Behavioral/Systems/Cognitive

GABA$_A$-Mediated Inhibition of Basolateral Amygdala Blocks Reward Devaluation in Macaques

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Amygdala ablation disrupts reinforcer "devaluation" in monkeys (Malkova et al., 1997). Here, we tested the hypothesis that transient inactivation of amygdala by the GABA$_A$ agonist muscimol (MUS), specifically during the period of reward satiation, would have a similar effect. Six pigtail macaques were trained on a visual object discrimination task in which 60 objects were associated with one of two specific food rewards. Subsequently, we evaluated the selective satiation-induced change (devaluation) in object preference in probe sessions. We also examined the effect of the amygdala inactivation during the probe sessions to determine whether the inactivation limited to the testing period (and not during the satiation period) is sufficient to impair the expression of reinforcer devaluation. MUS infusions were aimed at basolateral amygdala (BLA) in a pseudorandomized design; each monkey received MUS or saline either before or after selective satiation with each of the two food rewards (six infusions total). Under the control (saline) condition, the monkeys significantly shifted their preference from objects representing the sated food rewards to those representing the nonsated rewards (30% change). When BLA was inactivated during selective satiation (i.e., MUS infused before satiation), this devaluation effect was blocked. In contrast, MUS infusion after satiation, so that it was present just during the testing period, did not impair the shift in object preference (27% change). Thus, BLA is necessary for the appropriate registration of the change in the reinforcer value but not for the subsequent expression of the devaluation involving its transfer to secondary reinforcers.

Key words: **amygdala; muscimol; reward; satiation; GABA$_A$; nonhuman primate**

Introduction

The amygdala functions as part of a network responsible for associating sensory stimuli with their affective and motivational significance (Weiskrantz, 1956; Pribram, 1986; Davis, 1992; LeDoux, 1995; Everitt et al., 2000). These associations include those required for fear conditioning as well as those involved in processing reward and linking sensory stimuli with positive reinforcement (for review, see Baxter and Murray, 2002). By registering the current value of positive reinforcement, the amygdala may guide goal-oriented behavioral action and permit shifts in behavior in response to outcomes (Hatfield et al., 1996; Malkova et al., 1997; Schoenbaum et al., 1998, 1999, 2003; Blundell et al., 2001, 2003; Parkinson et al., 2001; Balleine et al., 2003). Under conditions in which animals are required to adjust behavior based on changes in the value of the reinforcer, the integrity of the amygdala has been found to be critical. For example, after amygdala ablation, monkeys selecting objects baited with specific treats failed to adjust their object choices when one of the treats was devalued by "selective satiation" (Malkova et al., 1997). Moreover, amygdala activity in humans responds to "devaluation" of a food reinforcer as assessed by functional magnetic resonance imaging (Gottfried et al., 2003).

Failure to adjust behavior according to change in reward value can result from a failure to (1) form representations of cues associated with their incentive properties, (2) register and encode a change in reward value in a manner that can guide subsequent decisions, (3) update the representation of secondary reinforcers with new values of primary reinforcers, or (4) apply the changed representations to direct behavioral choices. The components that account for deficits after amygdala impairment have not been identified. One study in rats concluded that amygdala removal after initial learning of a conditioned stimulus–unconditioned stimulus (CS–US) association, but before devaluation of the US, was without effect on the subsequent devaluation of the CS (Pickens et al., 2003). In contrast, Salinas and McGaugh (1995) found that drug-induced inactivation of rat basolateral amygdala (BLA), timed to disrupt encoding of a decreased reward value, blocked expression of the devaluation 24 h later.

The purpose of the present study was to test the hypothesis that devaluation of conditioned reinforcers (i.e., objects) in nonhuman primates depends on amygdala activity during satiation with the primary reinforcer (food) but not thereafter. This hypothesis predicts that (1) transient inactivation of amygdala during satiation will interfere with subsequent devaluation of objects associated with the sated food, and (2) transient inactivation of amygdala after satiation and during object selection (i.e., probe trials) will not impair the expression of devaluation. We trained animals to associate individual objects with one of two specific

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food rewards and then evaluated the change in object preference after devaluation. We assessed the effect of amygdala inactivation [by focal muscimol (MUS) infusion] during satiation and compared it to the effect of amygdala inactivation after satiation and before testing object preference. The BLA was targeted based on rodent studies indicating that BLA, and not the central nucleus, is crucial for processing associations between stimulus and reward value (Hatfield et al., 1996).

Materials and Methods

Subjects

Six pigtail macaques (*Macaca nemestrina*), three females (OG, OH, JN) and three males (OK, GR, ZC), were subjects in this study. They were 2–4 years of age and weighed 2.8–5.5 kg at the beginning of this study. All subjects had been trained previously on a crossmodal auditory–visual matching task. Two of them (OH and OK) also participated in a previous experiment in which they were trained on visual delayed nonmatching-to-sample tasks and received bilateral reversible inactivations of perirhinal cortex by focal drug infusions (Malkova et al., 2001). All monkeys were housed in pairs in a room with regulated lighting (12 h light/dark cycle) and maintained on primate LabDiet (number 5049; PMI Nutrition International, Brentwood, MD) supplemented daily with fresh fruit. Water was available *ad libitum* in the home cage. 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.

The monkeys were implanted with stereotaxically positioned chronic-infusion platforms, which allowed a removable injector, fitted with an infusion cannula of adjustable length, to be inserted into predetermined sites in the brain through the guiding channels of the platform.

Infusion platform

The design and construction of the platform assembly are a modification of the method of Dooley et al. (1981). A custom mold was fabricated (Elmeco Engineering, Rockville, MD) with a total of 12 rows by 14 columns spaced 2 mm apart to maximize the number of sites for injections. The holes were arranged into two groups of seven columns, and the two groups were separated at the midline by a 5.5-mm-wide section in which two threaded holes were located for placement on a stereotaxic holder (Fig. 1*A*). Stainless-steel pins were placed in the holes and then liquid acrylic (Kooliner; Patterson Dental Supply, St. Paul, MN) was poured into the mold to form the platform. Once the platform had cured, the pins were removed, and any irregularities were sanded until smooth.

Injector

A custom-built telescoping infusion apparatus (Elmeco Engineering) was designed to fit snugly into the infusion platform and allow an easy adjustment of infusion cannula length. The apparatus consists of a series of three lengths of stainless-steel tubing, each positioned in a solid stainless-steel disc (Small Parts, Miami Lakes, FL). The discs are serially interlocked, with the central tubes acting as a guide. The three lengths of tubing are of successively larger diameters, allowing for concentric placement (Fig. 1*B*). The 27 ga tube of the topmost disc serves as the infusion cannula and is connected via polyethylene tubing (BioLab, Decatur, GA) to a 50 μl Hamilton syringe. To perform the infusion, the telescoping apparatus is slowly inserted through the chronic platform until the discs are flush with each other and the platform and the drug is infused (see below, Intracerebral drug infusions) using an injection pump (model 341; Sage Instruments; Orion Research, Cambridge, MA).

