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Transient inactivation of basolateral amygdala during selective satiation disrupts reinforcer devaluation in rats

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Abstract

Basolateral amygdala (BLA) function is critical for flexible, goal-directed behavior, including performance on reinforcer devaluation tasks. Here we tested, in rats, the hypothesis that BLA is critical for conditioned reinforcer devaluation during the period when the primary reinforcer (food) is being devalued (by feeding it to satiety), but not thereafter for guiding behavioral choices. We used a spatially-independent task, which employed two visual cues, each predicting one of two foods. An instrumental action (lever press) was required for reinforcer delivery. After training, rats received BLA or sham lesions, or cannulae implanted in BLA. Under control conditions (sham lesions, saline infusions), devaluation of one food significantly decreased responding to the cue associated with that food, when both cues were presented simultaneously during extinction. BLA lesions impaired this devaluation effect. Transient inactivation of BLA by microinfusion of the GABA_A agonist muscimol resulted in an impairment, only when BLA was inactivated during satiation. When muscimol was infused after satiation and, therefore, BLA was inactivated only during the choice test, rats showed no impairment. Thus, BLA is necessary for registering or updating cues to reflect updated reinforcer values, but not for guiding choices once the value has been updated. Our results are the first to describe the contribution of rat BLA to specific components of reinforcer devaluation, and are the first to show impairment in reinforcer devaluation following transient inactivation in the rat.

Keywords

muscimol; pharmacological inhibition; lesion; reward; goal-directed behavior

Introduction

The basolateral amygdala (BLA) is critical for forming and modifying associations between sensory stimuli and salient events (Holland and Gallagher 1999). The role of BLA for behavioral adaptation has been well documented using the reinforcer devaluation task in rats (e.g. Hatfield et al., 1996, Pickens et al., 2003), monkeys (e.g., Malkova et al., 1997), and humans (e.g. Gottfried et al., 2003). In this task, subjects associate specific cues with primary reinforcers (e.g. foods), with each cue predicting one particular food. One of the foods is then “devalued” by selective satiation (feeding to satiety) or taste aversion (pairing

with illness). Following devaluation, subjects typically shift behavioral responses away from cues associated with the devalued food, even without an opportunity to experience the cue in the presence of the devalued food. This shift reflects a cognitive linking between the cue and the devalued outcome. When rats are required to form two distinct cue-reinforcer associations, this shift is impaired by BLA lesions introduced after initial cue-reinforcer training (Balleine et al., 2003; Ostlund and Balleine, 2008; Johnson et al., 2009).

Thus, once initial cue-reinforcer associations have been formed, an inability to shift behavioral choices following selective satiation could reflect failure to: (1) update the incentive value of the primary reinforcer (food), (2) link the internal representation of the cue to the reduced value of the food it predicts, and/or (3) maintain the revised cue representation to guide subsequent choices. While the necessity of BLA for these components has been examined in monkeys (Wellman et al, 2005), it has not yet been studied in rats.

To determine whether one or more of these functions is dependent on BLA in the rat, we used focal drug treatment to transiently inactivate this structure during the satiation period or during the subsequent choice of cues. Based on our previous findings in monkeys (Wellman et al., 2005), we hypothesized that, in rats, the cognitive process of linking the food devaluation to the representation of the predictive cue requires active processing in BLA during the period of selective satiation. We predicted that BLA inactivation, using the GABA_A agonist muscimol (MUS), would lead to outcomes similar to those previously observed in monkeys: (1) inactivation of BLA *during satiation* will disrupt the subsequent shift away from cues that predict the devalued food, without impairing the devaluation of the food itself and (2) inactivation of BLA after satiation, *during cue presentation*, will not alter the devaluation-induced shift in choice. In order to test the rats under conditions that approximate the task used for monkeys (Malkova et al, 1997), we used a task that we recently developed for rats that relies on visual cues, in which two different visual cues predict two different foods, with an instrumental action (lever press) required for reinforcer delivery (West et al., 2011a).

To compare our results with those previously obtained with post-training lesions of BLA in tasks that did not depend solely on visual cues (Balleine et al., 2003; Ostlund and Balleine, 2008; Johnson et al., 2009), we also tested a group of BLA-lesioned rats in our task.

Materials and Methods

Subjects

109 male Long-Evans rats (Charles River, Frederick, MD) weighing approximately 300–350 g at the start of the study were used. Rats were pair housed in the Division of Comparative Medicine at Georgetown University Medical Center in climate controlled rooms on a standard light:dark cycle (lights on 0600:1800). To provide motivational incentive for subsequent behavioral testing, rats were food restricted to 85–90% of their free-feeding body weight (15–20g chow/day/rat) with water available *ad libitum*. Food restriction began 5 days prior to the start of behavioral training and continued throughout training and testing. The study was conducted under a protocol approved by the Georgetown University Animal Care and Use Committee and in accordance with the *Guide for Care and Use of Laboratory Animals* adopted by the National Institutes of Health. Rats were trained pre-operatively using a reinforcer devaluation task that we have previously described in detail (West et al., 2011a).

Materials

Apparatus—Standard rat operant chambers (Habitest, Coulbourn Instruments, Whitehall, PA) were used for instrumental training, cue training and the instrumental probe of reinforcer devaluation. Graphic State software (Coulbourn Instruments, Whitehall, PA) was used to program the training and testing sequences and to collect and record the rats' responses. Each chamber contained two levers (Coulbourn H21-03R), with a light panel located above each lever. Each light panel consisted of three Light Emitting Diodes (LEDs) in a row. Each LED emitted a specific color light, but only two lights were used as cues in these experiments, a green light and a red light. The first cue (Cue 1) was a green light flashing once per second and the second cue (Cue 2) was a red light flashing 5 times per second. Thus, color and flash frequency distinguished the two lights. Each of the two cues appeared either above the right or the left lever, in a balanced order across trials. A food tray was located in between the levers. Both levers were present throughout training and testing. Each operant chamber was located inside a standard isolation cubicle (Coulbourn H10-24).

