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Effects of Lesions of the Central Nucleus of the Amygdala on Anxiety-Like Behaviors in the Rat

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KOPCHIA, K. L., H. J. ALTMAN AND R. L. COMMISSARIS. *Effects of lesions of the central nucleus of the amygdala on anxiety-like behaviors in the rat.* PHARMACOL BIOCHEM BEHAV 43(2) 453–461, 1992.—The effects of lesions of the central nucleus of the amygdala on anxiety-like behaviors in the rat were determined using two animal models, the conditioned suppression of drinking (CSD) and defensive burying paradigms. For CSD conflict testing, water-restricted rats were trained to drink water from a tube that was occasionally electrified (0.25 mA); electrification was signaled by a tone. CSD test sessions were 10 min in duration and were conducted 4 days per week. After at least 3 weeks of conflict testing, both punished (30–40 shocks per session) and unpunished (10–12 ml water per session) responding had stabilized. Subjects then received bilateral electrolytic lesions of the central nucleus of the amygdala or sham lesions. After a 1-week recovery period, CSD conflict testing was reinstated and continued for 20 weeks. Amygdaloid-lesioned subjects accepted significantly more shocks than did sham controls. In addition, acute challenges with the benzodiazepine chlordiazepoxide (2.5–10 mg/kg, IP, 30-min pretreatment), the barbiturate phenobarbital (20 mg/kg, IP, 10-min pretreatment), and carbamazepine (10 mg/kg, IP, 10-min pretreatment) produced an increase in punished responding in both amygdaloid-lesioned and sham-treated subjects. Analysis of covariance (ANCOVA)-based adjusted means for the change in shocks received were not significantly different between the two groups. Following completion of the CSD studies, subjects were tested in the defensive burying paradigm. Although there was no significant difference between lesioned and sham-treated subjects on the percent of animals that exhibited burying, subjects with lesions of the central nucleus of the amygdala exhibited a significantly greater latency to initiate defensive burying. Lesioned subjects also exhibited a shorter duration of defensive burying than sham-treated subjects; however, this difference was not statistically significant. These data suggest that although the central nucleus of the amygdala contributes to baseline anxiety-like behaviors the anxiolytic-like effects of chlordiazepoxide, phenobarbital, and carbamazepine do not appear to be dependent upon the integrity of this amygdaloid nucleus.

Amygdala Anxiety Anxiolytics Benzodiazepines Carbamazepine Chlordiazepoxide
Conflict behavior Defensive burying behavior Phenobarbital

TRADITIONALLY, benzodiazepines such as diazepam, chlordiazepoxide, and related agents have played a major role in the management of anxiety states. These agents generally are effective following acute administration; however, the side effect profile of benzodiazepines often limits their use (3). Perhaps, identification of the specific neuroanatomical area(s) of the brain involved in the expression of anxiety would allow for the development of more selective drugs with less severe side effects.

Several neuroanatomical areas are currently being investigated as possible sites necessary for the expression or control of anxiety and anxious behavior. The limbic system has been traditionally accepted as being the "emotional" portion of the brain. One area of the limbic system that has been implicated in the expression of emotional behavior, both in humans and in animals, is the amygdala. Stimulation of the amygdala results in fear-like autonomic responses in rabbits (2) and cats (36), whereas lesions of the amygdala reduce or abolish anxiety-like behavior in several animal models (13,15,16,30). In humans, electrical stimulation of the amygdala produces a subjective sensation of fear and anxiety accompanied by autonomic responses indicative of fear, such as tachycardia, muscle tension, and increased blood pressure (5). There is evidence that the amygdala is an important site of action for the anti-anxiety effects of benzodiazepines in that this brain structure contains a high density of benzodiazepine receptors (21,40).

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In addition, intracerebral administration of benzodiazepines into the amygdala exerts anxiolytic-like effects (20,27,29). Furthermore, the results of several studies have indicated that the central nucleus of the amygdala is critical for the expression of fear and/or anxiety (15,30,38,39). The role of this nucleus in the anxiolytic-like effects of benzodiazepines and other antianxiety agents is less clear, however. Early studies by Shibata et al. (29) suggested that lesions of the central nucleus of the amygdala blocked the anxiolytic-like effects of benzodiazepines, but more recent studies have challenged this finding (13,38).

