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PhD Thesis:

Dissecting the neural circuitry behind social decision-making processes by direct and observational learning

BIO/14

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ABSTRACT

Humans are social animals that rarely live in complete isolation. Thus, human decisions often take the form of social decisions, regarding other social agents and possibly influenced by them. Social decision-making processes can lead, in turn, to relevant social manifestations such as altruistic or selfish behaviors that significantly determine our society. Humans can learn these behaviors by direct experience (ie, direct learning) or through the observation of others (ie, observational learning). However, the neurobiology underlying altruistic or selfish choices, either by direct or observational learning, is still unclear. In recent years, many studies described social decision-making processes and associated behaviors such as altruistic-like behaviors in nonhuman animals, including rodents. Moreover, rodents are able to learn spatial and affective tasks also by observing their similars. Thus, rodents offer the possibility to dissect in detail the neural bases of social decision-making processes either when these are learned by direct or observational learning.

For my PhD project, we developed a social decision-making task (SDM) in which mice can decide whether to keep for themselves (ie, selfish choice) or share a reward with their conspecifics (ie, altruistic choice). We found that adult male mice overall preferred to share food with their similars. However, preference for altruistic choices was modulated by internal and external determinants such as sex, familiarity, motivation, empathy and social dominance. Besides, our inhibitory chemogenetic manipulations demonstrated that the basolateral amygdala (BLA) is involved in the establishment of altruistic decisions. In particular, BLA neurons projecting to the prelimbic (PL) region of the prefrontal cortex mediated the development of a preference for altruistic choices, whereas PL projections to the BLA produced a drift towards selfish acts.

We also revealed that mice were able to acquire the SDM through observation. In particular, observer mice showed an enhanced performance compared to their conspecific demonstrators. At the neural level, we found that the dorsal region of the hippocampus (dCA1) was fundamental for the acquisition of social decision-making processes through others' observation. Indeed, dCA1 silencing, while did not compromise the procedural learning of the SDM task, considerably slowed the establishment of a clear social preference through observation. Then, this PhD project sheds light on the neural circuitry underlying social decision-making processes either by direct or observational learning, with relevance to pathologies such as neuropsychiatric or neurodegenerative disorders often associated with social dysfunction, including social decision-making deficits.

RIASSUNTO

Gli esseri umani sono animali sociali che raramente vivono in completo isolamento. Pertanto, le nostre decisioni spesso prendono la forma di decisioni sociali, che riguardano altri interlocutori e possibilmente vengono influenzate da essi. I processi decisionali possono portare, a loro volta, a manifestazioni sociali rilevanti come i comportamenti altruistici o egoistici, i quali determinano in modo significativo la nostra società. Gli esseri umani possono apprendere questi comportamenti attraverso l'apprendimento diretto o attraverso l'osservazione degli altri, che prende il nome di apprendimento osservativo. Tuttavia, i fondamenti neurobiologici alla base delle scelte altruistiche o egoistiche, sia attraverso l'apprendimento diretto che attraverso l'osservazione, non sono ancora chiari. Negli ultimi anni, molti studi hanno descritto in animali non umani, inclusi i roditori, comportamenti che possono assomigliare all'altruismo. Inoltre, i roditori sono in grado di apprendere task spaziali e affettivi anche osservando i loro simili. Pertanto, i roditori offrono la possibilità di analizzare in dettaglio le basi neurali dei processi decisionali sociali sia quando queste vengono apprese tramite apprendimento diretto sia tramite osservazione.

Per il mio progetto di dottorato, abbiamo sviluppato un task decisionale in ambito sociale (SDM) in cui i topi possono decidere se condividere (scelta altruistica) o no (scelta egoistica) una ricompensa con i loro conspecifici. Abbiamo osservato che i topi maschi adulti, nel complesso, preferivano condividere la ricompensa con i loro conspecifici. Tuttavia, la preferenza per le scelte altruistiche è stata modulata da fattori interni ed esterni come il sesso, la familiarità, la motivazione, l'empatia e la dominanza sociale. Inoltre, le nostre manipolazioni chemogenetiche inibitorie hanno dimostrato che l'amigdala basolaterale (BLA) è coinvolta nella formazione di una preferenza altruistica. In particolare, i neuroni BLA che proiettano alla regione prelimbica (PL) della corteccia prefrontale hanno mediato lo sviluppo di una preferenza per le scelte altruistiche, mentre le proiezioni da PL a BLA hanno determinato decisioni più egoistiche.

Abbiamo anche dimostrato che i topi sono in grado di acquisire il task del SDM attraverso l'osservazione dei loro simili. In particolare, i topi che hanno osservato il task hanno mostrato prestazioni migliori rispetto ai loro compagni senza osservazione. A livello neurale, l'ippocampo dorsale (dCA1) si è dimostrato fondamentale per l'acquisizione dei processi decisionali sociali attraverso l'osservazione degli altri. Infatti, il silenziamento della regione dCA1, nonostante non abbia compromesso l'apprendimento procedurale del nostro task, ha rallentato considerevolmente la creazione di una preferenza sociale attraverso l'osservazione. Quindi, questo progetto di dottorato fa luce sui circuiti neurali alla base dei processi decisionali sociali tramite l'apprendimento diretto o osservazionale, con rilevanza per patologie come i disturbi neuropsichiatrici o neurodegenerativi spesso associati a disfunzioni sociali, inclusi i deficit decisionali sociali.

INTRODUCTION

1. AN INTRODUCTION TO HUMAN SOCIAL DECISION-MAKING

1.1 Social decision-making description, key aspects and potential deficits

Human beings are for their nature social animals that interact with each other on a daily basis and create shared social spaces where every individual can find their identity and personal growth. From an evolutionary perspective, social living and social interactions have significantly favored single individuals in their access to resources and goods otherwise limited or out-of-reach, in this guaranteeing their survival (Alberts, 2019; Maestripieri, 2010; Silk, 2007a,b). Beyond survival reasons, the social encounter with others has also permitted important steps for the human species such as shaping the current societies (Kendal et al., 2018). In this scenario, humans rarely find themselves isolated, rather they are constantly surrounded by their similars that fall within the same social matrix. Therefore, human decisions are often configured as decisions occurring in the social setting or social decisions regarding others (ie, social decision-making), influencing and/or being influenced by them (Lee, 2013; Seo and Lee, 2012; Terenzi et al., 2021). These social decisions affect others as well as the individual itself and for this reason embody the combination of both self- and other-related interests. Choices favoring self- or other-interests can generate different types of social or antisocial behaviors significantly influencing our society.

Social decision-making processes are continuously determined by a variety of either external (ie, the environment) or internal (ie, the individual) factors, or the combination of both, that need to be considered (Seo and Lee, 2012; Suzuki and O'Doherty, 2020; Terenzi et al., 2021) (**Fig. 1**). The external aspects influencing social decision-making can be either social or non-social. The latter mostly refers to context characteristics such as uncertain or risky conditions, ambiguity or scarcity present in the surrounding environment (Doya, 2008; Hansson, 2007; Spohn et al., 2022). The external social aspects instead involve the presence of others with their bodies, emotions, intentions and their actions (Gu et al., 2019; Rilling and Sanfey, 2011; Terenzi et al., 2021). In particular, emotions exert a strong influence on the individual's decisional capacity. For instance, emotions can be stronger in the presence of familiar and in-group members, due to greater empathy (Bartal et al., 2011; Ferretti and Papaleo, 2019; Preston and de Waal, 2002), this producing biased decisions towards them

(Cronin, 2012; Decety et al., 2016; Scheggia et al., 2020). Besides, the individual has to carefully consider the consequences of its decisions for others (Lee and Harris, 2013; Rilling et al., 2008). Furthermore, the others can also serve as a model from which learning something useful for future decisions (ie, observational learning), even though the gains from this learning strategy can depend on additional social attributes such as others' reputation and their trustworthiness (Suzuki and O'Doherty, 2020; Terenzi et al., 2021).

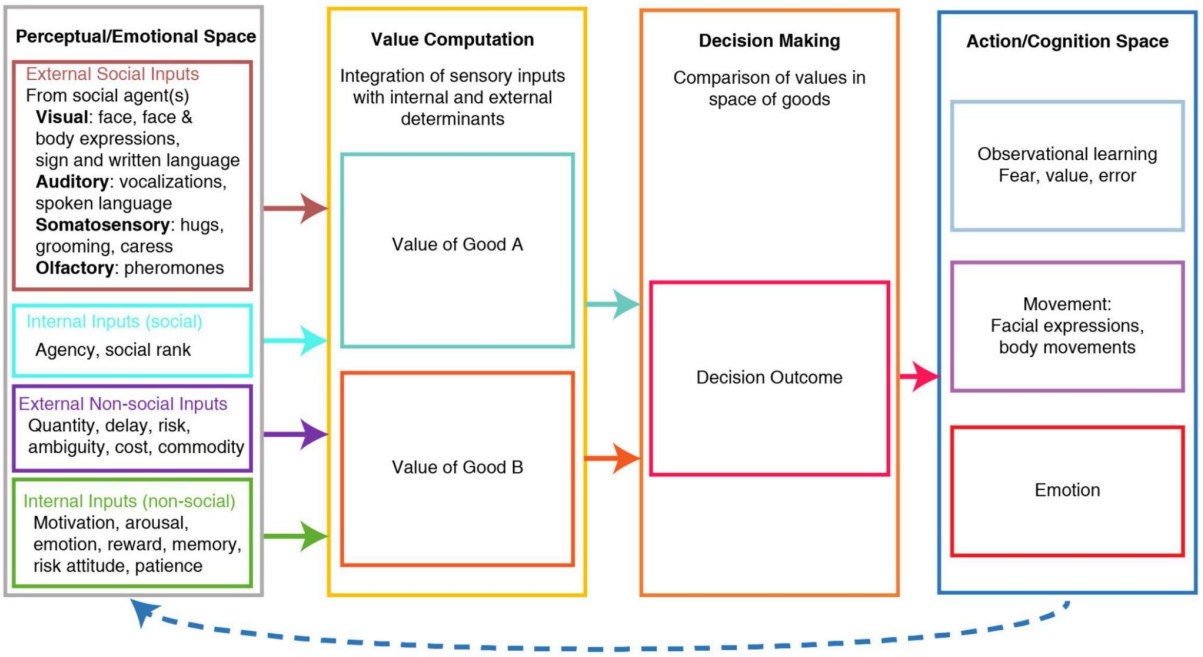


Fig. 1 | Internal and external modulators of social decision-making (Terenzi et al., 2021).

Beyond external factors, there are internal modulators of social decision-making to keep in mind. As for the external, also internal factors can be social or non-social (Terenzi et al., 2021). The internal non-social aspects are mainly resumed in the psycho-physical conditions of the individual agent such as arousal state, motivation, cognitive resources and emotions that can strongly affect its social decisions. For instance, the current emotional state associated with fluctuations in hormonal and neurotransmitter levels can dramatically bias how the individual sees others, interprets their actions and prepares for a decision regarding them (Maltese and Papaleo, 2020). Moreover, the internal social determinants regard how the individual sees itself in society meaning how they are positioned with their social status in society structure, whether they start from a higher or lower social ranking. In fact, the

majority of humans live in extended social structures that are highly organized. In these, the social status of other group members and the social hierarchy that follows represent an important variable in further interactions with them. For instance, the presence of dominant members and the relationship with them can strongly affect an individual's cognitive apparatus and their following course of actions in the social context (Ligneul et al., 2017; Qu et al., 2017).

For all these reasons, social decision-making is configured as an extremely complicated process involving multiple networks and components (Báez-Mendoza et al., 2021; Rilling and Sanfey, 2011; Terenzi et al., 2021). Indeed, the individual continuously needs to perceive and update the information arriving from the external social environment (ie, social perception), attribute a value to this information (ie, social evaluation) and finally elaborate a response in the form of a decision and a further action in the social arena (ie, social response) (**Fig. 2**).

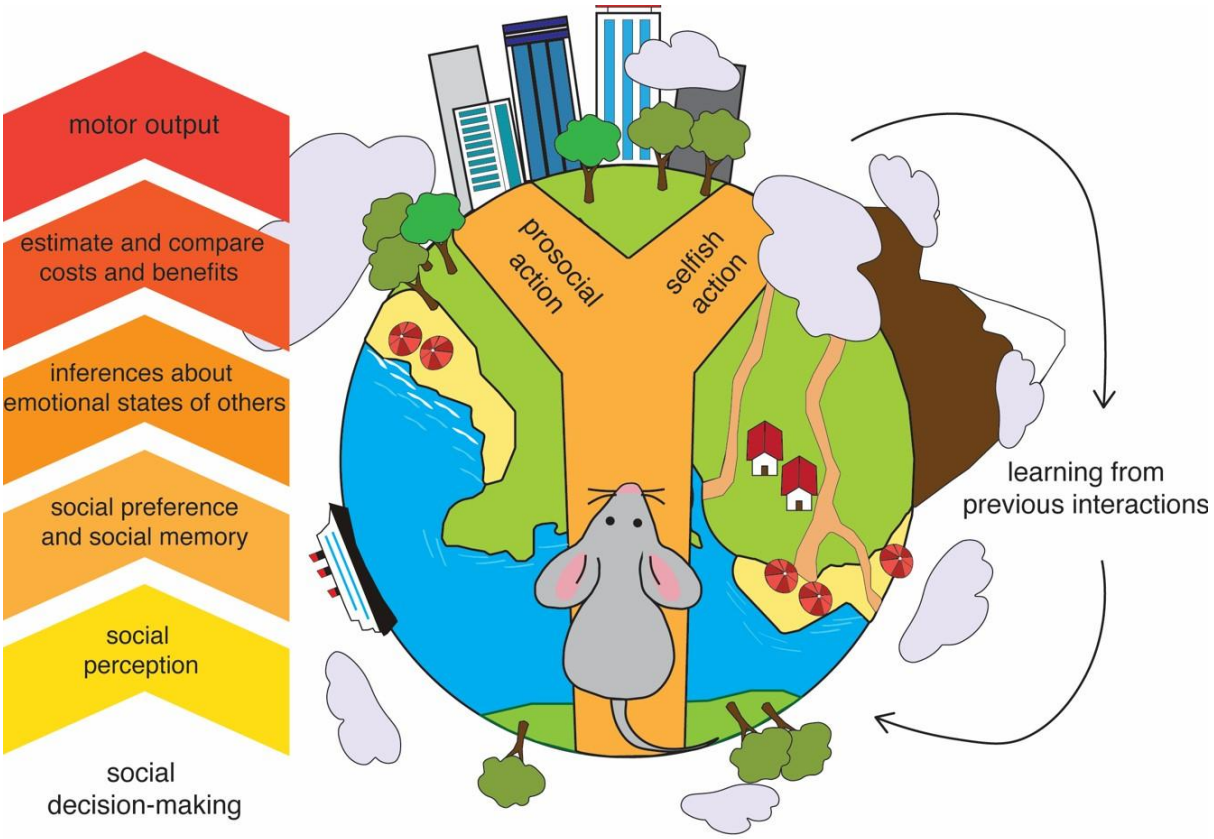


Fig. 2 | The major steps involved in social decision-making (Coccia, La Greca et al., 2022, *under revision*).

In this framework, social decision-making processes require the recruitment and the exact coordination of many cognitive and socio-emotional skills which allow the individual to produce effective and appropriate decisions in the social context. Executive functioning, social memory, emotional discrimination, emotional contagion and mentalization/Theory of Mind (ToM), among others, appear essential abilities for the expression of social decision-making processes (Arioli et al., 2018; Lee and Harris, 2013; Terenzi et al., 2021). Indeed, action planning and strategy evaluation, memory of others' identities and previous interactions with them, others' emotional recognition and sharing (ie, empathy) plus the capability of inferring their intentions (ie, ToM) or even learning from their actions (ie, observational learning) are all considered unavoidable steps for the correct execution of decisions regarding others in the social arena (Arioli et al., 2018; Lee and Harris, 2013; Terenzi et al., 2021). Deficits at the level of one or more of these skills or at the level of one or more networks (ie, social perception, social evaluation and social response) involved in social decision-making processes are commonly observed in psycho-pathological, neuropsychiatric or neurodegenerative conditions.

In 2016, Besnard and colleagues conducted a study on fifteen patients affected by prefrontal lobe damage (Besnard et al., 2017). Through cognitive and socio-cognitive behavioral measures, the authors found important deficits in elements necessary for social decision-making, but not in general decision-making detached from the social context. Specifically, patients with prefrontal lesions displayed difficulties in processing and reusing external information from other social agents (ie, ToM deficits) for solving different social situations presented (Besnard et al., 2017). More recently, Woodcock et al. described twenty autistic adolescents, between eleven and eighteen years old, being impaired in self- and parent-reported measures of executive functioning, ToM and emotion regulation during social decision-making processes (Woodcock et al., 2020). In particular, autistic adolescents exhibited a major difficulty in reading others' intentions, producing more frequent antisocial decisions in a specific social decision-making behavioral paradigm. Moreover, they also showed a marked lack of control on their emotional response towards their partners (Woodcock et al., 2020). Similarly, Yang and collaborators examined social decision-making ability in thirty-five patients affected by schizophrenia in a social decision-making task (Yang et al., 2017). The patients mostly exhibited an anomalous social behavior during the test, the authors partly attributing this to specific ToM deficits affecting the capability of inferring and integrating others' intentions that is necessary for effective social decision-making (Yang et al., 2017).

Dysfunctional social decision-making also extends to neurodegenerative disorders. Indeed, relevant aspects of social decision-making such as emotional recognition, empathy and ToM abilities are often described as fragmented in a variety of neurodegenerative diseases including frontotemporal dementia (FTD) and Huntington's disease (HD) (Christidi et al., 2018; Manuel et al., 2020; Mason et al., 2021). The study from Eddy and Rickards has closely analyzed social decision-making ability in HD (Eddy and Rickards, 2012). In fact, the authors tested sixteen patients with HD in a classic social decision-making behavioral paradigm. Eddy and Rickards reported inappropriate social decisions mainly due to emotion dysregulation, that is a feature very common in HD. Other interesting studies are those from Eslinger et al. and Mendeza with Shapira dealing with twelve and twenty-one patients affected from FTD, respectively, and presenting them with different types of social-moral dilemmas in conjunction with assessments of empathy and ToM abilities (Eslinger et al., 2007; Mendeza and Shapira, 2009). Both studies recognize impairments in the decisional capacity of FTD patients when dealing with social-moral dilemmas, especially when emotional or mentalization aspects are required for expressing their decisions. In conclusion, these studies highlight how the disruption of one or more skills within the multiple networks (ie, social perception, social evaluation and social response) sustaining social decision-making processes can dramatically affect how social decisions and behaviors are expressed in our society.

1.2 Social decision-making learning strategies

In the social context, humans can learn how to express their decisions through different learning strategies representing alternative decision-making systems. Indeed, social decisions can be reached through personal experience via a trial-and-error strategy (ie, direct learning), but also through the observation of other agents performing in the social environment (ie, observational learning) (Fryling et al., 2011; Seymour, 2009; Yoon et al., 2021) (**Fig. 3**).

Social decision-making by direct learning mainly indicates the process of acquiring information through first-hand experience (Seymour, 2009). Here, the individual is called in first person to act, update its performance and reach the best possible solution in terms of available payoffs (Zonca et al., 2021). While embracing the direct learning system, the individual carries out many attempts, continuous errors and adjustments are made before the prefixed goal is achieved. After this series of trials-and-errors, the individual finally learns, the successful response can be reinforced and become a habit (Seymour, 2009). In this

framework, prediction error and interactive learning become fundamental skills for learning how to make a decision in the social environment (Seo and Lee, 2012). Indeed, deviations from predicted outcomes significantly modify the way the individual will select the next moves. Moreover, the information incoming from external physical stimuli such as the other social agents or other objects in the environment can be used by the decision maker to update its current decisional scheme (Meltzoff et al., 2009; Zonca et al., 2021). Besides, information incoming from other social agents can also serve as a model for the decision-maker to learn new skills or behaviors without the need of acquiring these first-hand (Zonca et al., 2021).

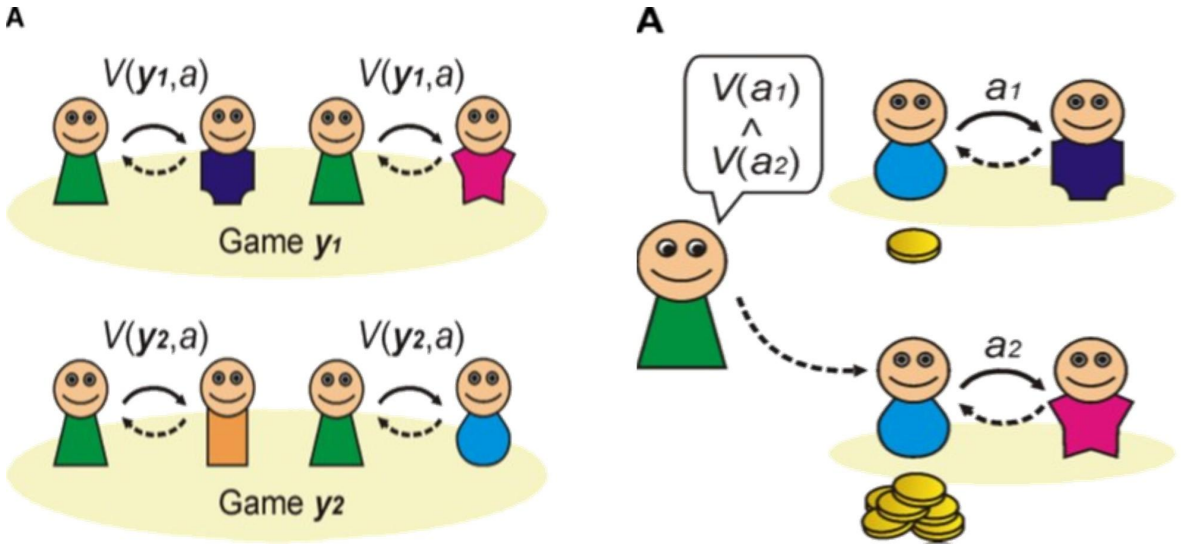


Fig. 3 | Direct and observational learning strategies in a social decision-making experimental task (adapted from Seymour, 2009).

The ability of observing others and learning from them is called "observational learning" and it is very widespread among humans especially due to their innate social nature (Bandura and Walters, 1977; Meltzoff et al., 2009; Leblanc and Ramirez, 2020). Indeed, humans can learn from others their movements, emotions and even complex social behaviors such as social decision-making processes (Yoon et al., 2021). Observational learning is a very robust type of learning that can overcome mere imitation providing the basis for the flexible replication of others' behavior (Zonca et al., 2021). This form of learning is part of the Bandura's social learning theory that conceives humans as social animals principally learning from the social context they live in (Bandura and Walters, 1977). In particular, Bandura and colleagues showed through the famous "Bobo doll" experiment how children often observe

and learn from people around them, even in the case these display negative behaviors such as aggressive ones (Artino, 2007; Bandura et al., 1961). Then, observed individuals represent an influential model to replicate and either the personal characteristics such as the sex or the age of the model or the current observer's psycho-physical situation like their attentional or motivational state strongly influence the future outcome.

The two decisional learning strategies mentioned above do not necessarily overlap in all situations. An individual can express a decisional process without the need of a previous demonstration from the other social agents or the social information (Reader and Leris, 2014). Indeed, the individual can already succeed through the use of his personal experience or the individual information to unveil all the uncertain aspects linked to a particular social situation (Reader and Leris, 2014). However, the two learning strategies are also not directly in opposition, meaning they are not immediately autoexclusive because of their definition. In fact, both can integrate or support each other at different levels of the social decision-making process (Fryling et al., 2011; Reader and Leris, 2014; Seymour et al., 2009). For instance, learning by experience can lead to the acquisition of a specific behavior at first and then the subsequent observation of others can inform the single individual on how to further ameliorate this. Viceversa, learning by observation can inform and give initial cues about a certain behavior that then the individual can refine and better adapt to its needs through the trial-and-error learning strategy (Yoon et al., 2021; Zonca et al., 2021). Finally, both the decision-making learning systems (ie, by direct or observational learning) originate relevant social behaviors that are often viewed as the main driving force behind social interactions shaping our society.

1.3 Behavioral economic games and social decision-making processes

In the social sphere, humans often choose to help others, reciprocate previously received help from them or cooperate with them for reaching a common goal. Social decisions like this can lead to relevant social manifestations known as prosocial behaviors that are meant for others, benefitting them and increasing their life wellbeing (Batson and Powell, 2003; Marsh et al., 2021; Trivers, 1971). Prosocial behaviors are not the only expression of social decision-making processes, in fact social decisions can also produce egoistic or antisocial behaviors (Marsh et al., 2021; Terenzi et al., 2021). However, prosocial behaviors are undoubtedly one of the most extraordinary and commonly reported phenomena when it comes to social decision-making processes (Marsh et al., 2021). Despite their apparent

irrationality in terms of evolutionary fitness and economic predictions (Rilling and Sanfey, 2011; Sanfey, 2007), many scientists consider prosocial behaviors at the foundations of human society because these bring together many different individuals, continuously creating a connection between them and fuelling social interactions (Cronin, 2012; Marsh, 2016; Silk and House, 2016). Prosocial behaviors based on social decision-making processes can take many facets such as reciprocation, cooperation and other-regarding preference, the latter often being configured as helping or altruistic behavior (Cronin, 2012; Marsh et al., 2021). Prosocial behaviors can directly be measured through tasks readapted from the economic field (Lee, 2008; Sanfey, 2007; Van Dijk and De Dreu; 2021). Presenting the task as a behavioral economic game resembling real life situations, game theory offers the possibility to study in detail decisional processes and associated behaviors occurring in the social context.

Reciprocation refers to the decision and the act of returning a favor or a payback based on the outcome of past interactions (Trivers, 1971). This prosocial behavior can be driven by expectations of getting future help from the reciprocated one/s. Reciprocation is very ancient, having strong evolutionary roots behind (Trivers, 1971). Indeed, reciprocation offers a huge evolutionary advantage to the agent in the self-return, the future payoff they can get with their action (Trivers, 1971). Individuals can reciprocate either in negative or positive terms but the emphasis of most literature is on the latter (Dolivo and Rutte, 2016). In fact, positive reciprocation might open the possibility for prolonged and more stable relationships such as reciprocal cooperation. Cooperation is a peculiar prosocial behavior that sees individuals interacting with each other for achieving common goals (Dugatkin, 1997). Cooperation is often studied in the specific form of reciprocal cooperation. Indeed, the fact individuals can reciprocate opens to the possibility for their mutual cooperation in the long-term. Moreover, cooperation involves advanced cognitive and social skills such as memory of previous interactions and continuous adaptation to the strategies of other social agents (Lopuch and Popik, 2011).

Reciprocation and cooperation have often been studied through the use of the prisoner's dilemma, the trust and the ultimatum game (Lee, 2008; Terenzi et al., 2021; Van Dijk and De Dreu; 2021) (**Fig. 4**). In these behavioral paradigms, players often are asked to choose whether to mutually cooperate with their partners (ie, prisoner's dilemma) or equally divide a certain amount of money with them (ie, the ultimatum game) or trust them for a common investment (ie, the trust game) (Lee, 2008; Sanfey 2007). In many cases, the

prosocial decision to cooperate, share and trust their partner can result extremely disadvantageous compared to other possibilities more rewarding, hence more rational, for the single individual and its own interests (Sanfey 2007). Despite that, many studies report the significant occurrence of reciprocating and cooperative behaviors towards others both in the experimental setting and in society (Rilling and Sanfey, 2011; Silk and House, 2011, 2016).

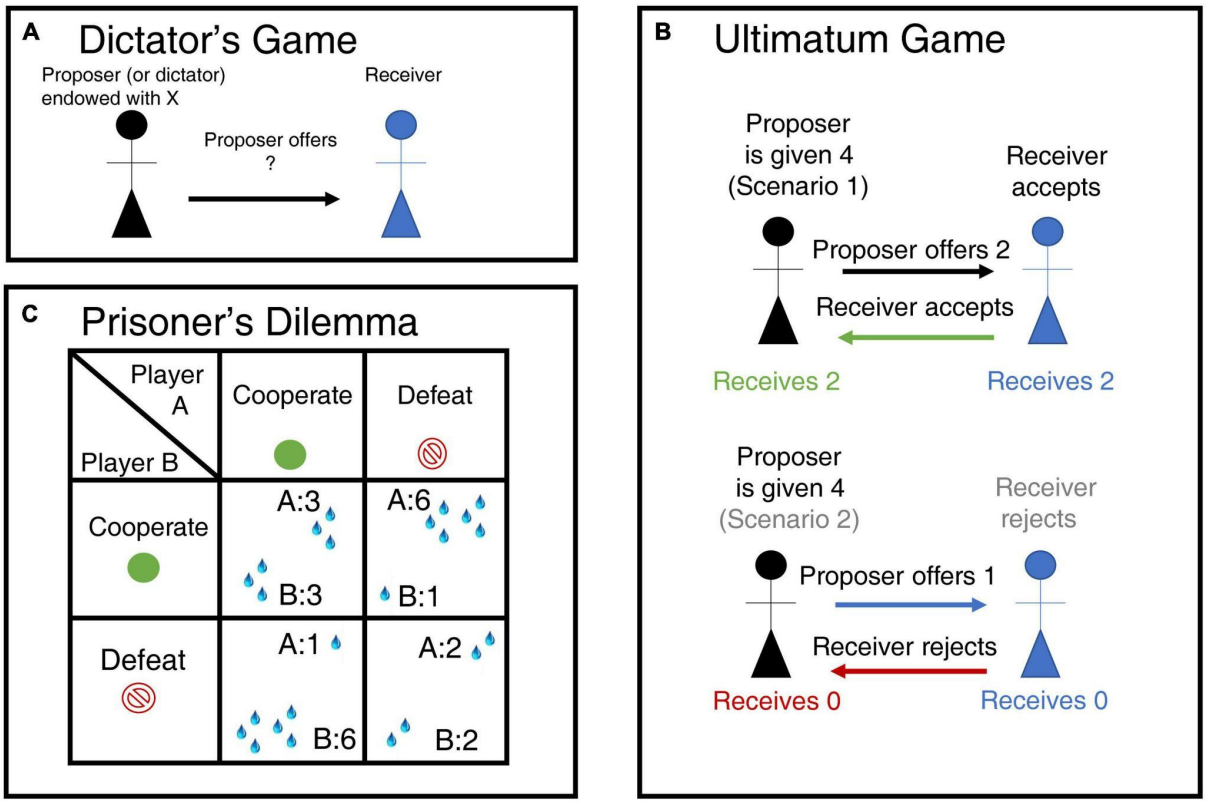


Fig. 4 | Behavioral economic games and social decision-making processes (Terenzi et al., 2021).

Other-regarding preference refers to a category of prosocial behaviors where the concern for others and their wellbeing is at the first place. Other-regarding preference represents a genuine and immediate preference for other individuals that can be in need, pain or distress (Bartal et al., 2011; de Waal, 2008; Dal Monte et al., 2020). Helping behavior and particularly altruism are often direct emanations of this genuine preference for others. Differently from helping behavior and other prosocial behaviors that could still hide underlying egoistic/selfish motives, altruism represents the pure sacrifice of one's own resources for the primary benefit of others without necessarily a self-return (Ben-Ner and Kramer, 2011; Marsh, 2016). In the real world, the resources devoted to others can include

personal time, material goods such as money or food (eg, charity donations) or even extreme body donations (eg, organ donations) (Brethel-Haurwitz et al., 2018). From an evolutionary perspective, altruism likely evolved to promote survival through actions associated with kin selection, reciprocity and parental care (Ben-Ner and Kramer, 2011; Hamilton, 1964; Trivers, 1971). However, many say empathy (ie, reading and sharing others' emotions) probably has guaranteed the successful transmission of the behavior till modern society. Indeed, empathy is often the most immediate and proximate cause behind altruistic acts and this might have promoted the perpetuation of the altruistic behavior through evolutionary history (Bartal et al., 2011; Decety et al., 2016; de Waal, 2008; de Waal and Preston, 2017) (**Fig. 5**). Besides, empathy-driven altruistic behavior can even be stronger in the presence of familiar, in-group or reciprocating members, even though altruistic acts also extend towards unrelated members or complete strangers (Brethel-Haurwitz et al., 2018; de Waal, 2008).

Through psychological and self-reported measures, altruism can be measured by asking individuals how they would act in extremely urgent social situations such as when others are in need or in danger (Ben-Ner and Kramer, 2011). In particular, altruism has often been analyzed using the dictator game where subjects need to choose whether to keep for themselves or share with others a certain amount of money (Ben-Ner and Kramer, 2011; Sanfey, 2007). The shared amount is taken as a direct manifestation of the dictator's altruistic behavior. In contrast with predictions from game theory, many experiments in the lab and observations from society show individuals often prefer sharing money or other kinds of goods with their similars in need (Ben-Ner and Kramer, 2011; Marsh et al., 2021). In this scenario, other motivations beyond rational ones might come to play a part in eliciting the altruistic behavior such as others' identity (eg, familiar or in-group members) or their emotional state (eg, others' pain or distress) (Cronin, 2012; Keysers et al., 2022; Marsh, 2016).

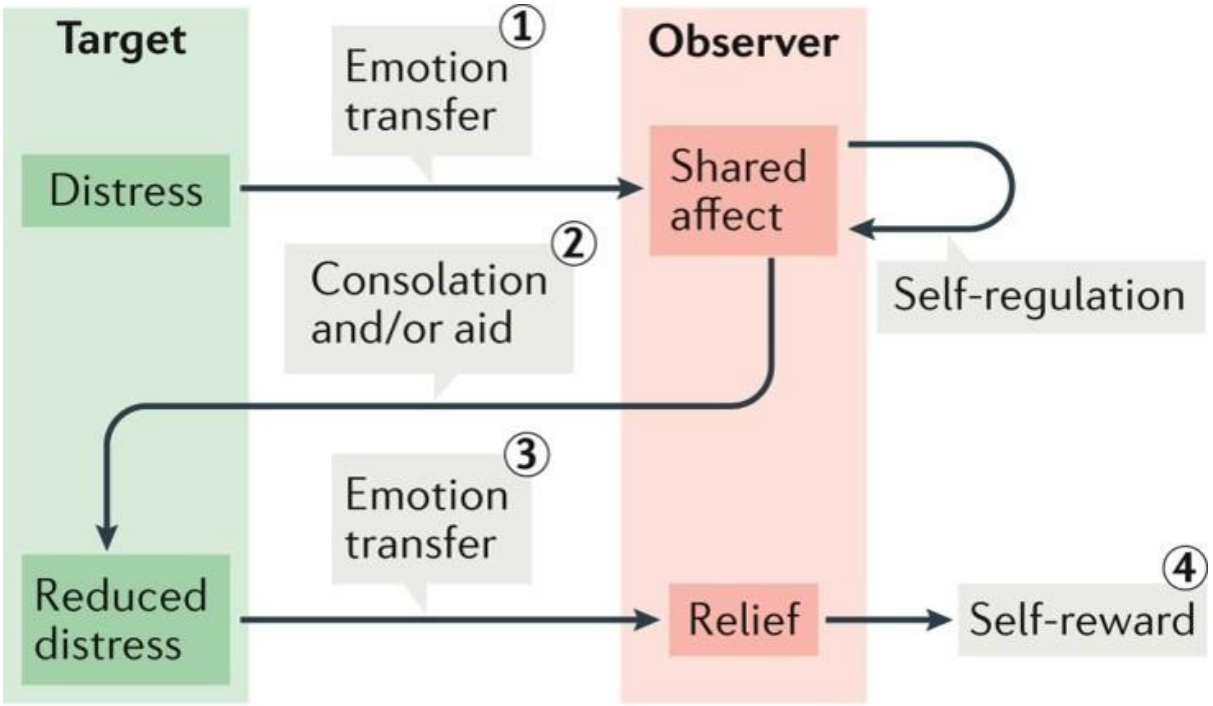


Fig. 5 | How empathy might guide altruistic behavior towards others (de Waal and Preston, 2017).