Standard cages (25 cm X 18 cm X 13 cm) were used for selective satiation and consummatory probe of reinforcer devaluation.

Food reinforcers—For all behavioral testing, rats received two different types of pellets, chocolate purified formula and sugar dustless precision pellets (45 mg, BioServ, Frenchtown, NJ). These two were different from each other in both flavor and nutritional content. The choice of pellets remained constant across all experimental procedures. Responses to Cue 1 consistently resulted in the delivery of a sugar pellet, whereas responses to Cue 2 consistently resulted in the delivery of a chocolate pellet.

Preoperative Behavioral Training

Instrumental training—Rats were trained to press a lever in order to receive a food pellet. The duration of each session was limited to 30 min. In the first stage of instrumental training, rats were trained on a fixed ratio (FR) schedule where one response on either lever resulted in a delivery of one food pellet (FR1). In the second and the third stage of instrumental training, rats were trained on a FR schedule where every third (FR3) and every fifth (FR5) response, respectively, led to the delivery of a food pellet. Criterion to advance to the next stage of training was set at obtaining 50 reinforcer deliveries (25 pellets of each reinforcer) per session.

Cue training—Rats were trained to press a lever with either Cue 1 or Cue 2 flashing above it, in order to receive a food pellet. There were four stages of cue training. In the first stage, rats were required to respond by pressing the lever with a cue light flashing above it (active lever) on a FR5 schedule in order to receive a food reinforcer. The cue light above the other lever was not illuminated (inactive lever). The position of the active and inactive levers alternated pseudorandomly so that the cue light could be above either the left or right lever on a given trial, in a balanced order. Each rat was allowed 60 s to respond on the active lever. In the second stage, the time to respond on the active lever was reduced to 30 s. In the third stage, each rat was allowed 30 s to respond on the active lever with a variable ratio schedule (VR9), with pellets delivered in response to between 5 and 13 lever presses. During these three stages, responses on the inactive lever were without effect and the criterion to move to the next stage was set at 200 responses on each lever (left or right). In the final stage of cue training, each rat was allowed 30 s to respond on the active lever with the pellet delivered on a VR9 schedule. At this stage, incorrect responses (i.e., responses on the inactive lever) resulted in a 10 s timeout period signaled by illumination of the house light. The percent of correct responses was determined using the formula: (number of reinforcers delivered/total number of trials)*100. Criterion level of performance was set at

85% correct over three consecutive days. When the rats reached criterion, they underwent a surgical procedure.

Surgical Procedures

Rats received either BLA lesions (n=13), BLA sham operations (n=11), or were implanted with cannulae placed in BLA (n=85) (Table 1). For the surgery, they were anesthetized with equithesin (a combination of sodium pentobarbital, chloral hydrate, magnesium sulfate, ethanol, and propylene glycol) (2.5ml/kg, i.p.) or isoflurane (1%), hair shaved, skin sterilized with betadine and ethanol, and placed into a stereotaxic frame. All stereotaxic coordinates were determined using the rat brain atlas of Paxinos and Watson (2007).

Experiment 1: BLA Lesions—Bilateral, excitotoxic lesions of the BLA were made by intracerebral infusion of *N*-methyl-D-aspartic acid (NMDA, Sigma, St. Louis, MO; 12.5 µg/µl, dissolved in saline), following the procedure previously described by Pickens et al., (2003). NMDA was infused through a 27-gauge cannula connected by polyethylene tubing to a Hamilton syringe, using an infusion pump (New Era Pumps Systems Inc, Farmingdale, NY), at a rate of 0.1 µl/min. 0.2 µl of NMDA was infused at the coordinates: 2.7 mm posterior to bregma, 5.0 mm lateral to midline, and 8.7mm ventral to the surface of the skull. An additional 0.1 µl of NMDA was infused 0.3mm dorsal to the first infusion site (i.e., 8.4mm ventral to the surface of the skull). Sham animals received identical procedures but were infused with saline rather than NMDA.

Following the completion of the drug infusion, the cannula was left in place for an additional minute to allow drug diffusion away from the cannula tip. Skin was sutured and the wound dressed with triple antibiotic ointment. Following surgery, rats were treated with carprofen (5 mg/kg, s.c.) and saline (1ml, s.c.).

Experiment 2: BLA Cannulae Placement—Rats were implanted with guide cannulae (22 gauge; Plastics One, Roanoke, VA) fitted with 28 gauge internal cannulae that extended 1 mm beyond the tip of the guide. Cannulae were placed bilaterally into BLA located at: 2.8 mm posterior to bregma, 5.2 mm lateral to midline, and 7.5 mm ventral to dura. The cannulae were fixed to skull by screws using dental acrylic (Kooliner, GC America Inc, Alsip, IL). Twenty-eight-gauge dummy cannulae (Plastics One, Roanoke, VA) cut to the same length as the guide cannulae were inserted to maintain cannula patency. Following surgery, rats were given carprofen (5 mg/kg, s.c.) and saline (1ml, s.c.).

Drugs—For all intracerebral infusions, muscimol (Sigma, St. Louis, MO) was dissolved in saline to make a 2mM solution. 1 nmol of muscimol in a volume of 0.5 µl was infused into BLA in each hemisphere. This dose and volume were previously used to transiently inactivate rat amygdala (Blair et al., 2005, Forcelli et al., 2012).