Conflict paradigms in the rat have been used by many investigators in the study of anxiety and antianxiety drugs. One animal model that has been used extensively in the study of anxiety and antianxiety agents is the conditioned suppression of drinking (CSD) (8,9,18,19), a modification of the Geller-Seifter conditioned conflict test (10–12) and the Vogel acute conflict test (37). The CSD procedure has been used in numerous studies investigating the anxiolytic-like effects of benzodiazepines (19), barbiturates (18), buspirone (19,28), and antidepressant agents (8,9). Consistent with its utility in the treatment of some anxiety disorders (25), the anticonvulsant agent carbamazepine also has been reported to increase punished responding in the CSD (14) and other conflict paradigms (1). The effects of amygdaloid lesions on CSD behavior have not been investigated.

Recent studies by Treit and others (4,7,33,35) have suggested that the defensive burying paradigm also may serve as a behavioral model for the study of anxiety and antianxiety agents. While both traditional and novel anxiolytic agents have been shown to decrease both the frequency of occurrence and duration of burying behavior in rats, the effects of amygdaloid lesions in this model have not been reported.

Therefore, the present study was designed to determine the effects of bilateral electrolytic lesions of the central nucleus of the amygdala on behavior in the CSD conflict and defensive burying paradigms. In addition, the influence of lesions of the central nucleus of the amygdala on the anxiolytic-like effects of the benzodiazepine chlordiazepoxide, the barbiturate phenobarbital, and the anticonvulsant agent carbamazepine in the CSD were determined.

METHOD

Animals

Female Sprague-Dawley rats (Charles River Farms, Cambridge, MA), 225–275 g at the start of the experiment, were housed in groups of four in a climate-controlled room with a 12 L : 12 D cycle (lights on 0700–1900 h). Initially, food and water were continuously available. Following a 2-week accommodation period and continuing throughout CSD testing, all animals were placed on a restricted water schedule, as described below. Food continued to be available in the home cage.

CSD Testing—Apparatus

CSD conflict testing was conducted in an apparatus similar to that described previously (6,9). The testing chamber was a rectangular box with Plexiglas sides and a metal floor and top. Recessed into one wall was a metal drinking tube to which a calibrated (0.5-ml units) length of polyethylene tubing was attached for measuring the volume of water consumed. Programming for the test sessions was controlled by solid state modular programming equipment (Coulbourn Instruments Co. Inc., Lehigh Valley, PA).

CSD Testing—Procedure

For the first few sessions, water-restricted (24-h deprivation) subjects were placed in the CSD experimental chamber and allowed to consume water freely without the shock contingency. After 1 week of nonshock sessions, the tone/shock contingency was initiated. The 7-s tone periods were presented at regular (23 s) repeating intervals to the subjects. During the latter 5 s of each tone period, contact between the floor and the metal drinking tube completed a circuit that resulted in the delivery of a 0.25-mA shock to the rat. The duration of the shock received was equal to the duration of tube contact (less than 200 ms). Shocks were delivered by a Coulbourn Instruments Two-Pole Small Animal Shocker (Model E13-02). Tube contact during the silent (intertone) period resulted in water intake without any shock (unpunished responding).

Initially, the shock inhibited fluid consumption in the test chamber. After several days, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and brief contacts with the tube during the tone periods. The volume of water consumed (unpunished responding), as well as the number of shocks received (punished responding), were recorded each session. All subjects achieved stable control values (day-to-day coefficients of variation of approximately 30% for individual rats) for punished and unpunished responding by the end of the second week of CSD sessions with the alternating tone-no tone periods. Subjects were tested individually in 10-min sessions at the same time of day (0800–1200 h) Tuesday–Friday, and were allowed free access to water from Friday afternoon until Monday morning.

