The good, the bad and the toy

On the Behavioral and Neural Bases of Pro-social Choice in Rats

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Abstract English

Pro-social choices are decisions that yield benefit for other individuals. Using comparative psychology and neuroscience, the objective of my thesis was to investigate the behavioral and neural bases of such choices in a rodent model. First, we established a sound and controlled Pro-social Choice Task testing pro-social preferences in rats (***Chapter I***). In a subsequent project, we lesioned the basolateral amygdala (BLA), a structure known to be involved in social behavior, with the hypothesis that BLA-lesioned rats would not acquire pro-social preferences (***Chapter II***). In a third study, we destroyed the lateral orbitofrontal cortex (LO) in rats after having established baseline individual pro-social preference levels and hypothesized that LO lesion would abolish social preference (***Chapter III***). Finally, we investigated the presence of social learning in the PCT (***Chapter IV***).

We first demonstrate that rats prefer mutual rewards and thus behave pro-socially. We further show that BLA-lesions abolish pro-social preferences in rats and report a LO damage-related impairment in social context-specific choice allocation. Finally, we show that choice allocation evolves across trials in both social and non-social contexts, suggesting the presence of social learning in the PCT.

Keywords: pro-social choice; rat; basolateral amygdala nucleus, lateral orbitofrontal cortex; social learning.

Abstract German

Prosoziale Entscheidungen sind vorteilhaft für andere Individuen. In meiner Dissertation untersuche ich die neuronale Basis solcher Entscheidungen bei Ratten, mittels Methoden der vergleichenden Psychologie und Neurowissenschaften. Zunächst entwickelten wir ein aussagekräftiges, kontrolliertes Paradigma zur Untersuchung prosozialer Präferenzen - den "Pro-social Choice Task" (**Chapter I**). In einer zweiten Studie untersuchten wir die Auswirkung von Läsionen der basolateralen Amygdala (BLA) - einer nachweislich wichtigen Struktur im "sozialen Netzwerk" des Gehirns - mit der Annahme, dass die Entstehung prosozialer Präferenzen beeinträchtigt wird (**Chapter II**). Anschließend erforschten wir den Effekt von Läsionen des lateralen orbitofrontalen Kortex (LO) auf prosoziales Verhalten mit der Hypothese, dass solche Läsionen vorab etablierte prosoziale Präferenzen aufheben (**Chapter III**). Letztlich beleuchteten wir die sozialen Lernprozesse welche im Rahmen des PCT stattfanden (***Chapter IV***).

Wir konnten zeigen, dass Ratten gemeinsame Belohnungen gegenüber einer ausschließlich eigenen Belohnung bevorzugen. Läsionen der basolateralen Amygdala heben ebendiese Präferenzen auf. Läsionen des orbitofrontalen Kortex bewirken eine Beeinträchtigung der Diskriminierung zwischen sozialem und nicht-sozialem Kontext. Letztlich konnten wir zeigen, dass sich das Entscheidungsverhalten, sowohl im sozialen als auch im nicht-sozialen Rahmen, dynamisch verändert und damit auf das Vorhandensein sozialer Lernmechanismen im PCT hindeutet.

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Discussion

Abbreviations

ACC	Anterior Cingulate Cortex
B	Basal Amygd. Nucl.
BLA	Basolateral Amygdala
BM	Basomedial Nucleus
BR	Both Reward Choice
CI	Confidence Interval
CM	Central Amygd.Nucl.
CTA	Conditioned Taste Aversion
DA	Dopamine
DLO	Dorsolateral Orbital Cortex
DLPFC	DorsoLateral Prefrontal Ctx
e.c.	External capsule
FL	Frontal Lobe
INS	Insula
LO	Lateral Orbitofrontal Cortex
L	Lateral Amygd. Nucl.
MD	Medial Thalamus Nucl.
MO	Medial Orbital Cortex
MPFC	Medial PreFrontal Cortex
NAcc	Nucleus Accumbens
$NAcc_{Sh}$	Nucleus Accumbens Shell
OFC	OrbitoFrontal Cortex
OMPFC	Orbitomedial Prefrontal Ctx
OR	Own Reward Choice
PCC	Post. Cingulate Ctx
PBS	Phosphate Buffer Solution
PCT	Pro-social Choice Task
PDG	Prisonner's Dilemma Game
PFA	Paraformaldehyde
PFC	PreFrontal Cortex
PirCtx	Piriform Cortex
OT	Oxytocin
STS	Superior Temporal Sulcus
TG	Trust Game
USV	UltraSonic Vocalizations
VLO	VentroLateral Orbital Ctx
VO	Ventral Orbital Cortex

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We all want to help one another.
Human beings are like that.

We want to live by each other's happiness,
not by each other's misery.

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[...] just a story about people and rats.
And the difficult part of it was deciding who the people were, and who were the rats.

Terry Pratchett

The Amazing Maurice and His Educated Rodents

Introduction

(Pro-)Social Choice:

From Humans to Animals – Filling the Gap

Most people care about their lovers, friends and relatives. A great majority of us would incur a high cost in order to protect and help not only the ones we love but also individuals we do not know (e.g., donations). Showing what is commonly referred to as "pro-social skills" is generally appreciated and rewarded in human society (Miller et al., 1991; Wilson, 2015a) whereas lacking such abilities typically leads to isolation, contempt and reclusion (Rose-Krasnor, 1997; Anderson and Kiehl, 2012). As the evolutionist David Sloan Wilson notes, "*thanks to the* [highly pro-social individuals] *of the world, our families, neighborhoods, schools, voluntary associations, businesses, and governments work as well as they do"* (Wilson, 2015b; p. 141).

Beyond mere dyadic synergy, humans maneuver in a multifactorial, rich and extremely complex social environment. Think about it: we interact with countless individuals every day, a cognitive task which requires interpretation (and often prediction) of others' actions and subsequent behavioral adaptation. This task is overwhelming by its complexity. Let me

share a personal experience as an example. About ten years ago, I celebrated my birthday with some friends, and really wanted this particular girl to show up. Unfortunately for me, I was as capable to confidently invite her to the party as an oyster is capable of growing legs and start riding a seahorse. My great aptitude for never-failing plans kicked in: I was going to organize my own surprise party. My best friends would know about it and would invite people on my command while I would pretend to not know anything. Genius, isn't it? Obviously, most people (including the special girl) were invited in that way, otherwise suspicions may have risen. Well, although I had no doubt that my friends would help me as I requested, I am still astonished of how far they went to do as I wished. They played their respective roles with everyone involved in the evening, organized the whole logistic of the party, prepared actual surprises that I was not aware of, and even today some of them would still swear it was a real surprise party if one would ask. Thus, if no one suspected anything of my Machiavellian plan, it is predominantly due to the effort my friends went through to make me happy. Coming back to our matter at hand, I invite the reader to consider the amount of (social) information that needs to be integrated in order to organize such an event. I myself had to anticipate and interpret reactions and behaviors from others in order for the plan to work. I needed to simulate complete lack of awareness about the incoming evening and I even decided not to tell a great friend because I was conscious of his complete disability to keep secrets! But my friends also accepted to go through particular costs of organizing the evening and playing their roles. Now, as Forrest Gump would put it, "that is all I have to say about that".

Through this example, I want to emphasize how incredibly complex decision making and behavior in social contexts can be and yet, how capable humans are at dealing with such choices. As I mention throughout this thesis, other species can show remarkable social choice behavior as well.

Addressing social choice: from humans to animals and back

"*Man is a social animal*". As far as we know, this quote, stated more than two thousand years ago, is the oldest written record formulating the basic principle of the influence of social context on behavior. Through it, Aristoteles outlines the belief that human beings are made to live in groups. This matter became largely discussed in philosophy and is nowadays a central topic of discussion in psychology, physiology, economics and sociology among other fields. This disciplinary crossroad, albeit complex to combine, presents a unique opportunity to test the robustness of theories of social choice behavior in different fields of research. Here is an example:

The self-interest hypothesis, inspired from economic theory, states that decisions should be evaluated on the personal gain (or outcome) received (Fehr and Schmidt, 2006). Thus, according to this theory, individuals should maximize their benefits, even if this yields a cost to others in social contexts. However, experimental research shows that people actively and spontaneously share acquired goods with other individuals (Koch and Normann, 2008) and might even be willing to incur a cost to benefit conspecifics (Fehr and Fischbacher, 2003), thus violating the self-interest assumption. This example, one among many, illustrates how different tools, theories and data from diverse fields can be combined to refine our understanding of a particular process (in our case, social choice).

Rather than economics and experimental psychology, as used in the previous example, my thesis builds upon the combination between behavioral and cognitive neuroscience. These fields of study aims at (among other goals) understanding the input (stimuli) / output (behavior) relationships by addressing the underlying brain processes. To illustrate this aspect, let me share a personal work experience I had a few years ago when I had the chance to participate in a neuroscientific experiment investigating the neural bases of charitable donations in the Life & Brain Institute, in Bonn (Germany). Together with Klaus Fliessbach and Katarina Kuss, we wondered whether mental effort would have a significant impact on donating behavior. To address this question, we asked participants to solve different arithmetic calculations that were monetarily rewarded if correctly solved. Subsequently, we gave them the possibility to donate some or all of their newly acquired money to a charity they had previously chosen. Participants were told that one split among the trials would be randomly implemented and the money would be divided and transferred accordingly. As other experiments had shown, we saw that most participants did willingly donate part of their pay-off. However, without telling the participants, we categorized calculations as “difficult”, “easy” or requiring “no effort” and saw that the percentage of money donated decreased with increasing amount of effort needed to solve the equation. Thus, different features of the equation (input) such as reward magnitude (amount of money) and effort furnished (difficulty of the calculus) had an impact on the behavior (output). This finding suggests that pro-social choice magnitude (i.e. amount of the donation) can be contingent on situational factors, in our case cognitive effort. So far, this experiment used basic behavioral economics, but its result motivated the integration of cognitive neuroscience with the perspective of identifying brain structures involved in the observed behavior. Therefore, we

used a similar design together with functional magnetic resonance imaging (*f*MRI[1]), a technique that indirectly measures brain activity through blood flow in neural tissue, to observe which brain areas were involved in the computation of effort-based charitable donations. Discussing the main results would be too extensive here[2]; rather, my purpose is to introduce naïve readers to how neuroscientific tools can increase our understanding of social decision making. Moreover, a better comprehension of the neural bases of social decision making is a promising avenue for treating conduct disorders and more generally socially-impaired individuals (Paxton and Greene, 2010).

To this day, most research investigating the neural bases of pro-social choices has been carried out on a species that can verbally confirm to be endowed with such attributes, i.e. ourselves. Due to ethical factors, studies typically explore such decisions using non-invasive approaches, i.e. based on techniques that do not harm brain tissue. Maybe the most famous technique used is *f*MRI (see above), although several additional methods are being increasingly used in cognitive neurosciences (see Rilling and Sanfey, 2011; Crockett and Fehr, 2014). Although the use of such techniques has produced a formidable amount of data, the main limitation of these procedures is the lack of causal evidence for the necessity or contribution of a given neural structure in the process of social decision making. As such, animal models of (social) decision making can complement human research at two different levels. First, they allow the use of neuroscientific methods that go beyond large-scale neural recording techniques in humans by providing direct access to neural

[1] Terms written in blue are further explained in the Glossary

[2] Results can be found in : J. Hernandez Lallement, K. Kuss, P. Trautner, B. Weber, A. Falk, and K. Fliessbach, "Effort increases sensitivity to reward and loss magnitude in the human brain.," Soc. Cogn. Affect. Neurosci., Jan. 2013.

activity with high temporal and spatial resolution. Thus, such approaches offer opportunities for causal interventions in the anatomy, activity, connectivity, genetics and neurochemistry of the neural circuits implicated in social decision making processes (Kalenscher and van Wingerden, 2011). Second, through experimental analysis of behavior, such models provide a unique chance to compare the evolution of social decision making across species (Crowley and Zentall, 2013) and sample the spectrum of social behavior from markedly individualistic to highly social species. Therefore, animal models present essential tools to precisely delineate the neural pathways and mechanisms involved in social decision making and provide a method to carry out between-species comparisons that are ultimately relevant for a better understanding of human social cognition.

An interesting epiphrasis of Aristotle's sentence quoted above would be "not only *man is a social animal*". Although it is surely true that humans are social beings, so are other species, from insects to mammals. Thus, in order to understand how social cognition evolved to its current state in human beings, it is important to investigate its current state in other species. This approach, typically called comparative psychology, provides tools and methods to address between-species behavioral differences that are ultimately relevant for the apprehension of human cognition (Brinck, 2008). Joan Silk and Bailey House stated, "*there is currently considerable interest* [referring to pro-social sentiments] *in how we came to be such unusual apes*" (Silk and House, 2011; p.1), but one could also wonder how we came to be such *unusual mammals* and therefore address the (potential) evolution of pro-social choice in non-primate mammals. It is true that generally, non-human primates are the reference model for the investigation of pro-social choice, but recently,

rodents have emerged as a reference model of social decision making. Note that an incredible amount of work on eusocial insects and other animals is of crucial relevance as well, and readers who want to develop a complete view on comparative work of social choice could refer to Schneider and Bilde, (2008), Hou et al., (2010), Meunier et al., (2011) and Strassmann and Queller, (2011).

During my four years as a PhD student, I investigated the behavioral and neural bases of pro-social choice in a rodent species: the rat (*Rattus norvegicus*). If I did my job well, the reader should be convinced by the end of this thesis that understanding how rats (and other animals) perform social choice is highly informative and relevant for human research.

On the definition of pro-social choice

> "***Pro-***": From Latin *pro* for 'in front of, on behalf of, instead of, on account of'. This particle is generally used for strengthening the favoring and supporting aspect of a term.
>
> "***Social***": **1.** Relating to society or its organization; **2.** Needing companionship and therefore best suited to living in communities; **3.** (Zoology) Gregarious; breeding or nesting in colonies.
>
> *"**Choice**":* An act of choosing between two or more possibilities.
>
> Oxford Dictionary, *2015*

So far, I have introduced how animal social choice behavior can contribute to a better understanding of human cognition and how neuroscientific tools can help us in our quest. It is now important to define the exact behavioral and cognitive process I addressed during my PhD: pro-social choice. A common definition promoted in the literature characterizes pro-

social choice as "*any behavior performed by one individual to alleviate another's need or improve their welfare*" (Cronin, 2012; p.1). This definition, formulated in the realm of primate research, implies that pro-social choice must be a goal-directed behavior, the goal being the improvement of other's welfare or well-being (note the particle *"to"* in the quote above). Thus, it implies other-regarding preferences, i.e. an interest in the state of others (Burkart et al., 2007). However, according to the box above, pro-social choice can also be defined as "*any act that supports and favors other individuals' well-being or welfare*", thus unrestricted to other-regarding preference processes. This alternative rationale is not novel and can be found in previous work from Miller and colleagues (1991) who noted that "*acting prosocially does not always require the ability to take the perspective of another; other motivations* […] *may be sufficient for acting prosocially and different types of perceptive taking may be relevant for certain prosocial behaviors but not others"* (Miller et al., 1991; p.56). More recently, in his book "*Does altruism exist?*", the evolutionist David Sloan Wilson proposed a similar definition, agnostic about the psychological motivation and the amount of sacrifice required to help others, and similarly to Miller and colleagues, noted that "*behaving prosocially does not necessarily require having the welfare of others in mind*" (Wilson, 2015a; p. 129).

In our investigation of pro-social choice in rats, my co-workers and I decided to adopt the second, less stringent definition, because we believe it provides a more general framework when addressing the question of pro-sociality in animals. In other words, we think that framing our approach to other-regarding preferences would be deleterious (in a first time) for our purpose. Let me explain. One crucial aspect in comparative psychology is to understand how similar behaviors might differ and relate between species. In

humans, a large amount of pro-social choices are goal-directed behaviors (e.g., donations, the goal being to increase other's benefit and/or your own satisfaction). However, choice allocation patterns that benefit others can evolve based on mechanisms that do not require for an actor to have the concern for others in mind (West et al., 2007a; Strassmann and Queller, 2011). Thus, screening for behaviors obeying to a less stringent criterion might allow to detect possible "*behavior polymorphisms*" or different (and potentially parallel) evolutions of a particular behavior in different species. In other words, pro-social choice defined as other-regarding preferences, as often characterized in primates, might be a particular but different modality or branch of pro-sociality than the one at work in rodents (but again, it is not necessarily the case). Thus, we believed that screening for behaviors that increased other's benefit, regardless of underlying psychological motivations, would be a valid first approach to our research question. Certainly, if yielding positive results, further research should aim at refining our understanding of this particular choice process, by for example, investigating whether this behavior would be instrumental on the goal (see general discussion). To sum up this paragraph, I would like to insist that the definition we adopt here makes only very liberal claims about the underlying motives and psychological processes of pro-social choice. Therefore, any choice that produces benefit for another individual is labeled as "pro-social" as long as that behavior happens in a genuinely social context and is driven by social motives, whatever they are. I believe that these limitations are fruitful in the first approaches to a particular behavior, and can be overcome by slowly refining our definition based on empirical findings, such as the ones I present in the chapters of this thesis.

Making the case for rodents

"The second rat gets the cheese!"

Paraphrase of Terry Pratchett, in The Amazing Maurice and His Educated Rodents

A note on the comparative approach

Before providing general information on rodent social behavior, it is important to discuss the limitations inherent to addressing social choice in other species. Phylogenetic analysis suggests that rodents diverged from other placental mammals during the Cretaceous period, i.e. from 65 to 140 million years ago (Asher et al., 2005). Determining homologies of distantly related species relies to some extent on comparative knowledge in regard to the ancestral situation. However, while fossils allow us to infer the modifications of morphological traits between species, the extrapolation to cognitive and behavioral processes is far less promising. Thus, is if often assumed that social cognition in rodents and other mammals evolved in parallel upon lineage split in the evolutionary tree. This is a first potential limitation of the comparative approach: it generally considers that modalities present in descendants were most likely also present in a common ancestor, therefore disregarding potential *de novo* development of these modalities. Although studying rodents cannot inform us on what happened during the time after their lineage split from ours on the evolutionary tree, it can provide important knowledge on the possible alternative development of social behaviors. Indeed, despite these limitations, the fact that countless other species from insects and arachnids to non-mammalian vertebrates show social behavior suggests that comparative studies can provide a great amount of evidence on how these processes evolved, potentially uncloaking the question of their origin. It should also be

noted that phylogenetic relationship does not always explain the link between species showing comparable morphological and behavioral features or cognitive abilities. Rather, socio-ecological crossroads such as sharing similar environmental conditions, can explain why certain features might emerge in different species exposed to particular ecological and social constellations. For example, convergence is a phenomenon describing that distantly related species independently develop similar adaptations (e.g., the independent ontogenesis of wings in insects and birds or development of flippers in penguins, dolphins and fishes). Note that psychological convergence can also emerge, as recently suggested in domestic dog (*canis familiaris*; Hare and Woods, 2013). Thus, studying the socio-ecological factors to which different species where and/or are submitted can be, to some extent, a way to include evolutionary origins of social cognition in a given study.

Socio-ecology of rats

Rats are nocturnal mammals belonging to the rodent order. These animals live up to three to four years and reach a body weight of 450-520g in males and 250-300g in females. A pregnant female will give birth to 4-8 babies which go through monogamous (one male, one female) or polygamous (1 male, 2/6 females) breeding. Adulthood is reached within 50 ± 10 days. Social experience during the time before adulthood has drastic impact on subsequent behavior (Lukkes et al., 2009) as well as brain development (Fone and Porkess, 2008). Rats are highly gregarious animals which imply abounding interaction with conspecifics. Generally, social behavior happens extremely fast in the first encounters between individuals. In males, these interactions determine future hierarchy (Baenninger, 1966): alpha (leaders), beta (submissive) and omega (persecuted) individuals. During interaction, rats mostly rely on auditive and

olfactive input rather than visual one (the vibrissae are extremely useful for rats, but are rather used in navigation, orientation and balance). Although several differences have been observed from their free-ranging wild counterparts (earlier sexual maturity, no reproductive seasonal cycle, smaller adrenal glands), laboratory rats soon display social behaviors observed in wild rats. For instance, social ranking (indicated by the degree of submissiveness, see above), auto- and allogrooming or play fighting are regularly observed under laboratory conditions (Whishaw and Kolb, 2005), indicating that the behavior of captive laboratory rats can serve as a proxy for wild-type animals' social behavior.

Laboratory experiments to study behavior

The main advantage provided by laboratory experiments is the capacity to place animals in minimalistic situations that allow precise quantification of specific parameters (Skinner, 1953; Zentall, 2011). In the investigation of social choice mechanisms, paradigms typically aim at identifying the influence of social interaction(s) on a series of behavioral parameters. For example, animals are put in presence of another individual and asked to perform decisions whose outcome influences and/or is influenced by the partner. Alternatively, other studies quantify the influence of an individual's experience on an observer's behavior or choice allocation.

Pioneering studies on social behavior in rats reported seemingly contradictory findings which might arise from methodological aspects (see Box 1: "*Methodological considerations on pro-social choice*"). Despite these discrepancies, stable evidence shows that rats' behavior is influenced by conspecifics' experience through the so-called social transmission of information (Church, 1959).

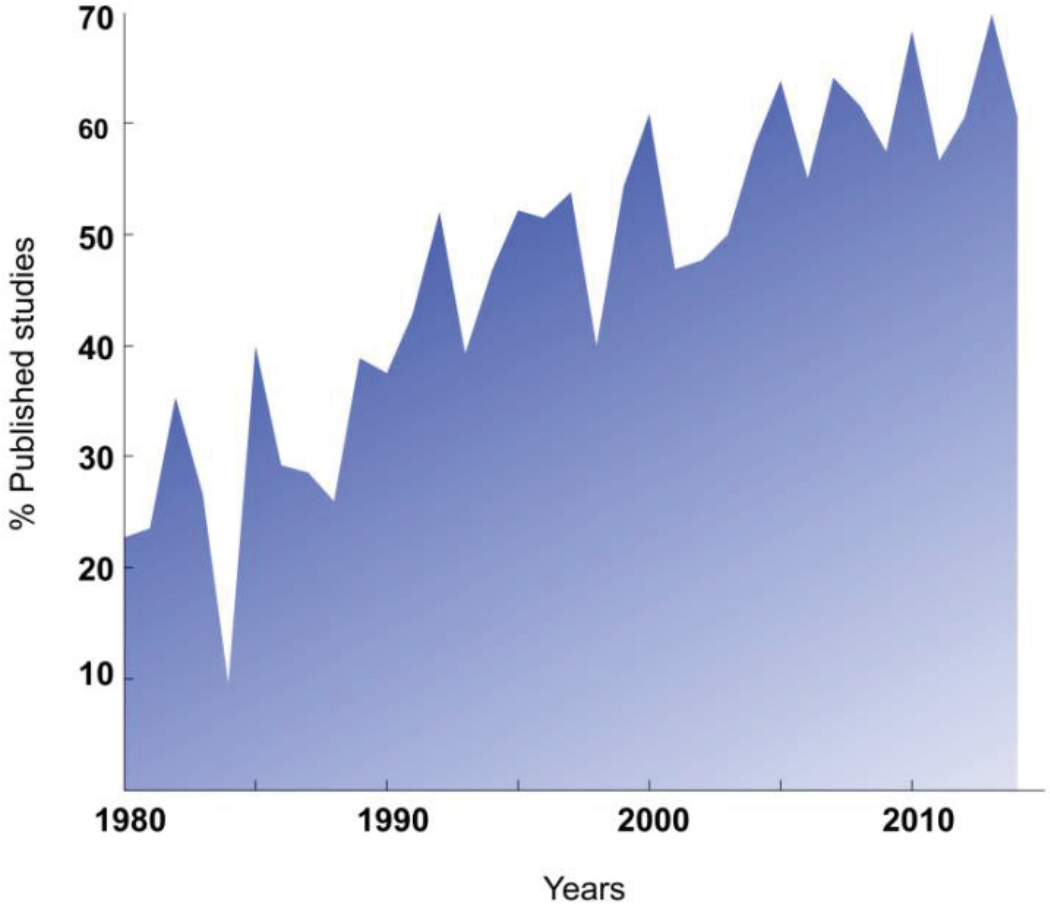

Figure 1A | Number of studies on rat and social behavior indexed on Web of Science between 1980 and 2015. Ratio between number of neuroscientific publications on rat's social behavior and studies investigating social behavior in rats (Rat * Social * Neurosciences / Rat * Social). See furnished Media Supplementary Materials (USB key) for dataset.

For instance, social interaction modulates foraging behavior (Galef, 1985; Galef and Whiskin, 2008) and motor learning (Zentall and Levine, 1972) as well as avoidance (Masuda and Aou, 2009) and fear-related behavior (Kim et al., 2010).

Box 1

Methodological considerations on pro-social choice

One of the main goals of investigating the impact of social context on behavior in controlled laboratory experiments is to isolate the source of reinforcement from subsequent behavioral responses. Importantly, in minimalistic situations where operant behavior takes place, slight modifications of the experimental design can have drastic impact on behavioral measures. For example, cooperative behavior emerged in rats (Daniel, 1942) but disappeared when the chamber length increased (Daniel, 1943) or when physical contact became impossible (Marcuella and Owens, 1975). Similarly, empirical evidence suggests that deprivation levels can influence the establishment of social behavior in rats (Taylor, 1975; Viana et al., 2010), higher deprivation levels being correlated with decrease in pro-social behavior. Finally, a decrease of social learning rates (Bunch and Zentall, 1980) and cooperative moves after abolishment of visual communication (Gardner et al., 1984) has been shown in rats.

These considerations do not suggest that animals do not use social cues to guide decisions. For example, a naïve rats learn faster when put in presence of an experienced conspecific than with a naïve partner (Zentall and Levine, 1972), and animals show pro-social choice contingent on the partner identity (Rutte and Taborsky, 2007a), demonstrating that social cues can influence behavior. Rather, they suggest that social and non-social stimuli might compete in the acquisition of behavior. Thus, depending on task contingencies such as experimental design, physical structure or sensorial communication possibilities, non-social reinforcement might compete and eventually overcome social-cue based behaviors. The potential competition between social (using the partner to direct decisions) and non-social (using non-social cues) behavior reinforcement has been addressed in a recent study in which pairs of rats performed cooperative coordinated moves to access reward (Schuster, 2002). In one condition, rats were informed of their correct or incorrect position by a light cue,

whereas the complementary condition did not use such non-social signaling. Interestingly, results showed that the establishment of cooperative coordination in rats increased when mediated by a non-social light cue, and was abolished when reward was delivered regardless of the partner's move. As pointed out by the author, "*it seems likely that learning to use the reliable and unvarying nonsocial light cue was easier than using the variety of possible cues from a partner's presence and behavior*" (p.60), suggesting a competition between social and non-social cues on behavior acquisition.

Furthermore, recent work shows that rats reciprocate help to partners that previously helped them (direct reciprocity; Rutte and Taborsky, 2007a) and provide help if they received assistance from others in the past (generalized reciprocity; Pfeiffer et al., 2005; Rutte and Taborsky, 2007b). Moreover, helping behavior is modulated by social experience, that is, actor rats preferentially assist helpful partners they had previously been in contact with (Ben-Ami Bartal et al., 2014). Finally, helping behavior can depend on the current satiation state (Schneeberger et al., 2012) as well as food-seeking behaviors of the partner (Márquez et al., 2015).

Altogether, these evidences suggest that rodents can contribute to a better understanding of pro-social choice dynamics. This is corroborated by the dramatic increase in neuroscientific publications investigating social behavior in rodents in the last decades (Figure 1A). Interestingly, other rodent species, such as mice and voles have also received particular attention in neuroscientific research contrary to limited increase in non-human primate species (Figure 1B). Note that ethical restrictions might account for these

discrepancies; nonetheless, the major role of rodent models in this field is undeniable.

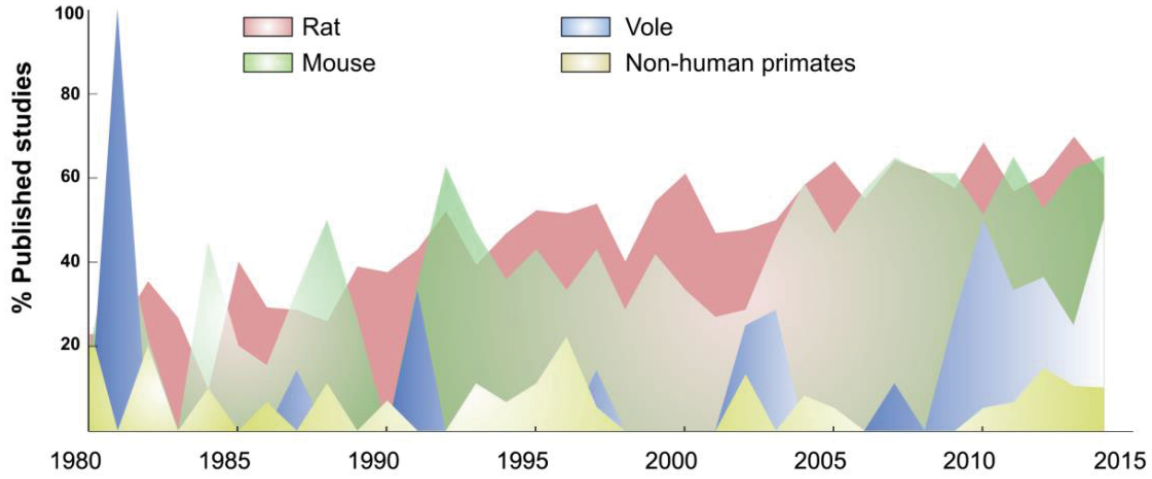

Figure 1B | Model-based publication levels. Ratio between number of neuroscientific studies published on social behavior in a given model (rat, mouse, vole and non-human primate) and all studies using the same model published on social behavior (Model * Social * Neurosciences / Rat * Social). Non-human primate species screened were: chimpanzees, macaques, marmosets, capuchin monkeys, cotton-top tamarin monkeys.

The Neural bases of pro-social choice

The brain consists of two major morphological and functional units (cells): the neurons and the glia. One of the scopes of neuroscience is to explain how these units assemble to control behavior and how they are influenced by other surrounding networks and environmental stimuli. Thus, neurobiology helps us to understand how neuronal networks interact to generate complex behavior.

So far, the common approach to examine decision processes is to investigate the neural correlates of choice behavior, i.e. to focus on the neural signature associated with the properties of the options at stake. In humans, a great amount of evidence has been collected using non-invasive approaches where typically the brain of participants is being scanned while decisions are made. The large majority of studies investigating choice behavior uses “simple” contexts in which generally one agent is involved in- or affected by- the choice. However, in social contexts, more than one person is part of the choice process and/or influenced by the decision’s outcome. Although controversy still exists on the precise neural bases of decision making, it is a now common view that decisions are computed and implemented through cortico-striatal pathways involving mainly the prefrontal (PFC) and parietal cortices as well as striatal and limbic structures (Knutson and Cooper, 2005; Delgado, 2007; Plassmann et al., 2007; Kable and Glimcher, 2009). Here, it is necessary to provide a short introduction on the discovery of the brain reward system and its role in social decision making.

The reward system

One of the greatest neuroscientific breakthroughs in the last decades is the discovery of the reward system. Historically, reward processing mechanisms were suggested by studies on animals investigating drug addiction such as the pioneering work from Hoebel, 1985. Typically in these experiments, it was shown that rats voluntarily self-administer intra-cranial infusions of amphetamine (later studies replicated the effect using other addictive solutions such as cocaine).

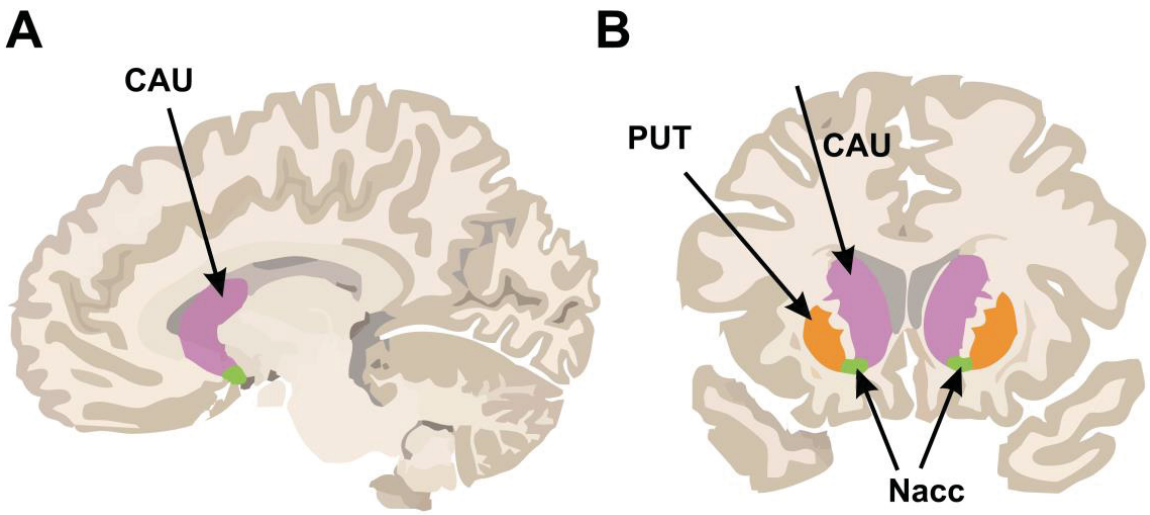

Figure 2 | The subdivisions of the striatum. A. Sagittal and **B.** coronal section shows the position of the caudate nucleus (CAU), putamen (PUT) and Nucleus Accumbens (NAcc), altogether forming the striatum. Modified from Sanfey, 2007.

Because amphetamine increases levels of a particular neurotransmitter called dopamine (DA), it was suggested that primary targets of these fibers, mainly a specific region located at the base of the forebrain (the striatum; Figure 2), might be a crucial area in the establishment of the self-administration behavior. In another major study from Schultz et al., (1997) carried out in non-

human primates, it was shown that midbrain dopaminergic neurons adapt their firing rate to the expectancy of a liquid reward, thus linking DA to the anticipation of rewards and unraveling this neurotransmitter as a cornerstone of the reward system.

It is now known that the firing of these neurons (as well as the *f*MRI BOLD signals measured in their primary target, the striatum) reflects a signal used to update current environmental contingencies to ultimately drive behavior through trial and error, a learning process also known as Reinforcement Learning (RL; Barraclough et al., 2004). The neural underpinnings of this phenomenon have been largely addressed by using recordings in behaving animals in so-called operant conditioning (Skinner, 1953), such as Pavlovian (stimulus-outcome) or instrumental learning (response-outcome). Typically, implanted animals learn that a particular stimulus (tone or light) or response (e.g., movement, eye movement) produces a reward. For example, an animal has to find the correct solution (yielding food reward) among a certain set of possibilities which it does through the process of trial and error learning. The neural signatures show that whereas DA activity is initially observed at reward reception, this signal gradually shifts in time until DA activity is related to the predictive stimulus rather than the actual reward. Through this mechanism, decision making strategies evolve and improve with experience by constantly updating the environmental stimuli value contingent on reward.

Social choice in the brain

It is strongly believed that the brain translates stimuli belonging to different modalities (e.g., food, money or sexual desire) into a single scale, which allows organisms to weigh and compare reinforcers of different nature.

Given that the mesolimbic DA system scales to several modalities of rewards in both rodents and primates, it is a strong candidate for such processes. Therefore, the reward system described above[3] is not only involved in the processing of primary rewards (or reinforcers) such as food but is also recruited during the treatment of secondary reinforcers such as money (Zink et al., 2004) and arousing sexual stimuli (Prévost et al., 2010), and, to a certain extend, responds as well to social stimuli (Ruff and Fehr, 2014).

A recent endeavor building upon the work in reward processing has been focusing on multi-agents situations and the representation of social stimuli, and decision making in social contexts (Behrens, Hunt, Woolrich, & Rushworth, 2008; Bhanji & Delgado, 2013; Izuma, Saito, & Sadato, 2008). Most initial studies inquired whether positive social stimuli would recruit the reward system as primary reinforcers do. Particularly, a subset of these studies have used game theory, a combination of tasks and models that attempt to explain how decision makers proceed when they interact with one another, in order to understand how social decision making is implemented in the human brain (Fehr and Camerer, 2007). Although controversy still exists (Ruff and Fehr, 2014), most of these studies report that so-called "social rewards", i.e. rewards obtained through social interactions and/or rewarding feelings related to conspecifics' experience, also recruits the reward system. For example, one study used *f*MRI to relate brain activations to the various outcomes of a Prisonner's Dilemma Game (PDG). In the typical version of this game, two individuals have the choice between two alternatives: cooperating and defecting. If both players consistently cooperate, they receive a higher payoff in the long run whereas mutual defection leads to the lowest payoff possible.

[3] Note that additional structures participate in decision making and value-based learning. Additional information can be found in Hikosaka et al., 2014.

However, a temptation to defect is always present because defecting while the opponent cooperates leads to the highest payoff. The main finding was that the striatum shows greater activity for reciprocated than unreciprocated cooperation (Rilling et al., 2004). In addition, this structure shows higher activation for rewards obtained through reciprocated cooperation than by identical, but non-social windfall gains (Rilling et al., 2002; Suzuki et al., 2011). In the Trust Game (TG), another classic scenario of game theory, a player (investor) is given the possibility to invest some money in a partner (the trustee); once transferred, the money is multiplied by a certain factor. Then, the trustee can decide to return part of the money to the investor or not. Thus, if the trustee refunds part of the money, both players will end up with higher endowment; however, the trustee can as well defect, and keep all of the endowment. In a multiple round TG, the activity in the trustee's caudate nucleus scaled with how much reciprocity the investor had shown in previous interactions (King-Casas et al., 2005). Moreover, prior knowledge about the trustee decreased caudate activity in investors responding to their trustees (Delgado et al., 2005), suggesting that neural correlates of factors clearly influencing social choice can be found in brain tissue. In an additional study, investors were given the possibility to punish defectors at their own cost. In such trials, increased activity was observed in the caudate nucleus for real rather than symbolic punishment (de Quervain et al., 2004). Finally, a group of studies has particularly focused on the neural processing of charitable donations. One study found that the striatum was engaged in the reception as well as donation of money (Moll et al., 2006) whereas another study found that this activity was enhanced when decisions were voluntary (Harbaugh et al., 2007).

