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Ibotenic Acid Lesions of the Basolateral, but Not the Central, Amygdala Interfere With Conditioned Taste Aversion: Evidence From a Combined Behavioral and Anatomical Tract-Tracing Investigation

Renée Morris, Stephen Frey, Tafadzwa Kasambira, and Michael Petrides
McGill University

Rats (*Rattus norvegicus*) with almost complete ibotenic acid lesions (at least 90%) of the basolateral amygdaloid complex (BLA) failed to learn a conditioned taste aversion (CTA; Experiment 1A). In these same BLA rats, the bidirectional parabrachial–insular pathway that courses through the central nucleus of the amygdala (Ce) was shown to be spared (Experiment 1B), indicating that the BLA per se is critical for CTA learning. In contrast to the deleterious effect of BLA lesions on CTA, ibotenic acid lesions of the Ce did not block CTA learning (Experiment 2). Nonreinforced preexposure to the gustatory stimulus attenuated CTA acquisition in normal rats, and, under these conditions, rats with BLA lesions were no longer impaired (Experiment 3). Thus, ibotenic acid lesions centered over the Ce, sparing a considerable extent of the BLA, together with the testing procedure used in previous experiments (e.g., L. T. Dunn & B. J. Everitt, 1988), led to the belief that the CTA deficits reported after electrolytic lesions of the amygdala were the result of incidental damage to fibers of passage.

Dramatic emotional changes that include dietary alterations have been reported in the monkey after temporal excisions that included the amygdala (Aggleton & Passingham, 1981; Jones & Mishkin, 1972; Klüver & Bucy, 1939; Weiskrantz, 1956). In rodents, the amygdala has been shown to be critically involved in passive avoidance learning (Cahill & McGaugh, 1990; Dunn & Everitt, 1988; Richardson, 1973). In view of the role of the amygdala in both ingestive and avoidance behavior, a large body of research has focused on the role of this limbic structure in conditioned taste aversion (CTA) learning. CTA refers to the acquired avoidance of the ingestion of a flavored substance that has been previously paired with gastrointestinal malaise. Electrolytic lesions of the amygdala have been repeatedly shown to disrupt the acquisition of a CTA (Aggleton, Petrides, & Iversen, 1981; Bermudez-Rattoni, Grijalva, Kiefer, & Garcia, 1986; Borsini & Rolls, 1984; Fitzgerald & Burton, 1983; Kolb, Nonneman, & Abplanalp, 1977; Lasiter & Glanzman, 1985; Nachman & Ashe, 1974; Rolls & Rolls, 1973). More recently, however, ibotenic acid, fiber-sparing lesions of the amygdala have failed to interfere with CTA learning (Dunn & Everitt, 1988). In light of this finding, which was subsequently confirmed by others (e.g., Bermudez-Rattoni & McGaugh, 1991; Hatfield, Graham, & Gallagher, 1992), the previously observed CTA learning deficit produced by electrolytic lesions of the amygdala was interpreted as the result of incidental damage to fibers of passage.

To our knowledge, the only fiber system that courses through the amygdala that could possibly be involved in CTA learning is the bidirectional parabrachial–insular pathway. In the rodent, the parabrachial nucleus, where gustatory and visceral responses can be recorded (Norgren, 1976; Norgren & Leonard, 1973), projects to the insular cortex (Lasiter, Glanzman, & Mensah, 1982; Moga et al., 1990; Saper, 1982a, 1982b). Likewise, both the gustatory and visceral portions of the insular cortex (Cechetto & Saper, 1987; Yamamoto, Matsuo, & Kawamura, 1980) project back to the parabrachial nucleus (Halsell, 1992; Moga et al., 1990; Saper, 1982a, 1982b). As it passes through the amygdala, this bundle of axons remains confined within the limits of the central nucleus (Ce) (Frey, Morris, & Petrides, 1997). The latter observation suggests that electrolytic lesions to the basolateral amygdaloid complex (BLA; e.g., Aggleton et al., 1981) that impair CTA learning are not likely to sever this bundle. This consideration, together with recent reports that ibotenic acid lesions of the BLA may interfere with CTA (Yamamoto, 1993, 1994; Yamamoto & Fujimoto, 1991; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994), has reopened the "BLA–fiber-of-passage controversy." Parts of this study have been previously published in abstract form (Frey, Morris, Kasambira, & Petrides, 1996; Frey, Morris, & Petrides, 1996).

Experiment 1

This experiment tested the possibility that the previously reported failure to observe a CTA deficit after ibotenic acid lesions of the amygdala (see introduction) might have been due to the limited damage induced in the BLA and not to sparing of the fibers of passage. This alternative explanation

Renée Morris, Stephen Frey, Tafadzwa Kasambira, and Michael Petrides, Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Correspondence concerning this article should be addressed to Renée Morris, who is now at the Department of Biomedical Science, The University of Wollongong, Northfields Avenue, Wollongong, New South Wales 2522, Australia. Electronic mail may be sent to Renee_Morris@uow.edu.au.

Table 1
Stereotaxic Coordinates and Volume of Ibotenic Acid (IBO) Injected to Create Lesions and of Wheat Germ Agglutinin–Horseradish Peroxidase (WGA–HRP) Injected to Trace the Fibers Passing Through the Amygdala

Injection	AP	ML	DV	Volume
IBO lesions to the BLA for Experiment 1A and Experiment 3				
First lowering	−2.4 to −2.6	4.9 to 5.0	−6.8 to −7.5	0.1 to 0.15 μl
Second lowering	−2.7 to −3.0	4.9 to 5.0	−6.8 to −7.0	0.1 to 0.15 μl
IBO lesions to the Ce for Experiment 2				
One lowering	−2.4	4.1	−7.0	0.5 μl
WGA–HRP injections in the parabrachial pontine nucleus for Experiment 1B				
One lowering	−8.8	2.0 to 2.2	−6.9 to −7.2	0.5 to 1 μl

Note. Stereotaxic coordinates (in millimeters) used for the IBO-induced lesions of the basolateral amygdaloid complex (BLA) and central amygdaloid nucleus (Ce) and for the WGA–HRP injections within the parabrachial pontine nucleus. The actual coordinates, adapted from Paxinos and Watson (1986), are a combination of one particular value falling within the range indicated for the three axes (AP = anterior–posterior; ML = mediolateral; DV = dorsoventral). In making the lesions of the basolateral amygdaloid group, two lowerings per hemisphere were performed to encompass the entire basolateral complex. The injected volumes of IBO and WGA–HRP fall within the volume ranges indicated.

was partly suggested by the finding from Hatfield et al. (1992, p. 287) that "the acceptable lesions ranged in size from 30–80%" of the BLA. Only the observation of a CTA impairment after virtually complete ibotenic acid damage to the BLA, coupled with the demonstration (in the same animals) that the bidirectional parabrachial–insular fibers had been spared, could reestablish the role of the BLA in this type of learning.

Experiment 1A: The Effects of Ibotenic Acid Lesions to the BLA on the Acquisition of a CTA

The aim of this experiment was to evaluate the effect of almost complete ibotenic acid lesions (at least 90%) of the BLA on the acquisition of a CTA. Twenty rats were used in this experiment. Of these, 13 received bilateral ibotenic acid lesions to the BLA, and 7 served as unoperated controls. Rats in both the BLA and control groups were tested for their ability to acquire a CTA.