Intracerebral infusions—Intracerebral infusions were performed following the procedures previously described by Forcelli et al. (2012). Internal infusion cannulae were attached to 10.0 µl Hamilton syringes via polyethylene tubing, which was filled with saline. A small air bubble separated the saline from the drug or vehicle. Drug was infused bilaterally into BLA at a rate of 0.1 µl/min using an infusion pump (New Era Pumps Systems, Inc Farmingdale, NY), 10 minutes before behavioral testing.

The amount of muscimol (1 nmol) in the volume used (0.5 µl) has been shown to spread approximately 1mm over the course of an hour (Allen et al., 2008; Martin & Ghez, 1999). The time from infusion to completion of all experiments for any given rat was always less than 1 hr, thus, we expected that during the testing BLA would be effectively inactivated.

Postoperative Behavioral Training and Testing

Postoperatively, rats were retrained to criterion on the final stage of the cue training. Further experimental testing followed a timeline, which is outlined for each group in Figure 1 and is further described below. The numbers of rats used in each experimental group are shown in Table 1.

Selective satiation—For selective satiation, each rat was placed in a standard cage, presented with a Petri dish filled with 25 g of one of the two foods, and monitored until it ceased eating. All rats finished eating the food within 30 minutes. The amount of food each rat consumed was measured by weighing the remaining food and subtracting it from the original amount. Satiation was followed either by an instrumental probe (described below) or a consummatory probe (described below).

Instrumental probe of reinforcer devaluation—Following selective satiation with one of the food reinforcers (e.g., chocolate pellets), rats were given a 5 min instrumental probe during which the cues above both levers were illuminated simultaneously. Cue 1 and Cue 2 were counterbalanced across the lever positions so that Cue 1 could be located over the right lever (Cue 2 on left) or over the left lever (Cue 2 on right). Rats were allowed a maximum of 15 s to respond in each trial on a VR9 schedule. Once the rat responded on the lever, the pellet dispenser was activated, but no pellet was delivered. If the rat did not respond within 15s, another trial was initiated. During each trial, the cue light was active until the end of the trial (i.e., pellet dispenser activation or 15s). To prevent rats from experiencing the current (i.e., decreased) value of the food reinforcer in the presence of the cues, the probe was conducted under extinction (i.e., no pellets were delivered during the test). The total number of lever presses associated with each cue (Cue_D or Cue_{ND}) was recorded. After finishing one session of the instrumental probe, rats were retrained to criterion (85% correct) in the final stage of the cue training. Subsequently (at least 2 days later), the alternative reinforcer (e.g., sugar pellets) was devalued and rats were tested again on the instrumental probe. The order of the food used for the devaluation was counterbalanced within each treatment group.

The cumulative number of responses on the lever associated with each cue was recorded across each instrumental probe session. Two-way ANOVA, with instrumental probe (satiation with sugar pellets versus satiation with chocolate pellets) and devaluation status (responses to Cue_D versus Cue_{ND}) as repeated measures, showed no significant effect of the probe ($F_{1,9}=1.16$, $p=0.31$; $F_{1,12}=0.68$, $p=0.42$, for BLA shams and saline infusions, respectively) or probe by devaluation status interaction ($F_{1,12}=4.38$, $p=0.07$; $F_{1,12}=3.49$, $p=0.09$, for BLA shams and saline infusions, respectively). Therefore, the cumulative number of responses on the lever associated with each cue was summed across both instrumental probe sessions. Thus, the total number of responses to Cue_D equaled the number of responses to Cue 1 (paired with sugar pellets) when sugar pellets were devalued plus the number of responses to Cue 2 (paired with chocolate pellets) when chocolate pellets were devalued.

Consummatory probe of reinforcer devaluation—10 minutes following selective satiation, each rat was given access to two Petri dishes, one filled with 25 g of the satiated food (e.g. chocolate pellets) and the other filled with 25 g of the non-satiated food (sugar pellets). Rats were given 30 min to consume the food. The amount of each type of food pellets consumed was measured by subtracting the food remaining from the original amount. At least 2 days later, rats were satiated on the other food. The total amount of devalued (satiated) food pellets (in grams) consumed after each devaluation was then summed for

each rat across the two sessions and compared with the total amount of non-devalued food pellets consumed across the two sessions.

Experimental Schedule

Experiment 1: BLA lesions—Figure 1A shows the experimental timeline for rats with BLA and BLA sham lesions. Rats in these two groups were tested in two instrumental probe sessions, each preceded by satiation with one of the two foods, in a counterbalanced order. Between the two instrumental probes, rats were retrained on the final stage of the cue training until they re-attained criterion (85%), which took between 17 days. Following the second instrumental probe, they underwent two consummatory probe sessions, one with each of the two foods, separated by at least two days. The order of the food choice for the selective satiation was counterbalanced across rats.

Experiment 2: BLA inactivation—For the inactivation experiments, all rats were implanted with bilateral cannulae aimed at BLA and were divided in seven groups as outlined in Table 1. All groups received either MUS or saline infusions; four groups were tested only in the instrumental probe sessions and three groups only in the consummatory probe sessions. Rats in each group were tested in two probe sessions, each following satiation with one of the two foods. Figure 1B shows the experimental timeline for the four groups that were tested in the instrumental probes. Figure 2 outlines the timing of the drug/saline infusions with respect to the probe sessions. Before the instrumental probe session, three groups received MUS infusions in BLA, each group following one of the time schedules: (A) MUS infusions before satiation, (B) MUS infusions after satiation with no delay, (C) MUS infusions after satiation with a 30-minute delay. As shown in Figure 2 there was an equivalent duration of time between drug infusion and the probe session for the groups that followed the time schedules (A) and (C) allowing for the drug to spread within the same extent of the brain tissue in BLA in both groups. The group tested under the time schedule (B) matched (more closely) the group tested under (A) with respect to the time after satiation. The fourth group consisted of all rats that received only saline infusions, each animal following one of the three time schedules, pooled together.

