Neurobiology of Learning and Memory 128 (2016) 40–45

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Rapid Communication

Acquisition of specific response–outcome associations requires NMDA receptor activation in the basolateral amygdala but not in the insular cortex

Shauna L. Parkes [a,b,c,*], Guillaume Ferreira [a,c,1], Etienne Coutureau [b,c,1]

[a] INRA, Nutrition and Integrative Neurobiology, UMR 1286, 33076 Bordeaux, France
[b] CNRS, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, UMR 5287, 33076 Bordeaux, France
[c] Université de Bordeaux, 33076 Bordeaux, France

ARTICLE INFO

Article history:
Received 13 November 2015
Revised 17 December 2015
Accepted 20 December 2015
Available online 29 December 2015

Keywords:
Goal-directed behavior
Instrumental
Conditioning
Acquisition
Amygdala
Insular cortex

ABSTRACT

The basolateral amygdala (BLA) and the gustatory region of the insular cortex (IC) are required for the encoding and retrieval of outcome value. Here, we examined if these regions are also necessary to learn associations between actions and their outcomes. Hungry rats were first trained to press two levers for a common outcome. Next, specific response–outcome (R–O) associations were introduced such that each response now earned a distinct food outcome. Prior to each specific R–O training session, rats received a bilateral infusion of the N-methyl-D-aspartate (NMDA) receptor antagonist, DL-APV, into either the BLA or the IC. One of the two outcomes was then devalued immediately prior to a choice test. Inhibition of NMDA receptor activity in the BLA, but not the IC, during the acquisition of specific R–O associations abolished selective devaluation. These results indicate that the BLA is critical for learning the association between actions and their specific consequences.

The basolateral amygdala (BLA) plays a pivotal role in reward learning (Balleine & Killcross, 2006; Wassum & Izquierdo, 2015) and modulates both stimulus-guided reward-related behavior (Baxter & Murray, 2002; Hatfield, Han, Conley, Gallagher, & Holland, 1996; Johnson, Gallagher, & Holland, 2009; Pickens et al., 2003; Schoenbaum, Chiba, & Gallagher, 1998) and response-guided choice behavior (Johnson et al., 2009; Ostlund & Balleine, 2008), via its connections with the nucleus accumbens core (Shiflett & Balleine, 2010) and gustatory region of the insular cortex (IC; Parkes & Balleine, 2013). The relationship between the BLA and the IC is of particular interest given that these regions share dense, reciprocal connections (Krettek & Price, 1977; McDonald, 1998; Pitkanen, 2000; Price, 2003; Shi & Cassell, 1998; Sripanidkulchai, Sripanidkulchai, & Wyss, 1984) and appear to play dissociable roles in goal-directed instrumental actions.

Following specific-satiety induced outcome devaluation, rats with pre-training lesions of the BLA (Balleine, Killcross, & Dickinson, 2003; Corbit & Balleine, 2005; Coutureau, Marchand, & Di Scala, 2009) or the IC (Balleine & Dickinson, 2000) appear insensitive to the change in outcome value and fail to selectively decrease instrumental responding for the now devalued reward. Interestingly, when tested under rewarded conditions, IC-lesioned rats show selective devaluation (Balleine & Dickinson, 2000) whereas, in BLA-lesioned rats, selective devaluation only emerges across the session (Balleine et al., 2003). While these studies have indeed provided clear evidence that the BLA and IC are involved in goal-directed actions, they do not reveal the precise role of these structures. More recently, temporally restricted manipulations before or after outcome devaluation have revealed that the BLA is involved in the encoding (Parkes & Balleine, 2013; Wassum, Cely, Balleine, & Maidment, 2011; Wassum, Ostlund, Maidment, & Balleine, 2009; West et al., 2012) and the IC in the retrieval (Parkes & Balleine, 2013; Parkes, Bradfield, & Balleine, 2015) of changes in outcome value, i.e. incentive memory. Surprisingly, the direct involvement of the BLA and the IC during the acquisition of response–outcome (R–O) associations has not been investigated. Such knowledge is crucial in order to progress our understanding of the neural circuitry mediating decision-making processes.

