 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.

Ultrasonic vocalisations: we used the paired-sample t test to assess differences in the USV rate between a focal rat and its recipient, or between prosocial and selfish trials in separate groups (dominant focals,

submissive focals, submissive recipients and dominant recipients). The strength and significance of correlation between USV rate and prosocial choice were assessed with Bivariate Pearson Correlation.

Social dominance: we applied the paired-sample t test to study differences in the number of pellets eaten between dominant and submissive animals.

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.

General Linearized 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 S4**) for regressor details) using the formula

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

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[66], 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.

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.

Supplemental Information

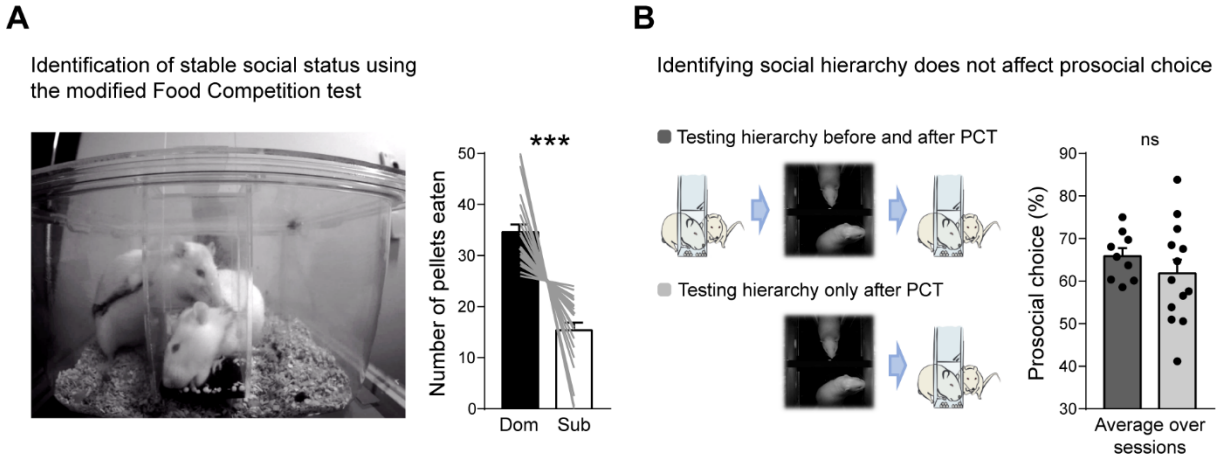


Figure S1. Identification of stable social hierarchies in pairs of cagemates. Related to Figure1.

(**A**) (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) Average 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 categorised as the dominant ('Dom') and the rat eating less pellets as the submissive ('Sub') of the pair (paired-sample *t* test for food consumption of dominant against submissive: $t_{(21)} = 6.442$, $p = 0.000002$).

(**B**) 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: $t_{(20)} = 1.083$, $p = 0.293$). Data are represented as group MEAN ± SEM of the prosocial levels of the 5 testing sessions. *p<0.05, **p<0.01, ***p<0.001.