Of the three groups that were tested only in the consummatory probe sessions, two received MUS infusions, one group before satiation and one group after satiation, and one group received saline infusions. Because the conditions MUS before satiation and MUS after satiation with a 30-minute delay were matched with respect to the time allowed for the drug spread within BLA, we did not include a separate group for the latter condition. Rats in the saline group received infusions either before satiation or after satiation and their data were pooled together.

Histological verification of lesions and cannulae placement

Following the completion of behavioral testing, rats with BLA and BLA sham lesions were perfused transcardially with saline followed by 10% formalin, rats with cannulae were decapitated, both procedures performed under equithesin anesthesia. Coronal cryosections (40 μ m thick) were cut on a cryostat. The sections were stained with cresyl violet acetate. Microscopic examination was used to evaluate the extent of lesions and the placement of the infusion cannulae.

Data Analysis

All data analyses were performed using SPSS software. In the instrumental probe, nine trials were the minimum number of trials that all rats completed. Thus, the cumulative number of responses to Cue_D and Cue_{ND}, respectively, across trials 1–9 were recorded. For all groups, the data were analyzed by within-subject ANOVA with repeated measures for the

devaluation status (Cue_D and Cue_{ND}), followed by Bonferroni-Holm step-down corrected planned comparisons (one-tailed).

In the consummatory probe, the total amounts of the non-devalued food and the devalued food, respectively, consumed after selective satiation, were recorded. For all groups, the data were analyzed by within-subject ANOVA with repeated measures for the devaluation status (Cue_D and Cue_{ND}), followed by Bonferroni-Holm step-down corrected planned comparisons (one-tailed).

Devaluation index—To overcome the variability in the total number of lever presses between rats, we transformed the data for each rat into a devaluation index. It was calculated according to the formula: (Responses to Cue_{ND} minus responses to Cue_D) / (Total responses). Thus, if a rat responded equally to both cues, the devaluation index would be 0. If a rat responded only to Cue_{ND} or only to Cue_D, the devaluation index would be 1 or -1, respectively. According to the formula, if a rat showed a devaluation effect, its devaluation index was positive. The devaluation index was also calculated for the consummatory probe according to the formula: (Total amount of Food_{ND} consumed minus total amount of Food_D consumed) / (Total amount consumed). For each group, devaluation indices were analyzed using a one-sample t-test comparing the scores to 0. Scores that were significantly higher than 0 indicated that the group showed a devaluation effect. Devaluation indices were also used for between-group comparisons. For the BLA lesion analysis, these were made by Student's t-test. For the BLA inactivation analysis, a one-way ANOVA, followed by the Bonferroni-Holm corrected planned comparisons (one-tailed), was used.

Results

Experiment 1: The effects of BLA lesions on reinforcer devaluation

Histological verification of lesions—Figure 3 shows the extent of BLA lesions, with all cases superimposed on drawings of coronal sections of the rat brain. BLA lesions were largely as intended, including damage to the lateral, basal, and accessory basal nuclei and some damage to the anterior and posterior basomedial nuclei. Unintended damage was observed unilaterally in the dorsal and ventral endopiriform areas (n=1) or the lateral portion of central nucleus (n=1) or both (n=1). Of the 13 BLA-lesioned rats, 3 rats were excluded due to insufficient bilateral lesion; two rats sustained only a unilateral lesion and one rat less than 50% damage, bilaterally.

The total number of rats that were included in each group is given in Table 1.

Cue training—The mean percent correct the rats in each group achieved during the last three days of cue training are shown in Table 1. Preoperatively, BLA sham and lesioned rats did not differ in performance during cue training, as confirmed by Student's t-test ($t=1.01$, $p=0.32$).

Selective satiation—BLA sham and lesioned rats did not differ with respect to the amount of food consumed (a mean of 15.5 ± 2.4 and 14.7 ± 1.7 g, respectively) during selective satiation, as shown by Student's t-test ($t=0.3$, $p=0.77$).

Instrumental probe of reinforcer devaluation following BLA lesions—In the instrumental probe session we assessed whether devaluation of a food reinforcer was transferred to the associated cue. Successful transfer would manifest as fewer lever presses to the cue associated with the devalued reinforcer (Cue_D) than the cue associated with the non-devalued reinforcer (Cue_{ND}). In this session, the cues above both levers were

illuminated simultaneously (Cue 1 was illuminated above one lever and Cue 2 was illuminated above the other). To prevent rats from experiencing the current (i.e., decreased) value of the food reinforcer in the presence of the cues, the probe was conducted under extinction (i.e., no pellets were delivered during the test). As shown in Figure 4A, rats with sham lesions responded significantly less on the lever corresponding to the Cue_D as compared to Cue_{ND} ($p < 0.05$, Bonferroni-Holm). Rats with BLA lesion responded equally to both Cue_D and Cue_{ND} ($p = 0.63$, Bonferroni-Holm), showing no devaluation effect. ANOVA with repeated measures showed no significant effect of treatment (sham vs lesion, $F_{1,19} = 0.018$, $p = 0.9$) or devaluation status (Cue_{ND} vs Cue_D, $F_{1,19} = 2.8$, $p = 0.1$), but a significant devaluation-status by treatment interaction ($F_{1,19} = 5.3$, $p < 0.03$).

Despite the impairment in devaluation, lesioned rats displayed an equivalent number of total responses to both cues combined as did the sham-operated controls (mean $\pm$ SEM: sham=92.9 $\pm$ 12.4; lesion=90.3 $\pm$ 14.9; t-test: $t = 0.14$, $p = 0.89$). These data indicate that lesions of BLA did not change the overall level of instrumental responding.

