Neuroscience 303 (2015) 73–81

FUNCTION OF THE CENTROMEDIAL AMYGDALA IN REWARD DEVALUATION AND OPEN-FIELD ACTIVITY

K. KAWASAKI, [a] **A. C. GLUECK,** [b] **I. ANNICCHIARICO** [b] **AND M. R. PAPINI** [b]*

[a] *Department of Psychology, Hoshi University, 2-4-41 Ebara, Shinagawa, Tokyo 142-8501, Japan*

[b] *Department of Psychology, Texas Christian University, Fort Worth, TX 76129, USA*

Abstract—The present research aimed at determining the role played by the amygdala in reward devaluation using transient inactivation induced by lidocaine microinfusions into the centromedial region. Two situations involving reward devaluation were tested in rats: consummatory successive negative contrast (cSNC) and anticipatory negative contrast (ANC). In cSNC, rats exposed to a downshift from 32% to 4% sucrose consume less 4% sucrose than rats always exposed to 4% sucrose. Extensive evidence suggests that reward devaluation in the cSNC situation is accompanied by negative emotion. In ANC, rats consume less 4% sucrose when each session is closely followed by access to 32% sucrose rather than by 4% sucrose. Evidence suggests that reward devaluation in the ANC situation does not involve negative emotions; rather, ANC appears to involve Pavlovian anticipation of the higher value solution. To test the effects of lidocaine microinfusions in a situation known to induce negative emotion, but unrelated to reward devaluation, animals were also exposed to a lighted open field. Centromedial amygdala inactivation reduced the cSNC effect and increased exploratory behavior in the open field, both effects consistent with a reduction in negative emotional state. However, no detectable effects of amygdala inactivation were observed in the ANC situation. These results suggest that, first, the function of the amygdala is not unique to reward devaluation and, second, it is concerned with tagging the devaluation experience with aversive valence. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: incentive contrast, reward devaluation, amygdala, consummatory successive negative contrast, anticipatory negative contrast, open-field activity.

INTRODUCTION

Reversible lesions produced by infusions of the sodium-channel blocker lidocaine in several amygdala locations disrupt the effects of reward devaluation on instrumental behavior. In one experiment (Salinas et al., 1993), rats exposed in a runway to a 10-to-1 pellet downshift decreased running speed relative to rats always reinforced with 1 pellet—an effect known as instrumental successive negative contrast (iSNC; Flaherty, 1996). While both lidocaine and vehicle rats exhibited comparable response latencies on the first downshift session, lidocaine-treated animals recovered faster from reward devaluation in the following sessions. In another experiment using the same procedure, Salinas and McGaugh (1996) infused bicuculline, a $GABA_A$-receptor antagonist, immediately after the first downshift session into the amygdala and observed an enhancement of the iSNC effect. Furthermore, restricted neurotoxic lesions uncovered differential effects. Whereas lesions of the central nucleus of the amygdala (CeA) enhanced the iSNC effect, lesions of the basolateral nucleus of the amygdala (BLA) reduced the iSNC effect (Salinas et al., 1996). Interestingly, none of the two lesions affected the initial response to the downshift. Consistent results were reported with similar manipulations of the amygdala in a related form of incentive contrast—consummatory successive negative contrast (cSNC). In cSNC, consummatory behavior for a small reward is reduced by prior access to a large reward, relative to unshifted controls always receiving the small reward (Flaherty, 1996). Large centromedial amygdala lesions reduced or even eliminated the cSNC effect (Becker et al., 1984), whereas intraamygdala infusion of the $GABA_A$ agonist diazepam reduced the size of the cSNC effect (Liao and Chuang, 2003).

These results suggest that output from the amygdala is a critical component of the negative emotional state induced by reward devaluation in both the iSNC and cSNC situations. Moreover, $GABA_A$ receptors are involved in the modulation of the response to reward devaluation in both situations, as also shown by systemic treatments with benzodiazepines (for cSNC: Flaherty and Driscoll, 1980; Flaherty et al., 1990; Pellegrini et al., 2004; Freet et al., 2006; Ortega et al., 2014; for iSNC: Rosen and Tessell, 1970; Vogel and Principi, 1971). However, these effects of amygdala manipulations on iSNC and cSNC situations differ in one respect. Whereas disruption of amygdala output did not seem to affect the initial response to the reward devaluation in the iSNC situation (Salinas et al., 1993), the cSNC effect was disrupted on the first downshift session

*Corresponding author. Tel: +1-817-256-6084.
E-mail addresses: kkawasak@hoshi.ac.jp (K. Kawasaki), amanda.glueck@tcu.edu (A. C. Glueck), i.annicchiaricoiseda@tcu.edu (I. Annicchiarico), m.papini@tcu.edu (M. R. Papini).

Abbreviations: ANC, anticipatory negative contrast; BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; cSNC, consummatory successive negative contrast; GABA, gamma-aminobutyric acid; iSNC, instrumental successive negative contrast; PBS, phosphate-buffered saline.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.053
0306-4522/

(Becker et al., 1984). Such differential effects are not surprising since these contrast situations respond differentially to a number of behavioral and neurobiological manipulations (Flaherty, 1996). For example, lesions of the hippocampus and nucleus accumbens disrupt iSNC without apparently affecting cSNC (Flaherty et al., 1998; Leszczuk and Flaherty, 2000), whereas lesions of the gustatory thalamus disrupt cSNC without affecting iSNC (Sastre and Reilly, 2006). But the experiments involving the amygdala were based on different manipulations (i.e., lidocaine infusions vs. electrolytic lesions). Thus, the present experiment sought to understand the role of the amygdala in the cSNC effect by producing a reversible inactivation of the centromedial region just before the first reward devaluation experience. Compared to pretraining irreversible lesions, the current approach has the advantage that the consummatory behavior develops under normal amygdala conditions before and after disruption of its activity.