Magnetic resonance imaging

Approximately 2 weeks before the surgery, each monkey received a T1-weighted magnetic resonance imaging (MRI) structural brain scan to calculate stereotaxic coordinates for the platform implantation. Postoperatively, each monkey received at least one T1-weighted scan to verify the coordinates for the position of the platform. For each scan, the monkey was anesthetized with a 4:1 (v/v) mixture of ketamine (ketamine hydrochloride, 10–20 mg/kg, i.m.) and xylazine (0.2–0.4 mg/kg, i.m.) and placed in a nonferrous stereotaxic frame (David Kopf Instruments, Tujunga, CA). MRI was performed in a 1.5 T Vision scanner (Siemens Magnetom Vision, Munich, Germany) using a human head coil. MR images were obtained using a three-dimensional FLASH (fast low-angle shot) pulse sequence (repetition time, 25 ms; echo time, 5 ms; field-of-view, 20 cm; slice thickness, 1 mm). The frame and the monkey were aligned in the MR unit using a landmark laser-alignment system and bubble levels to ensure that the planes of the MR unit were parallel to those of the stereotaxic instrument. The MRI scans were used to obtain the coordinates of the basolateral amygdala relative to both the interaural plane (marked by the ear bars) and the midline, which were visible on the scan (Saunders et al., 1990). Based on these measurements, coordinates for positioning the infusion platform and coordinates for the infusion sites were calculated. Postoperatively, the position of the predetermined infusion sites was validated by another MRI scan using either 19 ga plastic tubes filled with vitamin E or tungsten microelectrodes (FHC, Bowdoinham, ME), which were visible on the scan. One of the tubes or electrodes was inserted, via the guiding channels and predrilled holes in the skull, into a specified location in each hemisphere at least 15 mm above the intended infusion site. The final coordinates for the drug infusions were determined with respect to the position of the tubes (or electrodes) and the ear bars.

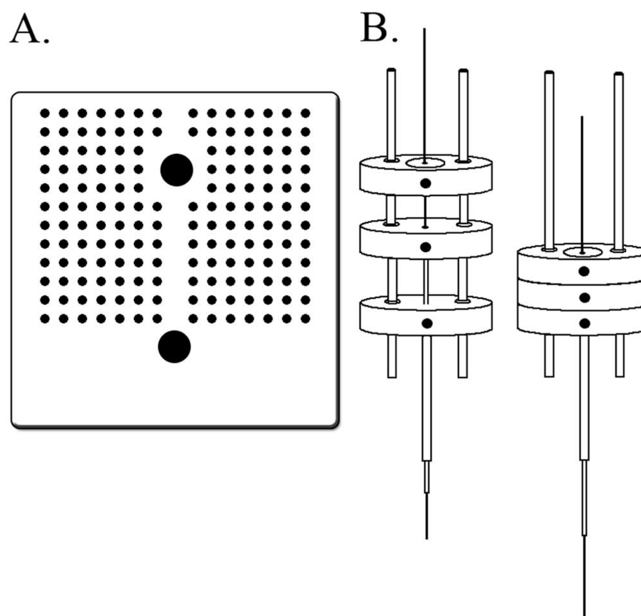


Figure 1. ***A***, The infusion platform. A top view of the platform, which contains holes 2 mm apart in 12 rows by 16 columns. The holes are separated at the midline by 5.5 mm, where two threaded holes were located for placement on a stereotaxic holder. ***B***, The injector apparatus. The left picture shows the separation of the three parts, and the right picture shows the three disks flush together as they appear during infusion.

Surgery for implantation of the infusion platform

After first sedating the monkey with ketamine, a surgical level of anesthesia was established and maintained with isoflurane gas (1–2%, to effect). Atropine (0.1 ml/kg, s.c.) was administered to counteract the decrease in heart rate caused by the anesthetics and maintain cardiac output close to normal. Throughout the aseptic procedure, the monkey received an intravenous drip solution of isotonic fluids; heart rate, respiration rate, blood pressure, expired CO_2, and body temperature were monitored.

For the surgery, the monkey was placed in a stereotaxic headholder, and a sterile field was established. The skull was exposed over the area of the cranium, where the placement of the platform was determined based on the preoperative MRI. The platform (with stainless-steel pins placed into the guide tubes to prevent their closure) was positioned stereotaxically, four plastic anchor bolts were inserted under the skull, and an oval wall was created with Palacos R bone cement (BioMet Orthopedics, Warsaw, IN). Kooliner acrylic was poured around and under the prefabri-

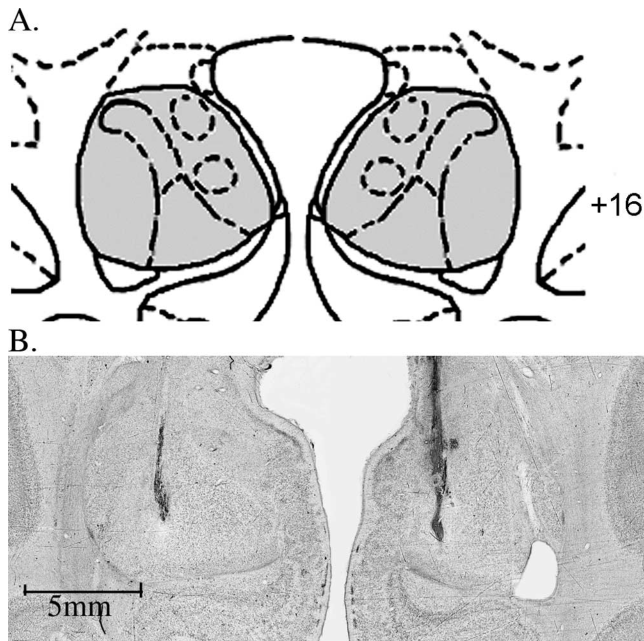


Figure 2. ***A***, Coronal sections showing the intended infusion site (shaded) in both the left and right basolateral amygdala. Numerals indicate the distance in millimeters from the interaural plane. ***B***, Photomicrograph of Nissl-stained coronal sections through the basolateral amygdala of the animal OH, which was used for histological evaluation (see Materials and Methods), at approximately the same level as that in ***A*** showing the infusion cannula tracts bilaterally. The additional cannula tracts visible laterally on each side are from drug infusions into perirhinal cortex done in a previous experiment (Malkova et al., 2001).

cated platform. Once the acrylic cured, the pins were removed from the platform, and a removable acrylic cap was secured with screws to cover the platform and protect the guide channels when not in use. All monkeys received postoperative analgesics as determined in consultation with the facility veterinarian. Approximately 1 week after the surgery, the channels intended for drug infusions were opened by hand-held drill under anesthesia and aseptic conditions.

Intracerebral drug infusions

The $GABA_A$ agonist muscimol was used to transiently suppress synaptic activity in the vicinity of the focal infusion site. Previous work (Martin, 1991; Martin and Ghez, 1999) showed that a 1 μl infusion of MUS affected a sphere of tissue ~2 mm in diameter surrounding the site of infusion. In other studies in our own laboratory, MUS in the same volume and concentration as used in the present studies, when placed into sites 2 mm apart, induced distinct changes in motor function (Dybdal et al., 1997) or social behavior (Lower et al., 2002; Wellman et al., 2004).

The infusions in these studies were aimed at the BLA (Fig. 2*A*), which includes basal, accessory basal, and lateral nuclei (Amaral et al., 1992), bilaterally. MUS (9 nmol in 1 μl; Sigma, St. Louis, MO) or sterile saline (0.9% NaCl solution) of the same volume was infused at a rate of 0.2 μl/min. The drug infusions were performed using an aseptic technique while the monkey was seated in a primate chair (Crist Instrument Company, Hagerstown, MD) with minimal restraint. The entire infusion procedure lasted ~15 min.