Method

Subjects and housing. All procedures complied with the Canadian Council of Animal Care guidelines. Twenty experimentally naive male hooded rats (*Rattus norvegicus;* Long–Evans), weighing 310–336 g at the time of surgery, were used in this study. The rats were individually housed in wire cages in the animal colony under a 12-hr light–dark cycle. Throughout the study, the rats were allowed free access to Rat Chow (Ralston-Purina, St. Louis, MO). Water was also continuously available, except for the duration of the behavioral testing, when the rats were maintained on a 23-hr 40-min water deprivation schedule (see the *CTA testing* section). Rats were acclimatized to these conditions at least 4 days before the beginning of any procedures.

Surgical procedure and animal care. Surgery was performed under aseptic conditions. The rats were deeply anesthetized with sodium pentobarbital (65 mg/kg ip) and placed on a heating pad, in a stereotaxic apparatus. A small incision was made on the rat's skin to expose the skull. The head was positioned so that the lambda and bregma points were horizontally aligned, and small holes were drilled in the rat's skull over the regions of interest. Injections of either neurotoxin or anatomical tracers were performed bilaterally, after which the wound was cleaned and the skin incision sutured. The rats were removed from the stereotaxic apparatus and injected with lactate ringer (0.5 cc sc) to prevent dehydration. The rats were monitored until fully recovered from anesthesia. Aspirin administration (100 mg/kg orally) and topical application of lidocaine ointment (5%) were provided on the 1st day after surgery. Rats were given a minimum of 10 days of recovery before the onset of the behavioral training.

Injections of ibotenic acid. To induce cell loss, a 10 μg/μl solution of ibotenic acid in 0.1 M phosphate buffer (pH 7.4) was infused in the BLA through a glass micropipette attached to an infusion pump. Each microliter of ibotenic acid was delivered over 1 min. After the completion of the injections, the micropipette was left in place inside the rat's brain for an additional 2 min to minimize diffusion. Table 1 shows the stereotaxic coordinates as well as the volume of ibotenic acid used to perform the lesions of the BLA.

CTA testing. The rats were tested in their home cages in the animal quarters. A 100-ml Richter tube was attached to the front wall of each cage with the spout protruding into the cage. The rats were placed on a 23-hr 40-min water deprivation schedule during these experiments. Fluid consumption was measured to the nearest milliliter throughout the study. On the first 5 days, water was available for 20 min per day. On Day 6, the rats were presented with a 2% sucrose solution (wt/vol) for 20 min, immediately after which they received an intraperitoneal injection of 0.15 M LiCl (20 ml/kg) to induce sickness. On Days 7 to 9, the rats received water for 20 min per day, and, on Day 10 (test day), they were reexposed for 20 min to the sucrose solution to test for any acquired aversion.

Experiment 1B: The Bidirectional Parabrachial–Insular Pathway After Ibotenic Acid Lesions of the BLA, as Revealed by Anterograde and Retrograde Wheat Germ Agglutinin–Horseradish Peroxidase (WGA-HRP) Tracing

Ibotenic acid has occasionally been reported to damage the fibers of passage (Coffey, Perry, Allen, Sinden, &

Rawlins, 1988; Frey et al., 1997; Jarrard, 1989). It was therefore critical to rule out any possibility that, if an impairment were observed in Experiment 1A, it would not be due to damage to the fibers passing through the lesioned area. Thus, the aim of the second part of this experiment (Experiment 1B) was to see, in the same BLA rats, whether the bidirectional bundle that links the parabrachial pontine nucleus and insular cortex (and passes through the amygdala) was spared by the lesions. The compound WGA-HRP was selected as the tracing agent because of its anterograde and retrograde labeling properties. When injected in the parabrachial nucleus of a normal rat, WGA–HRP is anterogradely transported to reveal the course and the termination of the fibers originating from the injection site (i.e., the parabrachial–insular connection). Likewise, WGA–HRP is retrogradely transported and labels the neurons located in the insular cortex that project to the parabrachial nucleus (i.e., the insular–parabrachial connection; see Frey et al., 1997; Halsell, 1992; Moga et al., 1990). After the completion of the CTA testing (Experiment 1A), the BLA-lesioned rats, along with 7 intact rats, received bilateral injections of WGA-HRP in the parabrachial nucleus. If ibotenic acid lesions of the BLA spare the reciprocal parabrachial–insular pathway, then the labeling resulting from the tracer injections in the BLA rats should be essentially comparable to that of the controls.

Method

General considerations. Two rats at a time, selected randomly (i.e., experimenters were unaware of their performance on the CTA) from the lesioned rats used in Experiment 1A, were subjected to the present tract-tracing experiment. After the injections of tracer, a 48-hr survival period was allowed for transport of the tracing compound, after which the two brains were processed for WGA-HRP histochemistry, and the assessment of the lesions was made. Any rat with unsatisfactory lesions was discarded at this stage of the experiment. This process was repeated daily until a sample of 5 animals with at least 90% bilateral lesions of the BLA and extensive bilateral filling of the parabrachial nucleus with WGA–HRP was obtained. The remaining BLA rats then had their brains examined to reveal the location and extent of the lesions. The 7 control rats were subsequently assigned to the WGA-HRP tracing method.

Injections of tracer. WGA-HRP (2% in 0.1 M phosphate buffer, pH 7.4) was injected into the parabrachial nucleus. Each microliter of WGA–HRP was delivered over 1 min, through a 20-gauge cannula (1.5 mm external diameter, 0.75 mm internal diameter) connected to a 10-μl microsyringe. After the injections, the micropipette was left in place inside the rat's brain for an additional 2 min to minimize diffusion. Table 1 shows the stereotaxic coordinates as well as the volume of tracer injected in the parabrachial nucleus.

WGA–HRP histochemistry and processing of the tissue. Forty-eight hours after the injections of WGA–HRP, the rats were overdosed with sodium pentobarbital and perfused transcardially with a 500-ml aldehyde solution (2% paraformaldehyde, 2.5% glutaraldehyde, 10% sucrose in 0.1 M phosphate buffer, pH 7.4). The brains were subsequently cryoprotected overnight in a 30% sucrose solution (in distilled water), frozen, and sectioned coronally at 50 μm with a sliding microtome. A series of brain sections was collected in polyester mesh-bottom well plates in cold (4 °C) phosphate buffer (pH 7.4) and processed for histochemistry with a modified version of the tetramethylbenzidine technique (Mesulam, 1978). The sections were mounted from dilute acetate buffer (pH 7.4) onto chrome–alum subbed microscope slides. After drying, brain sections were dehydrated in cold graded ethanols (4 °C) and coverslipped with Permount. A second series of brain sections was collected in well plates, in 4% paraformaldehyde at room temperature. The tissue was floated in phosphate buffer (pH 7.4) and mounted on chrome–alum subbed slides. Finally, the tissue was stained for both cell bodies and fibers by use of the metachromatic properties of thionin (i.e., the formol-thionin procedure of Donovick, 1974) and coverslipped with Permount.