To test for the boundary of the effects of lidocaine on reward devaluation, amygdala inactivation was also studied in the anticipatory negative contrast (ANC) situation and in the open field. The ANC task involves the same rewards used in the cSNC situation, but delivered in a different arrangement (Flaherty, 1996). In the ANC effect, consumption of 4% sucrose is suppressed in a group for which each trial is followed shortly thereafter by access to 32% sucrose (4–32 condition), relative to a group for which both trials provide access to 4% sucrose (4–4 condition). Such consummatory suppression does not depend on the last reward received a day earlier, but on the relative value of the forthcoming reward (Flaherty et al., 1995). The ANC effect develops over sessions and it is immune to pharmacological manipulations that eliminate, reduce, or exacerbate the cSNC effect, including treatments with benzodiazepine anxiolytics (Flaherty and Rowan, 1988) and corticosterone (Ruetti et al., 2009). There is also an unpublished report suggesting that electrolytic lesions of the central nucleus of the amygdala do not affect ANC (Coppotelli and Flaherty, cited in Flaherty, 1996, p. 121). Flaherty (1996) suggested that ANC is an anterograde phenomenon, that is, that consumption of the first reward is inhibited by anticipation of a forthcoming reward of a greater value. Thus, although the ANC effect involves reward devaluation, there is no evidence that the effect is accompanied by negative emotion. It was expected that amygdala inactivation would not affect ANC.

The effects of amygdala inactivation were also tested in the open-field situation. This task was chosen because it is known to induce behaviors indicative of negative emotion (Suarez and Gallup, 1981; Pare, 1994; Ramos, 2008). Rats exposed to a well-lighted open-field arena showed reduced activity in the central area, an indication of heightened unconditioned fear (Bouwknecht et al., 2007), and increased c-Fos expression in the BLA (Hale et al., 2006). These results, therefore, lead to the expectation that inactivation of the amygdala before open-field testing would enhance activity, especially in the central area of the arena.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 48 male Wistar rats, experimentally naïve and about 90 days of age at the start of the experiment. They were bred from animals purchased at Harlan Labs (Indianapolis, IN, USA), maintained in same-sex groups in polycarbonate cages after weaning, and moved to individual wire-bottom cages around postnatal day 40. The colony room was maintained at a relatively constant temperature (18–23 °C) and humidity (50%), and subject to a 12:12-h light cycle (lights on at 07:00 h). Rats were tested during the light portion of the daily cycle. Water was freely available throughout their lives. Food was freely available until they were approximately 90 days of age. In preparation for surgery (see below), all animals were food deprived to 90% of their free-food weight. After recovery from surgery and in preparation for behavioral testing, animals were further deprived to an 81–84% of their original free-food weight. This stepwise deprivation procedure was implemented to reduce the number of postsurgical days and thus minimize the risk of loose cannula implants. Supplemental food was given every day at least 15 min after behavioral sessions; the amount of food was determined by an empirically derived formula aimed at keeping animals within the preestablished range of food deprivation. While on deprivation, animals were weighed daily.

Apparatus

cSNC and ANC testing took place in eight conditioning boxes (MED Associates, St. Albans, VT, USA) made of aluminum and Plexiglas, and measuring 29.4 × 28.9 × 24.7 cm (L × H × W). The floor was made of steel rods, 0.5 cm in diameter and 1.2 cm apart, placed perpendicular to the feeder wall. A bedding tray filled with corncob bedding and placed underneath the rods collected fecal pellets and urine; the bedding was replaced as needed. An elliptical opening 1 × 2 cm (W × H), 3.5 cm from the floor and located on the feeder wall served to present a sipper tube (diameter: 1 cm). When fully inserted, the sipper tube was flush against the wall. A house light (GE 1820) located in the center of the box's ceiling provided diffuse light. A computer in an adjacent room controlled the presentation and retraction of the sipper tube, and recorded contacts with the sipper tube. Each conditioning box was placed in a sound-attenuating chamber containing a speaker (white noise) and a fan (ventilation), and producing masking noise with an intensity of 80.1 dB (SPL scale C).

Open-field testing was carried out in three units (MED Associates, St. Albans, VT, USA), between 9:00 and 15:00 h. The dimensions of each chamber were 43 × 30 × 43 cm (L × H × W). Rats were tested in squads of three whenever possible. A light bulb (100 W) was suspended on top of each field, above the central area. The open field was cleaned immediately after each session.

Surgical and infusion procedures

Surgeries were performed over a period of 4 weeks. As a result, animals started the experiment at different times. Assignment to the conditions of the experiment (contrast and lidocaine) was done so as to run them concurrently as far as possible. In preparation for surgery, animals were anesthetized (5%) and maintained (1–2%) with inhalation isoflurane. Once anesthetized, the animal's head was shaved and cleaned with betadine and alcohol 70%. To prevent eye dryness, a drop of mineral oil was applied to each eye. Animals were then set in the stereotaxic frame and a midline incision was made, the skull was cleaned, and bregma was located. Four bone anchor screws were placed in the skull to provide stability to the cannula implant. Cannula guides were placed bilaterally at the following coordinates (Paxinos and Watson, 2007): −2.4 AP, ±4.2 ML, and −7.0 D/V. When inserted for microinfusion, cannulae were lowered for an additional millimeter. Dental cement was used to provide stability to the implant. Buprenorphine (0.4 mg/kg, sc) was administered to alleviate pain induced by the surgery. Animals were then housed individually in polycarbonate cages until fully recovered from the anesthetic and then placed back in their home cages. Rats were allowed 5–8 days for recovery from surgery. During this period, their weight was gradually brought to the target 81–84% deprivation.

Lidocaine hydrochloride (Sigma–Aldrich, Saint Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) (10 μg/0.25 μL, 40 μg/μL) was bilaterally infused before key sessions in each of the three testing procedures (see below). This lidocaine dose was chosen following Parent and McGaugh (1994). Animals were placed in a polycarbonate cage without the lid and 10-μL syringes (Hamilton, Reno, NV, USA) attached to polyethylene tubing were connected to the cannulae. The cannulae were then inserted into the cannula guides and lidocaine (or an equal volume, equal speed infusion of PBS) was administered by activating an infusion pump (KDScientific, Model KDS 232 CE, Holliston, MA, USA). The infusion pump was programed to deliver the lidocaine or PBS at a rate of 0.25 μL/min, for 1 min. An additional minute was allowed for the fluid to diffuse in brain tissue. The cannulae were then removed, the guides were blocked with an occluder, and the animal was placed in the conditioning box or open field. The session started no more than 3 min after the cannulae were removed from the guides.