Regarding animals, a set of studies that received particular attention from the media demonstrated that mesolimbic DA plays a role in the establishment of monogamous pair bonds in prairie voles (*Microtus ochrogaster*), a rodent species characterized by a life-lasting partner choice (Aragona et al., 2006). Particularly, it was suggested that the co-localization between striatal DA and neuroendocrine receptors could mediate the establishment of pair bonding, because such receptors, involved in complex social behavior, are co-localized with reward processing areas in the prairie voles (Young et al., 1998). Strikingly, a close species, the montane vole (*Microtus montanus*), characterized by a relative asocial and promiscuous life rhythm, does not show co-localization between reward mediators and hormonal receptors in the brain (McGraw and Young, 2010).

In addition to the "basic" reward system depicted in Figure 2, the large majority of studies investigating social decision making reports the activation of additional structures during social decision making (Figure 3). It has been proposed that this "social matrix" would reflect the additional emotional processing and network complexity required in social contexts in which other individuals are involved (Sanfey, 2007). A review of the brain social matrix is far beyond the scope of this thesis and would cloud the clarity of this section. Therefore, in the following paragraphs, I will exclusively focus on two brain areas that I investigated during my PhD: the basolateral amygdala (BLA) and the lateral orbitofrontal cortex (LO). Appendix A provides short information about the additional structures presented in Figure 3. I finish this general introduction by providing a short description of the anatomy and functionality of these two structures.

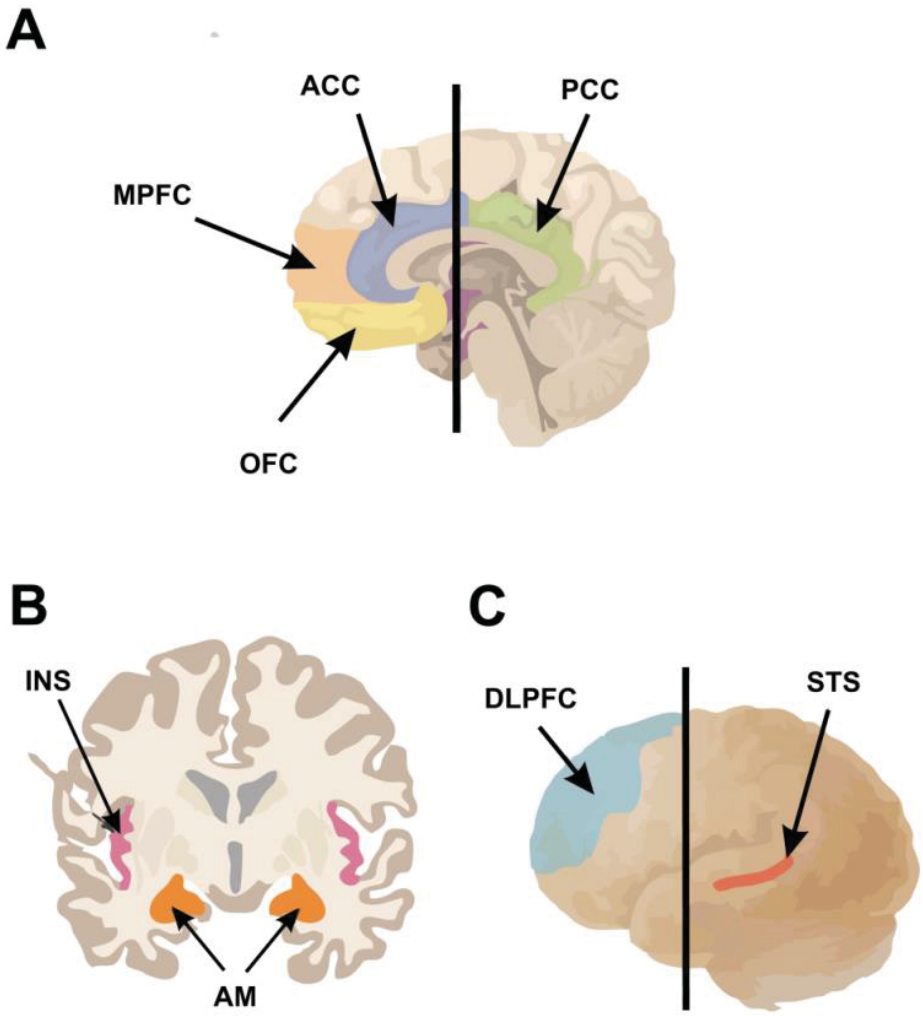

Figure 3 | Map of brain areas typically activated in social decision making studies. A. Sagittal section showing the orbitofrontal cortex (OFC) position, **B.** coronal section showing the bilateral amygdala (AM) location and **C.** lateral view of the human brain. ACC, Anterior Cingulate Cortex; PCC, Posterior Cingulate Cortex; MPFC, Medial Prefrontal Cortex; STS, Superior Temporal Sulcus; DLPFC, DorsoLateral Prefrontal Cortex. Modified from Sanfey, 2007.

The (basolateral) amygdala

The amygdala (AM; also known as amygdaloid body or amygdala nuclear complex) is a brain structure located in the mammal temporal lobe involved in emotional processing and social perception (Amaral, 2006).

Evolutionary homologous of the two[4] major AM neuronal clusters, the corticomedial (CM) and basolateral amygdala (BLA) are found in most mammals including primates and rodents (Figure 4) but also in birds, reptiles and fish (Scalia and Winans, 1975; Jarvis et al., 2005).

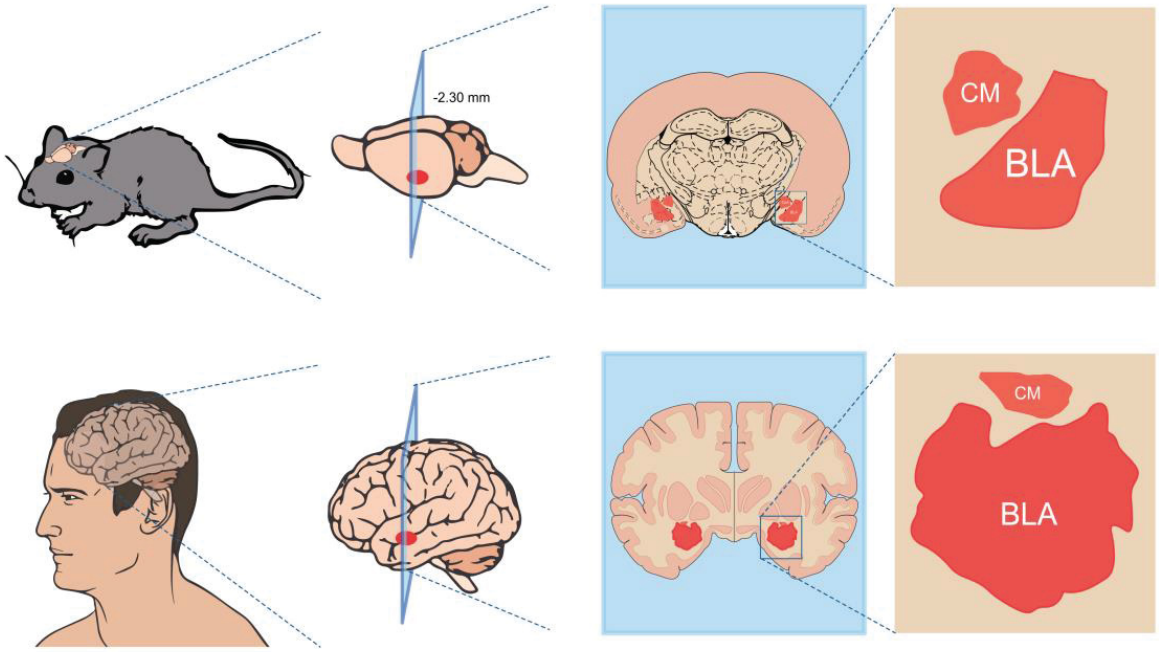

Figure 4 | Comparison of the BLA between rats and humans. The basolateral amygdala is conserved across species in its anatomical location. The BLA and central nucleus of the amygdala (CM) are depicted next to a coronal brain section from a rat (up) and a human (down). From left to right: organism – brain – coronal section – AM sub-nuclei. Modified from Janak and Tye, 2015.

Moreover, functional evidence (see below) suggests that AM functions and circuitry are well conserved across vertebrates (McDonald, 1998). In the following section, I largely focus on the BLA, although occasional references are made to the adjacent CM cluster. Before describing major BLA functions, I focus on its anatomical and hodological characteristics.

[4] Occasionally, a third cluster, the cortical nucleus is included in the AM. The subdivisions defined here are based on the descriptions by Baxter et al. (2002).

Basolateral amygdala neuroanatomy and connectivity

The BLA includes three strongly interconnected clusters (Savander et al., 1997): the lateral, basal and basomedial nuclei (Figure 5).

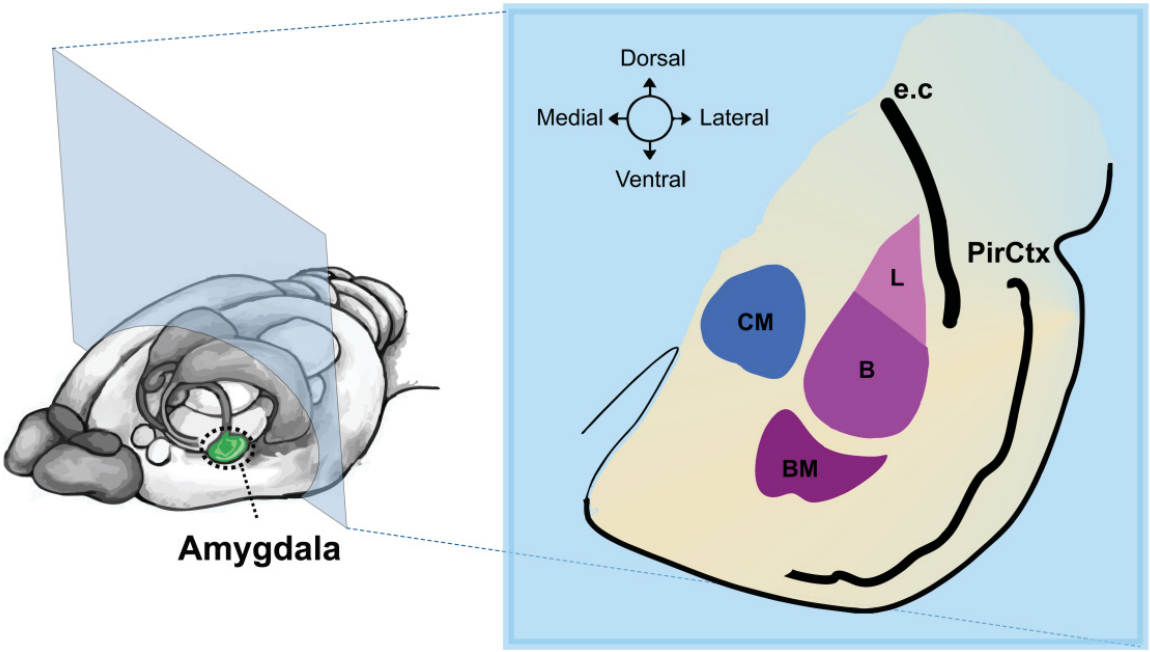

Figure 5 | Nuclei of the rat BLA complex. Left: Whole rat brain illustration, with the left amygdala highlighted in green. **Right:** Coronal sections at bregma -2.30mm. Areas in violet form part of the basolateral group (BLA), area in blue represents the corticomedial group (CM). The BLA nuclei are divided into three groups as described in text. L, lateral amygdala; B, basomedial amygdala; BM, basal amygdala. Other abbreviations: PirCtx, piriform cortex; e.c, external capsule. Modified from Sokolowski and Corbin, 2012.

Each of these nuclei can be further subdivided based on histochemistry and functional anatomy characteristics, but for the sake of clarity, I will hereafter refer to the BLA as the formation of the three aforementioned regions and will discuss its characteristics in rats if not mentioned otherwise. The BLA is laterally surrounded by the external capsule and lies ventromedial to the caudate putamen complex (striatum) and anterior to the ventral part of the

hippocampus. Most neurons in the BLA are pyramidal cells (that resemble the pyramidal neurons found in the cerebral cortex) that represent the main output projections innervating other brain areas. Remaining neurons are non-pyramidal cells that establish amygdalo-amygdalar connections and do not extend beyond the structure's borders (interneurons). The BLA is connected to a large number of structures (Figure 6).

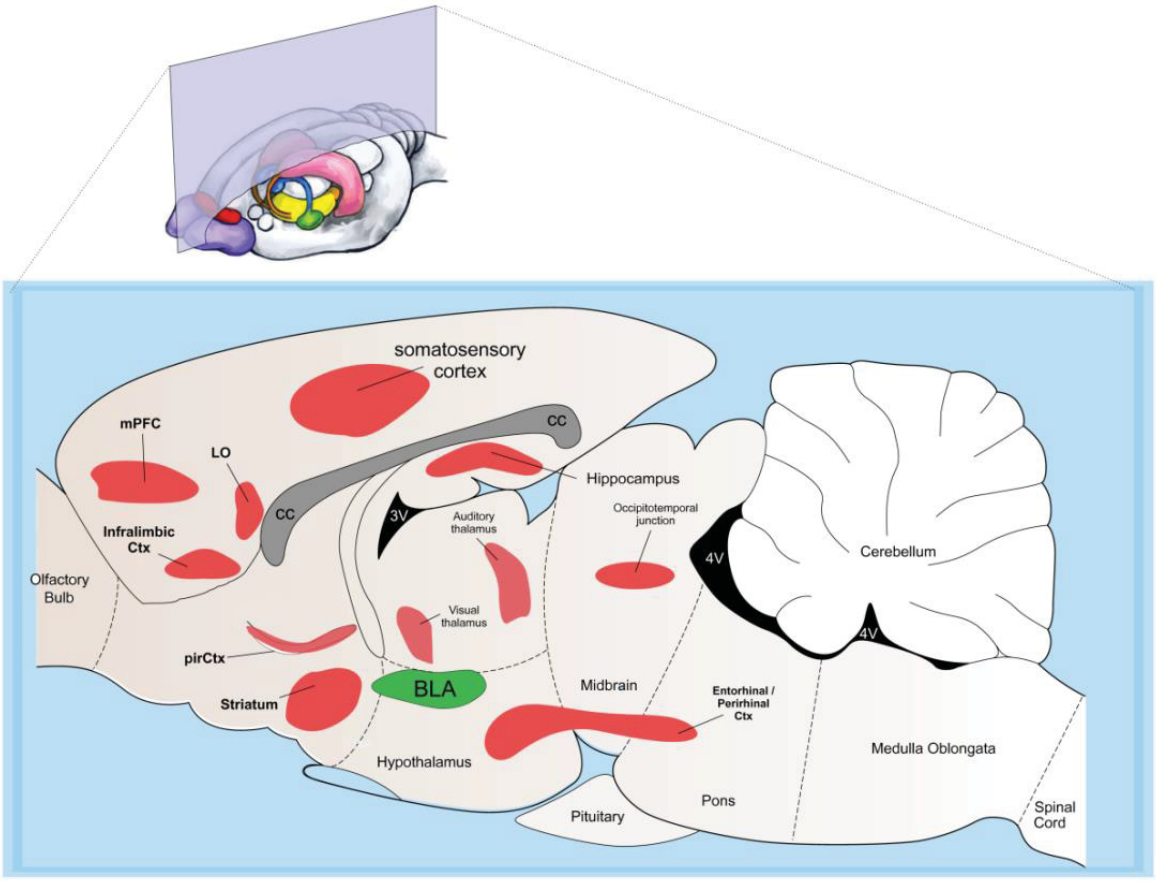

Figure 6 | Rodent BLA connectivity. Up: Whole rat brain illustration (green: amygdala; violet and red: olfactory bulbs; pink: hippocampus; yellow: hypothalamus). **Down:** Sagittal section of the rat brain. Red areas represent neuronal clusters that project to the BLA (green). BLA, basolateral amygdala; LO, lateral orbitofrontal cortex; mPFC, medial prefrontal cortex; pirCtx, piriform cortex. Modified from Paxinos and Watson, 1998.

For instance, strong projections from sensory areas (McDonald, 1998) such as insular (gustatory and proprioceptive areas) and parietal structures (somatosensory) as well as occipito-temporal (visual information; McDonald and Mascagni, 1996) and temporal networks (auditive pathways; Herbert et al., 1991) innervate the BLA. Moreover, the BLA receives robust afferences from the auditive and visual thalamus (see LeDoux and Farb, 1991 and Vaudano et al., 1991 for auditive and visual thalamic projections, respectively). Olfactory inputs originating in the primary olfactory cortex and the main accessory olfactory bulb target mainly the CM nucleus (Scalia and Winans, 1975), sparing the BLA (McDonald, 2009). Thus, most of the BLA sensory input appears to be non-olfactory. Additionally, the BLA receives strong projections from the perirhinal and entorhinal cortices (Krettek and Price, 1978; McDonald, 1998), the piriform cortex (Majak et al., 2004) as well as the hippocampus (Canteras and Swanson, 1992), suggesting that contextual and memory pathways convey to the BLA. The BLA shares robust reciprocal connections with the PFC, mainly with the insular (see text above; Allen et al., 1991), infralimbic (Hurley et al., 1991) and lateral orbital cortices (Krettek and Price, 1977; Ongür and Price, 2000), which form the cortico-amygdalar pathways thought to be involved in decision making and other high cognitive processes. Note that in primates (still unclear in rodents), the BLA-PFC reciprocal connections show higher topographical organization (medial and orbital networks project preferentially to the ventrolateral and ventromedial BLA, respectively; Carmichael and Price, 1995). Finally, very robust BLA projections innervate the ventral and medial striatum, which are known to be involved in instrumental response based on stimulus reinforcement associations (Cador et al., 1989).

Functional evidence

Historically, Brown and Schafer (1888) reported general learning impairment after lesion of the temporal lobe in monkeys. Later, Klüver and Bucy (1937) made similar observations and extended these results to fear processing as well as eating and sexual behavior. With the refinement of lesion methods, a pioneering study found that BLA lesion led to a general impairment in the behavioral and emotional response of visual stimuli (Weiskrantz, 1986). Since then, most of the understanding of concrete amygdalar functions built upon work performed in rodents investigating the neural bases of emotions and related learning, such as conditioned taste aversion (CTA) or fear conditioning, forms of emotional learning in which a given stimuli leads to a defensive response (behaviorally and physiologically) through its association with an aversive event (Phelps and LeDoux, 2005). For example, animals are brought to associate a neutral conditioned stimulus (CS) with an aversive unconditioned stimulus (US) through associative learning, which in turn triggers measurable fear-related behavior (e.g., freezing behavior, see Kim et al., 2010). For example in CTA, the CS (e.g., a light or a tone) is first associated with the delivery of food reward (e.g., sugar pellets). After repetitive exposure to this stimulus, food-related behavior contingent on the ignition of the CS can be observed in the animal (i.e. Pavlovian or classical conditioning). In a second step, the food reward is paired with an aversive state (for example intra-peritoneal injection of lithium; US) and the animal typically shows a reduction in the response to the CS (Holland and Rescorla, 1975; Holland and Straub, 1979).

Several researchers have proposed that the aforementioned reduction in CS response represents the decrease in food reward value. This view implies

that the CS evokes a representation of the food reward in the brain. Interestingly, BLA (but not CM) lesions completely abolish or attenuate the decrease in CS response (i.e. response to reinforcer devaluation; Hatfield et al., 1996), but do not affect the initial CS-reward pairing, as lesioned rats show CS-related behavioral responses (see also Gallagher et al., 1990). Note that BLA-lesioned animals also avoid eating US-paired reward (e.g., pellets presented in the home cages), as observed in non-lesioned individuals (Hatfield et al., 1996). Therefore, BLA lesions seem to specifically disrupt the CS-triggered update in reward representation.

Studies using second-order conditioning support this idea. Here, a CS (referred to as *second-order CS*) presented with an initial, different CS paired with reward (e.g., food; *first-order CS*) acquires so-called second order reinforcing properties, i.e. animals will perform a conditioned response upon ignition of the second-order CS, which was not directly associated with food. Studies show that while both sham-operated and BLA-lesioned rats acquire conditioned response to the first-order CS (as mentioned above), BLA lesions (Hatfield et al., 1996; Holland and Gallagher, 1999) and inactivation (Gewirtz and Davis, 1997) disrupt second-order conditioning in contrast to sham-operated animals. Note that BLA-lesioned rats can sometimes develop normal second-order Pavlovian conditioning if the first-order CS has been established pre-operatively (Setlow et al., 2002), an effect also observed in non-human primates (Málková et al., 1997) relatively specific to BLA lesions (see Thornton et al., 1998).

In non-human primates, studies using selective devaluation of one of two food rewards demonstrated that BLA-lesioned macaques choose both rewards equally often, whereas healthy individuals show a bias towards the non-devaluated, remaining reward (Málková et al., 1997). In line with these

results, BLA lesions disrupt phenomena that depend on the ability to represent the properties of rewards (Blundell et al., 2001), and more precisely of motivationally significant compared to rather neutral events (Dwyer and Killcross, 2006). Note that differential (if not opposite) impact of BLA lesion on reversal and other forms of learning have been reported, from increase (Izquierdo et al., 2013) to decrease (Churchwell et al., 2009)[5] of behavioral flexibility, as well as normalization (Stalnaker et al., 2007). However, such differential effects might be due to experimental protocols (Ochoa et al., 2015). Therefore, altogether, these findings suggest that the BLA, from rodents to non-human primates, is important for the rapid update of stimulus-value associations under specific contextual circumstances.

Until recently, fear conditioning epitomized the role of the BLA (and more generally the AM; see Phelps and LeDoux, 2005) but an increasing number of studies suggest that this area does not specifically compute negative stimulus valence, but also plays a role in reward driven behavior (Janak and Tye, 2015). For example, BLA lesion (Everitt et al., 1991) or reversible inactivation (Fuchs and See, 2002) prevents cue-based memory formation of stimuli of positive valence (see Sacchetti et al., 1999 for similar results using fear conditioning). Furthermore, exciting novel evidence of BLA involvement in the treatment positive valence stimuli computation comes from optogenetics, a powerful light-based tool that allows to specifically (de-) activate neurons in a given structure. For instance, photoactivating BLA neurons increased anxiety in mice, measured as a decreased time spent in the open arm of an elevated plus maze (classical test to measure anxiety levels in

[5] This study used a disconnection approach rather than a lesion.

rodents). However, the same study showed that activating particular BLA pathways (for example the BLA-CM connections) induced anxiolysis (Tye et al., 2011). Thus, diverse BLA connectivity patterns can mediate opposite behavioral effects.

In addition to participating in non-social decision making, the AM (and to a certain extend the BLA) is strongly recruited for choices in social contexts, suggesting that this structure is important for social cognition (Adolphs, 2009). In humans, amygdala volume is correlated with social network size and complexity (Bickart et al., 2011), and is involved in various social processes such as face recognition (Breiter et al., 1996; Morris et al., 1996; Fried et al., 1997), eye contact (Spezio et al., 2007), group affiliation (Bavel et al., 2008), and social judgement (Koscik and Tranel, 2011). In rodents, the AM is involved in rank-related behavior (Rosvold et al., 1954) as well as interaction with a distressed conspecific (Knapska et al., 2006). In monkeys, recruitment of the AM is observed during eye contact (Mosher et al., 2014), face coding (Hasselmo et al., 1989) and social decision making (Chang and Platt, 2013). Finally, substantial electrophysiological data show that AM neurons respond to a large range of social calls in bats (*Pteronotus parnellii*; Naumann and Kanwal, 2011; Gadziola et al., 2012b). This finding receive support a study performed in rats showing an increase and decrease in firing rate contingent on hearing positive and aversive social calls, respectively (Parsana et al., 2012).

The body of evidence shortly reviewed here demonstrates an involvement of the BLA not only in emotional learning, but also in social decision making (see Chapter II). Importantly, this body of evidence suggests that the BLA participates in the treatment of social stimuli carrying both positive and negative valence. Thus, rather than a fear-specialized structure,

the BLA is increasingly seen as a vigilance device based on cortico-striato-amygdalar loops that mediate the binding between sensory stimuli with their emotional significance, to ultimately contribute to the behavioral output (Schoenbaum et al., 1999, 2003; Janak and Tye, 2015).

The (lateral) orbitofrontal cortex

In rodents, the frontal lobe (FL) can be divided in three sections (Heidbreder and Groenewegen, 2003): (*i*) dorsal (including the dorsal prelimbic and anterior cingulate areas), (*ii*) ventral (including the ventral prelimbic and infralimbic areas) in the medial part of the FL and (*iii*) orbital area (OFC) that encompasses the tissue situated on the FL ventral surface.

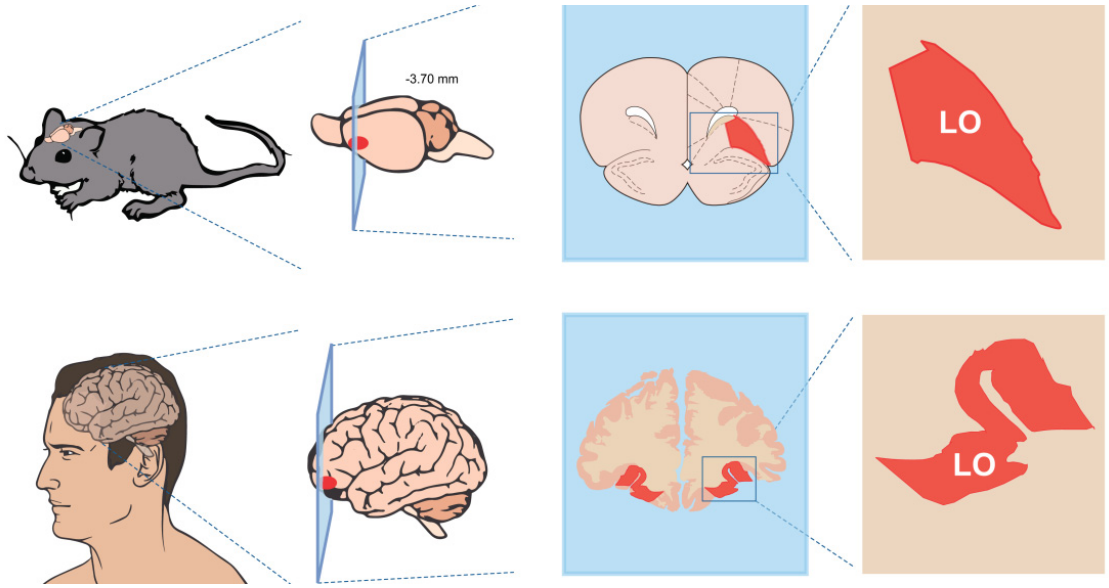

Figure 7 | Comparison of the LO in rats and humans. The LO shows several differences between rats (up) and humans (down). The LO is depicted next to a coronal brain section from a rat (up) and a human (down). From left to right: organism – brain – coronal section – LO sub-nuclei. Modified from Janak and Tye, 2015.

The OFC (together with the whole FL) shows important alterations in size and cytoarchitecture from rodents to primates, which urged some

scientists to put in doubt the validity of between-order/species comparisons (Preuss, 1995). Indeed, beside the important size difference, the most striking distinction between rodents and primates lies in cytoarchitectonic features, mainly the lack of granular cortex (specific cortical layer characterized by the presence of neurons with distinctive "granulated" appearance) in rodents as compared to primates (Price, 2007). To circumvent this issue, it has been proposed that connectivity and hodological analysis, rather than anatomical analogies, could reveal evolutionary homologous areas of the OFC. This approach established cautious but validated between species comparison of the OFC (Figure 7; Ray and Price, 1993; Carmichael and Price, 1995b; Wallis, 2011).

In rodents, the medial and orbital portions of the FL are classically pooled and referred to as the orbitomedial prefrontal cortex (OMPFC; Figure 8A), as first discussed in the seminal paper by Ongür and Price (2000), who described the OMPFC anatomy and input/output relationships in several species. Four (Reep et al., 1996) and more recently five subdivisions (Van De Werd et al., 2010) of the OMPFC can be distinguished (from medial to lateral; Figure 8B): the medial (MO), ventral (VO), the ventrolateral (VLO), the lateral (LO) and dorsolateral orbital cortices (DLO). The recent endeavor promoting the OFC as a constellation of neural clusters playing distinct roles rather than a unique homogeneous structure is receiving empirical support in cognitive science (see Chapter III). Of particular interest in this thesis is the LO, which seems to play a particular role social behaviors in humans. The LO encompasses the brain tissue extending from (lateral axis) the external border of the VO to the internal border of the DO (rostrally) and insular cortex (caudally) and follows (medial axis) the infralimbic and prelimbic areas.

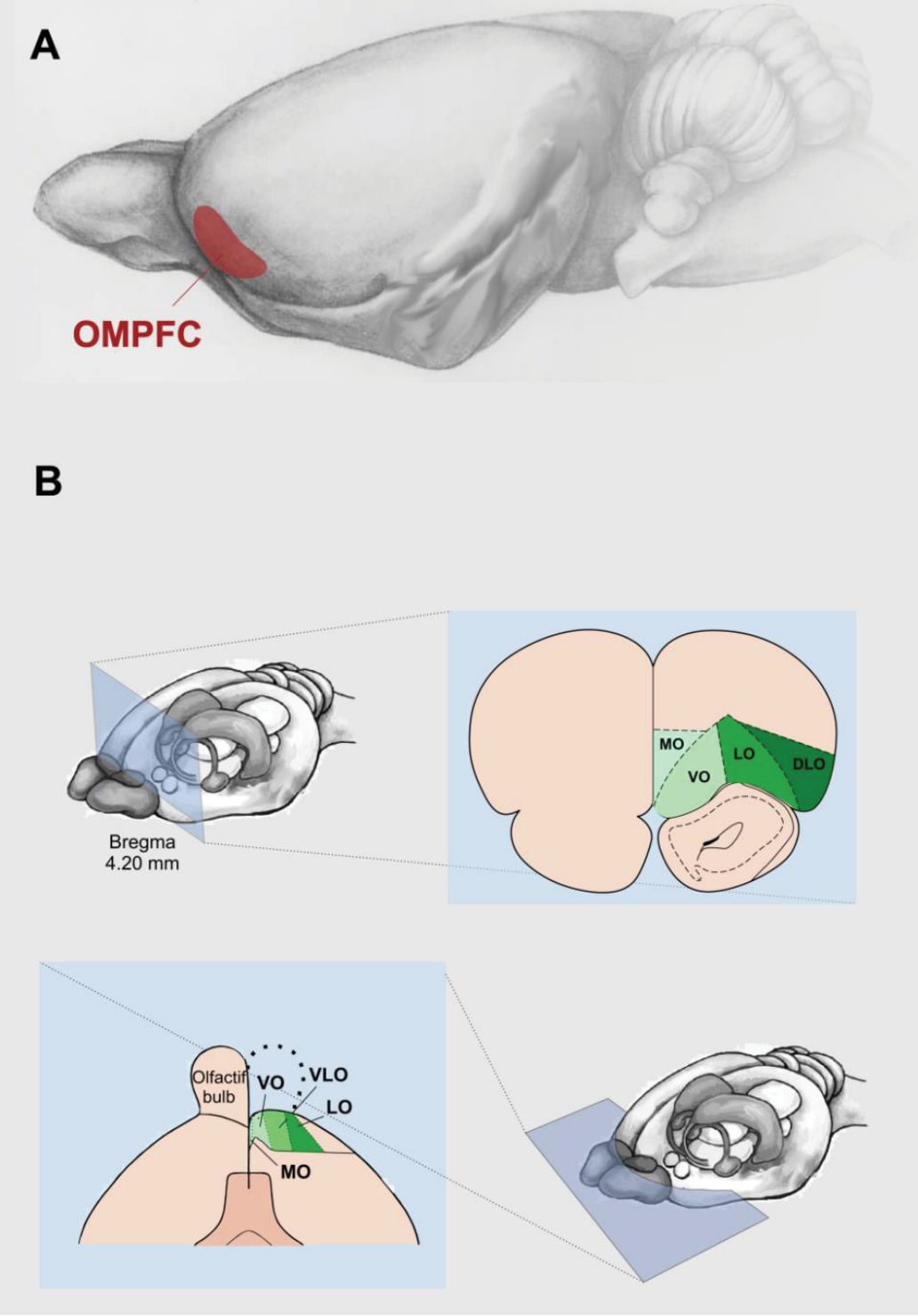

Figure 8 | The rat OMPFC. A. Lateral view of a rat brain. The OMPFC is depicted in red. **B. Major sub-divisions of the OMFPC in rats.** Coronal (up; bregma -4.20mm) and axial (down) sections of the rat frontal lobe. The OFC subdivisions are highlighted on the lateral panels. MO, medial orbital; VO, ventral orbital; LO, lateral orbital; VLO, ventrolateral orbital; DLO, dorsolateral orbital. Modified from Sokolowski and Corbin, 2012 and Paxinos and Watson, 1998.

(Lateral) Orbitofrontal cortex neuroanatomy and connectivity

Because the large majority of studies investigate the role of the OFC as a whole structure, specific LO connectivity studies are rather scarce[6]. According to the delimitation of the LO described previously, I intersected several tracing studies that reported afferences to- and efferences from- this region (Figure 9). The LO (as well as the whole OMPFC) shows intense reciprocal connections to the medial nucleus (MD) of the rodent thalamus (Ray and Price, 1993; Jankowski et al., 2013), which is also the case in primates (Goldman-Rakic and Porrino, 1985; Ray and Price, 1993). Note that MD-LO projections are topographically organized (Figure 9; more medial MD portions project on caudal LO areas; see Ray and Price, 1992; Reep et al., 1996). Using anterograde transport tracing, Sesack and colleagues (1989) reported that mPFC injections of a traceable marker in the rat brain labelled several structures including the deep LO and VO layers (as well as the insular, perirhinal and entorhinal cortices). Interestingly, the infralimbic cortex, an anatomically close region to the LO, projects preferentially to the MO and VO but sparing the LO (Hurley et al., 1991), suggesting that the LO reciprocal connections are rather selective even in its close surrounding. The LO also shares reciprocal connections with the striatum (Selemon and Goldman-Rakic, 1985). More specifically, the LO preferentially projects to the Nucleus Accumbens' shell ($NAcc_{Sh}$) and to the lateral portion of the caudate-putamen complex (Schilman et al., 2008).

[6] Note that recent reviews suggest that most studies performed in monkeys generally assess neural correlates within its lateral portion (areas 11 and 13; Wallis, 2011; Stalnaker et al., 2015).

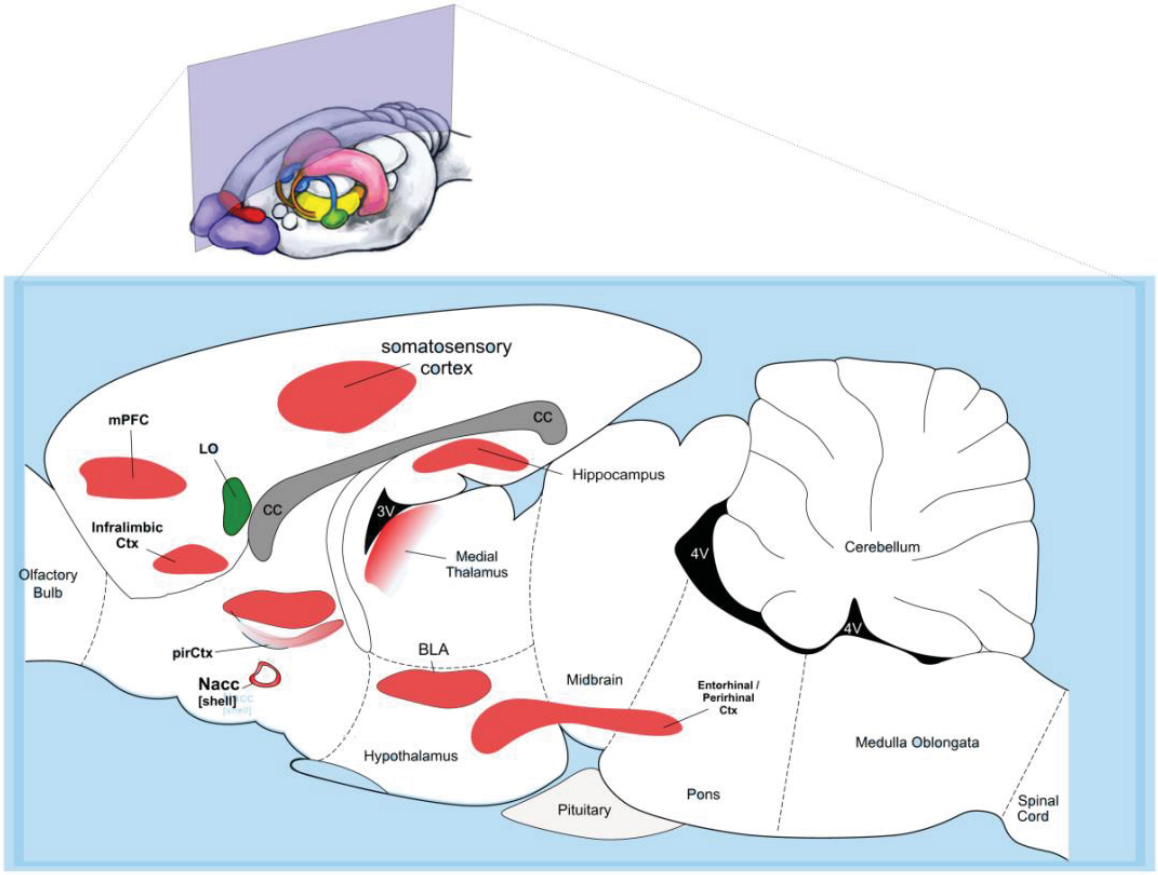

Figure 9 | Rodent LO connectivity. Sagittal section of the rat brain. Red areas represent neural clusters that project to the LO (green). BLA, basolateral amygdala; LO, lateral orbitofrontal cortex; mPFC, medial prefrontal cortex; pirCtx, piriform cortex. Color gradient (NAcc and PirCtx) represent topographical projection strength to the LO. Modified from Paxinos and Watson, 1998.