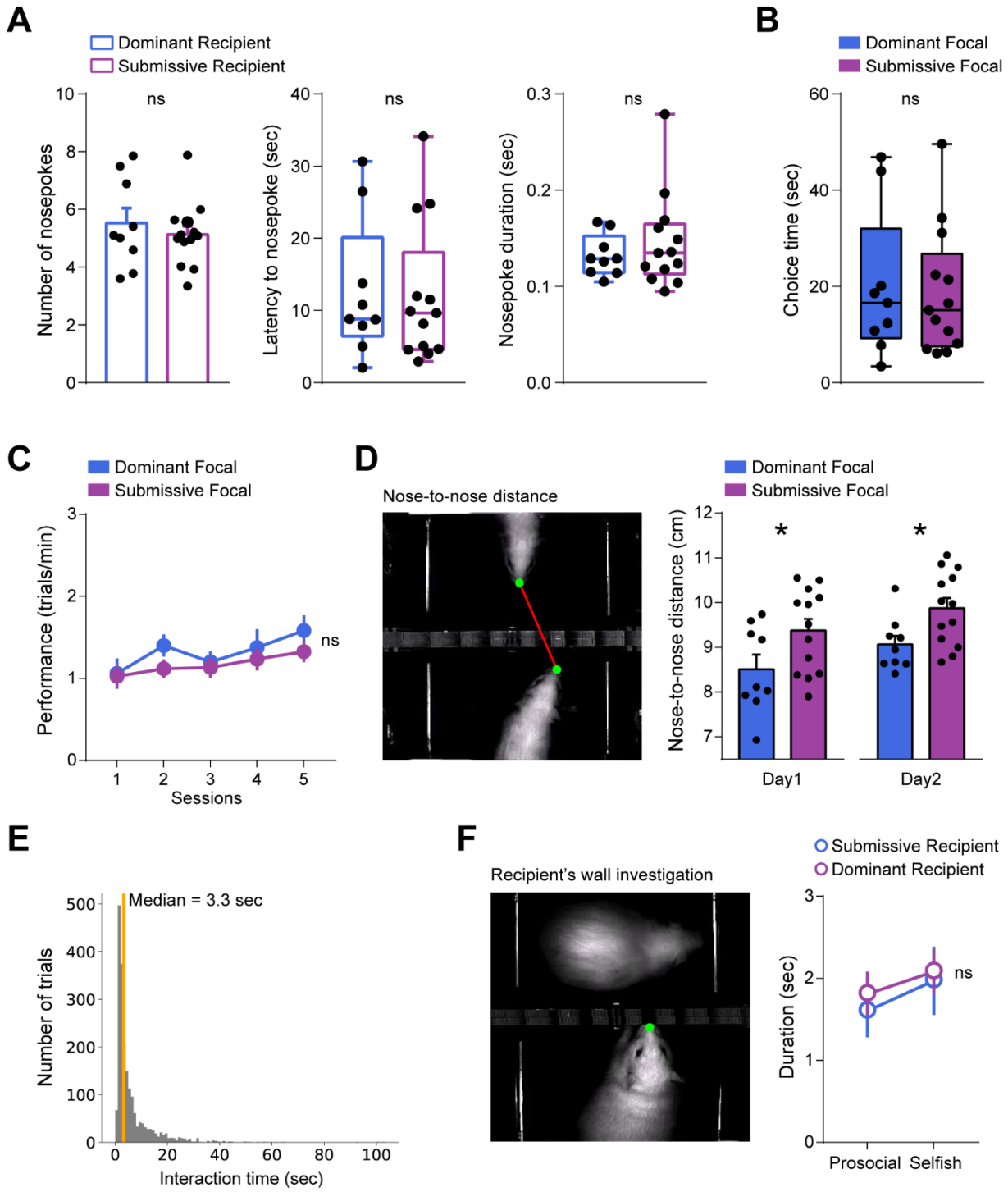


Figure S2. Effects of dominance status on recipient's nosepokes, choice time, task performance, nose-to-nose distance, and recipient's wall investigation. Related to Figure2.

(**A**) Social hierarchy of the recipients does not affect displays of food seeking behaviour. 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: $t_{(20)} = 0.707$, $p = 0.488$; Mann-Whitney U Test for latency to nosepoke and nosepoke duration: $U = 63$, $p= 0.794$; $U = 54$, $p = 0.794$).

(**B**) The duration of the choice period was similar between dominant and submissive focals (Mann-Whitney U Test for choice time: $U = 61$, $p = 0.896$).

(**C**) Dominance status did not affect the performance in the PCT, measured as number of trials per minute (a repeated-measure ANOVA with "session" as within-subjects factor and "hierarchy" as between-subjects factor revealed a significant effect of "session" ($F_{(4,80)}=5.554$, $p=0.001$), no significant interaction between "session" and "hierarchy" ($F_{(4,80)}=0.710$, $p=0.587$) and no significant effect of "hierarchy" ($F_{(1,20)}=0.819$, $p=0.376$)).

(**D**) The median nose-to-nose distance prior to choice (left image) was lower in pairs with dominant focals than in pairs with submissive focals, in both the first and second session of the PCT (independent sample t test for day1: $t_{(20)} = -2.109$, $p = 0.048$; for day2: $t_{(20)} = -2.538$, $p = 0.02$).