Injection site verification

We performed a detailed histological analysis on one monkey (OH), to validate the accuracy of the MRI-based calculation of injection sites. For histological processing, the monkey was given an overdose of barbiturate (sodium pentobarbital, 100 mg/kg, i.p.) and perfused through the heart with normal saline, followed by aldehyde fixatives. The brain was removed from the skull, and the tissue was sectioned in the coronal plane at 50 μm on a freezing stage microtome. Every section through the amygdala was mounted, defatted, stained with thionin, and coverslipped. A photomicrograph of a representative section showing cannula placement is shown in Figure 2*B*.

The placement of the cannula documented in the histological sections was coaligned with the placement of the microelectrode registered on the MRI scan. After adjustments were made for the tissue shrinkage associated with fixation, the target site as determined on the MRI and the cannula artifact visualized on tissue sections coincided with an error of less than ±0.5 mm. (The remaining five animals are still undergoing additional testing and were not available for histological processing.)

Apparatus and materials

The monkeys were trained in a Wisconsin General Testing Apparatus located in a darkened sound-shielded room. The test compartment was illuminated with a 15 W fluorescent bulb (Philips F15T8-CW; Philips Electronics, New York, NY), but the monkey's compartment was always unlit. The test tray, which was located at the level of the floor of the monkey transport cage, contained three food wells spaced 18 cm apart, center to center on the midline of the tray. The wells were 25 mm in diameter and 5 mm deep. For the present task, only the two outer wells were used. The stimuli were 120 junk objects that differed widely in shape, size, color, and texture. There were two different food rewards. One was one-half of a "fruit snack" (food 1), a chewy candy made from fruit juice, ~10 mm in size (Safeway, Pleasanton, CA), and the other was one-half of a peanut (food 2). Based on previous studies (Malkova et al., 1997), these two food rewards were found to be equally palatable. In addition, before initiation of the behavioral training, we tested that the monkeys were readily taking both rewards and replaced any reward if it appeared undesirable. For one monkey, the fruit snack was replaced by a piece of dried pineapple of the same size (Brown's Fruit Bites, Sinking Spring, PA) and for two monkeys, the peanut was replaced by a single Cheerio (Cheerios; General Mills, Minneapolis, MN).

Behavioral testing procedure

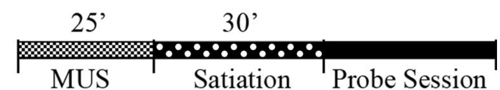
Overview. Postoperatively, all monkeys were trained on a task described previously by Malkova et al. (1997). The behavioral procedure has three phases. In phase I, the animals are trained to form an association between objects and food rewards. One food is presented with a set of 30 objects that are always baited with that specific food and are each simultaneously presented with one of a set of 30 nonrewarded objects; this is done for two separate foods, each with its own set of 30 object pairs. The animals are trained to criterion on the two different sets of food–object associations, intermixed within a session. Once they have been trained to criterion on the object discrimination, their object preference under baseline conditions is evaluated in a "probe session" in which the animals are allowed to chose between two objects, each of which is derived from one of the two sets (e.g., yellow block covering peanut vs blue bowl covering fruit snack). By repeating this choice with 30 different rewarded object pairs, it is then possible to generate a preference score for each food–object set without repeating any objects. In phase II, the animals are given unlimited access to one of the two foods in their home cage until they refuse additional offers of that food. This procedure in monkeys and in humans is called "selective satiation" (Malkova et al., 1997; Thornton et al., 1998; Baxter et al., 2000; Gottfried et al., 2003; Izquierdo and Murray, 2004a,b; Izquierdo et al., 2004) [a similar procedure in rats has been called "specific satiety treatment" (Balleine and Dickinson, 2000)], an operational term defined as the process of allowing the monkeys to eat *ad libitum* only one of the two food rewards. This procedure results in the loss of interest in whichever food was presented during selective satiation; this is referred to as "devaluation." The devaluation of a food transfers from the home cage to the testing apparatus: when the monkeys are presented with 30 pairs of food rewards (food 1 vs food 2), they avoid the devalued food rewards in favor of the nondevalued reward (Izquierdo et al., 2004). In phase III, the animals are evaluated in a probe session for object preference. Under control conditions, many of the objects associated with the sated (devalued) food are rejected in favor of the objects associated with the nonsated food, resulting in a clear shift in the preference score. This shift occurs regardless of which food is used for selective satiation. The rejection of an object associated with the sated food indicates that the devaluation of the food has successfully been transferred to the object. In

Day 1	Day 2	Day 3	Day 4	Day 5&6-7
Food Object Assoc. Task	Baseline Probe Session	Food Object Assoc. Task	Infusion Probe Session (drug/ saline)	OFF

Day 4 procedures:
a. MUS infusion→FOOD 1 satiation→Probe session
b. MUS infusion→FOOD 2 satiation→Probe session
c. FOOD 1 satiation→MUS infusion→Probe session
d. FOOD 2 satiation→MUS infusion→Probe session
e. Saline infusion→FOOD 1 or 2 satiation→Probe session
f. FOOD 1 or 2 satiation→Saline infusion→Probe session

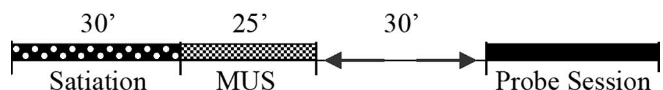
Figure 3. Behavioral testing schedule. Days 1–7 represent a sequence of behavioral testing for each of the six infusions (a–f).

A. *Infusion BEFORE Satiation*



B. *Infusion AFTER Satiation*

C. *Infusion AFTER Satiation:* Increased Time



D. *Infusion AFTER Satiation:* 18nmol MUS

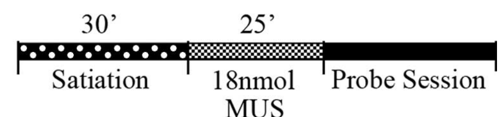


Figure 4. Time schedule for infusion sessions. ***A***, ***B***, Timing of the infusion sessions when the muscimol or saline was infused before (***A***) and after (***B***) selective satiation. ***C***, ***D***, Timing of the follow-up control experiments.

our experimental design, we inactivated BLA either just before the satiation session (i.e., during phase II) or immediately after satiation and before the testing of preference in the probe session (i.e., during phase III). We reasoned that, if the BLA is necessary for the registration or encoding of the devaluation in a manner that can be subsequently transferred to the objects, then inactivation during satiation in phase II will impair the shift in object preference measured in phase III. Furthermore, if the BLA is necessary for either the memory of the devaluation or the transfer of the devaluation to the objects, then inactivation during phase III will impair the shift in object preference. Conversely, if BLA inactivation during phase III does not impair the shift in object preference, then it will indicate that BLA is not required for either the memory of the devaluation or its association with the objects.

Phase 1: object discrimination training. Monkeys were first trained to discriminate 60 pairs of objects. In each pair, one object was designated as positive (i.e., baited with a food reward) and the other was designated as neutral (i.e., unbaited), with one-half of the positive objects (30) baited with food 1 and the other half baited with food 2. The monkeys were trained at a rate of one session per day, 5 d/week, until they reached criterion, which was set at a mean of 90% correct responses over 5 consecutive days (i.e., 270 correct responses of 300).

