Research

Temporary basolateral amygdala lesions disrupt acquisition of socially transmitted food preferences in rats

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Lesions of the basolateral amygdala (BLA) have long been associated with abnormalities of taste-related behaviors and with failure in a variety of taste- and odor-related learning paradigms, including taste-potentiated odor aversion, conditioned taste preference, and conditioned taste aversion. Still, the general role of the amygdala in chemosensory learning remains somewhat controversial. In particular, it has been suggested that the amygdala may not be involved in a form of chemosensory learning that has recently received a substantial amount of study—socially transmitted food preference (STFP). Here, we provide evidence for this involvement by pharmacologically inactivating the basolateral amygdala bilaterally during STFP training. The same inactivation sites that impaired taste aversion learning eliminated the normally conditioned preference for a food smelled on a conspecific's breath. Impairments of learned preference persisted even in testing sessions in which BLA was not inactivated, and learning was normal when the BLA was inactivated only during testing sessions; thus, the impairment was a true acquisition deficit. In conjunction with previous results from other paradigms, therefore, our data suggest that the amygdala is vital for learning procedures involving pairings of potent and arbitrary chemosensory stimuli.

In rodents, the amygdala is one of the major recipients of gustatory and olfactory information. The basolateral amygdala (BLA) receives olfactory projections from the olfactory piriform cortex and gustatory projections from the parabrachial nuclei and insular cortex (Pare 2003). It is unsurprising, therefore, that single-unit and field potential electrophysiology studies of BLA in awake rats demonstrate responses to both gustatory (Yasoshima et al. 1995; Nishijo et al. 1998) and olfactory stimuli (Rosenkranz and Grace 2002; Sevelinges et al. 2004). These findings make the amygdala a probable neural substrate for sensory processes involving gustation and/or olfaction.

Permanent lesion studies support this prediction, providing evidence that the BLA plays an important role in taste- and olfaction-related classical learning behaviors. Excitotoxic BLA lesions significantly alter taste palatability (Ganaraja and Jegannathan 1999, 2000; Touzani et al. 1997) and attenuate taste preference learning (Gilbert et al. 2003). BLA ablation also impairs learning of conditioned taste aversions (CTA) (Rollins et al. 2001; Mickley et al. 2004; Reilly 2005) and taste-potentiated odor aversions (Hatfield et al. 1992; Ferry et al. 1999; Ferry and Di Scala 2000; Sakai and Yamamoto 2001).

Despite these findings, however, the involvement of BLA in basic chemosensory aversion and preference learning remains a topic of some controversy (Yamamoto et al. 1994; Reilly 2005). This is partially because the BLA dependence of CTA may vary as a function of exact experimental protocol; there is some evidence that the amygdala may be vital for the formation of aversions to tastes delivered to a passive rat (i.e., via intra-oral cannulae, or IOC), but not for similar aversions formed to tastes sampled actively (i.e., via a lick spout; Schafe et al. 1998).

Another reason for the controversy has to do with social transmission of food preference (STFP), a learning paradigm that

involves both olfactory and taste stimuli. This paradigm requires that rat subjects learn to associate the scent of a conspecific's breath with that of a food recently consumed by that conspecific; following exposure to the odor mixture, preference for the demonstrated diet is developed over alternatives (Galef Jr. and Whiskin 1997). Recently, Burton et al. (2000) suggested no BLA involvement in this task. In this study, however, even control rats learned poorly (see Alvarez et al. 2001), perhaps because rats were exposed to the unconditioned stimulus (UCS, carbon disulfide in rat breath) multiple times; it is possible that a learning decrement caused by repeated UCS exposure (e.g., Nakajima et al. 2006) could serve to mask amygdalar involvement in learning, as it appears to have masked hippocampal involvement (Bunsey and Eichenbaum 1995; Alvarez et al. 2001).

Here we revisited this issue, investigating amygdalar involvement in STFP using temporary BLA inactivation (via intracranial infusion of the GABA α agonist muscimol). Past amygdala lesion studies have used electrolytic or excitotoxic methods, permanently destroying the structure (Touzani et al. 1997; Clark et al. 2002; Reilly 2005); such lesions necessarily conflate an area's involvement in learning and performance, and leave open the possibility that observed effects are the result of secondary damage to other structures connected to the BLA. We therefore infused muscimol into the BLA during STFP training (and also during CTA training, using both IOC and a one-bottle lick spout for taste delivery).

Our results demonstrate that BLA is crucial for acquisition of STFP. We show that inactivation has no impact on expression of a learned preference, and that the impact of the muscimol infusion on acquisition is smaller the further the infusion site is from the center of the BLA. We further confirmed the validity of our techniques and results by showing that, as expected, BLA inactivation attenuates CTA learning when taste is administered through an IOC. These results expand our understanding of emotional chemosensory learning, offering clues as to more precise characterizations of amygdalar function.

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Results

Experiment 1: STFP acquisition

Initial testing in the STFP paradigm was performed 24 h after the training (i.e., social preference transmission session) day; the 24-h delay ensured, in Experiment 2 (see below), that the initial infusion of muscimol had long since dissipated by T1. As there was no significant difference between the proportion of demonstrated diet consumed for different diets in control rats ($t_{[14]} = 0.91$, $P > 0.35$), we compiled the data across diet for all analyses. The testing sessions examined STFP learning 1 d (T1) and 1 wk (T7) after training. We will discuss the results of each testing session in turn.