(**E**) Histogram including all trials of pairs with dominant and submissive focal, highlighting the median time of interaction 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 Figure3.

(**F**) Submissive and Dominant recipients spent similar amount of time investigating the wall during the interaction time, both in prosocial and selfish trials (a repeated-measure ANOVA with "choice" as within-subjects factor and "hierarchy" as between-subjects factor revealed no significant effect of "choice" ($F_{(1,20)} = 2.139$, $p = 0.159$), no significant interaction between "choice" and "hierarchy" ($F_{(1,20)} = 0.049$, $p = 0.827$) and no significant effect of "hierarchy" ($F_{(1,20)} = 0.167$, $p=0.687$)). Bar graphs in (A) and (D) show MEAN ± SEM and individual values; line plots in (C) and (F) show MEAN ± SEM; box plots in (A) and (B) show median, first and third quartiles, with whiskers indicating maximum and minimum values. Individual values correspond to the median over days for each animal. *p<0.05, **p<0.01, ***p<0.001. ns: not significant.

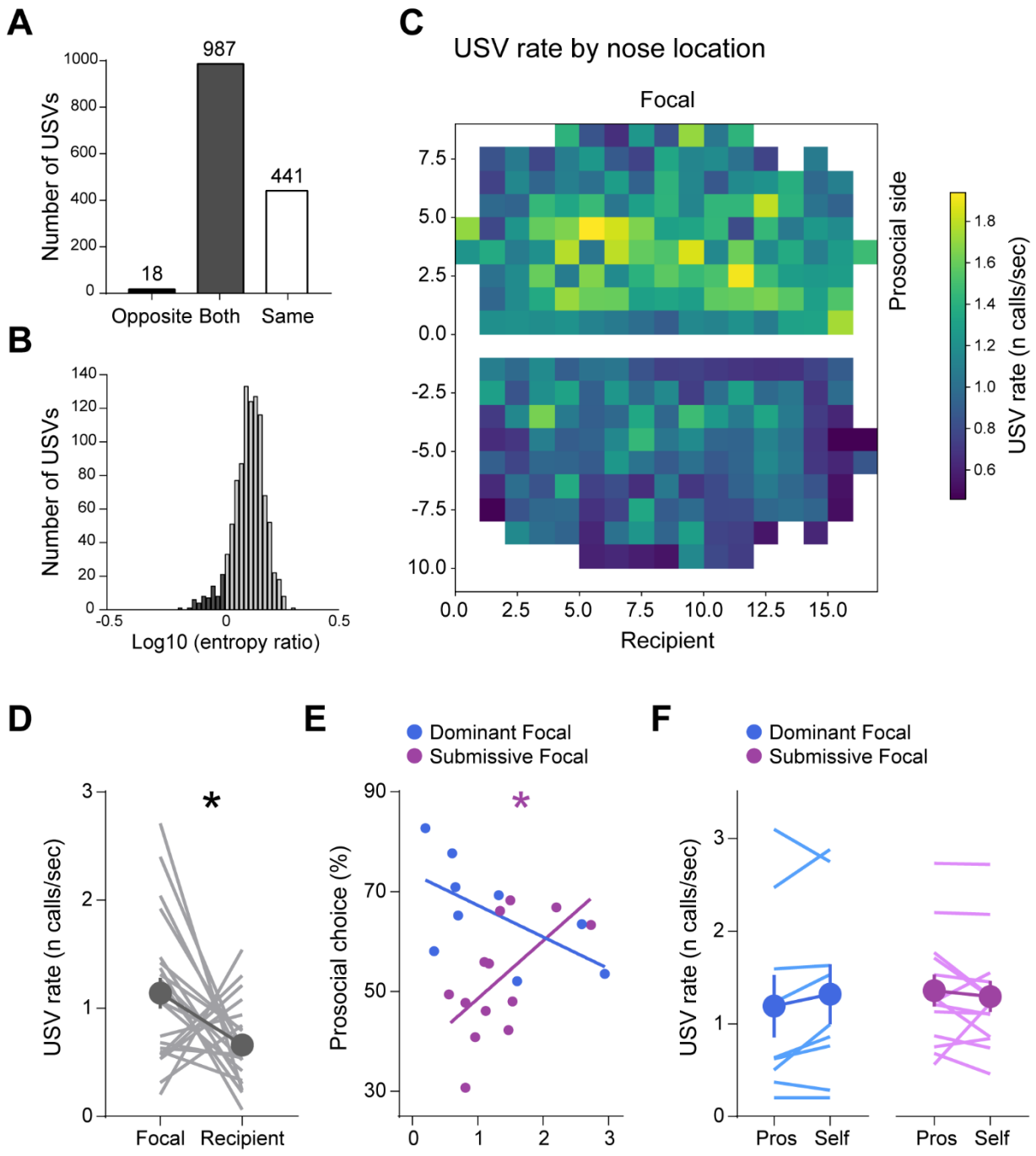


Figure S3. Validation of the USV assignment to individual animals within a pair and profile of USVs rates. Related to Figure 4.

(**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 ($\log_{10}$(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) normalised by the time spent in each nose location. 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**) Focal animals vocalised at higher rate than their recipients, during the interaction time (paired sample *t* test of focal's against recipient's USV rate: $t_{(20)} = 2.694$, $p = 0.014$).

(**E**) Correlation between focal's USVs rate during interaction and prosocial choice preference. A significant positive correlation was observed for submissive focals, while no significant correlation was present for dominant focals (Pearson correlation between USV rate and prosocial choices for dominant focals: $r = -0.601$, $p = 0.087$; for submissive focals: $r = 0.598$, $p = 0.031$).

(**F**) No significant effect of trial type on USV rate was seen for dominant focals, neither for submissive focals (paired sample *t* test for dominant focals: $t_{(8)} = -1.496$, $p = 0.173$; for submissive focals $t_{(12)} = 0.772$, $p = 0.455$).

