Neurobiology of Learning and Memory 127 (2016) 1–9

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Basolateral amygdala lesions abolish mutual reward preferences in rats

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

Comparative Psychology, Institute of Experimental Psychology, Heinrich-Heine University Düsseldorf, Universitätstrasse 1, 40225 Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 14 October 2015
Accepted 8 November 2015
Available online 17 November 2015

Keywords:
Basolateral amygdala
Rat
Pro-social
Lesion
Pro-social Choice Task

ABSTRACT

In a recent study, we demonstrated that rats prefer mutual rewards in a Prosocial Choice Task. Here, employing the same task, we show that the integrity of 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 Prosocial 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.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Humans have prosocial sentiments (Silk & 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, Decety, Mason, & Bartal, 2011; Decety, 2011). In support of this idea, recent research on social decision-making in rodents (Hernandez-Lallement, van Wingerden, Marx, Srejic, & Kalenscher, 2015; Márquez, Rennie, Costa, & Moita, 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, Lichtenberg, Chen, Cheer, & Roesch, 2014; Willuhn et al., 2014). Human neuroimaging studies show that decisions that benefit others typically recruit limbic and prefrontal brain areas (Behrens, Hunt, & Rushworth, 2009; Bickart, Dickerson, & Barrett, 2014; Ruff & Fehr, 2014). Particularly, the amygdala, a temporal structure involved in emotion (Phelps & LeDoux, 2005), face recognition (Adolphs, Tranel, Damasio, & Damasio, 1994; Breiter et al., 1996; Fried, MacDonald, & Wilson, 1997; Morris et al., 1996), group affiliation (Van Bavel, Packer, & Cunningham, 2008) and social network management (Adolphs, Tranel, & Damasio, 1998; Bickart, Wright, Dautoff, Dickerson, & Barrett, 2011; Kennedy, Gläscher, Tyszka, & Adolphs, 2009) has been proposed to regulate perception, affiliation and avoidance in social contexts (Bickart et al., 2014). Notably, psychopathy, a clinical condition characterized by anomalies in affective processing and empathy, has been linked to altered amygdala functionality (Blair, 2012; Decety, Chen, Harenski, & Kiehl, 2013; Kiehl et al., 2001) and volume (Yang, Raine, Narr, Colletti, & Toga, 2009). In rodents, amygdala lesions lead to an increase in the frequency of several social behaviors in novel environments (Wang, Zhao, Liu, & Fu, 2014), disruption of socially transmitted food preference (Wang, Fontanini, & Katz, 2006), impairment in sexual behavior (Harris & Sachs, 1975; Kondo, 1992; Newman, 1999) and possible alteration of social recognition (Maaswinkel, Baars, Gispen, & Spruijt, 1996 but see Wang et al., 2014). We thus hypothesized that BLA lesions

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.

* Corresponding author.
E-mail address: julien.her@gmail.com (J. Hernandez-Lallement).

http://dx.doi.org/10.1016/j.nlm.2015.11.004
1074-7427/© 2015 Elsevier Inc. All rights reserved.

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., 2015) 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. Methods

2.1. Subjects and housing

Thirty-six adult male Long-Evans rats (*Charles River, Italy*) weighing between 250 and 450 g 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 h 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).

2.2. Behavioral testing

2.2.1. Apparatus

We used a double T-Maze setup described previously in detail (Hernandez-Lallement et al., 2015). Briefly, the setup consisted of a custom made double T-Maze apparatus (Fig. 1(A)) with the choice compartments in both mazes facing each other. Animals could enter one of the two choice compartments (Fig. 1(A), *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 (Fig. 1(A), *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.

2.2.2. Experiment timeline and task design

The timeline of the experiment is shown in Fig. 1(B).

Preparation phase: Upon completion of initial habituation procedures (see Appendix and Hernandez-Lallement et al., 2015), 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 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 Appendix).

Prosocial Choice Task (PCT): The general principles of the task are described in detail in Hernandez-Lallement et al. (2015). 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 (Fig. 1(C), 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 0 s, $t0$) 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 Appendix). 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.

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 (Fig. 1(C), 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.