Finally, prosocial behaviors often involve multiple brain areas and higher-order cognitive functions in humans (Arioli et al., 2018; Marsh, 2016; Rilling and Sanfey, 2011). Due to cognitive skills like mentalization and social tools such as language and culture, many have considered for long adult humans the only ones capable of prosocial behaviors (Silk and House, 2011, 2016). However, a growing amount of studies has identified examples of prosociality, including altruism, in living forms such as human infants (Lucca et al., 2018; Schmidt and Sommerville, 2011). Thus, adult human prosociality is perhaps an extended version of something already existing in "simpler" social entities such as human infants and other nonhuman species.

2. SOCIAL DECISION-MAKING BEYOND HUMANS

2.1 Social decision-making in nonhuman animals

Despite the high cognitive and socio-emotional demands, increasing evidence from the elds of behavioral ecology, comparative psychology and biology suggests that nonhuman animals are capable of sophisticated prosocial behaviors, based on elaborate social decision-making processes, such as cooperation, helping behavior and altruism. Bonobos share food with groupmates and non (Tan and Hare, 2013; Tan et al., 2017) although this might be cognitively demanding (Krupenye et al., 2018). Vampire bats take care of starving members of their group (Wilkinson, 1988; Carter and Wilkinson, 2013) also translating these social relationships into the wild (Ripperger et al., 2019). Humpback whales risk their life for helping other species such as seals or sea lions against predators (Pitman et al., 2017). African gray parrots cooperate and reciprocate each other indicating prosociality might even surpass the mammal reign (Brucks and von Bayern, 2020). Although probably not as extended as the human version and with significant intra- and inter-species differences, it is hard to deny that prosocial behaviors also exist in the nonhuman animal world.

Among the other nonhuman animals, rodents, particularly mice and rats, represent a great candidate for studying prosociality. Indeed, rodents are a highly social species that often prefer group living and feeding together in the wild (Kondrakiewicz et al., 2019; Schweinfurth, 2020). In the laboratory setting, these groups are often characterized by a variety of social behaviors such as aggression, mating, dominance, parental care and other approach-avoidance strategies (Kondrakiewicz et al., 2019; Schweinfurth, 2020; Schweinfurth and Taborsky, 2016). Moreover, specific studies focusing on social dominance reveal the organization of rodent groups is rather complex and they can form linear and stable hierarchies over time (Fan et al., 2019; Varholick et al., 2019). Besides, rodents can successfully recognize and remember conspecifics they have met in the past (Okuyama, et al., 2016; Okuyama, 2018).

Increasing evidence also demonstrates rodents are able of emotional discrimination (Ferretti and Papaleo, 2019; Ferretti et al., 2019; Scheggia et al., 2020) (**Fig. 6, top**) and emotional contagion (Allsop et al., 2018; Jeon et al., 2010; Scheggia and Papaleo, 2020) (**Fig. 6, bottom**). There are studies showing rodents can consciously recognize others' affective

state/s such as fear, stress or relief (Burkett et al., 2016; Ferretti et al., 2019; Scheggia et al., 2020). Emotion discrimination appears fundamental for emotional contagion ability. Most of the evidence on emotional contagion is obtained by using the observational fear conditioning paradigm where individuals learn the emotions of fear and sufferance through others (Allsop et al., 2018; Jeon et al., 2010; Keum and Shin, 2019). Taken together, all these pieces of evidence might reveal the presence of empathy (Keum and Shin, 2019; Scheggia and Papaleo, 2020) that, in turn, might drive rodents to express more sophisticated forms of social behaviors towards others such as prosocial behaviors (Bartal et al., 2011; Keysers et al., 2020; Kim et al., 2021).

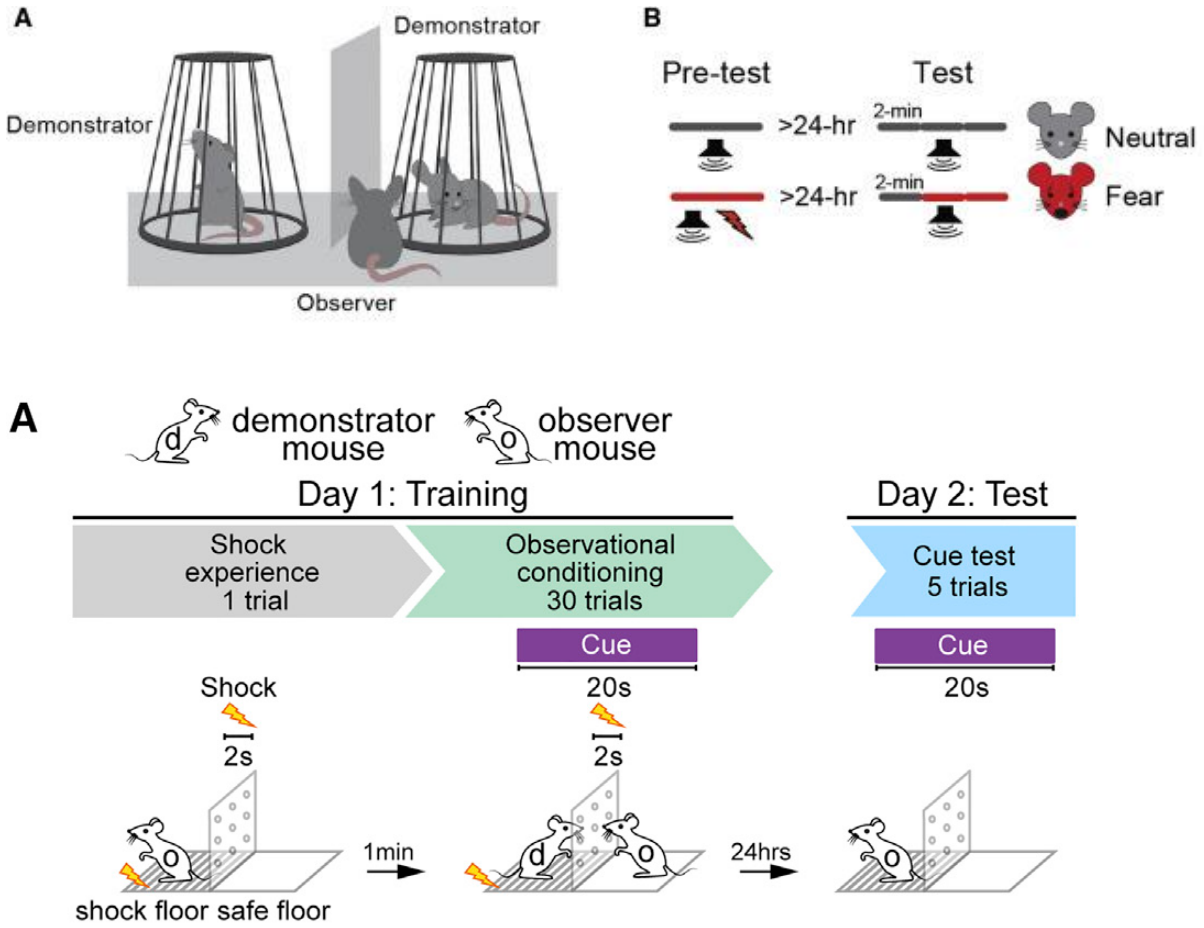


Fig. 6 | Behavioral paradigms for assessing emotional discrimination (top) and emotional contagion (bottom) in rodents (adapted from Ferretti et al., 2019 (top), Allsop et al., 2018 (bottom)).

2.2 Reciprocation and cooperation behaviors in rodents

A demonstration of reciprocity in rodents comes from the studies of Rutte and Taborsky (Rutte and Taborsky, 2007, 2008). They used an adapted version of the prosocial choice task, that is greatly used in human and nonhuman primate research (de Waal, 2008), where rats after a previous interaction can choose to pull a stick for delivering food to a conspecific. The past interaction can be either cooperative when the focal rat received food or defective when the focal rat did not. Rutte and Taborsky demonstrated the existence of reciprocation behavior in female rats highlighting the importance of prior social experience such as prior cooperation or defection episodes (Rutte and Taborsky, 2007, 2008). Female rats can remember specific information regarding a previous partner and then precisely reciprocate based on the outcome of their previous interaction (Rutte and Taborsky, 2007, 2008). Besides, rodent reciprocity can be possible even in the presence of multiple social partners as a recent study by Kettler and colleagues showed (Kettler et al., 2021). In this specific case, rats were able to remember each individual among others who have previously cooperated or not with them, also reciprocating the exact quantity of food previously received (Kettler et al., 2021).

Beyond the partner's identity and the quantity of the help received from them, other factors influence the actor's choice to reciprocate. By manipulating the attractiveness of the reward (ie, carrots vs bananas), Dolivo and Taborsky demonstrated how unrelated female rats not only reciprocate according to the quantity but also based on the quality of the previous help received (Dolivo and Taborsky, 2015). Moreover, Schneeberger and colleagues highlighted that rodent prosociality also depends on the actor's motivation and the partner's physical condition (Schneeberger et al., 2020). By increasing the resistance of the pulling stick and playing with the partner's physical status, the authors showed how unrelated female rats are more willing to reciprocate hungrier partners that were cooperative in the past, even when this was associated with greater effort (Schneeberger et al., 2020). Then, the condition of need or distress (ie, the "smell of hunger") expressed by their partners might have guided the prosocial actions of female rats (Schneeberger et al., 2020). In a very fascinating experiment, Gerber and colleagues also identified the "smell of cooperation", in addition to the smell of hunger, as a leading factor in rats' reciprocation (Gerber et al., 2020). By performing the task of Rutte and Taborsky (Rutte and Taborsky, 2007) in two separate rooms, the authors provided to one room the odor of two rats reciprocating in an adjacent room (ie,

the “smell of cooperation”) showing how female rats reciprocated more consistently in this condition even without a history of positive outcomes behind (Gerber et al., 2020).

By using an adapted version of the human prisoner’s dilemma in a double T-maze, Viana and colleagues also showed rats can successfully understand the structure of positive and negative outcomes associated with their choices (ie, the payoff matrix) mostly preferring mutual cooperation above defection on the long-run (Viana et al., 2010). In particular, they used sated rats with food and water ad libitum avoiding a primordial confounder such as food competition (Viana et al., 2010). Despite this result, Delmas and colleagues reported successful cooperation is possible even when rats are mildly food-restricted (Delmas et al., 2018). However, other confounders might have altered the results by Viana and colleagues like the fact that in their maze individuals had all the time to copy the others’ movements. That is why other studies have preferred the use of operant chambers on mazes that allow a tighter control of rats' intentions by introducing time constraints on these. In particular, Wood and colleagues showed that rats are able to cooperate in the prisoner’s dilemma even when the time for this prosocial decision is constrained (Wood et al., 2016). The studies using operant chambers also revealed other factors play a role in rodent prosociality such as sex differences (Wood et al., 2016) and the other’s social perception (Lopuk and Popik, 2011). For instance, Lopuch and Popik reported visual and auditory stimuli altered rodent cooperative behavior in their instrumental task (Lopuk and Popik, 2011).

Despite all the positive evidence presented above, the tasks using mazes or operant chambers remain non fully automated and often characterized by the use of conditioned stimuli such as noise and lights that might impact the actor’s choice. Thus, more recent experiments tried to implement social cooperation tasks through the use of advanced technology (Avital et al., 2016; Shin and Ko, 2021). Due to their design, these experiments allow for more careful evaluation of previously mentioned factors such as sex and perceptual information. Avital et al. conceived an apparatus where rats had to move in synchrony to get a common reward (Avital et al., 2016). A video tracking system joined with a machine algorithm kept track of the movements and the exchange between the actor rats while two automated peristaltic pumps delivered the reward (ie, water with sucrose). Through this advanced setting, Avital and collaborators showed, once more, how rats can successfully cooperate (Avital et al., 2016). Furthermore, Avital and collaborators not only confirmed how different levels of sensory modalities (ie, visual and auditory stimuli) modulate rodent prosociality but also there can be nonsocial cognitive aspects such as attentional resources

influencing this (Avital et al., 2016). Finally, Shin and Ko implemented the behavioral paradigm by Avital and colleagues (Avital et al., 2016) and also validated this in mice (Shin and Ko, 2021). This result is in agreement with another recent study that sees mice using cooperation even for solving conflictual situations (Choe et al., 2017). Then, the authors (Shin and Ko, 2021) suggested how this experimental paradigm might be extremely useful for investigating specific prosocial behaviors deficits like impaired social cooperation even in animal models of neuropsychiatric disorders such as autism (Han et al., 2020).

2.3 Helping and altruistic-like behaviors in rodents

As shown in the previous paragraph, nonhuman animals such as rodents engage in complex social decision-making processes such as reciprocation and cooperation that can eventually benefit their conspecifics. However, these prosocial behaviors can often be biased by kin and reciprocity motives or, in general, by selfish intentions (de Waal, 2008; Scheggia and Papaleo, 2020). Besides this, rodents appear also able of prosocial behaviors such as helping or altruism-like behaviors that are often driven by a genuine preference for others (ie, other-regarding preference) (**Fig. 7**), that should be without expectations and mostly linked to empathy (de Waal, 2008; Decety et al., 2016; Keysers et al., 2022). These prosocial behaviors do not necessarily rely on kin or reciprocity motives and can be even directed towards unfamiliar, out-group or non-reciprocating individuals (Bartal et al., 2014; Marsh, 2016). Many literature studies have already reported that rodents display either consolatory (Burkett et al., 2016) or empathy-like (Allsop et al., 2018; Jeon et al., 2010; Scheggia and Papaleo, 2020) behaviors towards their similars. Furthermore, rodents, either mice or rats, are also capable of more refined prosocial acts such as helping conspecifics that are trapped (Bartal et al., 2011; Ueno et al., 2019a), have been harmed (Greene, 1969; Hernandez-Lallement et al., 2020) or are seeking food (Hernandez-Lallement et al., 2015; Marquez et al., 2015).

The most famous experiments on helping behavior come from Bartal and colleagues. In their experiments, rats are willing to free trapped cage mates even when there is no immediate self-benefit for the helper such as a following social interaction (Bartal et al., 2011). Helping behavior is also carried out when there are other more attractive options such as chocolate chips next to the restrained partner (Bartal et al., 2011). This study has also been replicated in mice from Ueno and colleagues (Ueno et al., 2019a). However, other studies

suggests caution regarding rodents' helping behavior because this might be driven by additional motives not directly related to other-regarding preference such as social interest or attentional biases (Heslin and Brown, 2021; Ueno et al., 2019b). Rats' helping behavior has also been addressed as an expression of altruism in an old experiment by Greene (Greene, 1969). In this experiment, observer rats voluntarily stop their conspecifics' pain, due to an electrical shock, by pressing a lever (Greene, 1969). This is maintained even when becoming more costly for the observer in terms of physical demands such as pressing the lever twice for preventing the shock delivery to the partner (Greene, 1969). The experiment by Greene has also been recently replicated and implemented by Hernandez-Lallement and collaborators (Hernandez-Lallement et al., 2020). Despite significant individual differences, the researchers showed once more rats prefer avoiding harm to conspecifics and, subsequently, sharing food with them (Hernandez-Lallement et al., 2020). Nonetheless, Hernandez-Lallement et al. also reported an alternative explanation for rats' harm aversion that might be caused by selfish motivations like not to suffer the unpleasant feeling of witnessing the other's pain (Hernandez-Lallement et al., 2020).

In two recent works, a modified and automated version of the prosocial choice task was used for assessing altruistic-like behavior in rats, avoiding possible confounders such as food competition (Hernandez-Lallement et al., 2015; Marquez et al., 2015). In their experiments, the authors showed rats more frequently prefer sharing food with others, hence improving their current condition (Hernandez-Lallement et al., 2015; Marquez et al., 2015). Moreover, rats intentionally avoided food sharing when a toy instead of a real partner was placed (Hernandez-Lallement et al., 2015). Rats' prosocial choice also takes into account the needs of the receiver, indeed the amount of sharing increases in the presence of animals displaying an increased food-seeking behavior (Marquez et al., 2015). However, these works, due to the setting design, seem to measure cooperative behavior rather than altruistic-like behavior. Furthermore, other relevant conditions such as testing the prosocial behavior under increased effort or without the presence of a concurrent reward (ie, without self-benefit) were missing. Along with this, an analysis of the factors characterizing social decision-making processes, such as social dominance and empathy, was not conducted. A recent study, using the same task design, has started to fill this gap giving a more comprehensive depiction of prosocial or altruistic-like behaviors that can be found in rodents (Gachomba et al., 2022).

In conclusion, a study directly and precisely addressing altruistic behavior and its involved components such as social dominance and empathy in rodents, especially in mice, is

still missing. Indeed, altruism is typically characterized by a direct personal cost to the altruistic agent with no conceivable long-term benefit (de Waal, 2008; Marsh, 2016). This capacity might differ from that to help or to prevent pain in others captured by current animal paradigms (Bartal et al., 2011; Hernandez-Lallement et al., 2015), hence the need for further exploration of altruistic-like behaviors in rodents that could contribute to a better understanding of the neurobiology underlying social decision-making processes.

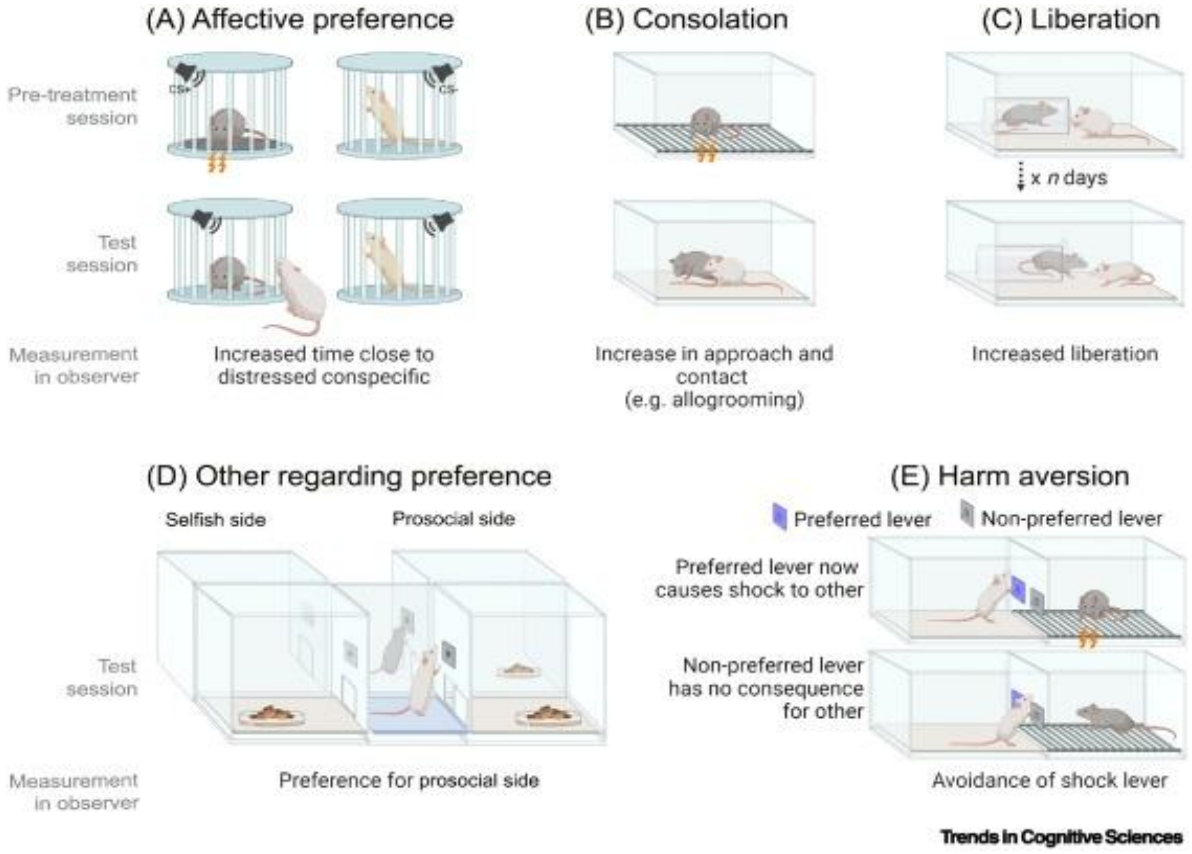


Fig. 7 | Examples of prosocial behaviors found in rodents (Keysers et al., 2022).

3. THE NEURAL CIRCUITRY BEHIND SOCIAL DECISION-MAKING

3.1 The neural circuitry underlying human social decision-making

Many areas, either cortical or subcortical, participate to social decision-making and probably are included in an extended circuitry known as the social decision-making network (Báez-Mendoza et al., 2021; Gangopadhyay et al., 2021; Rilling and Sanfey, 2011; Rilling et al., 2008; Tremblay et al., 2017). The areas within the network maintain their specialization yet their crosstalk guarantees the coordinated execution of fundamental nodes of the social decision-making process such as social perception, social evaluation and social response that in turn require many cognitive and socio-emotional skills (**Fig. 8**).

Studies have shown the importance of the prefrontal cortex (PFC), mainly the orbitofrontal (OFC), the medial (mPFC) and the ventromedial (vmPFC) areas, for perceiving and processing others' facial expressions and social interactions that can represent critical information for the decision-maker in the social context (Báez-Mendoza et al., 2021; Tremblay et al., 2017). The involvement of the PFC and the anterior cingulate cortex (ACC) appears essential when monitoring and interpreting others' expressions, intentions and movements (Báez-Mendoza et al., 2021). In particular, the integration and the further evaluation of others' actions would be possible thanks to the recruitment of the vmPFC together with the striatum (Báez-Mendoza et al., 2021; Rilling and Sanfey, 2011). Additionally, the mPFC has been implicated in the detection of prediction errors and mismatch especially in the situations where the outside environment presents uncertain and conflictual information (Báez-Mendoza et al., 2021; Terenzi et al., 2021). Other areas such as the amygdala and the insula have also been found to characterize decision making in the social context (Rilling and Sanfey, 2011; Tremblay et al., 2017). Moreover, the amygdala together with other cortical areas such as the mPFC also convey relevant information about self and others' position in the social hierarchy, another important determinant of the social decision-making process (Báez-Mendoza et al., 2021; Ligneul et al., 2017).

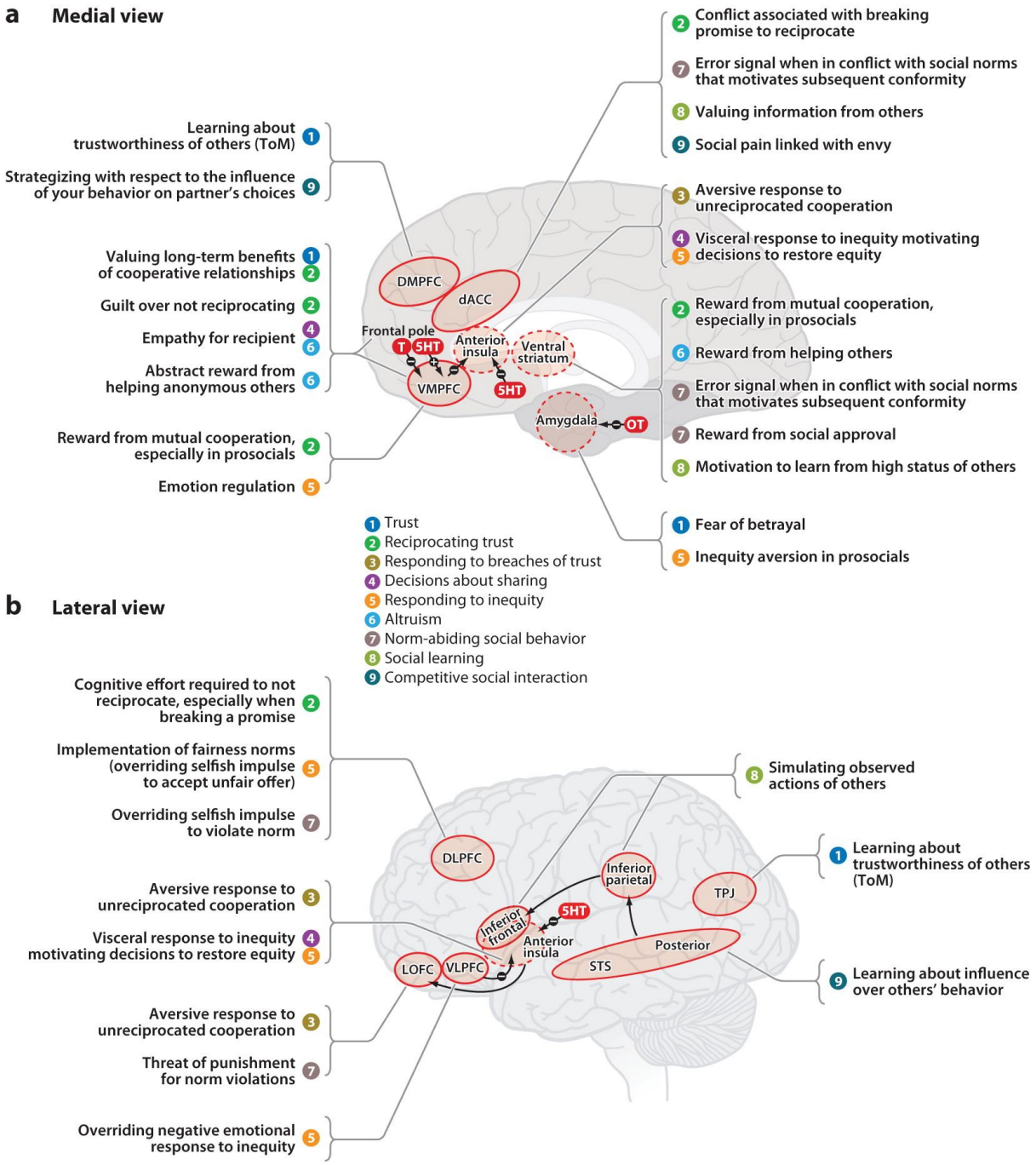


Fig. 8 | Neural areas involved in social decision-making (adapted from Rilling and Sanfey, 2011).

The areas mentioned above are also involved in situations when social decisions directly generate prosocial behaviors in the laboratory setting or in society. For instance, striatal and PFC activity are found to be fundamental when people donate to charity (Marsh, 2016; Marsh et al., 2021). Decisions to donate mainly recruit the ventral striatum whereas decisions to oppose require the PFC intervention (Behrens et al., 2009; Seo and Lee, 2012).

Hackel et al. showed greater activation in the ventral striatum when participants had to share a reward with others during an fMRI session, the neural response being particularly evident in the case of closer individuals such as in-group members (Hackel et al., 2017). More recently, Saulin and colleagues also recognized the need for dorsal-striatal activation in a prosocial choice task guided by multiple motives such as empathy and reciprocity (Saulin et al., 2022). Furthermore, PFC activation is particularly evident when unfair offers are presented during the ultimatum game (Rilling and Sanfey, 2011). Indeed, disrupting PFC activity can mean participants' higher acceptance of unfair offers (Rilling and Sanfey, 2011; Seo and Lee, 2012). Besides, activity in these areas often correlates with that of others implicated in empathy such as the ACC and the amygdala, that are responsible for modulating social preference and relationships with other similars (Báez-Mendoza et al., 2021; Dal Monte et al., 2020). During the trust game, the functioning of the amygdala and the insula can strongly affect how players' faces and emotions are processed, with a serious bias on further decisions regarding them (Báez-Mendoza et al., 2021; Mars et al., 2021).

In this framework, the discovery of nonhuman prosociality is particularly relevant for improving our further comprehension of the above-mentioned neural machinery underlying human social decision-making processes. Indeed, animal models such as nonhuman primates or rodents offer a higher range of possibilities in terms of experimental techniques and therapeutic applications (Gangopadhyay et al., 2021; Ko et al., 2017). Besides, research models display similarities with the human brain in terms of genetic background, structural anatomy, biological mechanisms and cognitive functions, in this representing a precious resource for translating research findings to humans (Bicks et al., 2015; Cacioppo and Decety, 2011; Rilling et al., 2008). Then, by combining advanced behavioral testing with neuroscientific tools, it is possible to dissect more in detail the neurobiology behind social decision-making processes including when these originate prosocial behaviors.

3.2 The contributions of the prefrontal cortex and the amygdala to nonhuman social decision-making

Specific brain areas including the PFC and the amygdala have been identified as fundamental for planning social actions, the regulation of individual emotions, the recognition and sharing of those of others (Gangopadhyay et al., 2021; Ko, 2017; Yizhar and Klavir, 2018) (**Fig. 9**). These cognitive and socio-emotional skills are often essential for the initiation, monitoring

and completion of a decision that concerns other individuals and takes place in the social context.

The massive and spread connections with other brain areas such as the amygdala, the ACC and the hippocampus (HPC) make the PFC a special hub for directing and modulating advanced expressions of social cognition including social decision-making processes (Dal Monte et al., 2020; Gangopadhyay et al., 2021). The PFC, in particular, seems strongly recruited at the beginning (ie, social perception), in the middle (ie, social evaluation) and at the end (ie, social response) of a social decision (Gangopadhyay et al., 2021). For instance, it has been found that neurons in the inferior temporal and the orbitofrontal cortex (OFC) of the macaques' brain preferentially fire for others' facial features and their identity (Gangopadhyay et al., 2021). In addition, Azzi and colleagues recorded OFC neurons, at single and population level, in macaques that had to choose whether to keep a reward for themselves or share it with others (Azzi et al., 2012). During the experiment, the OFC neuronal activity was directly connected to the reward evaluation and the motivational aspects underlying the individual's choice, including when this choice regarded others (Azzi et al., 2012). Furthermore, the authors suggested that specific OFC populations activity also provide information regarding social identity and rank that can significantly affect the individual's decision to approach or even share with others (Azzi et al., 2012). In line with the previous results, Chang et al. identified specific neuronal responses in the ACC and the OFC for processing social outcomes in the monkeys' brain. Using a modified version of the dictator's game, in which a monkey can keep or donate a reward to others, the authors showed that self-referenced neurons are mainly present in the OFC while other- and both-referenced neurons in the ACC gyrus (Chang et al., 2013).

Beside the OFC, a mouse genetic study showed that new protein synthesis in the medial PFC (mPFC) is crucial for forming and especially consolidating a conspecific's memory or social recognition memory (Sakamoto and Yashima, 2022). Following this, Selimbeyoglu and colleagues highlighted that the excitation-inhibition balance within the mPFC is particularly relevant for the decision to socially approach or avoid other individuals (Selimbeyoglu et al., 2017). Indeed, the authors used a mouse model of autism lacking the CNTNAP2 gene and exhibiting hyperexcitability at the level of the mPFC (Selimbeyoglu et al., 2017). By optogenetically manipulating the mPFC excitation-inhibition, the social interaction deficits present in the autistic model were rescued (Selimbeyoglu et al., 2017). Moreover, Noritake et al. demonstrated the importance of the mPFC in the macaques' brain when monitoring and evaluating self- and other-related rewards (Noritake et al., 2021). In

particular, the authors showed how the mPFC is able to modulate the activity of other areas such as the dopaminergic midbrain nuclei altering the subjective value the individual attributes to a specific reward when others are present (Noritake et al., 2021). Besides, the neuronal oscillatory synchronization between the mPFC and the basolateral complex of the amygdala (BLA) is enhanced during social decision-making processes driven by other-regarding preference (Dal Monte et al., 2020). Dal Monte and colleagues tested pairs of rhesus macaques in a prosocial choice task where actors were asked to choose whether to donate juice to others vs a bottle or to both others and themselves. Other-regarding preference was differently modulated by the communication between the mPFC and the BLA. In particular, the uncoupling between the two areas was noted in the case of negative other-regarding preference or not donating the reward to others (Dal Monte et al., 2020). Finally, correlated neural activity is also present not only intra- but also inter-brain of different socially interacting individuals. Kingsbury and colleagues recognized, by using microendoscopic calcium imaging, a similar activity pattern in the dorsomedial PFC (dmPFC) of two mice closely interacting (Kingsbury et al., 2019). Hundreds of dmPFC neurons were simultaneously recorded in a pair of individuals freely interacting in a social arena and highly correlated neural activity was found in their brains. In particular, social interaction interruption significantly reduced inter-brain synchronization at the level of the dmPFC, suggesting that correlated neural activity was intimately dependent on the direct social interaction between the two individuals (Kingsbury et al., 2019). dmPFC neuronal activity, both at single-cell and population level, was also essential for tracking social partners' behavior, especially in the presence of dominant or competitive members (Kingsbury et al., 2019).

A subcortical region deeply connected to the PFC and the ACC is the amygdala, an important brain area for valence attribution, social salience and emotional processing, especially in the case of negative or aversive stimuli (Gangopadhyay et al., 2021; Knapska et al., 2006; Ko, 2017; Yizhar and Klavir, 2018). The amygdala receives information about the environment from the thalamus and sensory cortices and then it takes part in several circuits communicating with other areas including the PFC, the ACC and the HPC (Meisner et al., 2022). The amygdala is mainly divided into two subregions that are the basolateral complex (BLA) and the central nucleus (CeA) (Meisner et al., 2022). These, in particular, appear involved in important social components of social decision-making such as emotional discrimination (Ferretti et al., 2019) and contagion (Allsop et al., 2018). In their paper, Ferretti and colleagues (Ferretti et al., 2019) showed how the CeA is fundamental for rodents'

discrimination of conspecifics characterized by altered affective states such as fear or stress (ie, emotional discrimination). In particular, oxytocin signaling seems essential for this capability of the CeA as Ferretti and colleagues reported with their manipulation in a mouse model of schizophrenia affected by socio-emotional deficit/s (Ferretti et al., 2019). In fact, the neuropeptide oxytocin, the hormone most robustly associated with parental care (Marsh, 2016), seems also deeply involved in relevant prosocial manifestations in humans such as trust (Kosfeld et al., 2005), generosity (Zak et al., 2007; Domes et al., 2007), cooperation (De Dreu, 2012) and social bonding (Lim and Young, 2006).