Phase II: reinforcer devaluation by selective satiation. Approximately 24 h after the last feeding, a food box attached to the monkey's home cage was filled with one of the food rewards (either food 1 or food 2) of measured quantity while the monkey was in its home cage. The monkey was allowed to eat the food without being directly observed for 25 min. The experimenter then entered the room and checked the amount of food eaten. In all cases, there was food still remaining in the food box. The experimenter then observed the monkey from outside of the housing room until the monkey did not take any more food for a 5 min period. At that time, the food box was removed, and the amount of remaining food was measured. In all cases, 30 min was a sufficient time to complete this procedure. Each monkey received each of the following treatments before phase II: (1) MUS, (2) saline, and (3) "no infusion." MUS was infused with both food 1 and food 2 sessions; saline was infused once with one of the foods, food 1 in OH, OK, OG, and ZC and food 2 in JN and GR. As indicated in Figure 3, each of the two foods was used for satiation on three occasions. Within the randomized schedule, selective satiation with food 1 and food 2 alternated between weeks so that at least 2 weeks intervened between repeated satiation with the same food.

Phase III: experimental probe sessions. All phase II treatment conditions are tested in phase III. Each monkey received each of the following treatments before phase III: (1) MUS, (2) saline, and (3) no infusion. For the no-infusion treatment, the probe sessions (phase III) followed immediately after the satiation. For MUS and saline, the intracerebral infusions were administered just before the probe sessions. MUS was infused with both food 1 and food 2 for each animal, and saline was infused with food 2 in OH, OK, OG, and ZC and food 1 in JN and GR.

The timing schedule for the two different infusion probe sessions is presented in Figure 4, *A* and *B*. As indicated, the selective satiation procedure lasted ~30 min; the time required for transport and infusion was ~25 min, of which 15 min was infusion time.

Experimental testing schedule. As presented in Figure 3, on day 1, the monkey was given a practice session on the food–object association task with the original 60 pairs of objects. On day 2, the monkey was given a baseline probe session with the rewarded objects (each choice was between a food 1 rewarded object and a food 2 rewarded object) to determine the baseline number of choices of food 1 and food 2 objects. On day 3, the monkey was again given a practice session on the food–object association task with 60 pairs of objects. On day 4, according to a randomized schedule, the monkey was infused with MUS or saline either in phase II or in phase III and subsequently given an infusion probe session to determine the number of choices of food 1 and food 2 objects. The monkey was then given at least 2 d of rest without any testing before this testing schedule began again. Each monkey completed six of these testing schedules (Fig. 3, a–f), typically over a period of 6 weeks.

The practice sessions (days 1 and 3) with the original 60 pairs of objects were administered to ensure that the effects of the satiation procedure did not carry over to the subsequent probe sessions. Furthermore, it was important to be sure that the repeated devaluations of each food did not alter the monkey's choices of objects during the baseline probe sessions. Therefore, each infusion probe session (day 4) was compared with its preceding baseline probe session (day 2). In addition, to avoid the possibility that the repeated devaluation might affect the monkeys' choices and also their willingness to eat a large amount of the same food repeatedly, the saline infusions were limited to one infusion in phase II with one of the foods and one infusion in phase III with the other food.

"Difference score" for measuring devaluation. Previous studies have found that monkeys often consistently prefer one type of food reward versus the other when the same task is used [e.g., mean ratio of choices for

Table 1. Saline infusions

	Saline BEFORE satiation			Saline AFTER satiation					
Monkey	Baseline probe	Infusion probe	Difference score	Baseline probe	Infusion probe	Difference score	Cumulative baseline	Cumulative infusion	Cumulative difference score
OH	28	22	−6	3	2	−1	31	24	−7
OK	27	18	−9	6	2	−4	33	20	−13
OG	30	19	−11	0	0	0	30	19	−11
JN	28	19	−9	3	0	−3	31	19	−12
ZC	1	0	−1	30	28	−2	31	28	−3
GR	3	1	−2	21	11	−10	24	12	−12
Average	19	13	−6	11	7	−4	30	20	−10

The number of objects rewarded with the devalued food that the monkeys chose in the infusion probe session subsequent to saline infusions either BEFORE or AFTER selective satiation and in the preceding baseline probe session. Difference score represents the numerical difference between number of objects chosen in the baseline probe session and in the infusion probe session. In the condition of saline infusion BEFORE satiation, monkeys OH, OK, OG, and JN were satiated with food 1, and monkeys ZC and GR were satiated with food 2. In the condition of saline infusion AFTER satiation, the monkeys were sated with the other food, respectively.

control and operated monkeys, respectively: 22:8 and 19:11 in Malkova et al. (1997); 23:7 and 25:5 in Thornton et al. (1998); and 21:9 and 25:5 in Baxter et al. (2000)]. Because the number of possible choices in a probe session is constant (i.e., 30), the number of chosen food 1 objects limits the choice of food 2 objects. Given an asymmetrical baseline, satiation with one ("preferred") food can result in a stronger devaluation effect. To overcome the asymmetry, ensure maximum statistical power, and conform to the previous literature (Malkova et al., 1997; Baxter et al., 2000; Izquierdo and Murray, 2004a,b; Izquierdo et al., 2004), we combined the difference scores obtained from each devaluation to generate cumulative difference scores. We recognize that one food may contribute significantly more to the overall effect than the other food. However, if, under control conditions, the devaluation effect shows the same trend for each of the foods, then the cumulative effect will be a valid representation of the response to satiation. In view of this fact, we planned our analysis to take the possible asymmetry into account.

Thus, in each probe session, the number of food 1 and food 2 objects chosen was recorded for each monkey. The number of objects rewarded with the devalued (sated) food chosen in each drug-treatment probe session was then subtracted from the number of objects rewarded with that same food chosen in the preceding baseline probe session, resulting in a difference score (expressed as a negative number to reflect the direction of the change). A greater devaluation effect is reflected in a larger difference score. For MUS infusion in phase II, the difference score was assessed for each satiation (food 1 and food 2) separately, and the two values were then added to obtain a cumulative difference score. Similarly, the cumulative difference score was obtained for MUS infusion in phase III. For the purpose of data analysis, all saline infusion results for each animal (both phase II and phase III) were pooled to generate a cumulative difference score; this ensured that both foods were represented in the control condition. The differences between the MUS infusion in phase II, phase III, and saline were analyzed by within-subject ANOVA with repeated measures. All statistical comparisons were within subjects.

Results

Histological verification

Figure 2 presents a photomicrograph of a brain section through the amygdala showing the placement of the infusion cannula bilaterally in BLA as intended. Histological evaluation demonstrated that the cannula was placed within the intended area of BLA and thus confirmed the accuracy of the MRI-guided stereotaxic positioning of the infusion platform and the postoperative verification of the cannula placement by MRI. The histological verification makes us confident that all of our infusions were in BLA in all cases. At this stage of the analysis, we did not attempt to make additional differentiation between individual nuclei within BLA.

Pretraining on object discrimination

The monkeys reached the criterion in an average of 36 sessions (range, 26–44) and made an average of 486 errors (range, 406–639).