An ANOVA of the T1 data revealed that muscimol in the BLA inhibited STFP learning. More demonstrated diet was consumed than undemonstrated (main effect $F_{[1,18]} = 4.9$, $P < 0.04$), and there was a significant interaction between diet and drug ($F_{[1,18]} = 10.3$, $P < 0.005$). Tests of simple effects revealed that control rats learned to prefer the demonstrated diet over the undemonstrated diet ($F_{[1,7]} = 10.4$, $P < 0.02$), but that rats with BLA inactivation did not consume significantly more demonstrated diet ($F_{[1,11]} < 1$, $P > 0.7$): Saline-infused rats took $74.7 \pm 6.9\%$ (mean $\pm$ SEM) of their total diet from the demonstrated diet, while rats with BLA inactivation ate $50.6 \pm 8.2\%$ demonstrated diet.

Figure 1A shows these results graphically. Rats that received bilateral saline infusions (i.e., the control group, right side of figure) showed robust STFP learning on T1: Most of these rats' ingested diet consisted of the diet that had previously been consumed by the demonstrator (white bar), and only ~20% consisted of the alternate diet (gray bar). In contrast, most of the rats that received bilateral muscimol infusions into the BLA failed to develop a preference for the demonstrated tastes. These rats ingested about equal amounts of demonstrated and undemonstrated diets on T1 (left side of figure).

We observed similar significant retention effects on T7: A mixed-effect ANOVA revealed no overall effect of drug ($F_{[1,18]} < 1$), a significant effect of diet ($F_{[1,18]} = 7.1$, $P < 0.02$), and a diet $\times$ drug interaction ($F_{[1,18]} = 5.8$, $P < 0.03$). Saline-infused rats showed long-term retention of learned preference ($80.6 \pm 4.9\%$ of consumption was demonstrated diet, $F_{[1,7]} = 20.5$, $P < 0.005$), whereas rats receiving muscimol infusions continued to show a lack of significant preference for the demonstrated diet ($53.7 \pm 8.3\%$ of consumption was demonstrated diet, $F_{[1,11]} < 1$, $P > 0.6$; see Fig. 1B).

Our T1 and T7 results show that BLA inactivation inhibits development of STFP. The absence of a main effect of drug ($F_{[1,18]} = 1.9$, NS), meanwhile, demonstrates that muscimol and saline-infused rats consumed similar amounts of food overall (BLA-inactivated rats ate 6 ± 0.54 SEM g, while control rats ate 7.5 ± 0.98 SEM g)—muscimol infusions did not interfere with the animals' eating.

Experiment 2: STFP acquisition vs. STFP expression

While the above experiment suggests that BLA inactivation during training and/or testing sessions makes a rat incapable of demonstrating an STFP, it does not wholly clarify the role of the BLA in acquisition and maintenance of the learned preference. It might be argued that inactivation of the BLA may have impaired preference expression despite leaving overall consumption intact, and that this performance impairment is what was observed in T1 and T7. Alternatively, it might be argued that BLA inactivation on T1 and T7 impaired maintenance of a memory trace that was successfully laid down on the training day.

In a second experiment, therefore, we evaluated STFP learning in two groups of rats: One received BLA muscimol infusions

A

B

Figure 1. BLA inactivation impairs STFP. The y-axis shows milligrams of each diet consumed. (A) For animals with the BLA inactivated (left bars), the demonstrated diets (white bar) were not preferred over undemonstrated diets (gray bar). Rats with an intact BLA (right bars), meanwhile, clearly show a preference for the demonstrated diet, which made up almost 80% of their total consumption during T1. * $P < 0.02$. (B) STFP learning was maintained in saline-infused rats. As on T1, on T7 saline-infused rats strongly preferred the demonstrated over the undemonstrated diet. Animals in the muscimol-infused group, meanwhile, ingested equal amounts of the demonstrated and undemonstrated diets. * $P < 0.005$. In this and all later figures, error bars = SEM.

solely during the interaction training session ($n = 7$), and the other solely during the session T1 ($n = 8$). In all other ways, this experiment was identical to the previous experiment, with saline vehicle infused during all non-muscimol sessions.

A three-way mixed ANOVA of the data from Experiment 2 revealed, similar to Experiment 1, a lack of difference between T1 and T7 performance ($F_{[1,36]} = 2.2$, $P > 0.1$); we therefore collapsed across testing sessions to summarize the effect of BLA inactivations. A significant diet $\times$ group interaction ($F_{[1,36]} = 16.0$, $P < 0.001$) revealed that muscimol had different effects when infused on training day and T1. Subsequent Simple Effects tests revealed that rats receiving BLA muscimol infusions on the interaction day failed to consume different amounts of the demonstrated and undemonstrated diets ($56.5 \pm 7.1\%$ demonstrated diet, $F_{[1,20]} = 1.4$, $P > 0.2$), but that rats receiving saline infusions on the training day showed strong STFP learning ($81.9 \pm 6.6\%$ demonstrated diet, $F_{[1,20]} = 26.7$, $P < 0.0001$) despite having received BLA muscimol infusions before that session itself. Figure 2 shows these data graphically: The left pair of bars shows the performance of rats deprived of BLA function during the training session, and the right pair of bars shows the performance of rats deprived of BLA function during T1. Inactivating BLA during the interaction training session inhibited acquisition of STFP, but rats receiving saline infusions during the training session and

Figure 2. BLA is necessary for acquisition but not expression of STFP. The y-axis shows milligrams of each diet consumed. During T1, rats for which the BLA was inactivated during training (*left bars*) consumed equal amounts of the demonstrated (white bar) and undemonstrated (gray bar) diets; i.e., they failed to show learning even when tested with an intact BLA. Rats trained with an intact BLA (saline-infused, *right bars*) showed a strong preference for the demonstrated diet, despite being tested with inactivated BLA. * $P < 0.001$.

muscimol infusions during T1 showed normal learning, and normal retention of learning on T7.