Besides the CeA, also the BLA appears involved in social behavior. Indeed, many studies using the observational fear learning paradigm pointed to the BLA as essential for emotional contagion ability. In 2010, Jeon and colleagues showed that mice can learn fear by observing others receiving foot shocks and this social process is mainly vehiculated by the activity of the ACC (Jeon et al., 2010). Nonetheless, Allsop et al. demonstrated that also the BLA is required for this form of social learning (Allsop et al., 2018). Indeed, the selective inhibition of the BLA and its connections with the ACC specifically impairs the acquisition and the transfer of the fear state from other conspecifics (Allsop et al., 2018). Furthermore, BLA projections to the mPFC have a causal role in the modulation of anxiety-related and social behaviors (Felix-Ortiz et al., 2016). In fact, activating the BLA-mPFC pathway increases anxiety-like behaviors and social avoidance in the resident-intruder test (Felix-Ortiz et al., 2016), although this effect may be strongly subjected to oxytocin's influence (Gangopadhyay et al., 2021). Other studies indicate the involvement of the BLA in more sophisticated expressions of social behaviors such as prosociality. Following a previous experiment (Chang et al., 2013), Chang and colleagues reported that BLA activity was crucial for monkeys exhibiting a prosocial choice during a modified version of the dictator game (Chang et al., 2015). In particular, BLA neurons sustained other-regarding preference both across trials and days. Furthermore, unilateral infusion of oxytocin into the BLA significantly increased both the attention to recipients and the frequency of prosocial acts (Chang et al., 2015). Besides, the connectivity between the amygdala and other cortical-subcortical areas appears essential for refined social expressions (Dal Monte et al., 2020), in fact, the disruption of this connectivity is often considered responsible for the social deficits observed in clinical disorders such as autism, schizophrenia and social anxiety disorder (Meisner et al., 2022).

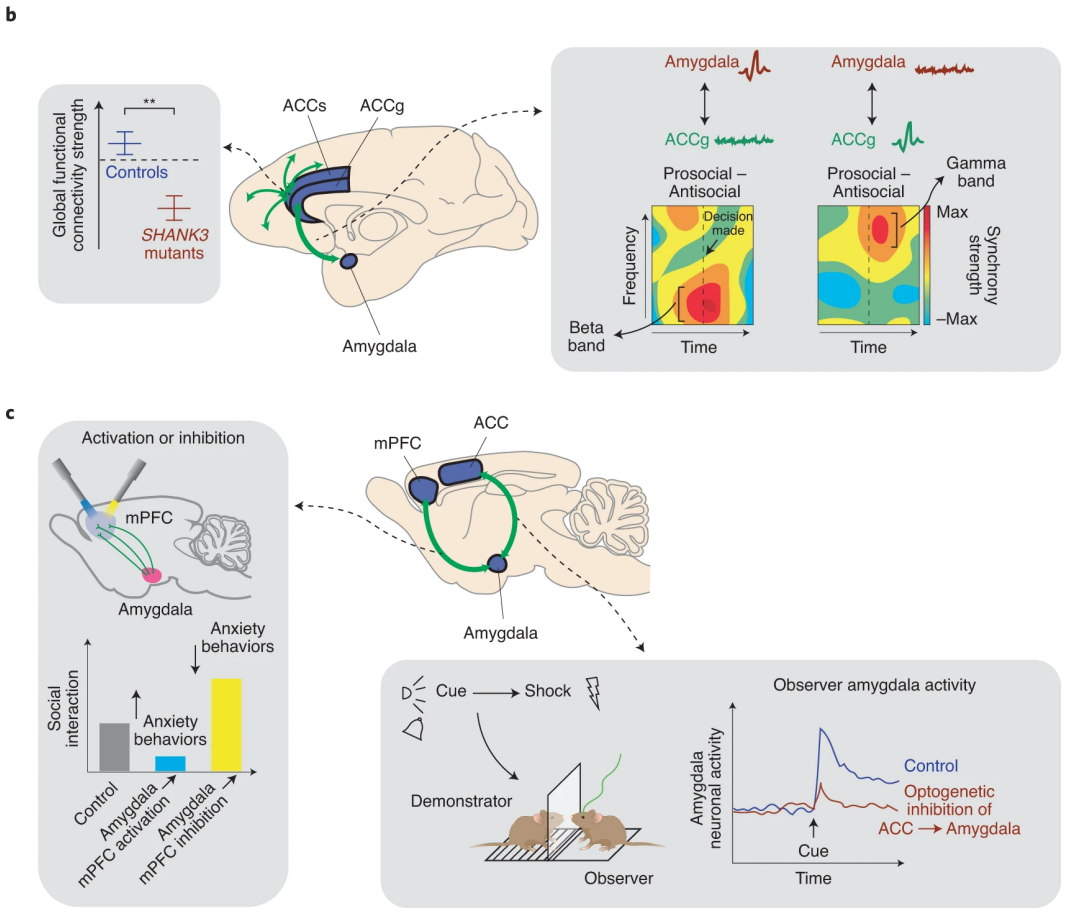


Fig. 9 | The importance of the prefrontal cortex and the amygdala in nonhuman social decision-making (adapted from Gangopadhyay et al., 2021).

3.3 The involvement of the dorsal hippocampus in nonhuman social decision-making

Recent research identifies the HPC as a key area not only for spatial but also for social cognition and behavior. Indeed, many studies show the HPC is deeply involved in social recognition and memory (Okuyama, 2018; Rao et al., 2019; Smith et al., 2016). In 2016, Okuyama and colleagues reported the ventral CA1 (vCA1) region of the hippocampus covering a major role in social memory (Okuyama et al., 2016). Greater activation in vCA1 cells during exposure to a familiar mouse was reported in a social discrimination task (Okuyama et al., 2016). Furthermore, optogenetic inhibition of vCA1 neurons and vCA1-to-NAcc projecting neurons resulted in social recognition deficits in both the social discrimination task and the resident-intruder assay. However, vCA1 optogenetic reactivation restored the social memory engram for the familiar mouse (Okuyama et al., 2016). Besides, also the hippocampal CA2 area, particularly the dorsal region (dCA2), has gained attention

for its role in social memory (Okuyama, 2018). In fact, Smith and colleagues excited, through optogenetics, CA2 vasopressin-expressing neurons receiving projections from the hypothalamic paraventricular nucleus in a direct interaction test with a familiar mouse at different time intervals (Smith et al., 2016). After optogenetic stimulation, the authors reported an enhanced social recognition memory for the familiar mouse even seven days later (Smith et al., 2016). These findings are in line with previous experiments specifically targeting CA2 and showing how lesioning this area leads to important social memory deficits in behavioral tasks such as the three-chamber task (Hitti and Siegelbaum, 2014). More recently, also the CA3 area has received attention for its involvement in social cognition. In 2018, Chiang et al. showed that knockout mice lacking the NMDA receptor subunit 1 gene in CA3 pyramidal neurons displayed impairments in synaptic plasticity with associated social recognition memory deficits (Chiang et al., 2018). Furthermore, chemogenetic inhibition demonstrated that ventral CA3 is fundamental for the encoding of social memories (Chiang et al., 2018).

However, social recognition memory is just one of the components involved in social decision-making. Other elements might play a role such as the capacity to replay past interactions with others or the ability to learn from them (ie, observational learning). Many studies indicate the crucial involvement of the hippocampus, specifically the CA1 region, in awake replay of past experiences (Carr et al., 2011). Awake or online replay consists in the hippocampal reactivation of place cells encoding previously met locations or paths particularly relevant in the context of spatial navigation (Carr et al., 2011). The mechanism of replay has been proposed as fundamental for a variety of cognitive functions such as memory consolidation, spatial working memory and also decision-making processes (Ólafsdóttir et al., 2018). Even though the hippocampal replay is not always directly implicated in trial-by-trial decision-making (Gillespie et al., 2021), still there is evidence of a crucial role of this mechanism in decisional planning also because of its link with areas devoted to working memory and executive functioning such as the PFC. For instance, Shin and colleagues found that hippocampal replay was essential for rats' decisions during navigation in a W-track spatial task (Shin et al., 2019). In particular, the researchers recorded neural ensembles in the dorsal CA1 (dCA1) and the PFC revealing how the coordinated activity between the two areas was fundamental for rats' reward retrieval inside the maze (Shin et al., 2019). According to the authors, this result would show how the hippocampal replay mechanism can inform and guide decisions through the recall of past experiences (Shin et al., 2019). More recently, Igata and colleagues designed an original spatial task in which rats had to learn new

paths each time for collecting a reward (Igata et al., 2021). The authors showed how hippocampal replay at the dCA1 level, measured as place cells increased theta-sequences and sharp wave ripples, was necessary for updating and improving rats' behavioral strategy across trials. Furthermore, online disruption of dCA1 replay resulted in decisional impairments negatively influencing rats' spatial navigation (Igata et al., 2021).

Beyond the hippocampal replay mechanism, social place cells have been recently identified in the dorsal CA1 region (dCA1) of the hippocampus either of bats or rodents (**Fig. 10**). A pioneering work led by Omer found a subset of neurons in the dCA1 of an observer bat specifically encoding the position of a conspecific during a spatial task, demonstrating the role of dCA1 in observational learning (Omer et al., 2018).

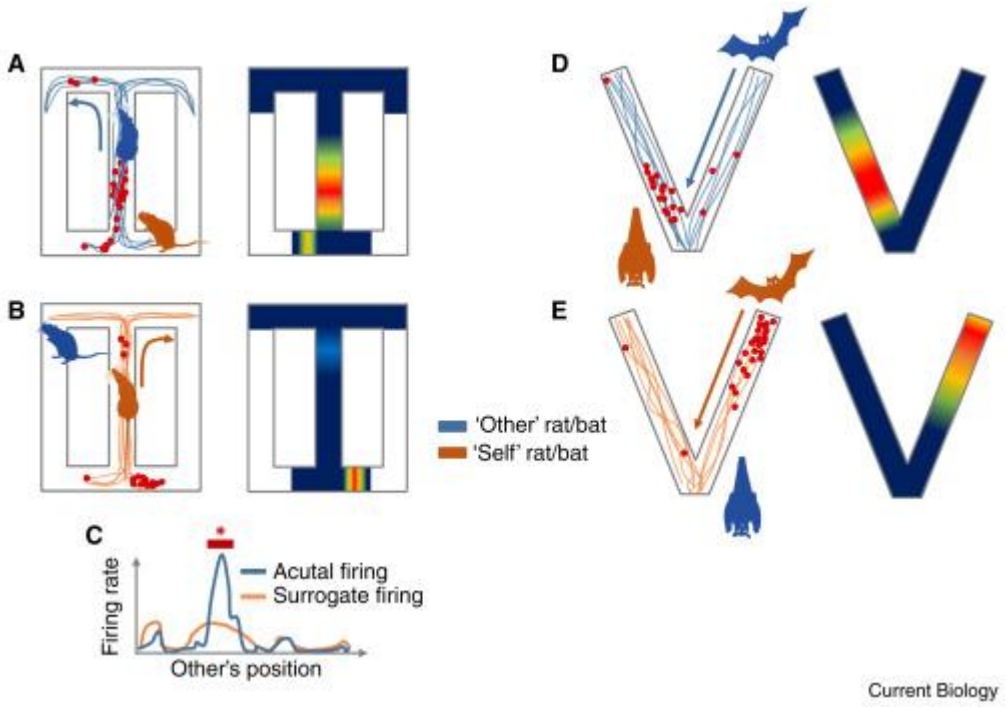


Fig. 10 | Social place cells and observational learning in the dorsal hippocampus (Duvelle and Jeffery, 2018).

Furthermore, social place cells are involved in mapping the other not only from a spatial perspective but also during goal-directed behavior (Danjo, 2020). For example, in an observational T-maze task in which one rat observes another rat's trajectory to earn a reward, a subset of dCA1 pyramidal cells showed spatial receptive fields that were identical for the self and the other, demonstrating that hippocampal cells integrate representations for both self and others (Danjo et al., 2018). These studies open to the possibility of dCA1 not only

capable of mapping spatial information but also social information pertaining to the other social agents that can help carry out a decision in the social context (Eichenbaum, 2017; Schafer and Schiller, 2018; Montagrin et al., 2018: Duvelle and Jeffery, 2018).

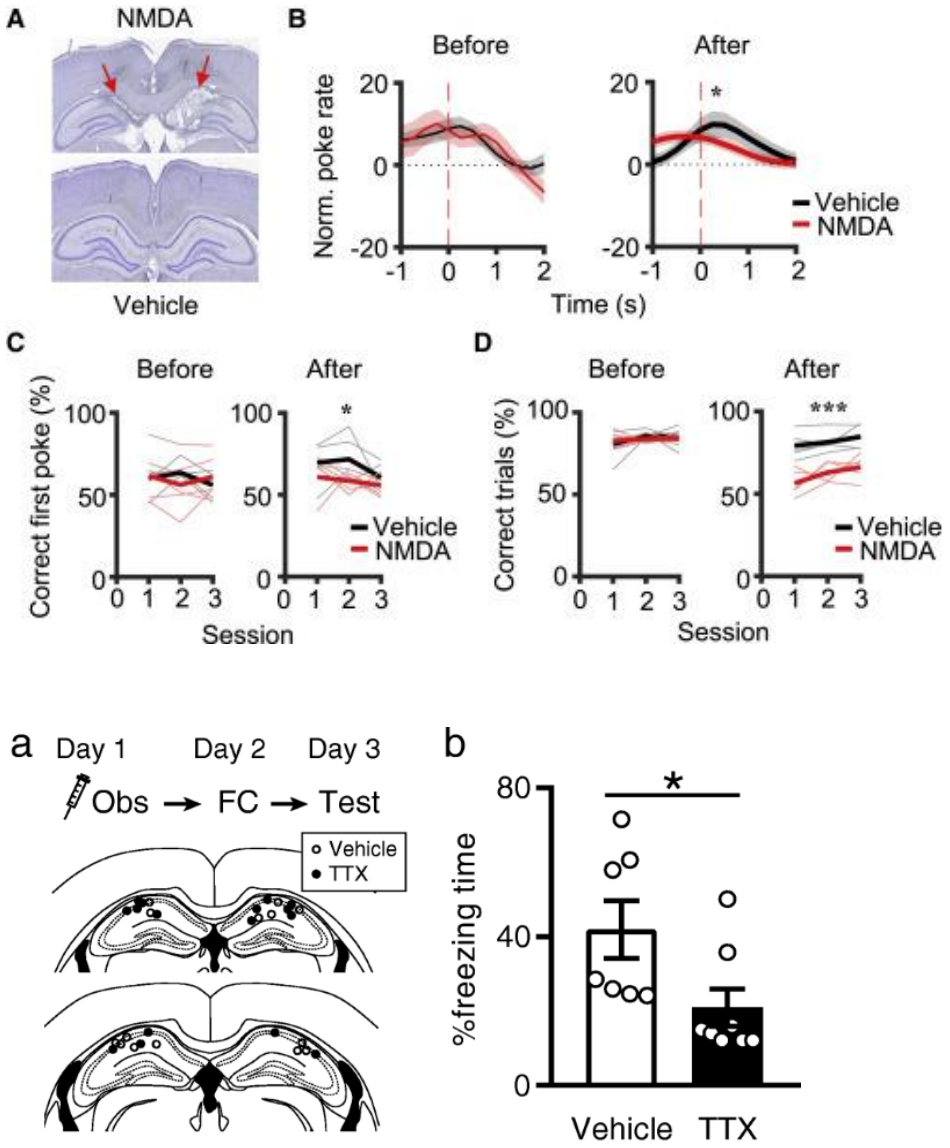


Fig. 11 | dCA1 inactivation and observational learning deficits (adapted from Mou et al., 2022 (top) and Nomura et al., 2019 (bottom)).

Others' observation can also produce a facilitatory effect on the performance and literature findings reported that this facilitation involves the dCA1 region (Mou and Ji, 2016; Danjo et al., 2018; Fujisawa and Ouchi, 2022). Recently, Mou and colleagues showed that rats can learn a T-maze spatial task by observing their conspecifics (Mou et al., 2022).

Thanks to observation, rats' performance was consistently enhanced and the authors suggested the involvement of hippocampal replay behind this facilitation (Mou et al., 2022). Following this view, others' observation would direct the process of awake replay to guide future decisions in the spatial context. However, lesioning the dCA1 through the chemical neurotoxic NMDA reversed this situation, preventing observer rats from spatial learning (Mou et al., 2022) (**Fig. 11, top**). Besides, Nomura et al. also demonstrated the involvement of dCA1 activity in fear acquisition through others' observation (Nomura et al., 2019). The authors reported that prior observation of conspecifics, receiving foot shocks in the fear conditioning apparatus, promoted and increased subsequent fear learning in observers (Nomura et al., 2019). Particularly, hippocampal neurons active during others' observation were also active during self-experienced fear conditioning promoting an enhanced fear memory (Nomura et al., 2019). Furthermore, dCA1 inhibition through bilateral infusion of the neurotoxin tetrodotoxin blocked the observation-induced enhancement of fear learning previously described (Nomura et al., 2019) (**Fig. 11, bottom**).

MAJOR RESEARCH AIMS

Aim 1: Understand whether mice are able of social decision-making by direct learning

Due to the presence of necessary skills for social decision-making processes and prosocial behaviors in rodents, the first major aim of this PhD project was testing the ability of mice to make social decisions regarding their conspecifics by direct learning, that is through a trial-and-error decisional strategy. Recognizing the lack in literature of a specific behavioral paradigm capable of testing altruism in rodents, we devised an original social decision-making task (SDM), modeled on the human dictator game (Lee, 2008; Sanfey, 2007; van Dijk and De Dreu, 2021), to specifically investigate altruistic behavior in the mouse model. Modeling the SDM in this way, it gave us the opportunity to test key attributes of the altruistic behavior such as other-regarding preference for individuals in need, sacrifice of one's own resources and associated costs behind the altruistic decision (Ben-Ner and Kramer, 2011; Brethel-Haurwitz, et al., 2018; Marsh et al., 2021).

In this framework, our study is rather different from others in literature that mainly considered reciprocation, cooperation and helping behavior manifestations in rodents. In fact, these studies were not adapting behavioral paradigms such as the dictator game specifically designed for testing altruism. The studies were driven by other experimental objectives such as analyzing the importance of previous reciprocation history (Dolivo and Taborsky, 2015), observing the need of coordinated actions for social cooperation (Shin and Ko, 2021) and showing how individuals are motivated to help restrained companions (Bartal et al., 2011) or avoid harming them (Hernandez-Lallement et al., 2020). Their results do not necessarily imply altruistic behavior and key features such as an individual's own sacrifice and the cost for its altruistic action. Furthermore, many of the above studies preferentially used rats instead of mice (Bartal et al., 2011; Dolivo and Taborsky, 2015; Hernandez-Lallement et al., 2020). We preferred using mice in our SDM, basing our decision on preexisting literature evidence demonstrating these, similarly to rats, are capable of emotional discrimination (Ferretti et al., 2019), emotional contagion (Keum et al., 2018), social memory (Okuyama, 2018) and observational learning (Nomura et al., 2019) that, as already explained, are essential abilities for social decision-making processes.

Besides, altruistic behavior is a complex multifactorial process that involves internal and external modulators such as familiarity, individual motivation, empathy and social dominance (Suzuki and O'Doherty, 2020; Terenzi et al., 2021). Thus, our aim was also trying to explain the individual differences in social performance based on these influential determinants. More detailed information about these aspects might help us highlight the reason why a social agent should choose to benefit others in the social context, although this prosocial choice might be particularly disadvantageous for them.

Aim 2: Understand whether mice are able of social decision-making by observational learning

The second major aim of the current PhD project was to understand whether mice, similarly to humans, can learn social decisions by observing their conspecifics (ie, by observational learning). Observational learning in humans is very widespread, many are the examples where humans can learn movements, emotions and even complex social behaviors from others (Fryling et al., 2011; Seymour, 2009; Yoon et al., 2021). According to this view, observational learning is an alternative decision-making system next to direct learning where the subject primarily relies on other social agents' performance and, based on this, shapes his own course of actions in the social sphere. Despite the high socio-cognitive demands, literature evidence clearly demonstrates the presence of observational learning in rodents including mice (Danjo et al., 2018; Keum et al., 2018; Nomura et al., 2019). In fact, rodents can learn spatial (Mou et al., 2021) and affective (Nomura et al., 2019) tasks through others' observation and this often gives them an advantage. However, a study showing the possibility of observational learning also for sophisticated social behaviors in rodents is still missing.

For all these reasons, we chose to study observational learning in mice for the acquisition of complex social expressions such as social decision-making processes. Our main assumption was that recipients/observers were able to display a consistent social performance in our SDM after previous observation. This might be a direct measure of the SDM learning acquisition mainly realized through others' observation rather than direct experience. This also would tell us about an enhancing effect on performance due to observational compared to direct learning. Together with this, we also wanted to check other parameters possibly influencing observational learning (Fryling et al., 2011; Selbing et al., 2014; Zonca et al., 2021). Indeed, characteristics related to the model or proper of the

observer might bias observational learning. For instance, a bad demonstration from the observer or even the quality of its actions might influence the observer's performance. Moreover, the observer's performance might just be a mere imitation of what previously observed, lacking flexibility and adaptability. The analysis of these additional parameters would give a better depiction of the complexity of the observational learning phenomenon in the mouse model. This improved characterization could also help in revealing the neurobiology behind observational learning even when this ability favors the expression of social decision-making processes such as altruistic behavior.

Aim 3: Understand the neural circuitry underlying social decision-making by direct and observational learning

The third aim of this PhD project was investigating the neural circuitry underlying social decision-making processes, including when these take the form of prosocial behaviors such as altruism. A similar study might be tremendously important for situations such as neuropsychiatric and neurodegenerative disorders often characterized by social dysfunctions including social decision-making deficits (Christidi et al., 2018; Yang et al., 2017; Woodcock et al., 2020). Social decision-making deficits are often associated with deficits at the level of one or more areas included in the social decision-making network. The neural deficits are connected with impairments in cognitive and socio-emotional abilities such as executive functioning, social memory, empathy and observational learning that are essential for the manifestation of a social decision (Báez-Mendoza et al., 2021; Rilling and Sanfey, 2011; Terenzi et al., 2021). Then, reconstructing the circuitry behind social decision-making processes and elucidating what occurs at the behavioral level when one or more of these areas are compromised appears extremely relevant. It is true we used the mouse model for studying the circuitry behind social decision-making that has its limitations if someone would try to directly translate our results to humans. Still, as already explained, rodents present similarities to humans in terms of structural and functional aspects. In fact, rodents share with humans different areas that strictly regulate necessary cognitive and socio-emotional components of the social decision-making process (Allsop et al., 2018; Bicks et al., 2015; Gangopadhyay et al., 2021; Terburg et al., 2018). In particular, three major areas were selected, according to literature evidence, for better characterizing the circuitry behind social decision-making processes such as altruistic behavior.

The PFC, BLA and HPC appeared great candidates for the study of the social decision-making network. Indeed, both the PFC and the BLA are deeply involved in the initiation, modulation and execution of many social and prosocial behaviors. Nonhuman animal studies report the involvement of the PFC in social perception, action planning and the decision to approach-avoid other individuals (Ko, 2017; Yizhar and Klavir, 2018; Sakamoto et al., 2022), while the BLA is often mentioned when it comes to emotional recognition and sharing, valence attribution and the processing of negative stimuli such as others' fear (Felix-Ortiz et al., 2016; Ferretti et al., 2019; Knapska et al., 2006; Scheggia et al., 2020). Furthermore, the PFC and the BLA are intimately linked with important factors of social decisions such as social dominance or hierarchical status (Ligneul et al., 2017; Wang et al., 2011). Besides, the reciprocal communication between the two areas has been reported as fundamental for regulating prosocial behaviors in nonhuman animals (Allsop et al., 2018; Dal Monte et al., 2020; Gangopadhyay et al., 2020). Despite all this evidence, few studies have directly analyzed the specific contribution of the BLA and the PFC in prosocial behaviors reminiscent of human altruism in the mouse model. Filling this gap, the current study would give the chance to shed more light on the neurobiological machinery involved when the individual chooses to prioritize other-related interests over his, including situations such as in pathological conditions where this possibility might be biased or even denied.

The HPC, instead, is not only involved in social recognition memory (Okuyama, 2018), that remains very important when dealing with other individuals' identity (eg, familiar or previously met individuals), but also the existence of the replay mechanism (Ólafsdóttir, et al., 2018) and the social place cells (Danjo et al., 2018; Omer et al., 2018) appear relevant factors for the social decision-making process. Particularly, the presence of social place cells in the dorsal HPC (dCA1) would make human and nonhuman animals capable of tracking others' movements and actions in the surrounding environment, with the possibility of replaying and learning what is observed (ie, observational learning). Furthermore, dCA1 activity is not only fundamental when rodents learn spatial information from their conspecifics (Mou et al., 2021), but also when they acquire affective states from them (Nomura et al., 2019). Besides, lesioning or inhibiting dCA1 reduces the performance advantage produced by observational learning (Mou et al., 2021; Nomura et al., 2019). However, a direct assessment of the specific contributions of dCA1 when the individual learns from others not only spatial and affective skills but also social decision-making processes such as altruism is still missing.

MATERIALS AND METHODS

1. Animals

All procedures were approved by the Italian Ministry of Health (permits n. 107/2015-PR and 749/2017-PR and 191/2020-PR and 200/22-PR) and local Animal Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the European Community Council Directives. Routine veterinary care and animals' maintenance was provided by dedicated and trained personnel. Two to six-month-old males and females C57BL/6J animals were used. Distinct cohorts of naïve mice were used for each experiment. Animals were housed two to four per cage in a climate-controlled facility (22±2 C), with ad libitum access to food and water throughout, and with a 12-hour light/dark cycle (7pm/7am schedule). Experiments were run during the light phase (within 10am-5pm). All mice were handled on alternate days during the week preceding the first behavioral testing.

2. Behavioral tasks

2.1 Social decision-making task (SDM) design

Experiments were conducted in a standard operant chamber (ie, the actor's compartment, Length: 24cm x Width: 20cm x Height: 18,5cm; ENV-307W-CT; Med Associates, Inc.) fused with a custom-made small triangle-shaped chamber (ie, the recipients' compartment, Length: 18cm x Width: 14cm x Height: 18,5cm). The separation wall between the two compartments consisted into a metal mesh with 1cm holes that allowed social exploration and nose-to-nose interaction. The actor's compartment was equipped with two nose poke holes and a food magazine between them, for delivery of food rewards (14mg; Test Diet, 5-TUL). The recipient's compartment presented only a food magazine connected to a food dispenser. The setup was placed inside a sound attenuating cubicle (ENV-022V, Med Associates, Inc) homogeneously and dimly lit (6 ± 1 lux) to minimize gradients in light, temperature, sound and other environmental conditions that could produce a side preference. All the setup was controlled by custom scripts written in MED-PC IV (Med Associates, Inc.). Furthermore, a

digital camera (Imaging Source, DMK 22AUC03 monochrome) was placed on top of the setup to record the test using a behavioral tracking system (Anymaze 6.2, Stoelting, UK).

During the SDM, the actor mouse (ie, the dictator) could determine to receive a food reward for himself, namely the selfish choice, or to allocate the reward also to his companion (ie, the recipient), namely the altruistic choice (**Fig. 12a**). Both choices were reinforced on fixed ratio (FR) 1, such that one nose poke either to the left or to the right side corresponded to one food reward delivery. After one nose poke, an intertrial interval of 5 seconds occurred allowing the actor mouse to retrieve his food pellet. The recipient was a passive player and only received food rewards upon actor choices. The actors were tested for five days in 40min sessions and were always paired with the same recipient throughout the same experiment. Actor and recipient were mildly food-restricted to 90% of their baseline weight to encourage task engagement in the initial phase and were housed together for at least two weeks before the experiment unless it was the unfamiliar condition (ie, unfamiliar recipient). Finally, the location of the altruistic choice was counterbalanced between left and right nose pokes across mice, but never changed across test sessions of the same mouse.

2.2 SDM behavioral validation

For primarily validating our SDM and demonstrating mice social decisions were really dictated by the presence of their conspecifics, the testing subjects (ie, the actors) were tested in three different conditions: 1) with recipient, in which a real cage mate was placed in the adjacent compartment; 2) no recipient, the compartment of the recipient was empty; 3) with toy, the recipient was replaced with an inanimate object (**Fig. 12a**). The task structure and design were identical across all the test conditions for controlling potential confounders such as lights, sounds, odors, so the food regimen (ie, mild food restriction for both actor and recipient) and the identity of the social companion (ie, familiar recipient) were unaltered. However, in the toy condition, actors were tested for five days with a partner and the day following the last session (day 5), the recipient was replaced by an inanimate object (day 6) to check possible changes in the mouse's decisional performance.

2.3 SDM under costly conditions

To test whether mice made voluntary choices to benefit others under costly conditions, we tested mice using an increasing FR schedule for altruistic decisions from FR2 to FR8 (**Fig. 14a**). In this condition, the number of operant responses required to dispense food to the recipient is increased on each day (from 2 to 8). Selfish responses remained on FR1 throughout the experiment. In the no recipient condition, for each actor the preferred nose poke was reinforced using the increasing FR schedule and the other nose poke was kept on FR1.

For further assessing altruistic behavior under costly conditions, mice were trained in the SDM and then the paradigm was modified such that one nose poke resulted in food rewards for themselves only (ie, selfish choice) and the other to the recipient only (ie, altruistic choice without concurrent reward). Mice were tested in a longer session (120min) to observe possible effects of satiety on their choices.

2.4 SDM different experimental conditions

For evaluating the role of visual cues and social contact (ie, nose-to-nose interaction) within the SDM, an opaque or transparent partition, differently from the original metal mesh, dividing the recipient and the actor was placed (**Fig. 15a**). The opaque partition blocked both visual cues and social contact, while the transparent partition allowed visual cues but not social contact. The task design and protocol was the same as above except the selected pairs started with the designated partition (ie, metal, opaque or transparent) from the beginning. Additionally, social proximity/exploration of the dictator towards the recipient was recorded through a digital camera (Imaging Source, DMK 22AUC03 monochrome) mounted on top of the apparatus and further measured using the behavioral tracking Anymaze software (Anymaze 6.2, Stoelting, UK).

For evaluating the effect of familiarity in our SDM, mice could also be tested with unfamiliar peers and their performance analyzed. In this case, the actor and the recipient were never housed together and they were mildly food-deprived separately. After the test sessions, the actor and the recipient were always returned to their separate cages and no direct interaction outside the task was possible.

For proving altruistic behavior is particularly evident when others are in need, two hours before each session of the SDM task some of the recipients were separated from their cage mates actors and lead to satiety giving free access to food and reward pellets in their cage. Then, both actors and recipients mice were transferred to the operant chamber and

tested. In a different cohort of mice, we tested satiety-induced reward devaluation in the recipients mice after standard training in the SDM task for five days. In this condition one group of actor mice was tested with food-restricted recipients and one group with sated recipients following satiety-induced reward devaluation on day 6 (**Fig. 16d,e**).

2.5 Tube test

The tube test was performed in a transparent Plexiglas tube (Length: 30cm, inside diameter 3cm) as described in previous studies (Fan et al., 2019; Wang et al., 2011) (**Fig. 17a**). For habituation, the tube was placed inside the cage for three consecutive days. After habituation, mice were trained to run inside the tube. Each mouse was released at alternating ends of the tube and was allowed to run through the tube. We used a plastic stick to guide the mouse to the end of the tube if needed. Each animal was given ten training trials on two consecutive days. For the test, two mice were simultaneously released into the opposite ends of the tube and care was taken to ensure that they met in the middle of the tube. The first mouse that retreated and placed its two rear paws outside the tube was recorded as the loser of the trial and the other mouse the winner. Between each trial, the tube was cleaned with 50% ethanol. Mice were tested pairwise using a round robin tournament, on daily sessions. Each pair of cage-mates was tested in consecutive trials, alternating the starting side of the tube. The test was performed until all the ranks were stable for at least 4 consecutive days.

In hM4D-expressing animals, the tube test was performed in different cohorts of mice with or without the SDM task. The training in the tube for habituation was performed before the SDM task, then the tube test started and the SDM task started on the same day. The tube test was performed at least one hour after the SDM task (with CNO injection). In hM4D-expressing animals that did not perform the SDM task, after reaching stable ranking, mice received CNO or vehicle and were tested at different time points following injection (1-2 hours, 6-8 hours, 24 hours). For BLA silencing, one mouse received CNO and the other cage mates received vehicle. In control cages, all the animals received vehicle.

2.6 Observational fear conditioning task

The apparatus consisted of two identical and adjacent fear conditioning chambers (Ugo Basile, Length: 24cm, Width: 20cm, Height: 30cm) separated by a transparent Plexiglas partition (**Fig. 17j**). Visual, olfactory and auditory cues could be transmitted between the

chambers but no social contact was possible between the two mice. A demonstrator mouse (ie, previously recipient in the SDM) and an observer (ie, previously actor in the SDM) were individually placed in the two chambers. The same pairs of mice tested in the SDM were used. We adopted a behavioral protocol based on a previous study (Jeon et al., 2010). The two mice were allowed to explore the chambers for 5min (ie, baseline). Then, a 2-s foot shock (0.7mA) was delivered every 10s for 4min to the demonstrator mouse (ie, training/conditioning) using the behavioral tracking software Anymaze (Anymaze 6.2, Stoelting, UK). The test lasted a total of 9min (ie, baseline+training/conditioning). At the end of the procedure mice returned to their home-cage. For BLA silencing (ie, hM4D-expressing animals), the observer mouse was injected with CNO 30min before test starts. In control cages, the observers received vehicle.

2.7 SDM observational learning task

We devised an additional phase next to the original SDM for specifically measuring mouse observational learning capacity. In detail, we start with a five-day observation phase where the recipient (ie, the observer) observes and, possibly, learns the SDM from their dictator (ie, demonstrator). After that, a five-day testing phase begins where the observer is placed in their previous dictator's compartment and now they are called to act by expressing a social decision towards their companion (**Fig. 21a**). During this second phase, mouse SDM learning capacity after previous observation (ie, observational learning) is specifically assessed. The observation phase plus the testing phase lasted a total of 10 days. The daily test sessions lasted 40min. As the original SDM, mice were mildly food-restricted (ie, 90% of their baseline weight), familiar pairs (ie, cage-mates) were used for the entire test duration. Other parameters related to the experimental apparatus such as lights, sounds and nose pokes were the same as in the original SDM. Finally, the location of altruistic choice was counterbalanced between left and right nose pokes across demonstrators, but never changed across test sessions of the same mouse in the observational phase. The same was done for observers in the additional testing phase for assessing the role of imitation and flexibility in their observational learning capacity.