As shown in Figure 4B, sham-lesioned rats displayed a robust devaluation effect (devaluation index significantly greater than zero; one sample t-test; $t = 2.82$; $p < 0.05$). In contrast, BLA-lesioned rats displayed no devaluation effect (devaluation index not significantly different from zero; one sample t-test; $t = 1.2$; $p = 0.26$). Furthermore, devaluation index for BLA-lesioned rats was significantly less than that of sham-lesioned rats (Student's t-test, $t = 2.6$; $p < 0.05$).

Consummatory probe of reinforcer devaluation following BLA lesions—The consummatory probe was used to determine if the rats, following selective satiation (e.g., with chocolate pellets), successfully devalued the particular food reinforcer, i.e. whether they consumed less of the devalued food (Food_D; chocolate pellets) as compared with the non-devalued (Food_{ND}; sugar pellets). As shown in Figure 5A, both rats with sham lesions and BLA lesions ate significantly more of the non-devalued food than the devalued food ($p < 0.05$, Bonferroni-Holm's step down test). ANOVA with repeated measures showed a significant effect of devaluation status (Food_{ND} vs Food_D, $F_{1,18} = 44.4$, $p < 0.05$) but no significant effect of treatment (sham vs lesion, $F_{1,18} = 0.02$, $p = 0.9$) or devaluation-status by treatment interaction ($F_{1,18} = 0.57$, $p = 0.46$). Thus, BLA lesions did not affect the performance under the conditions when, after selective satiation, rats were given the choice between the non-devalued and the devalued food in the absence of the visual cues.

As shown in Figure 5B, the mean devaluation index both for rats with sham lesions and BLA lesions were positive values, significantly different from 0 ($t = 4.7$, $t = 5.4$, respectively, $p < 0.05$), indicating a clear devaluation effect in both groups. In addition, there was no significant difference between the two groups ($t = -0.57$, $p = 0.58$).

Experiment 2: The effects of BLA inactivation on reinforcer devaluation

Rats were trained and tested using the same reinforcer devaluation paradigm as described in Experiment 1.

Histological verification of cannula placement—Figure 6 shows the placement of cannulae in BLA. Of the 85 rats that were implanted, 70 had correct bilateral cannulae placement. 15 rats were excluded from further analysis due to a misplaced cannula, either unilaterally or bilaterally.

Cue Training—The mean percent correct the rats in each group achieved during the last three days of cue training are shown in Table 1. Preoperatively, there were no significant differences between groups, as confirmed by a one-way ANOVA ($F_{3,35} = 0.2$, $p = 0.9$).

Selective satiation—During selective satiation, rats treated with saline ate statistically equivalent amounts of food as those treated with MUS, 10.8 g $\pm$ 1.3 and 13.3 $\pm$ 1.5 g, respectively. There was no significant difference between the groups (*t*-test: *t*=-1.3, *p*=0.21). This result indicates that the presence of MUS in BLA during satiation did not affect the amount of food the rats consumed.

Instrumental probe of reinforcer devaluation following BLA Inactivation—As in Experiment 1, we assessed whether devaluation of a food reinforcer was transferred to the associated cue in the instrumental probe. As shown in Figure 7A, rats treated with saline responded significantly less to Cue_D than to Cue_{ND} (*p* < 0.05). Both groups that received MUS after satiation, i.e. group with no delay and group with a 30-minute delay before the probe, also responded significantly to Cue_D than to Cue_{ND} (*p* < 0.05). Because these two groups did not significantly differ from each other (ANOVA; treatment: *F*_{1,15}=0.37, *p*=0.55; treatment by devaluation status: *F*_{1,15}=0.79, *p*=0.39), they were combined for further analyses as one group, MUS after satiation (Figure 7A). In contrast, rats treated with MUS before satiation responded equally to both cues (*p*=0.85). ANOVA revealed a significant main effect of treatment (*F*_{2,36}=11.12, *p*<0.0005), a significant effect of devaluation status (*F*_{1,36}=11.84, *p*<0.001), and a significant devaluation-status by treatment interaction (*F*_{2,36}=3.45, *p*<0.05). Bonferroni-Holm step down planned comparisons showed that MUS infused into BLA before satiation, but not after satiation, disrupted the devaluation effect.

As shown in Figure 7B, the mean devaluation indices for rats treated with saline and rats treated with MUS after satiation (collapsed across no delay and the 30-minute delay) were positive values, significantly different from 0 (*t*=2.5, *p*<0.05, and *t*=5.2, *p*<0.001; respectively). As before, MUS after satiation groups were collapsed with respect to devaluation index, because they did not differ significantly from each other (*t*=0.89, *df*=15, *p*=0.39). In contrast to saline-treated, and MUS-after-satiation-treated animals, the mean devaluation index for rats treated with MUS before satiation was not significantly different from 0 (*t*=0.12, *p*=0.91), showing that rats responded equally to both cues. Between-group one-way ANOVA showed a significant main effect of treatment (*F*_{3,35}=5.2 *p*<0.05) and a Bonferroni-Holm test showed that the devaluation index for MUS before satiation was significantly different from both saline and MUS after satiation (*p*<0.05). In addition, the devaluation index for saline-treated rats did not differ from MUS after satiation group.