Training procedures

cSNC. Training started once animals recovered from surgery and reached the 81–84% target deprivation weight. Animals were tested in three tasks administered in the same order: cSNC, ANC, and open-field activity. Before the start of cSNC training, animals were randomly assigned to one of two conditions, depending on the sucrose concentration during preshift sessions. Half the animals (n = 24) received access to 32% sucrose on sessions 1–10 and then were downshifted to 4% sucrose on sessions 11–15. The rest of the animals (n = 24) received access to 4% sucrose on sessions 1–15. Animals were transported to a waiting room in squads of four. The composition of each squad and the assignment to a training box were maintained constant, but the order in which squads were run was changed randomly across days to minimize the effects of cues related to the sequential regularity of running (e.g., individual odors). Each session started and ended with the sipper tube retracted during an interval averaging 30 s (±15). All sessions lasted 5 min starting from the first recorded contact with the sipper tube. During the session, the house light, white noise, and fan were on continuously. At the end of a session, animals were placed back in their cages and the conditioning boxes were wiped with a damp paper towel, feces removed, and bedding material replaced as needed. Sufficient food to maintain target body weights was delivered in the home cage not less than 15 min after the squad ended its daily training session. Sucrose solutions were prepared weight by weight by mixing 32 (or 4) g of commercial sugar for every 68 (or 96) g of distilled water. Lick frequency (i.e., total number of licks in the 5-min session) was the dependent variable.

In preparation for the microinfusion procedure, animals were placed under the same conditions before each session, but not given any infusions. This was done to habituate rats to the handling and general stimulation that was to occur during the microinfusion procedure. Before session 11, all animals received a microinfusion of either lidocaine or PBS (equal volume and delivery rate).

ANC. Training in the ANC situation started a day after the last session of the cSNC task with a subset of all the animals. Animals showing apparent signs of implant deterioration were removed from the experiment and prepared for histological analysis immediately after cSNC training. As a result, only a subset of 19 animals received training in the ANC situation. Nonetheless, histological analysis showed that there were no differences between the brains of animals sacrificed early and those sacrificed later. Animals were randomly assigned to the new conditions (i.e., irrespective of prior assignment to the cSNC situation). Of the 11 animals assigned to Group 4–32, six had downshift experience and five had unshifted experience in the previous cSNC task. Of the eight animals assigned to Group 4–4, two had downshifted experience and six had unshifted experience in the previous cSNC task. Unequal sample size across groups resulted from the fact that animals started the experiment not all at the same time and from the results of the histological analysis of cannula placements.

Animals were trained in the same conditioning boxes used for cSNC training. An additional sipper tube was placed in the front wall, to the right of the sipper tube used during cSNC training. The original sipper tube always delivered 4% sucrose, whereas the new sipper tube delivered 32% or 4% sucrose depending on the group. There were two groups in this phase of the experiment. Group 4–4 received access to two solutions

per day, each lasting 3 min from the first recorded lick and separated by a 30-s intersolution interval. Animals had access to 4% sucrose in both bottles. Group 4–32 received the same arrangement, except that the second daily bottle provided access to 32% sucrose. After 10 daily sessions, all animals received one infusion of lidocaine and one of PBS in counterbalanced order, immediately before the session and with the same general procedure used in the previous phase. One day without infusions was interpolated between these two infusion days. Thus, whereas the contrast condition (4–4 vs. 4–32) was a between-subject factor, the infusion condition (lidocaine vs. PBS) was a within-subject factor. Lick frequency was the dependent measure.

Open field. The day after the end of the ANC training phase, the same subset of animals was exposed to the open field for a single 20-min session. Immediately before this session, each animal received an infusion of either lidocaine or PBS. Of the 12 animals assigned to the LID condition, eight had been in Group 4–32 and four in Group 4–4 during the previous ANC task. Of the seven animals assigned to the PBS condition, three had been in Group 4–32 and four in Group 4–4 during the previous ANC task. At the start of the trial, the rat was placed in the center of the open field and allowed free movement. Distance traveled (cm), the dependent measure, was recorded automatically in 5-min bins by a computer located in an adjacent room.

Histology

The day after the last training session, either in the cSNC phase or in the open-field phase, all animals were sacrificed with an overdose of CO_2 and the brains were immediately extracted and embedded in 4% paraformaldehyde for at least 3 days. Brains were then embedded in 30% sucrose for at least 2 days and sectioned with a cryostat in 40-μm slices. Slices were stained with cresyl-violet and photographed in an Olympus CX41 light microscope with Q-Color 3 digital camera; an adaptor and Image-Pro Express were used for image capture and analysis.

Statistics

The dependent variables were subjected to an analysis of variance with an alpha value set at the 0.05 level. Pairwise comparisons using the LSD test were derived from the main analysis whenever justified by appropriate significant interactions. The IBM SPSS package (Version 21) was used to compute all the statistics. For brevity, only significant F and p values are reported in the text.

RESULTS

Cannula placement

Fig. 1 shows the placements of the cannula tips. For 15 rats (six in lidocaine groups, nine in PBS groups), the cannulae were located in the wrong position in one or both hemispheres, and therefore their behavioral data were not included in the analyses. Thus, a total of 33 animals were included in the behavioral analyses with cannulae located in the centromedial amygdala in both hemispheres.

cSNC

Fig. 2 shows the results of the cSNC phase of training with the final sample size for each group. Preshift performance was nondifferential across groups assigned to the sucrose and infusion conditions. A Sucrose (32%, 4%) × Infusion (LID, PBS) × Session (1–10) analysis revealed only a significant increase across sessions, $F(9,261) = 18.535$, $p < 0.001$. Fig. 2 only shows the final preshift session; an analysis of just these data also indicated nonsignificant differences.