2.8 Object location displacement test

The procedure for the object location displacement test was adapted from a previous study (Barker and Warburton, 2011) (**Fig. 28a**). The task involved an habituation phase of 40min/1h to the apparatus, a sample/familiarization phase (10min) and a test/recall phase (10min), separated by a 24h delay (ie, long-term memory). Between sessions, all the objects were cleaned with 50% ethanol. Mice were tested in a standard open field arena (UgoBasile, 44 × 44cm) with black PVC walls. The stimuli were objects constructed from Duplo blocks (Lego) and varied in shape, color, and size, and were too heavy to be displaced. A digital camera (Imaging Source, DMK 22AUC03 monochrome) was placed above the apparatus to record the test using a behavioral tracking system (Anymaze 6.2, Stoelting, UK). These videos were also used offline by experimenters blind to the manipulations for a posteriori scoring of the time spent in the different zones of the apparatus and exploratory behavior, which was defined as the animal directing its nose toward an object at a distance of at least 2cm.

Specifically, two identical objects were placed near the corners on one wall in the arena (10cm from the walls) in the acquisition phase. In the test phase, one object was left in the same position, the other one was displaced to the corner adjacent to the original position, such that the two objects were diagonal from each other. The positions of the objects in the test were counterbalanced between the animals.

2.9 Three-chamber task

We adapted a standardized procedure from a previous study (Rein et al., 2020) (**Fig. 28c**). Actor mice (ie, test subjects) were tested in a standard three-chamber sociability cage (Ugo Basile, 60x40x22cm) equipped with transparent PVC walls and two cups that could host either a mouse or an object (15cm in height, diameter 7cm). After each test, the apparatus was cleaned with 50% ethanol and allowed to air dry. All test stages were carried under dimly lit (6 ± 1 lx). Digital cameras (Imaging Source, DMK 22AUC03 monochrome) were placed above the apparatus to record the test using a behavioral tracking system (Anymaze 6.2, Stoelting, UK). The three-chamber task consisted of four experimental steps/phases. Habituation (10min) to the apparatus occurred on the day before the experiment. On the day of testing, after a second habituation to the apparatus with empty cups (10min), an adult conspecific mouse (ie, mouse 1 (M1) or familiar), that was not a cagemate to the actor mouse (ie, the test subject), was placed in one of the cups at one side of the apparatus, whereas the other cup contained a novel object built with Duplo blocks (Lego). Following the sociability

test, after a 2min delay (ie, short-term memory), a novel mouse (ie, mouse 2 (M2) or stranger), that was not a cagemate, was placed into the cup instead of the object and the actor mouse was tested for another 10min to assess the preference for the new mouse over the familiar one (ie, social novelty/memory).

3. Chemogenetics/viral injections

3.1 Chemogenetics overview

For dissecting the specific contribution of the selected areas to the social decision-making strategies (ie, direct or observational learning) and its behavioral manifestations (ie, altruistic behavior), we chose to use the neuroscientific tool of chemogenetics. Similarly to other available tools (ie, optogenetics) in the modern field of social behavioral neuroscience, chemogenetics allows researchers to investigate the specific role of one or more areas in a behavior of interest (Roth, 2016). Although optogenetics offers a more precise temporal control of in vivo neuronal activity, we chose chemogenetics for our cause because it is less invasive, not requiring the chronic implantation of optic fibers and at the same time maintaining the ability to control neural activity. Indeed, through the combination of chemistry and genetics knowledge, chemogenetics offers the possibility to manipulate, either exciting or inhibiting, selected areas when a certain behavior is enacted (Roth, 2016).

Chemogenetics specifically adopts designed engineered protein receptors that exclusively respond to the administration of designed drugs or ligands (DREADDs) (Zhu and Roth, 2014; Campbell and Marchant, 2018). Indeed, DREADD receptors lack pharmacological activity without their designed ligands and ligands are pharmacologically inert without their specific receptors. DREADDs can be either excitatory or inhibitory, such as the muscarinic receptors coupled to the Gαq (eg, hM3Dq) or Gαi (eg, hM4Di) intracellular signaling pathways, and selectively respond to specific small molecule ligands like Clozapine N-oxide (CNO) (Zhu and Roth, 2014). DREADDs are usually enveloped into viral vector systems such as adeno-associated viruses (AAVs), capable of infecting different human-nonhuman species and eliciting a mild immune response from the host. DREADDs can also be coupled with fluorescent tags like mCherry, a member of the mFruits family of monomeric red fluorescent proteins, for further detection. In fact, these fluorophores are chemical compounds that can re-emit light after light excitation at the microscope. Ligands

like CNO for activating DREADDs can be administered, among other ways (eg, intracerebral infusion), through intraperitoneal injection (i.p.) (Roth, 2016). Via i.p., CNO is primarily metabolized by the liver, then reaches the nervous system and activates the receptors in the chosen area/s.

3.2 Viral vectors

The viral vectors carrying DREADDs used for the chemogenetic experiments in the current PhD project were: 1) AAV5-CamKIIa-mCherry (114469, titer: ≥ 7×10^{12} vg/mL); 2) AAV5-CamKIIa-hM4D(Gi)-mCherry (50477, titer ≥ 3×10^{12} vg/mL); 3) AAV5-Syn-DIO-hM4D(Gi)-mCherry (44362, titer ≥ 7×10^{12} vg/mL). The viral vectors were purchased from Addgene. CAV2 equipped with Cre recombinase (titer ≥ 2.5×10^{11} vg/mL) was purchased from the Institute of Molecular Genetics in Montpellier CNRS, France.

3.3 Surgical procedures

C57BL/6J mice were naïve and 2 months old at the time of surgery. All mice were anesthetized with a mix of isoflurane/oxygen 2%/1.5% by inhalation and mounted onto a stereotaxic apparatus (Stoelting) linked to a digital micromanipulator. Brain coordinates of viral injection were chosen in accordance with the mouse brain atlas (Paxinos and Franklin, 2019): 1) BLA, AP: -1.7 mm, ML: ± 3 mm, DV: -4.5mm; 2) PL, AP: +1.9mm, ML: ± 0.25 mm, DV: -2.4 mm; 3) dCA1, AP: -2 mm, ML: ± 1.5 mm, DV: -1.3 mm; 4) vCA1, AP: -3.16 mm, ML: ± 3.10 mm, DV: -4.55 mm and -4.30. The volume of AAVs injection was 400 nL and 150 nL for CAV2-cre (injection rate 200 nL/min), per hemisphere. We infused virus through a 10-µL Hamilton syringe. After infusion, the pipette was kept in place for 3 min (ie, diffusion time). Specifically, for CAV2-cre injections optimal spreading, two DV coordinates were used per hemisphere either for the BLA (-4.6 and -4.3) and the PL (-2.6 and -2.3) and 150 nL for each DV coordinate was delivered, waiting for 3min only after the second infusion (ie, the higher DV coordinate) per hemisphere. To investigate the functional role of BLA→PL connections, we injected a retrogradely transported canine adenovirus (CAV-2) engineered to express Cre recombinase (CAV2-Cre) into the PL and also injected the BLA with an AAV carrying a cre-dependent hM4D(Gi)DREADD receptor and mCherry (hM4D BLA→PL) (**Fig. 20a**). With this combination, we achieved DREADD(Gi)-mCherry expression exclusively in BLA neurons projecting to the PL. We used the same approach to

study PL neurons projecting to the BLA (hM4D PL→BLA). After virus injection mice were allowed 4 weeks to recover and for the viral transgenes to adequately express before behavioral experiments.

3.4 Drugs

For hM4D activation we used i.p. administration of Clozapine N-Oxide (CNO) dihydrochloride (water soluble) (HB6149, Hello Bio) dissolved in physiological saline (0.9% NaCl) at a dose of 3 mg/kg in a volume of 10 ml, 30min before the behavioral experiments. All mice (control CNO, hM4D CNO) received i.p. CNO injection.

4. Tissue-slice preparation and immunohistochemistry

Mice were transcardially perfused with 40ml of 0.1 M phosphate buffered saline (PBS) and then with cold paraformaldehyde (PFA, 4% in PBS). The brain was removed from the skull and post-fixed with 4% PFA in PBS for 2-to-6 h at 4°C. The brain was sliced in 50 μm coronal sections using a vibratome 1000 Plus Sectioning System (3 M). Brain slices were incubated in 1% Triton X-100 in PBS (1% T-PBS) supplemented with 10% normal goat serum (NGS) for 1-2 h at room temperature (RT), shaking. After permeabilization and blocking, slices were incubated with primary rabbit anti-dsRED polyclonal antibody (dilution 1:1,000) in 0.3% PBS (0.3% T-PBS) supplemented with 1% NGS overnight at RT or 48h at 4 °C, shaking. The appropriate Alexa Fluor-conjugated secondary antibody (ie, goat anti-rabbit Alexa Fluor™ 568 IgG (H+L), dilution 1:1,000) in 0.3% PBS (0.3% T-PBS) with 1% NGS were applied for 2 h at RT followed by nuclei staining with the blue-fluorescent DNA stain 4′,6-diamidino-2-phenylindole (DAPI, dilution: 1:50,000 in PBS; Thermo Fisher Scientific). Labelling in the BLA, PL and dCA1 was visualized with a confocal microscope (Zeiss) with a 10x/20x objective and analyzed using Fiji (ImageJ) software. To detect native fluorescence of the mCherry-fused hM4D, BLA- and dCA1-containing brain slices were acquired with Nanozoomer S60 (Hamamatsu), using constant settings.

5. Antibodies

For immunohistochemistry analyses related to PFC-BLA reciprocal neuronal projections, the following primary antibody was used: rabbit anti-DsRed polyclonal antibody (632496, Takara/Clontech; dilution: 1:1,000). The following secondary antibody was used: goat anti-rabbit Alexa Fluor™ 568 IgG (H+L) (A-11011, Invitrogen; dilution: 1:1,000).

6. Statistics and analyses

6.1 Behavioral-related analyses

The number of nose poke responses was counted by a software (MED-PC V, Med Associates, Inc) and then imported by using the MED-PC To Excel tool (MPC2XL, Med Associates, Inc) into a Microsoft Excel spreadsheet for better data visualization. To quantify daily individual social preference for altruistic over selfish responses we calculated a decision preference score as following:

$$Decision\ preference\ score\ = \frac{(number\ of\ altruistic\ responses - number\ of\ selfish\ responses)}{total\ number\ of\ responses}$$

Besides the decision preference score, we also conceived an additional index called the learning index for specifically measuring the SDM learning capacity/ability of mice. This index builds upon the decision preference score expressed on the final test day (ie, test day 5) when mouse social performance is considered definitive and checks whether and how well mice are consistent with their final social performance since they begin the task. The learning index is calculated as follows:

$$Learning\ index\ = \frac{(number\ of\ responses\ to\ the\ choice\ of\ election - number\ of\ responses\ to\ the\ other\ option)}{total\ number\ of\ responses}$$

For better clarifying how this index works, two examples are further provided. 1) In the SDM by direct learning, an actor mouse expresses an altruistic preference on the last day (ie, test day 5). We keep this altruistic preference as a starting point for measuring how stable and consistent the actor mouse is in his altruistic behavior since the SDM starts. Initially, the actor mouse will not show a high learning index as it is still learning the SDM. After this first acquisition phase, the dictator will probably start to act in accordance with his final decision,

then showing a higher learning index. 2) In the SDM by observational learning, an observer, after previous task observation from others, is now called to act and expresses an altruistic preference on the last SDM day (ie, test day 5). Starting from this, we check whether the observer mouse acts and shows agreement to his altruistic behavior since the test starts (ie, from day 1 onwards). Differently from the SDM by direct learning, the observer due to previous observation should already exhibit a high learning score since the initial SDM stage.

Video images from the SDM were automatically analyzed a posteriori for scoring exploratory behavior (ie, social proximity/exploration behavior) using the Anymaze video tracking system (Anymaze 6.2, Stoelting, UK). Specifically, we measured the time spent either by the actor or the recipient in social exploration when entering a designated region of interest (ROI) in proximity of the adjacent companion's compartment. Videos from the observational fear learning task were, instead, manually scored for (vicarious) freezing behavior using the Behavioral Observation Research Interactive Software (Boris 8.7, Università di Torino) (Friard and Gamba, 2016).

In the tube test, to assign each animal social rank we used the normalized David's score (DS) for dominance. The score was calculated from the individual proportion of wins and losses in all the trials, in relation to the wins and losses of its opponents, as reported in a previous study (De Vries et al., 2006). We then normalized the score to be between 0 and *N-1* (where *N* is the number of subjects in each cage), using the following formula:

$$Normalized\ DS = \frac{1}{n}\left(DS + \frac{n(n-1)}{2}\right)$$

In the object location displacement test, we measured the ability of mice to recognize an object that had changed location compared to the acquisition phase. To express the discrimination between the objects, we calculated a preference index as the absolute difference in the time spent exploring the displaced object and the familiar object divided by the total time spent exploring the two objects. The formula is the following:

$$Preference\ index: \frac{(time\ w/\ displaced\ object - time\ w/\ old\ object)}{total\ exploration\ time}$$

To measure sociability/social affiliation in the three-chamber task, we calculated a preference index as the absolute difference in the time spent exploring the mouse and the object divided by the total exploration time. The formula is the following:

$$Preference\ index: \frac{(time\ w/\ mouse\ -\ time\ \ w/\ object)}{total\ exploration\ time}$$

To assess social novelty/memory in the three-chamber task, we calculated a preference index as the absolute difference in the time spent exploring the mouse 2 (ie, stranger) and the mouse 1 (ie, familiar) divided by the total exploration time. The formula is the following:

$$Preference\ index: \frac{(time\ w/\ mouse\ 2\ stranger - time\ w/\ mouse\ 1\ familiar)}{total\ exploration\ time}$$

6.2 Statistics

Statistical analyses can be found below each figure or figure legend where results are graphically presented. Results are expressed as mean ± standard error of the mean (s.e.m.). For the analysis of decision preference score, learning index and percentage of altruistic responses we used two-way repeated measures ANOVA, followed by Bonferroni multiple comparison test. For the analysis of the number of nose poke responses, we used two-way ANOVA, followed by Bonferroni multiple comparison test. For the analysis of social exploration, we used two-way repeated measures ANOVA, followed by Bonferroni multiple comparison test. Two-tailed unpaired or paired t-test was used for statistical analysis of: number of responses, learning index, preference index shown in **Fig. 23d,f and Fig. 24a-d and Fig. 28b,d**. Mice were assigned to altruistic or selfish groups using one sample t-test to chance (50%). The accepted value for significance was $P < 0.05$. Statistical analyses were performed using GraphPad Prism 7. Numbers of mice are reported in the figure legends. Data distribution was tested using D'Agostino and Pearson normality test. No statistical methods were used to predetermine sample size for single experiments. The animal number was based on estimation from previous studies. Littermates were randomly assigned to the different groups. Experimenters were not blinded during experimental sessions and data acquisition, but they were during data analyses and all analyses were performed with blinding of the experimental conditions as stated in the methods section.

RESULTS

1. Mice express a refined social decision-making process such as altruism by direct learning

To test whether mice are capable of acting for the intentional benefit of other conspecifics, we first devised a SDM for mice that was equivalent to the human dictator game, one of the most prominent game-theoretical paradigms that have been designed to test altruism (Lee, 2008; Sanfey, 2007; van Dijk and De Dreu, 2021). We expanded a standard operant cage with an adjacent compartment, separated by a metal mesh, in which to host a recipient that would receive food rewards depending on the choice made by the dictator (ie, the actor mouse). The recipient was a passive player with a chance to receive a food reward from a magazine, depending on the actor's choice. To promote food-seeking behavior, at the start of the test, both the actors and the recipients were maintained at 90% of their free-feeding body weights. The actors were presented with a two-choice decision-making paradigm, in which nose poking resulted in either food rewards for themselves only (ie, the selfish choice) or for both themselves and the recipient (ie, the altruistic choice) (**Fig. 12a**).

Adult mouse littermates, three to six months-old, both males and females, were housed in same sex-pairs for at least two weeks before the start of testing. Animals were tested for five days, until they reached a stable performance for three consecutive days. We compared this condition against a control group of actor mice without the presence of a recipient (ie, no recipient condition) (**Fig. 12a**). The SDM structure and experimental design was identical between these three conditions. Thus, any differences in the response could be attributed to the influence of the recipient. At the group level, we found that actor mice with recipients preferred to share food rewards (ie, altruistic choices) more frequently than not (ie, selfish choices), exhibiting a positive decision preference score compared with that of mice in the no recipient condition, which did not display any choice preference (**Fig. 12b**). The location of the nose poke associated with the altruistic responses, shown in percentages, did not modify mouse social preference (**Fig. 12b**). Mice showed an increased number of altruistic over selfish responses when a recipient was present, whereas the mice in the no recipient condition mice chose equally between the two nose pokes (**Fig. 12c**). Following the last session (ie, day 5), we replaced the recipient mice with an inanimate object (ie, the toy condition) (**Fig. 12a,d**) and tested the actors to determine whether any changes to their

preference could be detected in the absence of a social partner. During this condition (ie, day 6), the actors decreased their decision preference score (both altruistic and selfish) in the presence of an inanimate object when compared against their behavior in the presence of the recipient (**Fig. 12d**). These results confirmed that the expression of the preference for altruistic or selfish choice was contingent on the presence of a conspecific.

We observed marked individual differences in the responses of the mice across days. We analyzed the performance of each actor separately and found that eleven of sixteen mice showed a significant increase in altruistic responses, more frequently than could be explained by chance (**Fig. 12e,f**), whereas the remaining five mice showed a significant decrease in altruistic responses (**Fig. 12e,f**). Altruistic and selfish mice in the test condition that included a recipient showed significantly different choices starting on the second day of testing (**Fig. 12g**). For the majority of altruistic mice, more than 80% of their total responses were of altruistic type (**Fig. 12h**).

Next, we asked whether sex influenced altruistic behavior during the SDM. We analyzed pairs of males and females separately. All actors, both males and females, displayed a clear social preference (**Fig. 13a**). At the group level, males displayed a significant preference for altruistic over selfish responses and only one male mouse of eight did not prefer to allocate food rewards to his recipient (**Fig. 13a,b**). In contrast, the females did not show an overall preferential choice (**Fig. 13a**). Among the eight tested pairs, half of the females displayed a preference for altruistic choices, whereas the other half made selfish choices (**Fig. 13a,b**). When the social partner was present, only males showed a preference for the altruistic responses (**Fig. 13c,d)**.

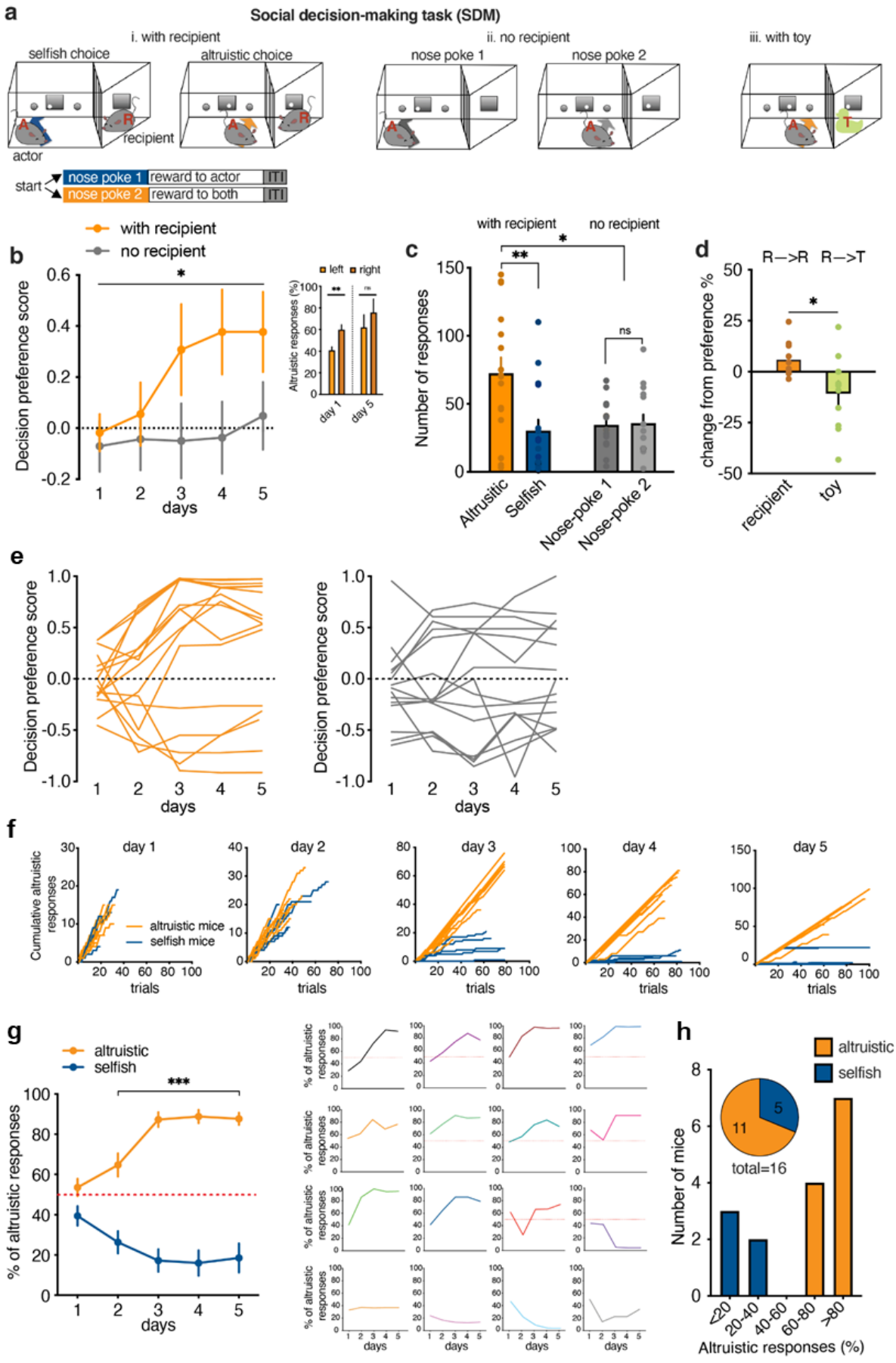

a
Social decision-making task (SDM)
i. with recipient
selfish choice
altruistic choice
recipient
actor
ii. no recipient
nose poke 1
nose poke 2
iii. with toy
start
nose poke 1
reward to actor
ITI
nose poke 2
reward to both
ITI
b
with recipient
no recipient
Decision preference score
days
left
right
Altruistic responses (%)
day 1
day 5
c
with recipient
no recipient
Number of responses
Altrusitic
Selfish
Nose-poke 1
Nose-poke 2
d
R—>R
R—>T
change from preference %
recipient
toy
e
Decision preference score
days
f
day 1
day 2
day 3
day 4
day 5
Cumulative altruistic responses
altruistic mice
selfish mice
trials
g
altruistic
selfish
% of altruistic responses
days
h
altruistic
selfish
Number of mice
total=16
Altruistic responses (%)

Fig. 12 | Mice are able of altruistic behavior by direct learning (adapted from Scheggia, La Greca et al., 2022). a, SDM experimental design. **b,** Decision preference score of mice tested with a recipient (orange) or no recipient (gray) (two-way repeated-measures (RM) ANOVA, group (with recipient, no recipient) × time (days 1–5): $F(4, 116) = 2.771$, $P = 0.0305$; the decision preference scores were found to fit a normal distribution across 5 days of testing (D'Agostino and Pearson normality test, with recipient: min $K2 = 3.122$, $P = 0.225$, $n = 16$; no recipient: min $K2 = 0.944$, $P = 0.623$, $n = 15$). Inset, altruistic responses on left ($n = 9$) and right ($n = 7$) nose pokes on day 1 (two-tailed unpaired t-test: $t = 3.37$, degrees of freedom (d.f.) = 14, $P = 0.0046$) and day 5 ($t = 0.79$, d.f. = 14, $P = 0.4419$). **c,** Number of nose pokes with a recipient ($n = 16$) and no recipient ($n = 15$; two-way RM ANOVA, group (with recipient, no recipient) × response (nose poke 1, nose poke 2): $F(1, 58) = 6.877$, $P = 0.0111$). **d,** Change of preference in an additional session with a recipient (R→R, $n = 10$) or with a toy (R→T, $n = 10$) (two-tailed unpaired t-test: $t = 2.24$, d.f. = 18, $P = 0.0374$). **e,** Individual decision preference scores in mice tested with (orange) or without (grey) recipient mouse over the five days of SDM task. **f,** Cumulative number of altruistic choices for each mouse (altruistic, orange; selfish, blue) during each daily session in the SDM task. **g,** Altruistic responses (in %) in altruistic ($n = 11$) and selfish ($n = 5$) mice (two-way RM ANOVA, group (altruistic, selfish) x time (days 1-5), $F(4, 56)=21.55$, $P < 0.0001$) and individual scores of altruistic responses across five days of SDM. d, Number of tested mice grouped by percentage of altruistic responses. [$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

We next analyzed the preference for altruistic or selfish choices in a larger group of animals (n = 52 actor–recipient pairs). We replicated the SDM task several times in naïve and virus-injected mice, for later chemogenetic experiments, and confirmed similar results to our initial findings (**Fig. 13e**). More than 70% of adult male mice displayed a preference for altruistic choices (**Fig. 13e**). However, the percentage of altruistic individuals drastically reduced when considering females (**Fig. 13e**). Taken together, these results highlight sex as a strong determinant for social decision-making processes.

2. Mouse altruistic behavior occurs even under costly conditions

To challenge the motivation of actor mice to allocate food rewards to their cage mates, we increased the cost of the altruistic decisions by reinforcing the responses at a fixed ratio (FR) of 2. Under this condition, two nose pokes were required to receive food together with the recipient, whereas only one nose poke was necessary for selfish responses (FR1) (**Fig. 14a**). We tested only those males and females mice that had previously demonstrated a significant preference above chance for altruistic responses after five days in the SDM (**Fig. 13a**). We similarly tested mice in the no recipient condition, in which their natural preference was set to FR2, whereas the other nose poke option was maintained at FR1 (**Fig. 14a**).

Both males and females displayed an increased number of altruistic responses over selfish responses, even when additional effort was required (**Fig. 14b,c**). Moreover, male FR2 responses (in percentage) were higher than those performed by mice tested without a recipient (**Fig. 14d**).

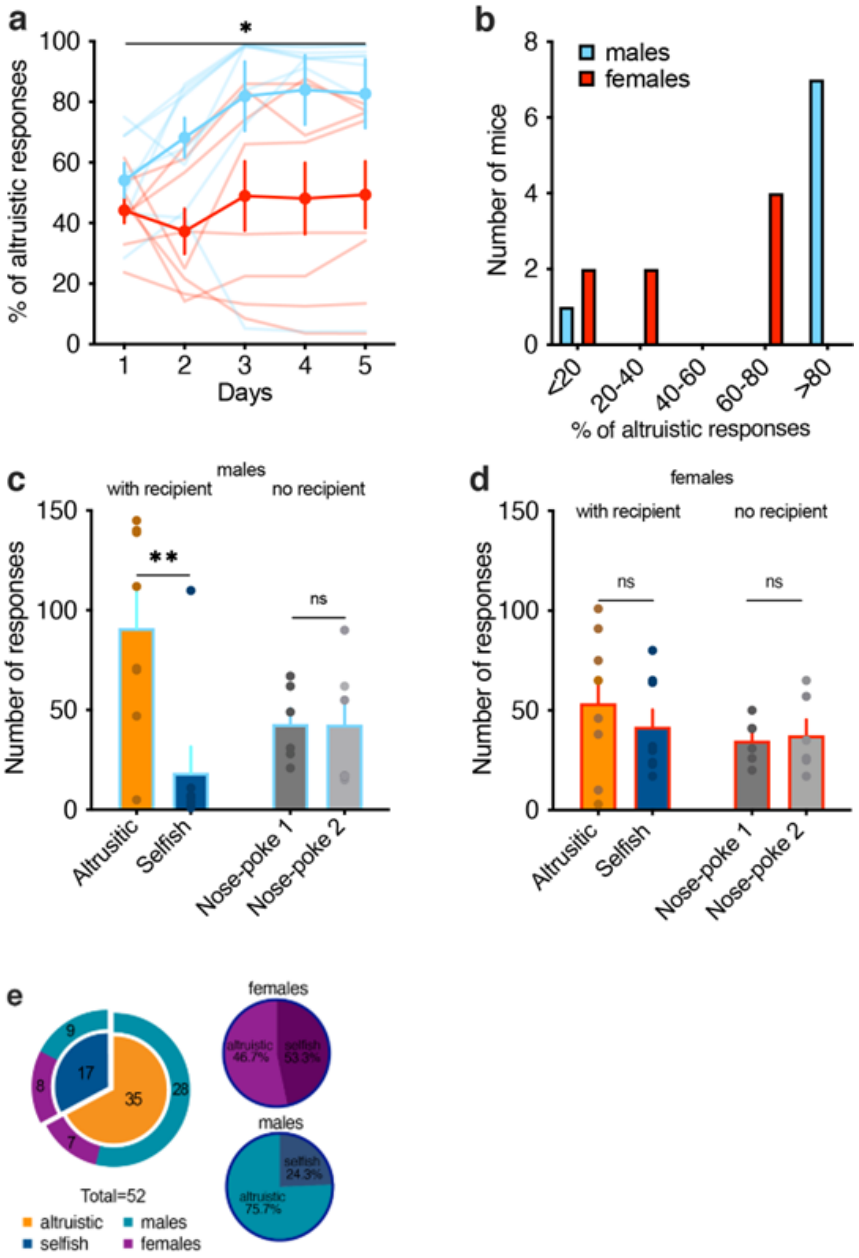


Fig. 13 | Adult male mice perform more altruistically than females (adapted from Scheggia, La Greca et al., 2022). a, Altruistic responses in males (n = 8) and females (n = 8) across five days of testing in the SDM (two-way RM ANOVA, gender, F(1, 14)=5.90, p = 0.0292; time (days 1-5), F(4, 56)=4.59, P = 0.0028). **b,** Number of tested mice grouped by gender and by percentage of altruistic responses. **c,** Number of nose pokes responses in male mice tested in the conditions with recipient (n = 8) and no recipient (n = 6) on day five of the SDM (two-way ANOVA, group (with recipient, no recipient) x response (nose-poke 1, nose-poke 2), F(1, 24)=6.2, P = 0.0199). **d,** Number of nose pokes responses in female mice tested in the conditions with recipient (n = 8) and no recipient (n = 6) (two-way ANOVA, group (with recipient, no recipient), F(1, 12)=4.1, P = 0.0630). **e,** The total number of mice grouped by preference and sex. [*P < 0.05, **P < 0.01,*** P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

This difference was not confounded by the baseline number of nose poke responses (**Fig. 14b**, **inset**). We then further increased the effort necessary to perform an altruistic action by

increasing these responses to a FR4. Under this condition, males showed increased altruistic responses compared to both females and to mice without recipients (**Fig. 14b,c**). Females did not show a preference between the two responses and mice without a recipient switched their preference to nose poke reinforced at FR1 (**Fig. 14b,c**). When the altruistic responses were reinforced to FR6, the females switched their preference to the nose poke that delivered food rewards more easily, whereas males continued to prefer altruistic responses (**Fig. 14b,c**). Finally, we tested males only in additional sessions to observe when a switch to the non-preferred response would occur. At FR8, male mice switched their preference to selfish responses, although they performed a similar number of nose pokes for both FR1 and FR8 (**Fig. 14d**). These results suggest that adult male mice preferred to share food rewards to benefit their cage mates, even under costly conditions.

We then tested whether actors would give food rewards to the recipients even if they did not receive a concurrent reward for themselves (ie, no concurrent reward). We first trained mice for five days in the SDM and then we modified, on day 6 and 7, the paradigm such that one nose poke resulted in food rewards for themselves only (ie, selfish choice) and the other to the recipient only (ie, altruistic choice) (**Fig. 14e**). We tested mice in two longer sessions (120min) to observe the effects of satiety on their choices. Although both groups of mice displayed a high percentage of selfish choices, this preference was reduced in altruistic mice (**Fig. 14e**). Moreover, while altruistic mice completed most of the altruistic choices in the first part of the session, selfish mice decided to give rewards later in the session (**Fig. 14f**), likely due to satiety. Altogether, these results suggest that mice were willing to help their conspecifics, even in the absence of a concurrent food reward for themselves.