All MUS-treated groups displayed reduced responding, as measured by the total number of lever presses (Cue_{ND} and Cue_D summed), for rats treated with MUS before satiation (19.4 $\pm$ 7.8), MUS after satiation with no delay (42.4 $\pm$ 15.7), and MUS after satiation with a 30-minute delay (31.1 $\pm$ 14.2), compared with saline (86 $\pm$ 10.9). A one-way ANOVA showed a significant effect of treatment (*F*_{3,35}=7.4, *p*<0.05). Bonferroni-Holm tests showed that whereas there were no differences between the MUS-treated groups, all these groups were significantly different from the saline-treated group (*p*<0.05). As mentioned above (in the Materials and Methods section) in order to control for the drug spread within the infused tissue over time, we included a group with MUS infusions after satiation with a 30-minute delay before the probe to match the time for drug spread in the group with MUS infusions before satiation. In these two groups there was an equivalent duration of time between drug infusion and the probe session, allowing for the drug to spread within the same extent of the brain tissue in BLA. The group with MUS after satiation with a 30-minute delay showed a similar decrease in responding as the group with MUS before satiation, but without the same impairment of the devaluation effect. Thus, despite the fact that MUS suppressed the overall response rates (in all group regardless of timing of the infusion), the devaluation indices showed clear differences between the conditions when MUS was infused either before satiation or after satiation. To determine if there was a relationship between degree of impairment in lever pressing, and devaluation index, we performed a correlation analysis.

We found that there was no correlation between the overall number of level presses and the devaluation indices, when analyzed either by groups (Saline, $R^2=0.1$, MUS before, $R^2=0.02$, MUS after, $R^2=0.17$), or for the data set as a whole ($R^2=0.01$, all p s >0.1).

Consummatory probe of reinforcer devaluation following BLA inactivation—

As shown in Figure 8A, rats treated either with saline or MUS (all conditions) ate significantly more of the non-devalued food than the devalued food ($p<0.05$, Bonferroni-Holms step down post-hoc). These effects were revealed by ANOVA with repeated measures, which showed a significant effect of devaluation status (Food_{ND} vs Food_D, $F_{2,18}=30.5$, $p<0.05$) but no significant effect of treatment (saline, MUS before satiation, MUS after satiation, $F_{2,28}=0.9$, $p=0.40$) or devaluation-status by treatment interaction ($F_{2,28}=0.9$, $p=0.42$). Thus, BLA inactivation did not affect the performance under the conditions when, after selective satiation, the rats were given the choice between the non-devalued and the devalued food in the absence of the cues.

As shown in Figure 8B, the mean devaluation index for all groups were positive values, significantly different from 0 ($t=4.5$, $t=4.3$, $t=7.3$, $p<0.05$), all showing a robust devaluation effect. A one-way ANOVA showed no significant difference across the devaluation indices of saline treated and MUS treated rats ($F_{2,28}=0.8$ $p=0.46$).

MUS infusions in BLA, regardless of treatment, did not affect the total amount of food consumed following selective satiation as measured by the total amount of food consumed (amount of Food_{ND} and Food_D consumed in grams summed) for rats treated with saline (6.7 $\pm$ 1.2), MUS before satiation (5.7 $\pm$ 1.1), and MUS after satiation (8.8 $\pm$ 2.2). There was no significant difference between the groups as shown by a one-way ANOVA ($F_{2,28}=0.9$ $p=0.4$).

Discussion

The data presented here support our hypothesis that, in the rat, BLA activity is required for the cognitive process of linking the devaluation of a primary reinforcer (i.e., a food) with the representation of the cue that predicts that specific food. This suggests that BLA activity is essential for updating cue value to align with the new value of the reinforcers. When BLA was inactivated by focal infusion of the GABA_A receptor agonist MUS during the satiation phase, rats failed to shift behavior to avoid the cue predictive of the devalued reinforcer. Rather, they responded equally to both cues during the probe session. In contrast, when BLA was inactivated only during the choice session, rats exhibited a normal devaluation shift, responding significantly less to the cue predicting the devalued outcome. This finding confirms our hypothesis that conditioned reinforcer devaluation does not require active processing in BLA to guide selection of cues during the choice period. Our study is the first to show 1) that transient inactivation of BLA is sufficient to impair the satiety-induced reinforcer devaluation effect in rats, 2) that BLA in the rat is essential for updating the value of the internal cue representation of the changed reinforcer value, but not for guiding subsequent behavioral choices.

These results agree with our previous findings in monkeys, in which inactivation of BLA during the period of selective satiation disrupted the devaluation effect, but inactivation of BLA limited to the post-satiety choice period was without effect on object selection (Wellman et al., 2005). While the regions with which the amygdala connects are preserved across species (Izquierdo et al., 2004; Rempel-Clower, 2007; Schoenbaum et al., 2007), the less extensive connections between prefrontal cortex and amygdala in the rat as compared with monkey (Ongur and Price, 2000; Uylings et al., 2003) might give rise to differences between the role of amygdala in rat and primate. However, our results indicate that in both

the rat and the monkey, the instantiation, but not the maintenance, of the devaluation effect is dependent upon BLA.

As in the monkey, neither lesions nor inactivation of BLA affected the amount of food consumed during the satiation sessions or during the consummatory probe sessions. Furthermore, the manipulations in BLA did not impair the devaluation of the primary (food) reinforcers, as indicated by a clear shift in food preference when the foods were presented during a consummatory probe in the absence of the cues. These findings are consistent with previous observations of no alterations in consummatory behavior after BLA lesions in rats (Johnson et al., 2009) and after MUS infusions in BLA in both rats (Simmons and Neill, 2009) and monkeys (Wellman et al., 2005). It is also consistent with observations in a reward inflation task (Wassum et al., 2009), in which infusion of naloxone, an opioid antagonist, into BLA prevented the typical increase in instrumental responding for sucrose in food-deprived rats, while increased consummatory responses to sucrose were still present. This indicates that active processing in BLA is not required for updating the value of the food itself (i.e., the primary reinforcer), rather, that BLA is selectively necessary for the cognitive modification of cue representations to reflect the decreased primary reinforcer value.