Similarly, the piriform complex (PirCtx; involved in olfaction) also projects topographically organized fibers to the LO in such a way that caudal parts of the pirCtx project to caudal LO regions (Ray and Price, 1992). Additionally, the LO is a highly poly-modal structure due to dense projections from sensory brain areas such as the primary taste cortex (gustatory) as well as the primary and secondary somatosensory cortex. Finally, it shows strong reciprocal connections within the OFC as well as with dorsolateral prefrontal and cingulate areas in the PFC (Ongür and Price, 2000).

Functional evidence

1. Decision making

The OFC has received particular attention because of its involvement in several forms of psychopathology such as drug addiction (Clark et al., 2013) and poor decision making (Bechara et al., 2000). The OFC is important for value-based decision making, (e.g., deciding whether to drink tea or coffee) and individuals with damage to this structure show a specific impairment in this choice category (Fellows and Farah, 2007). Neuroimaging studies confirm this view, showing a consistent OFC activation during such choices (Kable and Glimcher, 2007; Sescousse et al., 2010). Another set of studies show that OFC damage does not impair initial acquisition of stimulus-value learning but selectively affects novel contingencies acquisition in humans (Fellows and Farah, 2003) and animals (Pickens et al., 2003). Thus, the OFC might be particularly important for behavioral flexibility, i.e. the capacity to adapt behavior in the face of altering situations (Rushworth et al., 2007a). This finding has been widely replicated in the context of reversal learning deficit produced by OFC lesion or inactivation in animals. Typically, OFC-lesioned animals do not show any impairment in initial cue-based learning but show learning deficits when the cue/outcome contingencies are modified throughout the task (Chudasama and Robbins, 2003; Boulougouris et al., 2007). This type of behavioral flexibility might rely on connectivity patterns with other structures such as the BLA (Stalnaker et al., 2007; Churchwell et al., 2009).

Although the last decades brought notable progress to the understanding of OFC functions, several contradictory findings suggest that this structure plays a more subtle role in decision making (Stalnaker et al., 2015). For instance,

whereas some studies reliably demonstrate its role in value-based decision making (see above; De Martino et al., 2006) suggesting that OFC neurons represent the subjective value of rewards (Padoa-Schioppa and Assad, 2006), other studies emphasize that the OFC is important for the learning and storage of options (Kable and Glimcher, 2009). A recent view that might reconcile these contradictory findings proposes the OFC as a combination of sub-structures playing several distinct roles in decision making and learning, thus accounting for the result discrepancies (Wallis, 2011). According to this view, the OFC would be a highly complex modular structure that should be approached as such (Price, 2007). In line with this hypothesis, MO and LO activity (among other regions) is correlated with choices of immediate and delayed rewards, respectively (McClure et al., 2004, 2007) in humans. In macaques, lesions to the MO but not LO disrupt reward-based choices in macaques (Noonan et al., 2010). Finally, in rats, MO and LO lesions decreased and increased impulsivity in a delay discounting task, respectively (Mar et al., 2011).

2. Emotional regulation

Several studies have reported a specific role of the LO such as suppression of previously learned responses (Elliott et al., 2000), punishment magnitude (O'Doherty et al., 2001), and inhibitory control of emotions (Hooker and Knight, 2006). For instance, increased neural activity in the LO during anticipation of pain predicts a decreased pain-related activity in the insula, thalamus and the ACC (Wager et al., 2004; all three structures known to be part of the pain matrix in the brain). Additionally, the ventromedial prefrontal cortex (VMPFC), an anatomically related structure in humans, is recruited when subjects experience "social pain". In a study where subjects played a

game with other individuals, being excluded from the game (social distress) led to lower VMPFC activity, suggesting that this structure might regulate the activity related to the social distress of being excluded (Eisenberger et al., 2003). Interestingly, a role of the LO in the inhibition of pain perception is observed in rats (Zhang et al., 1997), although the pain used was not related to a social context.

Additionally, the LO also facilitates selective attention by inhibiting interference with other social stimuli. In a task where subjects had to focus on a particular stimulus (house) and ignore another one that could be either neutral or containing emotional value (neutral or fearful face), findings show that the LO was selectively engaged when the subjects had to ignore the fearful faces in order to make a decision about the houses (Vuilleumier et al., 2001). This result has been interpreted as evidence that the LO might be involved in inhibiting irrelevant information. Interestingly, LO activity is inversely correlated with activation in the AM during reappraisal of negative outcomes (Phan et al., 2005), suggesting a potential inhibitory effect on AM's emotion representation. Furthermore, Ochsner et al. (2004) found a higher LO activity, as measured with *f*MRI, when using reappraisal to decrease, as opposed to increase, negative affect; thus suggesting that this area might be involved in the inhibition of cortically represented feelings and concepts. The emotional regulation of LO has been extended to gambling behavior (Hooker and Knight, 2006) and depression (Elliott et al., 2002).

3. Involvement in social cognition

Finally, the LO is additionally recruited during self-regulation in social contexts. Numerous studies have reported alteration of social behavior in OFC lesioned patients such as avoiding eye contact, inappropriate teasing and

intimate behavior, suggesting that such individuals do not regulate their behavior using cue-based updating contingent on the current situation (Beer et al., 2003). It has been proposed that human beings possess a system specialized in social cue detection for behavioral adaptation (e.g., modifying the current behavior if being stared at with an angry expression; Blair and Cipolotti, 2000). One possibility is that the LO might be specifically engaged in such processes, as suggested by studies reporting higher activity in the area for angry expressions as compared to neutral ones (Blair, 2003). Thus, this structure might not be only directly engaged in inhibition processes (see previous paragraph), but might also register and implement social cues that indicate a need for inhibition. Finally, lower levels of neural activity in this region are associated with impairments in controlling aggressive tendencies towards others (Goyer et al., 1994), suggesting an role of the LO in the regulation of decision making in social contexts.

Unfortunately, research on specific LO functions in social behavior in rodents is extremely limited (see Chapter III). In rodents, lesion to the OFC increases male-male aggression (de Bruin et al., 1983). Disconnection and inactivation of the OFC and BLA increased impulsivity in rats (Churchwell et al., 2009), fueling the idea that such animals would show an impairment in pro-social behavior, as suggested by the negative correlation impulsivity and pro-social choice in humans (Harris and Madden, 2002; Yi et al., 2005; Curry et al., 2008). Note that this point remains, to this day, purely hypothetical. Recent research performed in non-human primates suggest that OFC neurons contribute to the acquisition of information about others and subsequent social choice (Watson and Platt, 2012), although additional findings propose the OFC as a network detecting own rewards (Chang et al., 2013). Moreover,

the OFC carries reward value altered by social context (Azzi et al., 2012). These discrepant findings might be related to the modular organization of the OFC previously discussed. Finally, the involvement of the LO in social behaviors is supported by the direct relationship between increased local cortex size and social network in humans (Lewis et al., 2011) and apes (Caldwell, 2008).

Content and Chapter contribution

As a neuroscientist and biologist, I aim to contribute to the understanding of social choice from two different perspectives: behavioral and physiological. To do so, my PhD aimed at developing a paradigm investigating pro-social choices in rats which allowed the use of neuroscientific approaches. My **first chapter** describes the basic results of this paradigm, the Pro-social Choice Task (PCT), which tests pro-social preferences in rats. By investigating the choice allocation between non-costly pro-social and selfish decisions, we compute baseline preferences and show that animals behave pro-socially. In the **second chapter**, we demonstrate that the BLA is crucial for the establishment of pro-social preferences measured in the PCT. The **third chapter** presents novel findings suggesting that LO might play a role in pro-social choice allocation in rats. Finally, in the **fourth chapter**, we discuss the dynamics of pro-social choice allocation and social learning within a social reinforcement learning framework.

Chapter I

Rats prefer mutual rewards in a Pro-Social Choice Task[7]

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Abstract

Pro-sociality, i.e. the preference for outcomes that produce benefits for other individuals, is ubiquitous in humans. Recently, cross-species comparisons of social behavior have offered important new insights into the evolution of pro-sociality. Here, we present a rodent analog of the Pro-social Choice Task that controls strategic components, de-confounds other-regarding choice motives from the animals' natural tendencies to maximize own food access and directly tests the effect of social context on choice allocation. We trained pairs of rats – an actor and a partner rat – in a double T-maze task where actors decided between two alternatives, only differing in the reward delivered to the partner. The "own reward" choice yielded a reward only accessible to the actor whereas the "both reward" choice produced an additional reward for a partner (partner condition) or an inanimate toy (toy Condition), located in an adjacent compartment. We found that actors chose "both reward" at levels above chance and more often in the partner than in the toy condition. Moreover, we show that this choice pattern adapts to the current social context and that the observed behavior is stable over time.

1. Introduction

Classic economic theory posits that decisions should be exclusively motivated by self-interest, and decision makers should therefore disregard other individuals' needs (Von Neumann and Morgenstern, 1994; Fehr and Schmidt, 2006). However, empirical evidence does not support this prediction and rather suggests that people actively and spontaneously share acquired goods (Koch and Normann, 2008; Muehlbacher and Kirchler, 2009; Hernandez-Lallement et al., 2013) and care about others (Bernhard et al., 2006). Furthermore, people are adept in detecting and responding to unfairness (Sanfey et al., 2003) and inequity (Sanfey, 2007), and engage in costly behaviors to punish social norm-violation and enforce social norm compliance (de Quervain et al., 2004).

Such behaviors are not just restricted to humans but can be found throughout the animal world, from social choice in our close primate relatives (Burkart et al., 2007; Yamamoto et al., 2009; Cronin et al., 2010; Horner et al., 2011) to the eusocial communities of the ants (Nowbahari et al., 2009). Although the non-human primate models yield important insights into the evolutionary roots of pro-sociality (Brosnan and de Waal, 2003; Cronin, 2012) and their neural underpinnings (Chang et al., 2013), other animals such as rats might offer an equally powerful model to investigate the evolution and neural substrates of social behavior (Kim et al., 2010; Atsak et al., 2011; Kashtelyan et al., 2014; Willuhn et al., 2014). Rats are ideally suited to study social choice behavior. For instance, rats often develop in social groups (Whishaw and Kolb, 2005), have clear hierarchical group organization (Baenninger, 1966) and prefer to eat close to conspecifics (Barnett and Spencer, 1951). Moreover, they are able to display cooperative coordination (Schuster, 2002) as well as direct

(Rutte & Taborsky 2007a) and generalized reciprocity (Pfeiffer et al. 2005; Rutte & Taborsky 2007b). Furthermore, it has been suggested that helping behavior might selectively be engaged depending on the state and bodily mass of a partner (Schneeberger et al., 2012), suggesting that social interaction patterns in rats are dynamic (Ben-Ami Bartal et al., 2014). Finally, it has been recently suggested that rats feel empathy (Ben-Ami Bartal et al. 2011; but see Silberberg et al. 2014). Thus, given that rats are capable of engaging in behaviors that produce benefits for conspecifics, this species is ideally suited to study the evolution, psychology and neuroscience of social behavior.

Hence, what is needed is a standardized, simple, fast and easy-to-train social choice task for rodents. This task should eliminate strategic, reciprocal or other egoistic motivational components and make the underlying cognitive choice mechanisms tractable. Moreover, a sound design should involve non-costly choices to de-confound pro-social motives from the animals' natural tendencies to maximize own-access to food as strong egoistic motives may compete, and thus obscure, pro-social sentiments (Silk et al. 2005). Insights gained from such a standardized animal model will facilitate cross-species comparison of pro-social behavior and will shed light on common evolutionary roots and factors favoring pro-social behavior (Dugatkin, 1997; Kalenscher and van Wingerden, 2011; Brosnan and de Waal, 2014a). Finally, a good paradigm should allow the full range of neurobiological manipulations, including behavioral, pharmacological and electrophysiological measurements, paving the path for manipulation and recording of neural activity during social decision making to enhance understanding and modeling of decision making in social contexts.

The scope of this study is to introduce a rodent analog of the Pro-social Choice Task (PCT; Silk et al. 2005; Horner et al. 2011; Marquez & Moita 2012), a simple and standardized behavioral experimental paradigm adapted from a well-established task in primates (Silk et al., 2005; Horner et al., 2011), to probe pro-social choice behavior. In line with definitions used in the literature (Miller et al., 1991), we define pro-social choice by its face validity as the preference for outcomes that produce a benefit for another individual. We hypothesized that rats behave pro-socially according to the above definition. In this task, rats (*hereafter: actors*) had to choose between two options yielding either only a reward for themselves ("own reward" OR; 1/0) or an additional reward to a partner ("both reward" BR; 1/1). Crucially, we compared the actors' BR preferences in a partner condition, in which the partner was an actual rat, with its BR preferences in a toy condition, where the partner was an inanimate toy rat of similar shape, size and color. We conjectured that, if a conspecific's access to food carries reinforcing value for actor rats (Kashtelyan et al., 2014), they should develop a preference for the "both-reward" alternative in the partner, but not the toy condition. Our main results confirmed this hypothesis: actors chose "both reward" at levels above chance and more often when paired with another rat than with an inanimate toy, suggesting that BR-preferences were dependent on social components of the task. Interestingly, there were large individual differences in the rats' propensity to choose the "both-reward" alternative. Moreover, we show that the rats' social-context-dependent preferences for the BR alternative remained stable after a repetition manipulation, suggesting that social preferences are stable over time.

2. Materials and Methods

2.1 Subjects and housing

Two batches (N = 20 and N = 48, respectively) of male Long-Evans rats (*Janvier Labs, St. Berthevin, France*) were used (see Supplementary Data). Animals were housed in groups of four rats per cage. In a study addressing the effect of food deprivation on choice in social context in rats, higher cooperation rates have been observed in sated rats compared to food deprived rats (Viana et al., 2010). Additionally, recent findings suggest that cooperation rates are influenced by multiple factors, including body weight (Schneeberger et al., 2012). Thus, we opted for a merely mild food deprivation schedule, and daily food intake was restricted to keep animals at >85% of free feeding body weight; to monitor the effect of body weight on social behavior, we included weight as a factor in our analyses to identify its putatively mediating effect on choice allocation (see below). Water was available *ad libitum* in the home cage at all time. All animal procedures adhered to the German Welfare Act and were approved by the LANUV (Landesamt für Natur-, Umwelt- und Verbaucherschutz North Rhine-Westphalia, Germany).

2.2 Experimental setup

Experiments were conducted in a custom-made double T-Maze (Figure 1), with the mazes' main compartments facing each other. The T-mazes were separated by a transparent and perforated wall allowing olfactory, auditory and visual communication. Each T-maze consisted of a starting box connected to two decision chambers by two independently operated doors, each leading to a choice compartment. To prevent the experimenters operating the setup

from influencing the rats' behavior, compartments and starting boxes were constantly covered using removable red tops.

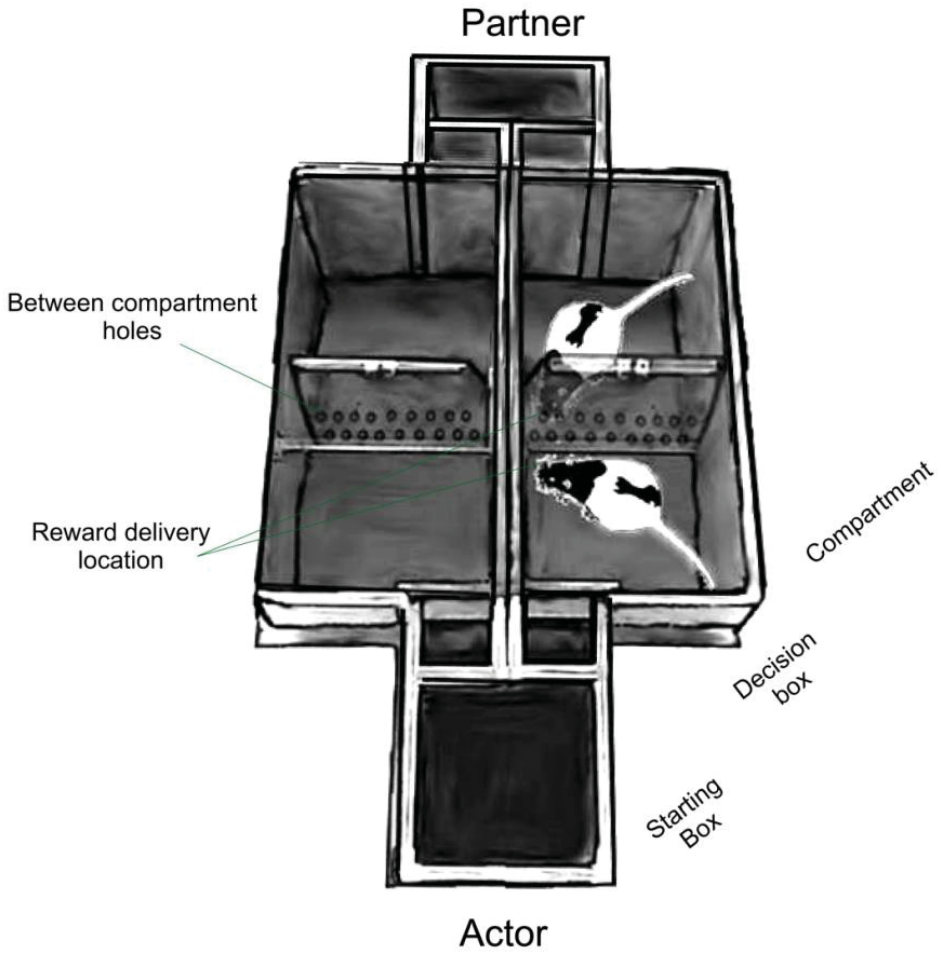

Figure 1 | Apparatus. Each T-Maze consisted of a starting box equipped with two independent doors that led to a decision box. A second door gave access to either compartment. Perforated and transparent walls were placed between compartments and between T Mazes to allow olfactory and auditory communication between rats. A semi-automatic reward delivery system was placed at the intersection of each perforated wall (not shown on figure).

The data from the first batch of rats was not collected using covers. Rewards (45mg dustless precision pellets, Bio-Serv, Germany) were delivered in the inner corner of the compartments through a funnel system, ensuring interaction-free pellet delivery. Food was hidden from the animals during the

decision phase, thus minimizing potential distractive or competitive motives (see Cronin 2012 for an extensive discussion of this point).

2.3 Experimental design

During the whole duration of the experiment, every actor was trained for one session a day on five consecutive weekdays for all habituation, training (see Supplementary Data) and testing sessions.

2.3.1 Groups and batches

The general structure of the experiment is described in Figure 2. In batch 1, four rats from the same cage were assigned to the "*partner*" group and sixteen rats were assigned to the "*actor*" group. In batch 2, sixteen rats were used as partners and thirty-two animals were used as actors. Actor and partner rats were never housed together. The actors were tested for four consecutive weeks paired with either a partner (partner condition) or a toy rat (toy condition), depending on the testing condition. Actors were always paired with the same partner.

2.3.2 General task design

Rats were tested in two main conditions: in the partner condition, both actor and partner rats were placed in the maze in their respective starting boxes; in the toy condition, a toy rat was used as partner. The actor always moved first and could enter either compartment. The partner never had a choice, i.e. the experimenter always directed the partner to the compartment facing the actor. After entering either compartment, actors received an identical amount of reward (three sucrose pellets), delivered after the same delay.

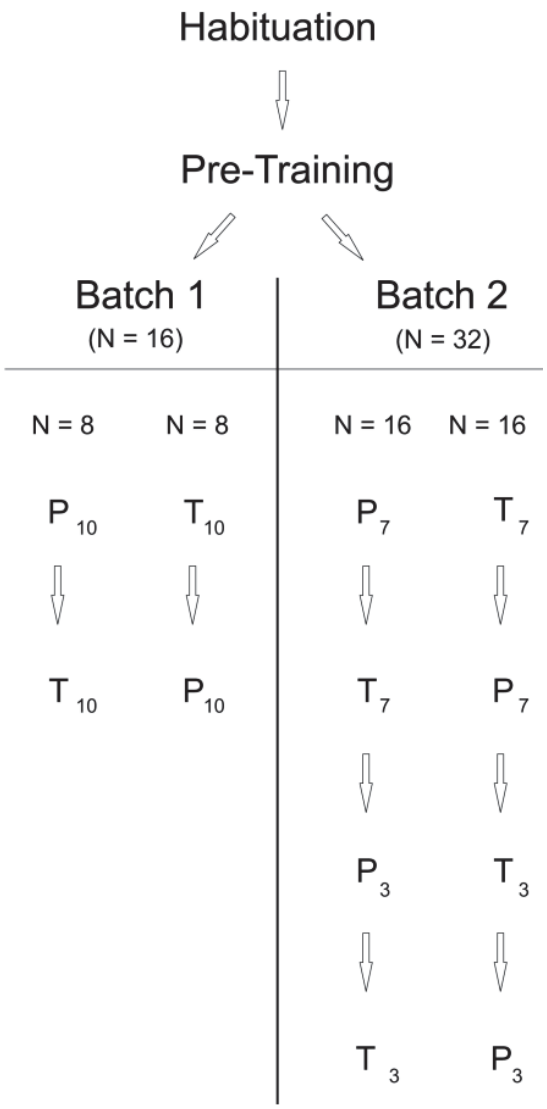

Figure 2 | Organization of groups and batches. All rats underwent habituation and pre-training procedures. Actors in batch 1 (N = 16) and batch 2 (N=32) were split in two groups, each starting in either the partner (P) or toy (T) condition. Each condition consisted of ten consecutive sessions in batch 1 (indicated by the subscript 10), or 7 sessions in batch 2 (pre-repetition), followed by three post-repetition sessions.

Importantly, entering one compartment (”both reward - BR” compartment) resulted in a reward delivery of same magnitude and delay in both the actor’s and partner’s compartments, whereas deciding for the alternative choice (the “own reward – OR” compartment) resulted in reward

delivery to the actor rat compartment only. In the toy condition, the partner was an inanimate toy rat of similar size, shape and color. The toy condition served as a control for pellet delivery sounds and potential secondary reinforcement effects of the food delivery. Importantly, the choice-reward payoff structure was identical across partner and toy conditions, i.e. rewards were delivered to the toy rat compartment with the same magnitude and delay as in the partner condition. Thus, any difference in choice allocation between the partner and the toy conditions could be attributed to the influence of social context on the actor's decisions. Note that a significant preference for the BR- or OR-alternative would suggest that the rats have some knowledge of the task structure, but it would not allow us to make inferences about the precise nature of their knowledge.

2.3.3 Typical trial structure

Each trial followed a strict time schedule (Figure 3) to guarantee invariant response times and reward delays. By doing so, we ensured that the actors' preferences for one compartment were not merely the results of asymmetrically timed reward deliveries. Both rats were placed in the respective starting boxes of the maze at the beginning of the session (Figure 3). The experimenter opened the actor's door and waited for the animal to enter one of the compartments. Door opening marked trial onset. For rats in batch 1, the rats had ten seconds to enter the compartment. Once a rat had fully entered a compartment with all four paws, the door was closed, "trapping" the rat inside the compartment (Figure 3; Actor enters, *t0*). Ten seconds later, the partner (or toy) was directed (by opening only one door) or placed (the toy was manually placed) by the experimenter into the compartment facing the actor rat's compartment (Figure 3; Partner enters, *t10*).

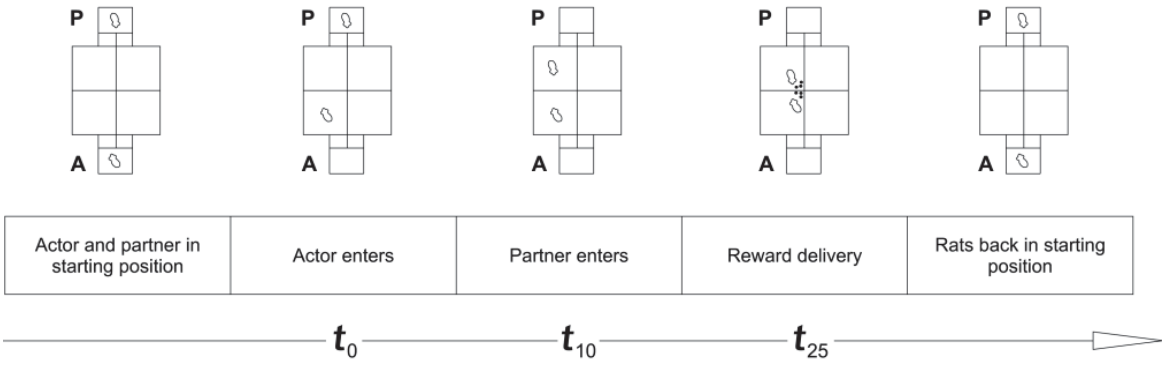

Figure 3 | Structure of a typical trial. The actor (A) always moved first into one of the two compartments (in free-choice trials), or into only one compartment (forced-choice trials; time 0 seconds; *t0*; trial onset). 10 seconds later (*t10*), the partner rat was directed into the compartment facing the compartment chosen by the actor. In the toy condition, the experimenter manually placed the toy in the respective compartment. 25 seconds after trial onset (*t25*) rewards were delivered to the actor only (after own-reward (OR) choices) or both rats (after both-reward (BR) choices). After reward consumption, rats were placed back in their start box positions, and a new trial started after a variable inter-trial interval (ITI; *t30-45)*.

Occasionally, partner rats were slow to enter the compartment, in which case the experimenter gently pushed the rat into the compartment, making sure that the strict time schedule was met. Reward was always delivered twenty-five seconds after trial onset (Figure 3; Reward delivery*, t25*) simultaneously into both compartments (after BR choices) or to the actor's compartment only (OR choices). After reward consumption, rats were manually replaced in their respective starting boxes to start a new trial. In the toy condition, the experimenter then removed the pellets delivered to the toy. The inter-trial interval (Figure 3; *t30-45*) duration was independent of the actor's choice.

2.3.4 Test sessions

A session began with ten (Batch 1) or eight (Batch 2) forced-choice trials (actors were forced to enter either compartment in a pseudo-randomized order) in order to allow sampling of the compartment / outcome contingencies. The forced-choice trials were followed by fifteen free choice trials in which the actors could freely choose which compartment to enter. Each rat was tested in ten (batch 1) or seven (batch 2; see next paragraph) consecutive sessions in the partner-condition, and the same amount of additional sessions in the toy condition. To control for potential order effects, half of the actors started testing in the partner condition, followed by the toy condition, with the reverse order for the other half of rats. We found no order effect on rats' between-condition preferences (see Chapter I - Supplementary Data).

To probe stability of preferences over time and social contexts, animals in batch 2 were tested in seven sessions (Pre-Repetition) in the partner condition, followed by the toy conditions (or vice versa), and were subsequently retested for three sessions in each condition again (Post-Repetition), thus amounting to a total number of ten sessions per condition.

To control for potential side biases, left and right compartments were pseudo-randomly assigned as BR or OR compartments within rats and across sessions; thus, BR and OR sides differed within and across rats and testing days. Moreover, all experiments were carried out in red light in a closed black curtain system, to minimize the influence of contextual cues on decision making. Throughout the experiment, the experimenter was positioned at the end of the maze during decision process and reward consumption. To prevent rats from moving towards or away from the experimenter, and thus creating an artificial side bias, the experimenter moved between trials, independently

of the BR or OR side allocation (see Supplementary Data). To control for social exploration motives, systematic approach / avoidance behavior as well as possible effects of closeness while eating (Barnett and Spencer, 1951), the partner was always directed into the compartment directly facing the compartment chosen by the actor, thus keeping the average distance between animals after entering the choice compartments equal and independent of the actors' choices.

2.4 Analysis

Social Bias: In addition to recording the percentage of BR choices relative to all choices, we calculate, for each rat, a social bias score (SB). The social bias score for rat *i* was expressed as the percent change in absolute BR choices in the partner condition [$BR(partner)_i$] relative to the BR choices in the toy condition [$BR(toy)_i$]:

$$SB_i = \left[\frac{\mathrm{BR(partner)}_i - \mathrm{BR(toy)}_i}{\mathrm{BR(toy)}_i}\right] * 100 \qquad (1)$$

Positive and negative SB-values quantify the tendency to choose the BR compartment more or less often in the partner condition relative to the toy condition.

Weight analysis: we related the actors' propensities to make BR choices to their body weights. Because rats in the two batches had different body masses, in order to establish commensurability between batches, the mean weights of each actor *i* were normalized to their initial weight in the first session using the following equation:

$$NormWeight_i = \left[\frac{MeanWeight_i - Sess1Weight_i}{Sess1Weight_i}\right] * 100 \qquad (2)$$

3. Results

We analyzed the actor rats' choice allocations in the two batches (Figure 2; N = 16 and N = 32, respectively) separately because of differences in the experimental design (see Materials and Methods). All rats completed all trials and sessions.

3.1 Actor rats have a preference for the BR compartment when paired with a partner rat

We first asked whether, at the group level, rats' preferences for BR or OR compartments were significantly different from chance, and furthermore, whether their preferences differed between partner and toy conditions. In batch 1 (Figure 4A; see below for batch 2 results), the proportion of BR choices was significantly above chance in the partner condition (One-sample Wilcoxon signed rank test; Z = 2.54 ; $p < .05$), and significantly below chance in the toy condition (Z = -2.95 ; $p < .01$). Accordingly, we found a significantly higher proportion of BR choices in the partner condition compared to the toy condition (Wilcoxon matched-pairs signed rank test Z = -3.41 ; $p < .001$).

3.2 Choice preferences are stable over time and faster re-acquired after repetition

To investigate whether the individual choice allocation pattern was stable over time, we tested the second batch of rats for seven sessions per condition, and then re-tested them in a repetition phase of three sessions per condition, thus repeatedly and successively alternating between partner and toy conditions (Figure 4B).

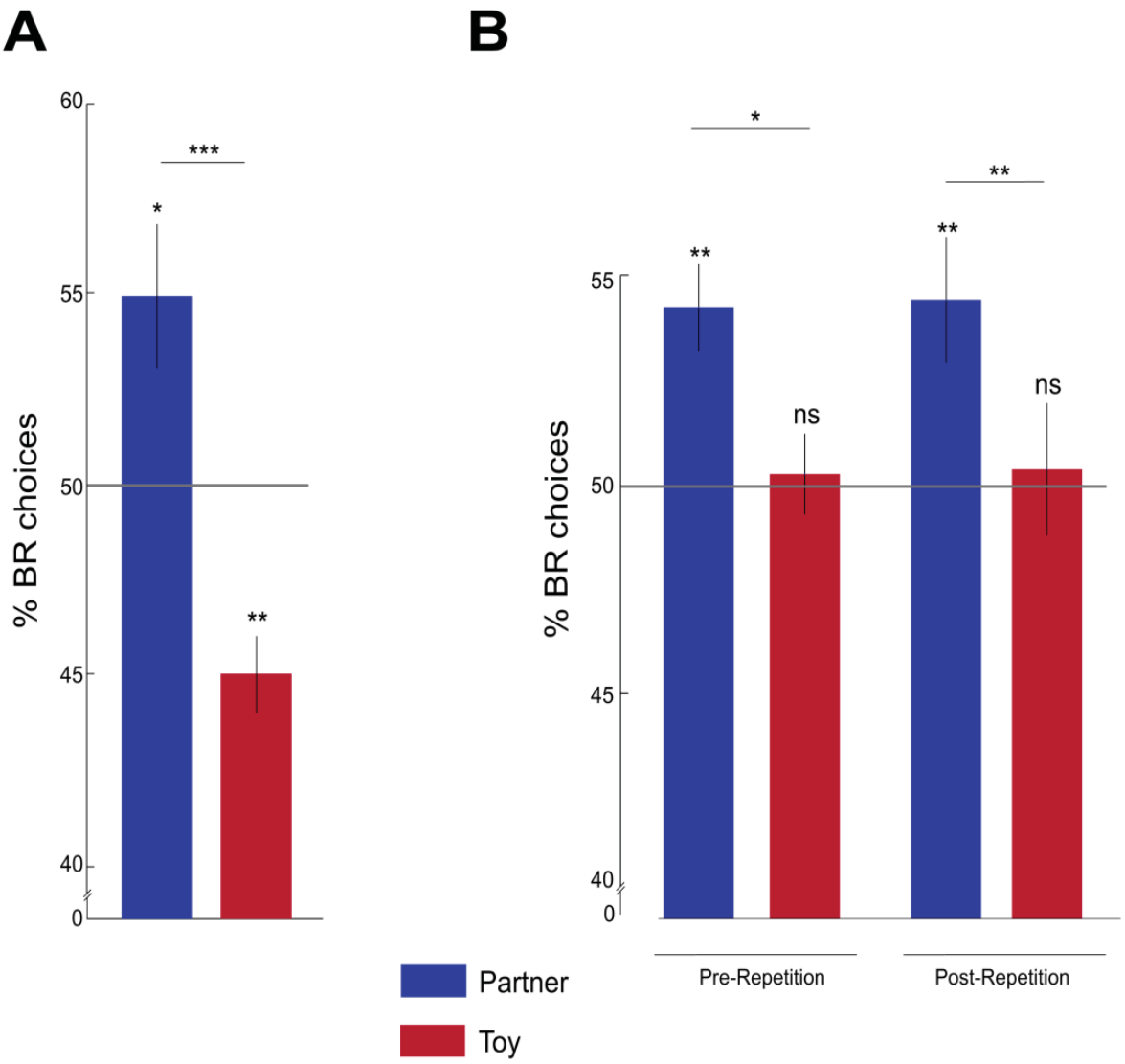

Figure 4 | Rats show pro-social behavior A. Percentage of BR choices for the partner (blue) and toy (red) conditions in Batch 1: the average percentage of BR choices was significantly higher in the partner compared to the toy condition and was different from chance levels. **B. Percentage of BR choices in Batch 2:** rats showed the same partner-toy-dissociation of pro-social behavior pre- and post-repetition. Y-axis is cut for demonstration purposes. *p < .05 ; **p < .01 ; ***p < .001 ; ns = not significant. Error bars represent the standard error of the mean, s.e.m.

The percentage of BR choices was significantly higher than chance for both Pre- and Post-repetition in the partner condition ([Pre] Z = 3.32 ; p < .01; [Post]

Z = 2.66 ; $p < .01$), but not in the toy condition ([Pre] Z = -.11 ; $p = .91$; [Post] Z = .10 ; $p = .92$). Moreover, we found a significantly higher percentage of BR choices in the partner compared to the toy condition in the Pre- (Z = -2.14 ; $p < .01$) and Post-repetition phases (Z = -2.42 ; $p < .01$). The percentage of BR choices did not significantly differ between Pre- and Post-repetition in neither condition ([Partner] Z = -.17 ; $p = .87$; [Toy] Z = -.13 ; $p = .90$). These results suggest that choice preferences were stable over time.

3.3 Individual differences in pro-social behavior

Overall, the above analysis, in which we pooled BR choices across all rats, has shown that the rats' frequency of choosing the BR compartment was significantly above chance in the partner condition, but the effect was relatively small (55% BR choices on average). However, the preference for the BR compartment greatly varied across rats, i.e. some rats showed substantially higher preference for the BR alternative in the partner condition compared to the toy condition (Figure 5 - left), whereas others neither developed a preference for the BR alternative, nor showed a condition-dependent choice pattern (Figure 5 - right). Thus, the overall mean fraction of BR choices may be diluted by the data from rats that did not display condition-dependent preferences. To determine the extent to which rats differed in their BR-preferences, we calculated a social bias score for each rat (SB, see section 2.4) reflecting the percent difference in BR-choices in the partner compared to the toy condition.

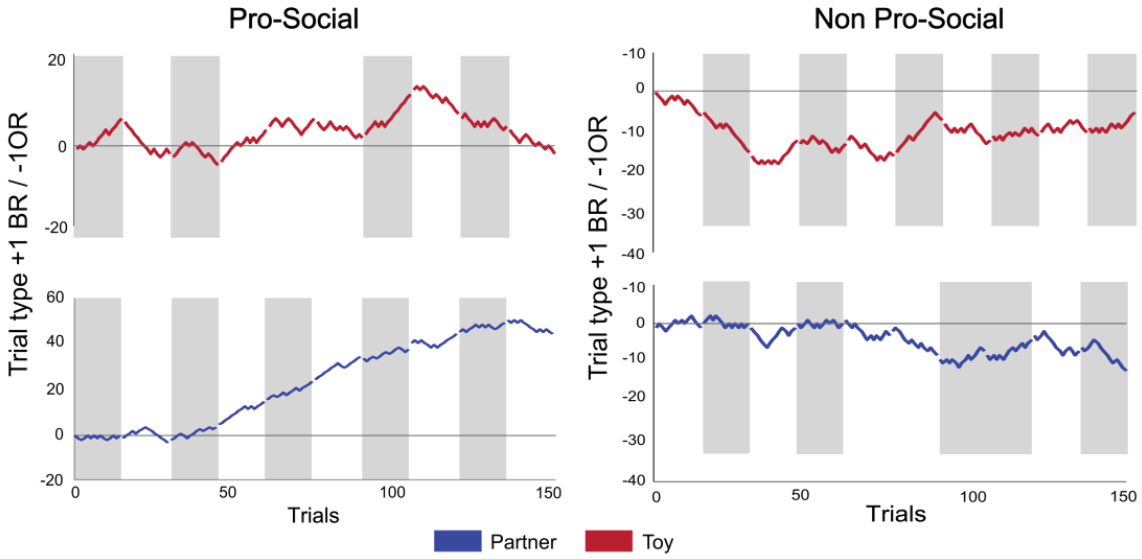

Figure 5 | Cumulative choice plots illustrate individual differences in pro-social behavior. For each trial, the running choice tally is incremented by +1 for each BR choice, and decremented by -1 for each OR choice. Thus, a monotonous upward slope indicates consistent BR choice across trials and sessions, neutral slopes indicate indifference, and negative slopes indicate consistent OR choices. Grey areas represent sessions where the BR compartment was on the left side. The two left panels show the cumulative choice plots of a rat classified as pro-social (performance in the toy condition indicated in red, upper left panel, performance in the partner condition indicated in blue in the second down left panel). The two right panels show the choice data from a rat classified as non pro-social.