Amygdaloid Central Nucleus Lesions

After at least 3 weeks of CSD training, subjects were assigned into one of two groups that were matched for comparable baselines, with one group receiving bilateral lesions of the central nucleus of the amygdala and the other group receiving sham operations. For surgery, animals were anesthetized with 0.1 mg/kg ketamine and received doses of xylazine (0.5 mg/kg) and atropine (0.1 ml/rat at 0.54 mg/ml). All lesions were bilateral, with lesion placement guided by coordinates from an atlas of the rat brain (22). Lesion coordinates (from bregma) were 2.0 mm posterior, ±3.7 mm lateral, and 8.5 mm deep (aiming toward the central nucleus). The lesion parameters were 1.6-mA cathodal DC current for a 20-s duration. In sham-operated animals, the electrode was placed as described above without any current being passed. Animals were allowed a 1-week recovery period, after which CSD testing was reinstated.

CSD Testing—Acute Drug Challenges

Control (i.e., nondrug) CSD testing was maintained for 12 weeks for baseline conflict determinations. Beginning at week 13, all subjects received a log-related range of chlordiazepoxide doses (2.5–10 mg/kg, IP) over the course of several weeks of testing. Each week, the effects of a different dose of chlordiazepoxide were determined; the order of the doses tested was randomized. Drug testing was conducted on Thursdays and Fridays each week using a standard crossover design. On the Thursday tests, half the subjects received the dose of chlordiazepoxide under investigation and half received saline.

These treatments were reversed on the Friday drug test. Thus, each animal served as its own control with respect to the effects of chlordiazepoxide. In this experiment, chlordiazepoxide or its vehicle were administered 30 min prior to CSD testing. In addition, the effects of single doses of phenobarbital (20 mg/kg) and carbamazepine (10 mg/kg) on CSD behavior were determined in amygdaloid-lesioned and sham-treated subjects. Both phenobarbital and carbamazepine were administered IP 10 min prior to testing using the crossover design described above for chlordiazepoxide.

Defensive Burying—Apparatus

Defensive burying testing was conducted in an apparatus as previously described (4). The testing chamber was a 40 × 30 × 40 cm Plexiglas box. The floor of the chamber was covered with clay bedding material (5 cm deep). In the center of one wall of the chamber, 2 cm above the level of the bedding material, was a small hole (diameter 0.5 cm) through which a wire-wrapped prod could be inserted.

Defensive Burying—Procedure

Beginning at week 21, animals were tested in the defensive burying paradigm. Habituation and testing sessions were conducted between 0800–1200 h using the procedure described by Beardslee et al. (4).

1. Habituation sessions. Animals were placed in groups of four or five into the chamber for 30-min sessions on each of four consecutive days. The wire-wrapped prod was not in place during these sessions.

2. Testing sessions. On the fifth day, prior to testing, the wire-wrapped prod was inserted through the wall to protrude 6 cm into the chamber. Animals were tested individually in the defensive burying testing sessions. Upon contact with the wire-wrapped prod (usually with the paw or mouth), the animal received a 3-mA shock for the duration of contact with the prod (less than 1 s). Animals that did not make contact with the electrified prod within 20 min were removed from the chamber and not included in the study. Only animals that received shocks were included in the analyses. Animals were observed for 20 min by a trained and blinded observer (K.L.K.). Three parameters were monitored in this period: a) the presence or absence of burying behavior (defined as the movement of bedding material toward the electrified prod by the rat), b) the latency from prod contact to the initiation of burying behavior, and c) the duration of burying behavior.

Drugs

Chlordiazepoxide HCl, phenobarbital HCl, and carbamazepine free base were obtained through Sigma Chemical Co. (St. Louis, MO); ketamine HCl was obtained through Aveco Co. (Fort Dodge, IA); atropine free base was obtained through Anpro Co. (Arcadia, CA); xylazine free base was obtained from Mobay Corp. (Shawnee, KS). Chlordiazepoxide and phenobarbital were dissolved in saline, while carbamazepine was suspended in 0.5% methylcellulose. All challenge drugs were administered IP in a volume of 1 mg/kg body weight.

Histology

Following behavioral testing, animals were anesthetized with pentobarbital (50 mg/kg, IP). Subjects were perfused with a 10% formalin solution via cardiac puncture and then killed by decapitation. Brains were removed and stored in dry ice for histological verification. Forty-micron frozen coronal sections were cut through the areas containing the lesions. These sections were mounted on glass slides and stained with cresyl violet. The extent of the lesions was determined by atlas sections taken from Paxinos and Watson (22). Histological verification of lesion status was determined by a trained observer (H.J.A.), who was blinded regarding the behavioral data. Only rats with verifiable bilateral lesions of the central nucleus of the amygdala were included in the statistical analyses. Based upon this criterion, 7 of the 10 (presumably lesioned) animals were ultimately included in the amygdaloid-lesioned group.