Downshifted animals infused with lidocaine before session 11 exhibited higher licking frequency than downshifted PBS controls, but the infusion manipulation did not affect the performance of unshifted controls. A Contrast (downshifted, unshifted) × Infusion × Session (11–15) analysis yielded a significant triple interaction, $F(4,116) = 2.750$, $p < 0.04$. There was also a significant contrast by session interaction, $F(4,116) = 6.168$, $p < 0.001$, and a significant change across sessions, $F(4,116) = 5.527$, $p < 0.001$. All other effects were nonsignificant. Pairwise LSD tests revealed that the source of the triple interaction was a significant difference between Groups 32/PBS and 4/PBS on session 11, $F(1,29) = 9.829$, $p < 0.005$. The same comparison between Groups 32/LID and 4/LID indicated nonsignificant differences for all postshift sessions. Further analyses were calculated separately for downshifted and unshifted groups. Thus, for Groups 32/LID and 32/PBS, there was a significant infusion by session interaction, $F(4,64) = 3.575$, $p < 0.02$, which originated from a higher lick frequency on session 11 by animals infused with lidocaine relative to PBS controls, as shown by LSD pairwise tests, $F(1,16) = 6.147$, $p < 0.03$. A similar comparison of Groups 4/LID and 4/PBS produced nonsignificant effects. Thus, as predicted, lidocaine infusions in the centromedial amygdala eliminated the cSNC effect. There was a hint of a retardation effect on subsequent recovery in lidocaine-treated, downshifted animals, but this trend was not supported statistically.

ANC

Fig. 3 shows the main results of this phase with the sample size for each group. All animals in Groups 4–32 and 4–4 received infusions of both lidocaine and PBS in counterbalance order. Contrast (4–32, 4–4) × Infusion analyses were calculated for the lick frequency during the first bottle (the critical bottle for an ANC effect) and the second bottle. There was a significant ANC effect on the first bottle with lower lick frequency in Group 4–32 than in Group 4–4, $F(1,17) = 5.458$, $p < 0.04$. However, the contrast by infusion interaction was negligible, $F < 1$. Thus, there was no evidence that the lidocaine infusion had an effect on ANC. A similar

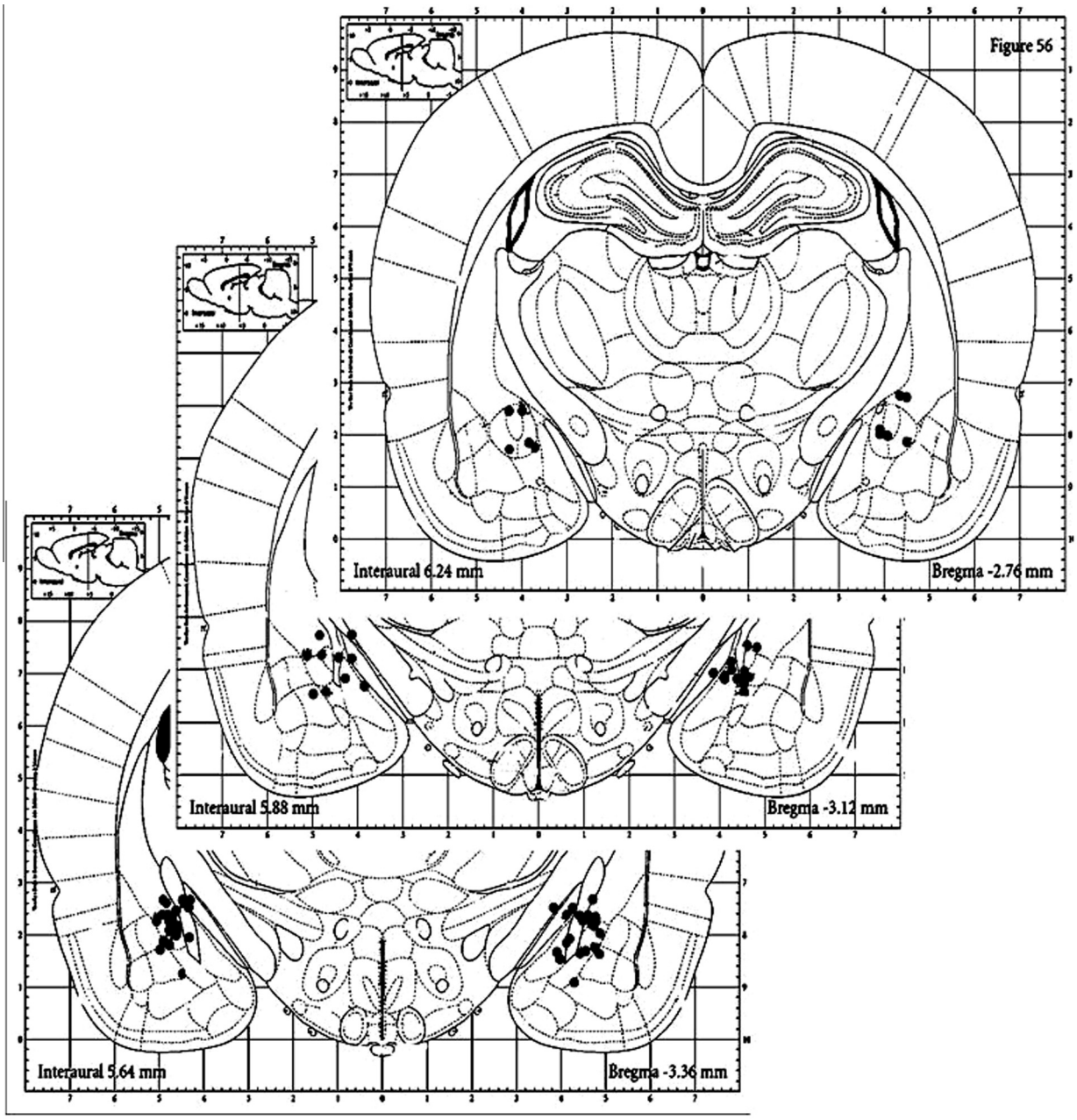

Fig. 1. Location of the infusion in all animals for which the tip of the cannulae was located in the centromedial amygdala in both hemispheres. Brain maps from Paxinos and Watson (2007).

analysis for the second bottle yielded nonsignificant effects.