Histology of rats not subjected to the WGA–HRP injections. The BLA rats that were not subjected to WGA–HRP histochemistry were anesthetized with a lethal dose of sodium pentobarbital and perfused through the heart with a 4% solution of paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were dissected out and cryoprotected overnight by immersion in a 30% sucrose solution (in distilled water). The brains were subsequently frozen and sectioned coronally at 50 μm with a sliding microtome. The brain sections were collected in phosphate buffer (pH 7.4), mounted onto chrome–alum subbed slides, and allowed to dry. Finally, the tissue was stained for both fibers and cell bodies with the formol-thionin procedure (Donovick, 1974), and the slides were coverslipped with Permount.

Assessment of the lesions. For all the rats, microscopic examination of the tissue was performed to see the location and extent of the lesions. The following histological criteria were used to define satisfactory BLA lesions: (a) bilateral and virtually complete cell loss of at least 90% of the lateral and basolateral nuclei of the BLA, and (b) no damage to the insular cortex. The lesions were subsequently reconstructed from enlarged projected images of the brain sections. Figure 1 compares an intact amygdala with a typical ibotenic acid lesion of the BLA (BLA 4, right hemisphere).

Results

Histological results. Of the original group of 13 operated rats, 3 were rejected because satisfactory lesions were present only unilaterally, and 1 was excluded because the lesions were too extensive, encroaching on the insular cortex. As a result, 9 lesioned rats were included in the statistical analysis of Experiment 1A (see below). Among these 9 rats, 5 (BLA 1 to BLA 5) had complete destruction of the lateral and basolateral nuclei of the BLA, including all their individual cytoarchitectonic subdivisions. The 4 remaining rats (BLA 6 to BLA 9) had complete lesions of the BLA, except for slight anterior sparing on both sides of the brain. In these 4 rats, however, the lesions encompassed a total of at least 90% (based on surface area of the lesions) of both the lateral and the basolateral nuclei of the BLA. As a result of this strict histological analysis, we are confident that the 9 rats included in the statistical analysis of the results (see below) represent an excellent sample of BLA lesions. Figure 2 shows the histological reconstruction of the lesions in the 9 BLA rats included in the statistical analysis of Experiment 1.

Behavioral results. The percentage of suppression used to evaluate the acquired aversion to the sucrose solution was calculated as follows for each rat in the experimental (BLA) and control groups: (sucrose intake on injection day − sucrose intake on test day) ÷ sucrose intake on injection day.

A complete suppression of sucrose intake would thus

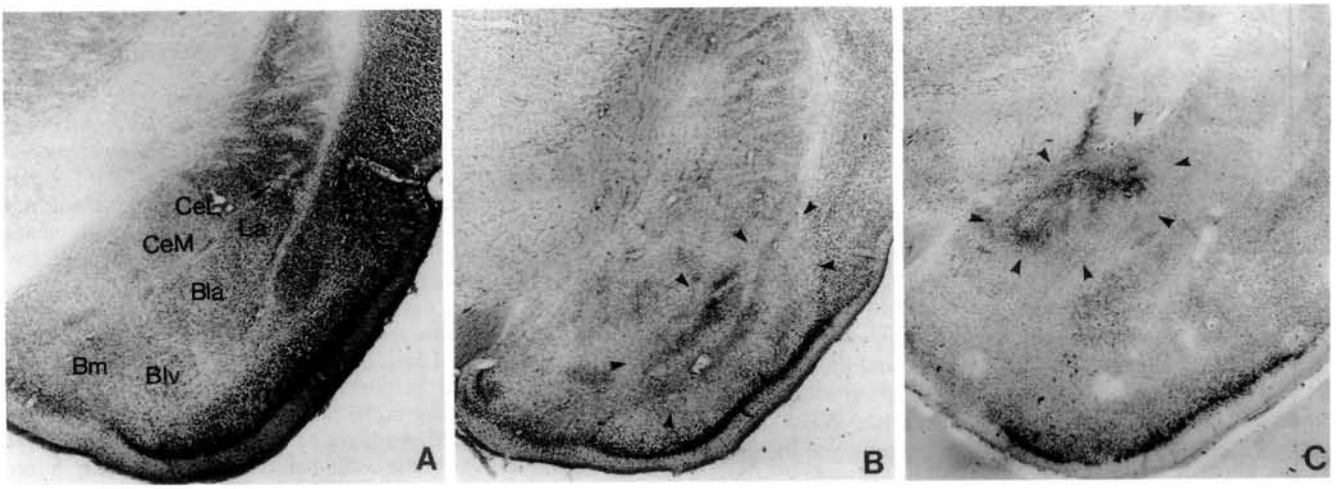


Figure 1. Light-field photomicrographs of Nissl-stained coronal sections through the amygdala in Panel A, an intact rat; Panel B, basolateral amygdaloid complex (BLA)-lesioned rat (BLA 4, right hemisphere); and Panel C, a central nucleus of the amygdala (Ce)-lesioned rat (Ce 3, right hemisphere). Magnification, ×20. The arrows indicate the limit of the lesions in B and C. Bla = basolateral amygdaloid nucleus; Blv = basolateral amygdaloid nucleus, ventral; Bm = basomedial amygdaloid nucleus; CeL = central amygdaloid nucleus, lateral; CeM = central amygdaloid nucleus, medial; La = lateral amygdaloid nucleus.

yield a ratio of 1.0. This value was then multiplied by 100 to provide the percentage of suppression from injection day to test day. As illustrated in Figure 3, there was a significant difference in the suppression ratios of the two groups, $t(13) = 3.74$, $p < .01$, indicating that rats with lesions of the BLA suppressed significantly less than did the control animals.

A two-way analysis of variance (ANOVA) with group as one variable and repeated measures on fluid intake on Day 5 (last habituation to water) and Day 6 (first exposure to the sucrose solution) was also carried out. This analysis was performed to test whether either of the two groups exhibited a reduction of intake on first exposure to sucrose. This analysis revealed that there were no effects of group, $F(1, 13) = 2.44$, *ns,* or Group × Day interaction, $F(1, 13) = 2.38$, *ns,* but that there was a significant effect of day, $F(1, 13) = 24.55$, $p < .001$. This indicates that all the rats increased their fluid consumption on Day 6 as compared with Day 5.

Anatomical results. Figure 4A shows the anterograde and the retrograde labeling at the level of the amygdala that resulted from an injection of WGA–HRP in the parabrachial nucleus of an intact rat. In their course to the insular cortex, the parabrachial efferent fibers congregate in a dense bundle that perforates the amygdala. Note that, in their course through the amygdala, these parabrachial fibers avoid the BLA (see light area on the figure) to remain confined within the limits of the Ce (see dark area on the figure). Note also the significant number of darkly stained neurons located mainly in the medial part of the Ce. These neurons were retrogradely labeled because they send a projection to the parabrachial nucleus. They are not part of the bidirectional parabrachial–insular fiber system. Figure 4B shows the labeling at the level of the amygdala after an injection of WGA-HRP in the parabrachial nucleus of a BLA-lesioned rat. The similarity of the labeling in both the normal (A) and the BLA (B) rat shows that even virtually complete damage to the BLA does not disrupt the fibers linking the parabrachial nucleus with the insular cortex. Figure 4C shows the labeling in the insular cortex that resulted from an injection of WGA–HRP in the parabrachial nucleus in a BLA-lesioned rat. These neurons were retrogradely labeled because they send a projection to the parabrachial nucleus. The presence of these heavily stained neurons in the insular cortex indicates that virtually complete lesions to the BLA do not disrupt the fibers linking the insular cortex with the parabrachial nucleus.