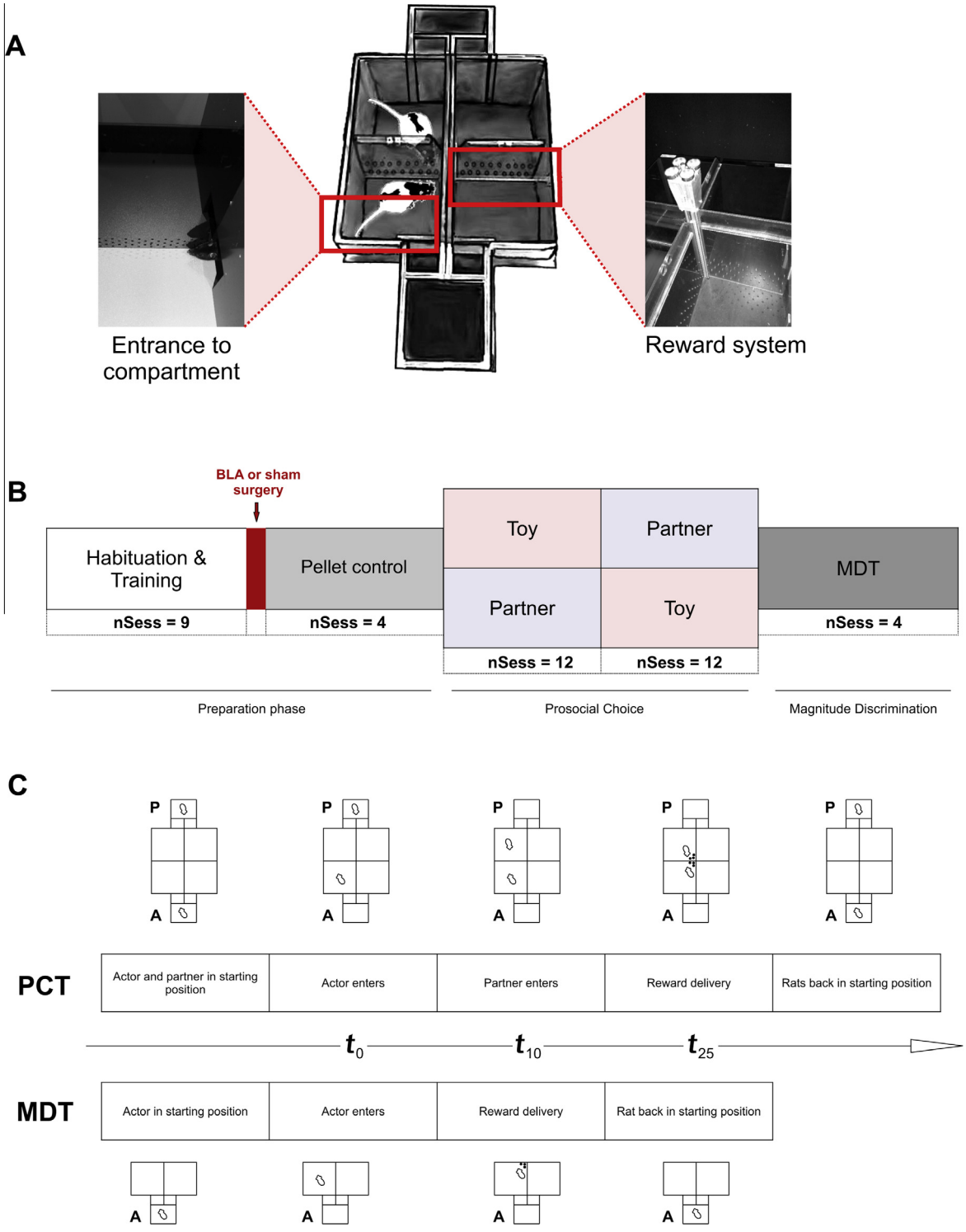

Fig. 1. Rodent Prosocial Choice Task: Apparatus and task design. (A) Double T-Maze apparatus: The setup consisted of a starting box equipped with two independently moveable doors that led to an intermediate box. A second door in each intermediate box gave access to the choice-compartments ("entrance 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. (B) Experiment timeline: *Preparation phase:* rats underwent habituation and training in the experimental setup (Appendix). 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. (C) Typical trial structure for PCT and MDT: *PCT:* both rats started in their respective starting boxes. Actors moved first (time 0 s, *t*0) into one of the two compartments. Ten seconds later (*t*10), the partner was directed to the opposite compartment, i.e. facing the actor. Rewards were delivered (*t*25) 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 (*t*0) and received either a small (3 pellets) or large (6 pellets) rewards (*t*10) before being replaced in the starting box for the following trial. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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., 2015). 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)$_i$]:

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

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., 2015) that allowed us to categorized animals according to a reference social bias scores 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) ± 4.8 mm, dorsoventral (DV) – 8. 6 mm; site 2: AP – 3.0 mm, ML ± 4.8 mm, DV – 8.8 mm. The AP and ML coordinates were relative to bregma, the DV coordinate was relative to the dura. Bilateral infusions were made using 0.3 mm injection needle (PlasticsOne) connected via polyethylene tubing to a 10 μl Hamilton syringe within a microinfusion pump (Harvard apparatus). Infusions were made using 0.2 μl 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/min, 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; 5 mg/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 (60 μm) 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 & 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 (Appendix), 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 (Fig. 2(A)) 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 gray areas, Fig. 2 (A) and (B)) into the lateral amygdaloid nucleus (LAVL) and the basomedial amygdaloid nucleus (BMP), sparing the central amygdaloid nucleus (CeN; Fig. 2(B)).

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 Section 2) 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 (Fig. 3(A), 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 (Fig. 3(A), right panel; $t_{(9)} = 2.37$, $p < .05$), replicating previous results with non-operated control rats (Hernandez-Lallement et al., 2015). By contrast, there was a near-significant trend toward 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.

We previously discussed (Hernandez-Lallement et al., 2015) 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

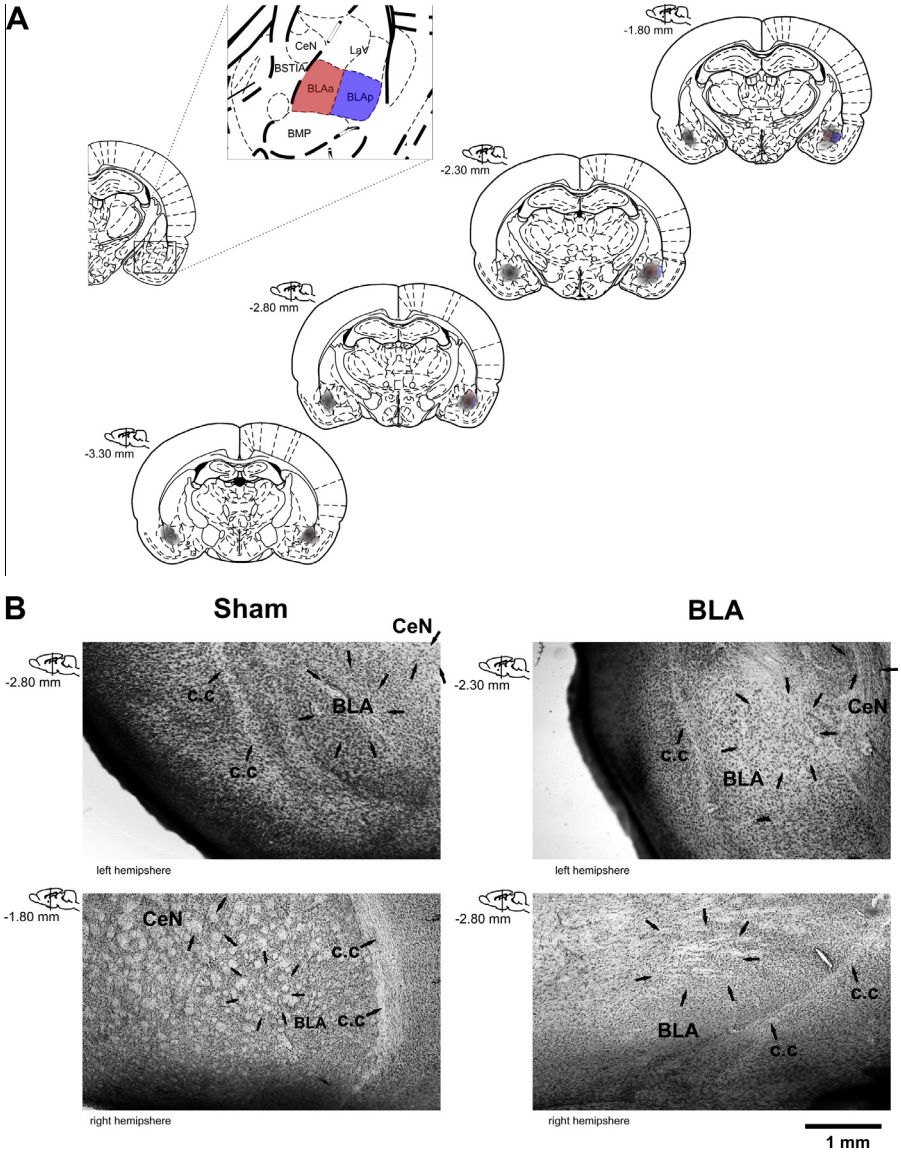

Fig. 2. Histology of BLA lesions. (A) Schematic illustration of the lesion spread for BLA lesions. Gray gradient represents lesion spread across all lesioned subjects ($n = 12$). Diagrams are adapted from Paxinos and Watson (1998). BLAa, basolateral amygdaloid nucleus, anterior part; BLAp basolateral 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. (B) 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). BLA, basolateral amygdaloid nucleus; CeN central amygdaloid nucleus; e.c, external capsule.

preferences between sham- and BLA-lesioned rats, we compared each rats' social bias score to a 95% confidence interval (Fig. 3(A), right panel; red vertical lines) obtained through a bootstrapped permutation analysis (see Section 2 and Hernandez-Lallement et al., 2015). 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 preference 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%; Fig. 3(B)) 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