Another possibility is that the disruption of reinforcer devaluation by BLA lesions may result from a generalization of the devaluation effect to both of the cues. Put another way, BLA lesions may impair the specific assignment of decreased value to the appropriate cue (i.e., CueD) and, instead, the state of satiety is applied non-specifically to both cues. Thus, embedded within the interpretation of “generalization” is an inability to update the value of the cue with decreased primary reinforcer value. One might expect that in the case of “generalization”, a reduction in overall responding, and/or a reduction in responding to the non-devalued cue, would occur. However, we found that the overall amount of responding was not reduced in our rats with BLA lesions; in fact, sham and lesioned animals had a statistically equivalent numbers of responses. In addition, there was no difference in the number of responses to Cue_{ND} between sham and lesioned rats. Thus, it seems unlikely that generalization of the decreased food value to both cues would account for the disrupted devaluation effect we report. Our results and previous studies have consistently shown that BLA is necessary for task performance only when multiple, distinct primary reinforcers are predicted by multiple specific cues (Johnson et al., 2009). This supports the idea that BLA is necessary for maintaining the specific sensory features of the rewards and associating those features with specific cues (Johnson et al., 2009). Therefore, it is possible that in the absence of BLA, rats cannot appropriately direct behavior to either cue. Because BLA is not necessary for performance on tasks in which the animals must associate only one reinforcer-cue pair (Hatfield et al., 1996, Pickens et al., 2003), the importance of BLA in conditioned reinforcer devaluation may be necessary for updating the specific sensory features of specific reinforcer-cue associations during selective satiation. In cases with only one cue-reinforcer pair, there is no ambiguity with respect to the stimulus to be updated.

The lack of reduction in responding we observed after BLA lesions differs from previous tests of devaluation in BLA-lesioned rats, as reported by Johnson et al., (2009) and Ostlund and Balleine (2008). Johnson et al. (2009) and Ostlund and Balleine (2008) found a general reduction in responding on both levers during instrumental testing, which reached almost a floor effect. Despite the reduced responding, in both studies the animals showed a clear disruption of the devaluation effect. Interestingly, Johnson et al., 2009 found decreased responding to the non-devalued cue, but not to the devalued, in BLA-lesioned animals using a Pavlovian reinforcer devaluation test; this decrease was not accompanied by an overall reduction in responding (food cup approach). One possible explanation for the difference between the findings of Johnson et al. (2009), Ostlund and Balleine (2008), and our present

findings may be the use of a cue in our instrumental task. In the present study, visual cues (separate from the operandum) signaled the start of a trial. The onset of the visual cues may have activated Pavlovian approach behavior and increased response vigor, as is seen in Pavlovian-to-instrumental transfer (PIT) paradigms, where the general PIT effect is unimpaired by BLA lesions (Corbit and Balleine, 2005).

In contrast to BLA lesions, MUS infusions in BLA in the present study did result in a significant decrease in total lever pressing. This decrease was, however, independent of the impairment in the devaluation effect because it was observed in all MUS treatment conditions, including conditions in which devaluation was not impaired. While this was apparent across all MUS treatment conditions, the comparison is especially compelling for the two groups matched for drug spread (i.e., MUS before satiation and MUS after satiation with a 30 minute delay prior to testing). In this case, both groups were matched for the amount of tissue inactivated, and showed similar low rates of responding, while differing with respect to the devaluation effect. Our findings are consistent with a previous report of a dose-dependent decrease in operant responding (lever presses for food) following MUS infusions in BLA, without affecting consummatory responses (Simmons and Neill 2009). Thus, the reduction in lever pressing could be due to deficit in general motivation or arousal following BLA inactivation, however, our findings suggest this effect is distinct from the specific effect of reinforcer devaluation.

The fact that BLA is not necessary for guiding the choices after devaluation of one of the foods suggests that the representations required for guiding response selection are maintained in one or more structures to which amygdala projects (Amaral, 1992; Baxter et al., 2000, Mitchell et al., 2007; Pickens, 2008). While neuronal activity in BLA has been shown to encode shifts in reinforcement contingencies in close alignment with, and anticipation of, shifts in behavioral responding (Paton et al., 2006), substantial evidence supports the OFC as the structure that is responsible for maintaining the representations of the cues and their relative values, and guiding behavioral selection based on these representations (Holland and Gallagher, 1999; Tremblay and Schultz, 1999; Holland and Gallagher, 2004). These data suggest that BLA-OFC interactions provide a node for rapid flexibility of associations (Saddoris et al., 2005; Stalnaker et al., 2007). Accordingly, disruption of OFC function after devaluation impairs the expression of devaluation behavior in rats and monkeys (Pickens et al., 2005; West et al., 2011b), in clear contrast to the lack of effect of BLA disruption at this time (Wellman et al., 2005). Thus, during satiation, BLA activity may be required to engage the OFC, enabling the modification values assigned to specific cue representations. Once this occurs, updated values in OFC can be used to guide subsequent behavioral choices. In apparent contrast to this suggestion, infusion of a protein synthesis inhibitor (anisomycin) in BLA following selective satiation disrupts the devaluation effect (Wang et al., 2005), suggesting that consolidation or storage may be occurring in BLA. However, the task employed by Wang et al does not require OFC for normal performance, while our task does (Ostlund and Balleine, 2007, West et al., 2011c). In addition, protein synthesis inhibition by anisomycin affects other neural functions, including c-fos activation (Edwards and Mahadevan, 1992), and neurotransmitter release (Canal et al., 2007). These additional effects of anisomycin (Helmstetter et al., 2008) and/or differences in task demands may explain apparently contrasting results with respect to BLA across laboratories.