Thus, SB scores can be interpreted as estimates of how much more (or less) a rat preferred the BR-alternative in the partner relative to the toy condition. Furthermore, we compared each rat's SB score to a benchmark SB score distribution obtained through a bootstrapped permutation analysis (see Supplementary Information and Figure 6; the red vertical line indicates the 95% confidence interval limit). We categorized all rats showing significantly higher SB scores than the upper confidence interval bound as pro-social (N = 29). All remaining animals were categorized as non-pro-social (N = 19). This analysis revealed a substantial degree of heterogeneity in preferences across rats, with SB scores ranging from -14.8 (14.8% more BR choices in the toy than

in the partner condition) to 45.6 (45.6% more BR choices in the partner than in the toy condition).

Figure 6 |Social bias scores of all rats. Colors represent rats classified as pro-social (green) and non-pro-social (grey). The vertical red line represents the upper 95% confidence interval threshold obtained from the permutation analysis.

Recently, body mass, already thought to reflect group hierarchy (Smith et al., 1994), has been shown to be a factor biasing rats' helping behavior towards lighter animals (Schneeberger et al., 2012). Therefore, we asked whether the individual differences in SB scores could be explained by the partners' individual weights. To this end, we correlated the SB scores with the normalized weight of the partners (the normalized weight parameter; see section 2.4). We found a negative correlation between normalized partner weight and SB scores (Figure 7; r = -.39, p = .006), suggesting that actors had a higher propensity to choose the BR alternative when paired with lighter partners. Interestingly, we also found a non-significant negative trend between average normalized actor weight and SB scores (r = -.25, p = .08), fueling the speculation that lighter actors may be more generous than heavier actors. We also computed the weight difference between individuals in each pair to investigate whether body mass differences between interacting animals could

affect choice allocation. We found no significant correlation between weight difference and SB scores (Spearman rank correlation, r = .21, *p* = .14).

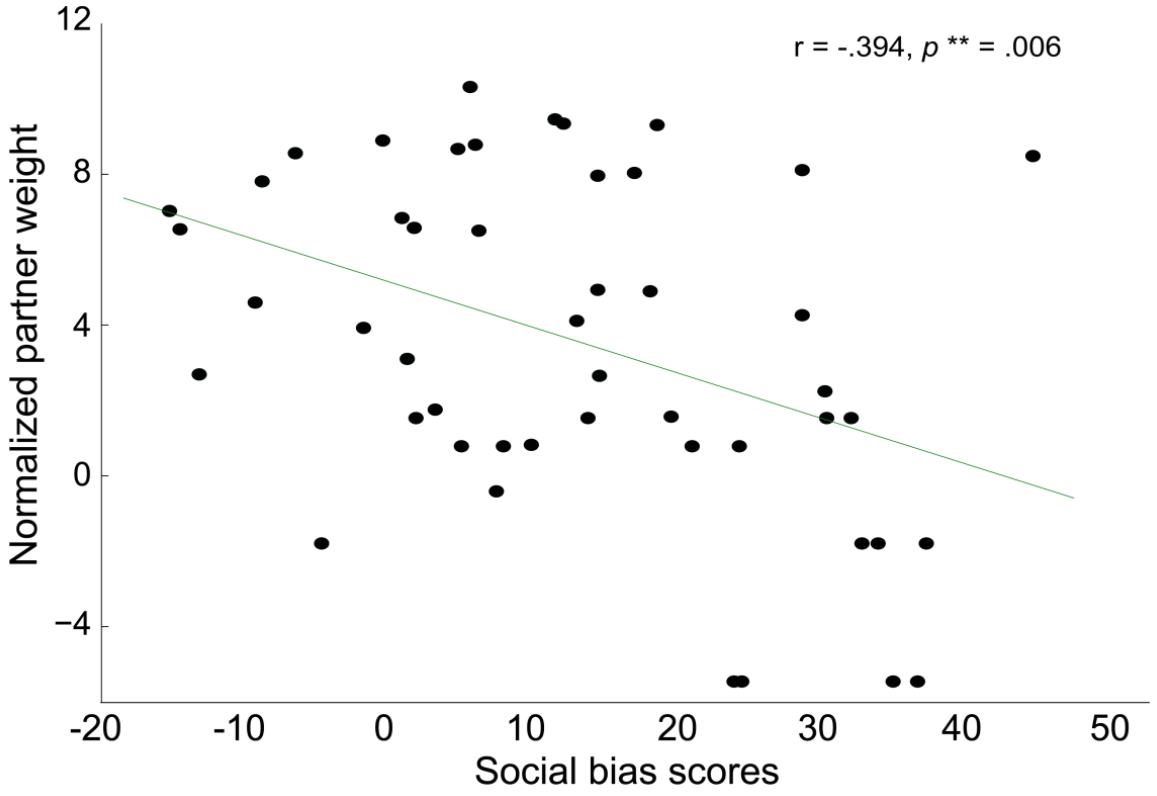

Figure 7 | Correlation between partner weight index and social bias scores. We found a negative correlation between the social bias score and the normalized partner weight

Finally, we explored the possibility that the mere identity of the partner, independent of its body mass, could be related to the choice preferences of the actors. In batch two, each partner was paired with two different actors. This allowed us to test whether the two actors paired with a given partner usually showed similar, or divergent, BR-preferences. To this end, we quantified, for each partner, how many of its paired actors were classified as pro-social or non-pro-social and compared these observed counts to the number expected by chance. The observed categorization frequencies were not significantly different from the frequencies expected by chance

(χ^2 = .00, p = 1.00), suggesting that the mere identity of the partner did not trigger pro-social tendencies.

4. Discussion

Using a novel, spatial rodent version of a Pro-social Choice Task (PCT; Silk et al., 2005), we tested whether rats showed non-costly helping behavior in a double T-Maze setup. Actor rats chose between two choice compartments yielding either just a reward for themselves, or an additional reward for a partner rat placed in an adjacent compartment. We contrasted the actors' percentage of BR choices in a partner condition with its BR choices in a toy condition, where the partner was an inanimate toy rat of similar shape, size and color. Importantly, the choice-reward payoff structure was identical across partner and toy conditions, i.e. rewards were delivered to the toy compartment with the same magnitude and delay as in the partner condition. Thus, any difference in choice distribution between both conditions would result from the influence of social context on decisions. If actors derive value from another rat's access to food, they should develop a preference for the BR alternative in the partner, but not in the toy condition. Our results confirmed that actors indeed revealed preferences for the alternative yielding food for their conspecific.

Importantly, we show that the BR-preferences were contingent on the social element of the task, and not merely driven by secondary reinforcement properties of pellet delivery, such as the sound, smell or sight of rewards. In addition, we controlled for additional motives by always directing the partner to the compartment facing the actor's compartment, independent of the

actor's choice; thus spatial proximity, social exploration motives, and approach/avoidance behavior are unlikely explanations of the actor's choices. Moreover, we found that BR-preferences quickly re-established after a repetition manipulation, suggesting that the observed behavior was stable over time. Finally, we found a negative correlation between the partner's weight and SB scores, indicating that actors had a higher propensity to choose the BR-alternative when the partner was light. Although the frequency of the rats' choices of the BR alternative was significantly above chance, the average fraction of BR choices was relatively small (Figure 4). We argue that the reason for the relatively subtle overall preference for the BR-alternative lies in the great individual variability in our rats' BR preferences: while some rats showed a very clear and marked distinction between partner and toy conditions, increasing their BR-choices by >45% when paired with a real rat relative to a toy, others selected the BR alternative equally often in both conditions. Based on their individual sensitivity to the social context, we classified approximately 60% of our rats as pro-social, showing a significantly – sometimes considerably – larger preference for BR choices in the partner than in the toy condition, and roughly 40% of our rats as non-pro-social, showing no or little difference in BR choices between conditions.

Interestingly, in a study using a primate analog of the PCT, the authors found comparable levels of pro-social preferences, which they interpreted as evidence for variable spontaneous pro-social choice (Horner et al., 2011). Reports of pro-social tendencies in non-human primates are highly variable (Silk et al., 2005; Horner et al., 2011; Cronin, 2012), which might be due to the great individual differences in pro-social behavior, but may also result from the lack of standardization of the experimental designs used (House et al., 2014) and/or from socio-ecological differences between animal species, such as

whether they engage in cooperative breeding or not (Burkart et al., 2014). Therefore, it is essential to establish a standardized social paradigm that allows for sound cross-species comparisons.

As mentioned above, we adopt a definition of pro-social choice promoted in the literature (Miller et al., 1991) as the preference for outcomes that produce a benefit for another individual. Importantly, to avoid any form of anthropomorphism and exercise interpretative caution (Morgan, 1903), we stress that this definition makes only very liberal claims about the underlying motives and mental mechanisms, and any behavior that increases the well-being of a conspecific would be labelled "pro-social" as long as that behavior happens in a genuinely social context and is driven by social motives, whatever they are. According to this definition, our rats' behavior qualifies as pro-social because the rats revealed a preference for outcomes that yielded food for another conspecific, and this preference was dependent on the social context (partner vs. toy). Our study was designed to demonstrate the proof-of-principle that rats have pro-social preferences according to above definition, but, admittedly, it offers little direct insight into the individual motives driving pro-social behavior. So, what are the putative mental and neural mechanisms underlying pro-sociality? We propose that pro-social choices can be understood within a social reinforcement framework (Ruff and Fehr, 2014) where BR- and OR-outcomes are associated with social reinforcement value. That is, an actor's pro-social choice might be driven by (i) the appetitive consequence of positive social reinforcement, i.e. animals may seek - possibly rewarding – communication signals emitted by the partner after having received a reward (Kashtelyan et al., 2014; Willuhn et al., 2014), or the pleasure of eating rewards together (Barnett and Spencer, 1951). In addition (ii), the rats may also be motivated by negative social reinforcement, i.e. they

may avoid the - putatively aversive – distress signals emitted from the partner missing out on reward after selfish choices. Positive and negative social reinforcement are not mutually exclusive, but could act in concert to produce pro-social choice. Importantly, despite the analogy with standard reinforcement learning, we maintain that preferences in the present task are genuinely social, i.e. dependent on social signals, such as the putative transmission and induction of affective states between actor and partner. Candidate substrates for a social transmission of affective states are ultrasonic vocalizations (USVs) which have been shown to reflect affective state in rats (Knutson et al., 1999; Wöhr et al., 2008; Takahashi et al., 2010). However, recent studies did not find evidence for a role for USVs in social transmission of fear (Pereira et al., 2012) or emotional contagion (Atsak et al., 2011). It is beyond the scope of this study to identify the actual communicative mechanisms driving pro-social behavior, but future studies should aim at isolating the motives underlying rodent pro-social choice.

The negative correlation between normalized partner weight and SB scores is also in line with the social reinforcement hypothesis: presumably, rats that are relatively hungrier might signal their state and/or respond more strongly to rewards bestowed on them, which might drive the actor's choice allocation towards the BR-compartment. Interestingly, pro-social behavior in non-human primates in possession of food seems to be fostered by begging (Gilby, 2006) and request (Warneken et al., 2007; Yamamoto et al., 2009, 2012; Melis et al., 2011) from conspecifics. However, recent results challenge this interpretation, as direct food sharing requests in chimpanzees did not trigger pro-social choice (Horner et al., 2011), nor did sympathy in great apes (Liebal et al., 2014). Interestingly, pro-social choice in long-tailed macaques has been shown to be related to hierarchy (Massen et al., 2011), i.e. dominant

individuals grant food to their partners whereas subordinate ones withhold its access to their conspecifics (Massen et al., 2010). In rodents, recent findings showed that rats preferentially helped sated, heavier, as well as lighter, hungrier partners (Schneeberger et al. 2012), thus suggesting a multi-factorial interaction between, at least rank position and hunger state in helping behavior in rodents. Therefore, future studies using a PCT design should parametrically vary rank position and deprivation state in individual pairing to explore their role in rodent pro-social choice. Interestingly, one recent study showed that rodent pro-social behavior was modulated by social experience (Ben-Ami Bartal et al., 2014), suggesting that potential pro-social preferences are influenced by social context. However, and importantly, our results also suggest that the partner's mere identity or behavior is not the single principal determinant of pro-social choice; it rather seems that the interaction between the partner's deprivation state and the actor's pro-social disposition is important for eliciting pro-social tendencies in the actor.

The current experimental design combines a series of advantages discussed elsewhere in the literature (see Cronin, 2012 for extensive discussion of this point). First, because pro-social choices were non-costly to the actor (Silk et al., 2005; Horner et al., 2011), we could de-confound pro-social motives from the rats' natural egoistic tendencies to maximize own payoff, which may have otherwise obscured any other-regarding consideration. Second, our task allowed the food to be hidden from the actors and partners during decision-making (Yamamoto et al., 2009, 2012), thus avoiding potential competitive or distractive influences on choice behavior. Third, partner rats could neither retaliate, nor return the favor, thus the actors' pro-social tendencies were not the result of strategic (tit-for-tat), reciprocal considerations. Finally, the toy condition was a crucial control manipulation: it allowed us to demonstrate that

pro-social choice was directly contingent on the social component of the task, i.e. the presence of a real partner (Silk et al., 2005; Burkart et al., 2007), and was not merely driven by secondary reinforcement mechanisms, such as the possibly motivational properties of the sensory features of the food rewards (sight, smell, dropping sound). Interestingly, animals in the first batch (but not the second batch) chose the BR alternative significantly below chance in the toy condition. One putative explanation to account for this counterintuitive result is that the rats showed a frustration effect, i.e. they assigned negative value to the pellets in the opposite non-social compartment that they could see and possibly also smell, but not access. Therefore, they might have avoided the delivery of such pellets by selecting the selfish option when paired with a non-social target. This explanation points towards multi-factorial effects: processes such as pellet delivery in the opposite compartment, or their inaccessibility in the non-social condition, might enter the decision process and reinforce subsequent behavior. Therefore, there might be multiple processes that promote or suppress the decision for the pro-social compartment. However, as this effect was not replicated in Batch 2, this explanation remains speculative.

In conclusion, we argue that pro-social preferences are the result of tractable social reinforcement mechanisms, which our experimental paradigm allows to trace down in future studies. For instance, it allows for behavioral, pharmacological and neurobiological interventions such as psychopharmacological manipulations of peptide- and hormone systems associated with pro-social behavior (Young et al., 1998), or manipulations of neural processes implicated in social behavior (Rushworth et al., 2007b), as well as electrophysiological recordings (Buzsáki, 2004). Finally, the fact that non-primate animals show pro-social behavior in the absence of strategic,

reciprocal or selfish motivations offers important insights into the evolution of pro-social behavior. Future studies could perform cross-species investigations (Brosnan and de Waal, 2014b) including the comparison of socio-ecological (Burkart et al., 2014) and methodological aspects of social behavior (Cronin, 2012) to reconcile diverging evidence on pro-sociality in the literature , and ultimately identify the factors driving its evolution.

Abbreviations: PCT, Pro-social Choice Task; BR: Both Reward; OR: Own Reward; CI: Confidence Interval.

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Author contributions: J.H-L. designed and performed the research, analyzed the data and wrote the paper. M.v.W. designed the research and analyzed the data and wrote the paper. C.M. wrote the paper. M.S. designed the research. T.K. acquired funds, designed the research, analyzed the data and wrote the paper. The study was supported by the Deutsche Forschungsgemeinschaft (DFG), grant n° KA2675 / 5-1.

Chapter I

Rats prefer mutual rewards in a Pro-Social Choice Task

Supplementary Information

1. Supplementary Data

No effect of condition order: We found no significant differences in social bias scores between rats that started in the partner versus toy condition in batch 1 (Mann-Whitney U Test; U = 28.00; p = .67) and batch 2 (U = 94.00; p = .20).

2. Supplementary Experimental Procedure

In the first batch, two partner rats did not reach the behavioral criterion of performing ten consecutive trials per session in step 3 and were excluded from the experiment schedule.

2.1 Subjects and housing

Two batches (N = 20 and N = 48, respectively) of non-castrated male Long-Evans rats (*Janvier Labs, St. Berthevin, France*) were used, all animals weighed between 190-300g at the beginning of the experiment. Animals were housed in groups of four animals per cage, under an inverted 12:12 hours light - dark cycle (lights off at 07:00), in a temperature- (20 ± 2°C) and humidity-controlled (60%) colony room.

2.2 Experimental design: Pre-Training

All rats were habituated to the transport procedure between their stables and the testing setup, as well as the double T-maze setup for two sessions, after which they went through three steps of pre-training. During pre-training, rats entered each compartment five times (ten trials per session) in pseudo-randomized order.

Step 1: the experimenter let the rats enter one compartment and waited for the animals to find the reward location. Rats were then manually replaced in the starting box, and could enter the opposite compartment on a following trial. All animals reached optimal criterion (all pellets eaten within the five seconds after entering the compartment) after two sessions.

Step 2: After entering a choice compartment, rats were trapped by closing the compartment door, and replaced in the starting box five seconds after reward

consumption. All rats reached criterion (all pellets eaten within the five seconds after entering the compartment) after two sessions.

Step 3: Forced trials were implemented as well as incremental delays (increasing step size of five seconds every session) between entering the compartment and reward delivery. Rats reached the behavioral criterion (same as step 2) for the final task after six sessions.

All rats accomplished all habituation and pre-training steps after twelve days of pre-training and started the Pro-social Choice Task. Before every session, partner and actor rats were allowed to physically interact for one minute.

2.3 Analysis and statistics

Group categorization: Animals were classified as pro-social if their bias score differed significantly from a bootstrapped reference permutation distribution. This permutation distribution of social bias scores consisted of N = 5000 draws of 10x2 sessions, with the percentage of BR choice of these sessions randomly assigned to partner and toy labels. For each of such draws, the resulting social bias score was calculated, generating a distribution of 5000 permuted social bias scores that followed a normal distribution. The 95% percentile of this distribution was selected as a benchmark social bias score, and subsequently the actual social bias score of each animal was then tested for significance against this condition-randomized social bias benchmark value.

Statistics: Group level analyses were carried out using non-parametric testing: Wilcoxon signed rank test (paired samples), Mann-Whitney U Test (independent samples) and one sample Wilcoxon signed rank test (one-sample tests). Correlation analysis employed two-tailed non-parametric testing

(Spearman's rho). Analysis carried on the second batch used one-tailed testing because of the directionality of the hypothesized effect. Individual analysis used pooled data from both batches. The following significance levels were used: $*p < .05$; $**p < .01$; $***p < .001$; ns = not significant. Multiple comparison are corrected using Bonferroni correction. All statistical analyses were carried using IBM SPSS Statistics 20 and MatLab 2013a (The MathWorks).

3. Media support

A video file of trials performed in the partner condition is available on the Media Supplementary Materials (USB key furnished with thesis).

Chapter II

Basolateral amygdala lesions abolish mutual reward preferences in rats[8]

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Abstract

In a recent study, we demonstrated that rats prefer mutual rewards in a Pro-social Choice Task. Here, employing the same task, we show that the integrity of the basolateral amygdala was necessary for the expression of mutual reward preferences. Actor rats received bilateral excitotoxic (n = 12) or sham lesions (n = 10) targeting the basolateral amygdala and were subsequently tested in a Pro-social Choice Task where they could decide between rewarding ("Both Reward") or not rewarding a partner rat ("Own Reward"), either choice yielding identical reward to the actors themselves. To manipulate the social context and control for secondary reinforcement sources, actor rats were paired with either a partner rat (partner condition) or with an inanimate rat toy (toy condition). Sham-operated animals revealed a significant preference for the Both-Reward-option in the partner condition, but not in the toy condition. Amygdala-lesioned animals exhibited significantly lower Both-Reward preferences than the sham group in the partner but not in the toy condition, suggesting that basolateral amygdala was required for the expression of mutual reward preferences. Critically, in a reward magnitude discrimination task in the same experimental setup, both sham-operated and amygdala-lesioned animals preferred large over small rewards, suggesting that amygdala lesion effects were restricted to decision making in social contexts, leaving self-oriented behavior unaffected.

1. Introduction

Humans have pro-social sentiments (Silk and House, 2011). It has recently been proposed that the mental and neural mechanisms underlying social preferences have their roots in evolution, and that rudiments of these preferences should be detectable in non-human animals too (Ben-Ami Bartal et al., 2011b; Decety, 2011). In support of this idea, recent research on social decision-making in rodents (Hernandez-Lallement et al., 2015a; Márquez et al., 2015) demonstrated that rats prefer mutual rewards, i.e. rewards delivered to them and a conspecific, over own-rewards only. Unfortunately, the neural bases of such decisions remain largely unknown, although recent efforts have started to shed light onto the potential underlying processes (Kashtelyan et al., 2014; Willuhn et al., 2014). Human neuroimaging studies show that decisions that benefit others typically recruit limbic and prefrontal brain areas (Behrens et al., 2009; Bickart et al., 2014b; Ruff and Fehr, 2014). Particularly, the amygdala, a temporal structure involved in emotion (Phelps and LeDoux, 2005), face recognition (Adolphs et al., 1994; Breiter et al., 1996; Morris et al., 1996; Fried et al., 1997), group affiliation (Bavel et al., 2008) and social network management (Adolphs et al., 1998; Kennedy et al., 2009; Bickart et al., 2011), has been proposed to regulate perception, affiliation and avoidance in social contexts (Bickart et al., 2014b). Notably, psychopathy, a clinical condition characterized by anomalies in affective processing and empathy, has been linked to altered amygdala functionality (Kiehl et al., 2001; Blair, 2012; Decety et al., 2013) and volume (Yang et al., 2009). In rodents, amygdala lesions lead to an increase in the frequency of several social behaviors in novel environments (Wang et al., 2014), disruption of socially transmitted food preference (Wang et al., 2006), impairment in sexual behavior (Harris and

Sachs, 1975; Kondo, 1992; Newman, 1999) and possible alteration of social recognition (Maaswinkel et al., 1996 but see Wang et al., 2014). We thus hypothesized that BLA lesions would selectively affect social decision making, while sparing self-oriented decision making abilities.

To test this hypothesis, we trained sham-operated and BLA-lesioned rats on a rodent Pro-social Choice Task (PCT; Hernandez-Lallement et al., 2015a) and a non-social Reward Magnitude Discrimination Task (MDT). In line with our hypothesis, we found that BLA-lesioned animals displayed lower levels of pro-social choice when paired with a partner rat, but not an inanimate rat toy, whereas sham-operated animals showed higher levels of pro-social choice when deciding for a partner rat, but not the inanimate toy. In contrast, both groups showed equally higher preferences for the larger reward in the MDT task.

2. Materials and Methods

2.1. Subjects and housing

Thirty six adult male Long-Evans rats (*Charles River, Italy*) weighing between 250-450g at the beginning of the experiment were kept at 85% of free feeding body weight with water available *ad libitum*. Upon arrival, animals were placed in groups of three individuals per cage, under an inverted 12:12 hour light - dark cycle, in a temperature- (20 ± 2°C) and humidity-controlled (60%) colony room. All animal procedures adhered to German Welfare Act and were approved by the local authority LANUV (Landesamt für Natur-, Umwelt- und Verbaucherschutz North Rhine-Westphalia, Germany).

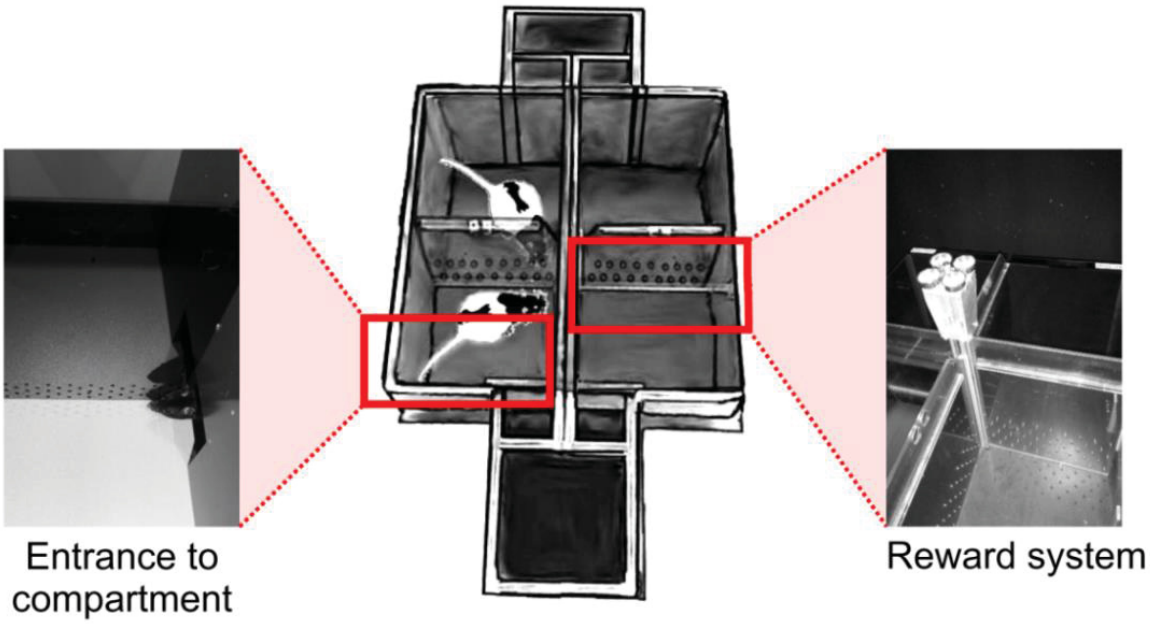

Figure 1 | Double T-Maze apparatus. The setup consisted of a starting box equipped with two independently moveable doors that led to an intermediate box. A second door in each intermediate box gave access to the choice-compartments ("entrance to compartment"). Perforated and transparent walls were placed between compartments and between T-Mazes to allow visual, olfactory and auditory communication between rats. A funnel reward delivery system ("reward system") was used to deliver rewards in the compartments in a spatially controlled fashion. All compartments were closed with red covers to isolate animals from distractive environmental cues.

2.2. Behavioral testing

2.2.1 Apparatus

We used a double T-Maze setup described previously in detail (Hernandez-Lallement et al., 2015a). Briefly, the setup consisted of a custom made double T-Maze apparatus (Figure 1) with the choice compartments in both mazes facing each other. Animals could enter one of the two choice compartments (Figure 1, *entrance to compartment*) to receive a reward. Rewards were identical in both choices (n = 3 sucrose pellets) and were delivered to the compartments through a funnel system (Figure 1, *reward*

system). All compartments were closed with red covers to isolate animals from distractive cues. Importantly, the between-compartment walls separating the two T-mazes allowed auditory and olfactory information transmission between rats. All sessions were carried out in a closed, red light illuminated curtain system during the rats' active period.

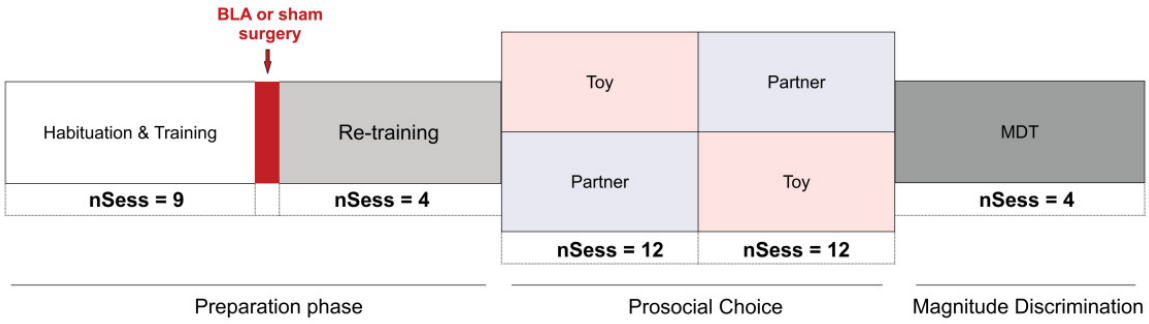

Figure 2 | Experiment timeline. *Preparation phase:* rats underwent habituation and training in the experimental setup. After surgical procedures, all actors underwent a pellet control task. *Pro-social Choice Task (PCT):* rats performed both partner and toy conditions in the PCT in pseudo-randomized order. *Magnitude Discrimination Task (MDT):* to control for reward discrimination abilities, all actors performed a MDT in the same experimental setup.

2.2.2 Experiment timeline and task design

The timeline of the experiment is shown in Figure 2.

Preparation phase: Upon completion of initial habituation procedures (see Supplementary Information and Hernandez-Lallement et al., 2015a), twenty-four randomly selected animals were assigned to an "actor" group and the remaining twelve animals were assigned to a "partner" group. Animals were housed in groups of four individuals but actors and partners were never housed together. Actor rats went through surgical procedure (see Supplementary Information) and were subsequently tested on a pellet control

task for four sessions. The pellet control task served as a control for the toy condition in the PCT (see below). It was identical to the toy condition in terms of task-structure and reward contingencies, except that pellets after BR-choices were delivered to an empty compartment (see Supplementary Information).

Pro-social Choice Task (PCT): The general principles of the task are described in detail in (Hernandez-Lallement et al., 2015a). Actor and partner rats were tested together. Actor rats decided between entering an "Own Reward" (OR 1/0) or a "Both Reward (BR 1/1) compartment. Both decisions resulted in the delivery of n = 3 sucrose pellets with identical delays into the respective actor's compartment but additional three pellets were delivered to the partner rat after BR choices only. Thus, there was no difference in the actor's reward after BR and OR choices, the choices differed only with respect to the partners' payoff.

The trial structure (Figure 3, upper panel) followed a strictly timed sequence of events to ensure invariant response times and reward delays. Actor and partner rats were put in their respective starting boxes at the beginning of each trial. The actor moved first (time 0s, *t0*) into one of the compartments, followed by the partner (or toy rat, see below; *t10*). In cases where the partner would not enter spontaneously, the experimenter gently pushed the animal in the compartment (pushing the partner had no effect on the actors' choices, see Supplementary Information). To control for social exploration motives, systematic approach/avoidance behavior as well as distance between rats, the partner was always, i.e. after OR- and BR-choices, directed into the compartment directly facing the compartment chosen by the actor by opening one door only, thus keeping the average distance between

animals constant for both choice alternatives (typically, rats ran to the reward delivery location and waited for the pellets to fall through the funnels). Reward(s) were delivered (*t25*) according to the actor's choice. All trials had identical length. In every session, actors started with n = 6 forced trials, half to the left and remaining half to the right side in a pseudo-randomized order, followed by n = 25 free choice trials.

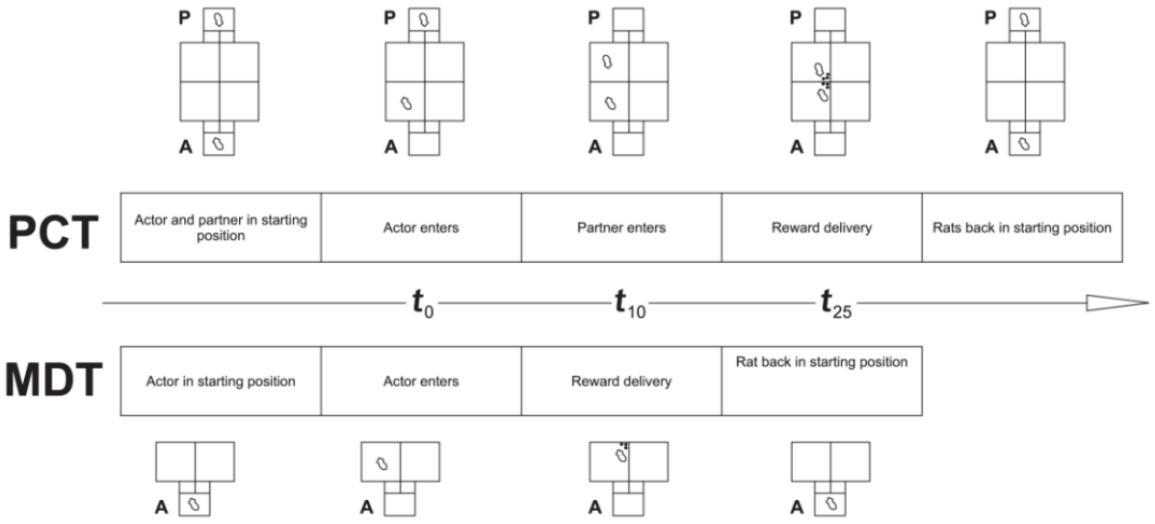

Figure 3 | Typical trial structure for PCT and MDT. *PCT:* both rats started in their respective starting boxes. Actors moved first (time 0s, *t0*) into one of the two compartments. Ten seconds later (*t10*), the partner was directed to the opposite compartment, i.e. facing the actor. Rewards were delivered (*t25*) either to the actor rat only after own-reward (OR) choices, or to both rats after both-reward (BR) choices. Rats were replaced in their respective starting box for the subsequent trial. The toy condition was identical, including reward delivery schemes, except that the partner rat was replaced by an inanimate toy. *MDT*: Actors moved into the left or right compartment (*t0*) and received either a small (3 pellets) or large (6 pellets) rewards (*t1*0) before being replaced in the starting box for the following trial.

All actors underwent both a partner (*# Sessions* = 12; paired with a real rat partner; actors were always paired with the same partner across sessions) and toy a condition (*# Sessions* = 12; paired with an inanimate rat toy puppet),

which served as a control for potential non-social motivational mechanisms, such as secondary reinforcement effects of the food delivery (magnitude, smell and sound). To control for side biases, left and right compartments were pseudo-randomly assigned as either BR (for half of the total session number, i.e. *# Sessions* = 6) or OR (*# Sessions* = 6) compartments across rats and sessions; thus, BR and OR sides differed across rats and testing days. Finally to control for potential order effects, the starting condition (partner *vs* toy) was randomized across actors; subsequently, after twelve sessions in their respective starting condition, the rat/condition assignment was reversed.

Magnitude Discrimination (MDT): Upon completion of the PCT, all actors performed a reward magnitude discrimination control task (MDT; (*# Sessions* = 4) to further test whether putative lesions effects in the PCT were due to general reinforcement impairments, such as reward devaluation or reversal deficits. Here, only one half of the double T-Maze was used (Figure 3, lower panel). In each session, one compartment was associated with the delivery of a large reward (LR; n = 6 pellets), and the other compartment with a small reward (SR; n = 3 pellets). The LR- and SR-compartment assignment was pseudo-randomized across sessions and rats; hence, as in the PCT, rats had to flexibly adjust to frequent contingency reversals across the four testing sessions. To ensure identical reward delivery time, all rewards were delivered ten seconds (*t10*) after the actors' choice. After reward consumption, the rat was replaced in the starting box for the next trial. The MDT sessions' structure was identical to the PCT structure, i.e. six forced trials to allow sampling the compartment's contingencies, followed by twenty-five free choice trials where rats could freely choose between left and right compartments.

2.3 Analysis and statistics

All analyses were performed using MatLab 2013a (The Mathworks) and IBM SPSS Statistics 20. Group analysis were made using average values across sessions (n = 12) and free choice trials (n = 25). Multiple comparisons are corrected using Bonferroni correction.

Social bias computation: To estimate differences in BR choices in the partner relative to the toy condition, we computed a social bias score (Hernandez-Lallement et al., 2015a). The social bias score (SB) for rat *i* was expressed as the percent change in BR choices in the partner condition [BR(partner)$_i$] relative to the BR choices in the toy condition [BR(toy)$_i$]:

$$SB_i = \left[\frac{\mathrm{BR(partner)}_i - \mathrm{BR(toy)}_i}{\mathrm{BR(toy)}_i}\right] * 100 \qquad (1)$$

Because the payoff to the actor rat was identical for all choices, and the difference between the partner- and the toy-condition was thus of social nature, a positive social bias score, i.e. more BR choices in the partner compared to the toy condition, can be interpreted as added positive social value placed on the partner's access to reward, a negative social bias score can be construed as the disutility of the partner's access to reward.

Permutation analysis: In order to explore individual differences in the social bias scores, we used a permutation analysis (Hernandez-Lallement et al., 2015a) that allowed us to categorize animals according to a reference social bias score distribution. To do so, we ran N = 5000 random permutations of the absolute percentage BR choice in each condition and across sessions. Each permutation generated a social bias score, which allowed us to compute the

95% confidence interval as a benchmark social bias score. Subsequently, individual social bias scores were tested for significance against this condition-randomized confidence interval.

Movement times: Movement times (delay between door opening and rat entering a given compartment with full body excluding the tail) of rats were extracted from recorded videos using Solomon (Solomon Coder beta 15.02.08 © András Péter). Individual BR/OR ratios were computed using average movement times across session and trials for each choice alternative.