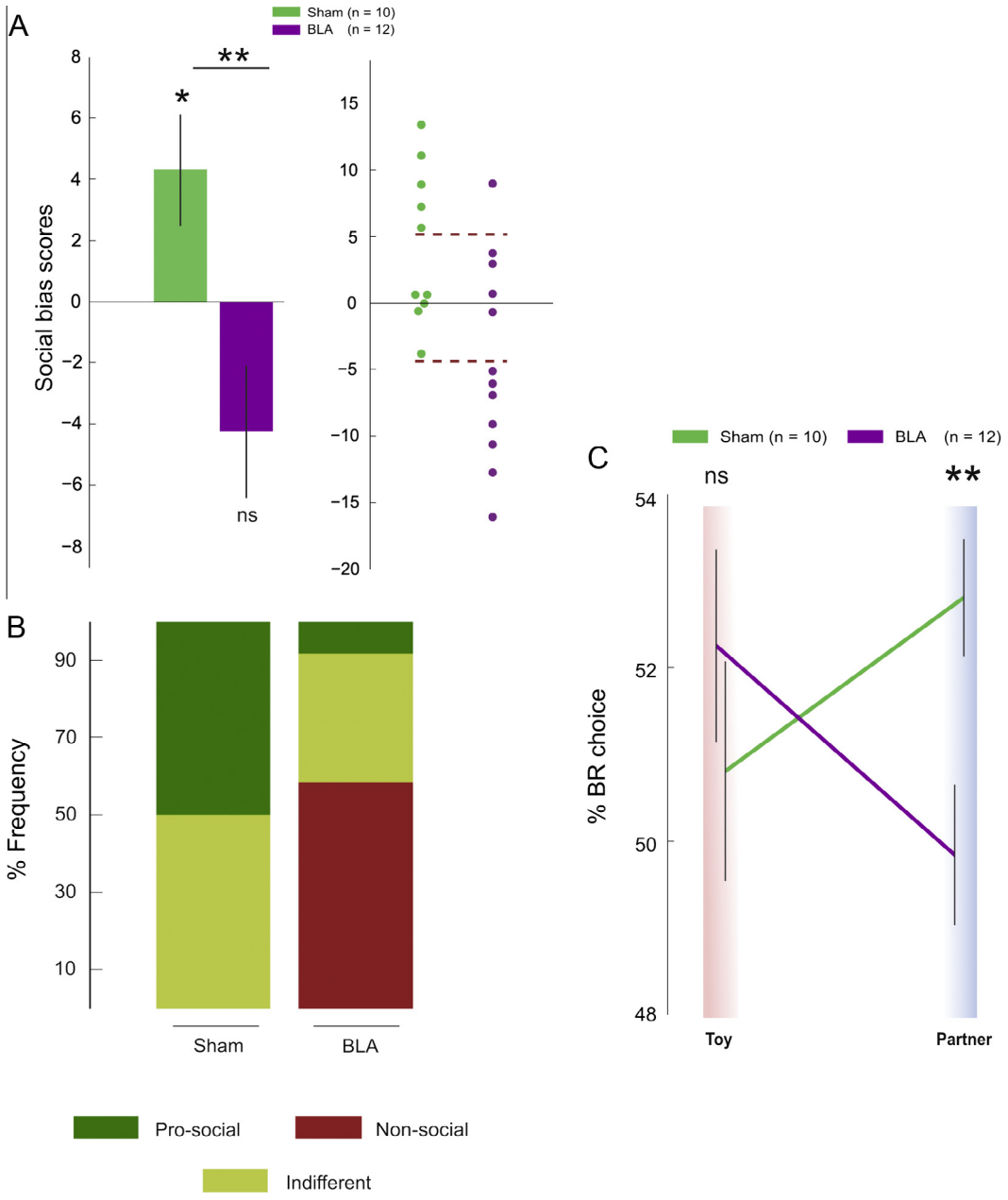

Fig. 3. BLA lesions abolish mutual-reward preferences in rats. (A) Social bias scores per group: The individual (dots) and mean (bar) social bias scores indicating the percent difference in BR choices in the partner-compared to the toy condition were significantly different between the sham (green) and 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 $\subseteq$ CI) and "*non-social*" (social bias scores < CI). (C) 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. $^{**}p < .01$, independent-samples *t*-test; ns, not significant. Error bars represent the standard error of the mean, s.e.m.

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. 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 (Fig. 3 (C); $F_{(1,20)} = 8.70$, $p < .01$). 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. However, the average weight of the animals was

not different between BLA- and sham-groups (Fig. 4(A); $t_{(20)} = .26$, $p = .80$), 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 (Fig. 4(B), $F_{(1,20)} = 0.01$, $p = .91$). 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 Appendix).

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, Fig. 1(C)) 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 (Fig. 4(C); 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 ($F_{(3,60)} = 1.47$, $p = .23$). These data suggest 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 Prosocial 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*