Here we have shown that, in rats, BLA is necessary for the cognitive process of reducing the value of (or preventing the generalization between) internal representations of visual cues, in order to reflect the decreased incentive value of the primary reinforcer. This process, which is disrupted by transient inactivation of BLA, takes place during selective satiation. Once the cue representations have been updated, BLA is not necessary for the subsequent guiding of

behavioral choice. Together with previous findings (e.g., Wellman et al., 2005), our study demonstrates a conservation of the role of the BLA across rats and monkeys with respect to reinforcer devaluation.

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Figure 1.
Experimental timeline for instrumental probes of reinforcer devaluation: A) BLA lesion/sham groups and B) BLA inactivation groups.

A. Infusion BEFORE satiation

B. Infusion *AFTER* satiation with no delay

C. Infusion *AFTER* satiation with a 30-minute delay

Figure 2.

Timing of the drug/saline infusions: A. MUS/Saline *before* satiation, B. MUS/Saline *after* satiation with no delay, C. MUS/Saline *after* satiation with 30-minute delay before the probe.

Figure 3. BLA lesion histology. A) All cases are superimposed on drawings of coronal sections, the darker the area, the more overlap across lesions. The numbers represent distance in mm from bregma, and approximately correspond to the appropriate planes of the atlas of Paxinos and Watson (2010). B) A representative photomicrograph from a sham-lesioned rat. C) A representative photomicrograph documenting a BLA lesion. LV - the lateral ventricle, CeA - central nucleus of the amygdala, BLA- basolateral subdivision of the amygdala, EC - external capsule.

Figure 4.

BLA lesion disrupts reinforcer devaluation in the instrumental probe A) Cumulative number of lever presses (mean+standard error) across the first nine trials for rats with BLA sham or BLA lesions. Solid bars indicate responses to the cue associated with the non-devalued food (Cue_{ND}), striped bars indicate responses to the cue associated with the devalued food (Cue_D). *denotes a significant difference ($p < 0.05$) between the responses to Cue_{ND} and Cue_D for sham lesioned rats. There was no significant difference between responses to Cue_{ND} and Cue_D for BLA lesioned rats. B) Devaluation index (mean+standard error) for cumulative number of lever presses across the first nine trials for sham and BLA lesions. * denotes a significant difference between groups ($p < 0.05$).

Figure 5.

Rats with BLA sham and BLA lesions show normal devaluation in the consummatory probe. A) Amount of food consumed in grams (mean+standard error) following devaluation by selective satiation. Solid bars correspond to the non-devalued food (Food_{ND}), striped bars correspond to the devalued food (Food_D). *denotes a significant difference ($p < 0.05$) between the amount consumed of the devalued and non devalued food for both sham and BLA lesions groups. B) Devaluation index (mean+standard error) for the consummatory probe. There was no significant difference between groups.

Figure 6.

Location of BLA infusions. A) Infusion sites plotted on drawings of coronal sections; x represents a correctly placed injection tip, o represents an incorrectly placed injection tip, rats with incorrect placement were excluded from behavioral analyses. The numbers represent distance in mm from bregma, and approximately correspond to the appropriate planes of the atlas of Paxinos and Watson (2010). B) A representative photomicrograph documenting cannula placement in BLA. MeA - medial nucleus of the amygdala, CeA- central nucleus of the amygdala, BLA-basolateral subdivision of the amygdala.

Figure 7. BLA inactivation before satiation disrupts reinforcer devaluation in the instrumental probe. A) Cumulative number of lever presses (mean+standard error) across the first nine trials for each group. Solid bars indicate responses to the cue associated with the non-devalued food (Cue_{ND}), striped bars indicate responses to the cue associated with the devalued food (Cue_D). *denotes a significant difference ($p < 0.05$) between the responses to Cue_{ND} and Cue_D for rats treated with saline in BLA and rats treated with MUS after satiation, either with no delay or with a 30-minute delay before the probe. There was no significant difference between responses to Cue_{ND} and Cue_D for rats that received a MUS infusion before satiation. B) Devaluation index (mean+standard error) for cumulative number of

lever presses across the first nine trials for sham and BLA lesions. * denotes a significant difference between groups. MUS before satiation was significantly different from all the other groups.

Figure 8.

BLA inactivation does not disrupt reinforcer devaluation in the consummatory probe. A) Amount of food consumed (mean+standard error) in grams following devaluation by selective satiation. Solid bars indicate the non-devalued food (Food_{ND}), striped bars indicate the devalued food (Food_D). *denotes a significant difference ($p < 0.05$) between the amount of devalued food and non devalued food consumed for saline and both MUS treated groups. B) Devaluation index (mean+standard error) for the consummatory probe. There was no significant difference between groups.

Table 1

Experimental Groups

Treatment	n included (n excluded)*	Tests each rats performed	Percent (%) correct on Cue Training**
Experiment 1: BLA Lesions			
BLA sham lesion	11 (0)	Instrumental and Consummatory probes	89.4 +/- 0.8
BLA lesion	10 (3)	Instrumental and Consummatory probes	88.3 +/- 0.7
Experiment 2: BLA Inactivation			
Saline in BLA	13 (0)	Instrumental probe	89.9 +/- 0.7
MUS in BLA before satiation	9 (5)	Instrumental probe	89.5 +/- 0.5
MUS in BLA after satiation with no delay	10 (2)	Instrumental probe	89.4 +/- 0.7
MUS in BLA after satiation with a 30-min delay prior to probe	7 (2)	Instrumental probe	89.2 +/- 1.3
Saline in BLA	13 (0)	Consummatory probe	
MUS in BLA before satiation	8 (2)	Consummatory probe	
MUS in BLA after satiation	10 (4)	Consummatory probe	

* (n excluded) represents the number of rats excluded from each group due to incomplete lesions or misplaced cannulae.

** Percent correct represents the mean performance (in %) on Cue Training across the last three days preoperatively for each group.