2.4 Surgery

Upon completion of habituation and training sessions, actors were pseudorandomly assigned to BLA or Sham group. Briefly, rats were anesthetized using inhalation of isofluorane (5% for induction, lowered to ca. 2.5% for maintenance), and positioned on a stereotaxic frame (David Kopf Instruments, USA). For each hemisphere, two holes were drilled in the skull at the following coordinates: site 1: anteroposterior (AP) - 2.4 mm, mediolateral (ML) ± 4.8mm, dorsoventral (DV) - 8. 6mm; site 2: AP - 3.0mm, ML ± 4.8mm, DV - 8.8mm. The AP and ML coordinates were relative to bregma, the DV coordinate was relative to the dura. Bilateral infusions were made using 0.3mm injection needle (PlasticsOne) connected via polyethylene tubing to a 10µl Hamilton syringe within a microinfusion pump (Harvard Apparatus) . Infusions were made using 0.2µl of 0.09 M quinolinic acid dissolved in 0.1 M phosphate buffer solution (PBS, *p*H value 7.4) at an infusion rate of 1 µl/mn, after which the needle was left in place for two minutes allowing the substance to diffuse away from injection site. Sham surgeries (n = 11) were

made by lowering the infusion needle to the same coordinates and injecting vehicle solutions (0.1 M PBS, *p*H value 7.4) according to the same protocol. After completion of the surgery, animals received injections of analgesic (Carprofen; 5mg/ml) for three consecutive days, and were given ten days of recovery followed by four re-training sessions (see above) before the experiment started. During training and testing, all experimenters were blind to the animals' sham/BLA group assignment.

2.5 Histology

After completion of the behavioral testing, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially using 0.01 M using phosphate buffer (PBS; 0.1 M, *p*H = 7.4) for three minutes followed by a fixating solution of paraformaldehyde (PFA 4%) for five minutes. Brains were immediately removed and stored in PFA solution for ten days at a temperature of 5°C. Coronal sections (60µm) of the BLA were obtained using a vibrotome (Leica, Germany) and stained with cresyl violet. Finally, injection sites and lesion extent were mapped using a rat brain atlas with standardized coordinates (Paxinos and Watson, 1998).

3. Results

Two animals (one in each group) died during recovery from the surgical procedure. All remaining actor rats (N = 22; N*[Sham]* = 10; N*[BLA]* = 12) completed all trials and sessions. There was no significant order effect of the starting-condition (animals starting training in the partner or toy condition) on social bias scores (ANOVA, $F_{(1,18)} = 2.61$, $p = .12$), and no significant order*lesion group interaction ($F_{(1,18)} = 1.61$, $p = .22$). We therefore pooled data from

animals across starting conditions in all following analyses. Finally, the actors' choice preferences did not differ from chance levels in a pellet control condition where no partner or toy was present (Supplementary Information), suggesting that BR-preferences in the toy or partner condition are unlikely to be driven by secondary reinforcement properties of the pellets per se.

3.1 Lesions and histology

Histological assessment of lesions (Figure 4) were performed by J.H-L. and confirmed by two additional individuals blind to the experimental manipulation. BLA lesions encompassed both anterior and posterior portions of the basolateral amygdala regions as defined by Paxinos and Watson (1998). Excitotoxic damage occasionally extended (see light shaded grey areas, Figure 5) into the lateral amygdaloid nucleus (LAVL) and the basomedial amygdaloid nucleus (BMP), sparing the central amygdaloid nucleus (CeN; Figure 5).

3.2 Basolateral amygdala lesions reduce social bias scores

To test if BLA-lesioned rats showed different preferences for mutual reward outcomes than sham-operated rats, we computed individual social bias scores (see Methods) which reflected the percent change difference in BR choice between partner and toy conditions. As indicated, social bias scores can be interpreted as a measure of the positive and negative social value placed on reward to others. We found a significant difference in social bias scores between the BLA-lesioned and sham-operated animals (Figure 6A, left panel; $t_{(20)} = 2.00$, $p < .01$), suggesting that BLA-lesioned rats valued mutual reward outcomes differently than sham-rats. Notably, the social bias scores between the groups had opposing signs: whereas social bias scores were, on average,

positive in the sham-group, they were negative in the BLA-animals. One-sample t-tests confirmed that social bias scores were significantly higher than zero in the sham group (Figure 6A; $t_{(9)}$ = 2.37, p < .05), replicating previous results with non-operated control rats (Hernandez-Lallement et al., 2015a). By contrast, there was a near-significant trend towards negative social bias scores in the BLA group ($t_{(11)}$ = -1.97, p = .07), suggesting that BLA-lesioned rats placed less value on the BR outcomes in the partner than in the toy condition.

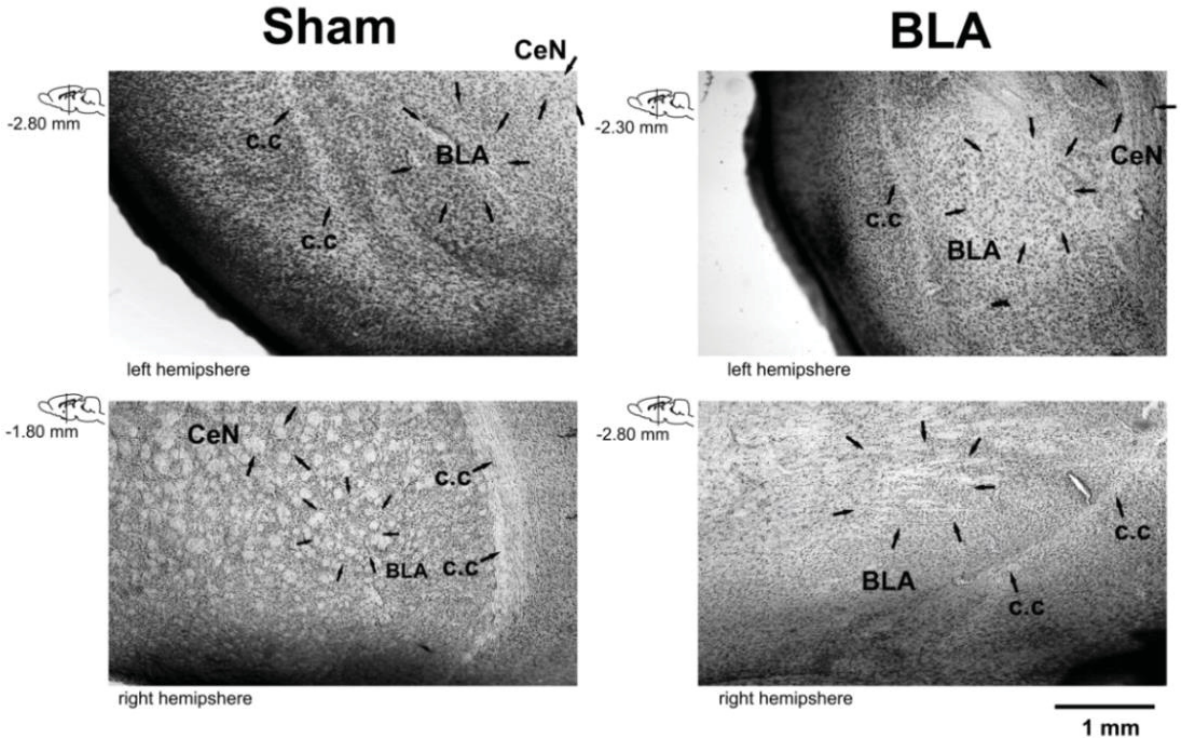

Figure 4 | Histology of BLA lesions. Photomicrographs depicting typical lesions of the BLA (left hemisphere, right up: rat #404; right hemisphere, right down: rat #401) and sham-operated control tissue (left hemisphere, left up: rat #397; right hemisphere, left down: rat #394). Numbers inform on distance from bregma. CeN, Central nucleus of the amygdala.

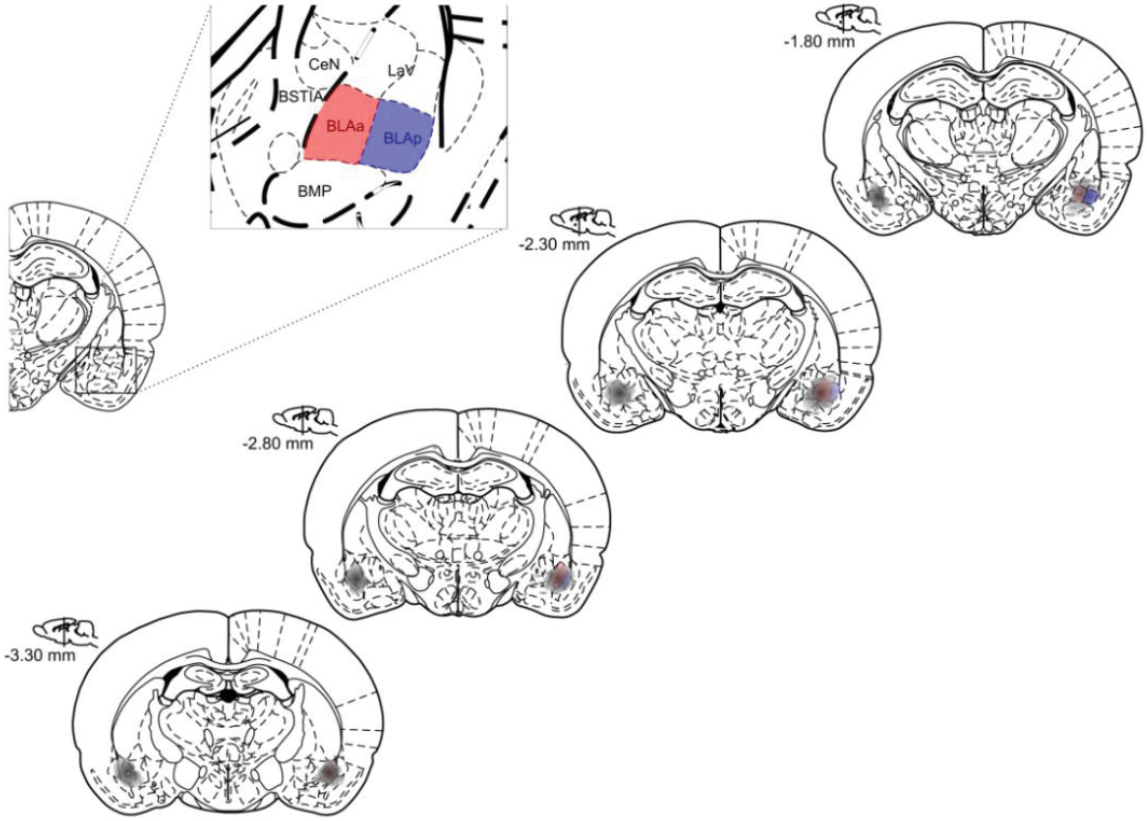

Figure 5 | Schematic illustration of the lesion spread for BLA lesions. Grey gradient represents lesion spread across all lesioned subjects (n = 12). Diagrams are adjusted from Paxinos & Watson, 1998. BLAa, basolateral amygdaloid nucleus, anterior part; BLAp basolateral amygdaloid nucleus, posterior part; BMP basomedial amygdaloid nucleus, posterior part; BSTIA bed nucleus of the stria terminalis; LaVL lateral amygdaloid nucleus; CeN central amygdaloid nucleus

We previously discussed (Hernandez-Lallement et al., 2015a) that averaged preference scores at the group level might be insufficiently informative of the choice allocation-dynamics and -levels because of large heterogeneity in mutual-reward preferences across rats. To better characterize the differences in mutual reward preferences between sham- and BLA-lesioned rats, we compared each rat's social bias score to a 95% confidence interval (Figure 6A, right panel; red vertical lines) obtained through a

bootstrapped permutation analysis (see Methods and Hernandez-Lallement et al., 2015a).

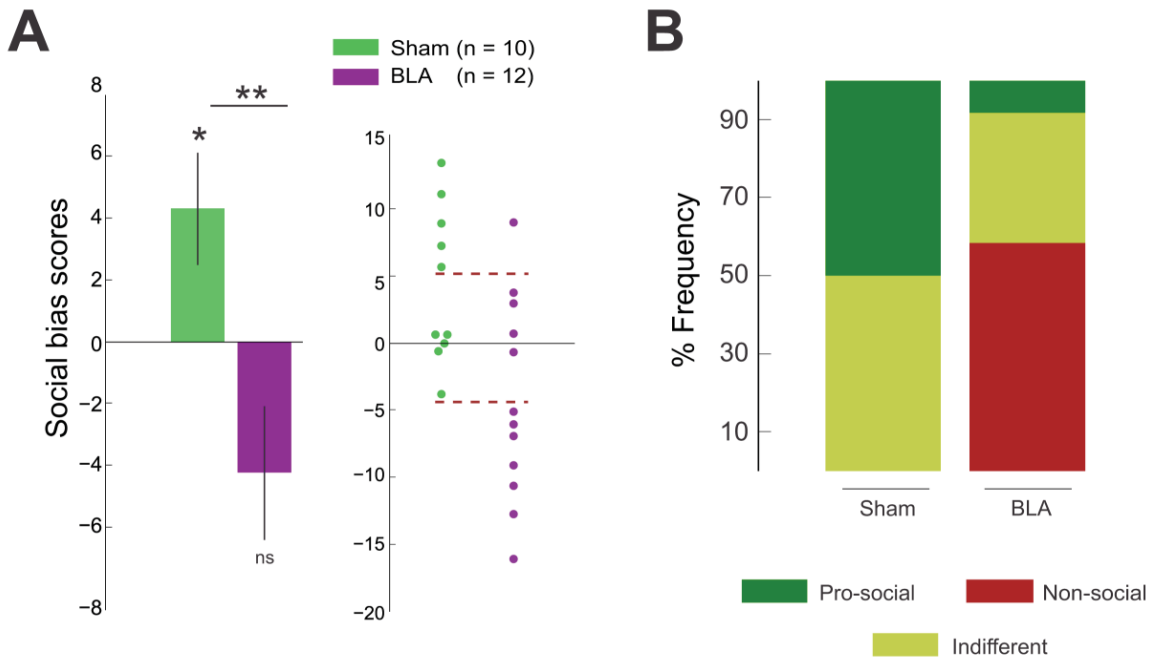

Figure 6 | BLA lesions abolish pro-social preferences in rats. A. Social bias scores per group. The individual (dots) and mean (bar) social bias scores indicating the percent difference in BR choices in the partner- compared to the toy condition were significantly different between the sham (green) and BLA rats (purple). Red vertical lines indicate the upper and lower bound of the 95% confidence interval (CI) computed from a reference permuted distribution. **B. Differential categorization between sham and BLA groups**. Using a reference social bias score distribution, rats from each group were categorized as "*pro-social*" (social bias scores > CI), "*indifferent*" (social bias scores ⊆ CI) and "*non-social*" (social bias scores < CI). ∗p < .05; ∗∗p < .01; ns, not significant. Error bars represent the standard error of the mean, s.e.m.

We categorized rats as "*pro-social*" if their social bias scores exceeded the upper confidence interval bound, as "*indifferent*" if their social bias scores were within the confidence interval and as "*non-social*" if their social bias scores were lower than the confidence interval's lower bound. Thus, in this categorization scheme, pro-social and non-social animals have respectively

higher or lower BR preferences in the partner than in the toy condition, whereas indifferent animals have no significant preferences.

This analysis revealed that in the sham group, half of the group (n = 5, 50%; Figure 6B) were classified as pro-social whereas the remaining half (n = 5, 50%) were classified as indifferent. Importantly, no sham-lesioned rat was classified as non-social. By contrast in the BLA group, n = 7 (60%) rats were classified as non-social, n = 4 (33%) were classified as indifferent, and only one animal (8%) was classified as pro-social. Accordingly, the frequency of rats classified as pro-social, non-social and indifferent was significantly different between sham and BLA rats ($X^2_{(2)} = 9.7$, $p < .01$). Further analysis revealed that the proportion of rats classified as non-social was significantly higher in the BLA-group than in the sham-group (z-test, Z = 2.93, $p < .05$), and the proportion of pro-social individuals was significantly lower in the BLA-group than in the sham group (Z = -2.19, $p < .05$).

3.3 Basolateral amygdala lesions abolish BR preferences in the partner condition

Social bias scores reflect the difference in BR-choices between the partner and the toy condition (see eq. 1). Thus, two different behavioral patterns might underlie the divergence of social-bias scores between sham and BLA groups. Lesion effects on social bias scores may either be due to the devaluation of mutual rewards in the partner condition, reflected by a lesion-related plunge in BR-preferences in the partner condition, or to an up-valuation of rewards to the toy rat, possibly through secondary reinforcement, leading to a rise in BR-preferences in the control condition.

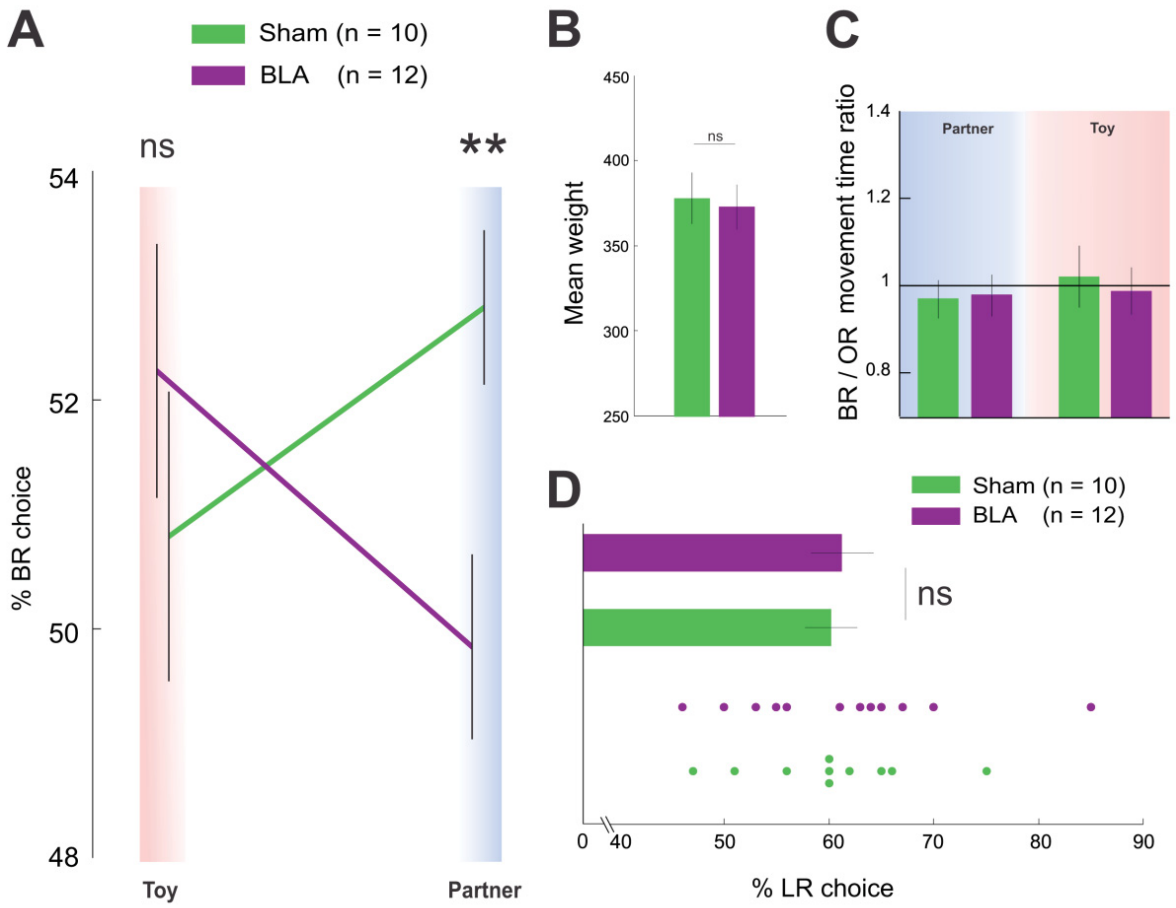

Figure 7| BLA lesion-induced learning impairment is social-context-specific. A. Percentage BR choices for sham (green) and BLA group (purple). BLA-lesioned animals made significantly less BR-choices than sham animals in the partner- but not the toy condition. Shading: blue partner; red toy condition. **B. Average weight per group.** The average weight did not differ between sham and BLA animals. **C. Movement time ratios.** The BR / OR movement time ratios were not significantly different from 1 in any group in any condition. Furthermore, direct comparisons between conditions or between groups were not significant either. **D. Performance in the MDT.** Individual (dots) and mean (bars) large reward preference in the MDT. Both groups of rats significantly preferred the LR alternative at levels above chance. There was no significant between group difference in large reward preference levels $**p < 0.01$; ns, not significant. Error bars represent the standard error of the mean, s.e.m.

To address this question, we computed a mixed ANOVA using %BR choice as dependent variable, and condition and lesion as within- and between-subject

factors, respectively. This analysis revealed a significant condition * lesion interaction on %BR choice (Figure 7A; $F_{(1,20)}$ = 8.70, p < .01). Post-hoc independent samples t-test revealed that, in the partner condition, the BLA group had significantly lower %BR choices than the sham group ($t_{(20)}$ = 2.76, p < .01, Bonferroni-corrected), whereas no significant lesion-effect on %BR-choice was found in the toy condition ($t_{(20)}$ = -.86, p = .40). This result suggests that the difference in social bias scores between BLA- and sham-lesioned animals was mainly due to the failure of BLA-rats to establish a BR preference in the partner condition, and to a lesser extent to differences in BR-choices in the non-social toy condition. Note that this behavior is not indicative of antisocial sentiments which would imply mutual-reward aversion in the partner condition – a tendency not shown by the BLA-lesioned rats.

Finally, we tested whether several putative confounds - body weight, motor parameters and experimenter intervention - that could potentially influence social decision making explained our lesion effects. The average weight of the animals was not different between BLA- and sham-groups (Figure 7B; $t_{(20)}$ = .26, p = .80), and there was no main effect of lesion on average movement time ratio, i.e. the ratio of movement times between OR and BR choices (Figure 7C, $F_{(1,20)}$ = 0.01, p = .91). Movement time ratios did not differ from chance levels in either group (Sham: Partner $t_{(9)}$ = -.71, p = .49; Toy $t_{(9)}$ = .29, p = .78; BLA: Partner $t_{(11)}$ = -.50, p = .63; Toy $t_{(11)}$ = -.21, p = .84), suggesting that all animals entered compartments comparably fast for both choice alternatives. Moreover, there was no correlation between social bias scores and movement time ratio (Sham: r = -.23, p = 0.52; BLA: r = .16, p = .62). Additional analyses showed that BLA lesion effects were not modulated by intervention of the experimenters who occasionally pushed the partner into the compartment (see Supplementary Information).

3.4 BLA lesions do not impair reward magnitude discrimination

It is possible that the BLA lesions induced general learning impairments so that the lesioned animals would be insensitive to any type of reinforcer, social or non-social. To exclude this possibility, all actors were tested in a reward magnitude discrimination task (MDT, Figure 3, lower panel) where the choice compartments in the same apparatus were now associated with the delivery of either three (small reward; SR) or six pellets (large reward; LR). Outcome discrimination and reversal learning deficits were both assessed by pseudo-randomizing the SR- and LR-compartment assignment across four testing sessions. The task had no social components, all rats were tested alone. Sham-operated as well as lesioned animals chose the LR compartment significantly above chance levels (Figure 7D; Sham*:* $t_{(9)}$ = 4.11; p < .01, BLA: $t_{(11)}$ = 3.74, p < .01), suggesting that both groups could still discriminate between reward magnitudes. Moreover, there was no significant difference in the percentage of large-reward choices between lesioned- and sham-animals ($t_{(20)}$ = -.27, p = 0.80). Finally, there was no significant interaction of session and group on LR choice ($F_{(3,60)}$ = 1.47, p = .23). This data suggests that animals in both groups could discriminate own-reward outcomes and flexibly adapt to reversing task contingencies. We therefore conclude that the BLA lesions specifically affected *social* aspects of the task.

4. Discussion

Rats have recently been shown to prefer mutual over own-rewards in a rodent Pro-social Choice Task. Here, we show that the integrity of basolateral amygdala (BLA) was necessary for the expression of mutual reward preferences. While 50% of the sham-operated animals showed mutual reward

preferences, 60% of the BLA animals behaved non-socially, i.e. made *less* mutual-reward choices in the partner compared to the toy control condition. Our results shed light on the putative neurobiological substrate of these social preferences.

We and others have recently discussed mutual reward preferences in light of a social reinforcement hypothesis (Chang et al., 2011; Ruff and Fehr, 2014; Hernandez-Lallement et al., 2015a) predicting that rats' choice allocation in the PCT is the consequence of social reinforcement learning. According to this view, social signals encoded at the neural level would reinforce individual's behavior towards pro- (or non-) social outcomes. More specifically, here, an actor's choice for mutual rewards could be driven by positive social reinforcement, i.e. through communication signals emitted by the partner that are perceived as rewarding by the actor (Seffer et al., 2014) or increased social interaction, e.g., pleasure derived from eating rewards in spatial proximity (Barnett and Spencer, 1951). Additionally, choice behavior could also be reinforced by negative social stimuli, i.e. putatively aversive distress signals produced by partners (Kim et al., 2010; Atsak et al., 2011) missing out on reward after OR choices. As previously noted (Hernandez-Lallement et al., 2015a), positive and negative social reinforcement learning are not mutually exclusive, but could act in concert to drive choice allocation. Interestingly, a recent study showed that positive and negative social stimuli (appetitive or aversive ultrasonic vocalizations, USVs) elicit opposite firing patterns in the rat amygdala (Parsana et al., 2012). Thus, USVs, which are known to carry affective state information (Knutson et al., 1999; Litvin et al., 2007) not only in rats (Wöhr and Schwarting, 2008; Seffer et al., 2014) but in also in other species (Sharp et al., 2005; Naumann and Kanwal, 2011; Gadziola et al., 2012a), are prime candidates for social stimuli driving choice in the PCT. This idea is

supported by a recent study showing that pro-social 50kHz USVs elicit phasic dopamine release in the nucleus accumbens (Willuhn et al., 2014), suggesting a functional link between social signals and reward processes.

The social reinforcement learning hypothesis provides a parsimonious framework that provides useful conceptual tools to describe and predict the rats' behavior in the PCT task as well as the role of the BLA in mediating mutual reward preferences and pro-social choice. The BLA has been proposed as a vigilance device, critical for linking the incentive properties of rewards and punishments to predictive sensory cues by enhancing their affective salience (Davis and Whalen, 2001; Schoenbaum et al., 2003). Thus, in social contexts, the BLA may be important for increasing an animal's sensitivity to the affective value of social information, and thereby drive social learning. According to this hypothesis, the BLA lesion effects in the present task would reflect deficits in representing and integrating social reinforcement values in the decision-making process. A deficit in attaching affective salience to social cues after BLA-lesions would then result in a general insensitivity to the affective value of social information, and consequently in the failure to acquire mutual reward preferences, as reflected by the large presence of non-social animals in the BLA group, which in contrast were absent among sham animals. This interpretation is particularly intriguing in light of psychopathic traits associated with amygdalar malfunction in humans (Anderson and Kiehl, 2012), possibly reflecting the psychopath's affective indifference to social cues and situations.

Abbreviations: BLA, Basolateral amygdala; PCT, Pro-social Choice Task; BR: Both Reward; OR: Own Reward; MDT: reward magnitude discrimination task; PBS: Phosphate Buffer Solution; PFA: Paraformaldehyde; CI: Confidence Interval; USV: UltraSonic Vocalization.

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Author contributions: J.H-L. designed and performed the research, analyzed the data and wrote the paper. M.v.W. analyzed the data and wrote the paper. S.S. analyzed the data and wrote the paper. T.K. acquired funds, designed the experiment, analyzed the data and wrote the paper.

Chapter II

Basolateral amygdala lesions abolish mutual reward preferences in rats

Supplementary Information

Julen Hernandez-Lallement*, Marijn van Wingerden, Sandra Schäble, Tobias Kalenscher

1. Supplementary Experimental Procedure

1.1. Experimental design

1.1.1. Initial training procedure

After habituation (*nSess* = 2) to the experimental setup during which animals were placed into the apparatus without any reward or conspecifics, rats learned to enter both compartments (training; *nSess* = 7; Hernandez-Lallement et al., 2015a) to collect a reward (three sucrose pellets). As soon as the behavioral criteria had been reached (consistently entering compartments, waiting for twenty-five seconds and consuming pellets within five seconds in at least two consecutive sessions), rats were randomly designed as actors or partners.

1.1.2. Pellet control condition

All actors were tested in a pellet control condition (*# Sessions* = 4) to control for secondary reinforcement properties of the sucrose pellets. The payoff matrix was identical to the pro-social choice task (PCT) with respect to reward contingencies (BR *vs* OR), but no partner or toy was present in the opposite chambers. Each session consisted of six forced and twenty five free choice trials. The first session was excluded from the analysis because rats showed poor performances in the task (animals failed to enter compartments), presumably because of direct or indirect (pause) surgery after effects. Thus, percentages of choice for a given compartment were computed over the last three sessions for all rats.

2. Supplementary Results

2.1. Pellet control condition

If the pellets delivered in the opposite compartments after BR choices had secondary reinforcement properties, actors should develop a preference for the BR compartment regardless of whether a partner or a toy was present (PCT task) or absent (pellet control condition). In the pellet control task, preferences for choices yielding additional reward in the opposite compartment (equivalent to the BR choice in the PCT) did not differ from chance levels in either group (Sham: $t_{(9)}$ = .30, p = .77 ; BLA: $t_{(9)}$ = .96, p = .36). Moreover, there was no significant difference in percent BR choices between BLA- and sham-rats ($t_{(20)}$ = -.17, p = .88).

2.2. Experimenter interventions

In on average 6% of all trials the partners had to be pushed in the compartments to ensure fixed trial length. It is possible that pushing the rats increased anxiety in the partners, and by social transmission of affective states, also in target rats. As partners were more likely to be pushed after OR- than BR-choices, this may be an important confound because actors may try to avoid the OR-option associated with transmission of fear. However, there was no effect of the proportion of push trials per session on actors' BR preferences ($F_{(1,19)}$ = 3.00, p = .10) , and there was no significant interaction between push trials and lesion condition ($F_{(1,19)}$ = 1.38, p = .54). Furthermore, there was no correlation between the pushes frequency and the BR preferences of the corresponding actors in either group (Sham: r = -.39, p = 0.26; BLA: r = -.36, p = 0.25).

Chapter III

Effect of lateral orbitofrontal cortex lesion on mutual reward preference in rats

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Abstract

The processes underlying pro-social choices (i.e. that yield benefit for others) have received a strong focus in the field of neurosciences. Particularly, animal models have provided powerful insights allowing between species comparisons at both behavioral and neural level. In a previous study, we showed that preference for mutual rewards in rats are abolished by lesion of the basolateral amygdala (BLA). Here, we present data suggesting that the lateral orbitofrontal cortex (LO) might additionally be involved in such behavior. Rats underwent a Pro-social Choice Task (PCT) where they decided between rewarding ("Both Reward" BR) or not rewarding a partner rat ("Own Reward" OR), both choices yielding an identical reward to themselves. Choices made in a social context (Partner condition) were contrasted to identical choice contingencies made in a non-social context (Toy condition). After establishment of baseline preferences, bilateral excitotoxic (*n* = 11) or sham infusions (*n* = 12) targeting the LO were performed, and animals were re-tested on the same design. Rats showed high BR preference in the partner condition but not in the toy condition before pharmacological intervention. However, while sham animals showed similar pre-/post-surgery between condition preferences, LO animals displayed high BR preference in both social and non-social contexts. However, this choice allocation pattern did not emerge in an additional batch of animals (*n*[Sham] = 6; *n*[LO] = 10). Moreover, both lesioned and sham groups in the second batch were impaired in reversal learning.

1. Introduction

Social behavior refers to interactions between individuals which can be either beneficial or costly (West et al., 2007b). Among these, pro-social choices (i.e. that benefit others; Miller et al., 1991; Hernandez-Lallement et al., 2015a) have been proposed as a major mechanism contributing to the development of modern human traits and society (Silk and House, 2011), and have consequently received particular attention in neuroscience (Moll et al., 2006; Harbaugh et al., 2007; Hernandez-Lallement et al., 2015b).

In the last decades, rodents have emerged as a promising model for the investigation of the neural bases of social decision making (Pfeiffer et al., 2005; Rutte and Taborsky, 2007a, 2007c; Sadananda et al., 2008; Atsak et al., 2011; Ben-Ami Bartal et al., 2011a, 2014; Seffer et al., 2014). Particularly, a rodent analog of the Pro-social Choice Task (PCT) has been used to show that rats prefer mutual rewards by providing food to conspecifics (Marquez and Moita, 2012; Hernandez-Lallement et al., 2015a). In an additional study, we have shown that rats with damage to the basolateral amygdala nucleus (BLA) do not establish pro-social preference (Hernandez-Lallement et al., 2015b). Here, using a similar design to measure pro-social decisions, we investigated the effect of damaging the lateral orbitofrontal cortex (LO), a prefrontal structure functionally and anatomically related to the BLA, on pro-social choice.

The LO, i.e. the lateral sub-cluster of the orbitofrontal cortex (OFC), is heavily involved in decision making (Wallis, 2011; Stalnaker et al., 2015). In humans, the LO is related to the computation of punishment magnitude (O'Doherty et al., 2001) and suppression of previously learned responses (Elliott et al., 2000). Additionally, the LO is selectively recruited in interactions

with other individuals as suggested by a study reporting higher activity in this area for angry expressions as compared to neutral ones (Blair, 2003). In rats, LO lesions induce increased impulsivity, as measured in a delay discounting task, whereas the opposite pattern was observed in medial OFC-lesioned animals (Mar et al., 2011). Unfortunately, there is limited information about LO involvement in social behavior in rodents. For instance, an increased male-male aggression was found in LO-lesioned rats (de Bruin et al., 1983), an effect also suggested in humans (Beer et al., 2003). A recent study performed in non-human primates reports that neurons in both MO and LO participate in the acquisition of information about conspecifics as well as in subsequent social choice behavior (Watson and Platt, 2012). Additional findings propose the OFC as part of a network devoted to the detection of own rewards (Chang et al., 2013). Finally, the OFC carries reward value altered by social context in primates (Azzi et al., 2012). Note that the last two studies performed recordings in the whole OFC and did not provide information allowing narrowing down the effect to LO-specific functions.

In order to investigate the effect of LO damage on pro-social preference, we trained rats in a PCT to extract baseline preferences and subsequently performed sham (n = 11) or excitotoxic LO lesion (n = 12) before re-acquisition of post-surgery pro-social preferences. Because of the involvement of the LO in social interactions, we hypothesized that pre-surgery preferences would be abolished by the LO lesion but would remain stable in post-surgery sessions in sham-operated animals. Furthermore, we trained an additional batch of rats (n[Sham] = 6; n[LO] = 10) in a PCT followed by a Magnitude Discrimination Task (MDT) to control for reversal learning impairments. In the PCT, we expected comparable results as previously hypothesized. Finally, based on previous findings suggesting reversal learning

impairments after OFC lesion, we expected LO-lesioned animals to not learn reversal contingencies in comparison to sham-operated rats.

2. Materials and Methods

2.1. Subjects and housing

Two consecutive batches of forty-eight (batch 1) and twenty-four (batch 2) male Long-Evans rats (*Janvier Labs, St. Berthevin, France*) were used, all animals weighing between 200-270g at the beginning of the experiment. Animals were housed in groups of four animals per cage, under an inverted 12:12 hours light - dark cycle (lights off at 07:00 a.m.), in a temperature- (20 ± 2°C) and humidity-controlled (60%) colony room. Daily food intake was restricted to keep animals at 85% of free feeding body weight. During the course of the experiments, weights were monitored daily to prevent severe weight loss. Water was available *ad libitum* in the home cage. The pre-lesion data from five animals in the sham group and seven animals from the LO group (batch 1) has been used in a previous study (Chapter I, Batch 2; Hernandez-Lallement et al., 2015a). All additional pre-lesion and all post-lesion data is completely novel and has not been used for publication. All animal procedures adhered to German Welfare Act and were approved by the local authority LANUV (Landesamt für Natur-, Umwelt- und Verbaucherschutz North Rhine-Westphalia, Germany).

2.2 Experiment and task design

2.2.1 Apparatus

Experiments were conducted in a custom-made apparatus (See Figure 1 of both Chapters I and II), previously described in detail (Hernandez-

Lallement et al., 2015a). In brief, each T-maze had a starting box attached to two separate compartments. All compartments were separated by a transparent and perforated wall allowing olfactory and auditory communication. Decisions were indicated by entering one of the compartments. Sucrose pellets were delivered in the inner corner of the compartments through a funnel system, ensuring interaction-free pellet delivery. Food was hidden from the animals during the decision phase, thus, minimizing potential distractive or competitive motives. The entire setup was covered with a red plexiglas roof. All experiments were carried out in red light in a closed black curtain system, to minimize the influence of contextual cues on decision making.

2.2.2 The Pro-social Choice Task

During the whole duration of the experiment, every rat was trained for one session a day on five consecutive weekdays for all habituation, training and testing sessions. Thirty-two (batch 1) and sixteen (batch 2) rats were assigned to the "*actor*" group whereas the remaining sixteen (batch 1) and eight (batch 2) were assigned to the *"partner"* group. Each actor received a pseudo-randomly generated partner number (actors and partners were never housed together) and interacted only with that conspecific during the PCT.