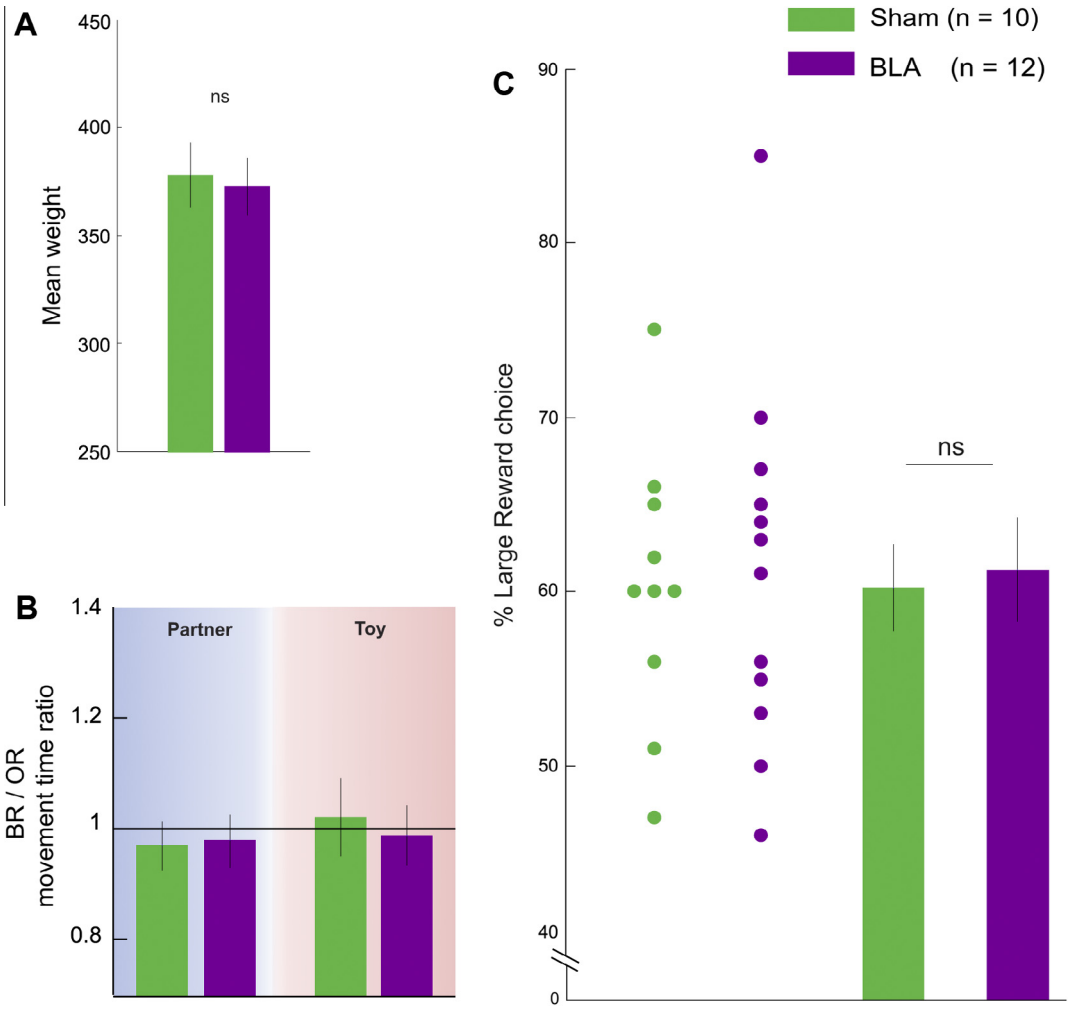

Fig. 4. BLA lesions do not affect bodily mass, response times or reward magnitude discrimination. (A) Average weight per group. The average weight did not differ between sham and BLA animals. (B) Movement time ratios. The BR/OR movement time ratios were not significantly different from 1 in any group in any condition. Furthermore, direct comparisons between conditions or between groups were not significant either. (C) Performance in the MDT. Individual (dots) and mean (bar) 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 preferences levels (mean ± s.e.m.). ns, not significant.

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, Winecoff, & Platt, 2011; Hernandez-Lallement et al., 2015; Ruff & Fehr, 2014) 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 toward 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, Schwarting, & Wöhr, 2014) or increased social interaction, e.g. pleasure derived from eating rewards in spatial proximity (Barnett & Spencer, 1951). Additionally, choice behavior could also be reinforced by negative social stimuli, i.e. putatively aversive distress signals produced by partners (Atsak et al., 2011; Kim, Kim, Covey, & Kim, 2010) missing out on reward after OR choices. As previously noted (Hernandez-Lallement et al., 2015), 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, Li, & Brown, 2012). Thus, USVs, which are known to carry affective state information (Knutson, Burgdorf, & Panksepp, 1999; Litvin, Blanchard, & Blanchard, 2007) not only in rats (Seffer et al., 2014; Wöhr & Schwarting, 2008) but in also in other species (Gadziola, Grimsley, Faure, & Wenstrup, 2012; Naumann & Kanwal, 2011; Sharp, McGowan, Wood, & Hatchwell, 2005), are prime candidates for social stimuli