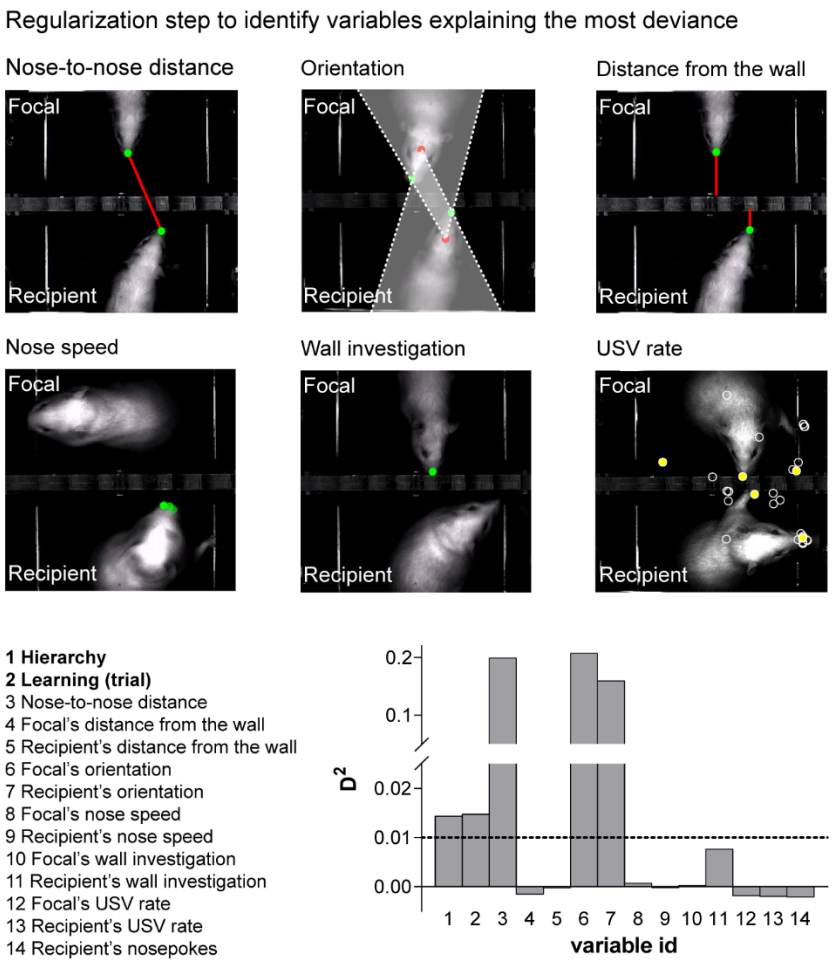


Figure S4. Regularisation step to identify variables explaining the most deviance. Related to Figure 5. 14 behavioural and categorical parameters were chosen as regressors and evaluated by their contribution to the explained deviance of the model. Pictures represent the 14 behavioural variables measured either in focal and recipient animals, listed on the middle panel. The graph on the right shows the mean proportion of deviance (D^2) computed for each regressor, after shuffling the remaining regressors 1000 times. 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.

Supplementary Table 1 | General Linear Model with all behavioural variables. Related to Figure S5.				
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	**