The PCT consisted of a partner (paired with the above mentioned partner rat) and a toy condition (actors paired with a toy rat). In a typical trial, both rats were placed in the starting box maze at the beginning of each session. Actors could enter either compartment (*t0*) which triggered the onset of the trial. The partner (or toy) was then directed into the compartment facing the actor's compartment (*t10*) and reward was delivered according to the choice's contingency (*t25*). For the actor, entering either compartment was

reinforced with an identical amount of reward (n = 3 pellets). However, entering the "Both Reward - BR" compartment resulted in an identical reward delivery in the opposite compartment for the partner, whereas entering the other compartment (the "Own Reward – OR" compartment) yielded reward delivery to the actor rat compartment only. Rats were replaced in their initial starting boxes for the subsequent trial. To control for potential side biases, left and right compartments were pseudo-randomly assigned to BR and OR choices across rats and sessions. In order to allow the sampling of the side-outcome contingencies, actors were given eight forced trials (counterbalancing side in a pseudo-randomized order) at the beginning of each session, followed by fifteen free choice trials.

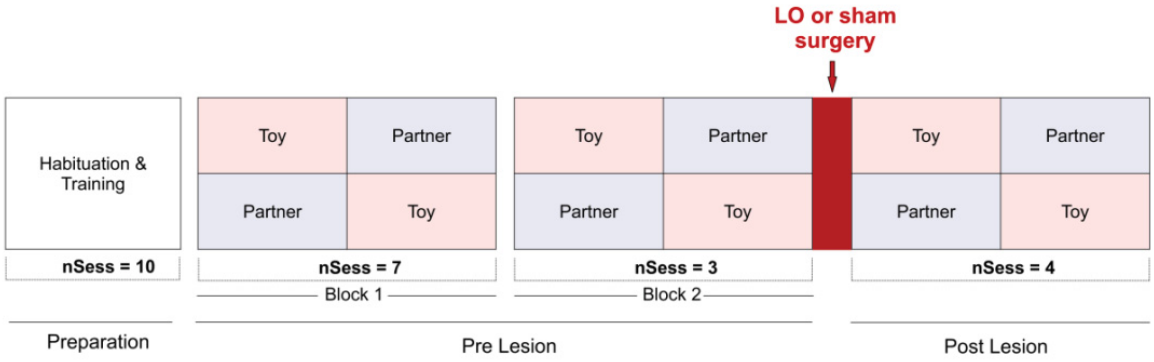

Figure 1 | Experimental timeline. *Preparation phase:* rats underwent habituation and training in the experimental setup. *Pre-Lesion:* rats performed both partner and toy conditions according to their starting condition for seven sessions in a first block (Block 1) and repeated each condition for three sessions in a second block (Block 2). *Post-Lesion:* after surgical procedure, rats were re-tested for four sessions per condition in the PCT. Animals in the second batch underwent a MDT directly following post-lesion PCT training (not shown in figure).

2.2.3 Experimental timeline

The timeline of the experiment is shown in Figure 1. All rats were habituated to the T-maze setup (*nSess* = 2) and trained to enter compartments for reward delivery (*nSess* = 8). Habituation and shaping procedures have been

described in detail elsewhere (Chapter I, Supplementary Information; Hernandez-Lallement et al., 2015a). In the PCT, actors spent seven sessions (Block 1) in each condition and were subsequently re-tested for three sessions per condition (Block 2), matching a total number of ten sessions per condition before pharmacological intervention (Figure 1, Pre-Lesion). This procedure was used to test for stability of choice preference over time. After surgical procedure (see below), actors were given ten days of post-surgery recovery time before being re-tested on the PCT for four sessions per condition (Figure 1, Post-Lesion). To control for potential order effects, half of the actors started in the social condition whereas the remaining half started in the non-social condition.

2.2.4 Magnitude Discrimination Task

In order to control for lesion effects on reversal learning, we trained an additional group of animals (Batch 2) in a PCT as described above, followed by a reward magnitude discrimination control task (MDT; *nSess* = 4). In the MDT, only one half of the double T-Maze was used (see Chapter II). Here, one compartment was associated with the delivery of a large reward (LR; *n* = 5 pellets), and the other compartment with a small reward delivery (SR; *n* = 1 pellets). The LR- and SR-compartment assignment was pseudo-randomized across sessions and rats; hence, rats had to flexibly adjust to frequent contingency reversals across the four testing sessions. Moreover, the magnitude contingencies were reversed within sessions. Thus, after one block of trials where LR and SR contingencies were associated with one compartment respectively, animals underwent an additional block of trials were contingencies were reversed. In each block, rats underwent four forced trials to allow sampling of the compartment's contingencies, followed by

fifteen free choice trials where rats could freely choose between left and right compartments. To ensure identical reward delivery time, all rewards were delivered ten seconds (*t10*) after the actors' choice. After reward consumption, the rat was replaced in the starting box for the next trial.

2.3 Surgery

Upon completion of both testing blocks (Figure 1, Pre-Lesion), actors were pseudo-randomly assigned to LO or Sham groups (Batch 1, LO: n = 16; Sham: n = 16; Batch 2, LO: n = 10; Sham: n = 6). All actors were anesthetized using mixture of isofluorane (5%) and oxygen, and positioned on the stereotaxic frame (David Kopf Instruments). Excitotoxic lesions targeting the LO were performed with 0.09 M of quinolinic acid dissolved in 0.1 M buffer solution, with 7.4 adjusted *p*H using 0.1 M NaOH. Bilateral infusions were made using 0.3mm diameter cannula (Plastics One) connected to a 10µl Hamilton microinfusion pump (Harvard Apparatus) through polyethylene tubes. Infusions were made using 3µl of quinolinic acid at an infusion rate of 1 µl/mn, after what the needle was left in place for three minutes allowing spread of the acid. The following coordinates were used: anteroposterior (AP) + 4.2 mm, mediolateral (ML) ± 0.6, dorsoventral (DV) – 4.3. The AP and ML coordinate where taken from bregma and the DV coordinate was taken from the dura. Sham surgeries were performed in a similar fashion, but infusing vehicle alone. During training, all experimenters were blind to the animals' group affiliation.

2.4 Analysis and statistics

All analyses were performed using IBM SPSS Statistics 20 or MatLab 2013a (The Mathworks). All multiple comparisons are corrected using Bonferroni correction.

Social bias scores: for each individual, we computed a social bias score (Hernandez-Lallement et al., 2015) that reflected the between condition BR preference difference. The social bias score for rat *i* was expressed as the percent change in absolute BR choices in the partner condition [$BR(partner)_i$] relative to the BR choices in the toy condition [$BR(toy)_i$]:

$$SB_i = \left[\frac{\mathrm{BR(partner)}_i - \mathrm{BR(toy)}_i}{\mathrm{BR(toy)}_i}\right] * 100 \qquad (1)$$

Social bias difference: In order to investigate potential pre- *vs* post-lesion social bias score difference, we computed for each rat *i* a social bias score difference reflecting the relative change in social bias in post-lesion relative to pre-lesion sessions:

$$SB\ Diff_i = [\mathrm{SB(PreLesion)}i - \mathrm{SB(PostLesion)}i] \qquad (2)$$

Lesion score: Any post-surgery social bias score alteration could be due to increase and/or decrease on BR preference in both partner and toy conditions. To further investigate a potential condition-specific effect of LO lesions, we computed an individual lesion score (LC) reflecting the relative BR preference pre-lesion relative to the same preference post-lesion in each condition:

$$LC_i = \left[\frac{\mathrm{BR(PostLesion)}_i - \mathrm{BR(PreLesion)}_i}{\mathrm{BR(PostLesion)}_i + \mathrm{BR(PreLesion)}_i}\right] \quad (3)$$

Thus, per condition, positive and negative deviations from 0 reflect an increase and decrease of BR preference post-lesion relative to pre-lesion baseline preferences.

3. Results

In the first batch, three animals in the LO group were excluded because of incomplete LO lesion and two animals died of post-surgery traumatism. In the sham group, two animals did not enter compartments after surgery and were excluded, and two animals died from surgery. In the second batch, one animal in the sham group died during surgery. All remaining actor rats (Batch 1, $n_{[Total]}$ = 23, $n_{[LO]}$ = 11, $n_{[Sham]}$ = 12; Batch 2, $n_{[Total]}$ = 15, $n_{[LO]}$ = 5, $n_{[Sham]}$ =10) completed all trials and sessions. There was no effect of starting condition on social bias scores in either batch and group (Batch 1, Sham: $t_{(10)}$ = .82; p = .43, LO: $t_{(9)}$ = -.31, p = .77; Batch 2, Sham: $t_{(8)}$ = .93; p = .38, LO: $t_{(4)}$ = -.18, p = .33). For clarity purposes, we first focus on the results obtained in batch 1 and delay additional results (batch 2) to the end of the result section.

3.1 Lesion analysis

Histological assessment of lesions (Figure 2) was performed by J.H-L. and confirmed by two additional individuals blind to the experimental manipulation. Lesions encompassed the orbitofrontal regions defined by Paxinos and Watson (1998). Occasionally, damage spread into edges of the

ventral orbital cortex (VO) and/or to the ventral part of the agranular insular cortex, sparing more dorsal portions of the OFC (Figure 3).

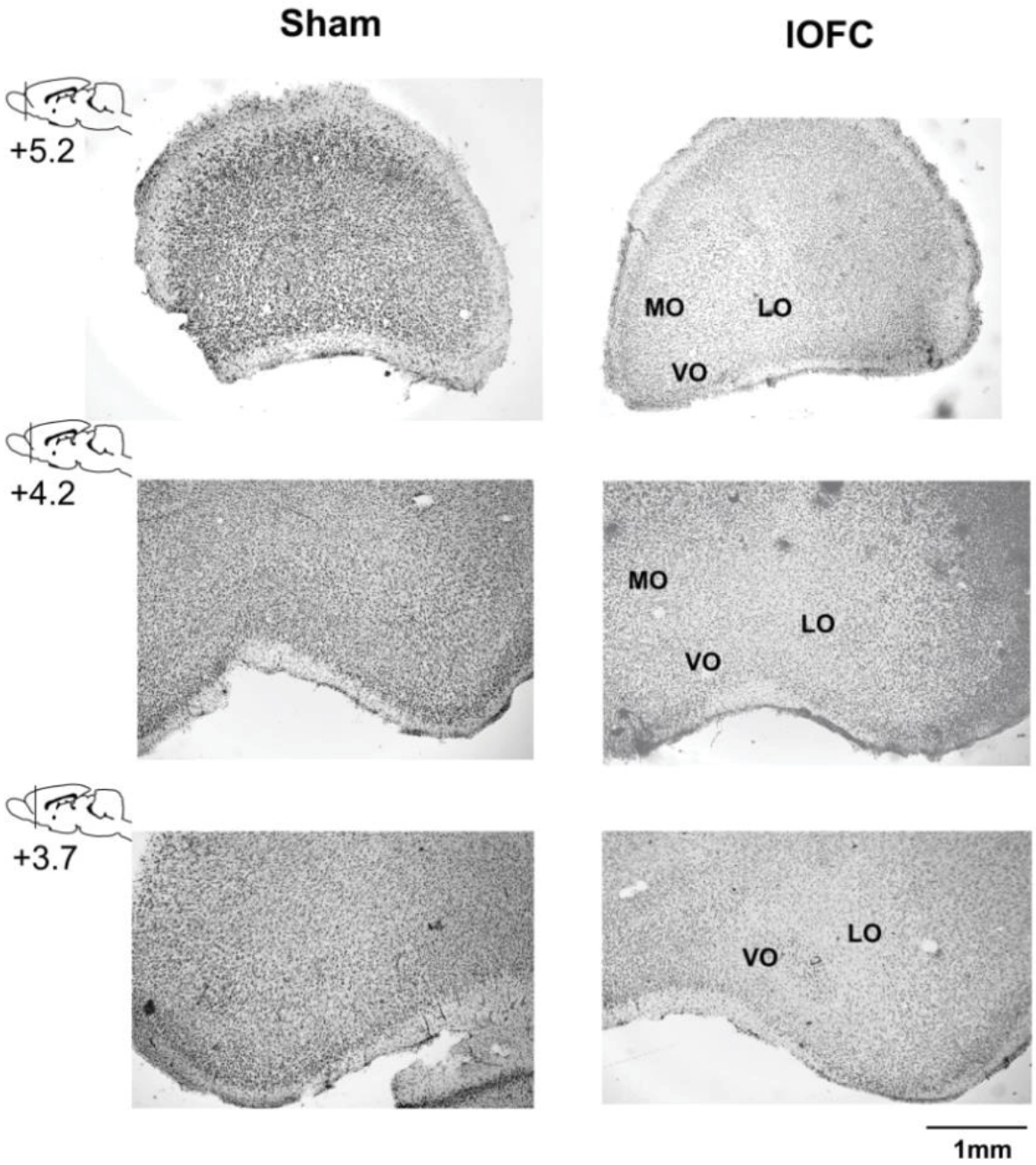

Figure 2 | Histological analysis of LO lesions. Photomicrographs depicting typical lesions of the lateral orbitofrontal cortex (right) and sham-operated control tissue (left). MO, medial orbital; VO, ventral orbital; LO, lateral orbital

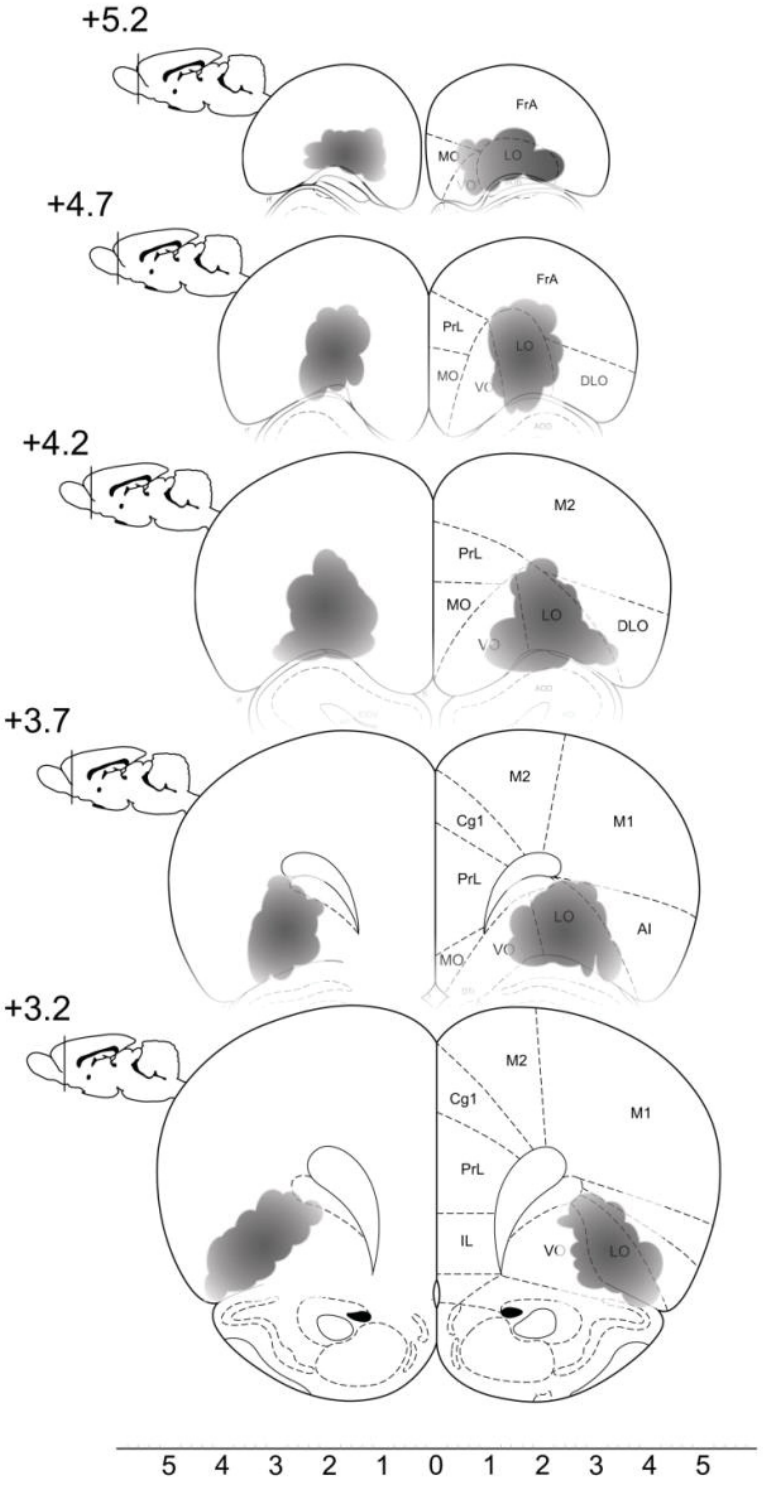

Figure 3 | Schematic brain slice illustration of LO lesion spread. Grey gradient represents lesion spread across subjects. Left numbers inform on distance from bregma (in *mm*). Diagrams are adjusted from Paxinos & Watson (1998). MO, Medial orbital cortex; VO, ventral orbital cortex; LO, lateral orbital cortex

3.2 Rats prefer mutual rewards in a Pro-social choice task

In a first time, we tested whether, as previously observed (Chapter I; Hernandez-Lallement et al., 2015a), rats in batch 1 would show higher BR preferences in the partner than in the toy condition in both pre-lesion testing blocks (Figure 4).

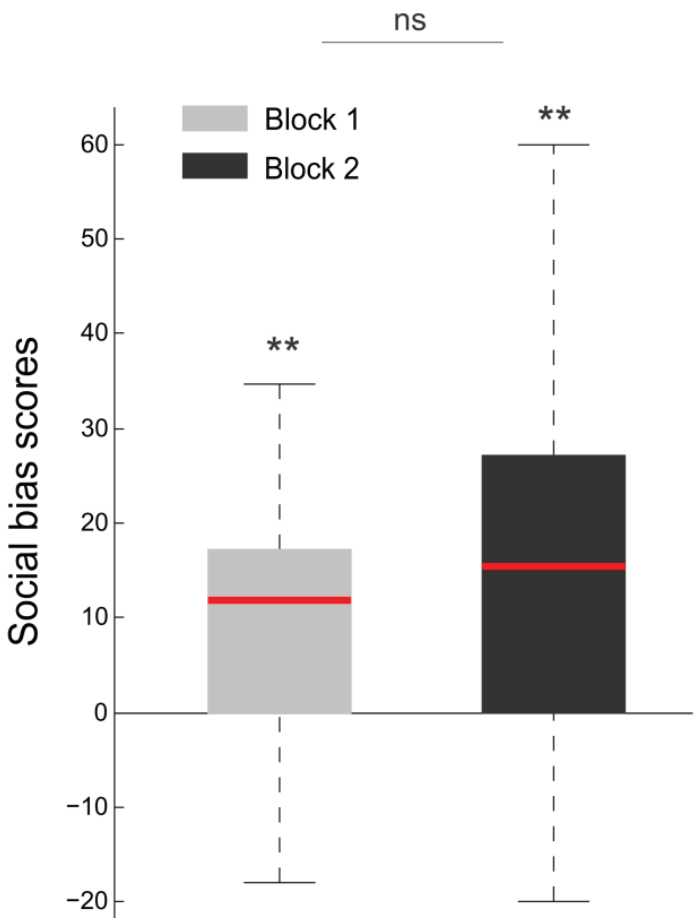

Figure 4 | Social bias scores per blocks. Before lesion, animals showed positive social bias scores in both testing blocks, suggesting that higher BR preference in the partner than toy condition was successfully established and stable over blocks. **p < .01, ns, non significant, Bonferroni corrected. Error bars represent the standard error of the mean, s.e.m.

Thus, we computed the individual social bias scores for each block and tested the distributions against chance levels. We found that in both pre-lesion

testing blocks, rats showed a significant positive deviation from zero (Block 1: $t_{(22)}$ = 3.01, p < .01; Block 2: $t_{(22)}$ = 3.47, p < .01), suggesting a higher BR preference in the partner than in the toy condition. Moreover, the social bias score distributions were not significantly different between block 1 and 2 ($t_{(22)}$ = -.89, p =.38). Thus, we pooled the data from both blocks in all subsequent analyses.

When distinguishing between LO and sham groups (Figure 5A), a repeated measures ANOVA showed a significant main effect of condition in pre-lesion sessions ($F_{(1,21)}$ = 16.31, p < .001), no significant condition*group interaction ($F_{(1,21)}$ = .12., p = .75), and no significant effect of group ($F_{(1,21)}$ = 3.45., p = .070). Post-hoc pairwise comparisons revealed a significant between-condition BR preference in both groups (Sham: MeanDiff. = -4.61, p < .01; LO: Mean Diff. = -5.39, p < .01) whereas there was no significant difference between Sham and LO groups in either condition (Partner: Mean Diff. = -2.47, p = .29; Toy: Mean Diff. = -2.47, p =.13). Furthermore, both groups showed a significant positive deviation from chance levels in the partner condition (Sham: $t_{(11)}$ = 3.01, p < .05; LO: $t_{(10)}$ = 4.13, p < .05) but not in the toy condition (Sham: $t_{(11)}$ = -.49, p = .17; LO: $t_{(10)}$ = -.39, p = .71). Finally, we found that social bias scores in both groups were significantly higher than zero (Figure 5B; Sham: $t_{(11)}$ = 3.51, p < .01; LO: $t_{(10)}$ = 3.04, p < .01) but no different from each other ($t_{(21)}$ =.12, p = .91).

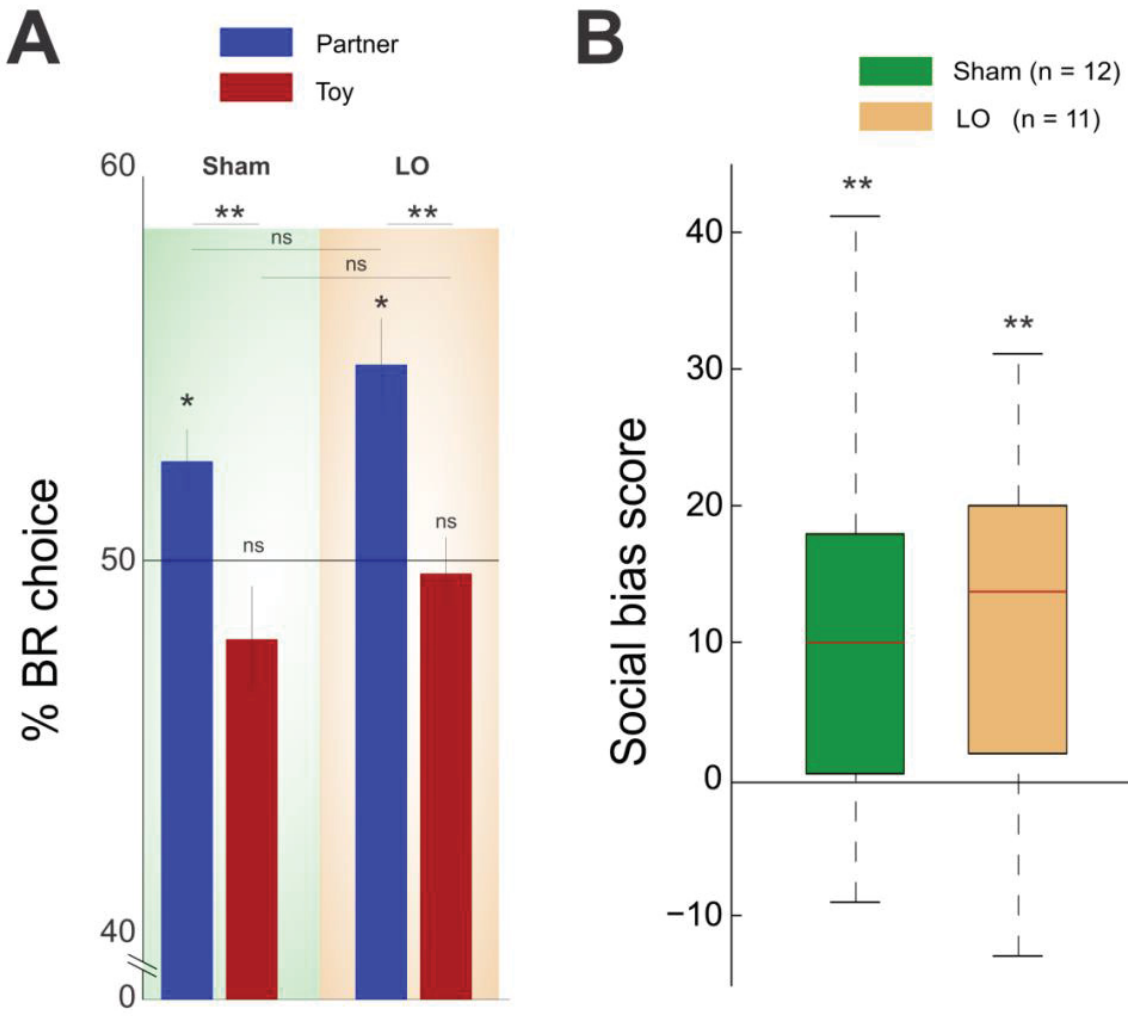

Figure 5 | Baseline preferences in the sham (green) and LO group (orange). A. Percentage BR preference per group per condition. For both sham (green background) and LO groups (orange background), we found a significant between-condition difference as well as a positive significant deviation of BR choices from chance levels in the partner (blue) but not in the toy condition (red). **B. Pre-lesion social bias scores per group.** The mean social bias scores were significantly different from chance levels for both sham (green) and LO group (orange). *$p < .05$, **$p < .01$, Bonferroni corrected.

3.3 Lateral orbitofrontal cortex lesion abolishes condition discrimination

In contrast to sham surgeries, we expected that LO lesion would suppress partner condition-specific BR preferences observed before surgeries,

thus, reducing the overall social bias scores. To this end, we computed the social bias score difference (Figure 6A; see Methods) for each group and tested the distributions against zero. We hypothesized that social bias score difference would fluctuate around zero in the sham group but would positively deviate from zero in the LO group. In line with our hypothesis, we found that social bias score differences did not differ from zero in the sham group ($t_{(11)}$ = .33, p = .75), but were significantly higher than zero in the LO group ($t_{(10)}$ = 2.05, p < .05), suggesting that the social bias scores of LO animals decreased after lesion. To further account for individual variation, we plotted pre- *vs* post-surgery social bias scores (Figure 6B) and quantified the number of animals showing a post-surgical social bias score decrease (dots under the diagonal line). We found a significant bias in decrease of social bias scores in the LO but not in the Sham group (Figure 6C; Sham: p = .01; LO: p = .77, binomial two-tailed)

3.4 LO-lesioned animals show increased BR preference in the toy condition

The reduction in social bias scores observed in the LO group could be due to a decrease or an increase of BR preference in the partner and toy condition, respectively. To test for this hypothesis, we computed a lesion score (LC; Figure 7A; see Methods) that individually evaluated the within-condition BR choice difference before and after lesion. Briefly, positive and negative lesion score represented higher and lower BR preferences post- than pre-lesion, respectively. As expected, LCs did not differ from zero in the sham group in either condition (partner: $t_{(11)}$ = -.33, p = .75; Toy: $t_{(11)}$ = .26, p = .80, Bonferroni corrected).

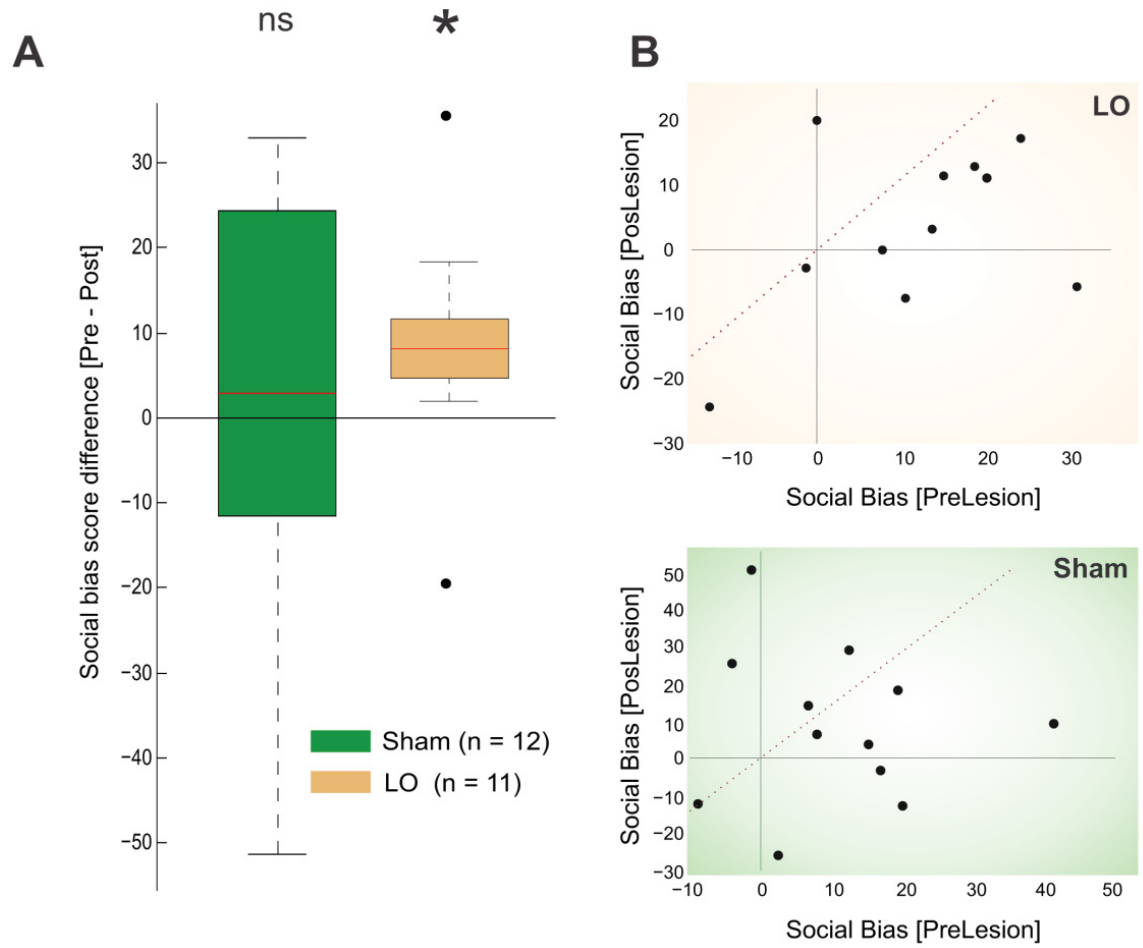

Figure 6 | Lesion effect on social bias scores. A. Pre- and post-lesion social bias score difference: Social bias scores did not differ from zero in the sham group, but were significantly higher than zero in the LO group. Dots are outliers detected by the boxplot algorithm. **B. Individual variation in social bias scores for the LO (up) and sham group (down).** The diagonal represents hypothetical identical pre/post lesion social bias scores for each individual (dots). Dots located under and above the diagonal represents rats whose social bias scores decrease and increase after surgery, respectively. **C. Number of animals under the diagonal.** In the LO group, there was a significant bias towards post-lesion decrease in social bias scores, which was not the case for the sham group. *p < .05

Interestingly, LCs in the LO group did not differ from zero in the partner condition ($t_{(10)}$ =.25, p = .81) but were significantly higher than zero in the toy condition ($t_{(10)}$ =2.64, p < .05), suggesting that LO-lesioned animals increased their BR preference in the toy condition only, whereas BR preference in the partner condition remained stable.

If BR preferences acquired before lesion were generalized to a non-social context, post lesion preferences might scale to pre-lesion basal levels. In line with this idea, we found a positive correlation between the LO animals' %BR post-lesion preference in the toy condition and %BR pre-lesion preferences in the partner condition (Figure 7B up; Pearson's coefficient; r =.71, p < .05*)* and toy condition (Figure 7B down; r =.63, p < .05). This was not the case in either condition in the sham group (Partner: r = -.44, p = .16 ; Toy: r = .004, p = .99).

3.5 Reversal impairments are no LO lesion specific

Although, reversal learning impairments epitomized OFC functions in the last decades (Stalnaker et al., 2007), successful reversal learning after OFC lesion has already been observed (Kazama and Bachevalier, 2009; Stalnaker et al., 2015). In order to confirm that LO-lesioned animals in this study could adapt choice allocation to between-sessions reversals in BR contingency, a second batch of animals (n[Sham] = 10 ; n[LO] = 5) was trained in an identical PCT design and subsequently in a MDT (see Methods). Briefly, in the MDT, each T-Maze compartment was associated with the delivery of a large (LR, n = 5 pellets) and a small reward (SR, n = 1 pellet). In each session, animals underwent two blocks of trials within which magnitude contingencies were

associated to each compartment but between which those contingencies were reversed. Hence, animals underwent within and between session reversals.

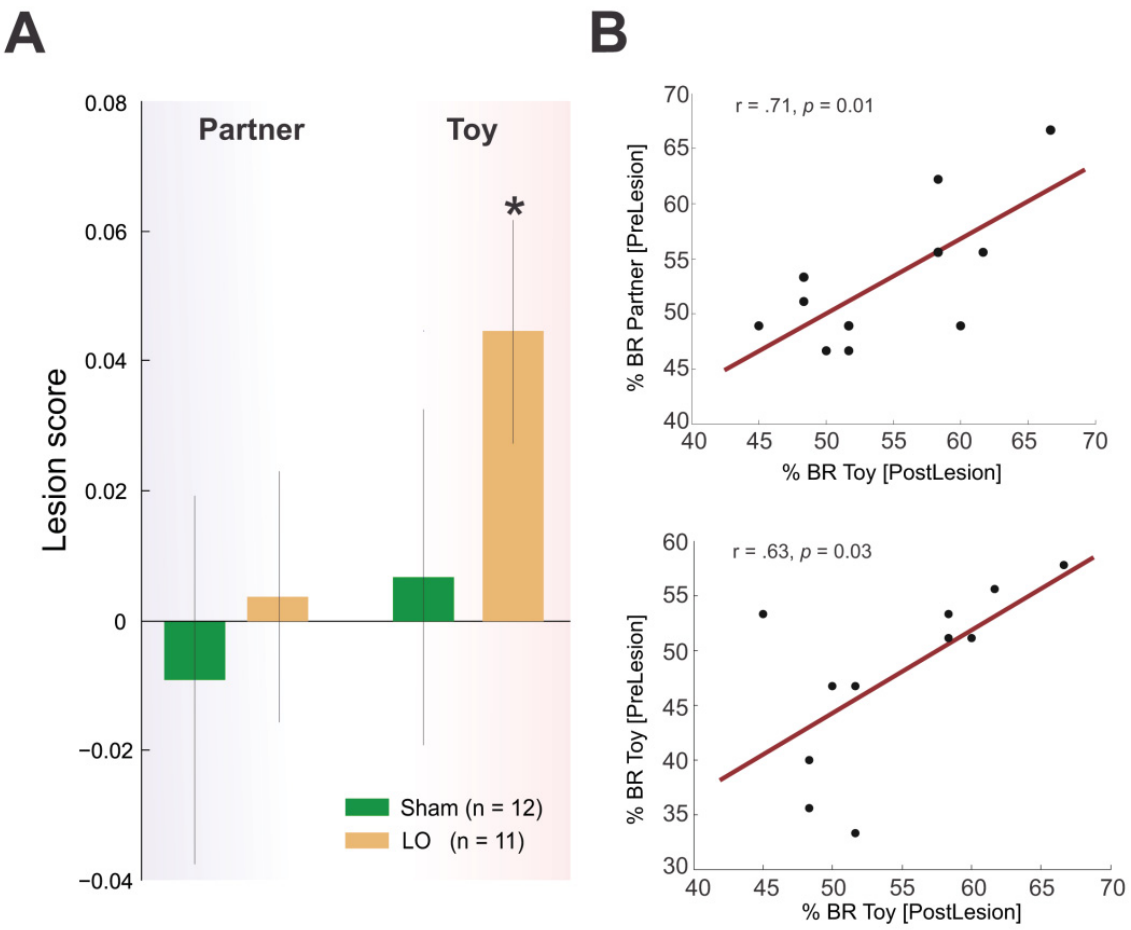

Figure 7 | Lesion to the lateral orbitofrontal cortex increase BR preferences in the toy condition A. Lesion score. The lesion score quantifies Pre/Post lesion difference in social bias scores for each rat. The lesion score did not differ from zero in either condition in the sham group (green). In the LO group (orange), there was no difference from zero in the partner condition but the lesion scores were significantly higher than zero in the toy condition. **B. Correlation between Pre/Post lesion BR preference in the LO group.** The percentage BR choice in both pre-lesion partner and toy condition were positively correlated with the post-lesion BR preference levels in the toy condition. *p < .05, Bonferroni corrected.

A repeated measures ANOVA (block and session as within subject factor and group as between subject factor) revealed a significant main effect

of block ($F_{(1,13)}$ = 18.80., p < .001), a significant block * group interaction ($F_{(1,13)}$ = 8.35., p < .05) and no effect of session ($F_{(3,39)}$ = .56., p = .64). Post-hoc pairwise comparisons showed a significant difference between blocks in the sham group (Figure 8; Mean Diff. = 16.67, p < .001) but not in the LO group (Mean Diff. = 3.34, p = .52). One sample t-tests against chance (50%) showed that the sham group had a significant preference for the LR option in block 1 ($t_{(9)}$ = 6.17, p < .001, Bonferroni corrected), whereas all other comparisons did not reach significance levels (Sham; block 2: $t_{(9)}$ = -2.36, p = .04; LO; block1: $t_{(5)}$ = 1.06, p = .35; block 2: $t_{(5)}$ = -.26, p = .81).