Experiments 3 and 4: CTA

As an additional test of our hypothesis that we impaired STFP learning through temporary inactivation of the BLA, we ran subsets of rats prepared with BLA cannula placements in two forms of conditioned taste aversion (CTA) paradigms. The results suggest that, as expected (see Schafe et al. 1998; Reilly 2005), temporary BLA inactivation using muscimol impairs classical conditioning of taste aversion, and does not impair a more operantly oriented CTA paradigm.

Experiment 3: IOC paradigm

In training sessions, all rats received an IOC infusion of sucrose or NaCl solution followed by ip injections of LiCl. They received another session with the taste the following day. Video recordings were coded for aversive behavioral responses to the tastes on both days. Numbers of gapes, head-shakes, and lateral arm flails were recorded and summed to quantify a simple, overall “aversion index”—a score of each rat’s aversion to the tastant (Fig. 3).

Because the variability differs widely between the two groups, a Wilcoxon test was performed on the Experiment 3 data. This test revealed a difference in aversion between the control and experimental groups (Wilcoxon $Z = 2.35$, $P < 0.02$). BLA inactivation attenuated the change in palatability of tastants caused by CTA training when the taste was delivered through an IOC.

The black bar in Figure 4 shows that rats receiving saline infusions into the BLA displayed a large aversion index during the testing session (18.5 ± 5.2). Rats never produced any aversive behaviors in the acquisition session; this, therefore, indicates that the previously palatable tastes had been made aversive by the training session—i.e., these rats had developed strong conditioned aversions. The white bar, meanwhile, shows that the testing session aversion index for rats receiving muscimol infusions during training sessions was less than half of that for control rats (8.6 ± 2.1).

Figure 3. Behaviors used to measure CTA for Experiment 3. (A) Gapes are a yawn-like opening of the rat’s mouth, in which the teeth are exposed and the jaw flexed up and down. (B) Head-shakes were recorded when the snout was moved quickly from one side of the body to the other. (C) Lateral arm-flails were high-frequency movements during which the rat’s forepaws were flung laterally and then brought together under the snout.

Experiment 4: One-bottle test

The one-bottle test is similar to the IOC paradigm in that both are taste aversion learning models, the only difference being that in the one-bottle test, the rat must actively approach a lick spout to receive the taste, as opposed to receiving the taste through the IOC while sitting passively. Since the taste is actively acquired as the rat consumes the stimulus from the lick spout, the learned aversion can be evaluated in terms of the reduction in the amount of fluid consumed from training to testing sessions. Previous studies suggest that learned aversions do not depend on the specific testing method, however—only on the training method (Schafe et al. 1998).

Rats drank sucrose or saline solution on the training day and received LiCl injections following consumption. On the testing day, the taste was presented once more; the reduction in consumption on the testing day is reported in Figure 5. Rats in both experimental and control groups showed CTA learning: Given only one bottle, rats will inevitably drink at least some small amount regardless of the aversiveness of the solution, but each group’s post-training consumption (control group = $4.3 \text{ mL} \pm 0.83 \text{ SEM}$; muscimol group = $4.2 \text{ mL} \pm 1.53 \text{ SEM}$) was around half of the amount consumed in the session preceding LiCl injection (control group = $8.5 \text{ mL} \pm 0.37 \text{ SEM}$; muscimol group = $8.3 \text{ mL} \pm 0.37 \text{ SEM}$). The training vs. testing differences were significant (control group: $t_{[5]} = 5.2$, $P < 0.005$; muscimol group: $t_{[5]} = 4.2$, $P < 0.01$), and the post-training difference between the groups was not ($t < 1$). This indicates that aversion in the BLA-inactivated group is strong and unattenuated.

The results from the two CTA experiments together make it clear that our muscimol infusions had the expected effects on rats’ behavior in a well-studied task. Previous studies have reported that amygdalar lesions cause impairments in CTA induced using IOC delivery of tastes, while sparing CTA induced when rats approach lick spouts for tastes. We have observed the same phenomenon here, which suggests that our muscimol infusions effectively inactivated BLA, thus adding additional evidence that it is BLA inactivation that caused STFP learning deficits in Experiments 1 and 2.

Histology and infusion placement

A sample of the histology is shown in Figure 6A; the cannula tract, which ends just above the BLA, is clearly visible. Figure 6B summarizes the histology from a random subset of 31 rats that received muscimol infusions. Only locations in the right hemisphere are shown, but data from the left hemisphere were qualitatively identical. Marks indicate the end of cannula tracks into the brain, where muscimol was infused. Almost all of the infusions were centered within the BLA (gray region, Fig. 6B).