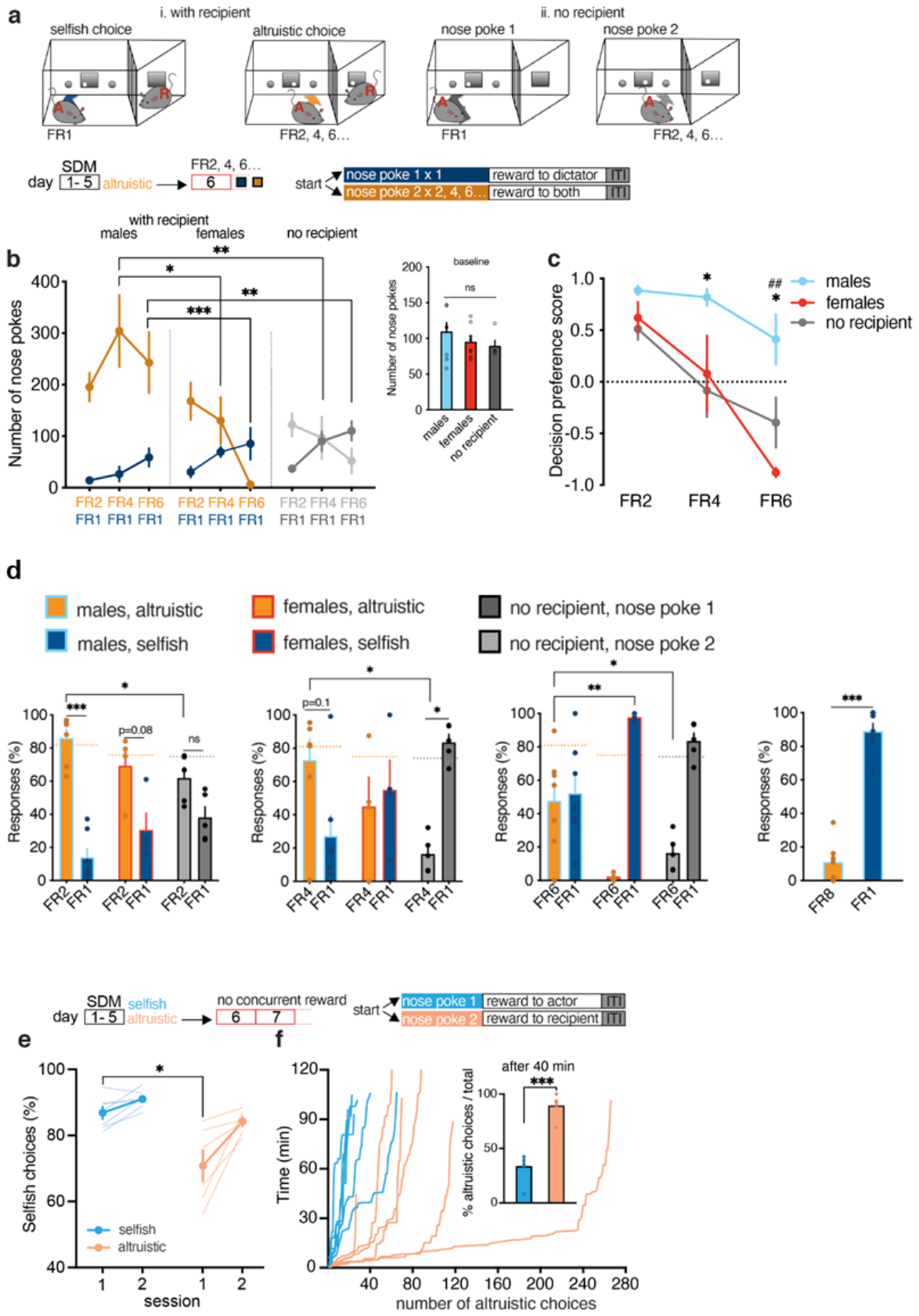


Fig. 14 | Altruistic behavior occurs even under costly conditions (adapted from Scheggia, La Greca et al., 2022). a, SDM experimental design with a different FR schedule. **b,** Left, the number of nose pokes on FR1 versus FR2, FR4 and FR6 in male (n = 7) and female (n = 4) actors and actors tested without a recipient (n = 5)

(between groups: two-way RM ANOVA, group (with recipient males, with recipient females, no recipient) × response (FR2, FR4, FR6): F(10, 52) = 4.25, P = 0.0002; within groups: two-way RM ANOVA, group (with recipient males, with recipient females, no recipient) × response (FR2, FR4, FR6): F(4, 26) = 4.48, P = 0.0069). Right, the number of nose pokes on SDM day 5 (one-way ANOVA: F(2, 13) = 0.67, P = 0.5270). **c,** Decision preference scores with FR2, FR4 and FR6, compared to FR1, in mice tested with a recipient (male, n = 7; female, n = 4) and without a recipient (n = 5) (two-way ANOVA, group (with recipient males, with recipient females, no recipient) × response (FR2, FR4, FR6): F(4, 26) = 3.55, P = 0.0193), P = 0.0265 (FR4) and P = 0.0678 (FR6) for males versus no recipient and P = 0.0010 versus females. **d,** Altruistic responses (orange) reinforced on FR2, FR4 and FR6 and selfish responses (blue) reinforced on FR1 expressed as percentage of the total in males (light blue, (n = 7) and females (red, n = 4) mice and responses on the preferred nose poke (NP1, dark grey) reinforced on FR2, FR4 and FR6 and responses on the non-preferred nose poke (NP2, light grey) reinforced on FR1 in mice tested without recipient (n = 6) (FR2: two-way RM ANOVA, group (with recipient males, with recipient females, no recipient) x response (FR1, FR2), F(2, 13)=3.5, P = 0.05. FR4: two-way RM ANOVA, group (with recipient males, with recipient females, no recipient) x response (FR1, FR2), F(2, 13)=5.1, P = 0.0192; FR6. two-way RM ANOVA, group (with recipient males, with recipient females, no recipient) x response (FR1, FR2), F(2, 13)=6.6, P = 0.0103. FR8: two-tailed unpaired t-test, t = 8.32, d.f.=6, P = 0.0002). **e,f,** Following SDM training, altruistic choices did not result in a concurrent reward for the actor. Shown are the percentage of selfish choices **(e)** (two-way RM ANOVA, group (selfish, altruistic) × time (sessions 1–2): F(1, 11) = 4.90, P = 0.0488) and the number of altruistic choices **(f)** over 120 min of SDM in mice grouped by selfish (n = 7) or altruistic (n = 6) preference (inset, percentage of altruistic choices in the first 40 min/total number of altruistic choices; two-tailed unpaired t-test: t = 8.17, d.f. = 10, P = 0.0001). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

3. Social contact, familiarity and recipient's hunger state strongly bias altruistic behavior

To test whether social contact or social interaction modulated the actors' social preference, we replaced the metal mesh with a transparent or an opaque partition dividing the two compartments. The transparent partition allowed visual, olfactory and auditory stimuli but prevented social contact. The opaque partition, instead, blocked either visual and social contact information, only allowing the passage of olfactory and auditory stimuli. We used a new cohort of mice for testing these conditions, one group with the metal mesh, another with the transparent partition and the last with the opaque partition. Mice tested in the presence of an opaque partition almost performed at chance, at the group level, during the SDM, with the majority changing many times their preference for the nose pokes (**Fig. 15a**). Instead, an analysis of individual performances revealed that mice tested with the transparent partition established a clear preference for one of the two options, even though the group splitted in half in social preference (ie, eight altruistic mice and eight selfish) (**Fig. 15a**). Additionally, to determine whether social interactions in the proximity of the divider between the actor and recipient compartments might have influenced the actors' decisions, we measured the time spent on social exploration in a previous cohort of mice tested with the metal mesh (ie, the original SDM design) and found that altruistic actor mice spent more time exploring their recipient than selfish ones (**Fig. 15b**). This was evident from the first session of testing, and

this pattern was maintained until the last session (Day 5). In contrast, we did not observe any differences in the social exploration by the recipients (**Fig. 15c**). Importantly, we found that social exploration of the actor mice during the first day of testing was positively correlated with the altruistic responses on the last day of testing, at which point the actors displayed a consistent behavioral preference (**Fig. 15d**). All together, these findings suggested that mice use both social visual cues and social contact to establish their social preference, in particular social contact and social interaction represent essential determinants for developing an altruistic behavior.

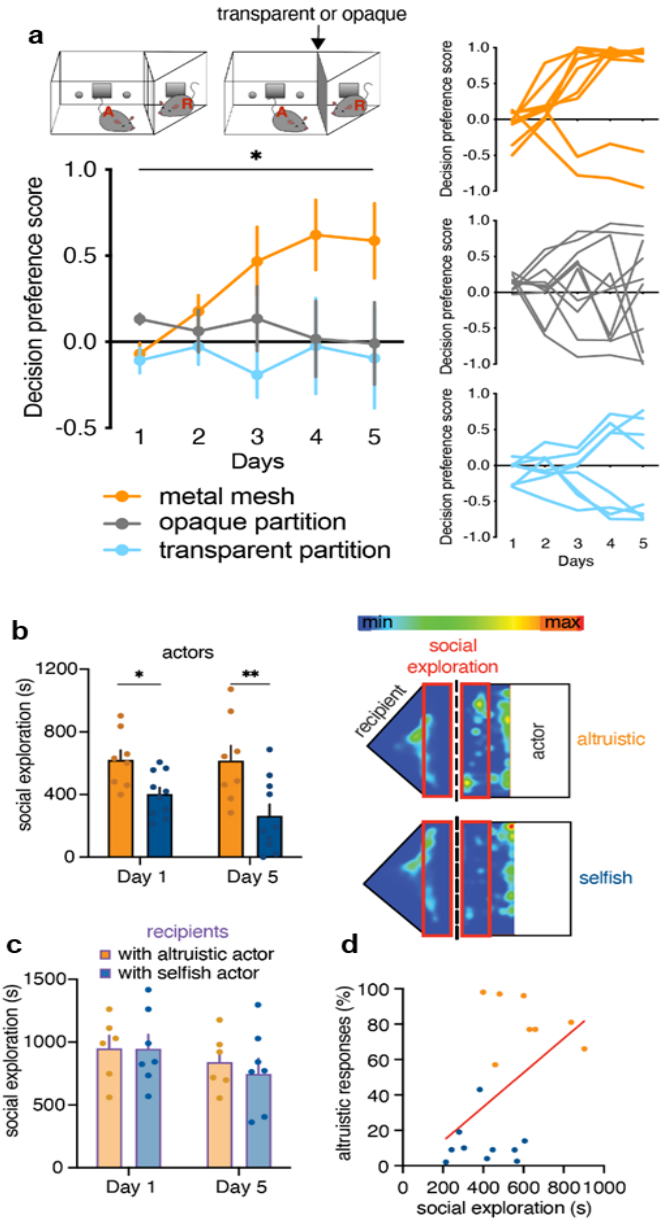


Fig. 15 | Social contact and partner's exploration drive altruistic behavior (adapted from Scheggia, La Greca et al., 2022). a, Left, decision preference scores in mice tested with a metal mesh (orange, n = 10), a

transparent partition (light blue, n = 8) or an opaque partition (gray, n = 8) (two-way RM ANOVA, group (metal mesh, transparent partition, opaque partition) × time (days 1–5): F(8, 100) = 2.037, P = 0.0494). Right, individual curves representing decision preference score. **b,** Left, social exploration of altruistic (orange, n = 8) and selfish (blue, n = 10) actors toward their recipients during SDM days 1 and 5 (two-way ANOVA, group (altruistic, selfish): F(1, 32) = 16.29, P = 0.0003). Right, schematic of the testing chambers. **c,** Social exploration of recipients toward altruistic (orange, n = 6) or selfish (blue, n = 7) actors during SDM days 1 and 5 (two-way ANOVA, group (altruistic, selfish): F(1, 11) = 0.16, P = 0.6902;). **d,** Correlation between social exploration on day 1 and preference for altruistic choices on day 5 (linear regression: r = 0.4890, P = 0.039, n = 18 pairs). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

Familiarity between individuals is known to amplify prosocial behaviors (Bartal et al., 2011; Burkett et al., 2016). To test whether social closeness affects the willingness to allocate food to others, in a new cohort of male mice we tested the actions of actors in response to unfamiliar recipients that were housed in different cages. We found that actors tested in the presence of unfamiliar recipients showed opposite choices compared with actor mice tested in the presence of familiar recipients (**Fig. 16a,b**). To determine individual differences in the responses across animals, we analyzed the performance of each actor. Under the condition featuring an unfamiliar recipient, we found that nine mice of fifteen showed a significant increase in the number of selfish responses (**Fig. 16c**), whereas only three mice acted altruistically. Three mice did not show any preference (**Fig. 16c**). The distribution of mouse preferences showed that fewer than 20% of responses were altruistic for the majority of mice (**Fig. 16c**). Thus, when the actor mice were presented with unfamiliar individuals, they acted more selfishly than actors paired with familiar. These data indicated that familiarity facilitates altruistic choices in mice.

To understand how the recipients' hunger state (ie, others' needs) could motivate altruistic behavior, we tested actor mice with sated or food-restricted recipients following the training in the SDM task (ie, test day 6) (**Fig. 16d**). Actor mice tested with sated recipients presented less altruistic responses (in percentage) compared to the day before when recipients were still food-restricted (ie, hunger state). In this sense, this group showed a greater change in their social performance or a greater difference in their altruistic behavior compared to the other group where dictators were performing with food-restricted recipients either on day 5 and 6 (**Fig. 16d**). After that, we also tested dictators, from a different cohort, either with food-restricted or sated recipients since the test started (**Fig. 16e)**. In line with the previous experiment, actor mice with food-restricted recipients (ie, hunger state) presented a greater altruistic behavior towards their companions in need immediately after the learning acquisition phase (ie, since day 3 onwards) (**Fig. 16e)**. Instead, mice with sated recipients decreased their altruistic tendency at the group level almost splitting half between the two

social outcomes (ie, altruistic and selfish behavior) (**Fig. 16e)**. These results suggest that the hunger state of the recipient is an important factor in the actor's decision to share food.

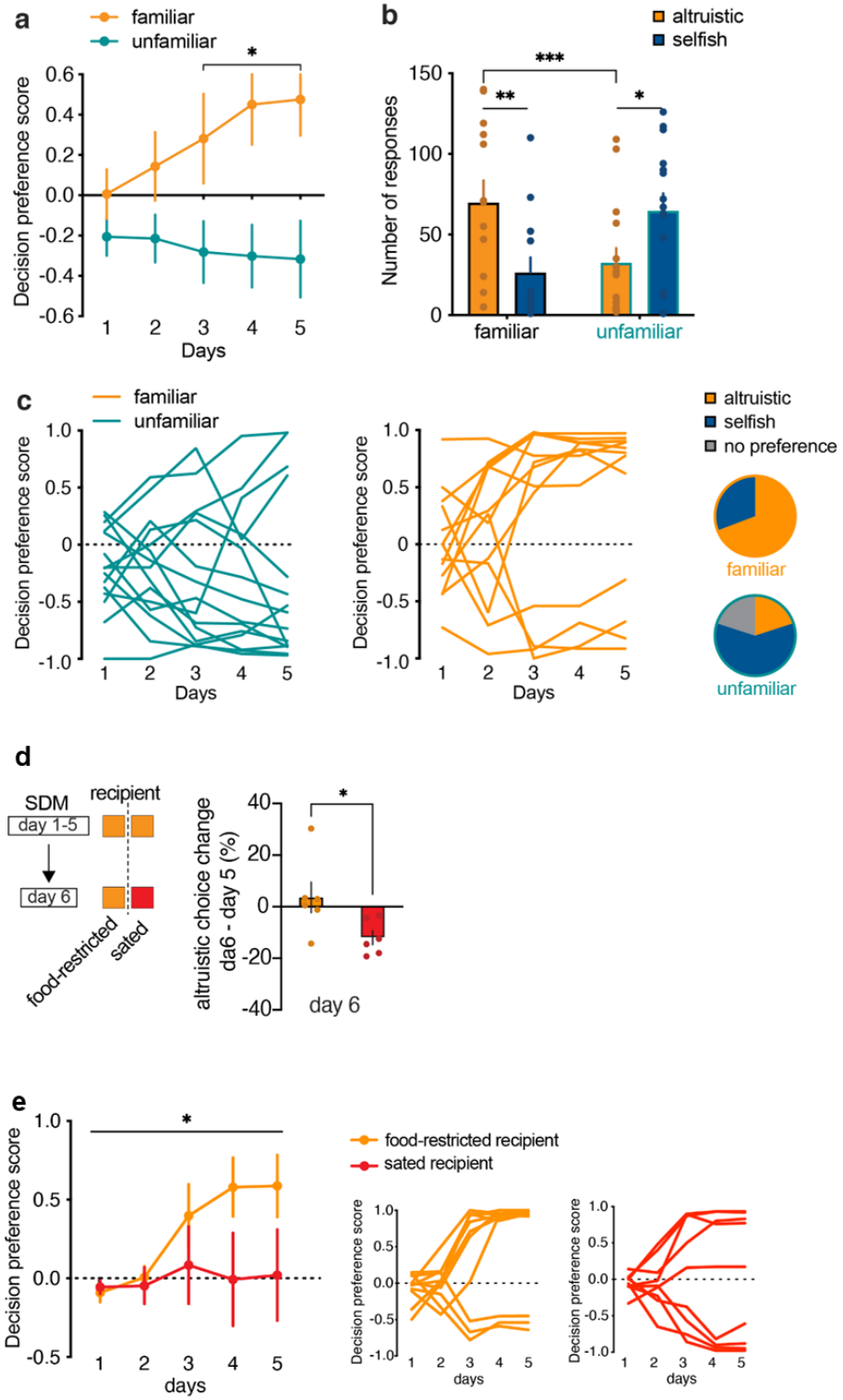


Fig. 16 | Recipient's familiarity and hunger state facilitate altruistic behavior (adapted from Scheggia, La Greca et al., 2022). a, Decision preference score in the 5 days of SDM in mice tested with familiar (orange; n = 13 (7 males, 6 females)) or unfamiliar (green; n = 15 (10 males, 5 females)) recipients (two-way RM ANOVA,

group (familiar recipients, unfamiliar recipients) × time (days 1–5): F(4, 104) = 2.707, P = 0.0342). **b,** Number of nose poke responses in the conditions with familiar (black border, n = 13) and unfamiliar (green border, n = 15) recipients (two-way RM ANOVA, group (familiar, unfamiliar) × response (altruistic, selfish): F(1, 52) = 12.03, P = 0.0011). **c,** Individual decision preference score in the SDM in mice tested with familiar or unfamiliar recipients and the number of mice tested with unfamiliar recipients (chi-square test: $\chi 2 = 5.99$, P = 0.0143). Mice were assigned as altruistic (orange), selfish (blue) or no preference (gray) using a one-sample t-test compared to chance (50%, red line). **d,** Following training in the SDM task actor mice were tested in an additional session with sated (red, n = 6) or food-restricted (orange, n = 6) recipient mice (two-tailed unpaired t-test: t = 2.37, d.f.=10, P = 0.0387). **e,** Left, decision preference score in mice tested with food-restricted (orange, n = 12) or sated (red, n = 9) recipient mice over the five days of SDM task (two-way RM ANOVA, group (sated, food-restricted) x time (days 1-5), F(4, 76)=2.62, P = 0.0409). Right, individual curves representing decision preference scores. [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

4. Social dominance and emotional contagion represent additional factors that motivate altruistic behavior

Social animals self-organize into hierarchies, where group members vary in their level of dominance, affecting social relationships (Cronin, 2012). To determine the impact of the hierarchical relation between animals within the same cage on the preference for altruistic choices we used the tube test, a robust assay in which one mouse forces its opponent out of a narrow tube and is classified dominant (**Fig. 17a**). Mice were tested pairwise using a round robin design, on daily sessions after the SDM task, and the social rank of each mouse was calculated, through the David's score (DS), on the basis of winning against the other cage mates, also considering the numerosity of each cage then the fights sustained. We analyzed the relationship between mouse altruistic behavior and social dominance in 39 actor-recipient pairs (**Fig. 17b**). In all cages the relation between mice was transitive and linear (α is more dominant over β, β more dominant than γ, γ more dominant over δ, then α is dominant over all the others) (**Fig. 17b, inset**). Overall, dominant actor mice displayed a higher decision preference score (ie, more altruistic choices) compared to subordinate actor mice (**Fig. 17c**). Specifically, the majority of actor mice being dominant were altruistic in the SDM (13 of 20), while the minority (7 of 19) belonged to the selfish group (**Fig. 17b**). Dominant altruistic actors had also higher DS than their recipients (**Fig. 17e**). By contrast, dominant selfish actors did not display a significant increase in DS compared to their recipients (**Fig. 17f**). Furthermore, dominant selfish mice suffered more losses in the tube test than altruistic (**Fig. 17f, inset**). Instead, subordinate mice either altruistic or selfish did not show any difference, both displaying lower social dominance compared to their recipients (**Fig. 17h,i**). Moreover, there was no difference in the number of losses between subordinate altruistic or selfish mice

(**Fig. 17i, inset**). These results indicate that mouse altruistic behavior is regulated by refined in-group dynamics involving the social status of each member.

Empathy refers to behavioral reactions to others' emotional states, including the motivation to help and the affective tendency to experience the emotions of others (Bartal et al., 2011; Decety et al., 2016; Scheggia and Papaleo, 2020; Keysers et al., 2022). Thus, we tested the hypothesis that the increased altruistic choices in familiar dominant mice could also relate to an increased affective state matching and comprehension regarding their subordinate individuals. To do this, we used an observational fear conditioning paradigm where mice can vicariously match the emotional state of their companion (**Fig. 17j**). Following the SDM task, actor mice and their recipients were placed in the two compartments of a double-chambered standard fear-conditioning apparatus, separated by a transparent partition. The actor mouse (ie, observer) was allowed to observe the recipient (ie, demonstrator) receiving repetitive foot shocks (**Fig. 17j**). We found that freezing behavior, which reflected the observational fear induced by social transmission, was higher in altruistic versus selfish mice (**Fig. 17j**). Both groups of mice spent a similar amount of time exploring the zone close to their conspecific demonstrator (**Fig. 17j**). Furthermore, the scores obtained in the observational fear learning positively correlated with social dominance (**Fig. 17k**). Altogether, these results indicate that altruistic mice, often occupying higher ranks in the social hierarchy (ie, a dominant position), are also moved in their altruistic tendency by greater emotional contagion and sharing towards their conspecifics, revealing empathy-like behaviors.

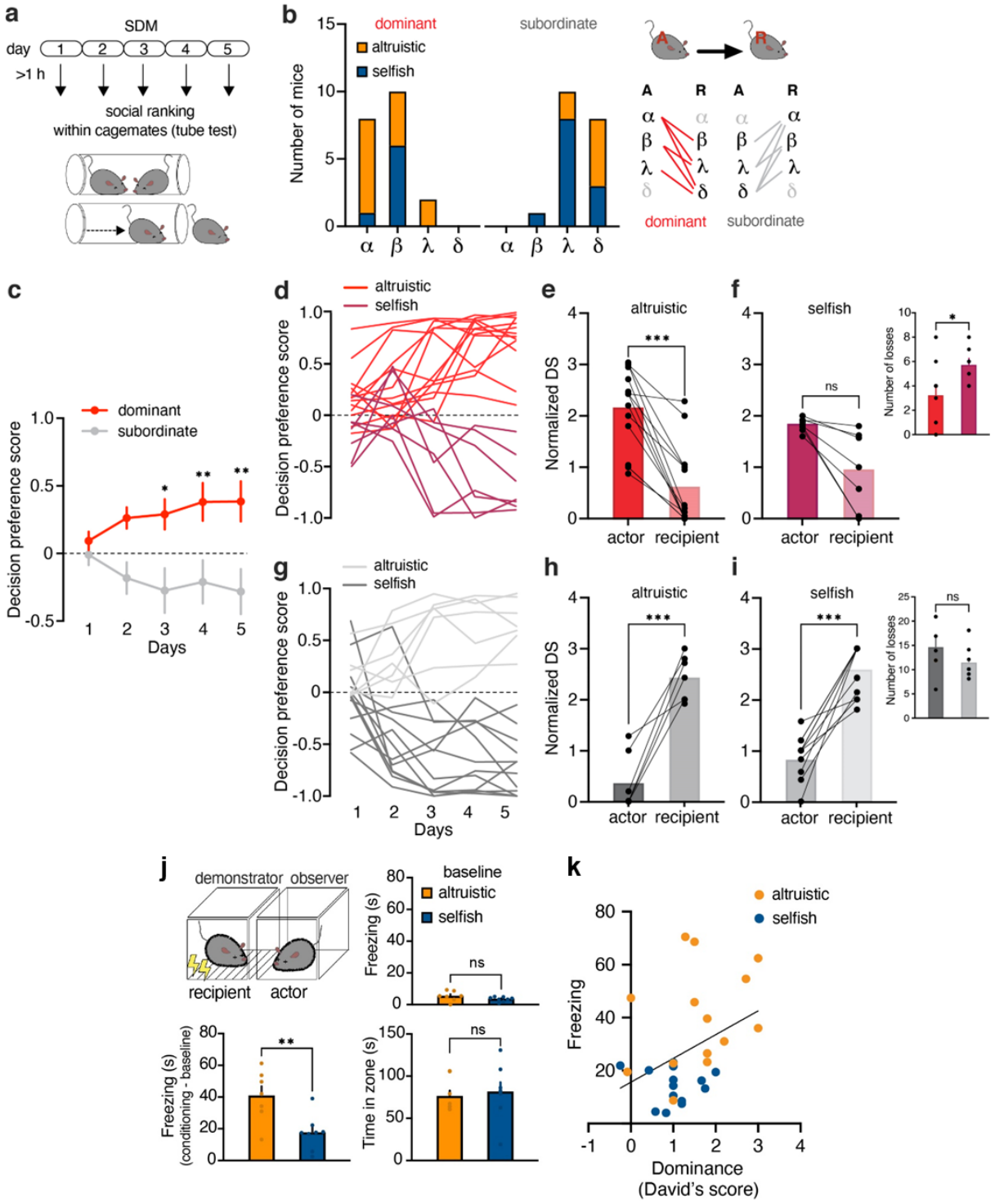


Fig. 17 | Social dominance and emotional contagion motivate altruistic behavior (adapted from Scheggia, La Greca et al., 2022). a, After the SDM daily session, mice were tested on the tube test (at least 1 h after SDM), to measure the hierarchical relationship of animals within the same cage. Actor and recipient mice were tested pairwise and using a round-robin design. **b,** Number of altruistic or selfish actor mice (A) that were dominant (red) or subordinate (gray) compared to the recipient (R) in the tube test (n = 39). **c,** Decision preference score of actor mice that were dominant or subordinate in the tube test compared to their recipient (two-way ANOVA: F(4, 148) = 3.46, P = 0.097; dominant, n = 20; subordinate, n = 19). d, Individual decision preference score in the SDM of dominant actor mice grouped by altruistic or selfish preference (n = 20). **e,f,** Social dominance (normalized DS) quantified based on the number and directionality of interactions in the tube

test in actor mice that were dominant compared to their recipient, grouped by altruistic (two-tailed paired t-test: t = 5.01, d.f. = 23,97, P < 0.0001; n = 13 pairs) **(e)** and selfish (two-tailed paired t-test: t = 2.27, d.f. = 6,87, P = 0.0576; n = 7 pairs) **(f)** preference. Inset, number of losses by dominant altruistic and selfish actor mice in the tube test (two-tailed paired t-test: t = 2.45, d.f. = 18, P = 0.0244). **g,** Individual decision preference score in the SDM of dominant actor mice grouped by altruistic or selfish preference (n = 19). **h,i,** Normalized DS in actor mice that were subordinate compared to their recipient, grouped by altruistic (two-tailed paired t-test: t = 7.66, d.f. = 11,39, P < 0.0001; n = 7 pairs) **(h)** and selfish (two-tailed paired t-test: t = 8.6, d.f. = 21,67, P < 0.0001; n = 12 pairs) **(i)** preference. Inset, number of losses by subordinate altruistic and selfish actor mice in the tube test (two-tailed paired t-test: t = 1.65, d.f. = 17, P = 0.1154). **j,** Top, Schematic representation of the observational fear learning and freezing behavior in actor mice, grouped by altruistic (n = 6) or selfish (n = 7) preference during baseline (two-tailed unpaired t-test: t = 15.31, d.f.=13, P = 0.1497). Bottom, freezing behavior (conditioning-baseline) in altruistic and selfish actors (two-tailed unpaired t-test: t = 3.30, d.f.=13, P = 0.0057) and total time spent in the proximity of the divider between the actor and recipient compartment (two-tailed paired t-test: t = 0.39, d.f.=13, P = 0.7021). **k,** Social dominance (normalized David's Score) predicts affective sensitivity (freezing behavior during observational fear learning) (linear regression, n = 27 mice, y = 8.971x + 15.61, F(1, 25)=4.47, P = 0.0446). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

5. BLA neuronal silencing reverts the preference for altruistic choices

As the BLA is strongly implicated in empathy-like and prosocial behaviors both in human and nonhuman animals (Allsop et al., 2018; Chang et al., 2015; Dal Monte et al., 2020; Ferretti et al., 2019; Gangopadhyay et al., 2021; Scheggia and Papaleo, 2020), we tested the effects of BLA silencing, via the chemogenetic tool, during the SDM task (**Fig. 18a,b**). For BLA neural silencing, we chronically administered CNO to both experimental groups (ie, control CNO and hM4D CNO group) for all the test duration, always 30min before starting the task as already explained before (see Materials and Methods section 3, par. 3.4) (**Fig. 18b**). We analyzed the decision preference score of the actor mice of both control CNO and hM4D CNO groups tested with familiar recipient mice.

We found that the control group showed higher decision preference score or greater altruistic behavior compared to the hM4D group (ie, the BLA-silenced group) (**Fig. 18c**). At the individual level, the majority of mice from the hM4D group (7 of 11) displayed selfish behavior, while most subjects from the control group (7 of 9) altruistic behavior (**Fig. 18c,d**). In particular, control mice showed an increased number of altruistic over selfish responses, whereas mice with BLA-silencing did not show any difference between the two choices (**Fig. 18e**). This result was particularly evident at the end of the training in the SDM task (**Fig. 18f**). Finally, BLA silencing did not affect the number of responses, latency to make a choice and locomotor activity during the SDM (**Fig. 18g-i**). Thus, these results indicate that the BLA is specifically required to express social decisions and associated (pro)social behaviors like altruism that greatly increase the benefit of others.

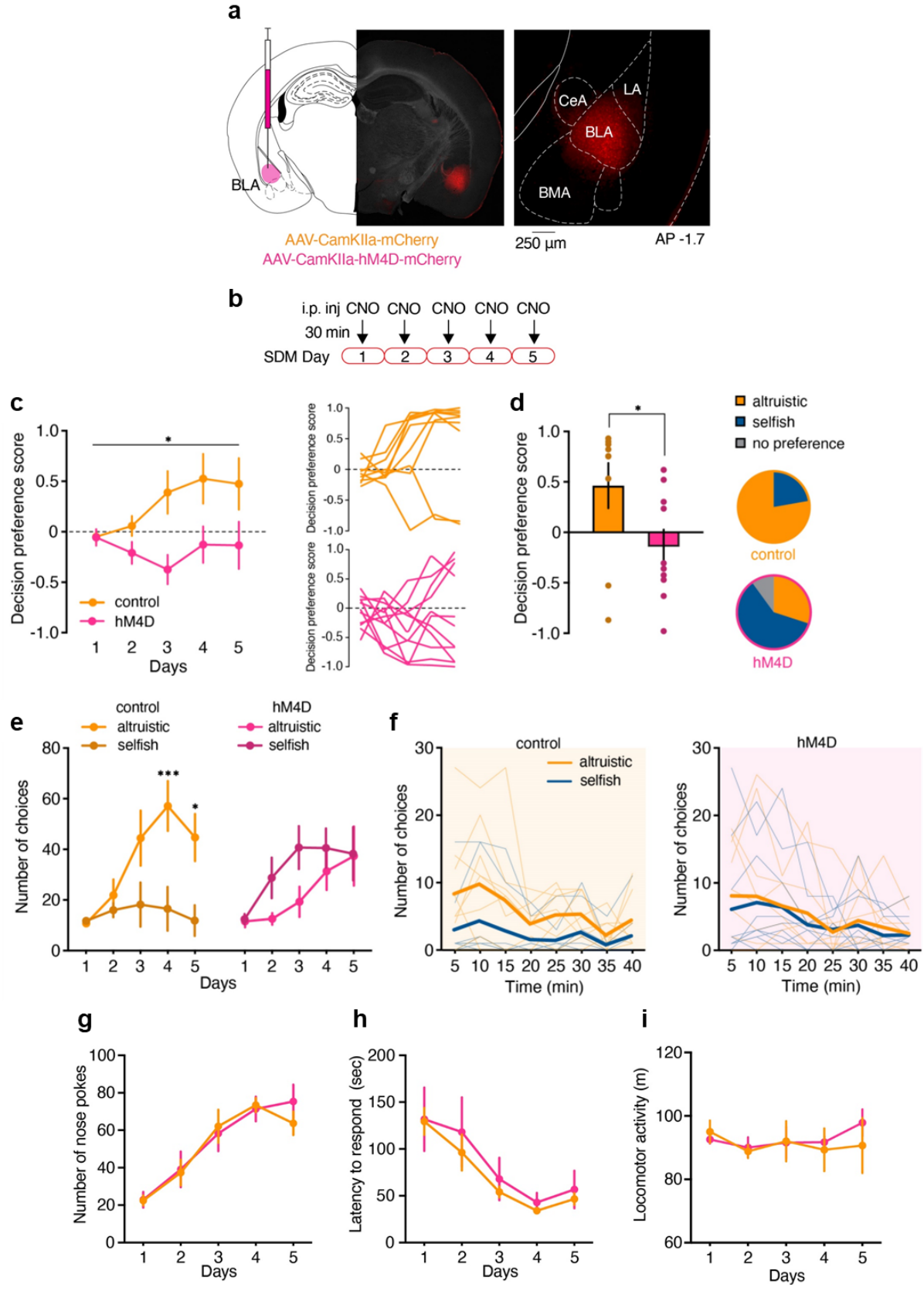


Fig. 18 | BLA neuronal silencing impairs mouse altruistic behavior (adapted from Scheggia, La Greca et al., 2022). a, Male mice were bilaterally injected in the BLA with AAV-CamKIIa-mCherry (control, orange) or

AAV-CamKIIa-hM4D-mCherry (hM4D, fuchsia). Representative image of a coronal section of BLA. **b,** Thirty minutes before the daily SDM session, control and hM4D mice received an intraperitoneal (i.p.) injection of CNO. As a control, we also tested hM4D animals that received vehicle. As we did not observe differences, we pooled the control animals together (two-tailed unpaired t-test: t = 0.927, d.f. = 8, P = 0.3810). **c,** Left, decision preference score in the 5 days of SDM in control (n = 9) and hM4D (n = 10) mice (two-way RM ANOVA, group (control, hM4D) × time (days 1–5): F(12, 140) = 1.981, P = 0.0301; one-sample t-test compared to chance (0.0): control: t = 3.146, d.f. = 44, P = 0.0030; hM4D: t = 1.730, d.f. = 49, P = 0.0899). Right, individual decision preference score in SDM of control and hM4D mice. **d,** Average decision preference score across 5 days of SDM (two-tailed paired t-test: t = 2.175, d.f. = 17, P = 0.0440) and number of control (n = 9) and hM4D (n = 10) mice displaying preference for altruistic or selfish choices. **e,** Number of altruistic and selfish choices in control (two-way RM ANOVA, choice (altruistic, selfish) × time (days 1–5): F(4, 64) = 5.0, P = 0.0013, n = 9) and hM4D (choice (altruistic, selfish) × time (days 1–5): F(4, 80) = 1.5, P = 0.2024, n = 10) mice over 5 days of the SDM task. **f,** Representation of altruistic and selfish choices at the end of the training in the SDM task (day 5) in control (left) and hM4D (right) mice. **g,** Number of nose pokes in control (n = 9) and hM4D (n = 10) mice (two-way ANOVA, group (control, hM4D), F(1, 13)=0.54, p = 0.4721). **h,** Latency to respond in control (n = 8) and hM4D (n = 9) mice (two-way ANOVA, group (control, hM4D), F(1, 11)=0.02, p = 0.877). **f,** Locomotor activity (two-way ANOVA, group (control, hM4D), F(1, 11)=0.10, p = 0.7566), during the five days of testing in the SDM task in control (n = 6) and hM4D (n = 7) mice. [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

6. BLA silencing reduces social dominance and abolishes emotional contagion

Social hierarchy influenced the preference for altruistic or selfish choices and the BLA is deeply involved in this aspect according to literature (Ligneul et al., 2017; Zhou et al., 2017, 2018). Thus, we tested whether the BLA could be linked also to the representation of social ranks. To do this, mice with CNO-induced BLA-silencing and control CNO mice were tested after the SDM in daily sessions in the tube test for the assessment of hierarchical relations (**Fig. 19a**). All the recipient mice received, as control, AAV-CamKIIa-mCherry virus and were injected with CNO (**Fig. 19a**). We found that silencing of the BLA in hM4D CNO mice significantly decreased the dominance compared to control CNO mice (**Fig. 19b,c**). Specifically, a higher number of hM4D CNO actor mice was subordinate to their recipient conspecific, differently from what occurred in controls (**Fig. 19d,e**). Consistent with our findings linking altruistic decision preference with hierarchy status, these experiments provide initial evidence of the BLA as a common hub in the determination of social dominance, that is an important modulator of social decision making processes.