The parabrachial fibers gather in a compact bundle as they run within the limits of the Ce. Further rostrally, however, they spread out considerably to innervate the large territory of the insular cortex. As a result, there is a marked reduction in the density of the parabrachial fibers at the level of the insula. However, this decrease in density is in no way correlated with a reduction in the number of fibers in the vicinity of the insular cortex. Rather, it is related to the fact that, as they approach their target, the fibers are no longer confined in a bundle (as is the case at the level of the Ce) but are now distributed onto a large cortical region. This peculiarity regarding the shape of the parabrachial–insular fiber pathway, along with the small diameter of the fibers within it, makes it difficult to illustrate it at the level of the insular cortex. That is why we chose to show the labeled pathway at the level of the amygdala, where the fibers form a bundle.

Experiment 2: The Effect of Ce Lesions on CTA

This experiment examined whether lesions restricted to the Ce would impair the acquisition of a CTA. It was

necessary to address this issue for two major reasons. First, in some earlier studies, the lack of CTA deficit after excitotoxic damage to the amygdala was correlated with lesions mainly involving the Ce. For instance, Dunn and Everitt (1988) stated that, although it "consistently included the lateral and the basolateral nuclei," the "lesion damage tended to be centered around the central nucleus of the amygdala" (p. 8). Likewise, in the investigation by Bermudez-Rattoni and McGaugh (1991), "the amygdala lesions were located mainly in the central nucleus of the amygdala" (p. 167). This experiment was therefore required to determine whether, in these above studies, the location of the lesions could be responsible for the lack of deficit observed in CTA. Second, the BLA is connected to the Ce (Krettek & Price, 1978; Ottersen, 1982), which is closely related to both the parabrachial nucleus (e.g., Frey et al., 1997) and the insular cortex (e.g., Saper, 1982a). We thus needed to determine whether the CTA deficit seen in Experiment 1A of the present study was due to damage to BLA per se, or to the disruption of its functional interaction with the Ce. Sixteen rats were used in the present experiment. Of these, 10 received bilateral ibotenic acid lesions aimed at the Ce, and 6 were used as controls. CTA learning was evaluated in both the operated and control groups.

Method

Subjects, surgical procedure, and CTA testing. Experimentally naive male hooded rats (Long–Evans) weighing 308–328 g at the time of surgery were used in this study. They were housed, operated, and tested as described in Experiment 1. The stereotaxic coordinates and the volume of ibotenic acid used to lesion the Ce (see Table 1) were the same as those used in the experiment by Frey et al. (1997) and created almost complete damage to the Ce while sparing the bidirectional parabrachial–insular pathway.

Results

Histological results. Of the original group of 10 operated rats, 4 were excluded from the statistical analysis because there was bilateral sparing of the lateral subdivision of the Ce. Among the remaining rats, 5 had virtually complete bilateral cell loss in the Ce, except for partial unilateral sparing of its lateral subdivision. One rat had complete lesions of the Ce. Figure 5 shows the histological reconstruction of the Ce lesions in the 6 rats included in the statistical analysis.

Behavioral results. The percentage of suppression used to measure the learned aversion was calculated as in Experiment 1. As illustrated in Figure 6, there was no significant difference in the suppression ratios of the two groups, $t(10) = 0.17$, *ns*. Thus, rats with lesions of the Ce were not impaired on CTA learning.

A two-way ANOVA with group as one variable and repeated measures on fluid intake on Day 5 (last habituation with water) and Day 6 (first exposure to the sucrose solution) was also carried out. The ANOVA was performed to test whether either of the two groups exhibited a reduction of intake on first exposure to sucrose. The analysis showed that there was no effect of group, $F(1, 10) = 2.64$, *ns*, but that there was a significant effect of day, $F(1, 10) = 10.79$, $p < .01$, and Group × Day interaction, $F(1, 10) = 10.79$, $p < .01$. The latter result reflects the fact that the Ce-lesioned rats increased their intake of the novel sucrose solution more than did control rats.

Experiment 3: The Effect of Nonreinforced Preexposure to the Conditioned Stimulus (CS) on CTA Learning in Rats With BLA Lesions

Under some conditions, rats may display a neophobic response to food, that is, hesitancy to ingest novel food (Barnett, 1963). Moreover, there is evidence that damage to the amygdala may alter this neophobic response (Aggleton et al., 1981; Borsini & Rolls, 1984; Kemble, Studelska, & Schmidt, 1979; Nachman & Ashe, 1974). On the basis of this observation, it has been proposed that the inability to form a CTA after lesions to the amygdala may reflect a failure to display an appropriate alerting response to the novelty of the CS (Aggleton et al., 1981; Nachman & Ashe, 1974). To be able to assess the effect of damage to the amygdala on neophobia separately from its effects on CTA, Nachman and Ashe (1974) and Dunn and Everitt (1988) introduced nonreinforced preexposures to the CS (i.e., a period of familiarization to the CS) in the classical CTA paradigm. In these studies, the performance of the amygdala-lesioned rats on CTA learning did not differ from that of control rats (Dunn & Everitt, 1988; Nachman & Ashe, 1974). It is not clear, however, whether the modified testing procedure or the fact that the lesions only partially invaded the BLA was the cause of the failure to observe a CTA deficit. The purpose of the present experiment was to examine this issue. Nine rats were subjected to almost complete bilateral ibotenic acid lesions of the BLA, and 7 rats served as controls. Both the BLA and control rats received nonreinforced preexposure to the to-be-conditioned stimulus and were subsequently tested for their ability to acquire a CTA.

Method

Subjects and surgical procedure. Experimentally naive male hooded rats (Long–Evans) weighing 308–328 g at the time of surgery were used in this experiment. They were housed and operated as described in Experiment 1A. The coordinates and the volume of ibotenic acid used to perform the lesions were the same as those used in Experiment 1A (see Table 1), which resulted in over 90% damage to the BLA.