Baseline object preference

Although at the beginning of the study both food 1 and food 2 appeared to be equally desirable, in the baseline probe sessions, all monkeys chose more objects rewarded with food 1 than objects rewarded with food 2. The average ratio between food 1 and food 2 objects was 27:3, and, even with repeated devaluations of both foods, this ratio remained stable across time. ANOVA with repeated measures did not show a significant change in the monkeys' choices across the six baseline sessions ($F = 1.002$; df = 5; $p = 0.437$), indicating that the baseline choice was independent of either the satiation procedures or the drug manipulations.

Because initial interest in the two foods appeared to be balanced, we were surprised by the extent of the differential baseline preference between the objects baited by food 1 and those baited by food 2. However, the monkeys' consistent preference for one type of food reward versus the other is in agreement with reports from other studies that used the same task (see Materials and Methods). Under control (saline) conditions (Table 1), devaluation of food 1 resulted in a significant decrease in choice of food 1 objects (paired t test; $t = 5.79$; $p < 0.05$), and devaluation of food 2 resulted in a significant decrease in choice of food 2 objects ($t = 3.05$, $p < 0.05$), indicating that both devaluations contribute to the total effect.

It is possible that the fact that the animals had no previous exposure to the fruit snacks allowed them to maintain their interest in this reward throughout the initial discrimination training period (phase I). This may also account for the observation that there were significantly fewer errors to criterion for object discrimination of the fruit snack-baited objects (180 ± 15) compared with peanut-baited objects (305 ± 24). Moreover, the preference for fruit snacks was manifest by a greater consumption (based on food weight) of fruit snacks (166 ± 17 g) compared with that of peanuts (75 ± 10 g) during satiety induction (devaluation).

Effect of devaluation

In the probe sessions, the monkeys are faced with a choice between two reinforced objects with equivalent reinforcement history (both objects consistently reinforced throughout phase I) so that devaluation-induced change in object preference is not driven by trained stimulus–reward (S–R) associations. The shift in object preference is instead dependent on stimulus–stimulus (S–S) associations between the objects and the particular food rewards. As a result, we found that when we obtained a drug-induced impairment of devaluation, it affected object choice without necessarily altering the loss of interest in the devalued food (see below).

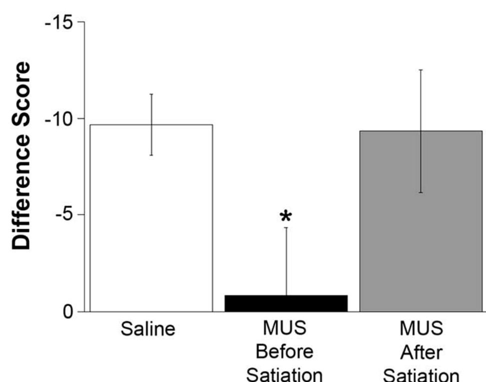


Figure 5. The average difference scores and SEM between object choice in baseline and infusion probe sessions in each infusion condition. Saline data were averaged for both BEFORE (phase II) and AFTER (phase III) conditions. The average change observed for infusion of MUS before satiation (phase II) was significantly different from both saline infusions and infusions of MUS AFTER satiation (phase III; $*p < 0.05$). Error bars represent SEM.

Effect of MUS in BLA: infusion before (phase II) versus after (phase III) satiation

As noted in Materials and Methods, each monkey received only one saline infusion before satiation (phase II) with one of the two foods and only one saline infusion after satiation (phase III) with the other food (Table 1). These two variants of the control conditions were not significantly different from each other with respect to difference scores (paired comparison; $t = -0.522$; $p = 0.624$) and were therefore combined (across both conditions and both foods) into cumulative difference scores. These cumulative difference scores were used for comparison with those from the two drug conditions.

Cumulative difference scores obtained in both experimental conditions, which included MUS infusions, and those obtained under saline infusions were compared by within-subject ANOVA with repeated measures, which resulted in a significant effect of the experimental condition (Fig. 5) ($F = 5.60$; df $= 2, 10$; $p < 0.05$). *Post hoc* pairwise comparisons (Bonferroni's; $p < 0.05$) showed that difference scores obtained under MUS infusion before satiation (phase II) were significantly lower than those obtained both under MUS infusion after satiation (phase III) and under saline. In contrast, difference scores obtained under MUS infusion after satiation (phase III) were not different from those obtained under saline condition.

Comparisons with nonsated (baseline) condition

Saline infusion in BLA in phases II and III combined

Under control conditions, analysis of object choices in the probe sessions revealed that the monkeys made significantly fewer choices of the objects baited with the devalued food after selective satiation compared with choices in the baseline condition (paired comparison; $t = 5.67$; $p < 0.05$). As indicated in Table 1, the monkeys chose on average 21 objects baited with the devalued food after selective satiation compared with 30 objects under baseline condition, yielding a difference score of -9 ($21 - 30$), which represents an average decrease of 30%.

MUS infusion in BLA in phase II

As indicated in Table 2, the monkeys receiving MUS in BLA just before satiation chose on average 31 objects baited with the devalued food compared with 32 objects under baseline (nonsated) condition, yielding a difference score of -1 (average decrease of only 3%). The monkeys' choices of objects baited with the devalued food in the infusion probe session and their choices under baseline conditions were not significantly different (paired comparison; $t = 0.236$; $p = 0.823$), indicating that selective satiation had no impact on object choice in this condition. By blocking the BLA activity, we prevented changes in the representation of the primary reinforcer, which thereby left the object preference unmodified by devaluation.

MUS infusion in BLA in phase III

As indicated in Table 3, the monkeys receiving MUS in BLA just before the experimental probe session (after selective satiation) chose on average 23 objects baited with the devalued food compared with 32 objects under the baseline condition, yielding a difference score of -9 (an average decrease of 28%). The difference between these two conditions was significant (paired comparison; $t = 2.935$; $p < 0.05$), indicating that devaluation of the objects had occurred. Moreover, the magnitude of the devaluation was equivalent to that obtained under saline control conditions (30%).

Food consumption during selective satiation

To rule out the possibility that the significant differences between the drug conditions are affected by the amount of food the monkeys ate under various drug conditions, we assessed the food consumption in each selective satiation. The amount of food the monkeys ate during the selective satiation changed significantly across sessions ($F = 4.78$; df $= 2, 8$; $p < 0.05$). They ate significantly more food during the first satiation (296 g; cumulative for both foods) than in the second satiation (211 g; paired *t* test; $t = 3.79$; $p < 0.05$), but with no significant change thereafter (217 g; paired *t* test; $t = 0.17$; $p = 0.87$). However, the amount of food eaten did not differ among the three experimental conditions: MUS infusion before satiation (phase II), MUS infusion after satiation (phase III), and saline (ANOVA; $p = 0.13$). Comparing each drug condition for food consumption based on food weight,

Table 2. MUS infusion BEFORE satiation

	Food 1 satiation			Food 2 satiation					
Monkey	Baseline probe	Infusion probe	Difference score	Baseline probe	Infusion probe	Difference score	Cumulative baseline	Cumulative infusion	Cumulative difference score
OH	28	28	0	1	3	2	29	31	2
OK	26	26	0	2	6	4	28	32	4
OG	29	23	−6	1	1	0	30	24	−6
JN	30	29	−1	1	9	8	31	38	7
ZC	30	24	−6	1	11	10	31	35	4
GR	23	13	−10	17	11	−6	40	24	−16
Average	28	24	−4	4	7	3	32	31	−1

The number of objects rewarded with the devalued food that the monkeys chose in the infusion probe session subsequent to MUS infusions BEFORE selective satiation and in the preceding baseline probe session. Difference score represents the numerical difference between the number of objects chosen in the baseline probe session and in the infusion probe session.