Open field

The same subset of animals assigned to the ANC task was also used in the open-field test. Animals were again assigned randomly with respect to previous experience, but because they did not become available for open-field testing at the same time and given the histological results, groups are not equal in sample size. Fig. 4 shows the final sample size and the results in terms of distance traveled. The location of the animal was taken into account by dividing the field into a central location and a peripheral area adjacent to the walls of the apparatus. Infusion (LID, PBS) × Bin (1–4) analyses were computed for each dependent variable. Distance traveled in the central area of the open field (Fig. 4, top) yielded a significant interaction, $F(3,51) = 4.18$, $p < 0.02$. The main effects for infusion and bin were also significant, Fs > 5.98, ps < 0.03. LSD pairwise tests derived from the main analysis indicated that the groups were different during the initial 5 min of open-field testing, $F(1,17) = 6.45$, $p < 0.03$. Distance traveled in the periphery yielded similar results, except that the infusion by bin interaction fell short of significance, $F(3,51) = 2.47$, $p < 0.08$. Overall, however, lidocaine animals showed more activity than

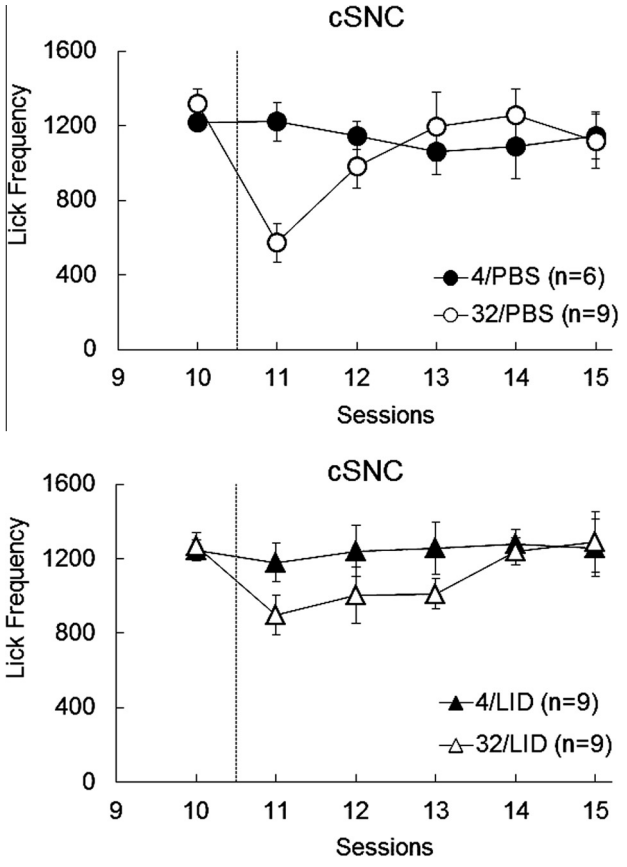

Fig. 2. Lick frequency during the last preshift session (10) and all postshift sessions (11–15) in groups receiving PBS (top) or lidocaine (LID, bottom) before session 11. PBS groups exhibited a significant cSNC effect on session 11 (the cSNC effect). All other comparisons were nonsignificant.

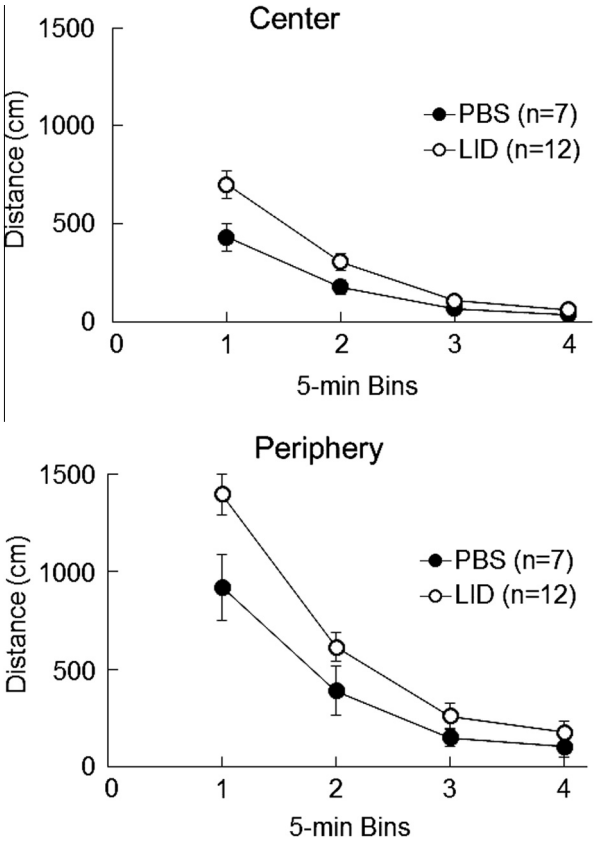

Fig. 4. Distance traveled during the 20-min open-field session presented in 5-min bins and separately for the central and peripheral areas in groups receiving a presession infusion of PBS or lidocaine (LID). Lidocaine caused a significant increase in distance traveled in the central area and in the periphery, especially during the initial 5 min of the session.

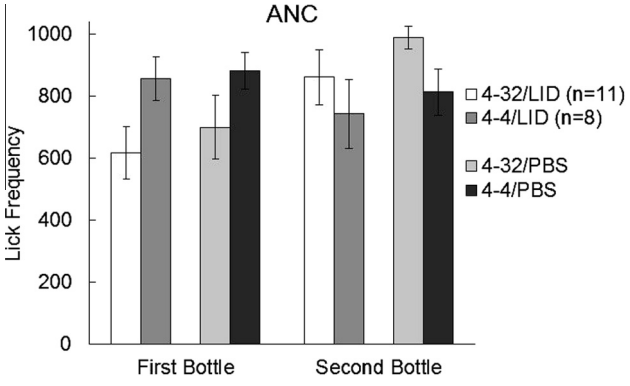

Fig. 3. Lick frequency during test sessions with either PBS or lidocaine (LID, within-subject factor), and shifted or unshifted (between-subject factor), on the first and second bottles. On the first bottle, there was a significant effect between groups (the ANC effect), but the infusion factor was not significant. Group differences were nonsignificant on the second bottle.

LPS animals, $F(1,17) = 6.87$, $p < 0.02$. There was also a significant reduction of activity across the session, $F(3,51) = 65.01$, $p < 0.001$. Thus, centromedial amygdala inactivation resulted in higher levels of locomotor activity in both the central and peripheral areas of the open field, although the effect was most pronounced during the initial 5 min of the session.