Encoding of information needed for social transfer have been reported to depend on neuronal projections from the anterior cingulate cortex to the BLA (Allsop et al., 2018; Chang et al., 2015). Thus, we first tested whether BLA downregulation could change the capacity to be affected by others affective state, using the observational fear conditioning paradigm (**Fig. 19f**). Similarly to the experiments on social dominance, we used a chemogenetic approach to target the glutamatergic neurons in the mouse BLA and through the use of inhibitory DREADDs (ie, hM4D) further silencing this. Control mice were injected

with AAV-CaMKIIa-mCherry but still receiving CNO (ie, control group). We found a significant reduction of freezing behavior during the conditioning phase in hM4D compared to control mice (**Fig. 19g,h**), suggesting reduced social transmission of emotions. Thus, consistent with a previous study (Allsop et al., 2018), we showed that the BLA is critically implicated in emotional state matching. Merging this result with those on social dominance, we revealed the BLA as an area of election for the conveying and the modulation of information regarding others' social status and affective states that represent essential information modulating social decision-making processes.

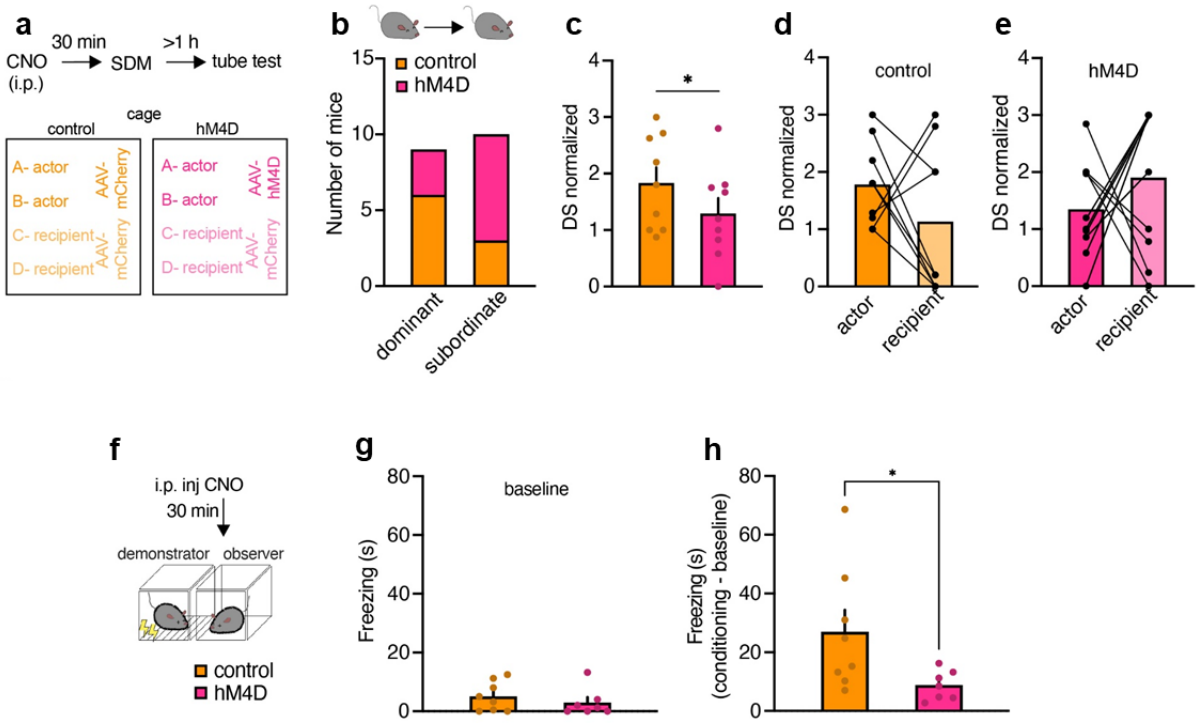


Fig. 19 | BLA silencing reduces social dominance and emotional contagion (adapted from Scheggia, La Greca et al., 2022). a, Left, control and mice that received hM4D for BLA silencing were injected with CNO (3 mg/kg) 30 minutes before the SDM task. At least 1 hour after daily session, mice were tested in the tube test for assessment of social ranking within cage mates. Right, cage composition. Each cage hosted 2 actor-recipient pairs. Actor mice received hM4D or control virus in the BLA. All the recipients received the control virus. **b,** Number of dominant or subordinate actor mice compared to their recipient conspecific (n = 19; two-sided Fisher's exact test p = 0.1789). **c,** Social dominance (normalized David's Score) quantified based on the number and directionality of interactions in the tube test in actor grouped by control (n = 9) and hM4D (n = 9) mice (two-tailed paired t-test: t = 2.15, d.f.=14, P = 0.0493). **d,e,** Social dominance (normalized David's Score) quantified based on the number and directionality of interactions in the tube test in **(d)** control (two-tailed paired t-test: t = 1.30, d.f.=16, P = 0.2120) and **(e)** hM4D actor mice (two-tailed paired t-test: t = 1.331, d.f.=14, P = 0.2045). **f,** Observers mice received intraperitoneal (i.p.) injection of CNO (3 mg/kg) and after 30 minutes were tested with their respective demonstrators on the observational fear learning paradigm. **g,h,** Freezing behavior displayed by actor mice, control (n = 8) and hM4D (n = 7), during baseline (**g,** two-tailed unpaired t-test: t = 0.83, d.f.=13, P = 0.4170) and conditioning phases of the test (**h,** two-tailed unpaired t-test: t = 2.22, d.f.=13, P = 0.0447). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

7. BLA-PFC reciprocal connections play different roles in social decision-making

The BLA has many inputs and outputs mediating different types of learning and supporting circuits involved in valence and emotional processing of external stimuli (Allsop et al., 2018; Chang et al., 2015; Felix-Ortiz et al., 2016; Gangopadhyay et al., 2021). Prefrontal cortex (PFC) subregions are among the major targets of the BLA, but also one of the major sources of top-down inputs (Ko, 2017; Yizhar and Klavir, 2018). We targeted the prelimbic (PL) region of the PFC, which supports goal-directed behaviors and is implicated in the initiation and modulation of different social behaviors, including those based on social decision-making processes such as other-regarding preference (Dal Monte et al., 2020; Gangopadhyay et al., 2021). For this reason, we chose to investigate via chemogenetic silencing either projections from the BLA to the PL (ie, hM4D BLA→PL) or vice versa (ie, hM4D PL→BLA) (**Fig. 20a**).

We found that silencing of BLA to PL projections abolished preference for altruistic choices (**Fig. 20b,c**), similarly to what we found following the silencing of the BLA. While in the control group the majority (7 of 10) showed a preference for altruistic choices (**Fig. 20c,d**), in hM4D BLA→PL mice the preference was equally distributed between selfish and altruistic choices, and in three mice we did not observe any preference (**Fig. 20c,d**). In line with these results, we found a significantly increased number of altruistic choices compared to selfish ones in control mice (**Fig. 20h**), which was not observable in hM4D BLA→PL mice (**Fig. 20h**), this result particularly evident at the end of the SDM (**Fig. 20e,f**). Besides, mice receiving hM4D in the PL for silencing projections to the BLA (ie, hM4D PL→BLA group) displayed a negative decision preference score (**Fig. 20b,c**), indeed the majority expressed a selfish preference (3 of 9) (**Fig. 20c,d**) and exhibited a significant increase of selfish choices (**Fig. 20h**), particularly evident at the end of the SDM (**Fig. 20g**).

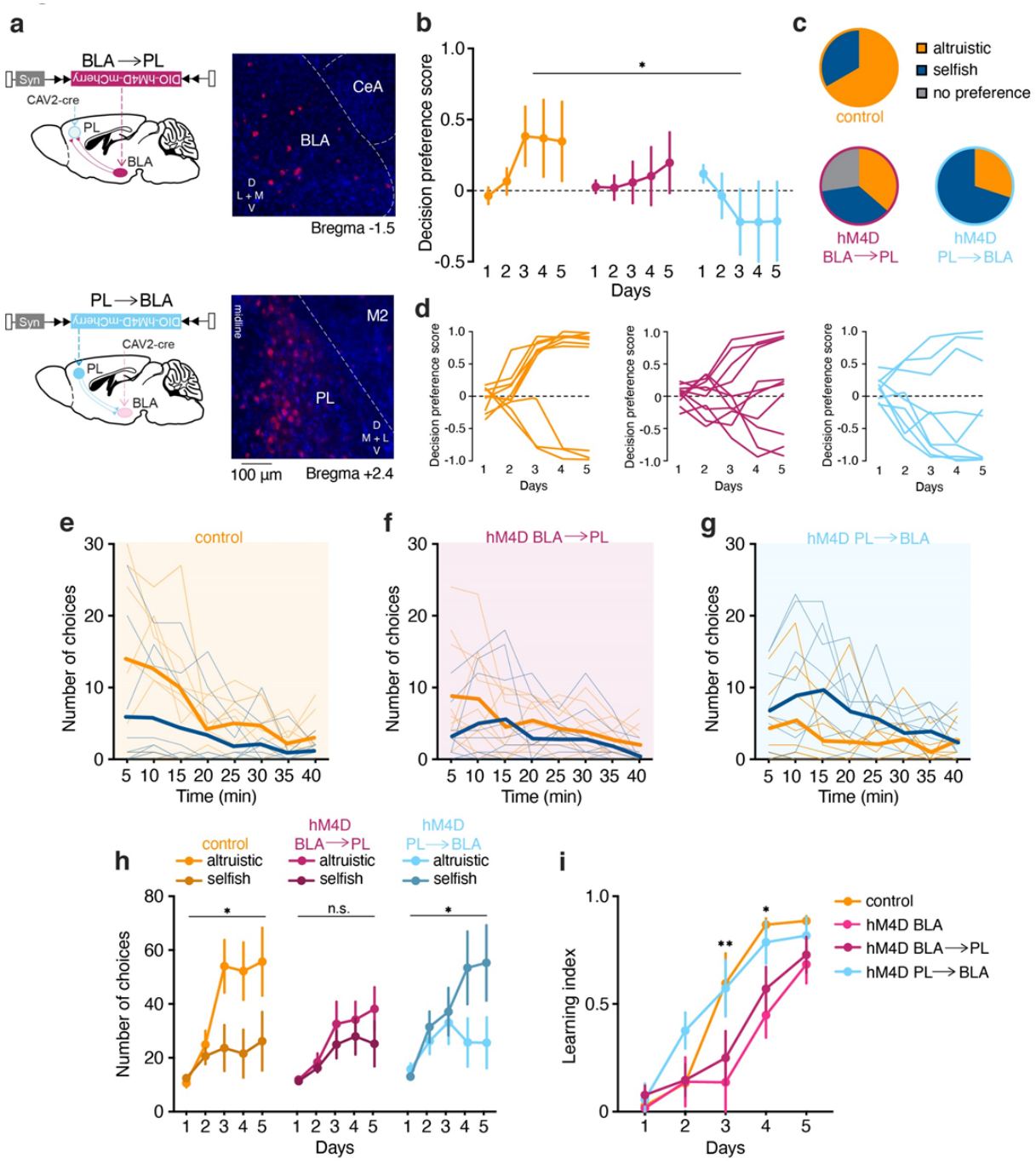


Fig. 20 | BLA-PFC reciprocal connections play different roles in social decision-making (adapted from Scheggia, La Greca et al., 2022). a, Schematic showing viral injection and projection areas and example images of coronal sections of BLA and PL. Mice received virus encoding Cre-dependent hM4D receptor in the BLA and CAV2-Cre in the PL or Cre-dependent hM4D receptor in the PL and CAV2-Cre in the BLA. With this combination, we achieved DREADD expression exclusively in BLA neurons projecting to the PL (hM4D BLA→PL) and vice versa (hM4D PL→BLA). CeA, central amygdala; M2, secondary motor cortex. **b,** Decision preference score in the 5 days of SDM in control CNO (orange, n = 10), hM4D BLA→PL (purple, n = 11) and hM4D PL→BLA (light blue, n = 9) mice (two-way RM ANOVA, group (control CNO, hM4D BLA→PL, hM4D PL→BLA) × time (days 1–5): $F(8, 108) = 2.03$, $P = 0.0493$). **c,** The number of mice displaying preference for altruistic or selfish choices. Mice were assigned as altruistic (orange), selfish (blue) or no preference (gray) by analyzing decision preference scores using a one-sample t-test compared to chance. **d,** Individual decision preference score in the SDM of control CNO and hM4D BLA CNO mice. **e–g,** Representation of altruistic and selfish choices at the end of the training in the SDM task (day 5). **h,** Number of

altruistic and selfish choices in control CNO (two-way RM ANOVA, choice (altruistic, selfish) × time (days 1–5): F(4, 72) = 3.6, P = 0.0088, n = 10), hM4D BLA→PL (choice (altruistic, selfish) × time (days 1–5): F(4, 64) = 2.6, P = 0.0401, n = 11) and hM4D PL→BLA (choice (altruistic, selfish) × time (days 1–5): F(4, 80) = 0.69, P = 0.5981, n = 9) mice over 5 days of the SDM task. **i,** Learning index representing the preference development in control CNO (n = 10), hM4D BLA (n = 10), hM4D BLA→PL (n = 11) and hM4D PL→BLA (n = 9) mice (two-way RM ANOVA, group × time: F(12, 140) = 1.91, P = 0.0376). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

Finally, to quantify the efficiency of the preference development, regardless its value (ie, positive or negative decision preference score), we calculated a learning index (see Materials and Methods section 6, par. 6.1) and we found that hM4D BLA→PL mice, similarly to mice that received hM4D in the BLA, displayed a significant reduction in their learning capacity of the SDM compared to control mice and to hM4D PL→BLA mice (**Fig. 20i**). This demonstrates that hM4D BLA→PL mice were slower in the acquisition and further development of their social preference, highlighting that the BLA represents a fundamental area for valence attribution to our decisions and actions including when these occur in the social dimension. Overall, these data suggest that PFC-BLA reciprocal connections have differential roles in social decision-making processes, differently affecting the establishment and the expression of decisions in the social context.

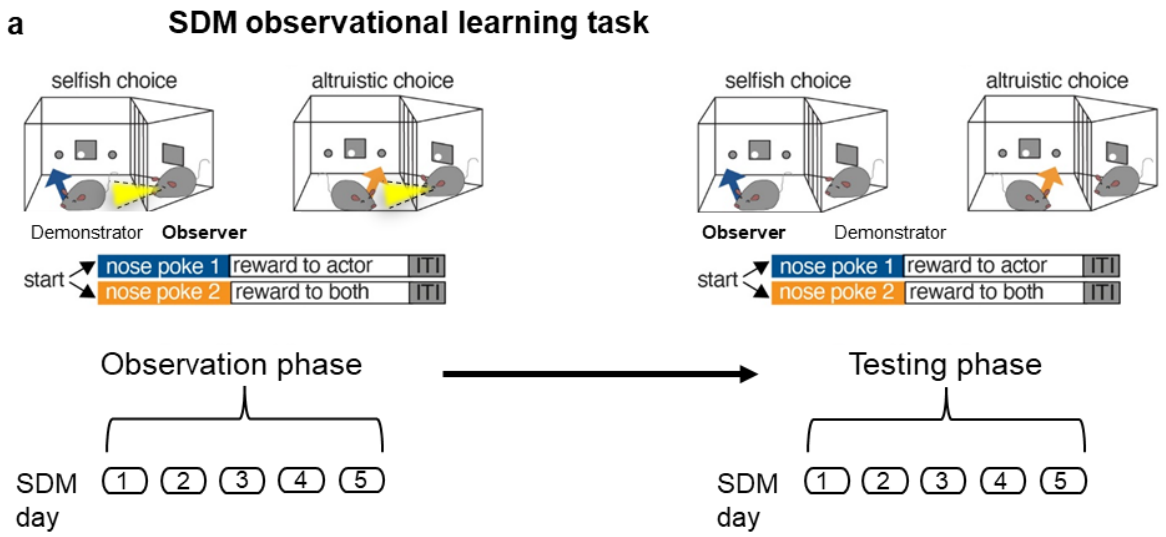
8. Mice learn social decision-making processes by observational learning

So far, we demonstrated that mice can learn social decision-making processes such as altruistic behavior by direct learning (ie, trial-and-error strategy). However, the human world has plenty of daily examples where subjects not only learn from themselves and their first-hand experience, but also from their conspecifics acting in the surrounding environment (Kang et al., 2021; Seymour, 2009; Yoon et al., 2021). This phenomenon is called observational learning and can also occur in the social sphere. Indeed, humans can learn social and prosocial behaviors, based on social decision-making processes, from others. Often, this previous observation even surpasses, in terms of performance accuracy and results, that obtained via direct learning, guaranteeing an advantage or an enhanced performance to the observer (Mou and Ji, 2016; Nomura et al., 2019; Yoon et al., 2021).

Leading from these considerations, we asked whether mice, similarly to humans, have the capability of learning how to make social decisions and express prosocial behaviors such as altruism through the observation of others (ie, by observational learning). To do this, we conceived an additional phase to the original SDM for specifically testing mouse

observational learning. Looking at the behavioral protocol, we start with a five-day observation phase where the recipient (ie, the observer) observes and possibly learns the SDM from their actor (ie, demonstrator) (**Fig. 21a**). After that, a five-day testing phase begins where the observer is placed in their previous demonstrator's compartment and now they are called to act by expressing a social decision towards their companion (**Fig. 21a**). We calculated a learning index for specifically assessing mouse observational capacity of the SDM. Through the learning index, we measured how consistent and coherent mice are with their final social preference since the test started. Here, our hypothesis is that mice receiving previous SDM demonstration from others (ie, observers) are able to show, once tested, greater understanding (ie, higher learning) since the beginning. Simplifying even more, the idea is that observers have already learned the SDM through previous others' observation and already know how to make a decision and express a social behavior once dealing with our task.

Our results showed that observer mice displayed higher learning capacity compared to the group acquiring the SDM by direct learning (ie, previous demonstrators) (**Fig. 21b**), this result being particularly evident at test start (ie, at test day 1) (**Fig. 21b**), also considering the individual learning performance (**Fig. 21c**). This is also evident for their number of task responses, for some observers doubled respect to their demonstrators (**Fig. 21d**). Furthermore, the time needed for expressing a social preference in the SDM (ie, latency to respond) was much lower for the observer compared to the demonstrator group (**Fig. 21e**). Again, this result was significant in the initial phases of the SDM (ie, day 1 and day 2), corroborating our thesis mice receiving previous observation of the task from others already know how to act since start. The increased learning capacity of observers was reflected in their enhanced social performance in the SDM. Indeed, observers already expressed a clear social preference at start that remained stable and coherent till the end (**Fig. 21f**). Instead, demonstrators performed inconsistently or even randomly at first, only expressing a stable social preference in the late stages when they finally learned the task (**Fig. 21f**). This result suggested demonstrators needed a couple of days for getting fully acquainted to the SDM while observers bypassed this learning acquisition step that was already realized via their prior conspecifics' observation.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SDM observational learning task
selfish choice
altruistic choice
Demonstrator
Observer
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reward to actor
ITI
nose poke 2
reward to both
ITI
Observer
Demonstrator
Observation phase
Testing phase
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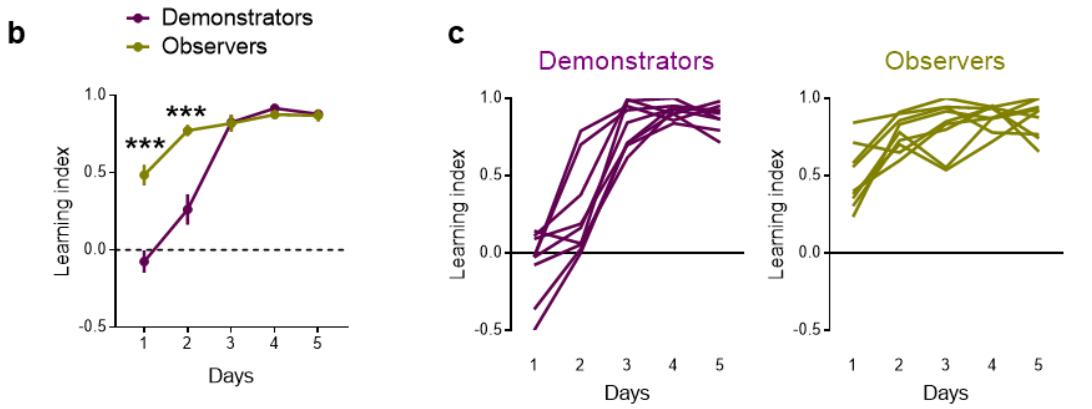
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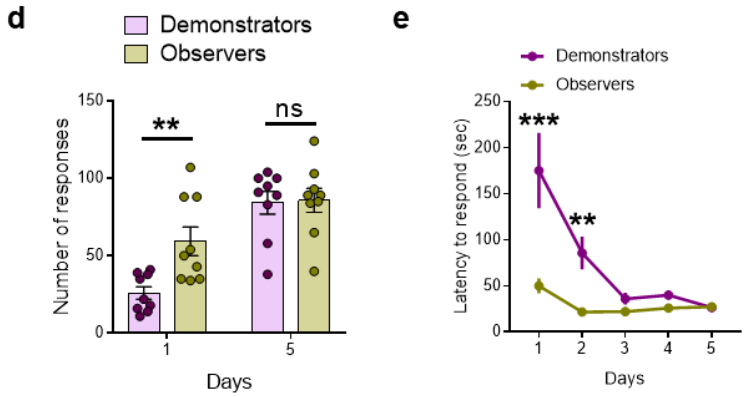
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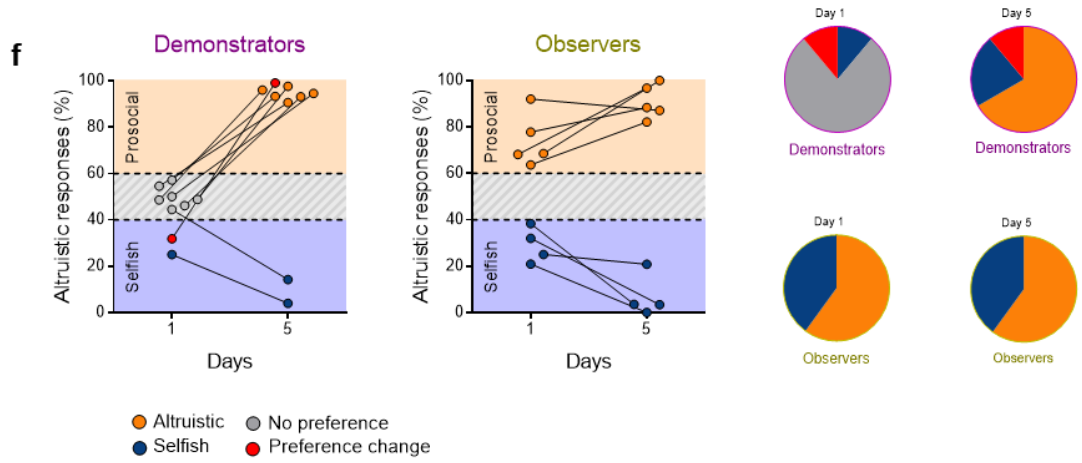
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Demonstrators
Observers
Altruistic responses (%)
Prosocial
Selfish
Days
Day 1
Day 5
Demonstrators
Observers
Altruistic
Selfish
No preference
Preference change

Fig. 21 | Mice learn social decision-making by observational learning (ie, through others' observation). a, SDM observational learning task design and experimental protocol. **b,** Left, learning index of Demonstrators (purple, n = 9) or Observers (green n = 9) (two-way repeated-measures (RM) ANOVA, group (Demonstrators, Observers) × time (days 1–5): $F(4, 64) = 17.73$, $P < 0.0001$; the learning indices were found to fit a normal distribution (D'Agostino and Pearson normality test, Demonstrators: min K2 = 3.3, P = 0.1920, n = 9; Observers: min K2 = 0.9294, P = 0.6283, n = 9). **c,** Individual learning indices in Demonstrators (purple) or Observers (green) over the five days of the SDM observational learning task. **d,** Number of responses in Demonstrators (purple, n = 9) or Observers (green, = 9) for the first and the last day of testing (two-way RM ANOVA, group (Demonstrators, Observers) × time (day 1, day 5): $F(1, 16) = 6.934$, $P = 0.0181$). **e,** Latency to respond (seconds) in Demonstrators (purple, n = 4) or Observers (green, = 6) across the five test days (two-way RM ANOVA, group (Demonstrators, Observers) × time (days 1-5): $F(4, 32) = 10.7$, $P < 0.0001$). **f,** Demonstrators (purple, n = 9) and Observers (green, n = 9) altruistic responses (in percentage) on the first (day 1) and the last (day 5) testing day. Inset, distribution pies for Altruistic, Selfish, Preference change or No preference labels of Demonstrators and Observers at day 1 and day 5. [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

We then replicated the SDM observational learning task several times in naïve and control-injected mice, for later chemogenetic experiments, and confirmed similar results to our initial findings. All observer mice, except one (1 of 39), displayed a higher learning index compared to their previous demonstrators (**Fig. 22a,b**). Furthermore, only a few observers (6 of 39) had a negative learning index or an index below the threshold of 0.20 that we set, corresponding to less than 60% of responses to the preferred choice on the first test day (ie, day 1), indicating the majority already understood the SDM thanks to previous others' observation and were ready to perform successfully since start (**Fig. 22a,b**). This was particularly evident when considering observers' social performance in the task compared to that of their demonstrators. We identified altruistic and selfish decision-makers from the two groups (ie, Demonstrators, Observers) and we found both altruistic and selfish observers gave a significantly higher number of responses (ie, equal to or more than 60%) to the choice of election on test day 1, demonstrating greater SDM comprehension already at start (**Fig. 22d**). Instead, both altruistic and selfish demonstrators did not show a significant difference between the two options at test start, the difference emerging only later during the task (ie, day 5) (**Fig. 22c**). Besides, observers exhibited a higher number of responses and, at the same time, lower response latency time compared to demonstrators, both parameters indicating stronger task learning acquisition (**Fig. 22e,f**). We also checked for individual and sex differences regarding observers' social performance in the task (**Fig. 22g**). We revealed, similarly to our results in the SDM by direct learning, the majority of mice (26 out of 39) preferred being altruistic towards their companions (**Fig. 22g**). In particular, both male (18 out of 26) and female (10 out of 13) observers mostly exhibited an altruistic preference (**Fig. 22g**). Regarding the learning index, there were no differences between male and female

observers (**Fig. 22l**). Both male and female observers showed greater learning capacity compared to their demonstrators in the initial phases of the SDM (**Fig. 22h,i**).

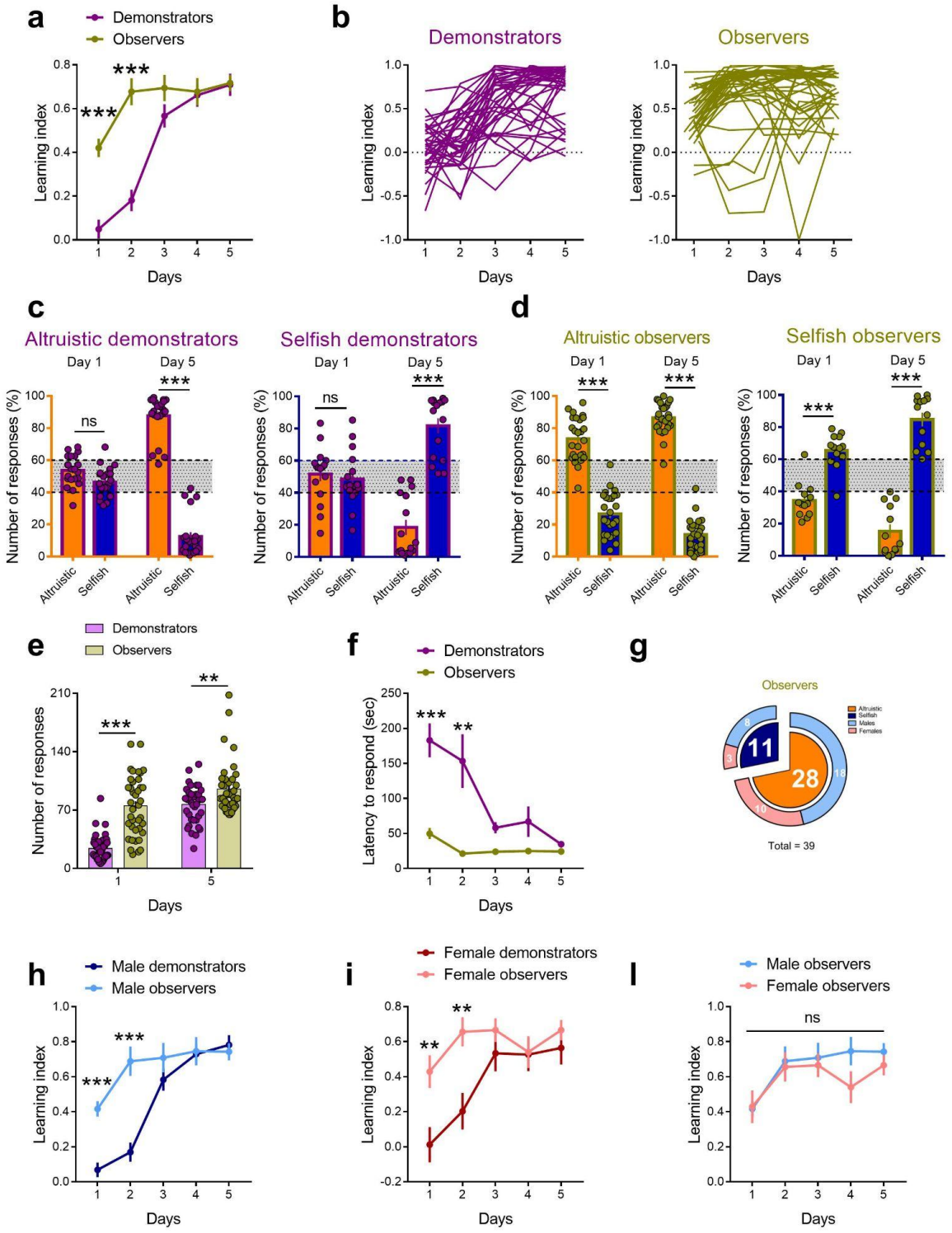

Fig. 22 | All data on observational learning pooled together. a, Learning index of all Demonstrators (purple, n = 39) or Observers (green, n = 39) pooled together (two-way repeated-measures (RM) ANOVA, group

(Demonstrators, Observers) × time (days 1–5): $F(4, 152) = 13.6$, $P < 0.0001$); the learning indices were found to fit a normal distribution (D'Agostino and Pearson normality test, Demonstrators: min K2 = 1.402, P = 0.4961, n = 39; Observers: min K2 = 0.1604, P = 0.9229, n = 39). **b,** Individual learning indices in Demonstrators (purple, n = 39) or Observers (green, n = 39) over the five days of the SDM observational learning task. **c,** Altruistic demonstrators (left, purple, n = 22) with their number of responses (in percentage) to the altruistic choice on the first and the last day of testing (two-way RM ANOVA, choice (Altruistic, Selfish) × time (day 1, day 5): $F(1, 21) = 62.6$, $P < 0.0001$); Selfish demonstrators (right, purple, n = 16) with their number of responses (in percentage) to the selfish choice on the first and the last day of testing (two-way RM ANOVA, choice (Altruistic, Selfish) × time (day 1, day 5): $F(1, 15) = 32.7$, $P < 0.0001$). **d,** Altruistic observers (left, green, n = 26) with their number of responses (in percentage) to the altruistic choice on the first and the last day of testing (two-way RM ANOVA, choice (Altruistic, Selfish) × time (day 1, day 5): $F(1, 25) = 29.4$, $P < 0.0001$); Selfish observers (right, purple, n = 13) with their number of responses (in percentage) to the selfish choice on the first and the last day of testing (two-way RM ANOVA, choice (Altruistic, Selfish) × time (day 1, day 5): $F(1, 48) = 27$, $P < 0.0001$). **e,** Number of responses in Demonstrators (purple, n = 39) or Observers (green, = 39) for the first and the last day of testing (two-way RM ANOVA, group (Demonstrators, Observers) × time (day 1, day 5): $F(1, 38) = 22.6$, P = < 0.0001). **f,** Latency to respond (seconds) in Demonstrators (purple, n = 39) or Observers (green, = 39) across the five test days (two-way RM ANOVA, group (Demonstrators, Observers) × time (days 1-5): $F(4, 210) = 7.49$, $P < 0.0001$). **g,** Total number of Observers analyzed divided by preference (Altruistic-Selfish) and sex (Males-Females). **h-l,** (Left) learning index of Male observers (n = 26) against their age- and sex-matched demonstrators (n = 26) across all test days (two-way RM ANOVA, group (Male demonstrators, Male observers) × time (days 1-5): $F(4, 100) = 9.96$, $P < 0.0001$); (center) learning index of Female observers (n = 13) against their age- and sex-matched demonstrators (n = 13) (two-way RM ANOVA, group (Female demonstrators, Female observers) × time (days 1-5): $F(4, 48) = 3.76$, $P = 0.0097$); (right) learning index of Male observers (n = 26) vs Female observers (n = 13) across the five test days (two-way RM ANOVA, group (Male observers, Female observers) × time (days 1-5): $F(4, 148) = 0.872$, $P = 0.4821$). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

Taken together, these results suggest previous SDM observation from others is sufficient for mice of both sexes to successfully understand how the task works and efficiently carry out this since they have the first opportunity to act. In particular, observational learning overall represents a more robust and efficient type of learning compared to direct learning. The advantage or facilitatory effect derived from prior observation of others is particularly evident in the opening bars of our task replacing the time a subject would spend learning the task by themselves. Indeed, most of the subjects who have already observed the performance of their peers already know how to behave and what to choose in the presented social context.

9. Observers do not imitate their demonstrators but can be influenced by their actions

Observing others' behavior does not necessarily imply making an exact copy of their actions, instead may result in flexible replication that adapts to different contexts (Fryling et al., 2011; Selbing et al., 2014). Furthermore, watching others performing in front of us exert an influence on our subsequent performance in the social environment. For instance, witnessing a bad model might bias the performance of the observer (Selbing et al., 2014). Thus to

evaluate observers' performance, we pooled together the data obtained from all naïve and control-injected animals tested in our laboratory.

Firstly, we asked whether mouse observational learning behavior was an exact copy of the movements or the sequential actions of the demonstrator, rather than a flexible replication of what was observed. For this reason, we analyzed whether the observers, at the group level, went more often to the same side of the SDM apparatus chosen by their previous demonstrators (**Fig. 23a**). We revealed a significant preference for the location of one of the two NPs in the demonstrators group, in particular demonstrators gave more responses to the right NP (**Fig. 23b**). This tendency was not present in the group of the observers that almost equally responded to both NP locations (**Fig. 23b**). We then deepened our analysis investigating whether each observer chose more frequently the same NP preferred by their demonstrator (**Fig. 23c**). In the observer group, no significant differences were found between the number of responses given to the preferred nose poke by the demonstrator compared to the one not preferred (**Fig. 23d**). Together, these results indicate observers do not necessarily copy the movements and the sequence of actions shown by their demonstrators, suggesting the possibility of flexible behavioral replication.