Table 3. MUS infusions AFTER satiation

	Food 1 satiation			Food 2 satiation					
Monkey	Baseline probe	Infusion probe	Difference score	Baseline probe	Infusion probe	Difference score	Cumulative baseline	Cumulative satiation	Cumulative difference score
OH	27	28	+1	2	2	0	29	30	+1
OK	28	17	−11	15	9	−6	43	26	−17
OG	28	20	−8	0	0	0	28	20	−8
JN	27	22	−5	1	0	−1	28	22	−6
ZC	29	17	−12	0	6	6	29	23	−6
GR	27	10	−17	9	6	−3	36	16	−20
Average	28	19	−9	4	4	0	32	23	−9

The number of objects rewarded with the devalued food that the monkeys chose in the infusion probe session subsequent to MUS infusions AFTER selective satiation and in the preceding baseline probe session. Difference score represents the numerical difference between the number of objects chosen in the baseline probe session and in the infusion probe session.

Table 4. Control infusions

	Increased time			MUS (18 nmol)		
Monkey	Baseline probe	Infusion probe	Difference score	Baseline probe	Infusion probe	Difference score
OH				29	26	−3
OG	27	16	−11	27	15	−12
JN	30	20	−10	30	23	−7
Average	28.5	18	−10.5	28.7	21.3	−7.3

The number of objects rewarded with the devalued food that the monkeys chose in the infusion probe session subsequent to either MUS infusions AFTER selective satiation with increased time or 18 nmol MUS infusions AFTER selective satiation and in the preceding baseline probe session. Difference score represents the numerical difference between the number of objects chosen in the baseline probe session and in the infusion probe session. Both control infusions were conducted by selectively sating with food 1, the preferred food.

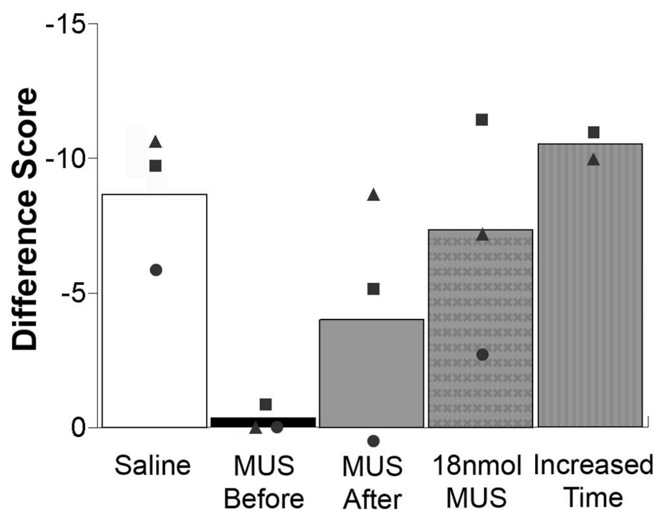


Figure 6. The average difference scores between object choice in baseline and infusion probe sessions in each infusion condition in the three monkeys that were used in the follow-up experiments. Circles represent monkey OH, squares represent monkey JN, and triangles represent monkey OG. No significant difference was found between 18 nmol of MUS and either the MUS after (phase III) or saline condition, whereas 18 nmol of MUS was significantly different from MUS before (phase II; $p < 0.05$). There were only two monkeys in the "increased time" condition, which precluded statistical comparison.

we found no significant difference between the three conditions in the amount of food eaten for peanuts, fruit snacks, or both cumulatively, indicating that the monkeys ate a comparable amount of food in each of the experimental conditions.

Primary reward consumption

During the testing of the monkeys in the devaluation condition, we observed that when the monkeys chose the object overlying the devalued food in the probe session, they often avoided retrieving the reward. After this observation, we began to record the number of food rewards eaten after the object choice. Because these records were started after the beginning of the study, this information is not available for all monkeys in all conditions. In the baseline probe sessions, the monkeys ate all rewards uncovered by the object selected. In contrast, in the probe sessions after selective satiation, the devalued food rewards were eaten in only one-half of the instances in which the monkeys chose the objects rewarded by it [this finding is consistent with those of Izquierdo and Murray (2004a)], and this amount was not significantly different across treatment conditions. Selective satiation also reduced the consumption of the nondevalued food, but to a lesser extent. Analysis of food consumption (devalued vs nondevalued) by treatment (saline, MUS before, and MUS after satiation) showed that the monkeys ate significantly more of the nondevalued food compared with the devalued food (ANOVA; $F = 5.86$; df = 1, 14; $p < 0.05$), but the effect of either the treatment or the interaction of the two factors was not significant ($F = 0.01$, df = 2, 14, $p = 0.99$; $F = 0.21$, df = 2, 14, $p = 0.82$, respectively). These results indicate that the effects of satiation are selectively attached to the foods when devalued (food 1 and food 2 in separate sessions) and that the drug infusions did not interfere with this effect.

Additional control experiments

One of the possible explanations for the differential effect between the two conditions, MUS infused in phase II versus phase III, is that the two conditions differed in the time elapsed between the drug infusion and the beginning of the behavioral testing in the probe session. When MUS was infused in phase II, the probe session started shortly after satiation (i.e., ~30 min after the MUS infusion), whereas when the MUS was infused in phase III, the probe session started immediately after the infusions (Fig. 4*A*, *B*). Thus, the lack of effect of MUS infused in phase III (immediately before the probe session) might have been attributable to an insufficient time allowed for the drug to spread over the same area of BLA as when the drug was infused before satiation. To rule out this possibility, we performed two additional control experiments with two and three of the same monkeys, respectively.

In the first experiment, we extended the MUS pretreatment time in phase III by 30 min (Fig. 4*C*). During that time, the monkey was placed back in its home cage, and, after 30 min, it was tested in the probe session. This experiment was done with two monkeys (OG and JN; the third monkey, OH, became ill after finishing the second experiment and could not be tested further) using devaluation of food 1 only. In the second experiment, we doubled the concentration of MUS infused in phase III to in-

crease the amount of $GABA_A$ receptor occupancy over the infused area of BLA. Thus, 18 nmol of MUS in 1 μl was infused (Fig. 4*D*). This experiment was done with three monkeys (OH, OG, and JN). The number of objects baited with the devalued food the monkeys chose in the infusion probe sessions, the number of objects baited with the same food the monkeys chose in the preceding baseline probe sessions, and the difference scores between the two values are presented in Table 4 for each of the two experiments.

In the first experiment, the difference scores of both monkeys were numerically higher than those under any other condition, indicating that MUS infused in phase III (after satiation) did not block the devaluation effect even with the extended time allowed after the infusion. Because there were only two monkeys available for this part of the experiment, this precluded statistical comparison (Fig. 6). Similarly, in the second experiment, the difference scores were higher than those obtained under MUS infusion in phase II (before satiation) ($t = 5.00$; $p < 0.05$) and not different from saline ($t = 0.28$; $p = 0.81$). Again, these results indicated that the increased dose of MUS given just before the probe session did not block the devaluation.