driving choice in the PCT. This idea is supported by a recent study showing that pro-social 50 kHz 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 & Whalen, 2001; Schoenbaum, Setlow, Saddoris, & Gallagher, 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 & Kiehl, 2012), possibly reflecting the psychopath's affective indifference to social cues and situations.

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.C. analyzed the data and wrote the paper. T.K. acquired funds, designed the research, analyzed the data and wrote the paper.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft (DFG) Grant no. KA 2675/5-1 to TK. MvW was supported by the Volkswagen Stiftung "Freigeist" fellowship, AZ 88216. The authors declare no competing financial interests. We are particularly thankful to Alexander Braun, Linus Roepert, Adrian Woelk and Maurice Zech for valuable help with the data acquisition. We thank Milan Srejic for his constant help on the practical aspect of the behavioral task as well as Günter Abel for his assistance with animal care.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nlm.2015.11.004.

References

Adolphs, R., Tranel, D., & Damasio, A. R. (1998). The human amygdala in social judgment. *Nature, 393*, 470–474.

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature, 372*, 669–672.

Anderson, N. E., & Kiehl, K. A. (2012). The psychopath magnetized: Insights from brain imaging. *Trends in Cognitive Sciences, 16*, 52–60.

Atsak, P., Orre, M., Bakker, P., Cerliani, L., Roozendaal, B., Gazzola, V., ... Keysers, C. (2011). Experience modulates vicarious freezing in rats: A model for empathy. *PloS One, 6*, e21855. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3135600&tool=pmcentrez&rendertype=abstract> Accessed 29.05.13.