An analysis of tip placement revealed the relationship be-

Figure 4. BLA inactivation impairs CTA learning using IOC taste delivery. The y-axis shows the number of aversive responses (i.e., sum of gapes, head shakes, and limb flails) produced in response to IOC-delivered sucrose during testing sessions. Rats that received BLA saline infusions during training (black bar) showed roughly twice as many aversive responses as experimental animals that received BLA muscimol infusions during training (white bar). Wilcoxon* $P < 0.02$.

tween muscimol infusion site and learning. We measured the horizontal distance between each infusion site and the center of the BLA (see Materials and Methods), and averaged the distances for the cannula in each hemisphere to obtain an average placement for each Experiment 1 rat. We then correlated this measure with the amount learned by each rat (the percentage of consumed food that consisted of demonstrated diet, averaged across T1 and T7). The Pearson product moment correlation between these two values was $r = 0.80$; this correlation is significantly different from 0 ($t_{[8]} = 3.75$, $P < 0.01$), providing still more evidence that BLA is a vital region for STFP learning, and further showing that our muscimol infusions did not, in and of themselves, impair STFP.

Discussion

In our hands, the BLA is necessary for STFP acquisition, but not for retention. The further that our muscimol infusions were from the center of the BLA, the less effective they were at blocking learning; this makes it clear that the act of infusing muscimol was not in and of itself the cause of learning impairments, but rather that proximity to the BLA strongly influences the effectiveness of the inactivation. The validity of our techniques was confirmed by replication of earlier work on the role of BLA in conditioned taste aversion (Schafe et al. 1998): Temporary BLA inactivation blocked CTA learning in the IOC paradigm and not in the one-bottle paradigm.

The use of temporary inactivation shows that our results cannot be explained as a mere performance deficit. It is true that amygdalar lesions can cause changes in naive eating behavior (Touzani et al. 1997; Y. Wang and D.B. Katz, unpubl.), but such did not occur here: Rats with an inactivated BLA consumed normal amounts of chow, and preference impairments were not observed in animals for which the BLA was inactivated only during testing sessions (T1), demonstrating that the BLA is not necessary for expression of learning. Rats that received muscimol infusions before training, meanwhile, showed no preference learning even when tested with an intact BLA. These data strongly suggest that the BLA is a vital node in the network specifically underlying the odor-dependent learning of food preferences.

Clearly, however, the amygdala is not ubiquitously involved in all forms of chemosensory learning. At the very least, one-bottle CTA training is less amygdala-dependent than many other forms of chemosensory learning, including CTA using an IOC for

taste delivery. In their examination, Schafe et al. (1998) suggested that the important difference between the two CTA paradigms might be the fact that rats must produce an approach behavior to receive fluid from a lick spout, whereas fluid is delivered to a passive animal through an IOC. While we can only offer conjecture at this point, it is possible that the CTA and STFP results may be related, in that the vital pair of associated stimuli in STFP—the smell of the diet and the smell of carbon disulfide on the demonstrator's own breath (Galef Jr. et al. 1988)—are delivered without the need of acquisitive behavior on the part of the subject rat; the same is true in CTA when IOC delivery is used, but not when a lick spout is used. While the subject rat may approach the demonstrator during the interaction, and may sample the demonstrator's odor actively during investigation of a conspecific (Luo et al. 2003), it is ultimately an unbidden exhalation by the demonstrator that delivers the stimuli to the vicinity of the nose, much as an IOC delivers a taste into the mouth. That is, the rat does not itself trigger stimulus delivery during STFP training, as it would if pressing a bar or licking a spout. Overall, then, our data suggest that the BLA is most deeply involved in learning about “found” stimuli—tastes and smells that come to a rat in the course of the day. (It is inevitable, of course, that other factors also play into the BLA dependence, such that rats' ability to learn some tasks that make use of “acquired” stimuli is impaired by BLA inactivation.)

If this speculation is correct, then it might be argued that amygdalar involvement in CTA, which is most noticeable in a paradigm with little ecological validity, may be an artifact of a system that is optimized for social learning. This speculative conclusion is consistent with a broad literature suggesting that the amygdala is a “socio-emotional organ” (Ochsner 2004; Phelps 2006).

Our results differ from those of an earlier study (Burton et al. 2000) in which permanent amygdalar lesions did not impair STFP. It is possible that the difference between inactivation and lesion explains the different results (but see Alvarez et al. 2001). Another explanation for the discrepancy may reside in the fact that Burton et al. (2000) ran each demonstrator–observer pair on multiple trials using different flavors, after first giving the pair several interactions for purposes of adaptation. Use of so many interactions could have caused a phenomenon akin to latent inhibition, wherein repeated exposure to the unconditioned stimulus (in this case, carbon disulfide on the demonstrator's breath) reduces that stimulus' efficacy in driving learning (see,

Figure 5. Inactivation of BLA does not impair CTA in the one-bottle test. The y-axis shows the difference between the amount of sucrose consumed during the session just before LiCl injection (most rats consumed the full 10 mL available) and the testing session. The reduction in consumption for each group is significantly greater than zero, demonstrating successful learning. The effect of BLA inactivation on this learning, meanwhile, was not significant, demonstrating a lack of amygdalar involvement in this particular form of CTA.