We then asked whether observers' performance was sufficiently adaptable to different or altered test conditions, specifically different from their demonstrator's initial setting. For this reason, we checked on our entire cohort of observers whether starting with the same or a different nose poke configuration from their previous demonstrators (ie, same side or different side, respectively) influenced their next observational learning capacity (**Fig. 23e**). This control was mainly for assessing whether mice could still learn through others' observation despite contextual references for their social decisions-actions were altered from the beginning. We did not find any difference in the learning performance between observers from the same side or the different side group (**Fig. 23f**), demonstrating how mouse observational learning adapted to different starting conditions. This result suggests that observers were able to flexibly develop their own repertoire of behavioral sequences to express their decisions in the SDM. Taken together, these results suggest that the observers understood the meaning of the actor's behavior, without necessarily copying that, and being able to replicate this in a dynamic manner.

It is true observers' performance was flexible enough and adaptable to different situations, however, we asked whether there were some internal-external factors significantly influencing this. In particular, we asked if witnessing a bad performance from a bad model was sufficient for compromising the future performance of the observers. We noticed from

our pooled data there were some demonstrators (8 of 39) exhibiting a bad performance (ie, below a learning index of 0.20) at the end of the task. We gathered the observers paired with these demonstrators and we confronted the performance of the two groups. We revealed there were no significant differences between the observers w/ bad demonstrators (8 of 39) and the bad demonstrators group in the learning performance across the test days (**Fig. 23g**). In contrast, observers coupled with good demonstrators (31 of 39) displayed a higher learning score in the first two test days (**Fig. 23g**), in line with our previous results on observational learning. We concluded a bad learning performance from observers might potentially be associated with an earlier bad demonstration from their demonstrators. A previous bad demonstration was then sufficient for lowering observers' learning capacity and compromising further performance in the SDM task. This result suggested, despite high flexibility and adaptability, observational learning can be subjected to other relevant factors, such as a bad model, that should be constantly kept in mind.

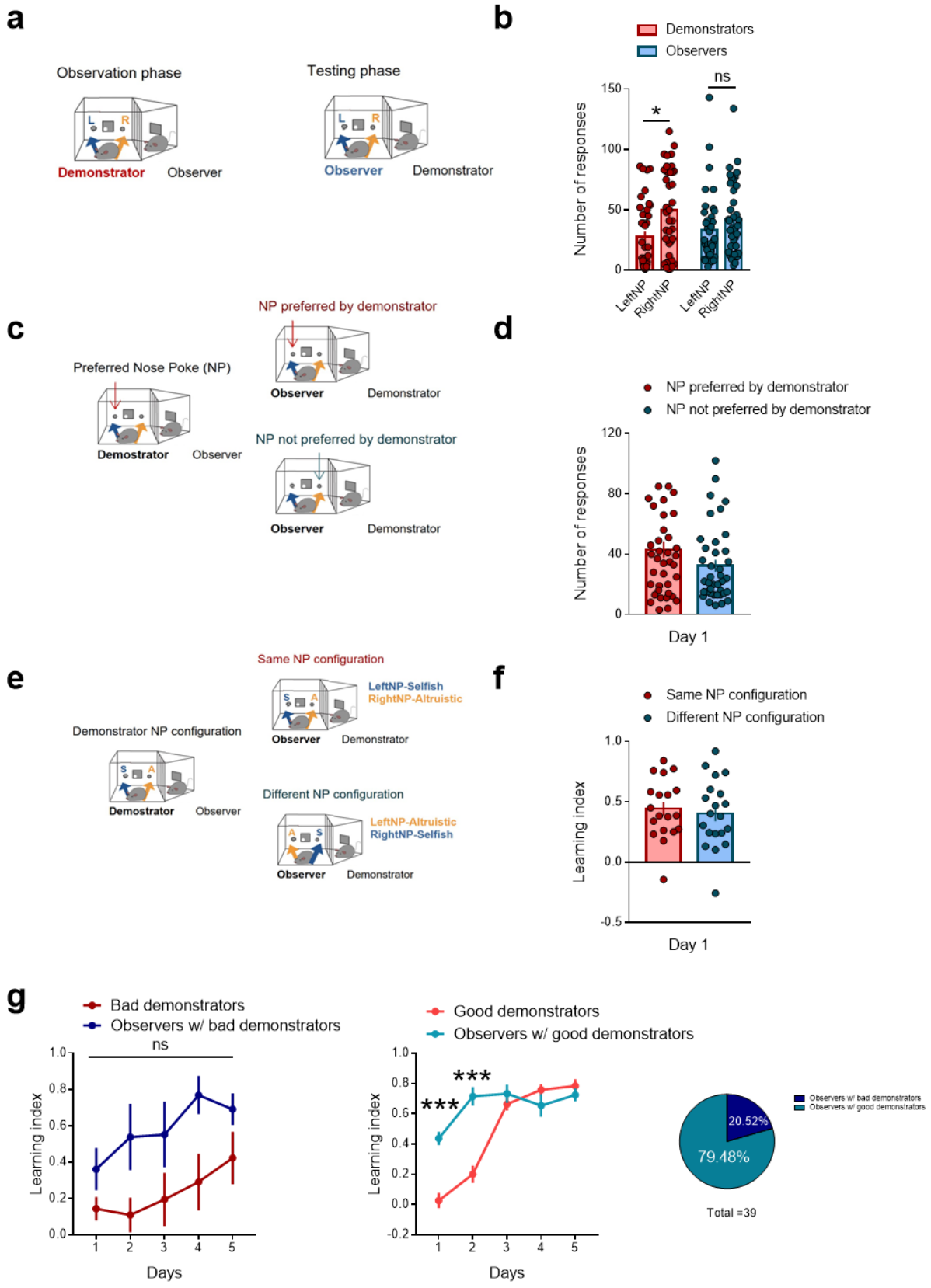

Fig. 23 | Observers do not imitate their demonstrators but can be influenced by their actions. a, Left, schematic representation of Demonstrators giving responses to Left or Right NosePoke (NP) during the SDM

task. Right, Observers giving responses to Left or Right NosePoke (NP) **b,** Number of responses to LeftNP and RightNP of Demonstrators (red, n = 39) or Observers (blue, n = 39), respectively (two-way repeated-measures (RM) ANOVA, group (Demonstrators, Observers) × side (LeftNP, RightNP): $F(1, 38) = 1.56$, $P = 0.2195$). **c,** Left, schematic representation of NP preferred by Demonstrator. Right, observer choosing NP preferred by demonstrator (top) or not (bottom). **d,** Observers number of responses to NP preferred by demonstrator (red, n = 39) or NP not preferred by demonstrator (blue, n = 39) (two-tailed paired t-test: $t = 1.44$, d.f. = 38, $P = 0.1589$). **e,** Left, schematic representation of Demonstrator NP configuration. Right, Observer starting with same NP configuration (top) or different NP configuration (bottom). **f,** Learning index of observers with Same NP configuration (red, n = 19) or Different NP configuration (blue, n = 20) (two-tailed unpaired t-test: $t = 0.468$, d.f. = 37, $P = 0.6423$). **g,** Left, learning index of Bad demonstrators (blue, n = 8) and Observers w/ bad demonstrators (bordeaux, n = 8) (two-way repeated-measures (RM) ANOVA, group (Bad demonstrators, Observers w/ bad demonstrators) × time (days 1–5): $F(4, 28) = 0.639$, $P = 0.6392$). Right, learning index of Good demonstrators (light blue, n = 31) and Observers w/ good demonstrators (red, n = 31) (two-way repeated-measures (RM) ANOVA, group (Good demonstrators, Observers w/ good demonstrators) × time (days 1–5): $F(4, 120) = 20.3$, $P < 0.0001$). Inset, total number (n = 39) of Observers divided by Observers w/ bad demonstrators (n = 8, in percentage) and Observers w/ good demonstrators (n = 31, in percentage). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

Following these considerations, we analyzed other aspects that could bias observers' performance. These aspects were either internal related to the observer or external related to the demonstrator (Suzuki and O'Doherty, 2020; Terenzi et al., 2021). In particular, we started considering whether the social preference of the demonstrator or the observer could exert some influence on the learning performance of the latter. We did not find any significant effect on both conditions. There were no learning differences in observers with an altruistic or a selfish demonstrator (**Fig. 24a**). Although we found no significant difference in observers that were altruistic or selfish, there was an interesting trend for altruistic observers being better learners (**Fig. 24b**). We also checked if social proximity/closeness might be a factor influencing observers' learning performance. We found no evidence for any improvement on observers' learning performance due to social proximity of either the demonstrator (**Fig. 24c**) or the observer himself (**Fig. 24d**). All together, these results suggest observers' learning capacity was flexible enough not to be influenced by external factors such as demonstrators' social preference or social proximity. However, this analysis on different parameters potentially influencing observers' learning should be taken as a reminder of the complexity of the phenomenon at stake (ie, observational learning) and how many factors either proper of the model or the observer could continuously influence this.

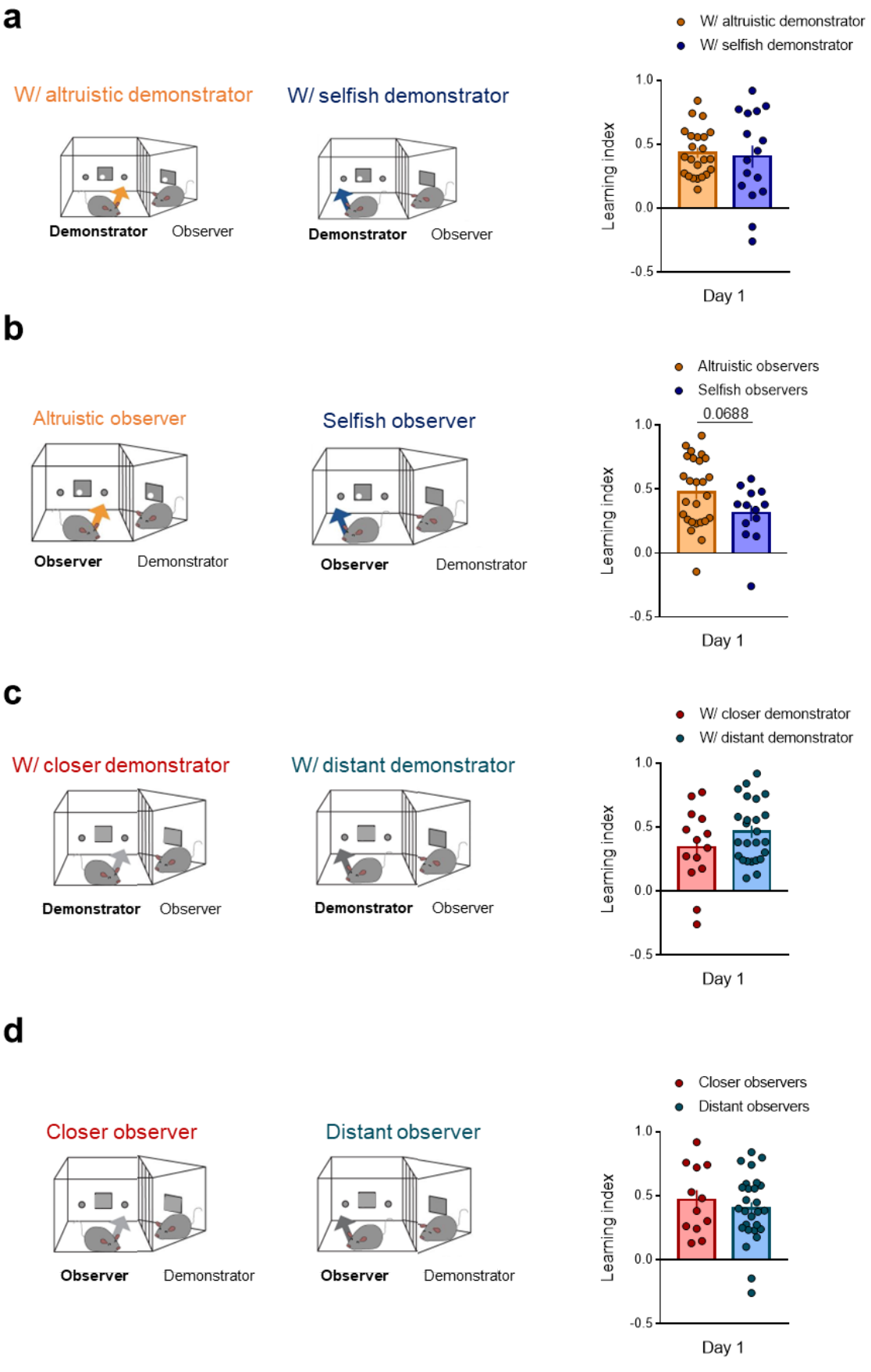
a
W/ altruistic demonstrator
W/ selfish demonstrator
Demonstrator
Observer
Demonstrator
Observer
W/ altruistic demonstrator
W/ selfish demonstrator
Learning index
1.0
0.5
0.0
-0.5
Day 1
b
Altruistic observer
Selfish observer
Observer
Demonstrator
Observer
Demonstrator
Altruistic observers
Selfish observers
0.0688
Learning index
1.0
0.5
0.0
-0.5
Day 1
c
W/ closer demonstrator
W/ distant demonstrator
Demonstrator
Observer
Demonstrator
Observer
W/ closer demonstrator
W/ distant demonstrator
Learning index
1.0
0.5
0.0
-0.5
Day 1
d
Closer observer
Distant observer
Observer
Demonstrator
Observer
Demonstrator
Closer observers
Distant observers
Learning index
1.0
0.5
0.0
-0.5
Day 1

Fig. 24 | Analysis of different parameters possibly involved in the SDM observational learning task. a, Left, schematic representation observer with (W/) altruistic (orange) or selfish (blue) demonstrator. Right, Learning index of observers W/ altruistic demonstrator (orange, n = 23) or W/ selfish demonstrator (blue, n = 16) (two-tailed unpaired t-test: t = 0.347, d.f. = 37, P = 0.7304). **b,** Left, schematic representation of Altruistic (orange) or Selfish (blue) observer with a conspecific demonstrator. Right, Learning index of Altruistic observers (orange, n = 26) or Selfish observers (blue, n = 13) (two-tailed unpaired t-test: t = 1.87, d.f. = 37, P = 0.0688). **c,** Left, schematic representation of observer W/ closer (red) or distant (light blue) demonstrator. Right, Learning index of observers W/ closer demonstrator (red, n = 14) or W/ distant demonstrator (light blue, n = 25) (two-tailed unpaired t-test: t = 1.39, d.f. = 37, P = 0.1732). **d,** Left, schematic representation of Closer (orange) or Distant (blue) observer with a conspecific demonstrator. Right, Learning index of Closer observers (red, n = 12) or Distant observers (light blue, n = 27) (two-tailed unpaired t-test: t = 0.754, d.f. = 37, P = 0.4557). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

10. Observers reciprocate altruistic acts previously received from their demonstrators

Reciprocation motives can often drive our decisions and behaviors towards others (Dolivo and Taborsky, 2015, 2016). For instance, witnessing a social act with a specific valence might push or even bias the observer to reciprocate what is observed in the exact same terms (ie, reciprocation). The reciprocation influence can be either for positive (eg, altruism↔altruism) or negative (eg, selfish↔selfish) acts. Differently, no reciprocation means a mismatch between the performance of the demonstrator and that of the observer (eg, altruism vs selfish, or vice versa). Thus, we investigated whether mice, similarly to humans, could be subjected to the influence of reciprocation behavior.

We found that half of our group of observers showed reciprocation (20 out of 39) and the other half did not (19 out of 39) at the end of the SDM task (**Fig. 25a**). A deeper analysis, however, showed marked individual and sex differences (**Fig. 25b**). Indeed, the majority of reciprocating observers were altruistic (15 of 20) rather than selfish, whereas in the no reciprocation group they equally splitted between the two choices (**Fig. 25b,c**). Furthermore, the reciprocation effect was particularly evident in male observers, in fact the majority of males (18 of 26) were reciprocating and, of these, many more (13 of 18) were reciprocating altruistic instead of selfish acts (**Fig. 25b,d**). Differently, the majority of female observers (11 of 13) belonged to the 'no reciprocation' group hence diverging in their social performance from their demonstrators (**Fig. 25b,d)**. Interestingly, the only female observers reciprocating to their demonstrators were altruistic (**Fig. 25b,d)**.

Taken together, these results highlight the importance of considering the reciprocation aspect when dealing with observational learning. In fact, what we observe and learn from others can have a strong influence on our following decisions and actions towards them.

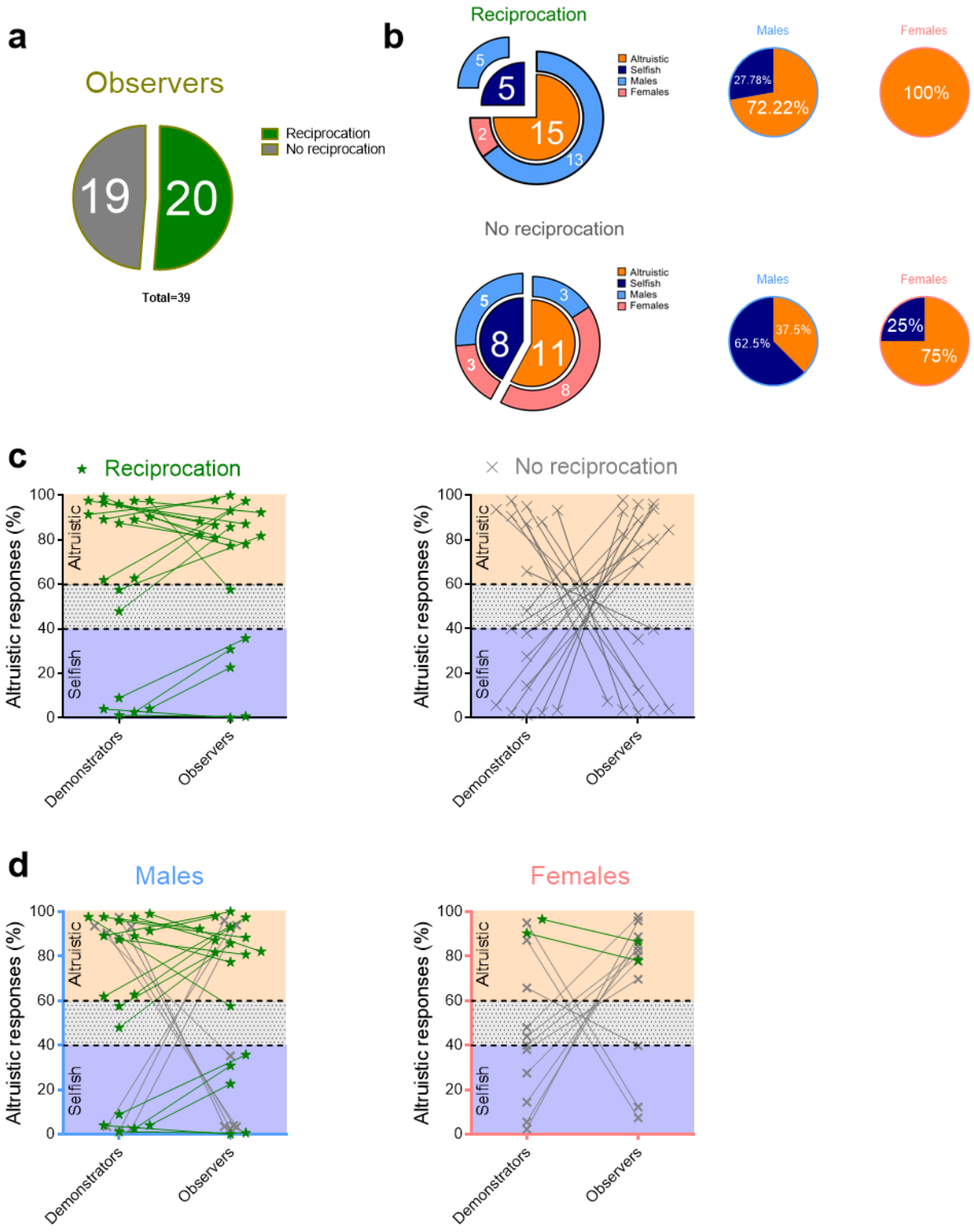

Fig. 25 | Observers mostly reciprocate altruistic actions from their demonstrators. a, Distribution pie for Reciprocation and No reciprocation in the Observers group. **b,** Reciprocation and No reciprocation pie for observers divided by preference and sex. **c,** Graphical representation of Reciprocation or No reciprocation conditions for Demonstrators and Observers. **d,** Graphical representation of Reciprocation or No reciprocation conditions for Demonstrators and Observers divided by sex (Left-Males, Right-Females).

11. dCA1 neuronal silencing blocks the formation of social behaviors through others

The HPC is a key area both for learning goal-directed behaviors by personal experience (ie, direct learning) and for learning these from others (ie, observational learning) (Mou et al., 2022; Ólafsdóttir et al., 2018; Shin et al., 2019). In particular, the social place cells in the dCA1 region makes this area the perfect candidate for studying the underlying circuitry behind mouse observational learning. In fact, according to literature, mice are able to track others' positions and actions in the surrounding environment, learning and adapting to these (Danjo et al., 2018; Fujisawa and Ouchi, 2022; Omer et al., 2018). Furthermore, lesioning or inhibiting the dCA1 has reported significant impairments in rodent observational learning ability of either spatial and affective tasks, suggesting this area is profoundly involved in learning from others (Mou et al., 2022; Nomura et al., 2019). However, as already stated in the aims section, a research investigating in detail whether this function of dCA1 is also involved in the acquisition of more refined behavioral expressions such as social decision-making processes (eg, altruistic behavior) is missing. That is the rationale to study the involvement of dCA1 through inhibitory chemogenetics in our SDM observational learning task (**Fig. 26a,b**).

To do this, we daily injected observer mice with CNO 30 minutes before the session for silencing dCA1 during observational learning behavior (**Fig. 26a,b**). As already explained (see Materials and Methods section 3, par. 3.1-3.4), we administered CNO to both experimental groups and, after the observation phase, observers were tested in the testing phase (**Fig. 26a,b**). Our results directly showed that dCA1 neuronal silencing impairs the formation of social decisions and behaviors through others' observation (ie, by observational learning). In fact, the learning index of the control compared to hM4D group was significantly different, with the latter group showing very low learning capacity at start, despite their previous SDM observation (**Fig. 26c**). This was in contrast with all our previous results showing that mice after others' observation are already able to express a social preference in the initial stages of the SDM task. That is also a sign of the facilitatory effect created by previous task observation that guarantees observers an easier start when it is their time to act.

Despite the learning deficit, there was no significant difference in the number of responses or latency time between the two groups (**Fig. 26d,e**), suggesting that dCA1 silencing did not affect learning of procedural aspects of the task, such as the

response-reward association, rather the social components necessary to carry out the task. This result suggests that dCA1 silencing blocked, at least initially, the integration of the social component of the task or the social outcomes/consequences associated with each response. Besides, as we did for naïve observer mice, we compared the learning index with the social performance in the task. Consistent with our previous data, observers in the control group already displayed a clear social preference either altruistic or selfish at start, and this was coherent across all the test days till the end (**Fig. 26f**). Instead, most hM4D observers (5 of 8) were initially blocked in the expression of their social preference, displaying a consistent social decision only later in the SDM (**Fig. 26f**). In the end, however, observers showed overall (5 of 8) a greater preference for altruistic behavior similarly to demonstrators (**Fig. 26f**).

Altogether, the data suggest that the dCA1 is deeply involved in the acquisition of social decision-making processes through others. Indeed, dCA1 silencing prevented the acquisition of this social information during the observation phase. When called to act (ie, testing phase), hM4D silenced observers presented a rather confused social performance with the preference for one of the two choices not being clearly separated. This was evident in the initial stages of the task that are also the critical time windows where the advantage due to previous observation should manifest more consistently. Besides, the fact dCA1 silencing did not affect the number of responses or the latency to respond indicates the impairment was not associated with the operational information related to the task, rather it was mainly on the social information gathered from the observation of others' performance. Finally, observers were not differing from demonstrators in their greater preference, at the group level, for altruistic behavior. Again, this reveals the most significant impairment created by our inhibitory manipulation on the dorsal region of the HPC (ie, dCA1) was not associated with changes in social preference (ie, becoming more altruistic or selfish), but it was mainly a matter of social learning and how this was initially disrupted.

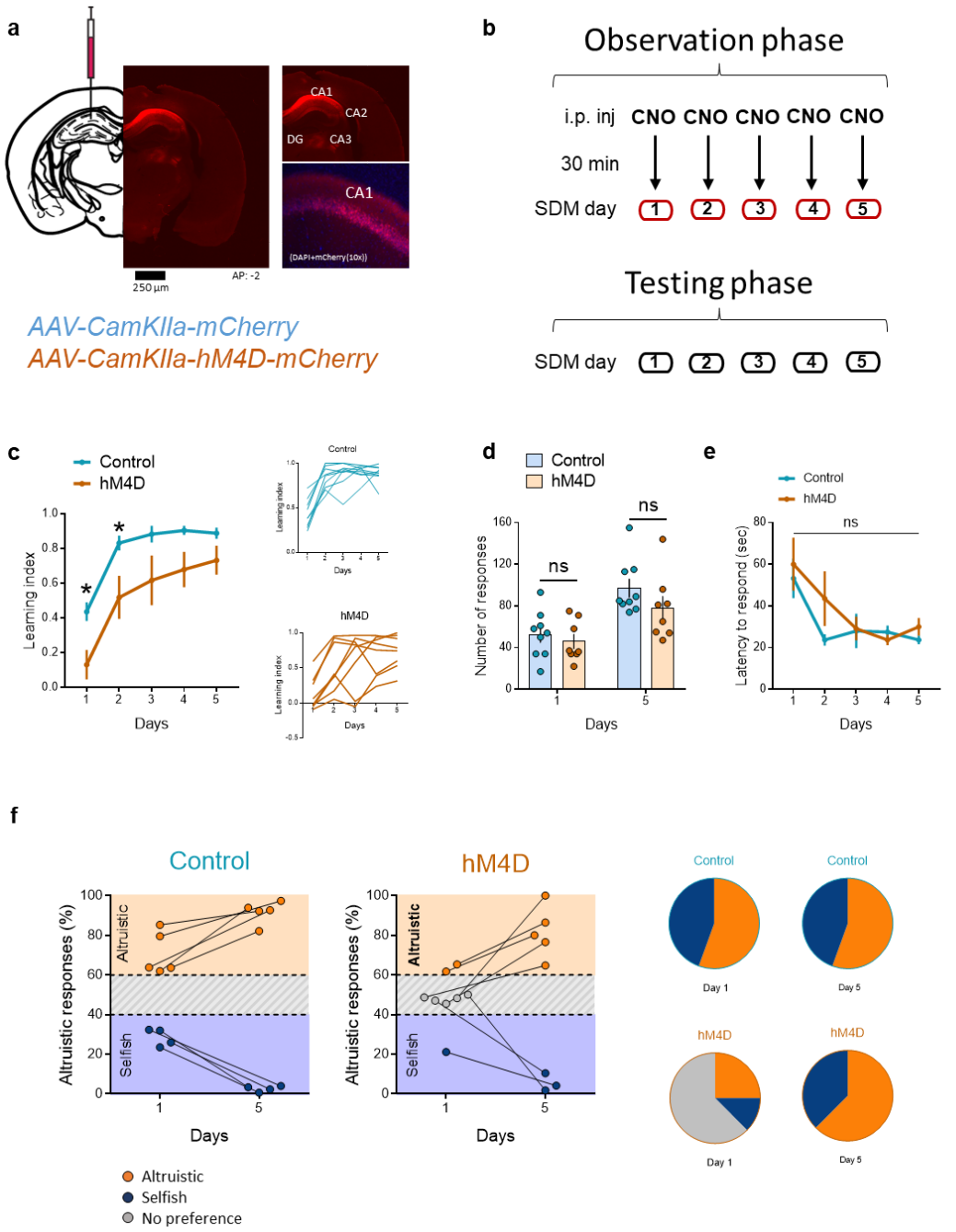

Fig. 26 | dCA1 neuronal silencing blocks the formation of social decisions and behaviors through others' observation (ie, by observational learning). a, Male mice were bilaterally injected in the dCA1 with AAV-CamKIIa-mCherry (Control, light blue) or AAV-CamKIIa-hM4D-mCherry (hM4D, light brown). Representative image of a coronal section of dCA1. **b,** Thirty minutes before the daily SDM session in the observational phase, Control and hM4D observer mice received an intraperitoneal (i.p.) injection of CNO. Control and hM4D observers did not receive CNO during the performance phase (ie, when observers are called to act). **c,** Learning index of Control (light blue, n = 9) or hM4D (light brown, n = 8) group across the five-day performance phase (two-way repeated-measures (RM) ANOVA, group (Control, hM4D) × time (days 1–5): F(1, 15) = 8.528, P = 0.0105). Inset, individual learning indices in Control (light blue, n = 9) or hM4D (light brown, n = 8) group over the five testing days. **c,** Number of responses in Control (light blue, n = 9) or hM4D (light green, = 8) group for the first and the last day of testing (two-way RM ANOVA, group (Control, hM4D) × time (day 1, day 5): F(1, 15) = 0.55, P = 0.4696). **d,** Latency to respond (seconds) in Control (light blue, n = 9) or hM4D (light brown, = 8) group across the five test days (two-way RM ANOVA, group (Control, hM4D) × time

(days 1-5): F(4, 60) = 0.849, P = 0.5000). **e,** Control (light blue, n = 9) and hM4D (light brown, n = 8) observers altruistic responses (in percentage) on the first (day 1) and the last (day 5) testing day. Inset, distribution pies for Altruistic, Selfish, Preference change or No preference labels of Control and hM4D group at day 1 and day 5. [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

12. dCA1 silencing, not vCA1, impairs the acquisition of social information from others

We then asked whether dCA1 was also involved in the retrieval of the acquired social information obtained from others and needed to perform the SDM task. To answer this question, mice were allowed to observe their demonstrators during the observation phase and then were administered with CNO for silencing the dCA1 in the testing phase (**Fig. 27a**). In the testing phase, we hypothesized that observers recalled information gathered through previous others' observation for successfully performing the task from the start. We specifically delivered CNO on day 1 and 2 of the testing phase (**Fig. 27a**) as these are the test days where we found more evident results with the hM4D dCA1 observer group. We found no evidence for negative effects on observational learning of dCA1 silencing during the retrieval phase (**Fig. 27b,e,f**). Observers from the dCA1 hM4D retrieval group were performing normally compared to controls both in terms of learning and social performance. Furthermore, the number of responses and the latency to respond were not affected (here, not shown). This might suggest that dCA1 has a major role in the acquisition phase of observational learning ability or when the information from others is primarily encoded and stored, but not in the recall phase when this social information needs to be retrieved. At this step, alternative neural structures might play a role, offering compensatory mechanisms that overcome the blockage of the dorsal HPC (Kumaran et al., 2015; Shin et al., 2019; Terranova et al., 2022). In contrast with this speculation, however, we identified some observer mice (3 of 7) in the hM4D dCA1 retrieval group not performing well at start. We checked if their bad learning was due to a bad performance previously shown from their demonstrators, but this was not the case. Even though the three observers' bad learning was not sufficient for irremediably affecting the learning group performance, we cannot entirely rule out, at this stage, the possibility that dCA1 retrieval silencing somewhat influences the observational learning process.