Here, we explicitly examined the role of the BLA and the IC in the acquisition of goal-directed instrumental actions. Given the important role of NMDA-dependent plasticity in acquisition of new information (Morris, 2013), we infused the NMDA receptor

* Corresponding author at: INRA, Nutrition and Integrative Neurobiology, UMR 1286, 33076 Bordeaux, France.
E-mail address: shauna.parkes@u-bordeaux.fr (S.L. Parkes).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.nlm.2015.12.005
1074-7427/

(NMDAR) antagonist, DL-APV, in the BLA or the IC prior to the acquisition of R–O associations and then tested performance during an outcome devaluation test. Critically, a behavioral paradigm was used that allowed us to dissociate the acquisition of lever pressing *per se* from the acquisition of specific R–O associations (Corbit, Leung, & Balleine, 2013; Yin, Knowlton, & Balleine, 2005).

Subjects and apparatus. Forty male Long Evans rats (Janvier, France) were housed in plastic boxes (2 rats per box) and maintained on a 12 h light/dark cycle. Training and testing occurred during the light portion of the cycle. Rats were put on a food-restricted schedule two days before the start of the behavioral procedures to maintain them at approximately 90% of their *ad libitum* feeding weight. All experiments were conducted in agreement with the French (council directive 2013-118, February 1, 2013) and international (directive 2010-63, September 22, 2010, European Community) legislations and received approval # 5012053-A from the local Ethics Committee.

Training and testing took place in 8 operant chambers (Imetronic, Pessac, France). Each chamber was equipped with a pump that was fitted with a syringe that, when activated, delivered a 20% polycose solution (0.1 ml) and two pellet dispensers that delivered grain or sugar pellets (45 mg; Bioserv Biotechnologies) into a recessed magazine. The chambers contained two retractable levers that could be inserted to the left and the right of the magazine. A house light provided illumination of the operant chamber.

Surgery and microinfusions. Rats were anaesthetized using Isoflurane (5% induction; 1–2% maintenance) and mounted on a stereotaxic apparatus (Kopf). Rats were subcutaneously injected with 0.1 ml solution of buprecare and the incision site was sprayed with the local anaesthetic, bupivacaine. Stainless steel 28 gauge guide cannulae (Plastics One) were implanted bilaterally in either the insular cortex (IC; anteroposterior +1.1 mm; mediolateral ±5.5 mm; dorsoventral −4.0 mm from skull surface) or the basolateral amygdala (BLA; anteroposterior −3.0 mm; mediolateral ±4.8; dorsoventral: −5.8 mm from skull surface). Cannulae were maintained in position with dental cement and dummy cannulae were kept in each guide at all times except during microinfusions. Rats were allowed at least 5 d to recover from surgery, during which time they were handled and weighed daily.

The NMDAR antagonist, DL-2-Amino-5-phosphonopentanoic acid (DL-APV; Sigma–Aldrich) was dissolved in aCSF (M Dialysis) to obtain a final concentration of 10 mg/ml. The vehicle solution was aCSF. DL-APV was infused by inserting a 30 gauge internal cannula into the guide cannula. The internal cannula was connected to a 10 µl glass syringe attached to an infusion pump (Harvard Apparatus) and projected an additional 2 mm ventral to the tip of the guide cannula. A total volume of 0.5 µl (IC) and 0.25 µl (BLA) was delivered at a rate of 0.25 µl/min. The concentration, rate and infusion volumes were chosen based on previous literature (e.g., Ferreira, Gutiérrez, De La Cruz, & Bermudez-Rattoni, 2002; Ferreira, Miranda, De la Cruz, Rodrigues-Ortiz, & Bermudez-Rattoni, 2005; Parkes, De la Cruz, Bermúdez-Rattoni, Coutureau, & Ferreira, 2014). The internal cannula remained in place for a further 1 min after the infusions and was then removed. One day prior to infusions, the dummy cannulae were removed, and the infusion pump turned on in order to familiarize the rats with the procedure.