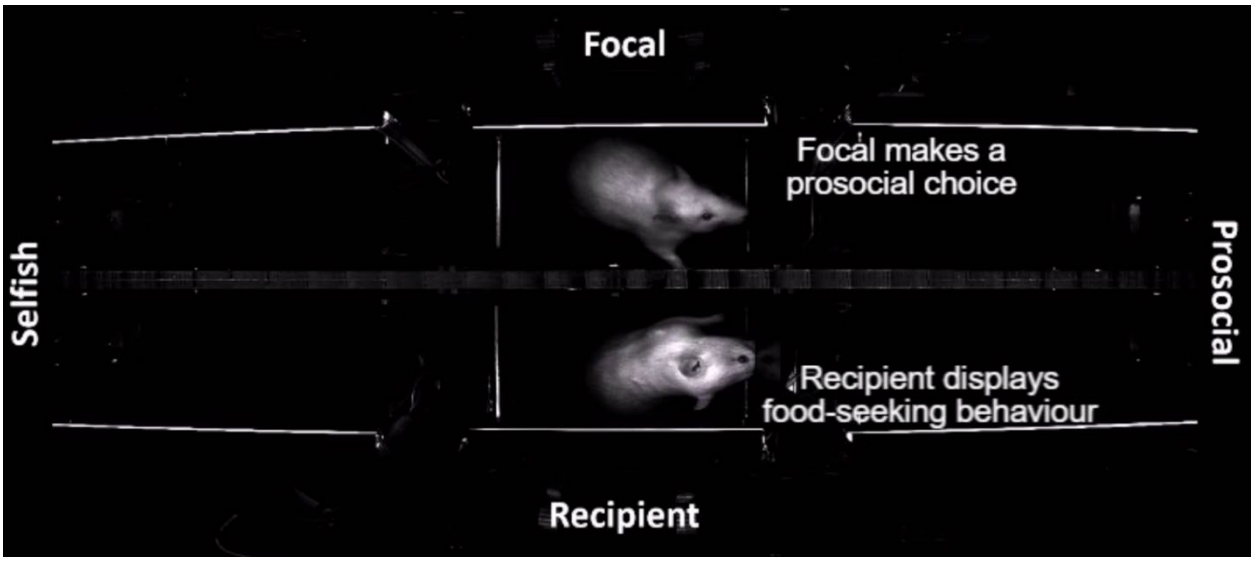
Prosocial choice is a binary variable (1:prosocial choice, 0:selfish choice). Trial is an ordinal variable indicating cumulative trial number over the first two days of the PCT. The dataset includes 1995 observations from 22 pairs of animals. *p<0.05, **p<0.01, *** p<0.001

SupplementaryTable 2 | Reduced GLM: pairs with dominant focal. Related to Figure5.				
Prosocial choice ~ trial * hierarchy * (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	***

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

Supplementary Table 3 | Reduced GLM: pairs with submissive focal. Related to Figure5.				
prosocial ~ 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	3.749	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	