Figure 6. The site vital for STFP acquisition can be localized to the BLA. (A) Sample histology, showing the placement of the cannula. The vertical lesions show the cannula tract, leading down toward the BLA. (B) A random subset ($n = 31$) of cannula tip sites are shown superimposed on a schematic representation of the rats' brain in the A-P plane (-2.80 mm from Bregma) of cannula implantation (Reprinted with permission from Elsevier © 1997, Paxinos and Watson 1997). (Gray shading, extent of left BLA).

for example, Nakajima et al. 2006). It's possible, therefore, that their animals learned such a mild preference that a lesion-induced impairment was not measurable. The fact that Burton et al.'s (2000) control animals conditioned much more poorly than ours (and more poorly than those conditioned by other researchers; see Bunsey and Eichenbaum 1995; Ganaraja and Jeganathan 2000; Alvarez et al. 2001; Clark et al. 2002) supports such an interpretation. In fact, since the learned irrelevance wrought by stimulus pre-exposure is itself dependent on the amygdala (Coutureau et al. 2001; Schaub and Koch 2000), any impairment of preference learning in Burton et al.'s (2000) lesioned rats may have been balanced out (or even overpowered) by an impairment in those animals' reduction of learned irrelevance. Such complex effects of brain lesions are not unheard of (see Stone et al. 2005).

Materials and Methods

Subjects

Thirty-six female Long-Evans rats (Charles River Laboratories) weighing 250–300 g at the start of surgery were used as subjects in the STFP experiments; 26 of these were also used for replication of previous findings regarding amygdalar involvement in CTA (see Schafe et al. 1998). An additional four rats were used in a pilot study to determine an appropriate dose of muscimol, and nine more females weighing 250–300 g served as “demonstrators” (see below). All animals were individually housed and kept on a 12-h light/12-h dark cycle (lights on at 7:00 a.m.), and testing sessions were run between 2:00 p.m. and 6:00 p.m. All methods complied with Brandeis University's animal use and care guidelines.

Surgery

Surgery was performed on observer and pilot study rats only. These rats were anesthetized via intraperitoneal (ip) injection of a ketamine/xylazine/acepromazine cocktail (ketamine, 100 mg/kg; xylazine, 5 mg/kg; acepromazine, 1 mg/kg) and given hourly update doses of the cocktail (20% volume of initial dose) to maintain deep anesthesia. An anesthetized rat was placed in a stereotaxic frame, the scalp was incised and retracted, and the skull was leveled. Small holes were drilled into the skull for five 0–80 screws and bilateral placement of guide cannulae (23-gauge, 15 mm in length) into basolateral amygdala by stereotaxic guidance (BLA coordinates relative to bregma: AP: -3.0 mm, ML ± 5.1 mm, DV -6.0 mm from dura; see Lalumière and McGaugh 2005; Touzani and Sclafani 2005). Cannulae were anchored to the skull

with dental acrylic. Stainless steel stylets (30-gauge, 15 mm in length) were inserted into the guide cannulae to ensure patency.

Each rat was also fitted with an intraoral cannula (IOC) for tastant delivery in conditioned taste aversion experiments. Thin polyethylene tubes were inserted between the first maxillary molar and the cheek, through the masseter muscle and inside the zygomatic arch, and out through the opening in the scalp, where they were cemented to the dental acrylic head-cap (see Fontanini and Katz 2005). Antibiotic ointment and iodine were applied liberally to the wound. Rats received subcutaneous injections of penicillin (30,000 units in 0.1 mL) and physiological saline (3 mL) immediately after surgery to aid recovery, and again 48 h later.

Statistics

Comparisons of diet consumption in the STFP tests were done using two-way mixed effects ANOVAs, with raw milligrams of consumed food serving as input to the analysis. Subsequent simple effects tests were run to explicate interaction effects; because controversy surrounds the use of pairwise post hoc tests (e.g., Scheffé, Tukey HSD) on mixed-effect ANOVAs, we used Simple Effects tests to further examine these data sets. Analysis of CTA results involved simple paired *t*-tests or their nonparametric equivalent.

The specific effect of infusion site on learning was evaluated as follows. From histology, cannula tip locations were marked on schematics of the amygdala (Paxinos and Watson 1997). The distance of these tips from the center of the BLA along a horizontal line was measured (drug diffusion is frequently extensive in the vertical direction due to both injection pressure and diffusion up the cannula track, so we focused on medial-lateral location for this analysis)—at the DV level of each tip site, the medial-lateral extent of BLA was measured and bisected, and the distance of the tip mark from that bisection point was noted. The pair of measurements from an individual surgery (one for each of two cannulae) was then averaged, giving a mean distance from the BLA center for each rat that received muscimol infusions in Experiment 1. A Pearson product moment correlation (*r*) was then calculated showing the relationship between tip location and learning (percentage of demonstrated diet in total consumption); this *r*-value revealed the relationship between the accuracy of BLA infusion and the impact of muscimol on learning.

Drug delivery

Rats were held in the experimenter's lap, and infusion cannulae were inserted into previously implanted guide cannulae. Infusion cannulae were connected to an infusion pump via polyethylene tubes. Muscimol or saline vehicle was infused into BLA at a rate of 25 μ L/min for 2 min (for a total of 50 μ L infusant). Infusion cannulae were left in place for an additional minute after the pump stopped, to allow infusant to diffuse away from the tips of the guide cannulae (Stone et al. 2005).