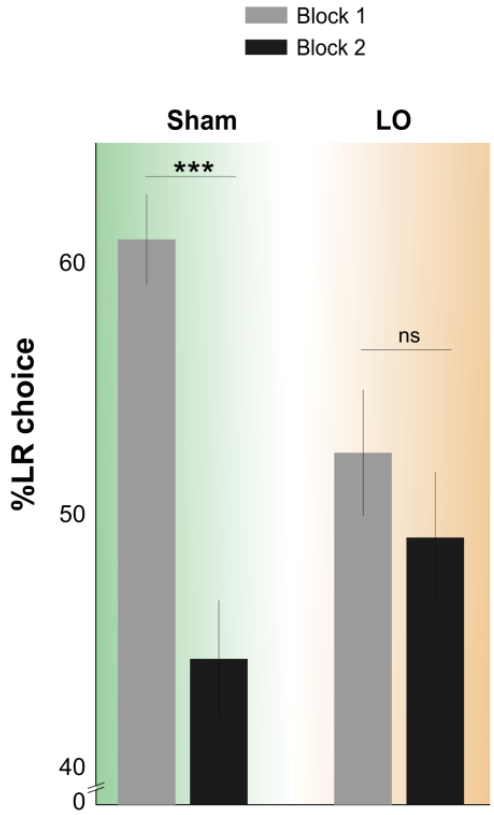

Figure 8 | Percentage of large reward choice per group in the MDT. There was a significant difference between block 1 (light gray) and block 2 (dark grey) in the sham (green, left panel) but not in the LO group (orange, right panel). ***p < .001, Bonferroni corrected.

These findings suggest that, although animals in the sham group showed higher preference for the LR alternative in block 1, they did not successfully reverse preferences between blocks of trials. Furthermore, the LO group was indifferent between both alternatives throughout the sessions. Additionally, animals in the second batch behave differently in the PCT as compared to rats in batch 1. A repeated measures ANOVA (condition and phase as within subject factors and lesion as between subject factor) showed no significant effect of condition in pre-lesion sessions ($F_{(1,13)}$ =.03, p = .85), no significant condition*group interaction ($F_{(1,13)}$ = .02., p = .88), and no significant effect of group ($F_{(1,13)}$ =.05., p = .83). Finally, social bias scores were not significantly different than zero in either group (Sham: $t_{(10)}$ = .22, p = .83; LO: $t_{(5)}$ =-.14, p = .89).

These results suggest that animals in this additional batch did not show any preference for the BR alternative in the partner condition. Thus, these findings strongly contrast with the findings obtained in batch 1 or results reported in Chapter I. Although this discrepancy could lie in the low sample size ($n_{[LO]}$ = 5), behavior observed in batch 2 raises questions regarding the replicability of results obtained in batch 1. Further experimentation is needed to validate the effect of LO lesion on pro-social choice in rats.

4. Discussion

The aim of this study was to investigate the impact of LO damage on pre-established pro-social preference in rodents. Results obtained in batch 1 show that before lesion, rats show stable pro-social choice by granting food-access to a conspecific. In comparison to sham animals who showed comparable post-surgery pro-social choice allocation, individuals with

damaged LO showed an increased in BR preference in the toy condition, whereas choice allocation in the partner condition remained stable in both groups. However, in a MDT controlling for reversal learning impairment, we show that both sham-operated and LO-lesioned animals from an additional batch showed a reversal learning impairment. These results contradict the data obtained in batch 1 showing that LO-lesioned animals had significant preference for the BR option in the toy condition, despite between session BR side reversals. Moreover, animals in this batch did not establish BR preferences before lesion.

The OFC in choice behavior

The OFC receives temporal and subcortical afferences from sensory association areas (Krettek and Price, 1977; McDonald, 1991, 1998; Stalnaker et al., 2007) and plays a major role in learning and goal-directed behavior (Schoenbaum et al., 2002; Winstanley et al., 2004; Roesch et al., 2006). Studies in primates have shown that the OFC is particularly relevant for the rapid and flexible update of cue/outcome associations (Procyk et al., 1993; Noonan et al., 2010; Walton et al., 2010), as seen in reversal learning tasks (Fellows and Farah, 2003; Bechara et al., 2009), and is important for updating the motivational value of a particular cue (Tremblay and Schultz, 1999). Similarly, the rodent OFC has been widely related to reversal learning (Schoenbaum et al., 2002) and is believed to keep track of stimulus/value associations (Roesch et al., 2006; van Wingerden et al., 2010a, 2010b). For instance, several studies reported that OFC-lesioned animals acquire initial cue/outcome association comparable to control individuals but require additional time to learn novel associations (Schoenbaum et al., 2002; Boulougouris et al., 2007). The results obtained in batch 1 are in line with these results. However, we could not

replicate these findings in an additional batch, which emphasizes that caution should be taken in interpreting these results.

A cortico-limbic network for social choice

The current results complement a recent report showing that BLA lesion impairs pro-social preference acquisition in a rodent PCT (Hernandez-Lallement et al., 2015b). The BLA and LO are known to show strong reciprocal projections (Barbas and De Olmos, 1990; Schoenbaum et al., 2000) and strong connectivity between these two structures predicts social network size in humans (Bickart et al., 2012). Furthermore, together with the BLA and other nuclei, the LO has been proposed as part of a social perception network in humans that would play a major role in the integration of social stimuli (Bickart et al., 2014b). Results from lesion studies in rodents suggested that the OFC could act as a cross-structure influence in the update of value computation in other areas such as the BLA (Schoenbaum et al., 1999, 2009a). Interestingly, OFC lesion-induced reversal learning deficit was suppressed by additional BLA lesion in rats (Stalnaker et al., 2007). In our paradigm, the updating of BR/ OR options in the PCT might rely on the reciprocal and functional BLA / LO interaction. Importantly, we found a positive correlation between pre-lesion pro-social preference levels (i.e. in the partner condition) and post-lesion BR preference in the toy condition. Thus, the generalization of BR preference to the toy condition might be contingent upon baseline (i.e. pre-lesion) pro-sociality levels. These results are in line with findings suggesting that basal preference levels can influence post-pharmacological intervention choice behavior (Zeeb et al., 2010).

Interestingly, LO-lesioned animals in batch 1 were able to adapt their choice allocation to successive reversal of the BR side in post-lesion sessions. This result is rather controversial given that reversal learning impairment after OFC lesions have been widely replicated (Schoenbaum et al., 2009b). However, one study reported that selective lesions to areas 11 and 13 in the macaque PFC, located between the lateral and medial OFC, did not impair reversal learning (Kazama and Bachevalier, 2009). Therefore, the results from Batch 1 suggest that the LO in rats is not critical for reversal learning. To test for reversal learning impairment triggered by LO lesion, we trained an additional batch of animals in a MDT where rats had to choose between a large and a small reward. Reversals were made within and between sessions (see Methods). We observed that reversal learning was impaired in both groups, suggesting that the impairment was not specific to the lesion. One possibility for this puzzling result is that reversals in the PCT were made between sessions whereas in the MDT, reversals were additionally performed within the sessions. Thus, it is possible that this design was too complex for the rats to perform successfully. Future designs should implement between session reversal to mirror the PCT design as closely as possible. Finally, one possibility is that the low sample size in batch 2 limited the emergence of a significant effect. This possibility should be tested in additional experiments.

In conclusion, our results suggest that the LO might be important in pro-social choice allocation in rodents. However, the discrepancy in behavior between different batches of animals emphasizes that additional experiments should be performed in order to reach a scientifically valid conclusion.

Abbreviations: PCT, Pro-social Choice Task; BR: Both Reward; OR: Own Reward; MDT: reward magnitude discrimination task; BLA, Basolateral amygdala; LO: Lateral Orbitofrontal Cortex; OFC: Orbitofrontal Cortex; PBS: Phosphate Buffer Solution; PFA: Paraformaldehyde.

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Chapter IV

A social reinforcement learning hypothesis of mutual reward preferences in rats[9]

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Abstract

Although the use of neuroimaging techniques has revealed much about the neural correlates of social decision making in humans, it remains poorly understood how social stimuli are represented, and how social decisions are implemented at the neural level in humans and in other species. To address this issue, the establishment of novel animal paradigms allowing a broad spectrum of neurobiological causal manipulations and neurophysiological recordings provides an exciting tool to investigate the neural implementation of social valuation in the brain. Here, we discuss the potential of a rodent model, Rattus norvegicus, for the understanding of social decision making and its neural underpinnings. Particularly, we consider recent data collected in a rodent Prosocial Choice Task within a social reinforcement framework and discuss factors that could drive social decision making in rodents.

A framework for understanding social decision making in animals

Animal choice behavior is often analyzed within a reinforcement learning framework (Schultz, 2006). According to the most basic reinforcement learning principles, action-outcome contingencies are learned through positive reinforcement (i.e., the likelihood of an operant behavior increases if it is followed by a reward) and/or negative reinforcement (i.e., the likelihood decreases if it is followed by an aversive event, such as an electric shock; Niv and Montague, 2008). Social decision making has been recently discussed in the light of a *social* reinforcement hypothesis (Chang et al., 2011; Hernandez-Lallement et al., 2015a) which states that animals' choices in social contexts are also affected by a process that updates the likelihood of some actions over alternative courses of action based on *social* outcomes. According to this view, any behavior that results in a social outcome that is perceived as appetitive, e.g., a friendly smile in humans, or putatively rewarding communication signals emitted by rats (Willuhn et al., 2014), will be reinforced. Correspondingly, any behavior that results in a social outcome that is perceived as aversive (e.g., swearing in humans) or negative (e.g., aggressive reactions of conspecifics in non-human animals) will less likely be repeated in the future. In the social reinforcement framework, social reinforcers are thus social stimuli that carry positive or negative reinforcement properties. There is indirect evidence for this hypothesis in rats. For instance, putatively rewarding 50 kHz ultrasonic vocalizations (USVs; see below) emitted by a conspecific rat trigger dopamine release in an observer rat's nucleus Accumbens (NAcc; Willuhn et al., 2014), a signal associated with reinforcement learning (Schultz et al., 1997). Furthermore, witnessing a reward delivered to a conspecific rat elicits

activation in an observer's NAcc – a possible mechanism for vicarious reinforcement (Kashtelyan et al., 2014). Finally, lesion to the basolateral amygdala nucleus, a neuronal clusters considered as a sensory gateway, abolishes mutual reward preferences in rats.

Recently, we used this framework to discuss the dynamics of pro-social choice behavior in a rodent PCT (Figure 1A; Hernandez-Lallement et al., 2015a). In this task, pairs of rats (an actor and a partner) are trained in a double T-maze setting. Actors are the first movers and choose to enter one of two different compartments, either choice leading to an identical reward for themselves. However, entering one compartment triggers the delivery of an additional reward for the partner rat (both-reward, BR; Figure 1A, upper panel), whereas entering the alternative compartment does not yield any additional reward to the partner (own-reward, OR; lower panel). To control for non-social secondary reinforcement effects, actor rats are also tested in a non-social toy condition. In this control condition, the partner rat is replaced by a toy animal of similar shape and size, while keeping all other task parameters are identical to the social condition, including the reward contingencies. Animals are allowed to sample both BR- and OR-outcomes for a certain number of forced trials (only one option available to the actor) followed by free choice trials (the actor can choose freely between OR- and BR-options) where their social preferences can be observed. Results show that rats prefer mutual rewards more in the partner condition than in the toy control condition (Hernandez-Lallement et al., 2015a). We interpreted this behavior as evidence for pro-social preference in rats because the actors' inclination for providing food access to the partners was driven by social factors beyond their own-payoff.

The social reinforcement learning hypothesis provides a useful and parsimonious framework that equips us with conceptual tools to describe and predict the rats' behavior in the PCT task. As pointed out, an actor's pro-social choice could be driven by (i) the consequence of positive social reinforcement (Figure 1A, "*Positive social feedback*"), e.g. rewarding communication signals emitted by the partner (Seffer et al., 2014) or pleasure derived from eating rewards in spatial proximity (Barnett and Spencer, 1951). Additionally, behavior could also be reinforced by (ii) negative social reinforcement (Figure 1A, "*Negative social feedback*"), e.g. potential distress signals produced by partners (Kim et al., 2010; Atsak et al., 2011) missing out on reward in OR choices. As previously noted (Hernandez-Lallement et al., 2015a), positive and negative social reinforcement are not mutually exclusive, but could act in concert to reinforce pro-social choices. If the social reinforcement hypothesis accounts for the choice allocation observed in the PCT, one should be able to find signatures of social learning in the choice dynamics of actor rats. To search for signs of social learning we exploited the reversal nature of the PCT task (see Hernandez-Lallement et al., 2015a for details). Briefly, to control for side biases and habit formation, the compartments associated with BR- and OR-outcomes were pseudo-randomized across testing sessions and rats. Thus, on nearly every session, the OR/BR-compartment assignments were reversed with respect to the previous session, and animals had to re-learn the compartment-outcome contingencies anew. It is important to note again that the outcome for the actor was identical for both choices; OR- and BR-choices differed only in the outcome to the partner rat. Hence, flexible adaptation to the frequent contingency reversals could only be driven by the social reinforcing component of BR-outcomes, not by absolute differences in outcomes. Using a large data set of rats tested on the PCT (N = 114 rats; data taken from different, partly

unpublished experiments), we divided the first eight sessions of testing (the number of training sessions differed across rats and experiments, but each animal in the data set was trained for at least 8 sessions per condition) in three blocks of five trials (each session consisted of 15 trials, which we subdivided into three blocks of five trials for analysis) and computed mean social bias scores across animals. Social bias scores quantify the normalized difference in mutual-reward choices between partner- and toy-conditions, i.e., how much more (or less) an actor chooses the BR-option in the partner- compared to the overall BR preference levels. Social bias scores can be construed as the added social value of a conspecific's access to food (see Hernandez-Lallement et al., 2015 for similar computation). The social bias score for rat *i* was computed with the following equation:

$$SB_i = \left[\frac{\mathrm{BR(partner)}_i - \mathrm{BR(toy)}_i}{\mathrm{BR(partner)}_i + \mathrm{BR(toy)}_i}\right] * 100 \qquad (1)$$

Note that in previous studies (Hernandez-Lallement et al., 2015a, 2015b), we used the BR preference in toy condition only in the denominator term of the social bias score equation which captured more directly the percent change from toy baseline levels. However, using only the percent change in the toy condition as normalization could potentially yield skewed distributions[10]. The formula used here, which produces strictly normalized values located between -100% and 100%, yields qualitatively similar results while retaining a normal distribution of social bias scores at the population level[11]. Accordingly, a positive social bias score for rat *i* (SB_i), i.e., higher BR

[10] This was not the case in previous studies from Hernandez-Lallement and colleagues, 2015a, 2015b.

[11] See General Discussion for a more elaborated approach on these two computations

preference in the partner than in the toy condition, reflects the added positive social value for a conspecific's access to food, whereas a negative social bias score can be interpreted as negative social value. Results are depicted in Figure 1B. A repeated measures ANOVA revealed a significant main effect of blocks on social bias scores ($F_{(2,226)}$ = 10.42, p < .001, η_p^2 = .08), indicating that social preferences (re-)emerged across trials within sessions. Post hoc pairwise comparisons showed a significant increase in social bias scores between blocks 1 and 3 ($t_{(113)}$ = 4.45, p < .001; CI_{99} = [-10.55, -2.73] ; Cohen's d = .54 ; Bonferroni corrected; α = .02), whereas no significant difference was found between blocks 2 and 3 ($t_{(113)}$ = 2.26, p = .03; CI_{99} = [-7.14, .54] ; d = .28) as well as between blocks 1 and 2 ($t_{(113)}$ = 2.38, p = .02; CI_{99} = [-7.02 ,.34] ; d = .28). Importantly, social bias scores quantify the normalized difference in BR preference between partner and toy condition (see eq. 1). Therefore, to break down the processes underlying the increase of social bias scores previously reported, we computed the average fraction of BR choices for the partner (blue) and toy (red) conditions, i.e., the percentage of BR choices out of all choices (Figure 1C). We found that rats were nearly indifferent between OR- and BR-alternatives at the beginning of a partner session, but their preferences for BR- over OR-options in the partner condition became increasingly pronounced as the session progressed. Surprisingly, this pattern was completely reversed in the toy condition, where animals decreased their preferences for BR over OR choices across trials within sessions. A repeated measures ANOVA (with condition and block as within subject factors) revealed a significant effect of condition on %BR-choices ($F_{(1,113)}$ = 13.23, p < .001, η_p^2 = .11) and a significant condition*block interaction ($F_{(2,226)}$ = 10.62, p < .001, η_p^2 = .09).

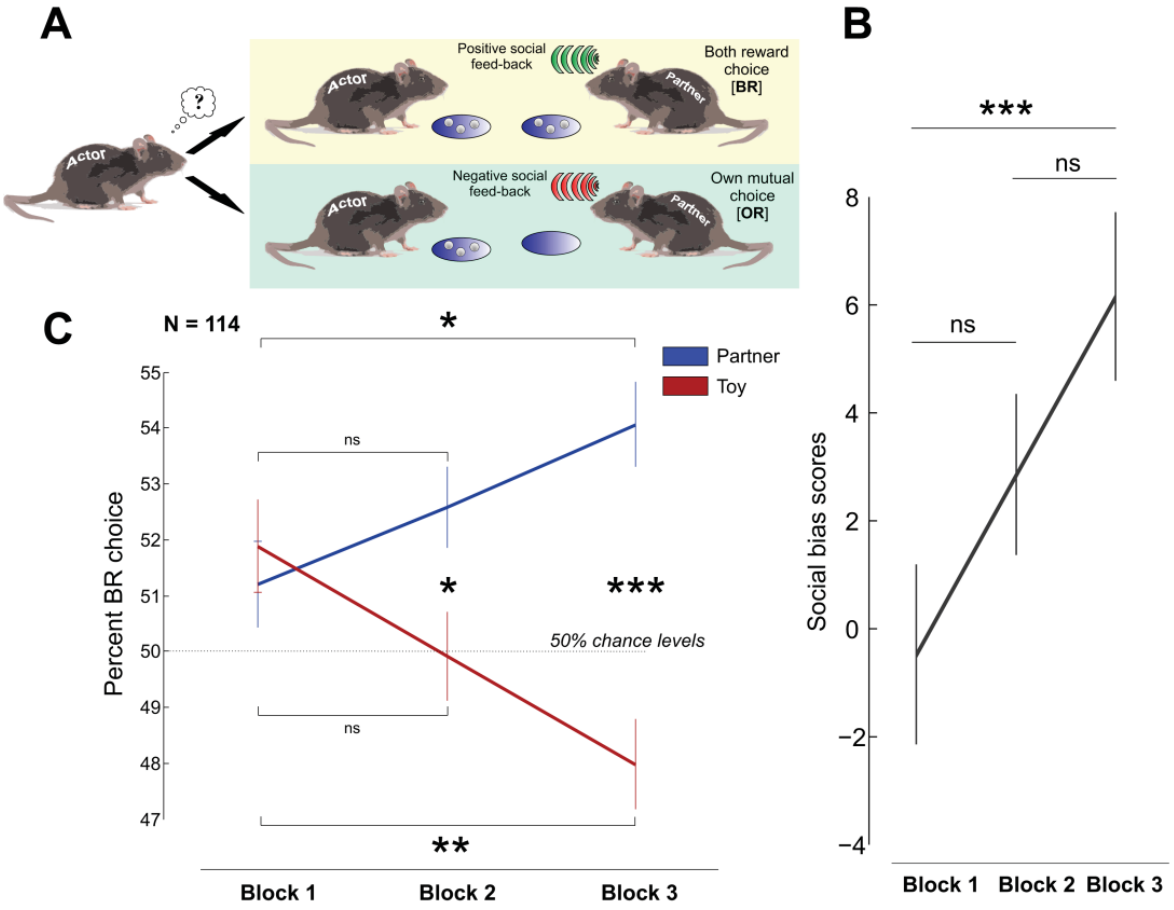

Figure 1 | Social reinforcement learning framework. A. Putative reinforcement mechanisms in a Pro-social Choice Task for rodents. An actor rat decides between rewarding (upper panel, yellow background) or not rewarding (lower panel, mint background) a partner rat at no cost to himself, while being identically rewarded for both choices as well. The reinforcement learning hypothesis implies that both outcomes can lead to positive and negative social feedback from the partner in cJulen Hernandez-Lallementase it gets access to food (upper panel), or not (lower panel), respectively. **B. Social bias scores increased within sessions.** Social bias scores computed across 114 rats, eight sessions and blocks of five trials. The distributions increased over blocks, and became significantly different from the precedent block from block 2 onwards. **C. %BR preference increased and decreased in the partner and toy conditions, respectively.** Preference for the BR alternative increased steadily across trials within sessions in the partner condition, and decreased in the toy condition. Error bars are s.e.m. * $p < .05$;** $p < .01$; *** $p < .001$, ns not significant; Bonferroni corrected

Further post-hoc pairwise comparisons revealed a significant difference in %BR choices between partner and toy condition for block 2 as well as block 3 (paired-samples *t*-test; Block 2: $t_{(113)} = 2.54$, $p < .05$, $CI_{99} = [-.09, 5.62]$; $d = .34$; Block 3: $t_{(113)} = 5.53$, $p < .001$, $CI_{99} = [3.23, 9.05]$; $d = .74$, Bonferroni corrected), but not in block 1 ($t_{(113)} = -.51$, $p = .58$, $CI_{99} = [-3.78 : 2.55]$; $d = -.07$). Moreover, %BR choices were significantly different between block 1 and 3 in both partner ($t_{(113)} = 3.00$, $p < .05$, $CI_{99} = [-.37 , -5.40]$; $d = .35$) and toy conditions ($t_{(113)} = 3.61$, $p < .01$, $CI_{99} = [6.68, 1.06]$; $d = .44$). No additional significant differences were found between blocks.

These findings have two important implications. First, they show that preference for the BR-option increased across trials in the partner sessions, a process which might reflect the updating of the social value of the choice outcomes. Second, we observed a within-session decrease of BR preference in the toy condition which suggests that animals developed an aversion against additional rewards delivered to the opposite compartment in a non-social context, possibly reflecting frustration effects related to rats' inability to access uneaten rewards in the opposite compartment. This bifurcating pattern implicates that 'baseline' preference levels in the PCT are dynamic; the actual preference for social outcomes should, therefore, not be compared to indifference levels (50%), but rather to the BR-choice levels observed in the non-social context control condition. This is precisely why social bias scores, i.e., the percent change of BR choice between partner and toy condition, in our opinion is a better estimate of mutual-reward preferences than comparison of BR-choices against chance. Overall, these data are consistent with the idea that the emergence of rats' social preferences reflects social learning.

Individual differences in social learning

An identical social context might affect individual animals in different ways. For instance, rat social behavior seems to be differentially influenced by group hierarchy (Baenninger, 1966) or social experience (Ben-Ami Bartal et al., 2014). Such inter-individual differences in social behavior should be prominent in PCT performance, too. To characterize individual differences in social preferences, we compared individual social bias scores to a bootstrapped reference distribution obtained through random permutation.

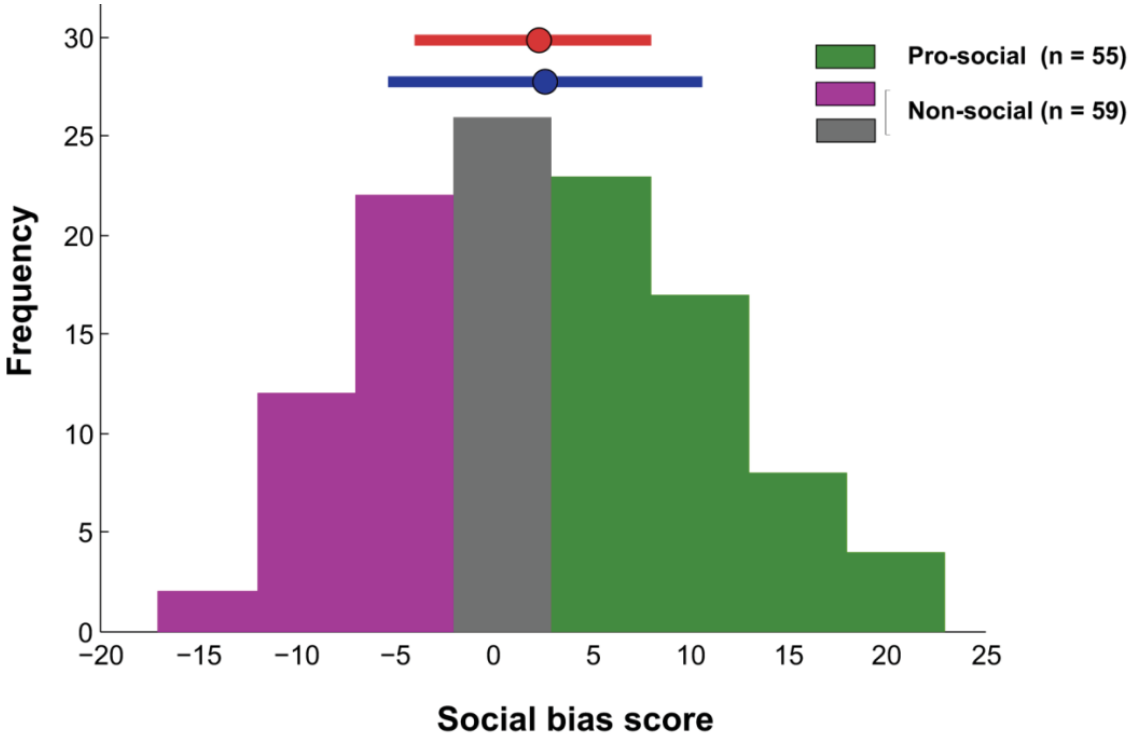

Figure 2 | Individual differences in social learning. Social bias scores exceeding the upper limit of confidence interval (upper limit: 5.47) were categorized as "Pro-social" (green; n = 55; 48% of all rats) and remaining animals were categorized as "Non-social" (violet/grey; n = 59, 52% of all rats). The grey bar represents animals from the non-social group located within the 95% confidence interval. Blue dot and line are the mean and standard deviation of the social bias score distribution, respectively. Red dot and line are the distribution's median and the 25% and 75% percentile values, respectively.

Briefly (see Hernandez-Lallement et al., 2015a for exact procedure), we generated a distribution of permuted social bias scores, computed by drawing scores (with replacement, N = 5000 times) from sessions in both partner and toy conditions while shuffling the session labels. We then compared actual social bias scores to the 95% confidence interval on this simulated distribution of social bias scores (Figure 2; confidence interval limits: *[-2.66 ; 2.66]*). Animals with social bias scores exceeding the upper limit of the confidence interval were categorized as "*Pro-social*" (n = 55; 48% of all animals) whereas all remaining animals were categorized as "*Non-Social*" (n = 59; 52%). Strikingly, in comparison to baseline levels (toy condition), pro-social animals had between 2 to nearly 21 more BR choices in the partner compared to the toy condition, illustrating that social preference levels varied substantially, even within the category of rats classified as pro-social. Additionally, animals classified as non-social included those that showed rather indifferent choice allocations across conditions (SB within the bootstrapped confidence interval; Figure 2, grey bar) and others that even showed "*anti-social*[12]*"* behavior, i.e., negative social bias scores reflecting lower BR preferences for a conspecific than for inanimate toys. Note that negative social bias scores reached only modest levels compared to the positive social bias scores of the pro-social group.

In order to further investigate whether non-social animals truly showed overall indifference and/or aversion towards mutual rewards across trials, we computed social bias scores in each block of trials for both pro-social and non-social groups. We hypothesized that, contrary to pro-social animals,

[12] The term "anti-social" needs to be interpreted with caution, because rats' choices may have been motivated by non-social factors that were unrelated to malicious, egocentric or other "anti-social" motives. We use the term "anti-social" agnostically to describe the negative effect of social context on social preferences.

rats in the non-social group would not show significant change in social bias scores across blocks (Figure 3A).

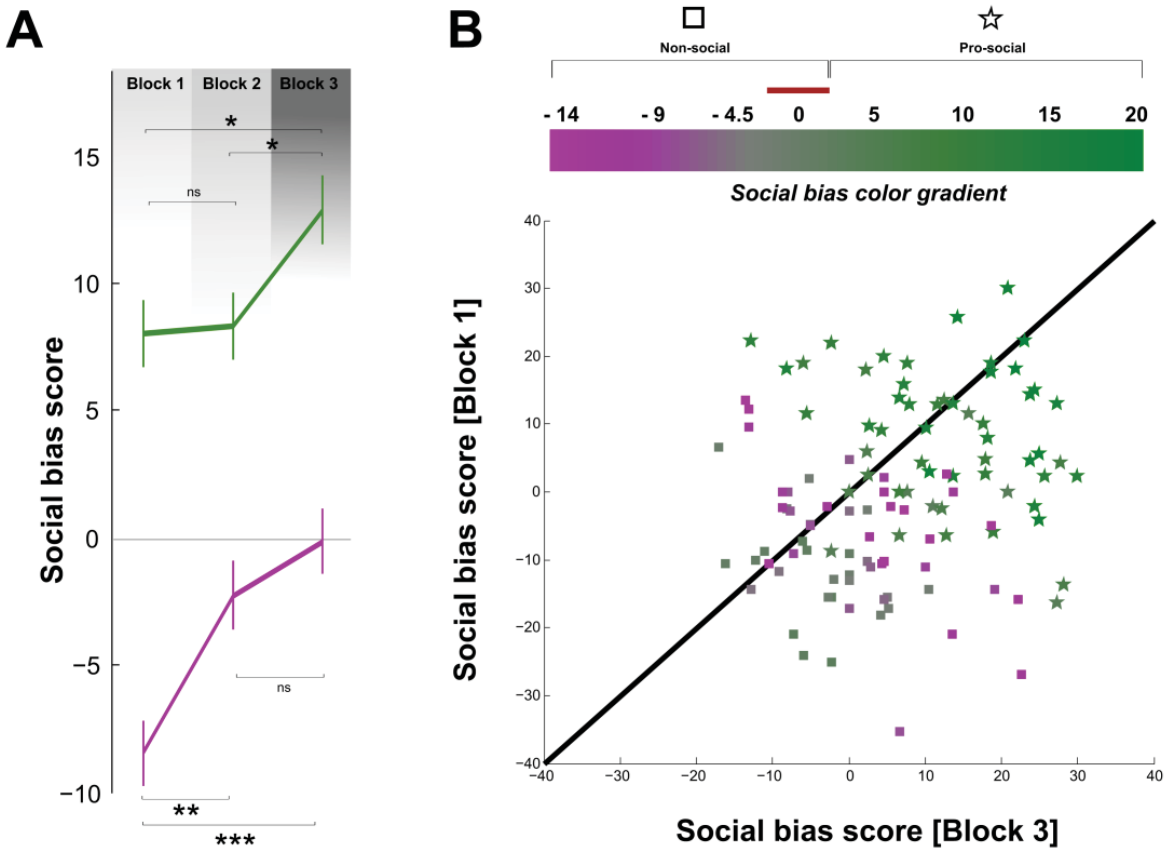

Figure 3 | Social bias scores across blocks per group. A. Social bias scores across blocks for pro-social (green) and non-social group (grey). Both groups showed significant increase in social bias score across blocks. **B. Increasing social bias scores from block 1 to block 3.** Scatter plot of individual social bias score levels in block 1 (y-axis) and block 3 (x-axis) for pro-social (star) and non-social animals (squares). Data points under the diagonal represent animals that had an increase in social bias score from block 1 to block 3. Color gradient inform on overall social bias score values (see panel A). The red horizontal line represents the 95% confidence interval.

A repeated measure ANOVA (blocks and group as within and between subject factors, respectively) revealed a significant main effect of block on social bias scores ($F_{(2,224)}$ = 10.25, p < .001, η_p^2 = .08), as well as a significant block*group interaction ($F_{(2,224)}$ = 4.07, p < .05, η_p^2 = .04). Moreover, there was a significant difference in social bias scores between block 1 and 3 ($t_{(54)}$ = 2.21, p < .05, CI_{99} =

[-10.72, 1.01] ; d = .47) as well as 2 and 3 in the pro-social group ($t_{(54)}$ = 2.07, p < .05, CI_{99} = [-10.44, 1.32] ; d = -.44), but not between blocks 1 and 2 ($t_{(54)}$ = .15, p = 1.00, CI_{99} = [-5.65, 5.06] ; d = .03), confirming that pro-social animals showed social learning. However, and crucially, we also found a significant difference in social bias scores between block 1 and 2 ($t_{(58)}$ = 3.17, p < .01, CI_{99} = [-11.27, -1.09] ; d = -.63) as well as between block 1 and 3 ($t_{(58)}$ = 4.00, p < .001, CI_{99} = [-12.70, -2.91] ; d = -.87) in the non-social group, although no difference was found between blocks 2 and 3 ($t_{(58)}$ = -.95, p = .74, CI_{99} = [-7.33, 3.09]; d = -.22). These results suggest that animals initially classified as non-social also showed social learning. While 64% of prosocial animals (n = 35) increased their social bias scores form block 1 to 3 (Figure 3B; stars under the diagonal), 70% of non-social animals (n = 41) showed a similar increase (squares under the diagonal), adding further support to the notion that non-social animals showed social learning, too. Therefore, although overall mean social bias scores differed between groups, the social learning rate might have been comparable across animals in both groups. To address this possibility, we computed the absolute difference in social bias scores between blocks 1 and 3 for every animal in each group. Direct comparison showed that rats in both groups showed comparable increases in social bias scores from block 1 to block 3 (Figure 4A; $t_{(112)}$ = -1.16, p = .25; CI_{95} = [-9.35, 2.47], d = -.21). Overall, this analysis suggests that animals classified as pro-social or non-social differed predominantly in their baseline social preference levels rather than in social learning capabilities, which were robust across the whole population. While the increase in social bias scores across blocks was comparable between groups, it is conceivable that pro-social and non-social animals differed in their social learning rate *within* the partner- and the toy-conditions.

To address this possibility, we regressed, for each condition separately, the rats' individual %BR-choices against block, and extracted the individual regression coefficients as estimates of the steepness of the slopes across blocks as a proxy of the rats' learning rates (linear fit of the %BR in each block, per animal; steeper slopes indicate higher learning rates).

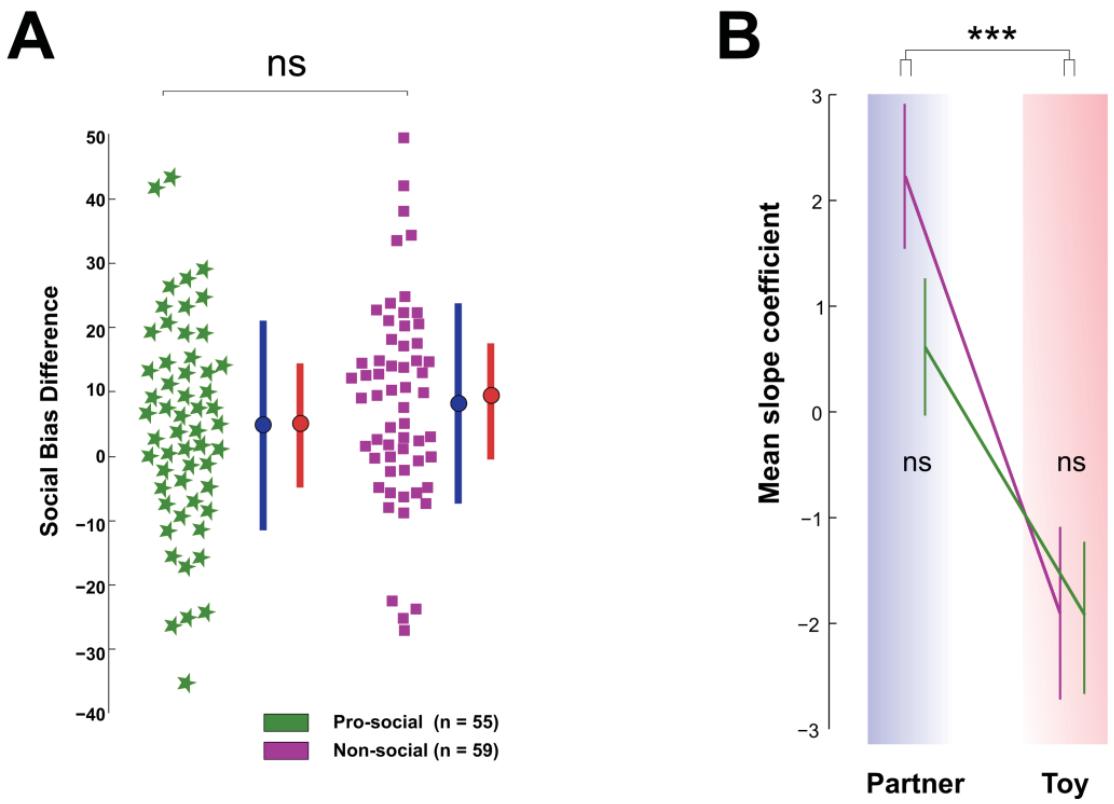

Figure 4 | Social learning within conditions. A. Social bias score difference. There was no difference in social bias score difference (Block 3 – Block 1) between groups. Blue dot and line are the distribution's mean and standard deviation, respectively. **B. Regression coefficient for %BR across blocks per group in the partner (blue background) and toy conditions (red background).** While both groups showed higher regression coefficients in the partner than in the toy condition (main effect of condition), there was no difference between groups in either condition. Magenta dot and line are the distribution's median and the 25% and 75% percentile values, respectively. Error bars are s.e.m. *** $p < .001$, ns not significant.

A mixed ANOVA revealed a significant effect of condition (Figure 4B; $F_{(1,112)}$ = 20.01, p < .001, η_p^2 = .15) but no condition*group interaction ($F_{(1,112)}$ = 1.23, p = .27, η_p^2 = .01). While both groups showed significantly higher slope values in the partner than in the toy condition (Prosocial: $t_{(112)}$ = 2.28, p < .05; CI_{99} = [-.43, 5.47], d = .46; NonSocial: $t_{(112)}$ = 4.12, p < .01; CI_{99} = [1.47, 6.88], d = .77), slope coefficients did not differ between groups in either condition (Partner: $t_{(112)}$ = 1.69, p = .10; CI_{99} = [-.89, 4.11], d = -.31; Toy: $t_{(112)}$ = -.05, p = .96; CI_{99} = [-2.88, 2.77], d = .01). Thus, this analysis also confirms that both pro-social and non-social animals showed comparable social learning in each condition. Altogether, these results show that pro-social and non-social rats show comparable social reinforcement learning capabilities, and that individual differences in initial social preference levels between animals can account for differences in pro-social preferences observed at the group level. Thus, considering learning rates next to preference levels is advisable when investigating social choice behavior in rodents. Regarding the PCT, one challenge for future research is to determine whether animals initially classified as non-social, i.e., rats that had lower social bias scores to begin with, would reach similar levels of mutual reward preferences as prosocial rats if they were trained more extensively. This possibility remains to be investigated.