Barnett, S. A., & Spencer, M. M. (1951). Feeding, social behaviour and interspecific competition in wild rats. *Behaviour*, 229–242. Available at: <http://psycnet.apa.org/psycinfo/1952-01970-001> Accessed 27.03.12.

Van Bavel, J. J., Packer, D. J., & Cunningham, W. A. (2008). The neural substrates of in-group bias a functional magnetic resonance imaging investigation. *Psychological Science, 19*, 1131–1139. Available at: <http://pss.sagepub.com/content/19/11/1131.short>.

Behrens, T. E. J., Hunt, L. T., & Rushworth, M. F. S. (2009). The computation of social behavior. *Science (New York, NY), 324*, 1160–1164. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19478175>.

Ben-Ami Bartal, I., Decety, J., Mason, P., & Bartal, I. B.-A. (2011). Empathy and pro-social behavior in rats. *Science, 334*, 1427–1430. Available at: <http://www.sciencemag.org/cgi/doi/10.1126/science.1210789> Accessed 08.12.11.

Bickart, K. C., Dickerson, B. C., & Barrett, L. F. (2014). The amygdala as a hub in brain networks that support social life. *Neuropsychologia, 63*, 235–248. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25152530> Accessed 16.12.14.

Bickart, K. C., Wright, C. I., Dautoff, R. J., Dickerson, B. C., & Barrett, L. F. (2011). Amygdala volume and social network size in humans. *Nature Neuroscience, 14*, 163–164. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3079404&tool=pmcentrez&rendertype=abstract> Accessed 12.01.15.

Blair, R. J. R. (2012). Neurobiological basis of psychopathy. *British Journal of Psychiatry, 182*, 5–7.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., ... Rosen, B. R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron, 17*, 875–887.

Chang, S. W. C., Winecoff, A. A., & Platt, M. L. (2011). Vicarious reinforcement in rhesus macaques (*Macaca mulatta*). *Frontiers in Neuroscience, 5*, 10.

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Molecular Psychiatry, 6*, 13–34. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11244481>.

Decety, J. (2011). The neuroevolution of empathy. *Annals of the New York Academy of Sciences, 1231*, 35–45.

Decety, J., Chen, C., Harenski, C., & Kiehl, K. A. (2013). An fMRI study of affective perspective taking in individuals with psychopathy: Imagining another in pain does not evoke empathy. *Frontiers in Human Neuroscience, 7*, 489. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3782696&tool=pmcentrez&rendertype=abstract>.

Fried, I., MacDonald, K. A., & Wilson, C. L. (1997). Single neuron activity in human hippocampus and amygdala during recognition of faces and objects. *Neuron, 18*, 753–765.

Gadziola, M. A., Grimsley, J. M. S., Faure, P. A., & Wenstrup, J. J. (2012). Social vocalizations of big brown bats vary with behavioral context. *PLoS One, 7*.

Harris, V. S., & Sachs, B. D. (1975). Copulatory behavior in male rats following amygdaloid lesions. *Brain Research, 86*, 514–518.

Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., & Kalenscher, T. (2015). Rats prefer mutual rewards in a prosocial choice task. *Frontiers in Neuroscience, 8*, 1–9. Available at: <http://www.frontiersin.org/Decision_Neuroscience/10.3389/fnins.2014.00443/abstract> Accessed 16.01.15.

Kashtelyan, V., Lichtenberg, N. T., Chen, M. L., Cheer, J. F., & Roesch, M. R. (2014). Observation of reward delivery to a conspecific modulates dopamine release in ventral striatum. *Current Biology, 24*, 2564–2568. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0960982214011361> Accessed 24.10.14.

Kennedy, D. P., Gläscher, J., Tyszka, J. M., & Adolphs, R. (2009). Personal space regulation by the human amygdala. *Nature Neuroscience, 12*, 1226–1227. http://dx.doi.org/10.1038/nn.2381.

Kiehl, K. A., Smith, A. M., Hare, R. D., Mendrek, A., Forster, B. B., Brink, J., & Liddle, P. F. (2001). Limbic abnormalities in affective processing by criminal psychopaths as revealed by functional magnetic resonance imaging. *Biological Psychiatry, 50*, 677–684.