DISCUSSION

Transient inactivation of the centromedial amygdala led to the following behavioral consequences. First, lidocaine infusions in the centromedial amygdala reduced the suppression of consummatory behavior induced by a 32% to 4% sucrose downshift, but had no noticeable effects in 4% sucrose unshifted controls. This reduction of consummatory suppression was detected both in terms of a significant difference between downshifted lidocaine and PBS groups, and in terms of a nonsignificant difference between downshifted and unshifted lidocaine groups (downshifted and unshifted PBS groups exhibited the cSNC effect). It should be noted that the cSNC effect was relatively short, lasting only one session. There is variability in the strength of this effect across experiment, a fact that might relate to the different recovery profiles recently identified in a reanalysis of published data using latent growth mixture modeling (Papini et al., 2014). Whether stronger results of centromedial amygdala inactivation would have been observed had the cSNC effect been longer lasting remains

to be determined. Second, there was no evidence that centromedial amygdala inactivation affected performance in the ANC situation. Third, animals infused with lidocaine in the centromedial amygdala also displayed a higher level of ambulatory behavior in the open field. Because the effects of lidocaine on open-field activity were observed after testing animals in the ANC situation, the lack of effect in the ANC test cannot be attributed to failures of lidocaine or amygdala damage during the cannulation process. The lack of a selective effect of lidocaine on central area activity may suggest that the effects were not specific to unconditioned fear. However, the open field was well lighted, a fact that may have made it difficult to discriminate effects on central vs. peripheral areas (Bouwknecht et al., 2007).

These results are consistent with the view that the amygdala is an important component of the circuit activated by reward devaluation, but mainly when such devaluation involves negative emotion. A reduction in the negative behavioral effects of reward devaluation may be accomplished by interfering with the reactivation of the preshift reward expectancy or by interfering with the output from the comparison between current and expected reward magnitudes. The present results do not distinguish between these possibilities; nonetheless, we center our discussion in the latter. There is substantial evidence that the cSNC effect is accompanied by a negative emotional response (Papini, 2003, 2014; Torres and Sabariego, 2014; Papini et al., 2015). In reward devaluation situations, changes in consummatory behavior are accompanied by the release of stress hormones (Mitchell and Flaherty, 1998; Pecoraro et al., 2009), changes in aggressive and sexual behavior (Mustaca et al., 2000; Freidin and Mustaca, 2004), hypoalgesia (Mustaca and Papini, 2005), and postsession increase in oral consumption of anxiolytics (Manzo et al., 2014, 2015). In turn, such suppression is modulated by opioids (Papini, 2009), cannabinoids (Genn et al., 2004), and benzodiazepine anxiolytics (Flaherty et al., 1986; Ortega et al., 2014), and influenced by posttraining administration of memory enhancers, including corticosterone and D-cycloserine (Bentosela et al., 2006; Ruetti et al., 2009; Norris et al., 2011).

By contrast, the ANC effect, which also involves a devaluation of 4% sucrose by anticipation of 32% sucrose, does not seem to be accompanied by negative emotion (Flaherty, 1996). Apparently, in the ANC situation the 4% sucrose solution becomes a signal for the higher value solution that follows. Thus, although involving the same solutions, the ANC situation may be described as an appetitive situation resembling Pavlovian conditioning. Consistent with this view, increasing the intertrial interval between access to 4% and 32% sucrose (analogous to increasing the delay between conditioned and unconditioned stimuli) reduces the ANC effect (Flaherty et al., 1991). The lack of evidence of an effect of centromedial amygdala infusions on ANC reported here must be taken with caution. In the present experiment, several factors may have conspired against detecting an effect, including prior experience in the cSNC situation. Moreover, the effects of centromedial amygdala inactivation were tested in the expression of ANC, rather than during its acquisition, a factor that may also explain the lack of effect (Groshek et al., 2005). Moreover, if the ANC effect represents a form of conditioning and given that the amygdala is involved in conditioning, why did we fail to observe any effects in this experiment? Evidence reviewed by Savage and Ramos (2009) suggests that the BLA is critically involved in situations demanding comparisons between rewards, but the role of the CeA is less clear. The CeA does not seem to be involved in simple appetitive conditioning situations (e.g., Tronel and Sara, 2002; Chang et al., 2012) or even in situations involving surprising increases in reward (Holland, 2006), both analogous to the ANC procedure used here. However, CeA lesions impair orienting responses to appetitive signals (Gallagher et al., 1990) and disrupt the ability of an appetitive Pavlovian signal to control appetitive instrumental behavior in a Pavlovian-to-instrumental transfer situation (Holland and Gallagher, 2003). Thus, the conclusion that centromedial amygdala infusions do not impair ANC is considered tentative at this point.

A significant role of the amygdala in the cSNC situation is also suggested by studies assessing cellular activity after a 32% to 4% sucrose downshift. Using c-Fos, a marker of cellular activity, Pecoraro and Dallman (2005) reported heightened activity after the first downshift session. More recently, elevated levels of pCREB, a marker of synaptic plasticity (Kida and Serita, 2014), were reported also after the first downshift session in the CeA (Glueck et al., 2014). The present results, however, caution against a selective role of the amygdala in the cSNC effect. In addition to being involved in fear conditioning (Helmstetter et al., 2008), the present results showed that the amygdala is also required for open-field performance. Activity in the open field, particularly in its central area, is usually viewed as indexing unconditioned fear, anxiety, or conflict between exploring and minimizing threat (e.g., Ramos, 2008). Thus, it is tempting to conclude that one critical role of the centromedial amygdala in the cSNC situation is to tag the downshift experience with negative emotional valence.

CONCLUSIONS

Prior research with the iSNC and cSNC situations suggested that the amygdala plays an important role in reward devaluation. However, the specific function was not clearly established because boundary conditions had not been explored. The present research made two novel contributions. First, it tested the effect of reversible amygdala inactivation of the centromedial region on the cSNC effect and, second, it extended the behavioral testing to two additional tasks, one involving reward devaluation in an appetitive Pavlovian context (ANC) and another involving negative emotion in the absence of reward devaluation (open-field activity). As expected, centromedial amygdala inactivation before the first 32% to 4% sucrose devaluation reduced the behavioral impact of the reward downshift. Moreover, the same treatment had no detectable effect on ANC, even though the same rewards were involved, albeit in a different arrangement. Finally, centromedial

inactivation increased activity in the central area of an open field, an indication of reduced unconditioned fear of open spaces. These results suggest that the amygdala's function is to assign negative emotional valence to the devaluation experience, a function that it may share with other situations inducing negative emotions, such as those involving threats.