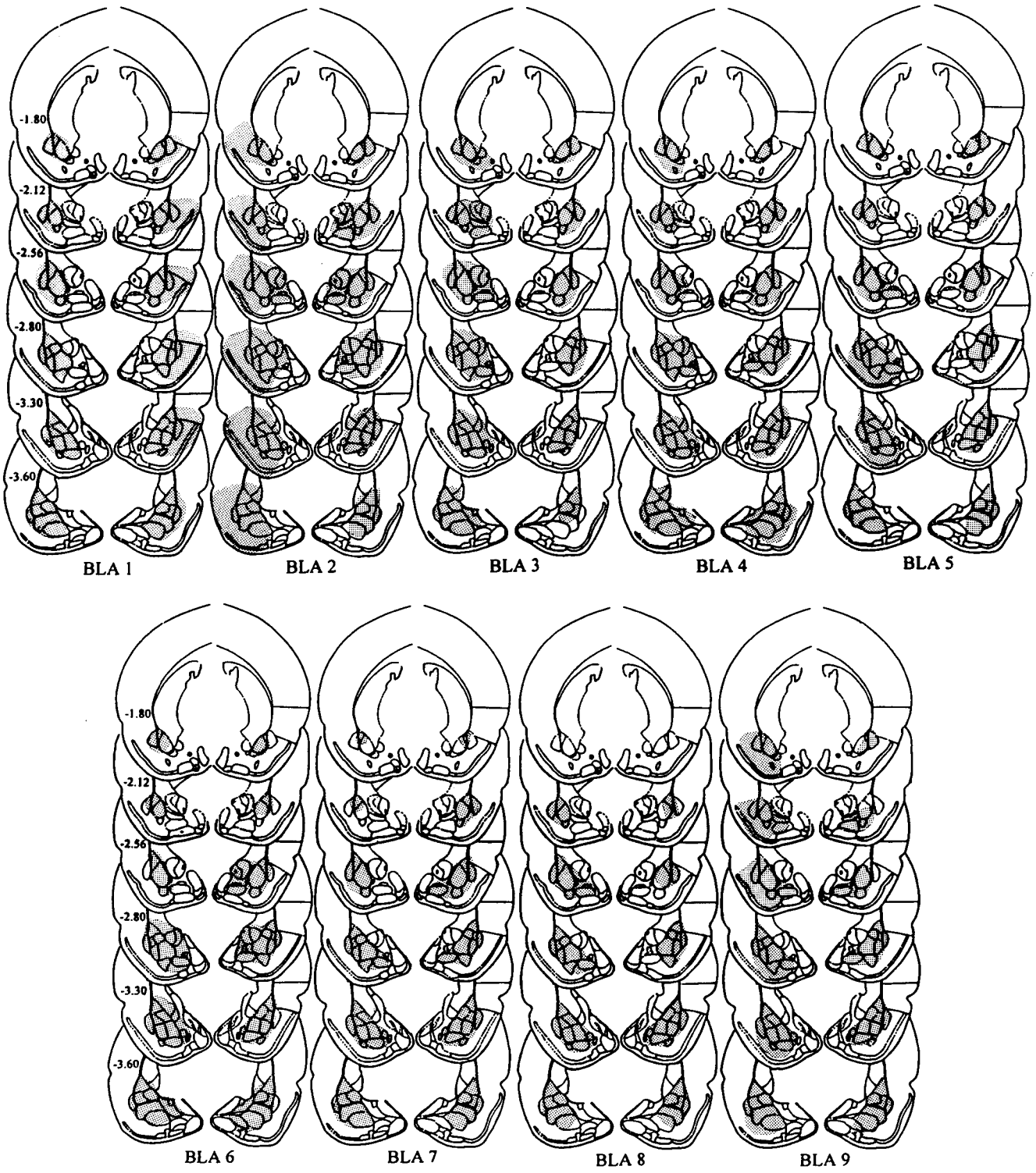
CTA testing. The rats were tested in their home cages in the animal quarters. A 100-ml Richter tube was attached to the front wall of each cage, with the spout protruding into the cage. The rats were placed on a 23-hr 40-min water deprivation schedule during the experiments. Fluid consumption was measured to the nearest milliliter throughout the study. On the first 5 days, water was available for 20 min per day. On Days 6, 7, and 8, the rats were given a 2% sucrose solution for 20 min. On Day 8, immediately after the exposure to the sucrose solution, the rats received an intraperitoneal injection of 0.15 M LiCl (20 ml/kg) to induce sickness. On Day 8, the volume of sucrose available to each rat was restricted to the volume that the rat had ingested on Day 7. After conditioning, water was presented for 24 hr to allow the rats to recover from any nonspecific effects of illness. After this period of free access to water, the rats were again placed on a 23-hr 40-min

water deprivation schedule, and the sucrose solution was presented every day for 20 min until the fluid consumption reached an asymptote.

Results

Histological results. All operated rats had satisfactory lesions of the BLA and were therefore included in the statistical analysis.

Behavioral results. The percentage of suppression used to evaluate the acquired aversion to the sucrose solution was calculated as in Experiment 1. Figure 7 illustrates the mean percentage of suppression for the BLA-lesioned and control groups. As shown on this figure, there was no significant difference in the suppression ratios of the two groups, $t(13) = 0.76$, *ns*, indicating that the BLA-lesioned rats did not suppress significantly less than did control rats.



A two-way ANOVA with group as one variable and repeated measures on Day 5 (last habituation day to water), Day 6 (first exposure to the sucrose solution), and Day 7 (second exposure to the sucrose solution) was computed. The ANOVA was performed to examine whether the drinking pattern of these two groups remained comparable over time. The analysis revealed that there were no effects of group, $F(1, 13) = 0.32$, *ns*, or Group × Day interaction, $F(1, 13) = 0.47$, *ns*, but that there was a significant effect of day, $F(1, 13) = 20.83$, $p < .001$. This reflects the fact that all rats increased their fluid consumption when exposed to sucrose.

Discussion

The purpose of the present series of experiments was to resolve the controversy as to whether the amygdala itself, that is, without damage to the fibers coursing through it, subserves CTA learning. The fibers passing through the amygdala that are likely to be implicated in CTA are those linking the parabrachial nucleus with the insular cortex. A resolution of this issue requires (a) examination of the effect of almost complete ibotenic acid lesions of the BLA on CTA acquisition, and (b) if an impairment were observed, the demonstration that these above fibers were spared. Experiment 1 of the present study achieved both these objectives. In Experiment 1A, rats with ibotenic acid lesions of the BLA exhibited a severe impairment on CTA learning. After the completion of the behavioral testing, the status of the bidirectional parabrachial–insular fiber bundle was assessed by means of WGA-HRP histochemistry and was shown to be spared (Experiment 1B). These data constitute the first demonstration, in the same living animals, of CTA impairment after ibotenic acid lesions of the BLA, along with

