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Basolateral amygdala lesions abolish mutual reward preferences in rats

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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.

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.

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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.