Discussion

Our results indicate that, during satiation (phase II), BLA is required to register a change in the incentive value of the reward and allow the subsequent adjustment in the monkeys' object preference during the probe session (phase III). When BLA was inactivated by focal infusion of the $GABA_A$ agonist muscimol during satiation, the monkeys continued to select the objects associated with the devalued food, although they showed reduced interest in that food. Our finding that transient inactivation of BLA during phase II interfered with the subsequent modification of object preference in phase III confirms our hypothesis that the devaluation of conditioned reinforcers in nonhuman primates requires active processing in the amygdala during the period of selective satiation.

Transient focal inactivation within BLA in the present study yielded results equivalent to those after complete amygdalectomy (Malkova et al., 1997). Thus, BLA in monkeys appears to have a role in registering the incentive value of the reinforcer and its changes, analogous to that described in conditioned appetitive behavior in rats (Everitt et al., 1989, 1991; Hatfield et al., 1996; Schoenbaum et al., 1999; Blundell et al., 2001, 2003; Balleine et al., 2003).

In our experiment, during the probe sessions, no new learning can occur because each baited object is presented only once while testing is underway. All learning of the predictive association between each object and its particular reward was established during the initial object discrimination training (in the presence of an intact amygdala) and maintained throughout the experiment, as verified by the monkeys' choice behavior under control conditions. Thus, the deficit we observed subsequent to BLA inactivation during satiation must reflect either an inability of the animals to make the stimulus–stimulus association between the objects and the revised reward value or an inability to selectively inhibit responding to (i.e., reaching for) the objects associated with the devalued food. Blundell et al. (2001) argue that BLA lesioned animals are not merely deficient in controlling their responses, but have deficits in the use of conditioned stimulus-evoked "representation of the sensory aspects of motivationally significant events."

When we examined the effect of BLA inactivation specifically during the probe session (phase III), our data demonstrated that it did not interfere with expression of the devaluation. The monkeys modified their object preference just as they did under no-drug conditions. These results indicate that once the devaluation of the primary reinforcer has already been registered and encoded (during phase II), BLA activity is neither required for reassigning value to the conditioned reinforcers nor required for the expression of the resulting behavioral preferences. Thus, the locus in which the devaluation is embedded must be somewhere other than BLA. This is consistent with one view of the role of the amygdala in fear conditioning, in which BLA enables the formation of associations that are stored elsewhere (McGaugh et al., 1996), whereas it is in contrast to the view in which amygdala is seen as a locus for memory encoding and storage (Fanselow and LeDoux, 1999). Consistent with the proposal of Cahill and McGaugh (1998), our data indicate that amygdala serves to facilitate the long-term storage of information at one or more distant loci, to which the amygdala projects (Amaral et al., 1992; Baxter et al., 2000). Such structures may include mediodorsal thalamus (Corbit et al., 2003; Izquierdo and Murray, 2004b), striatum (Yin et al., 2004), orbitofrontal cortex (OFC) (Baxter and Murray, 2002), and nucleus accumbens (Corbit et al., 2001) (but see de Borchgrave et al., 2002; Izquierdo and Murray, 2004b). Previous research indicates that OFC is especially suited for storing the representation of the devalued primary reinforcer and making the S–S links to the visual representations of the conditioned reinforcers (Porrino et al., 1981; Carmichael and Price, 1995; Tremblay and Schultz, 1999; Holland and Gallagher, 2004). This proposal is supported by observations in monkeys that lesions of OFC (Izquierdo et al., 2004) and surgical disconnection of the amygdala and OFC (Baxter et al., 2000) disrupt conditioned reinforcer devaluation. Moreover, neurons in OFC respond to the sight of a particular food in hungry subjects but not in subjects sated on that food (Rolls et al., 1989; Critchley and Rolls, 1996). The ability of these neurons to rapidly reevaluate and update their associations (Rolls, 2000) makes them ideal for updating the representation of the conditioned reinforcers. Furthermore, OFC neurons regulate the behavioral response to shifts in reward value and allow strategic decision-making based on expected outcomes (Schoenbaum et al., 1998, 1999, 2003). Together with our data, these findings suggest that, although activity in BLA is necessary for registering the devaluation (recalibrating the incentive value) of the primary reinforcer, it is not required for the subsequent application that guides behavior. This supports the prediction of Yin and Knowlton (2002) regarding BLA that "it may be possible to prevent changes in the US representation after training by inactivating this structure."

The fact that we observed a deficit in devaluation despite the integrity of the amygdala during initial training (phase I) is consistent with the findings by Baxter et al. (2000) using the same behavioral paradigm as in the present study and with those by Cador et al. (1989) and Burns et al. (1993) in which interventions in BLA were also done after initial training. These approaches are a clear departure from studies in which lesions or drug treatments were present during the first phase of training (Hatfield et al., 1996; Malkova et al., 1997).

Based on our evidence taken in the context of previous findings in the rat, we suggest that BLA serves to facilitate the representations of stimuli (possibly in OFC) in a manner that is flexible and amenable to subsequent modification in the absence of any S–R retraining. This function would apply equally to the initial training in phase I as it does to the updating in phase II. Although we did not test the effects of inactivation in phase I, previous results in rats have indicated that lesions or inactivation of BLA

done before phase I acquisition did not prevent first-order conditioning but did impair the ability of the first-order CS to support second-order conditioning (Setlow et al., 2002; Hatfield and Gallagher, 1995). BLA lesioned animals may acquire first-order conditioning via S–R associations (independent of OFC) rather than by more flexible cognitive representations of the food reinforcer (Setlow et al., 2002). Such associations would then not be accessible to S–S updating in the face of devaluation by satiety, extinction, or taste aversion (Hatfield et al., 1996).

Our results are in contrast to those of Pickens et al. (2003), who found that when BLA was lesioned after phase I in advance of devaluation in phase II, this intervention did not disrupt the subsequent devaluation of the conditioned stimulus. The authors suggested that BLA is critical for forming the representations that link cues with incentive properties of outcomes; once these representations are formed during the initial learning, BLA is not needed for maintaining or updating them with new information. Although the lack of effect of BLA lesions in that study may result from compensatory adaptation during the 2 week recovery period, it also may reflect the distinct type of devaluation used. In the study by Pickens et al. (2003), a repeated toxin-induced conditioned taste aversion was used to devalue the food. This long-lasting species-specific survival response may drive encoding of the devaluation at the level of OFC in the absence of enabling by the amygdala (the devaluation was highly sensitive to OFC lesions in the same study). On the other hand, in circumstances in which the devaluation is based on transient satiety, as in our study and in the human imaging experiments of Gottfried et al. (2003), or in reward reduction, as in the study by Salinas and McGaugh (1995), BLA may serve an amplification function necessary to enable the OFC to register and encode the sensory representation of the altered incentive value of the reward. In circumstances in which the change in reward value is threatening (e.g., illness), this amplifying/enabling function of the amygdala may be bypassed. Thus, we propose that the extent to which BLA function is required for the registration of a change in reward value depends on the extent to which amplification is needed to bring the change to the "attention" of the OFC.

Our data, using a GABA agonist to reversibly inactivate BLA in nonhuman primates, provide the first demonstration of a dissociation between the effects of amygdala inactivation during devaluation versus during the subsequent expression of that devaluation for an appetitive reinforcement. We can therefore conclude from our data using a GABA agonist to reversibly inactivate BLA in nonhuman primates that (1) the registration and encoding of a reduction in reward value depends on BLA activation specifically during the period of satiety-induced devaluation, and (2) once the devaluation has occurred, BLA activation is not required for the subsequent expression of the devaluation involving its transfer to secondary reinforcers. Thus, the BLA plays a vital, although transient, role in the process by which updating and revision of reward associations takes place.