Figure 2 (opposite). Histological reconstructions of each of the ibotenic acid-induced lesions aimed at the basolateral amygdaloid complex (BLA) used in Experiment 1. Coronal sections are adapted from Paxinos and Watson (1986). The stereotaxic coordinates corresponding to the different levels on the anterior–posterior axis are shown on the left side of BLA 1 and 6. BLA1, left side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and the posteriormost part of Bm. The lesion produced additional damage to DEn and VEn. BLA1, right side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and the posteriormost part of Bm. The lesion produced additional damage to DEn and VEn, and slightly encroached on the lateral aspect of the Pir and the ventralmost part of PRh. BLA2, left side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and a significant portion of Bm. The lesion encroached on the medial portion of CeL and CeM. The lesion produced additional damage to DEn, VEn, Pir, PRh, and the ventralmost part of Cpu. BLA2, right side: The lesion encompassed the full extent of La, Bla, Blp, Blv, Bm, CeL, and CeM. The lesion produced additional damage to DEn and VEn. BLA3, left side: The lesion encompassed the full extent of La, Bla, Blp, Blv, Bm, CeL, and CeM. The lesion produced additional damage to DEn, VEn, and the ventralmost portion of Cpu. BLA3, right side: The lesion encompassed the full extent of La, Bla, and Blv, and a significant portion of Blp and Bm. The lesion produced additional damage to DEn and VEn. BLA4, left side: The lesion encompassed the full extent of La, Bla, Blp, and Blv, a significant portion of Bm, CeL, and CeM. The lesion produced additional damage to DEn, VEn, and the ventralmost aspect of Cpu. BLA4, right side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and the posterior part of Bm. The lesion produced additional damage to DEn and VEn. BLA5, left side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and nearly all parts of Bm. It slightly encroached on CeL and CeM. The lesion produced additional damage to DEn, VEn, and the ventralmost aspect of Pir. BLA5, right side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and some portion of Bm. The lesion produced additional damage to DEn, VEn, and the ventralmost aspect of Cpu. BLA6, left side: The lesion encompassed the full extent of La and Blp, nearly all parts of Bla and Blv, and the posteriormost part of Bm. It slightly encroached on CeL and CeM. The lesion produced additional damage to DEn. BLA6, right side: The lesion encompassed the full extent of La, Blp, Blv, and nearly all aspects of Bla and Bm. It also encroached on CeL and CeM and produced additional damage to DEn and VEn. BLA7, left side: The lesion encompassed the full extent of La, Blp, Blv, a significant portion of Bla, and the posteriormost aspect of Bm. The lesions produced additional damage to DEn and VEn. BLA7, right side: The lesion encompassed the full extent of La, Bla (except for its anterior tip), Blp, Blv, and nearly all aspects of Bm. The lesion produced additional damage to DEn and VEn. BLA8, left side: The lesion encompassed the full extent of La and Bla (except for their anterior tips), Blp, Blv, and nearly all aspects of Bm. It also slightly encroached on CeL. The lesion produced additional damage to DEn and VEn. BLA8, right side: The lesion encompassed the full extent of Blp, nearly all aspects of La, Bla, and Blv, and the posteriormost aspect of Bm. The lesion produced additional damage to DEn and VEn. BLA9, left side: The lesion encompassed the full extent of La, Bla, Blp, Blv, and nearly all aspects of Bm. The lesion produced additional damage to DEn, VEn, and Pir. BLA9, right side: The lesion encompassed the full extent of Blp, Blv, Bm, and nearly all aspects of Bla. Bla = basolateral amygdaloid nucleus; Blp = basolateral amygdaloid nucleus, posterior; Blv = basolateral amygdaloid nucleus, ventral; Bm = basomedial amygdaloid nucleus; CeL = central amygdaloid nucleus, lateral; CeM = central amygdaloid nucleus, medial; Cpu = caudate-putamen; DEn = dorsal endopiriform nucleus; La = lateral amygdaloid nucleus; PRh = perirhinal cortex; Pir = piriform cortex; VEn = ventral endopiriform nucleus.

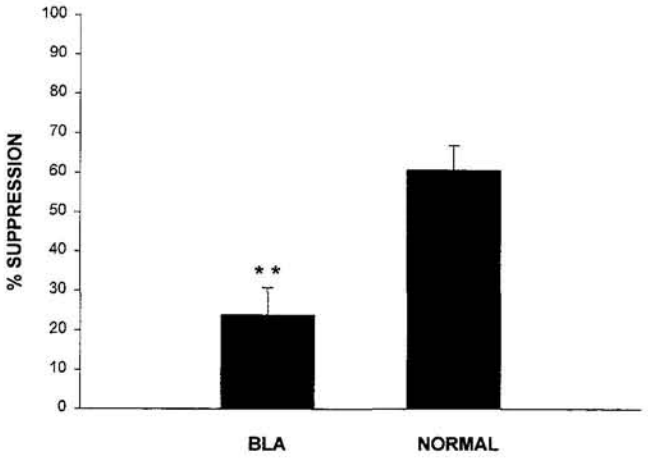


Figure 3. Mean percentage of suppression observed in Experiment 1 for the control group and for the experimental group that had ibotenic acid-induced lesions of the basolateral amygdaloid complex (BLA). The vertical bars mark the standard error of the mean. The asterisks indicate that the experimental group suppressed significantly less (**$p < .001$) than did the control group.

direct anatomical evidence that the above-described fibers of passage are intact.

The fact that electrolytic, but not ibotenic acid, lesions of the amygdala impair CTA learning has led to the belief that the deficit observed after electrolytic lesions of the amygdala is the result of the surgical manipulation that damages the fibers of passage (Dunn & Everitt, 1988). However, data gathered over the past decade no longer support the fiber-of-passage explanation. First, it has been repeatedly demonstrated that lesions to the insular cortex that would disrupt its connections with the parabrachial nucleus only produce a slight CTA impairment (Dunn and Everitt, 1988; Roldan & Bures, 1994; Yamamoto, 1993; Yamamoto et al., 1980; Yamamoto et al., 1994; Yamamoto & Fujimoto, 1991). Furthermore, no deleterious effect has been reported after electrolytic lesions of the Ce (Galaverna et al., 1993; see also Kemble et al., 1979), which would also interrupt the parabrachial–insular pathway (see Frey et al., 1997). Together, these data provide indirect evidence that the parabrachial–insular fiber pathway does not subserve CTA learning. Documentation of the exact course of the parabrachial–insular fiber pathway (i.e., confined within the Ce; Frey et al., 1997) strongly suggests that the CTA deficit reported after lesions of the BLA, even when these lesions were performed electrolytically (see Aggleton et al., 1981), may not have been secondary to the interruption of this fiber system, but that the deficit was directly related to the damage to the BLA itself.

What might explain the discrepancy between the above results and those from earlier studies in which no CTA impairment was reported after excitotoxin lesions of the amygdala (e.g., Dunn & Everitt, 1988)? In Experiment 1A of the present study, a severe CTA deficit was observed after almost complete bilateral ibotenic acid lesions of the BLA. Conversely, Experiment 2 demonstrated that virtually complete bilateral ibotenic acid lesions of the Ce had no effect on this type of learning. Together, Experiments 1 and 2 constitute strong evidence for a dissociation of the effects of BLA and Ce lesions on CTA learning. It is worthwhile to mention that this evidence is in accordance with the recent report of a dissociation of the effects of damage to the above-mentioned subdivisions of the amygdala on the