Acknowledgments—A.C. Glueck and I. Annicchiarico contributed equally to the research reported in this article. Partial support for this research was received from Sabbatical funds from Hoshi University, Japan, to KK, and from TCU/RCAF # 60672, to MRP. IA was supported by a Fulbright/Colciencias (Colombia) scholarship. The authors thank Brandon Grant for assisting with the maintenance of the animals and the histology portion of the study.

REFERENCES

Becker HC, Jarvis M, Wagner G, Flaherty CF (1984) Medial and lateral amygdala lesions differentially influence contrast with sucrose solutions. Physiol Behav 33:707–712.

Bentosela M, Ruetti E, Muzio RN, Mustaca AE, Papini MR (2006) Administration of corticosterone after the first downshift session enhances consummatory successive negative contrast. Behav Neurosci 120:131–136.

Bouwknecht JA, Spiga F, Staub DR, Hale MW, Shekhar A, Lowry CA (2007) Differential effects of exposure to low-light or high-light open field on anxiety-related behaviors; relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. Brain Res Bull 72:32–43.

Chang SE, Wheeler DS, Holland PC (2012) Effects of lesions of the amygdala central nucleus on autoshaped lever pressing. Brain Res 1450:49–56.

Flaherty CF (1996) Incentive relativity. Cambridge, UK: Cambridge University Press.

Flaherty CF, Driscoll C (1980) Amobarbital sodium reduces successive gustatory contrast. Psychopharmacology 69:161–162.

Flaherty CF, Rowan GA (1988) Effects of intersolution interval, chlordiazepoxide, and amphetamine on anticipatory contrast. Anim Learn Behav 16:47–52.

Flaherty CF, Grigson PS, Rowan GA (1986) Chlordiazepoxide and the determinants of contrast. Anim Learn Behav 14:315–321.

Flaherty CF, Grigson PS, Lind S (1990) Chlordiazepoxide and the moderation of the initial response to reward reduction. Quart J Exp Psychol 42B:87–105.

Flaherty CF, Grigson PS, Checke S, Hnat KC (1991) Deprivation state and temporal horizons in anticipatory contrast. J Exp Psychol: Anim Behav Proc 17:503–518.

Flaherty CF, Coppotelli C, Grigson PS, Mitchell C, Flaherty JE (1995) Investigation of the devaluation interpretation of anticipatory negative contrast. J Exp Psychol: Anim Behav Proc 21:229–247.

Flaherty CF, Coppotelli C, Hsu D, Otto T (1998) Excitotoxic lesions of the hippocampus disrupt runway but not consummatory contrast. Behav Brain Res 93:1–9.

Freet CS, Tesche JD, Tompers DM, Riegel KE, Grigson PS (2006) Lewis rats are more sensitive than Fischer rats to successive negative contrast, but less sensitive to the anxiolytic and appetite-stimulating effects of chlordiazepoxide. Pharmacol Biochem Behav 85:378–384.

Freidin E, Mustaca AE (2004) Frustration and sexual behavior in male rats. Learn Behav 32:311–320.

Gallagher M, Graham PW, Holland PC (1990) The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned performance. J Neurosci 10:1906–1911.

Genn RF, Tucci S, Parikh S, File SE (2004) Effects of nicotine and a cannabinoid receptor agonist on negative contrast: distinction between anxiety and disappointment? Psychopharmacology 177:93–99.

Glueck AC, Dennis T, Perrotti L, Torres C, Papini MR (2014) Brain expression of pCREB in rats exposed to reward devaluation. Neurosci Lett 587:93–97.

Groshek F, Kerfoot E, McKenna V, Polackwich AS, Gallagher M, Holland PC (2005) Amygdala central nucleus function is necessary for learning, but not expression, of conditioned auditory orienting. Behav Neurosci 119:202–212.

Hale MW, Bouwknecht JA, Spiga F, Shekhar A, Lowry CA (2006) Exposure to high- and low-light conditions in an open-field test of anxiety increases c-Fos expression in specific subdivisions of the rat basolateral amygdaloid complex. Brain Res Bull 71:174–182.

Helmstetter FJ, Parsons RG, Gafford GM (2008) Macromolecular synthesis, distributed synaptic plasticity, and fear conditioning. Neurobiol Learn Mem 89:324–337.

Holland PC (2006) Enhanced conditioning produced by surprising increases in reinforcer value are unaffected by lesions of the amygdala central nucleus. Neurobiol Learn Mem 85:30–35.

Holland PC, Gallagher M (2003) Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. Eur J Neurosci 17:1680–1694.

Kida S, Serita T (2014) Functional roles of CREB as a positive regulator in the formation and enhancement of memory. Brain Res Bull 105:17–24.

Leszczuk MH, Flaherty CF (2000) Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats. Behav Brain Res 116:61–79.

Liao R-M, Chuang F-J (2003) Differential effects of diazepam infused into the amygdala and hippocampus on negative contrast. Pharmacol Biochem Behav 74:953–960.

Manzo L, Gómez MJ, Callejas-Aguilera JE, Fernández-Teruel A, Papini MR, Torres C (2014) Anti-anxiety self-medication induced by incentive loss in rats. Physiol Behav 123:86–92.

Manzo L, Donaire R, Sabariego M, Papini MR, Torres C (2015) Anti-anxiety self-medication in rats: oral consumption of chlordiazepoxide and ethanol after reward devaluation. Behav Brain Res 278:90–97.

Mitchell C, Flaherty CF (1998) Temporal dynamics of corticosterone elevation in successive negative contrast. Physiol Behav 64:287–292.

Mustaca AE, Papini MR (2005) Consummatory successive negative contrast induces hypoalgesia. Int J Comp Psychol 18:255–262.

Mustaca AE, Martinez C, Papini MR (2000) Surprising nonreward reduces aggressive behavior in rats. Int J Comp Psychol 13:91–100.