Kim, E. J., Kim, E. S., Covey, E., & Kim, J. J. (2010). Social transmission of fear in rats: The role of 22-kHz ultrasonic distress vocalization. *PloS One, 5*, e15077. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2995742&tool=pmcentrez&rendertype=abstract> Accessed 14.06.11.

Knutson, B., Burgdorf, J., & Panksepp, J. (1999). High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. *Physiology & Behavior, 66*, 639–643. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10386908>.

Kondo, Y. (1992). Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. *Physiology & Behavior, 51*, 939–943.

Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22 kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research, 182*, 166–172. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17173984> Accessed 05.11.11.

Maaswinkel, H., Baars, A.-M., Gispen, W.-H., & Spruijt, B. M. (1996). Roles of the basolateral amygdala and hippocampus in social recognition in rats. *Physiology & Behavior, 60*, 55–63. Available at: <http://linkinghub.elsevier.com/retrieve/pii/0031938495022333>.

Márquez, C., Rennie, S. M., Costa, D. F., & Moita, M. A. (2015). Prosocial choice in rats depends on food-seeking behavior displayed by recipients. *Current Biology*, 1–10. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0960982215005928>.

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Yound, A. W., Calder, A. L., & Dolan, R. J. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature, 383*, 812–815.

Naumann, R. T., & Kanwal, J. S. (2011). Basolateral amygdala responds robustly to social calls: Spiking characteristics of single unit activity. *Journal of Neurophysiology, 105*, 2389–2404.

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Annals of the New York Academy of Sciences, 877*, 242–257.

Parsana, A. J., Li, N., & Brown, T. H. (2012). Positive and negative ultrasonic social signals elicit opposing firing patterns in rat amygdala. *Behavioural Brain Research, 226*, 77–86. http://dx.doi.org/10.1016/j.bbr.2011.08.040.

Paxinos, G., Watson, C. (1998). *The rat brain in stereotaxic co-ordinates*, 4th ed. Sydney.

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: From animal models to human behavior. *Neuron, 48*, 175–187. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16242399> Accessed 10.07.14.

Ruff, C. C., & Fehr, E. (2014). The neurobiology of rewards and values in social decision making. *Nature Reviews Neuroscience, 15*, 549–562. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24986556> Accessed 09.07.14.

Schoenbaum, G., Setlow, B., Saddoris, M. P., & Gallagher, M. (2003). Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. *Neuron, 39*, 855–867.

Seffer, D., Schwarting, R. K. W., & Wöhr, M. (2014). Pro-social ultrasonic communication in rats: Insights from playback studies. *Journal of Neuroscience Methods*, 1–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24508146> Accessed 03.04.14.

Sharp, S. P., McGowan, A., Wood, M. J., & Hatchwell, B. J. (2005). Learned kin recognition cues in a social bird. *Nature, 434*, 1127–1130. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15858573>.

Silk, J. B., & House, B. R. (2011). Evolutionary foundations of human prosocial sentiments. *Proceedings of the National Academy of Sciences of the United States of America, 108*(Suppl.), 10910–10917. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3131813&tool=pmcentrez&rendertype=abstract> Accessed 19.03.14.

Wang, Y., Fontanini, A., & Katz, D. B. (2006). Temporary basolateral amygdala lesions disrupt acquisition of socially transmitted food preferences in rats. *Learning & Memory, 13*, 794–800.

Wang, Y., Zhao, S., Liu, X., & Fu, Q. (2014). Effects of the medial or basolateral amygdala upon social anxiety and social recognition in mice. *Turkish Journal of Medical Sciences, 44*, 353–359.

Willuhn, I., Tose, A., Wanat, M. J., Hart, A. S., Hollon, N. G., Phillips, P. E. M., ... Wöhr, M. (2014). Phasic dopamine release in the nucleus accumbens in response to pro-social 50 kHz ultrasonic vocalizations in rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 34*, 10616–10623. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25100595> Accessed 07.11.14.

Wöhr, M., & Schwarting, R. K. W. (2008). Ultrasonic calling during fear conditioning in the rat: No evidence for an audience effect. *Animal Behaviour, 76*, 749–760. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0003347208002108> Accessed 11.12.11.

Yang, Y., Raine, A., Narr, K. L., Colletti, P., & Toga, A. W. (2009). Localization of deformations within the amygdala in individuals with psychopathy. *Archives of General Psychiatry, 66*, 986–994.