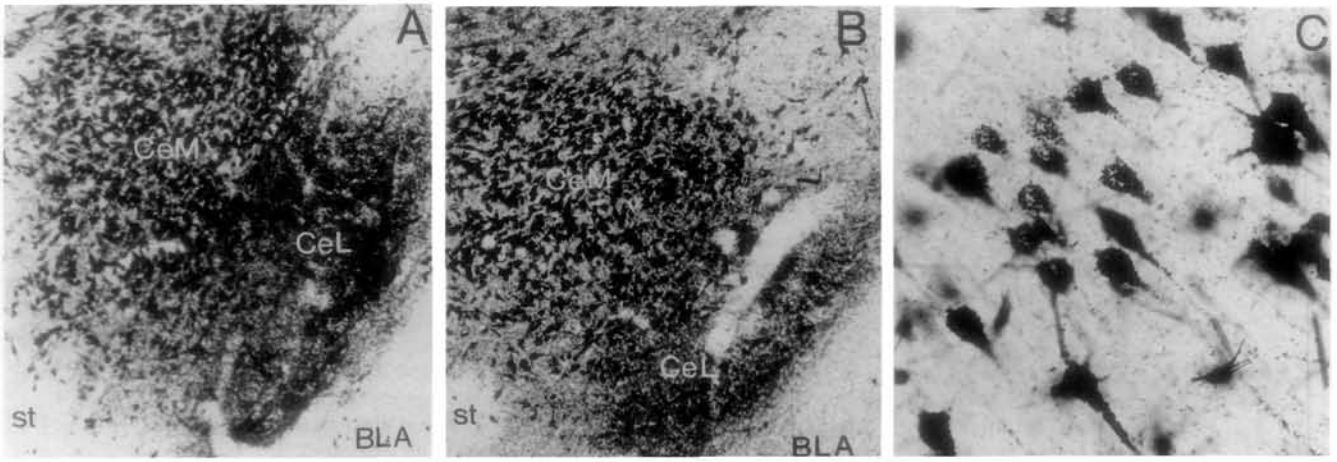


Figure 4. Light-field photomicrographs of coronal sections through the amygdala in Panel A, a normal rat, and Panel B, a BLA-lesioned rat, to show the retrograde and anterograde labeling resulting from an injection of wheat germ agglutinin–horseradish peroxidase (WGA-HRP) into the parabrachial nucleus. In A and B, note that the retrogradely labeled cells projecting to the parabrachial nucleus are concentrated in the medial subdivision of the central nucleus (CeM), whereas the dense punctate labeling of the fibers that originate in the parabrachial area and course through the amygdala is mainly confined within the lateral subdivision of the central nucleus (CeL). Magnification, ×90. In Panel C, some retrogradely labeled neurons that are present in the insular cortex in a BLA-lesioned rat are shown. Magnification, ×400. BLA = basolateral amygdaloid group; st = stria terminalis.

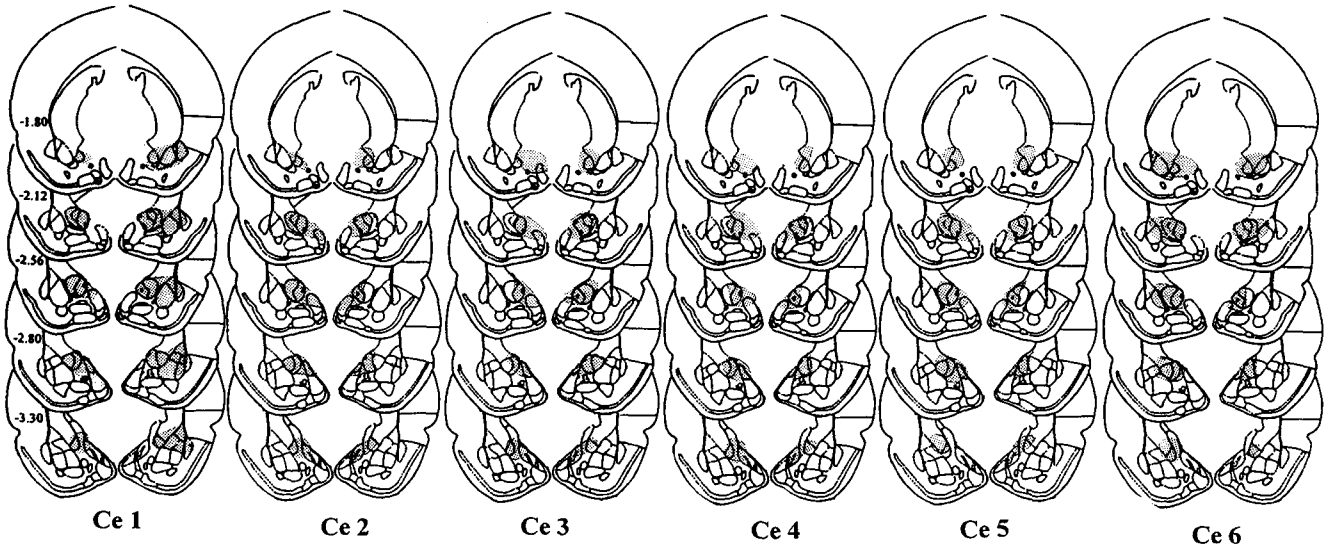


Figure 5. Histological reconstructions of each of the ibotenic acid-induced lesions aimed at the central amygdaloid nucleus (Ce) that were used in Experiment 2. Coronal sections are adapted from Paxinos and Watson (1986). The stereotaxic coordinates corresponding to the different levels on the anterior–posterior axis are shown on the left side of Ce1. Ce1, left side: The lesion encompassed the full extent of CeL, CeM, and the anteriormost aspect of Me. Ce1, right side: The lesion encompassed the full extent of CeL, CeM, La, DEn, and nearly all aspects of Bla. Ce2, left side: The lesion encompassed the full extent of CeL, CeM, and the anteriormost part of Me. Ce2, right side: The lesion encompassed the full extent of CeM as well as most aspects of CeL, except for its lateralmost part. Ce3, left side: The lesion encompassed the full extent of CeM and encroached on the anteriormost part of Me. CeL was mostly spared in this case. Ce3, right side: The lesion encompassed the full extent of CeL, CeM, and the anteriormost aspect of Me. Ce4, left side: The lesion encompassed the full extent of CeM and most of CeL, except for its posteriormost part. Ce4, right side: The lesion encompassed the full extent of CeM and encroached on the anteriormost part of Me. CeL was mostly spared in this case. Ce5, left side: The lesion encompassed the full extent of CeL, CeM, and the anteriormost part of Me. The lesion also produced slight damage to the medial part of both La and Bla. Ce5, right side: The lesion encompassed the full extent of CeM and encroached on the anteriormost aspect of Me. CeL was mostly spared in this case. Ce6, left side: The lesion encompassed the full extent of CeL, CeM, and the anteriormost part of Me. The lesion also produced slight anterior damage to the medial parts of La and Bla. Ce6, right side: The lesion encompassed the full extent of CeM and most of CeL, except for its posteriormost part. It also produced slight anterior damage to the medial part of La and Bla. Bla = basolateral amygdaloid nucleus; CeL = central amygdaloid nucleus, lateral; CeM = central amygdaloid nucleus, medial; DEn = dorsal endopiriform nucleus; La = lateral amygdaloid nucleus; Me = medial amygdaloid nucleus.

expression of aversive conditioning (Killcross, Robbins, & Everitt, 1997). These results provide an explanation why lesions mainly centered over the Ce (e.g., Bermudez-Rattoni & McGaugh, 1991; Dunn & Everitt, 1988) that spared a considerable extent of the BLA (Hatfield et al., 1992; Yamamoto & Fujimoto, 1991) failed to impair CTA. Likewise, it strongly suggests that the severe CTA deficit reported in Experiment 1A was due to the success of the surgical procedure in eliminating most (at least 90%) of the BLA.