In a pilot study, different dosages of muscimol (500 ng, 200 ng, 100 ng diluted in 0.9% saline) were bilaterally infused into the BLA to determine a dose that preserved normal behavior. Motor activity and powdered chow consumption in food-deprived rats were compared with that of similar rats which received saline infusion. A dose of 100 ng muscimol in 50 μ L of saline bilaterally infused into the BLA did not cause a noticeable difference in normal behavior (data not shown), and so this dose, which is comparable to that used in other, similar studies (Maren et al. 2001; Berla and McGaugh 2003; Zarrindast et al. 2004), was used for all experiments. Results reported below confirm that this dose of muscimol did not interfere with normal consumption or naive preference.

Histology

Cannula placements were confirmed histologically after the experiment. Rats were deeply anesthetized and perfused transcardially with 10% saline followed by 10% formalin. Brains were removed and refrigerated in 30% sucrose/10% formalin solution for several days. Coronal sections (40- μ m) were cut on a cryostat and mounted to slides. Sections were stained with cresyl violet to visualize cannula tracks (see Stone et al. 2005).

Experiment 1: Social transmission of food preference

Diets

Cocoa and cinnamon diets, which have been used in many previous STFP studies (Bunsey and Eichenbaum 1995; Galef Jr. and Whiskin 1997; Burton et al. 2000; Clark et al. 2002), were used in training here. Diets were made by adding either 1.0 g of ground cinnamon (diet Cin) (McCormick) or 2.0 g of Hershey's Cocoa (diet Coc) to each 100 g of powdered laboratory rat chow (Lab-Diet Formula 2008).

Adaptation

Rats (experimental $n = 12$, control $n = 8$) were placed on a daily regimen, in which they were familiarized with the behavioral setup and the feeding schedule over 4 d. Food was removed from both demonstrator (rats fed flavored chow and used to transmit the preference) and subject rats (hereafter referred to as observers) 23 h prior to the first adaptation session. Demonstrators were placed in test cages with plain ground chow for 1 h. Immediately after feeding, demonstrator–observer pairs were placed together in a test cage without bedding for 30 min. These single adaptation interactions help to minimize aggressive behavior during the experiment. At the end of the interaction, subject rats were moved to individual feeding cages for 1 h where they acclimated to eating from two adjacent porcelain cups containing ground plain rat chow. No additional food was given after each daily training session. Body weight was closely monitored to ensure maintenance of 85% free-feeding body weight. Only rats that ate ≥ 1 g of food on the last two training days were used for subsequent experiments.

Social preference transmission

On the day of the experiment, demonstrators were first placed in feeding cages for 1.5 h where they received either diet Cin or diet Coc (randomized and counterbalanced). Only demonstrators that ingested ≥ 1 g of food proceeded on to the interaction (nine of 10 rats initially tested met this criterion). While the demonstrators ate, observer rats received muscimol or saline infusions bilaterally into the BLA; they were then placed with their demonstrators in a test cage. Interaction was allowed for 30 min, after which the demonstrator was returned to its home cage. The interaction was monitored through Plexiglas cage tops. At the end of interaction, observer rats received 7 g of regular chow in pellet form in their home cages.

Preference testing

On T1 (i.e., 23 h after social preference transmission), observer rats once again received bilateral BLA muscimol infusions and were placed in feeding cages containing both diet Cin and diet Coc (cup positions were randomly assigned). This interpolation of a 1-d delay between training and test is ubiquitous in the STFP literature (Galef Jr. et al. 1988), as it is in CTA (e.g., Yamamoto et al. 1994; Schafe et al. 1998) and fear conditioning (Maren et al. 2001). Rats were allowed to eat for 1 h and were then returned to their home cages. The amount of flavored chow from each cup was recorded as a measure of taste preference. The depth of the feeding cups was such that chow spillage was minimal. On days 2–6, rats were kept on food restriction: Each rat was given ~ 7 g of pellet rat chow daily to maintain 85% of free-feeding body weight.

To test retention of learning, rats were again tested for food preference 7 d following training (T7), using procedures identical to that of T1 (e.g., muscimol was again infused into the BLA of

rats in the experimental group just before the session). Cup position was reversed from the earlier testing session.

Experiment 2: STFP acquisition versus expression test

Procedure

An additional 15 observer rats were prepared and run to compare the BLA's involvement in learning acquisition and maintenance. The procedures in this experiment were identical to those in Experiment 1, except that half (randomly selected) of the observer rats received muscimol infusions before the social preference transmission session and saline infusions before T1, and the remaining rats received the opposite treatment (saline infusions before the social preference transmission session and muscimol infusions before T1). No rats received muscimol infusions on T7. Previous studies (see, for example, Krupa et al. 1993; Stone et al. 2005) have demonstrated that 24 h is easily long enough for complete washout of the previous day's infusion.

Experiment 3: CTA—IOC delivery

Adaptation

For 3 d, rats were allowed to move freely for 10 min in a Plexiglas chamber (dia: 12", h: 12") with a syringe pump connected to their IOC via polyethylene tubing. Shortly after placement into the chamber, 5 mL of dH₂O was delivered, via the syringe pump, at 0.5 mL/min.