Subsequent to behavioral testing, subjects received a lethal dose of sodium pentobarbital. The brains were removed and sectioned coronally at 40 µm through the BLA and the IC. Every third section was collected on a slide and stained with thionine. The location of cannula tips was determined under a microscope by a blind observer using the boundaries defined by Paxinos and Watson (2006).

Instrumental training. Rats were given two 30 min sessions of magazine training during which a 20% polycose (0.1 ml) was delivered at random 60 s intervals. Rats were then trained to respond on two levers to earn a common outcome (20% polycose). During the session, each lever was presented twice for a maximum of 10 min each or until 20 outcomes were earned. This ensured that, despite differences in the rate of lever pressing, all rats received a similar number of outcomes. The inter-trial interval between lever presentations was 2.5 min. The order of the lever presentation was alternated and counterbalanced across rats and days. For the first 3 d, lever pressing was continuously reinforced. The probability of the outcome given a response was then gradually shifted using increasing random ratio (RR) schedules: a RR 2 schedule was used on days 4–5, RR 3 on days 6–7 and RR 4 on days 8–9. On day 10, rats were given food *ad libitum*, and underwent the surgical procedure described above. Following recovery, rats were food deprived for 2 days and then received 2 days of RR 4 training for polycose. On the next three days, two distinct rewards were introduced. These sessions were identical to the baseline training except now responding on one lever (e.g., left lever) delivered one pellet (e.g., grain) and responding on the other lever (e.g., right lever) delivered the other pellet (e.g., sugar) on a RR 4 contingency. Response–outcome (R–O) relationships were counterbalanced across groups. Fifteen minutes before each of these sessions, rats received an infusion of either DL-APV or vehicle.

Outcome devaluation tests. Twenty-four hours after the final specific R–O training session, rats received *ad libitum* access to one of the two outcomes for 1 h in distinct feeding cages. Immediately after, rats were given a 10 min choice unrewarded (extinction) test in which both levers were available but no outcome was delivered. The following day rats were re-trained (under either DL-APV or vehicle) and 24 h later were given a second devaluation test with the other outcome. Forty-eight hours later, rats were given a rewarded test. This test differed procedurally from the unrewarded test only to the extent that the two outcomes were delivered as a consequence of instrumental performance. Each outcome was delivered on an independent RR 4 schedule. Twenty-four hours later, rats were given a second rewarded test with the other outcome devalued.

Data analyses. All analyses were conducted using a mixed-model ANOVA followed by simple effects analyses to establish the source of any significant interactions. Statistical significance was set at $p \leqslant 0.05$. Data are presented as mean ± SEM.

Histological verification of the cannulae placements are presented in Fig. 1. Five rats were excluded because of incorrect location of one or both cannulae. This yielded the following group sizes: group vehicle ($n = 11$), group BLA ($n = 12$) and group IC ($n = 12$). Six of the animals in group vehicle had cannulae implanted in the BLA and five in the IC. For all analyses, there was no difference between vehicle-treated rats infused in the BLA and those infused in the IC, therefore the data were collapsed into a single group.

Lever pressing performance increased across baseline training for the common outcome and did not differ between groups (Fig. 2A). Statistical analyses confirmed a significant effect of session ($F_{(1,32)} = 158.18$, $p < 0.05$), but no effect of group nor any interaction between these factors (largest $F_{(1,32)} = 0.87$, $p > 0.05$). Across infusion sessions, vehicle-treated rats pressed significantly more than drug-treated rats ($F_{(1,32)} = 7.89$, $p < 0.05$), but there was no difference between rats infused with DL-APV in the IC and those infused in the BLA ($F_{(1,32)} = 3.09$, $p > 0.05$). Overall, responding increased across these training sessions ($F_{(1,32)} = 18.23$, $p < 0.05$) and there was no group by session interaction (largest $F_{(1,32)} = 0.86$, $p > 0.05$). Analysis of the magazine entries across the three infusion sessions revealed a significant linear trend (data not shown; $F_{(1,32)} = 50.07$, $p > 0.05$), such that magazine entries decreased across the training sessions, but no effect of group (largest $F_{(1,32)} = 2.17$, $p > 0.05$) nor any interaction between these factors (largest $F_{(1,32)} = 0.62$, $p > 0.05$).