To isolate the effect of damage to the amygdala on neophobia from its effect on CTA, a period of familiarization to the to-be-conditioned stimulus was introduced in the classical CTA paradigm (Dunn & Everitt, 1988; Nachman & Ashe, 1974). Experiment 3 was designed to see whether BLA lesions that are comparable in size to those of Experiment 1 would disrupt CTA learning when the rats were exposed to the sucrose solution before the conditioning session. In this experiment, no impairment was observed on CTA, in marked contrast to the clear impairment observed after similar lesions in Experiment 1A. It is important to point out that, in Experiment 3, CTA acquisition by the control rats was markedly attenuated (mean percentage of suppression = 49.1%). This confirms the widely accepted notion that preexposure to a gustatory stimulus that is not followed by negative reinforcement has a deleterious effect on subsequent attempts at conditioning (i.e., latent inhibition; Bennett, Wills, Wells, & Mackintosh, 1994; De La Casa & Lubow, 1995; Gallo & Candido, 1995; Kalat & Rozin, 1973; Reilly, Harley, & Revusky, 1993; Revusky, 1977; Revusky & Bedarf, 1967; Revusky & Garcia, 1970; Wittlin & Brookshire, 1968). Thus, under these conditions, BLA-lesioned animals were no longer impaired. In Experiment 1A of the present study, the animals received a single presentation of the sucrose solution immediately followed by LiCl-induced toxicosis. This experimental procedure,

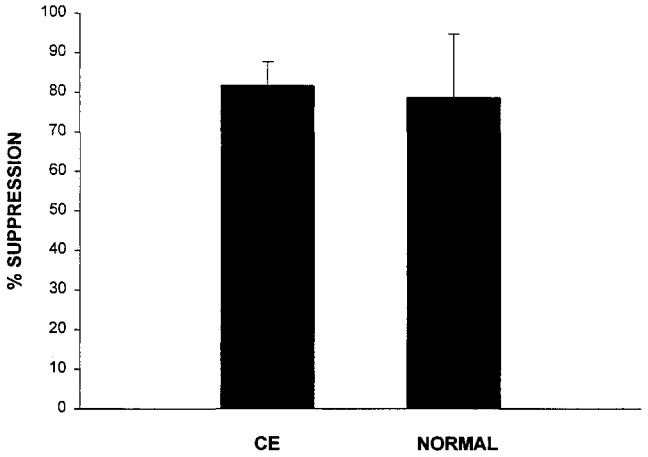


Figure 6. Mean percentage of suppression observed in Experiment 2 for the control group and the experimental group that had ibotenic acid-induced lesions of the central nucleus of the amygdala (Ce). The vertical bars mark the standard error of the mean. In this experiment, the experimental group did not suppress significantly less than the control group.

which has been repeatedly used over the past 2 decades (Aggleton et al., 1981; Bielavska & Roldan, 1996; Fitzgerald & Burton, 1981, 1983; Gallo, Roldan, & Bures, 1992; Ivanova & Bures, 1990a, 1990b; Nachman & Ashe, 1973, 1974; Nachman, Rauschenberger, & Ashe, 1977; Roldan & Bures, 1994; Swank & Bernstein, 1994; Swank, Schafe, & Bernstein, 1995; Yamamoto, 1993; Yamamoto & Fujimoto, 1991), produced a stronger CTA in control rats (mean percentage of suppression = 60.7%).

One could argue that the latent inhibition hypothesis does not account for the fact that, in the Dunn and Everitt (1988) study, the CTA was impaired after electrolytic but not after ibotenic acid lesions of the amygdala when the same experimental procedure was used. However, these a priori conflictual results could be explained in terms of the relative damage to the amygdala resulting from the two types of lesions. Indeed, a close look at the histology in the study by Dunn and Everitt reveals that the two types of lesions are by no means equivalent. For instance, the ibotenic acid lesions created partial damage to the BLA and the Ce, whereas the electrolytic lesions destroyed the Ce, the BLA, as well as an important part of the corticomedial amygdaloid group. On the basis of these observations, it is possible that the deleterious effects of the electrolytic lesions on the acquisition of a CTA might be correlated with the additional damage to the corticomedial amygdaloid group produced by this type of lesion. It is still possible, however, that the more severe impairment seen after the electrolytic lesions in the study by Dunn and Everitt might have been due to the combination of BLA damage plus the interruption of the parabrachial–insular pathway. Thus, the inability to form a CTA after large electrolytic lesions of the amygdala may reflect a more general learning impairment.

Although the Ce is closely related to both the insular cortex (Ottersen, 1982; Saper, 1982a) and the parabrachial nucleus (Bernard, Alden, & Besson, 1993; Jhamandas, Petrov, Harris, Vu, & Krukoff, 1996; Moga et al., 1990; Norgren, 1976; Ottersen, 1982; Saper & Lowey, 1980), damage to this amygdaloid nucleus does not alter CTA learning (Experiment 2). In contrast, lesions to the BLA, which projects to the Ce (Krettek & Price, 1978), do disrupt this type of learning (Experiment 1A). Together, these observations rule out the possibility that CTA learning might be sustained by the Ce or by its connections with the BLA. Moreover, these findings provide evidence that the BLA per se, along with its own anatomical connections with the rest of the central nervous system, is the critical structure for normal performance of this type of learning. For instance, the BLA is directly linked to the gustatory insular cortex (Allen, Saper, Hurley, & Cechetto, 1991; Ottersen, 1982; Saper, 1982a; Sripanidkulchai, Sripanidkulchai, & Wyss, 1984) and indirectly linked (Krettek & Price, 1977, 1978; Saper, 1982a) via connections with the mediodorsal nucleus of the thalamus. In addition, a vast array of connections link the BLA with the parabrachial region (Bernard et al., 1993; Moga et al., 1990; Saper & Lowey, 1980), the dorsal and ventral striatum (Carlsen, Zaborszky, & Heimer, 1985), and the motor cortex (Sripanidkulchai et al., 1984). This hodological mosaic suggests that the BLA occupies a privileged position for funnelling gustatory information into brain structures that modulate emotional, autonomic, and motor aspects of feeding behavior. The BLA could therefore constitute a critical node in the integration of the sensory quality of the sucrose solution with the visceral signal of poisoning and its affective consequences. Its anatomical relationship with the striatum and the motor cortex provides a route through which the motor aspects of avoidance behavior could be expressed. This view is in agreement with the position of Jones and Mishkin (1972), who proposed that the amygdala is interposed between cognition and affect. According to this view, the nature of the observed deficit is probably due to the dissociation between the sensory qualities of a stimulus and its affective value as registered from past experience.

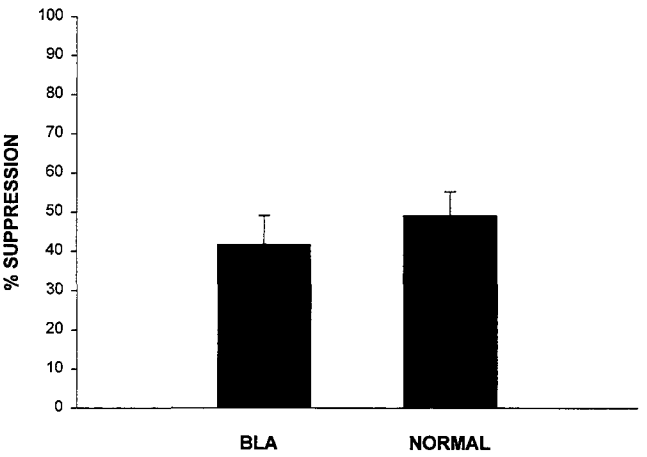


Figure 7. Mean percentage of suppression observed in Experiment 3 for the control group and the experimental group that had ibotenic acid-induced lesions of the basolateral amygdaloid complex (BLA). The vertical bars mark the standard error of the mean. In this experiment, the experimental group did not suppress significantly less than the control group.

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