CTA learning

Learning sessions took place the day after adaptation. In learning sessions, each rat received 5 mL of 0.1 M sucrose or 0.1 M NaCl solution through the IOC in place of dH₂O. Muscimol ($n = 10$) or saline ($n = 4$) was infused bilaterally into the BLA immediately following (i.e., 5 min after) drinking. An intraperitoneal injection of 0.15 M LiCl (20 mL/kg) was subsequently given (i.e., 10 min after muscimol infusion) to induce malaise.

Testing

On the following day, rats again received 5 mL of sucrose or NaCl solution in their daily session. The rats' behavior during the drinking session was videotaped and coded for aversive behaviors reflecting aversiveness of tastes. Numbers of gapes, head-shakes, and lateral arm flails, all of which increase when a rat encounters an aversive stimulus (Grill and Norgren 1978; Ossenkopp and Eckel 1995; Clarke and Ossenkopp 1998), were summed together as a simple measure of overall aversion to the tastants (see Fig. 3 for examples of each behavior); coding was done blind to experimental condition. Repeated responses separated by <1.5 sec were counted as single occurrences.

Experiment 4: CTA—One-bottle delivery

Adaptation

Rats were placed on a water-deprivation schedule such that at 10:00 a.m. on each of 3 d, rats were given 30 min of access to 10 mL of dH₂O from a single lick spout in a test chamber. At 5:00 p.m., rats were allowed to drink ad libitum from their home cage water bottles for 15 additional min (Stone et al. 2005).

CTA learning

During the AM drinking session, water was replaced with 10 mL of 0.1 M sucrose. Immediately after the session ended, muscimol ($n = 6$) or saline ($n = 6$) was infused bilaterally into BLA. An intraperitoneal injection of 0.15 M LiCl (20 mL/kg) was given to each rat immediately after drug infusion. Rats were given their normal PM water sessions.

Testing

On the next day, rats were again presented with 0.1 M sucrose during the AM drinking session. The difference in consumption compared with the training day was recorded to measure aversion.