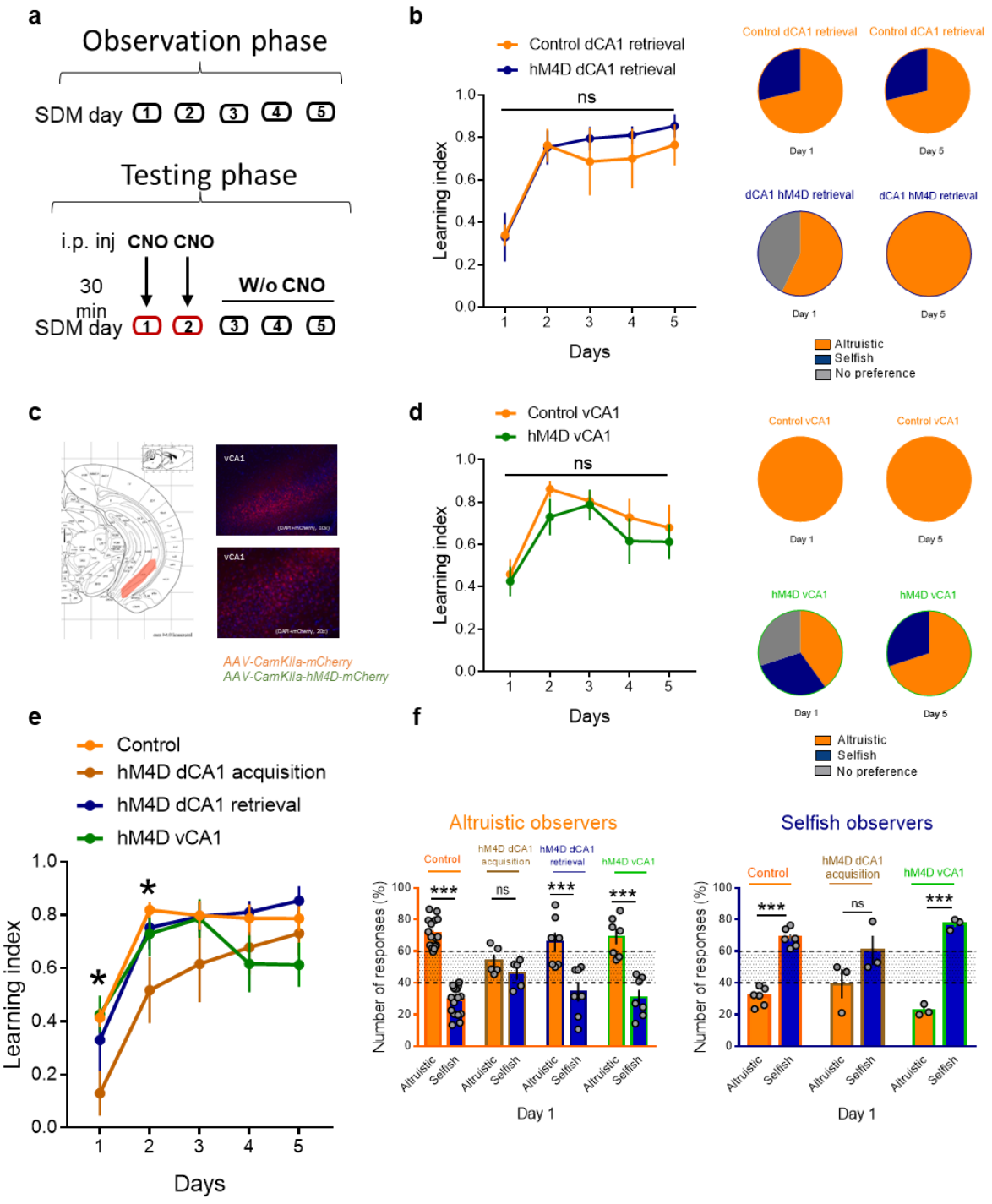

Fig. 27 | dCA1 silencing, not vCA1, prevents the acquisition of social information from others. a, Experimental design for experiment on dCA1 retrieval/recall. Male mice were bilaterally injected in dCA1 and received CNO 30 min before task during the first two days of the performance phase (ie, the retrieval/recall phase). The first acquisition phase (ie, the observational phase) was without (W/o) CNO. **b,** Learning index of Control dCA1 retrieval (bordeaux, n = 7) or hM4D dCA1 retrieval (blue, n = 7) group across the five-day performance phase (two-way repeated-measures (RM) ANOVA, group (Control dCA1 retrieval, hM4D dCA1 retrieval) × time (days 1–5): $F(4, 24) = 13.6$, $P = 0.7990$). Inset, pie with learning distribution for Control or hM4D dCA1 retrieval group at day 1 and day 5. **c,** For the experiment on vCA1, male mice were bilaterally injected in the vCA1 with AAV-CamKIIa-mCherry (Control vCA1, orange) or AAV-CamKIIa-hM4D-mCherry (hM4D vCA1, green). Then, mice received CNO 30min before task for all the observational phase (ie, the

acquisition phase). Representative image of a coronal section of vCA1. **d,** Learning index of Control vCA1 (orange, n = 7) or hM4D vCA1 (green, n = 10) group across the five-day performance phase (two-way repeated-measures (RM) ANOVA, group (Control vCA1, hM4D vCA1) × time (days 1–5): $F(4, 60) = 0.213$, $P = 0.9303$). Inset, pie with learning distribution for Control vCA1 or hM4D vCA1 group at day 1 and day 5. **e,** Learning index of Control (pooled together) (orange, n =), hM4D dCA1 acquisition (light brown, n = 8), hM4D dCA1 retrieval (blue, n = 7), vCA1 (green, n = 10) group across the five-day performance phase (two-way repeated-measures (RM) ANOVA, group (Control, hM4D dCA1 acquisition, hM4D dCA1 retrieval, hM4D vCA1) × time (days 1–5): $F(3, 43) = 2.72$, $P = 0.05$). **f,** Altruistic observers in all the experimental groups (Control (orange, n = 17), hM4D dCA1 acquisition (light brown, n = 5), hM4D dCA1 retrieval (blue, n = 7), hM4D vCA1 (green, n = 7)) with their altruistic responses (in percentage) on the first day of testing (two-way RM ANOVA, group (Control, hM4D dCA1 acquisition, hM4D dCA1 retrieval, hM4D vCA1) × choice (Altruistic, Selfish): $F(3, 64) = 6.26$, $P = 0.0009$); Selfish observers in all the experimental groups (Control (orange, n = 6), hM4D dCA1 acquisition (light brown, n = 3), hM4D vCA1 (green, n = 3)) with their altruistic responses (in percentage) on the first day of testing (two-way RM ANOVA, group (Control, hM4D dCA1 acquisition, hM4D vCA1) × choice (Altruistic, Selfish): $F(2, 18) = 5.4$, $P = 0.0145$). [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

Besides, as much evidence exists around the important role of ventral CA1 (vCA1) in social learning and memory (Okuyama, 2018; Rao et al., 2019), we decided to test whether the neural silencing of vCA1 brings similar results to dCA1 on mouse social decision-making processes by observational learning (**Fig. 27c**). For vCA1, we used the same experimental protocol for assessing the role of dCA1 in the acquisition of social information through others' observation (**Fig. 26b**). We chronically administered CNO to observers during the five-days observation phase for silencing vCA1 activity. We then analyzed observers' performance in the SDM during the testing phase to specifically assess their learning ability post observation. Similarly to results we obtained with dCA1 hM4D retrieval group (**Fig. 27b**), observers chemogenetically silenced for vCA1 did not show any difference either in social preference or learning performance from their age- and sex-matched control peers (**Fig. 27d,e,f**). Again, we detected some vCA1 hM4D observers (3 of 10) were performing badly in the initial test stages. However, in this case we were able to track down the bad performance of the three vCA1 observers to the bad performance of their previous demonstrators (here, not shown). As shown before (**Fig. 23g**), a bad demonstration from previous models can significantly compromise the following performance of observers. Said that, as it was for dCA1 retrieval, the bad performance of some individuals was not enough for compromising the overall learning ability of the vCA1 group that behaved at the same level of controls. After comparing all our chemogenetic manipulations against controls (**Fig. 27e,f**), it was clear only dCA1 silencing, and not vCA1, was significantly affecting mouse observational learning ability and the result was concentrated in the initial stages of the task, when the advantage due to previous observation should be more evident. Furthermore, the major role of dCA1 in observational learning should be primarily found in the acquisition phase when observer mice are learning for the first time the social information arriving from

the other social agents, and not in following stages where this acquired information should be retrieved.

Finally, we confirmed the role of dCA1 in the acquisition of both spatial and social aspects of the learning process, with consequences on short- and long-term forms of memory processes. We performed the object location displacement task and the three-chamber task, the two tests measuring spatial and sociability-social memory skills, respectively (**Fig. 28a,c**). We administered CNO for specifically silencing dCA1 during the acquisition/development of spatial and social information in the task. We found dCA1 silencing during the acquisition phase of the object location displacement task (ie, sample trial) significantly impairs long-term memory (ie, 24h retrieval) of spatial information already encountered in the test (ie, test trial) (**Fig. 28b**). Furthermore, dCA1 silencing also affects short-term social memory (ie, <30min) of familiar individuals, although it does not affect general sociability/social affiliation behavior (**Fig. 28d**). Besides, we also checked in our original batch of observers (**Fig. 26**) if our dCA1 manipulation was able to impact social and locomotor activity in the SDM observational learning task (**Fig. 28e**). Indeed, deficits in social exploration or locomotor activity could well have biased the results in the task and our following interpretation of these. We found no evidence for any effect of our chemogenetic manipulation either on social exploration or locomotor activity, suggesting our results were circumscribed to mouse observational learning ability and not biased by other confounding factors. Altogether, this evidence might suggest dCA1 is involved in the initial processing of both spatial and social information that represent fundamental aspects for observational learning ability. Indeed, the correctness of spatial information is crucial for tracking others' movements and their actions in the surrounding environment. Likewise, social information regarding others, such as their identity or our previous exchange with them, is fundamental when we face them or learn from them in the shared social context. In this sense, dCA1 dysfunction, by affecting both spatial and social components, might dramatically alter the individual capability of learning from others, including those situations where we learn social decisions and behaviors from them.

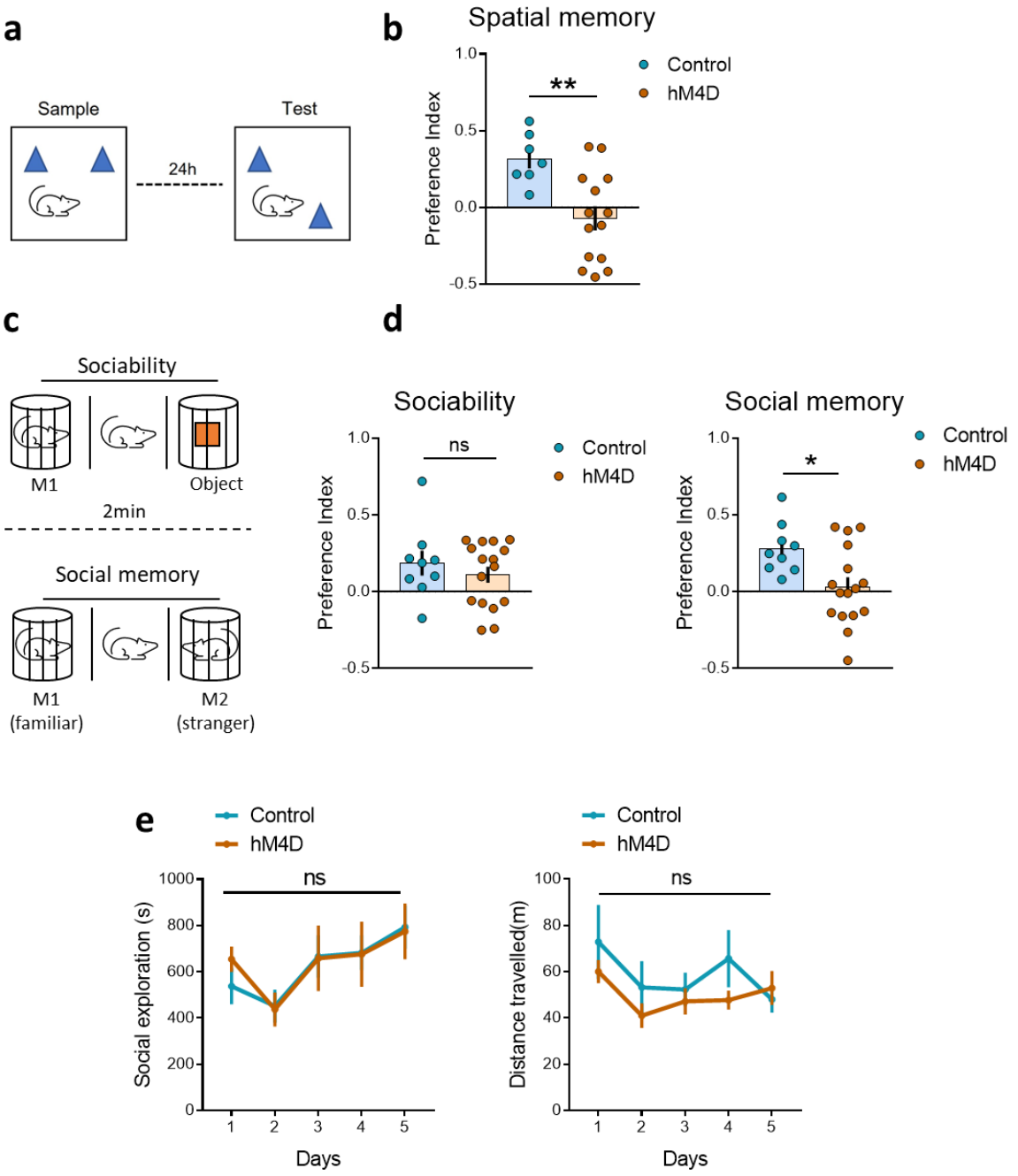

Fig. 28 | dCA1 activity is required for the development of spatial and social memory. a, Schematic representation of object location displacement test with 24h recall. **b,** Preference index of Control (light blue, n = 7) or hM4D (light brown, n = 14) in the object location displacement test (two-tailed unpaired t-test: t = 3.22, d.f. = 19, P = 0.0045). **c,** Schematic representation of the three-chamber task with the sociability and the social memory stage. **d,** Left, preference index of Control (light blue, n = 9) or hM4D (light brown, n = 16) in the sociability stage of the three-chamber task (two-tailed unpaired t-test: t = 0.81, d.f. = 23, P = 0.4263). Right, preference index of Control (light blue, n = 9) or hM4D (light brown, n = 16) in the social memory stage of the three-chamber task (two-tailed unpaired t-test: t = 2.64, d.f. = 23, P = 0.0147). **e,** Left, Social exploration (seconds) of Control (light blue, n = 9) or hM4D (light brown, n = 8) in the SDM observational learning task during the five days of testing in the SDM observational learning task (two-way ANOVA, group (Control, hM4D) x time (days 1-5): F(4, 60) = 0.486, P = 0.7461). Right, Distance travelled (meters) of Control (light blue, n = 9) or hM4D (light brown, n = 8) during the five days of testing in the SDM observational learning task (two-way ANOVA, group (Control, hM4D) x time (days 1-5): F(4, 60) = 1.34, P = 0.2656). [*P < 0.05, **P < 0.01, ***P < 0.001, ns/NS, not significant. Values are expressed as mean ± s.e.m.].

DISCUSSION

1. Social decision-making by direct learning in mice

In this study, we showed that mice are capable of refined social decision-making processes such as altruistic behavior in the presence of their similars. In particular, the majority of adult male mice were ready to share food with their conspecifics, even when this prosocial action came with a cost (ie, increased effort) or without any direct self-benefit (ie, no concurrent reward). Divergence in social decisions originated from differences in sex, emotional state matching and dominance hierarchy. We modeled our SDM on the dictator game, which is a specific behavioral paradigm used for assessing altruistic behavior in humans (Henrich et al., 2004; Lee, 2008; Sanfey, 2007), increasing the novelty and the validity of our results with the mouse model. We also tested different experimental conditions, involving either internal (ie, self-related) or external (ie, other-related) influential factors such as emotions, motivation and dominance, for exploring how decision-making processes operate within a socially interactive and dynamic environment. In the author's opinion, this is one of the few studies capable of reaching such complexity and detail in the analysis of decision-making processes in the social dimension. Thus, our original behavioral paradigm may allow for the examination of more refined social and prosocial behaviors (eg, altruistic behavior), based on social decision-making processes, in research models. This would give the chance for investigating the neural bases behind these sophisticated processes also in a clinical perspective where social decision-making deficits might be present (Arioli et al., 2018; Báez-Mendoza et al., 2021; Besnard et al., 2016; Meisner et al., 2022).

After an initial acquisition phase, mice learned via a trial-and-error strategy to choose between two options associated with different social outcomes (ie, selfish or altruistic choice) in our SDM. In the end, the majority of mice developed a clear social preference for one of the two choices. We confirmed that mouse social decision-making process was contingent on the presence of a conspecific, by testing actor mice in additional conditions where the recipient was not present (ie, without recipient) or replaced with an inanimate object (ie, with toy). Our results are consistent with recent studies performed in rodents, which demonstrated these engage in complex social and prosocial behaviors when others are present (Allsop et al., 2018; Gachomba et al., 2022; Hernandez-Lallement et al., 2015; Hernandez-Lallement et al., 2020). However, our SDM in its design and structure was more suitable for analyzing

altruistic behavior in rodents, particularly in mice. Indeed, we proved mice were ready to share food with their conspecifics even in the absence of any explicit return from their actions (ie, no concurrent reward), which suggested that the prosocial tendency persisted without evident self-benefit that is a critical factor that defines altruistic behavior (Brethel-Haurwitz et al., 2018; Marsh, 2016). Moreover, when altruistic decisions became more effortful and unfavorable in terms of energy requirements (ie, increased FR), adult male mice continued to display a preference for sharing food with their companions.

Besides, actor mice displayed an increased altruistic propensity towards familiar individuals, whereas selfishness increased in the presence of unfamiliar ones. These results are in line with literature findings where altruistic behaviors are thought to have primarily evolved for helping family or in-group members (Bartal et al., 2011; de Waal, 2008; Preston and de Waal, 2002). Our behavioral results also showed that dominance hierarchy between in-group members significantly influenced the preference for altruistic or selfish choice. In fact, social status is very important in a social group and can guide behavior and motivation (Juavinett et al., 2018; Ligneul et al., 2017). Here, we found that the majority of mice that displayed preference for selfish over altruistic choices were subordinate to their recipients. This could be interpreted as a competition for food in subordinate individuals, while dominant might benefit from easier access to food (Zhou et al., 2018). In nonhuman primates, where social ranks are very strict and stable, prosocial responses are more often directed from dominant towards subordinate members (Cronin, 2012). Thus, it is possible dominant individuals would behave in ways that benefit others in order to manifest their dominance. This is also in line with a recent study on rats (Gachomba et al., 2022) where dominant individuals were on average more prosocial.

Besides social dominance, we revealed additional driving forces such as empathy influencing, if not biasing, the development of altruistic behavior. Similar explanations for mouse altruism accompany or even overcome those from evolutionary theories explaining altruism through kin selection and individual fitness (Bartal et al., 2011; Batson, 2010; Preston, 2013). Indeed, in a laboratory setting where animals do not face such selection pressures, these additional determinants for altruistic behavior can provide a more exhaustive explanation why certain individuals engage in altruistic behavior, capturing the multifactorial nature of the process. Following these considerations, we found that altruistic mice were more interested than selfish in partner's social exploration. In fact, the social proximity between each other was a relevant determinant for mouse altruistic choices. The same was for the component of social contact. Indeed, when we denied that possibility, by placing a

transparent or an opaque partition, the altruistic propensity decreased considerably, suggesting social closeness and contact are fundamental ingredients for the altruistic recipe. Finally, according to literature, mice are able to discriminate (Ferretti et al., 2019; Scheggia et al., 2020) and share (Allsop et al., 2018; Scheggia and Papaleo, 2020; Keysers et al., 2022) the affective state of their conspecifics. In agreement, we found that mice that expressed preference for altruistic choices displayed higher affective state matching (ie, higher emotional contagion) with their familiar peers, suggesting more empathy-like behaviors. Consistently, we found that emotional contagion was also linked to the social rank of actor mice, suggesting multiple internal and external factors could drive altruism. In conclusion, our task was able to reveal different fundamental components of social decision-making processes such as motivation, familiarity, empathy and social dominance. This, in turn, would demonstrate that our SDM task was able to reveal the complexity of mouse social decision-making processes that, in a certain sense, could compete with that of humans.

2. Cortico-amygdala connections modulate social decision-making by direct learning

The role of the BLA in decision-making has been largely investigated in rodents under several conditions, such as risk-taking, punishments and threats (Killcross et al., 1997; Terburg et al., 2018; Wassum and Izquierdo, 2015). This evidence provides a robust picture of the critical role of the BLA in the integration of reward-related information and costs to guide decision-making. Association of these information are also integrated by the BLA with motivational and emotional inputs from the PFC and insular cortices (Balleine and Killcross, 2006; Ko, 2017). The preferential connection between the BLA and cortical structures, such as the PFC, has an important modulatory effect on social behavior and transmission of social cues (Allsop et al., 2018; Yizhar and Klavir, 2018). Furthermore, synchronization of neural activity between the BLA and the PFC is important for the establishment of prosocial behaviors such as other-regarding preference in nonhuman primates (Chang et al., 2015; Dal Monte et al., 2020). Thus, we hypothesized that the perturbation of the BLA neuronal activity during social-decision making might affect mouse social decisions towards their conspecifics. In agreement, we found that the downregulation of the BLA, through our chemogenetic manipulation, reduced the capacity of mice to show altruistic behavior towards their familiar peers. Besides, BLA silencing also brought to a reduction of mouse emotional contagion

respect to their conspecifics, which was correlated with their preference during our SDM. Thus, the establishment of a preference toward altruistic or selfish choices could in part be related to empathy-like capacity in mice. Altogether, the effects after neuronal silencing of the BLA might indicate that this structure could mirror the value of reward for self and for others, with relevant consequences on following decisions regarding them in the social context.

Downregulation of the BLA was also associated with lower social dominance. In nonhuman primates neural ensembles in the amygdala are correlated with the social rank of conspecific images (Munuera et al., 2018) and ablation of the amygdala caused a change from top to bottom of the dominance hierarchy (Rosvold et al., 1954). Previous studies also provided evidence pointing to the involvement of the PFC (Wang et al., 2011; Zhou et al., 2017, 2018), thus regulation of dominance behavior might be driven by the communication of multiple areas such as the BLA and the PFC. In the current PhD project, we particularly helped to complete previous research on the amygdala by indicating that the BLA is involved in the representation of social status that modulated the expression of social preferences in our SDM. Indeed, animals with silencing of the BLA displayed lower social rank and, at the same time, higher preference for selfish choices. Taken together, these data indicate that the BLA carries information about social rank that is critical for decision-making processes in the social arena. Considering the involvement of the PFC in the plastic modulation of social hierarchy, the role of the ACC in social information processing and empathy (Allsop et al., 2018; Jeon et al., 2010; Keum and Shin, 2019) and the reciprocal connections between the BLA and these cortical regions, the BLA might be considered as an hub where relevant cognitive and socio-emotional information is gathered to direct prosocial behavior.

As already introduced above, previous research has provided evidence pointing to the involvement of the PFC in social decision-making processes, including the modulation of relevant components such as social perception, social dominance and other-regarding preference (Bicks et al., 2015; Dal Monte et al., 2020; Zhou et al., 2018). Thus, the reciprocal communication between the PFC and the BLA might be extremely relevant for social decision-making processes like altruistic behavior. In line with this, we found that the effects of BLA→PL projections on altruistic choices were more similar to overall BLA silencing, producing an impairment in learning the SDM probably due to valence attribution and processing issues. Instead, the effects of PL→BLA silenced projections were more relevant to the development of selfish choices and behaviors towards others. Besides, silencing of the BLA was associated with rank changes down the hierarchy, suggesting that BLA→PL

projections could be more relevant for regulating hierarchy. In agreement with this, literature reports PL→BLA projections are not involved in the modulation of hierarchical dominance (Padilla-Coreano et al., 2022). Thus, the BLA, the PFC and their reciprocal connections distinctly modulate social decision-making processes and their key components such as empathy and social dominance, remaining an intriguing topic for future studies.

3. Social decision-making by observational learning in mice

Humans engage in different learning strategies when it comes to decisional processes. Indeed, decisions, including those made in the social context (ie, social decisions), can be either acquired through direct experience (ie, by direct learning) or through others' observation (ie, by observational learning) (Fryling et al., 2011; Seymour, 2009; Yoon et al., 2021). In particular, humans are able to learn movements, emotions and even complex social manifestations such as prosocial behaviors from their similars (Kang et al., 2021; Seymour, 2009; Yoon et al., 2021). Observational learning often produces a facilitatory/enhancing effect that can fasten the acquisition and the expression of the observed behavior (Yoon et al., 2021; Zonca et al., 2021). We have seen that nonhuman animals such as rodents, similarly to humans, can learn from their conspecifics either spatial or affective information. In fact, literature is becoming plenty of examples where rats or mice display a learning ability conveyed through others' observation and, starting from this, they successfully carry out behavioral tasks never performed before (Fujisawa and Ouchi, 2022; Mou et al., 2022; Nomura et al., 2019). As it happens with humans, this form of learning often can mean an advantage for the observer in their following task execution (Mou and Ji, 2016; Mou et al., 2022; Nomura et al., 2019).

In line with this evidence, we demonstrated mice can learn social decision-making processes such as altruistic behavior not only by direct but also through observational learning. Indeed, observer mice successfully learned the SDM from their previous demonstrators' observation and were already able to express their social decisions towards their companions. In particular, observers displayed greater understanding of the SDM at start, suggesting they already knew how to act in the social arena as soon as they were given the chance to perform. The observational learning phenomenon was confirmed by the fact that observers, differently from demonstrators, did not require any initial acquisition phase, but their social preference was already decided from the start. Meaning, all the observers'

learning of the SDM was already realized and achieved through their conspecifics' observation. Taken together, these results are in accordance with literature studies reporting rodents display successful and enhanced learning after observation of spatial and affective tasks (Fujisawa and Ouchi, 2022; Mou et al., 2022; Nomura et al., 2019). Our results add a further step to this pre-existing literature showing that mice can successfully exploit social information gathered from others also for the realization of more sophisticated behaviors in the social arena such as those based on social decision-making processes.

Observational learning also depends on additional internal or external factors (Terenzi et al., 2021, 2022; Tremblay et al., 2017). These are mainly related to characteristics proper of the self (ie, internal) or of the others (ie, external), respectively. For instance, the context where observational learning takes place can dynamically change, thus compromising the following expression of the observed behavior (Fryling et al., 2011; Zonca et al., 2021). Specifically, changes in the initial learning context might require enough flexibility from the observer to adapt with these new conditions. Moreover, observational learning often can fall into pure imitation behavior without the need from the observer to really understand or appreciate what is observed (Kang et al., 2021). In this scenario, observational learning might be configured as an exact repetition, without necessarily comprehending the meaning, of the movement sequences shown by the demonstrator. Following these considerations, we conceived different types of controls in our SDM observational learning task for specifically assessing the flexibility and adaptability of mouse observational learning capacity. Our analyses of the observers' preference for the nose poke side (ie, left vs right nose poke) or the nose poke choice (ie, preferred nose poke vs nonpreferred) of their previous demonstrators highlighted mouse observational learning was not an exact repetition of the observed movements. Rather, observers were following their own course of actions for solving the SDM. Besides, we found mice were able to display observational learning even when the context was modified from their first observation. Even though the initial contextual references were moved (ie, nose poke configuration), observer mice adapted to the new apparatus conditions and successfully performed our SDM. These results agree with existing literature on rodent observational learning that demonstrate the flexibility and adaptability of this behavior to changing conditions in the environment (Fujisawa and Ouchi, 2022; Troha et al., 2020).

Besides, observers might be affected by the actions or individual characteristics of their demonstrator (Selbing et al., 2014; Terenzi et al., 2022; Zonca et al., 2021). This could facilitate or damage the learning experience of the observer. For instance, a behavior with a

positive social valence such as altruistic behavior displayed by the demonstrator might favor the learning experience of the observer (Zonca et al., 2021). Or the fact the demonstrator is closer to the observer might influence the further performance of this. Viceversa, characteristics proper of the observer such as predisposition or proximity, either spatial or social, to the demonstrator might increase its learning experience of the behavior observed. For this reason, we analyzed parameters such as social preference or proximity and, for the most, we did not find any evidence that the following learning performance of observers was changed. Despite that, we found a tendency for altruistic observers being better learners compared to selfish ones. It might be altruistic observers were more engaged during the task, due to the retrieval of the reward shared by their demonstrator, and this might have favored their SDM learning. Instead, selfish observers were less engaged or active and this might have slowed down their learning performance. At the same time, we noticed a bad performance from demonstrators (ie, models) could compromise observers' learning experience and further task execution. This is in line with human literature that shows models with their bad demonstrations can influence observers in their acquisition and further realization of the behavior observed (Selbing et al., 2014; Terenzi et al., 2022; Yoon et al., 2021). This result also gives the idea, once more, of the complexity of social manifestations that can be found even outside the human reign.

We also investigated whether the social preference of the demonstrator could affect the social preference of the observer. In this framework, we thought the observation and the fruition of a social positive act such as an altruistic action might push observers to reciprocate with another positive action (ie, reciprocation) (Dolivo and Taborsky, 2015; Snippe et al., 2018). At the same time, reciprocation might also stand for negative actions previously received such as selfish ones. Despite only half of the observers displayed reciprocation motives, after digging into our data, we found very important sex- and social preference differences. Indeed, the reciprocation bias was almost entirely concentrated in the altruistic portion of the observers. Meaning, observers mostly reciprocated altruistic actions previously received. Reciprocation, in fact, has been fundamental for the evolution of prosocial behaviors such as altruism (Dolivo and Taborsky, 2015; Snippe et al., 2018; Trivers, 1971). This result also follows other findings in literature where rodents consistently reciprocate to their conspecifics, especially in the case of positive rewards previously received (Dolivo and Taborsky, 2015; Kettler et al., 2021; Rutte and Taborsky, 2007). Furthermore, the majority of reciprocating observers, either altruistic or selfish, were male observers. Despite much evidence exists regarding female rats' reciprocation (Dolivo and Taborsky, 2015), we did not

find this enhanced propensity to reciprocate in female observers. Just two female observers of thirteen were reciprocating and, intriguingly, they were reciprocating altruism. This might suggest female observers were less bound to their demonstrator's social performance, then their behavior would be more adaptive than males. Outside of the reciprocation argument, we also found that the majority of female observers, not necessarily reciprocating, were altruistic, even though according to our previous results they do not share the same altruistic predisposition of adult male mice. At this point, we might speculate that additional factors might have played a role in female observers' learning. As already introduced, social dominance or emotional factors might help explaining the transition from selfish demonstrators to altruistic observers. Or even biological determinants (eg, estrous cycle synchronization) might have altered the results we obtained with females (Misiolek et al., 2022).

In summary, we extended our original SDM with an additional testing phase capable of measuring observational learning ability in mice of both sexes. Through the use of a precise index such as the learning index, we assessed the existence of mouse observational learning also during a task that involved complex social operations such as social decision-making processes. We revealed previous others' observations brought a significant performance advantage in our SDM. Moreover, we demonstrated, by testing different conditions and parameters during the SDM, that mouse observational learning is not necessarily the mere imitation of their conspecifics' behavior, rather it configures as flexible and adaptable to changing conditions in the surrounding environment. However, as our analysis highlighted and in agreement with existing literature, reciprocation behavior remained a fundamental aspect of observational learning, an aspect that should be further investigated also in relation to sex differences.

4. The role of the dorsal hippocampus in social decision-making by observational learning

In the past, the HPC has been profoundly implicated in social learning and memory processes (Okuyama et al., 2016; Okuyama, 2018; Rao et al., 2019; Smith et al., 2016). More recently, the HPC has also been associated with goal-directed behaviors and decision-making processes. Specifically, the dorsal region of the HPC (dCA1) has been linked to abilities such as hippocampal replay (Carr et al., 2011; Ólafsdóttir et al., 2018) and observational learning

(Danjo et al., 2018; Duvelle and Jeffery, 2018; Omer et al., 2018) that can be crucial for guiding individual decisions in the social panorama. Specifically, the presence of social place cells in the dCA1 region has highlighted this area is crucial for tracking others' positions and movements in the surrounding environment (Danjo, 2020; Danjo et al., 2018; Omer et al., 2018). The area is also fundamental when rodents need to use this information to solve spatial and affective tasks (Mou et al., 2022; Nomura et al., 2019; Terranova et al., 2022). Besides, lesioning or inhibiting the area brings to observational learning deficits denying the performance advantage reached through prior observation (Mou et al., 2022; Nomura et al., 2019). Nonetheless, a study specifically addressing the involvement of the dCA1 in observational learning of more refined social expressions such as social decision-making processes is still missing.

For this reason, we chose to investigate the role of dCA1 in our SDM by observational learning. We used the chemogenetic approach because this modern neuroscientific tool allows the manipulation of a specific area when a certain behavior of interest is enacted (Roth, 2016; Zhu and Roth, 2014). By inhibiting or exciting the selected area, the chemogenetic instrument gives the chance to dissect the specific contribution of the area in the behavior under observation. For the specific aims of this PhD project, we applied chemogenetic neuronal silencing of the dCA1 while mice were observing the SDM from their conspecifics. We mainly worked on the acquisition phase when mice are learning, for the first time, the SDM through observation of their similars. We found that dCA1 neuronal silencing impairs the observational learning phase of social decision-making processes such as altruistic or selfish decisions. By silencing dCA1 during the acquisition stage, observers were not able to express a clear social preference when they were called to act. Differently from naïve mice and controls, the majority of dCA1 silenced mice did not take advantage of previous SDM observation from others and exhibited inconsistent social preference at start. dCA1 silenced observers were not affected in their number of responses during the task, this suggesting dCA1 silencing did not produce impairments at the operational level. Elaborating this, the observers' disrupted learning was not due to a difficulty in operationally giving the responses in the SDM. Rather, the inhibitory manipulation of dCA1 led to impairments in the association between the spatial and the social information obtained from others, that was necessary for the clear expression of social decisions since the earliest stages in the SDM. The temporary blockage of the dCA1, then, brought to the impossibility of acquiring, through others' observation, the right combination between the spatial location and the associated

social outcome since start. Learning this combination was, indeed, crucial for giving appropriate social responses from the start in our social task.

Additionally, we found not enough evidence for the effects of dCA1 silencing on observational learning during the retrieval/recall phase. Observer mice, for the majority, were not impaired in their social performance even though we administered CNO. They rather performed normally, suggesting that dCA1 involvement is crucial during the acquisition phase or the first encoding of the social information but might not be necessary for the retrieval of the social information. Indeed, other areas such as cortical or subcortical ones might be recruited during the retrieval phase (Kumaran et al., 2015; Terranova et al., 2022). This is in line with another study that found dCA1 having an effect on the acquisition and not at the retrieval phase of social behaviors (Chai et al., 2021). However, there were a few observers silenced during retrieval and showing bad performance at start. We were not able to connect the bad performance of observers with a bad demonstration from their demonstrators. Then, it is still possible dCA1 silencing during retrieval might bring impairments in social learning and memory processes. This conclusion would be supported by some literature studies (Nomura et al., 2019; Roy et al., 2017; Wilmot et al., 2019). Then, it is necessary to further dissect the involvement of the dCA1 area in the retrieval of social information acquired through others, eventually using a larger sample.

Besides, we checked whether the silencing of vCA1 area could bring similar results on observational learning. Indeed, vCA1 is known to be deeply involved in learning and memory processes that convey socio-affective information (Okuyama, 2018; Rao et al., 2019). However, we did not find enough evidence to impute a role to vCA1 on observational learning. Observer mice injected with inhibitory DREADDs in vCA1 (ie, hM4D vCA1) performed normally since they started the task. Their learning experience was not affected by our manipulation on vCA1, nor the type of their social preference. We did not silence vCA1 during the retrieval phase, so we cannot completely exclude an effect of vCA1 on this stage. Moreover, it might be that vCA1 mainly influences social decision-making when mice learn the task by direct learning, something we did not test in the current PhD project. Despite these limitations, we can say that, based on our current experiments, the vCA1 region was not essentially involved in the acquisition of the SDM by observational learning. It could be that this area, although its implications in social and affective tasks, is not directly required when learning others' movements and actions in the surrounding environment. Still, it might exert a certain influence when individuals learn the social task on their own. In the latter situation, the information from the dCA1 on other social agents might result less relevant compared to

the primary function carried out by the vCA1 in encoding and storing the socio-affective aspects related to the first-hand experience in the social setting.

Finally, our work on the dorsal region of the hippocampus (dCA1) highlights how deficits in this area slow down or even prevent observational learning from and through our peers in the social setting. Specifically, we found that dCA1 was fundamental for the acquisition of social decision-making processes through others' observation. Indeed, dCA1 silencing, without impairing procedural learning of the SDM task, significantly compromised the establishment of a clear social preference after observation. The disrupted acquisition of the social information gathered through others' observation would have important consequences on the following social performance of the individual. Indeed, the individual would no longer be able to make use of the observed social information and have difficulty in using this to guide its further decisions in the social arena.

5. Concluding remarks

In conclusion, we developed an original social decision-making task (SDM) enabling the detection of social preferences in mice for altruistic or selfish choices and the internal or external factors such as sex, familiarity, empathy and social dominance modulating those preferences. We found that mice, similarly to humans, are capable of refined social decision-making processes such as altruistic behavior towards their similars. Furthermore, mice can reach a social decision regarding other social agents either through direct or observational learning, the latter often guaranteeing a remarkable performance advantage. In this framework, mice represent a valuable model for dissecting more in detail the neural circuitry underlying sophisticated social manifestations such as social decision-making processes occurring in our society, either when the individual learns these first-hand or through the help of others.

In particular, our study highlighted critical neural substrates involved in social decision-making processes such as the amygdala (BLA), the prefrontal cortex (PL) and the dorsal hippocampus (dCA1), probably part of a more extended circuitry that is the social decision-making network. Through inhibitory chemogenetics, we revealed the specific contribution of each identified area in the learning and the expression of social decisions and behaviors. Finally, the current PhD project, by clarifying the neural circuitry underlying social decision-making processes, might help develop more targeted interventions in those

pathological conditions such as neuropsychiatric or neurodegenerative disorders often characterized by social decision-making deficits, that could have a real impact on the life quality and the social participation of the individuals affected.

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