Norris JN, Ortega LA, Papini MR (2011) Postsession D-cycloserine enhances the emotional memory of an incentive downshift event. Behav Brain Res 223:348–355.

Ortega LA, Glueck AC, Daniel AM, Prado-Rivera MA, White MM, Papini MR (2014) Memory interfering effects of chlordiazepoxide on consummatory successive negative contrast. Pharmacol Biochem Behav 116:96–106.

Papini MR (2003) Comparative psychology of surprising nonreward. Brain Behav Evol 62:83–95.

Papini MR (2009) Role of opioid receptors in incentive contrast. Int J Comp Psychol 22:170–187.

Papini MR (2014) Diversity of adjustments to reward downshifts in vertebrates. Int J Comp Psychol 27:420–445.

Papini S, Gallatzer-Levy IR, Papini MR (2014) Identifying profiles of recovery from reward devaluation in rats. Behav Brain Res 275:212–218.

Papini MR, Fuchs PN, Torres C (2015) Behavioral neuroscience of psychological pain. Neurosci Biobehav Rev 48:53–69.

Pare WP (1994) Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. Physiol Behav 55:433–439.

Parent MB, McGaugh JL (1994) Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. Brain Res 661:97–103.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. 6th ed. New York: Academic Press.
Pecoraro N, Dallman MF (2005) C-Fos after incentive shifts: expectancy, incredulity, and recovery. Behav Neurosci 119:366–387.
Pecoraro N, de Jong H, Dallman MF (2009) An unexpected reduction in sucrose concentration activates the HPA axis on successive post shift days without attenuation by discriminative contextual stimuli. Physiol Behav 96:651–661.
Pellegrini S, Muzio RN, Mustaca AE, Papini MR (2004) Successive negative contrast after partial reinforcement in the consummatory behavior of rats. Learn Motiv 35:303–321.
Ramos A (2008) Animal models of anxiety: do I need multiple tests? Trends Pharmacol Sci 29:493–498.
Rosen AJ, Tessel RE (1970) Chlorpromazine, chlordiazepoxide and incentive shift performance in the rat. J Comp Physiol Psychol 72:257–262.
Ruetti E, Justel N, Mustaca AE, Papini MR (2009) Postsession corticosterone administration enhances the effects of incentive downshift: exploring the boundaries of this effect. Behav Neurosci 123:127–144.
Salinas JA, McGaugh JL (1996) The amygdala modulates memory for changes in reward magnitude: involvement of the amygdaloid GABAergic system. Behav Brain Res 80:87–98.
Salinas JA, Packard MG, McGaugh JL (1993) Amygdala modulates memory for changes in reward magnitude: reversible post-training inactivation with lidocaine attenuates the response to a reduction in reward. Behav Brain Res 59:153–159.
Salinas JA, Parent MB, McGaugh JL (1996) Ibotenic acid lesions of the amygdala basolateral complex or central nucleus differentially affect the response to reductions in reward. Brain Res 742:283–293.
Sastre A, Reilly S (2006) Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats. Behav Brain Res 170:34–40.
Savage LM, Ramos RL (2009) Reward expectation alters learning and memory: the impact of the amygdala on appetitive-driven behaviors. Behav Brain Res 198:1–12.
Suarez SD, Gallup Jr GG (1981) An ethological analysis of open-field behavior in rats and mice. Learn Motiv 12:342–363.
Torres C, Sabariego M (2014) Incentive relativity: gene–environment interactions. Int J Comp Psychol 27:446–458.
Tronel S, Sara SJ (2002) Mapping of olfactory memory circuits: region-specific c-fos activation after odor-reward associative learning or after its retrieval. Learn Mem 9:105–111.
Vogel JR, Principi K (1971) Effects of chlordiazepoxide on depressed performance after reward reduction. Psychopharmacologia 21:8–12.

GLOSSARY

Amygdala: In mammals, a differentiated structure of the telencephalon, usually assigned to the limbic system and implicated in fear and fear conditioning, among other functions. Several regions can be distinguished anatomically, including the central, medial, basolateral, and intercalated cell masses, which may also be functionally differentiated

Anticipatory negative contrast (ANC): A consummatory task involving two trials per daily session. On trial 1, consumption of a small reward (e.g., 4% sucrose) is reduced when this trial is followed by access to a large reward (e.g., 32% sucrose) on trial 2, compared to a control receiving the small reward in both daily trials (4-to-4% sucrose). Although trial 1 involves the same reward in both conditions, the expectation of a large reward on trial 2 reduces consummatory behavior of the small reward relative to the unshifted control

Consummatory successive negative contrast (cSNC): A consummatory task involving a single session per day. Access to a large reward (e.g., 32% sucrose) during several daily sessions is followed by access to a small reward (e.g., 4% sucrose). The behavior of downshifted animals is compared to that of unshifted controls always receiving access to the small reward. During these final sessions, although both groups receive the same reward magnitude, downshifted animals exhibit a significant reduction in consummatory behavior relative to unshifted controls

Incentive contrast: A family of tasks sharing a process of comparison between rewards of different value. The typical outcome in these situations is that response to a given reward depends on other rewards that occur previously or simultaneously, or that are expected to occur in the current situation

Instrumental successive negative contrast (iSNC): An instrumental task involving one or several trials per daily session. Animals that learn to perform an instrumental response (e.g., running toward a goal box in a runway) for a large reward (e.g., 12 food pellets), later exhibit disruption of anticipatory behavior when the reward is downshifted (e.g., two food pellets), relative to the anticipatory behavior of an unshifted control always reinforced with the small reward

Open-field task: A task usually administered in a single session. The animal is released in an empty arena where it can move freely while its behavior is monitored. Rodents typically stay closed to the peripheral walls, a behavior called thigmotaxis, and avoid the central area of the field. Treatments that increase activity, especially in the central area, are usually interpreted as reducing unconditioned fear, anxiety, or conflict

Reward devaluation: A procedure involving a (usually unexpected) reduction to a nonzero reward magnitude. Provided the reduction is significant, such devaluation induces signs of negative emotion, including disruption of goal approach

(Accepted 24 June 2015)
(Available online 30 June 2015)