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References

- Alvarez, P., Lipton, P.A., Melrose, R., and Eichenbaum, H. 2001. Differential effects of damage within the hippocampal region on memory for a natural, nonspatial odor-odor association. *Learn. Mem.* **8**: 79–86.
- Berlau, D.J. and McGaugh, J.L. 2003. Basolateral amygdala lesions do not prevent memory of context-footshock training. *Learn. Mem.* **10**: 495–502.
- Bunsey, M. and Eichenbaum, H. 1995. Selective damage to the hippocampal region blocks long-term retention of a natural and nonspatial stimulus-stimulus association. *Hippocampus* **5**: 11.
- Burton, S., Murphy, D., Qureshi, U., Sutton, P., and O'Keefe, J. 2000. Combined lesions of hippocampus and subiculum do not produce deficits in a nonspatial social olfactory memory task. *J. Neurosci.* **20**: 5468–5475.
- Clark, R.E., Broadbent, N.J., Zola, S.M., and Squire, L.R. 2002. Anterograde amnesia and temporally graded retrograde amnesia for a nonspatial memory task after lesions of hippocampus and subiculum. *J. Neurosci.* **22**: 4663–4669.
- Clarke, S.N. and Ossenkopp, K.P. 1998. Taste reactivity responses in rats: Influence of sex and the estrous cycle. *Am. J. Physiol.* **274**: R718–R724.
- Coutureau, E., Blundell, P.J., and Killcross, S. 2001. Basolateral amygdala lesions disrupt latent inhibition in rats. *Brain Res. Bull.* **56**: 49–53.
- Ferry, B. and Di Scala, G. 2000. Basolateral amygdala NMDA receptors are selectively involved in the acquisition of taste-potentiated odor aversion in the rat. *Behav. Neurosci.* **114**: 1005–1010.
- Ferry, B., Wirth, S., and Di Scala, G. 1999. Functional interaction between entorhinal cortex and basolateral amygdala during trace conditioning of odor aversion in the rat. *Behav. Neurosci.* **113**: 118–125.
- Fontanini, A. and Katz, D.B. 2005. 7 to 12 Hz activity in rat gustatory cortex reflects disengagement from a fluid self-administration task. *J. Neurophysiol.* **93**: 2832–2840.
- Ganaraja, B. and Jeganathan, P.S. 1999. Increased sweet taste preference following the lesion of basolateral nucleus of amygdala (BLA) in rat. *Indian J. Physiol. Pharmacol.* **43**: 443–448.
- Ganaraja, B. and Jeganathan, P.S. 2000. Effect of basolateral amygdala & ventromedial hypothalamic lesions on ingestion & taste preference in rat. *Indian J. Med. Res.* **112**: 65–70.
- Galef Jr., B. and Whiskin, E. 1997. Effects of social and asocial learning on longevity of food-preference traditions. *Anim. Behav.* **53**: 10.
- Galef Jr., B.G., Mason, J.R., Preti, G., and Bean, N.J. 1988. Carbon disulfide: A semiochemical mediating socially-induced diet choice in rats. *Physiol. Behav.* **42**: 119–124.
- Gilbert, P.E., Campbell, A., and Kesner, R.P. 2003. The role of the amygdala in conditioned flavor preference. *Neurobiol. Learn. Mem.* **79**: 118–121.
- Grill, H.J. and Norgren, R. 1978. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res.* **143**: 263–279.
- Hatfield, T., Graham, P.W., and Gallagher, M. 1992. Taste-potentiated odor aversion learning: Role of the amygdaloid basolateral complex and central nucleus. *Behav. Neurosci.* **106**: 286–293.
- Krupa, D.J., Thompson, J.K., and Thompson, R.F. 1993. Localization of a memory trace in the mammalian brain. *Science* **260**: 989–991.
- Lalumiere, R.T. and McGaugh, J.L. 2005. Memory enhancement induced by post-training intrabasolateral amygdala infusions of β -adrenergic or muscarinic agonists requires activation of dopamine receptors: Involvement of right, but not left, basolateral amygdala. *Learn. Mem.* **12**: 527–532.
- Luo, M., Fee, M.S., and Katz, L.C. 2003. Encoding phomonal signals in the accessory olfactory bulb of behaving mice. *Science* **299**: 1196–1201.
- Maren, S., Yap, S.A., and Goosens, K.A. 2001. The amygdala is essential for the development of neuronal plasticity in the medial geniculate nucleus during auditory fear conditioning in rats. *J. Neurosci.* **21**: RC135.
- Mickley, G.A., Kenmuir, C.L., McMullen, C.A., Yocom, A.M., Valentine, E.L., Dengler-Criss, C.M., Weber, B., Wellman, J.A., and Remmers-Roeber, D.R. 2004. Dynamic processing of taste aversion extinction in the brain. *Brain Res.* **1016**: 79–89.
- Nakajima, S., Urata, T., and Ogawa, Y. 2006. Familiarization and cross-familiarization of wheel running and LiCl in conditioned taste aversion. *Physiol. Behav.* **88**: 1–11.
- Nishijo, H., Uwano, T., Tamura, R., and Ono, T. 1998. Gustatory and multimodal neuronal responses in the amygdala during licking and discrimination of sensory stimuli in awake rats. *J. Neurophysiol.* **79**: 21–36.
- Ochsner, K.N. 2004. Current directions in social cognitive neuroscience. *Curr. Opin. Neurobiol.* **14**: 254–258.
- Ossenkopp, K.P. and Eckel, L.A. 1995. Toxin-induced conditioned changes in taste reactivity and the role of the chemosensitive area postrema. *Neurosci. Biobehav. Rev.* **19**: 99–108.
- Pare, D. 2003. Role of the basolateral amygdala in memory consolidation. *Prog. Neurobiol.* **70**: 409–420.
- Paxinos, G. and Watson, C. 1997. *The rat brain in stereotaxic coordinates*. Elsevier Academic Press, Amsterdam.
- Phelps, E.A. 2006. Emotion and cognition: Insights from studies of the human amygdala. *Annu. Rev. Psychol.* **57**: 27–53.
- Reilly, S. 2005. Conditioned taste aversion and amygdala lesions in the rat: A critical review. *Neurosci. Biobehav. Rev.* **20**: 1–22.
- Rollins, B.L., Stines, S.G., McGuire, H.B., and King, B.M. 2001. Effects of amygdala lesions on body weight, conditioned taste aversion, and neophobia. *Physiol. Behav.* **72**: 735–742.
- Rosenkranz, J.A. and Grace, A.A. 2002. Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. *Nature* **417**: 282–287.
- Sakai, N. and Yamamoto, T. 2001. Effects of excitotoxic brain lesions on taste-mediated odor learning in the rat. *Neurobiol. Learn. Mem.* **75**: 128–139.
- Schafe, G.E., Thiele, T.E., and Bernstein, I.L. 1998. Conditioning method dramatically alters the role of amygdala in taste aversion learning. *Learn. Mem.* **5**: 481–492.
- Schauz, C. and Koch, M. 2000. Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. *Learn. Mem.* **7**: 393–399.
- Sevelinges, Y., Gervais, R., Messaoudi, B., Granjon, L., and Mouly, A.M. 2004. Olfactory fear conditioning induces field potential potentiation in rat olfactory cortex and amygdala. *Learn. Mem.* **11**: 761–769.
- Stone, M.E., Grimes, B.S., and Katz, D.B. 2005. Hippocampal inactivation enhances taste learning. *Learn. Mem.* **12**: 579–586.
- Touzani, K. and Sclafani, A. 2005. Critical role of amygdala in flavor but not taste preference learning in rats. *Eur. J. Neurosci.* **22**: 1767–1774.
- Touzani, K., Taghzouti, K., and Velley, L. 1997. Increase of the aversive value of taste stimuli following ibotenic acid lesion of the central amygdaloid nucleus in the rat. *Behav. Brain Res.* **88**: 133–142.
- Yamamoto, T., Shimura, T., Sako, N., Yasoshima, Y., and Sakai, N. 1994. Neural substrates for conditioned taste aversion in the rat. *Behav. Brain Res.* **65**: 123–137.
- Yasoshima, Y., Shimura, T., and Yamamoto, T. 1995. Single unit responses of the amygdala after conditioned taste aversion in conscious rats. *Neuroreport* **6**: 2424–2428.
- Zarrindast, M.R., Ahmadi, S., Haeri-Rohani, A., Rezayof, A., Jafari, M.R., and Jafari-Sabet, M. 2004. GABA(A) receptors in the basolateral amygdala are involved in mediating morphine reward. *Brain Res.* **1006**: 49–58.

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