Potential mediators of social reinforcement

Although consistent with the social reinforcement hypothesis, the results presented above do not inform on what kind of social reinforcement, negative and/or positive, underlies the within-session increase of BR-preference. Several social stimuli could drive the rats' choice allocation in the PCT. Prime candidates are auditory stimuli, mainly USVs, that are known to carry affective state information (Knutson et al., 1999; Litvin et al., 2007) not

only in rats (Wöhr and Schwarting, 2008; Seffer et al., 2014) but also in other species (Sharp et al., 2005; Gadziola et al., 2012a). Notably, substantial evidence obtained in big brown bats suggest that amygdala neurons discriminate between different social USVs (Naumann and Kanwal, 2011; Gadziola et al., 2012b; Peterson and Wenstrup, 2012; Grimsley et al., 2013). Similar results were obtained in rats showing that USVs reflecting negative (22 kHz) and positive (50 kHz) affective state can modulate approach behavior (Wöhr et al., 2008) and are coupled to tonic increase and decrease of amygdalar neuron firing rates, respectively (Parsana et al., 2012). Finally, the fact that 50kHz USVs elicit phasic dopamine release in the nucleus accumbens (Willuhn et al., 2014), as mentioned above, is consistent with the idea that USVs have social significance and qualify as social reinforcers. Other stimuli, such as odors (Wang et al., 2006; Wesson, 2013) might also carry reinforcing properties for rats. However, the idea that olfaction would drive pro-social choice allocation in the PCT would require highly dynamic chemical processes, which we believe unlikely given the trial-based design. Assessing the influence of several putative social signals in transmitting partner feedback and their effect on social decision making remains an unresolved issue for now.

In conclusion, we believe that the emergence of rodent models of social decision making provides exciting opportunities to study social choice using the full range of the neurobiological toolbox. Novel behavioral paradigms such as the PCT pave the way toward a mechanistic model of social decision making which would greatly contribute to a better understanding of the neural circuits that could be involved in non-human and human decision making in social contexts.

General Discussion

Pro-social Choice:

Advances, limitations and new horizons

Giving back what is due

"I think I smell a rat
Walking down the street
Carrying a baseball bat"

The White Stripes

Before starting with a review of the methods and main findings followed by a discussion of implications of our results, please let me introduce a little note on rats and their reputation. I hope the findings presented in the last chapters convinced naïve readers but also trained scientists that rats deserve some more respect and attention regarding their potential for social (neuro-)science. Maybe it is even time to adapt some of our language wording…

"*Être un rat*", literally "To be a rat" is a french expression for "being greedy", which in the english language, means to spy on and inform others about someone else's behavior. "*Una rata en un palacio sigue siendo una rata*" is spanish for "A rat in a palace remains a rat". I suppose many other languages

use such despising references to this particular rodent. Thus, don't be surprised if you smell a rat, walking down the street, carrying a baseball bat, if humans keep on talking in such condescending way about them. I often read that human beings despise rats because of their particular association with filth, but more particularly because of their bond to the pest. Although Camus only talked about rats in "*La peste*", fleas are responsible before them for the transmission of this disease (a flea in a palace remains a flea, until someone proves that fleas behave pro-socially, I guess). Let us hope that the recent surge of interest in rodent social decision making will improve mankind-rodent relationships in the future…

One a more serious note, despite my excitement over the rodent PCT, there are a few limitations to the work I presented in this thesis that I would like to consider in this discussion. I first shortly review the main findings of my PhD work and in a second time, I closely examine the Pro-social Choice Task and review its advantages and limitations. Finally, I emphasize a few aspects of this thesis where caution should be used, and finalize this thesis by discussing the social reinforcement learning framework at both behavioral and neural levels.

Review of the main findings

During my PhD thesis, my co-workers and I used behavioral and cognitive neuroscience to investigate the neural bases of pro-social choice in rats. In a first time, we established and validated a behavioral paradigm inspired from non-human primate studies adapted for rodents, the Pro-social

Choice Task (PCT; **Chapter I**). This paradigm, cornerstone of my thesis, allowed me to extract baseline pro-social preferences in successive batches of rats by comparing the decisions to provide additional food for a partner with the ones performed in a non-social context. Upon validation of the PCT, we performed excitotoxic lesions of two structures known to be recruited during decisions in social contexts: the basolateral amygdala (BLA) and the lateral orbitofrontal cortex (LO). In **Chapter II,** we showed that in contrast to sham-operated animals, BLA-lesioned rats did not show any pro-social preference. Results reported in **Chapter III** suggested that the LO plays a role in the specificity of pro-social preferences. While sham animals showed comparable pre- and post-lesion preference levels in each condition, LO-lesioned animals showed a generalization of pre-lesion preferences to the non-social context after lesion. However, these results could not be replicated in an additional batch. Finally, in **Chapter IV**, using a large data set of animals tested in the PCT, we demonstrated social learning and discussed its important aspect in pro-social choice allocation.

The Pro-social Choice Task

"*Sometimes I think that the act of serving food is one of the basic roots of relationships*"

Dalai Lama

An important contribution of the work presented here is the establishment and validation of the PCT for rodents. In the typical PCT, an "actor" individual performs dichotomous choices where either alternative provides him with an identical reward. In one option (named "Both Reward -

BR" in this thesis, also named "pro-social choice", see Sterck, Olesen, & Massen, 2015), a conspecific receives an additional reward, i.e. 1/1 (generally identical to the actor's reward, but see below) whereas the other alternative leads to the partner missing out on reward, i.e. 1/0 ("Own Reward - OR" or selfish choice; Silk et al., 2005).

The PCT was initially developed by primatologists who wanted to investigate whether non-human primates would provide food to conspecifics (Caldwell, 2008). In 2005, Silk and colleagues reported that chimpanzees (*Pan troglodytes*) were indifferent to conspecifics' payoff using a PCT design. Since then, most studies that tested chimpanzees on the PCT (or closely related variants) have shown similar results (Jensen et al., 2006; Vonk et al., 2008; Brosnan et al., 2009; Yamamoto and Tanaka, 2010), although one study showed spontaneous pro-social choice (Horner et al., 2011). Thus, primatologists are slowly agreeing that chimpanzees do not respond positively in PCT-like designs, although they show a wide range of additional helpful behavior such as food sharing, cooperation and assistance in item reaching under different conditions (Silk and House, 2011). In contrast, several other primates do show pro-social preferences in PCT-like designs such as marmosets (*Callithrix jacchus*; Burkart et al., 2007), long-tailed macaques (*Macaca fascicularis*; Sterck et al., 2015) as well as capuchin (*Cebus apella*; Lakshminarayanan & Santos, 2008) and tamarin monkeys (*Saguinus oedipus*; Cronin et al., 2010), even though not consistently replicated (Cronin et al., 2009). The work carried out in non-human primates shows that repetitive between- and within- species comparisons of pro-social preferences in closely

related (if not identical) designs is a formidable tool to understand social cognition from an evolutionary perspective[13].

Very few studies have observed pro-social behavior in such designs beyond the primate order. One study performed on corvids (*Corvus monedula;* Schwab et al., 2012) showed that pro-social preferences were contingent upon the partner's behavior (approach behavior and attempt to reach food reward) in a adapted PCT. To our knowledge, our work is the first report of pro-social choice preference in a PCT setting in rodents (*Rattus norvegicus*). Recently, our results have been complemented by findings showing that pro-social choice in rats might be contingent on food-seeking behavior from the partner rat (Márquez et al., 2015). This result is compatible with our findings: it is highly possible that reward-seeking stimuli initiated by the partner would have influenced the actor towards the BR compartment. Although a direct equivalent of our design did not yield significant preference in this study, several variables such as session numbers or deprivation levels could account for this discrepancy.

The PCT presents several advantages discussed in Chapter I and elsewhere in the literature:

1. Non-costly decisions enable to de-confound pro-social motives from the rats' egoistic tendencies to maximize own payoff.
2. Hidden rewards could modulate choice processes towards less impulsive decisions, as reported elsewhere (Stephens et al., 2002).

[13] A comparable work is currently being performed in dogs and related species. See for instance Hare and Woods, 2013

3. Partner rats never had a choice and could neither retaliate, nor return the favor; thus, the actors' preferences were not likely the result of strategic or reciprocal considerations (note that this cannot be ruled out).
4. Finally, the toy condition allowed us to demonstrate that pro-social choice was directly contingent on the social component of the task, i.e. the presence of a real partner.

Furthermore, variants of the PCT related to the previous points can be implemented with extraordinary facility. For instance, it is possible to modify the PCT payoff matrix (1) in order to induce inequality (higher reward for the partner than for the actor) or to test for altruistic behavior (granting food-access to a conspecific without receiving any). It would also be easy to manipulate the visibility of reward pellets by using transparent/opaque walls (2). Furthermore, switching the roles (3) in an actor/partner pair could inform on possible reciprocal motivations, as already observed in rats (Rutte and Taborsky, 2007a, 2007b). These manipulations have been already implemented in studies using non-human primates (for modifications of (1) see Brosnan and de Waal, 2003; for modifications of (2) see Horner et al., 2011; for modifications of (3) see Brosnan et al., 2009; Yamamoto and Tanaka, 2010). Note that our design received constant improvement throughout the experiments presented in these chapters. An overview of these improvements (and thus methodological differences between chapters) can be found in the Appendix B.

A few words of caution

On the interpretation of behavior

Notwithstanding the exciting advantages of the PCT, there are limitations in the interpretative power of the results presented in this thesis. Here, I cannot help but share a striking comment from a conference participant on a poster I presented about rats' pro-social preferences: "*How could…rats! … be pro-social??*". After some discussion with this person, I realized that my counterpart assumed that pro-social behavior implied other-regarding preferences as in "*I care about others, thus, I behave pro-socially*". Very often, the words "empathy" or "altruism" were recurrent in the people facing my posters and talks. Such semantic confusion can significantly impair and limit the understanding of a research topic as well as the communication between fields and scientists (West et al., 2007b), and it is important to stress once more that my co-workers and I do not claim that rats showing high pro-social preferences are generous towards others (having the well-being of others as behavior's motivation), that they share other's feelings (empathy) or that they are pure altruistic. Importantly, note as well as neither do our results invalidate these explanations. Our results merely show that rats preferred alternatives that provided food for others only when others were present. We qualified these preferences as pro-social because "*the term pro-social behavior is often used for all behaviors that benefit others*" (Hinde and Groebel, 1991, p.5; but see also Miller et al., 1991; Sterck et al., 2015; Wilson, 2015). Additional and exciting research will have to be (and is already being) carried out in order to elucidate the motivations of pro-social preferences as elicited in the rodent PCT.

Coming back to my conference participant's comment, I think it also emphasizes that caution needs to be taken in the interpretation we make of these results. Indeed, it is sometimes tempting to interpret patterns of behavior as resulting from complex cognitive processes, although alternative explanations might account for the observed decision patterns. For example, using an adapted PDG (see Introduction, p. 20) for rodents, Viana, Gordo, Sucena and Moita (2010) showed that rats reached stable levels of cooperation when a particular strategy was imposed to the partner (Tit for Tat - TFT - , which forces the partner to copy the previous decision of the actor; Axelrod & Hamilton, 1981). This exciting result led the authors to conclude that rats were able to understand the strategy of the opponent. However, in a classic PDG payoff matrix as used in this study, cooperative moves from the actors triggered identical subsequent choices for the partner (as imposed by the TFT strategy). Thus, on the long run, the cooperative compartment led to larger rewards than the defecting compartment. *Ergo*, non-social learning, independent from understanding the opponent's strategy, is a more parsimonious explanation for the choice allocation reported in this study (i.e. repetitive preference for the cooperative alternative).

These interpretational aspects are also relevant in research investigating altruistic behavior in animals. Defined as a costly behavior that confers a benefit to other individuals, altruism is by definition contingent on increasing another individual's benefit. Pioneering studies investigating altruism in rats suggested that aversive stimulation to one of the individuals could induce a pattern of behavior with face resemblance to altruism (Evans and Braud, 1969; Greene, 1969), that subsequently disappeared when no aversive stimulation was used (Taylor, 1975). In these studies, rats were given the possibility to block an electric shock delivered to a partner rat by entering a

particular compartment (Evans and Braud, 1969) or change previously established preferences from one lever to another lever, which required more physical effort to be operated, in order to stop the aversive stimulation to the partner (Greene, 1969). However, altruism per definition reduces the actor's benefit or current state (West et al., 2006); thus, it is possible that the actor felt discomfort because of being paired with a conspecific in pain, therefore reducing its current comfort. This hypothesis is a more parsimonious explanation for altruistic(-like) behaviors as reported in these studies.

Finally, similar observations can be found in the research for empathy in animals, i.e. the ability to share another individual's feelings, as recently suggested in both mice (Langford et al., 2006) and rats (Ben-Ami Bartal et al., 2011b). As noted by Atsak and colleagues (2011), "*In animals, it is* […] *often impossible to assess whether they are aware of the source of their emotions, and accordingly to disentangle models of emotional contagion from models of empathy*" (p. 1). Interestingly, more parsimony is found when similar results are being observed in phylogenetically more distant species (see Box 2: "*A tale of two studies*").

On the preference levels

Despite the promising possibilities of the PCT, it might already have come to the reader's mind that the preference levels reported in the previous chapters are rather small, never exceeding 55% at the group level. This comment, raised by numerous reviewers during the publication processes, deserves a comment in this section. Given the preference levels, how can we be confident that pro-social preferences are genuine?

I present four main arguments to defend the interpretation that rats show true pro-social behavior:

1. *Replication*: the effect was replicated across three batches of animals and a large number of experimenters. Note that batch 2 in Chapter III batch 2 did not show any pro-social preferences. As previously argued, the reduced sample size might account for this finding, although inter-individual differences could also lead to such results. Additional experimentation is required to investigate this aspect.

2. *No experimenter effect*: one possibility, given the effect size, is that experimenters unknowingly affected the animals' choices in the partner condition (Rosenthal and Fode, 1963). However, the experimenters were blind to the experimental / control group affiliation in Chapter II as well as in Chapter III. Thus, potential experimenter effects biasing rats towards BR preferences should have been observed in both lesioned and control groups, which was not the case. Moreover, we found no relation between experimenter's action and choice allocation (Chapter II).

3. *Large individual variability in pro-social preference levels*: As argued in Chapter II, group averages might not be as informative as individual preference levels. Thus, we computed social bias scores, a between condition percent change in BR preference, to extract individual levels of preferences. Typically, the distribution of social bias scores was spread over a large range of values, illustrating the large individual variability in preference levels. Thus, although group level analysis showed rather low levels of pro-social preferences, individual analyses revealed a more complex pattern.

Box 2

A Tale of Two Studies

In one study (Ben-Ami Bartal et al., 2011a), the authors trapped a rat in a particular retainer that could be opened by an actor. The actor had the choice between either eating an appetitive reward or freeing the entrapped partner. Results showed that actors preferred to free the entrapped partner than eating a reward. The authors implemented a series of control experiments where the restrainer was empty, contained an object or where the partner was not restrained but located across a perforated divider. The authors interpreted this exciting result as an evidence for empathy in rats (i.e. the ability to share another individual's feeling). Another study (Nowbahari et al., 2009) used a conceptually similar paradigm in ants by offering the possibility to help a trapped conspecific. Ants did not show any rescue behavior for individuals from other species, preys, control objects and even heterocolonial individuals, but strongly rescued homocolonial individuals.

The first study stated their results provided "*strong evidence for biological roots of empathically motivated helping behavior*" (p. 1), whereas no claim of psychological motives was made in the second study. The empathy-based interpretation in the first study implies that the actor's choices were driven by the conspecific's current distress. However, an additional necessary control would have been to place unstressed rats (for example anaesthetized) in the restraining chamber and observe whether the behavior was really contingent on the partner's distress or on the partner's mere presence. A more parsimonious explanation for these findings is that actors were driven by social exploration motives.

This tale of two studies illustrates that caution needs to be taken in the interpretation of behavior as well as in the tempting inference of high-cognitive abilities. Several authors have stressed the importance of parsimony in the interpretation of results because as pointed out by Vasconcelos and colleagues (2012), "*such proposals* [empathically motivated behavior] *deserve careful scrutiny because the field of comparative cognition is particularly vulnerable to unwarranted anthropomorphic interpretations*." (p.3).

4. *Social learning phenomenon:* In Chapter IV, we showed that rats increased and decreased their BR preferences over trials in the partner and toy condition, respectively. As a result, social bias scores significantly increased over blocks of trials. Moreover, we additionally showed that animals categorized as non-social, detected as such on the basis of their overall social bias scores, also showed an increase in social bias scores. Therefore, low preference levels at the group level might also be caused by animals starting at lower baseline levels.

On the social bias score computation

In Chapter IV, we modified our initial equation of social bias scores from a percent change (eq. 1) to a fully normalized computation (eq. 2).

$$SB_i = \left[\frac{\%\mathrm{BR[Partner]}i - \%\mathrm{BR[Toy]}i}{\%\mathrm{BR[Toy]}i}\right] * 100 \qquad (1)$$

$$SBi = \frac{\%\mathrm{BR[Partner]}i - \%\mathrm{BR[Toy]}i}{\%\mathrm{BR[Partner]}i + \%\mathrm{BR[Toy]}i} * 100 \qquad (2)$$

As previously argued, the fully normalized computation generates scores located between a [-100 ; +100] range, whereas the percent change computation is prone to producing a skewed distribution given that normalization is performed over one condition only ($\%BR_{[Toy]I}$ in the denominator). Here, I directly compare both computations using the data set presented in Chapter IV. Distributions can be seen in Figure 1.

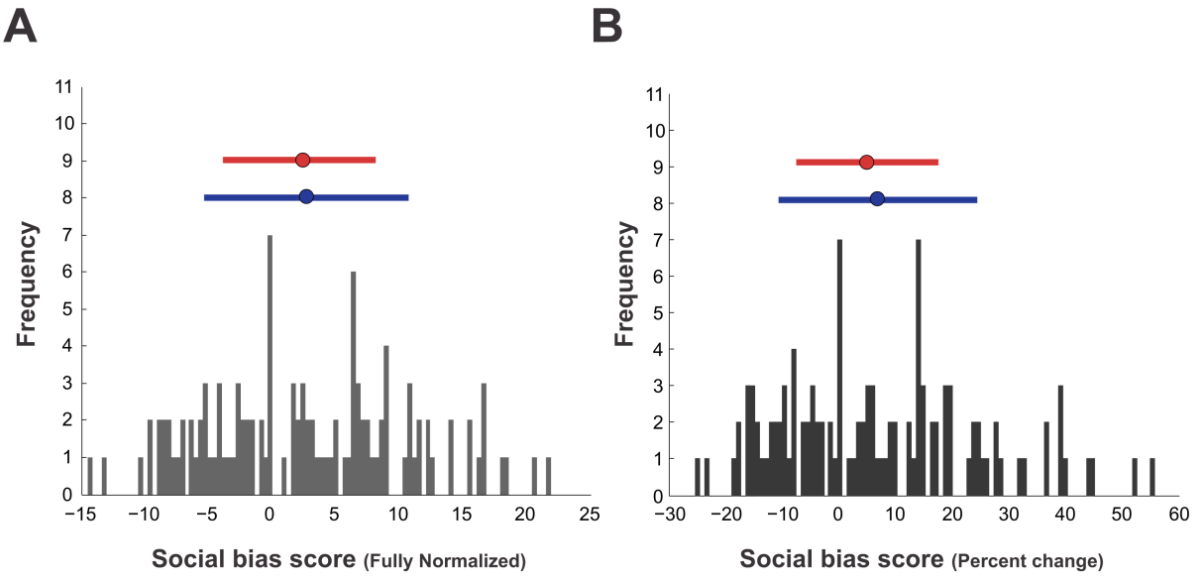

Figure 1 |Social bias score distributions. Distributions of the fully normalized (A) and percent change (B) social bias score computations. While the fully normalized distribution was normally distributed, it was not the case of the percent change one. Blue dot and line are the distribution's mean and standard deviation, respectively. Red dot and line are the distribution's median and the 25% and 75% percentile values, respectively.

While the fully normalized distribution was normally distributed (Figure 1A; Shapiro-Wilk test; $X_{(114)}$ = .99, p = .26), this was not the case for the percent change distribution (Figure 1B; $X_{(114)}$ = .97, p < .01). There were no outliers[14] in the fully normalized distribution and one outlier in the percent change distribution. However, the fully normalized (Fisher-Pearson coefficient of skewness; Sk_2 = .11; 90% expected range[15]: [-.352 ; +.352]) and percent change distributions were not skewed (Sk_2 = .35; [-.352 ; +.352]). Finally, both distributions were significantly higher than zero (*Percent Change*, one sample t-test; $t_{(113)}$ = 3.55, p < .001, CI_{95} = [1.17, 4.13] ; *FullNorm*, Wilcoxon sign rank

[14] Data points larger or smaller than [P_{75} + 1.5 * (P_{75} – P_{25})] and [P_{25} – 1.5 * (P_{75} - P_{25})], respectively; where q1 and q3 are the 25th and 75th percentiles

[15] 90% expected range for Pearson 2 skewness coefficient for n = 110. Extrapolated from Monte Carlo simulation estimates found in Doane and Seward, 2011.

test; Z = 3.16, $p < .01$). Note that both computations were tested in Chapter I, II and III and no differences were observed between the two computations. Indeed, both computations generated closely related scores when differences in BR choice levels between partner and toy condition are modest. However, while this analysis does not invalid previous results, we recommend using a fully normalized computation in future studies, as already performed in Chapter IV.

Extending the social reinforcement learning model

The social reinforcement learning model can be tested using several behavioral manipulations that could be tested in the PCT design. Here, I propose two possibilities:

1. Increasing the positive or negative affective states of a partner should reinforce pro-social preferences, contingent on whether positive or negative stimuli (if not both) carry reinforcement properties. One possibility based on the assumption that partner's positive affective states drive pro-social choice would be to propose preferred and non-preferred pellets to the partner, while the actor would receive identical pellets in both choices. Because the partner will show clear inclination for the preferred pellets, it is possible that different sensorial stimuli will bias the actor's choice allocation towards the "partner's preferred choice". A similar design could be tested using negative affective state in partners (e.g., foot shocks). Also, dynamically alternating the affective state of the partner (for instance: deprived, sated and anaesthetized) could reveal dynamic contingent pro-social choice allocation. Although design's details should still be discussed (for instance, it might be

important to control for inequity aversion), this design could potentially show instrumental behavior contingent on the preferences of the partner.

2. As discussed in Chapter IV, USVs are a likely candidate to carry information about partner's current affective state. Thus, quantifying the USVs elicited by partners receiving help from actors could provide great insight on the role of audition in pro-social choice allocation. Moreover, although playback studies often report lack of lasting behavior (Seffer et al., 2014), actor rats might prefer to enter a compartment paired with the delivery of positive USVs as compared to one associated with neutral or negative USVs. Note that comparable designs could test the role of additional sensorial stimuli, such as olfaction or vision.

A neural circuitry of pro-social choice?

In order to examine the neural bases of social decision making, Ruff and Fehr (2014) recently proposed a two folded framework (Figure 2) consisting of a (i) common currency and (ii) social-specific cognition schema. In the common currency schema, social and non-social information is processed by related areas in the brain; only the perceptual and cognitive information needed for social and non-social processing might differ. In the social-specific cognition schema, social and non-social processes are implemented by similar but anatomically distinct processes in the brain.

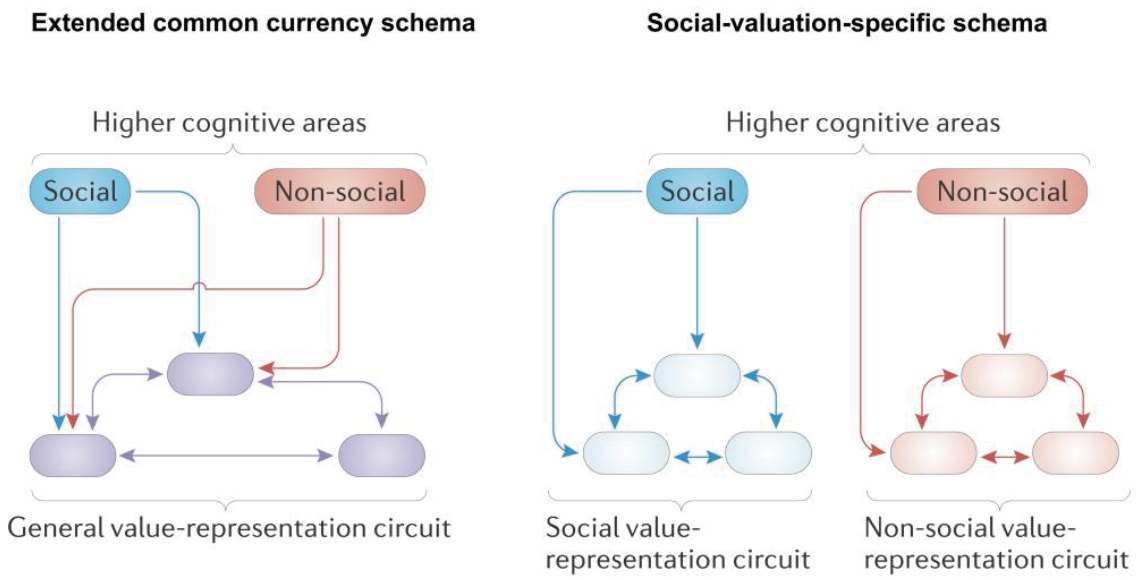

Figure 2 | Two frameworks for the computation of social and non-social context. A. The "extended common currency schema" proposes that a general value-representation circuit computes the salience and value of both social and non-social stimuli. Note that the perceptual and cognitive information of these stimuli might be computed by distinct brain areas (blue and red). **B.** The "social-valuation-specific schema" proposes that both social and non-social information are computed and represented in distinct neural networks; thus, this schema posits the existence of social- (blue) and non-social specific (red) neural networks. Note that the computation of both contexts can evolve following similar computational principles, but are anatomically different. Adapted from Ruff & Fehr, 2014.

The results presented in this thesis are consistent with the common currency schema. As discussed in the introduction, the BLA is involved in a large number of non-social decision making processes. In Chapter II, we showed that the BLA is important for the acquisition of pro-social preferences, providing additional evidence that this area is involved in the computation of social decision making. Given its innervations from visual, auditory and somatosensory tracts, as well as from olfactory and vomeronasal pathways in rodents, the BLA nucleus is often considered as the amygdalar sensory interface (Phelps and LeDoux, 2005; Brennan and Kendrick, 2006). It has been proposed that, in

general, the BLA may be a vigilance device critical for linking the incentive properties and outcome values of rewards and punishments to predictive sensory cues, and enhancing their affective salience (Schoenbaum et al., 1999, 2003). According to this view, the BLA would be critical for increasing an animal's sensitivity to the emotional value of social information, and thus, social learning. Furthermore, the rodent BLA is modulated by cross-structure influences from the OFC (including the LO) that would concur in the update of stimulus value (Schoenbaum et al., 2009a). According to this view, while the BLA might encode the emotional salience and relevance of (social) stimuli, the OFC could compute the information necessary for further behavioral adaptation (Schoenbaum et al., 1999).

Notably, lesion to the LO, a structure largely involved in learning processes and updating of stimulus value in non-social situations as well as social decision making (Blair, 2003), might have a significant effect on pro-social choice allocation in rats (Chapter III), although additional experiments should address this possibility. Nonetheless, the results presented in this thesis suggest that two structures involved in non-social decision making also participate in the guidance of social choices, as postulated by Ruff and Fehr's common currency scheme. Interestingly, the BLA and the LO (among other nuclei) have been recently proposed as a part of a social perception hub in humans necessary for managing social networks (Bickart et al., 2014a, 2014b). Additional experimentation should be carried out in order to validate this circuitry in rodent pro-social choice dynamics.

Conclusion

In this thesis, I have presented a novel research paradigm that allows extracting baseline pro-social preferences in rodents and reported exciting evidence of BLA and LO involvement in the acquisition / expression of such preferences. Rodents are increasingly used in research investigating the neural bases of social behavior. Their surprising social abilities combined with invasive tools to monitor neural activity and roles in social choice behavior make them an extremely promising model for further studies. Although the door has just begun to crack open, I believe that a close future will reveal that rodents in general have just begun to surprise us.

Düsseldorf,

The 27th of November, 2015

Glossary

functional Magnetic Resonance Imaging (*f*MRI): neuroimaging procedure that measures brain activity by detecting the hemodynamic response in brain tissues (Blood-Oxygen-Level Dependend or BOLD signal).

in-vivo: studies performed "in-vivo" report effects which are tested on the whole living organism (as opposed to partial organism, i.e. in-vitro). Such an approach is better suited to understand the overall effect of an experimental manipulation on a living individual.

Electrophysiology: electrophysiology is the science that addresses the electrical properties inherent to specific biological tissues and cells. First reported by Scribonius Largus (41 -54 b J.C.) and later by Volta and Galvani as well as Bois-Reymond, the observation that specific body parts were characterized by an electrical potential paved the path for the understanding of brain tissue and its functional organization.

Auto- / Allo-grooming: grooming (act of cleaning and brushing) of an animal by itself (auto-) and by others (allo-).

Optogenetics: scientific procedure mainly used in neuroscience that allows the activation and inhibition of specific neurons that have been previously genetically sensitized to light.

Cytoarchitecture: general organization of biological tissue. Generally used in the study of the central nervous system, the cytoarchitecture defines clearly distinct layers within a (brain) tissue.

Game Theory: combination of experimental tasks (or games) where several individuals interact under strict, known or unknown rules that dictate the final overall payoff of each player. These games provide a collection of empirical results from which the underlying neural activity can be investigated (see Fehr and Camerer, 2007).

Dopamine: neurotransmitter involved (among other functions) in reward-related behavior and learning.

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Appendix A

Reviewing the involvement of the brain structures thought to be involved in social decision making is the work of a lifetime. In this appendix, I propose peer-reviewed studies where readers can find information about potential roles for structures mentioned in the introduction, Figure 3, as well as related references for further literature search.

Structure	Article
Anterior Insula	recruited when experiencing unfairness (Sanfey et al., 2003). - Emotion of others (Gallese et al., 2004)
Dorsolateral Prefrontal Cortex	disruption of DLPFC functions decreased sensitivity to unfairness in humans (Knoch et al., 2006)
Medial Prefrontal Cortex	subjects with damaged MPFC showed an increased sensitivity to unfairness (Koenigs and Tranel, 2007)
Anterior Cingulate Cortex	encode rewards delivered to others in monkeys (Chang et al., 2013)
Posterior Cingulate Cortex	important for the processes of empathy (Bernhardt and Singer, 2012).
Superior Temporal Sulcus	important for the processes of empathy (Bernhardt and Singer, 2012).

Appendix B

Differences in PCT design between chapter I, II and III.

	Chapter I	Chapter II	Chapter III
Covers *	No (batch 1)/ Yes (batch 2)	Yes	Yes
Partner Pushing Device	Yes (Batch 1)	No	No
Computer interface**	No	No	Yes
Number of free trials	15	25	15
Number of forced trials	8	6	8
Block reversal	No (batch 1)/ Yes (batch 2)	No	Yes

* A 3D representation (screen shots & rotating video) of the social maze (i.e. double T-Maze) can be found in Media Supplementary Materials (USB key).

** The computer interface was a MatLab code programed by J.H-L. that allowed the experimenter to inform on the animal's behavior through a set of foot pedals. The script can be found in Media Supplementary Materials (USB key).

Appendix C

A large data set including different experiments performed during my PhD was used to investigate within session increase of preferences (*Chapter IV*). The table below gives more detailed information about each batch of animals.

	Animal #	Session #	Trial #	Provider
Batch 1	16	10	15	Jan Vier
Batch 2	48	10	15	Jan Vier
Batch 3	16	10	15	Charles River
Batch 4	10	12	25	Charles River
Batch 5	24	8	25	Charles River
Total	114			

Analysis: %BR choice was computed for three blocks of five trials each. Trials 16 to 25 for animals in batch 4 & 5 were excluded from the analysis.

Conference Proceedings and invited talks

Hernandez-Lallement J. On the behavioral and neural bases of pro-social choice in rats. Social Brain Lab, Netherland Institute of Neuroscience, December 2015.

Hernandez-Lallement J. The social rat. Comparative Psychology Lab colloquia, Düsseldorf, Germany. November 2015.

Hernandez-Lallement J, van Wingerden M, Schaeble S, Kalenscher T**.** Effect of lateral orbitofrontal cortex lesion on pro-social choice in rats. Society for Neuroeconomics, Miami. September 2014 (Poster)

Hernandez-Lallement J. Psychology of social behavior in rodents. PhD symposium, Düsseldorf, Germany. July 2013 (Invited speaker)

Hernandez-Lallement J. Temporal discounting and cooperative behavior. Onur Güntürkün's Lab. Ruhr-Universität Bochum, Bochum, Germany. May 2012 (Invited Speaker)

Publications

2015: **Hernandez-Lallement J**, van Wingerden M, Schaeble S, KalenscherT**.** A social reinforcement learning hypothesis of mutual reward preferences in rats.
Submitted to Current Topic in Behavioral Neurosciences.

2015: **Hernandez-Lallement J**, van Wingerden M, Schaeble S, Kalenscher T**.** Basolateral amygdala damage abolishes pro-social preference in rats.
Neurobiology of Learning and Memory.

2015: Oberliessen, L**, Hernandez-Lallement J**, van Wingerden M, Seinstra, M, Kalenscher T**.** Inequity aversion in rats
Submitted to Animal cognition.

2015: **Hernandez-Lallement J**, van Wingerden M, Marx C, Srejic M, Kalenscher. T. Rats prefer mutual rewards in a prosocial choice task*. Front Neurosci*

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Oh man… where to start? Well maybe where it all started, in the Center for Economics and Neuroscience in Bonn, Germany. I was working with Klauss Fliessbach on an fMRI project, and met Tobias Kalenscher at a conference (I learned some time after that he was dealing with a bad hangover that day). After a meeting for breakfast and another one for coffee at Düsseldorf University, I could start my PhD! But wait… there is no lab, or at least not yet? It took a few months before we could move into our experimental and office rooms, but that's when I got to know Tobias better who eventually became a friend of mine. I mean, who doesn't want to be friend with the PILF? (that is not in the glossary, you'll have to figure it out on your own). Really, thanks for letting me start this Lab with you![16]

During the time we waited for the lab to be built, I met a guy writing his PhD thesis in Tobi's office: Marijn, AKA The dude… Who would have told me I would go to the Fusion festival with that guy, that we would kinda share a flat for some time, and that we would become such great friends. Well we did ;) And I'm glad we did.

And well, that's how it all started! Oh boy, I still remember my office surrounded by skinner boxes and T-Mazes (which never worked, even after four years…), ordering computers for the lab and writing a grant (which I never got, sometimes you win, sometimes you learn…). Anyway, since the first day (although there were tough times…) I really felt grateful to work in the CompPsy lab with these awesome people. Not just T & M, but obviously Sandra, who taught me so much about surgeries and lab work. And tequila. Yeah, that's right. I'm not only gonna talk about how amazing colleagues you guys were! The world should also know about the amazing party people you guys still are! Which obviously brings me to my PhD colleagues, which I list in alphabetical order because none is "*awesomer*" than the other: Zsofia M, who lived at

[16] I decided to withhold party-related information concerning Tobias because, honestly, he is a very serious guy. More honestly, if you want more info, just pay me a beer ;)

my place for a while until the walls were so full of water she had to move out (home, sweet home…); Lina O (and as an extension the lab dog, Baloo!) who made it to the end of the Tough Mudder with the Tough Psychos!, and also drew the small cute rat on the book's side!; Maayke S, an amazing person with a great voice, with whom I played guitar and sang, between a Morris water maze and skinner boxes from the 60's, down in our basement; and Tina S, obviously, my ehemalige Büromate, specialized in cactüs annihilation. Here, about the new recruit in the PhD team: Adam! Actually, in earlier versions (you were not yet PhD), you had your own paragraph, but now you are pooled here dude. So copy/paste: a particular word for you man, who killed many of my working days by feeding me with alcohol. No great work or ideas I would have otherwise achieved during these dead days could have been worth the friendship we developed. Our talks and drunkenness are part of the best what happened in my time in Germany. But no, you won't get my hammock ;)

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There is one sentence I once read in a book from Isaac Asimov (thanks Linus): "*No one lives in splendid singleness*". That could not be more accurate for my PhD. To all the SHKs, interns, bachelor and master students: you made this happen, really. I would still be collecting data for chapter II if it wasn't for you. Again, in alphabetical order: Alex Braun, Verena Fischer, Cornelia Hoss, Maren Norpoth, Sonja Omlor, Linus Röpert, Lena Storzer, Juli Tkotz, Adrian Wölk and Maurice Zech. To all the ones that helped me on other tasks that are not reported in this thesis: Mareike Heller, Brian Daza, Thomas Carsten, Alexandra Shatliff and Zsofia Margittai. To all of you who hate me for my "extra work loads" and the ones that hate me even more for my "*Could you do me a little favor?*", thank you so much.

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Now I am really done. Oh boy, writing these last words feels so damn awesome!!

Thanks everyone, and keep rocking!