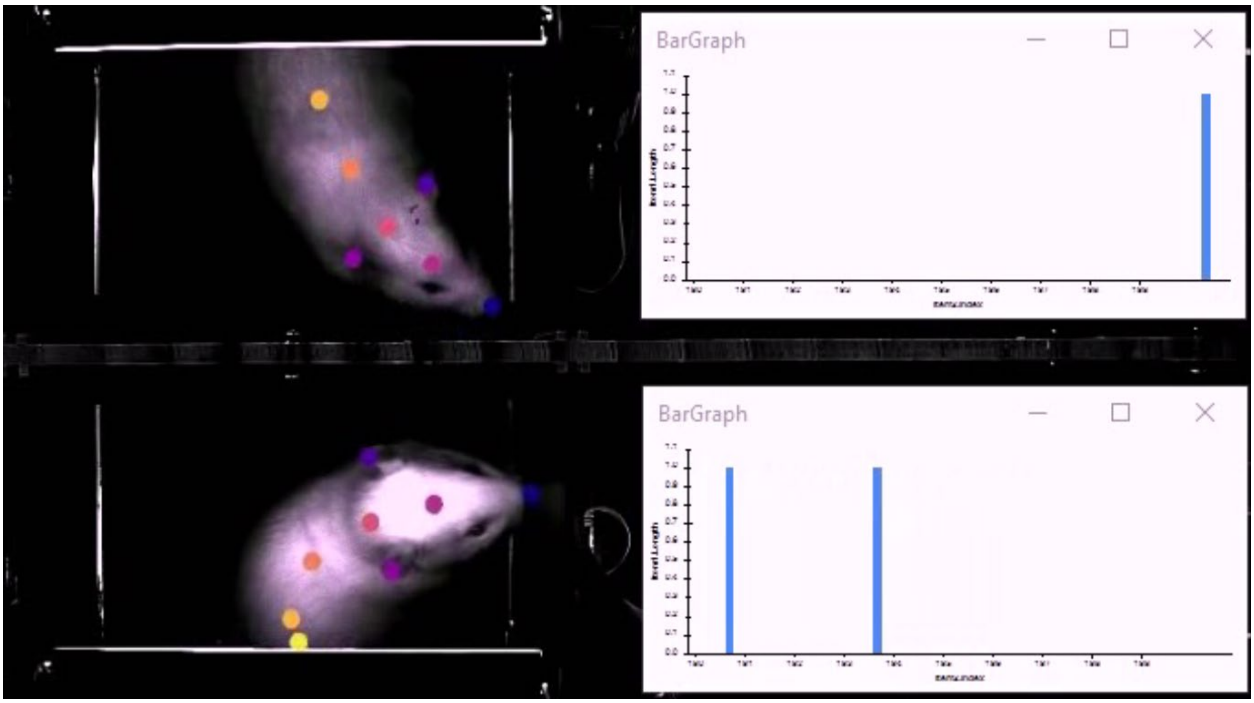
Prosocial choice is a binary variable (1:prosocial choice, 0:selfish choice). Trial is an ordinal variable indicating cumulative trial number over the first two days of the PCT. The dataset includes 1110 observations from 13 pairs of animals. #p<0.1, *p<0.05, **p<0.01, *** p<0.001



Movie S1. Representative trials of the Prosocial Choice Task. Related to Figure 1A-C.

The video shows representative prosocial and selfish trials, from start trial to reward delivery, of a pair of rats performing the Prosocial Choice Task. It can be observed the recipient rat displaying food-seeking behaviour (poking in the nose port on the preferred side), the focal rat making its choices, and how both animals visit the lateral arms where food is delivered.

https://drive.google.com/file/d/1-U1n485Uj79HF-5jN2Oxz0S8hs8dfZdW/view?usp=sharing



Movie S2. Representative trial with pose estimation and call events. Related to Figure 4.

Example video for illustrating tracked animals in the choice area and time of USV emission. Animal body parts are labelled using DeepLabCut. After synchronisation of video and audio data, we built for each rat a binary time-series, where each frame of the video was tagged with 1 if a USV was emitted or with 0 otherwise. We then used Bonsai to visualise the time of USV emission from each animal as train pulses (blue bars), in parallel with the video. To help visualise the video, we assigned different sounds to the USVs emitted by each one of the animals of the pair.

https://drive.google.com/file/d/1I78lFSftlxac-Um9lZUSLbf6VsgoY1cA/view?usp=sharing