References

Amaral DG, Price JL, Pitkanen A, Carmichael ST (1992) Anatomical organization of the primate amygdaloid complex. In: The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction (Aggleton JP, ed), pp 1–66. New York: Wiley.

Balleine BW, Dickinson A (2000) The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. J Neurosci 20:8954–8964.

Balleine BW, Killcross AS, Dickenson A (2003) The effects of lesions of the basolateral amygdala on instrumental conditioning. J Neurosci 23:666–675.

Baxter MG, Murray EA (2002) The amygdala and reward. Nat Neurosci 3:563–573.

Baxter MG, Parker A, Lindner CC, Izquierdo AD, Murray EA (2000) Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. J Neurosci 20:4311–4319.

Blundell P, Hall G, Killcross S (2001) Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. J Neurosci 21:9018–9026.

Blundell P, Hall G, Killcross S (2003) Preserved sensitivity to outcome value after lesions of the basolateral amygdala. J Neurosci 23:7702–7709.

Burns LH, Robbins TW, Everitt BJ (1993) Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. Behav Brain Res 55:167–183.

Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 30:77–86.

Cahill L, McGaugh JL (1998) Mechanisms of emotional arousal and lasting declarative memory. Trends Neurosci 21:294–299.

Carmichael ST, Price JL (1995) Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J Comp Neurol 363:615–641.

Corbit LH, Muir JL, Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: evidence of a functional dissociation between accumbens core and shell. J Neurosci 21:3251–3260.

Corbit LH, Muir JL, Balleine BW (2003) Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. Eur J Neurosci 18:1286–1294.

Critchley HD, Rolls ET (1996) Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol 75:1659–1672.

Davis M (1992) The role of the amygdala in conditioned fear. In: The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction (Aggleton JP, ed), pp 255–306. New York: Wiley.

de Borchgrave R, Rawlins JN, Dickinson A, Balleine BW (2002) Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. Exp Brain Res 144:50–68.

Dooley DJ, Dubach MF, Blake PH, Bowden DM (1981) A chronic stereotaxic guide-tube platform for intracranial infections in macaques. J Neurosci Methods 3:385–396.

Dybdal D, Dubach M, Vaurio R, Eastabrooke I, Heath A, Gale K (1997) Focal inhibition of primate substantia nigra: a new model for human dyskinetic and dystonic syndromes. Soc Neurosci Abstr 23:1963.

Everitt BJ, Cador M, Robbins TW (1989) Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience 30:63–75.

Everitt BJ, Morris KA, O'Brien A, Robbins TW (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 42:1–18.

Everitt BJ, Cardinal RN, Hall J, Parkinson JA, Robbins TW (2000) Differential involvement of amygdala subsystems in appetitive conditioning and drug addiction. In: The amygdala: a functional analysis (Aggleton JP, ed), pp 353–390. Oxford: Oxford UP.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229–232.

Gottfried JA, O'Doherty J, Dolan RJ (2003) Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science 301:1104–1107.

Hatfield T, Gallagher M (1995) Taste-potentiated odor conditioning: impairment produced by infusion of an *N*-methyl-D-aspartate antagonist into basolateral amygdala. Behav Neurosci 109:663–668.

Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996) Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci 16:5256–5265.

Holland PC, Gallagher M (2004) Amygdala-frontal interactions and reward expectancy. Curr Opin Neurobiol 14:148–155.

Izquierdo A, Murray EA (2004a) Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. J Neurophysiol 91:2023–2039.

Izquierdo A, Murray EA (2004b) Mediodorsal nucleus of the thalamus contributes to reinforcer devaluation effects in rhesus monkeys. Soc Neurosci Abstr 30:84.9.

Izquierdo A, Suda RK, Murray EA (2004) Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. J Neurosci 24:7540–7548.

LeDoux JE (1995) Emotion: clues from the brain. Annu Rev Psychol 46:209–235.

Lower LL, Silver CC, Maniatis PG, Clark C, Gale K, Malkova L (2002) $GABA_A$ receptor activation in the amygdala increases social interactions. Soc Neurosci Abstr 28:86.12.

Malkova L, Gaffan D, Murray EA (1997) Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. J Neurosci 17:6011–6020.

Malkova L, Barrow KV, Lower LL, Gale K (2001) AMPA receptor blockade in perirhinal cortex impairs visual recognition in monkeys. Soc Neurosci Abstr 27:455.5.

Martin JH (1991) Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neurosci Lett 127:160–164.

Martin JH, Ghez C (1999) Pharmacological inactivation in the analysis of the central control of movement. J Neurosci Methods 86:145–159.

McGaugh JL, Cahill L, Roozendaal B (1996) Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci USA 93:13508–13514.

Parkinson JA, Crofts HS, McGuigan M, Tomic DL, Everitt BJ, Roberts AC (2001) The role of the primate amygdala in conditioned reinforcement. J Neurosci 21:7770–7780.

Pickens CL, Saddoris MP, Setlow B, Gallagher M, Holland PC, Schoenbaum G (2003) Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. J Neurosci 23:11078–11084.

Porrino LJ, Crane AM, Goldman-Rakic PS (1981) Direct and indirect pathways from the amygdala to the frontal lobe in rhesus monkeys. J Comp Neurol 198:121–136.

Pribram KH (1986) The hippocampus system and recombinant processing. In: The hippocampus, Vol 4 (Isaacson RL, Pribram KH, eds), pp 329–370. New York: Plenum.

Rolls ET (2000) The orbitofrontal cortex and reward. Cereb Cortex 10:284–294.

Rolls ET, Sienkiewicz ZJ, Yaxley S (1989) Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. Eur J Neurosci 1:53–60.

Salinas JA, McGaugh JL (1995) Muscimol induces retrograde amnesia for changes in reward magnitude. Neurobiol Learn Mem 63:277–285.

Saunders RC, Aigner TG, Frank JA (1990) Magnetic resonance imaging of the rhesus monkey brain: use for stereotactic neurosurgery. Exp Brain Res 81:443–446.

Schoenbaum G, Chiba AA, Gallagher M (1998) Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci 1:155–159.

Schoenbaum G, Chiba AA, Gallagher M (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci 19:1876–1884.

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

Setlow B, Gallagher M, Holland PC (2002) The basolateral complex of the amygdala is necessary for acquisition but not expression of CS motivational value in appetitive pavlovian second-order conditioning. Eur J Neurosci 15:1841–1853.

Thornton JA, Malkova L, Murray EA (1998) Rhinal cortex ablations fail to disrupt reinforcer devaluation effects in rhesus macaques (*Macaca mulatta*). Behav Neurosci 112:1020–1025.

Tremblay L, Schultz W (1999) Relative reward preference in primate orbitofrontal cortex. Nature 398:704–708.

Weiskrantz L (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. J Comp Physiol Psychol 49:381–391.

Wellman LL, Clark C, Gaertner BG, Cole CE, Silver CC, Gale K, Malkova L (2004) Manipulation of $GABA_A$ receptors in central nucleus of amygdala: effects on social interactions. Soc Neurosci Abstr 30:215.3.

Yin HH, Knowlton BJ (2002) Reinforcer devaluation abolishes conditioned cue preference: evidence for stimulus-stimulus associations. Behav Neurosci 116:174–177.

Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.