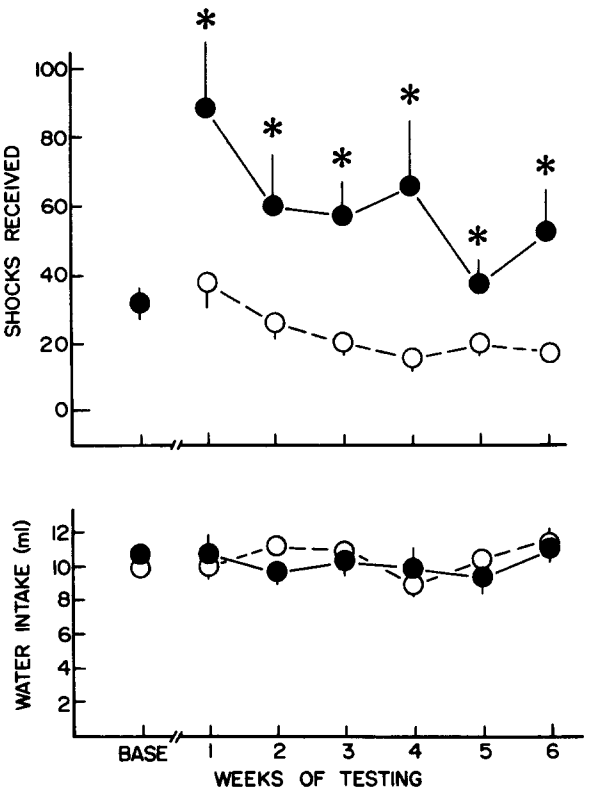

FIG. 1. Effects of lesions of the central nucleus of the amygdala on CSD conflict behavior. Plotted are the mean ± SEM number of shocks received (top panel) and water intake (in milliliters) for amygdaloid-lesioned (filled symbols; $n = 7$) and sham-treated (open-symbols; $n = 14$) subjects before (BASE) and for 6 weeks following sham/lesion treatment. *Amygdaloid-lesioned subjects accepted significantly more shocks than did sham-treated controls at the indicated test week, $p < 0.05$, LSD test following factorial ANOVA.

Statistical Analyses

Baseline (i.e., prelesion) CSD behavior was compared using *t*-tests for unpaired values. The time course for the effects of amygdaloid lesions or sham treatment on CSD behavior were analysed using two-way factorial analysis of variance (ANOVA) with repeated measures (main effects: sham/lesion, weeks of CSD testing). The effects of amygdaloid lesions on the response to acute challenges with chlordiazepoxide (chlordiazepoxide effect = acute chlordiazepoxide − acute vehicle) were determined using two-way ANOVA with repeated measures (main effects: lesion/sham, chlordiazepoxide doses)

TABLE 1

EFFECTS OF ACUTE PRETEST CHALLENGES WITH CARBAMAZEPINE AND PHENOBARBITAL ON CSD CONFLICT BEHAVIOR IN AMYGDALOID-LESIONED (CENTRAL NUCLEUS) AND SHAM-TREATED RATS

Treatment	Baseline Shocks Received*	Change in Shocks Received		Change in Water Intake (ml)§
		Absolute†	ANCOVA‡	
20 mg/kg phenobarbital (test week 18)				
Sham	23 ± 4	21 ± 9¶	+40	2.6 ± 1.2¶
Lesion	51 ± 9	114 ± 49¶	+76	2.6 ± 1.4
10 mg/kg carbamazepine (test week 19)				
Sham	23 ± 4	8 ± 2¶	+14	0.6 ± 0.5
Lesion	46 ± 7	33 ± 19¶	+21	1.0 ± 1.0

*Baseline values are reported as mean ± SEM shocks received.

†Values represent the absolute mean ± SEM (sham: $n = 14$; lesion: $n = 7$) change in shocks received (pretest agent–vehicle) during the punished periods.

‡Values represent the ANCOVA-based adjusted mean change in shocks received (covariate = the number of shocks accepted following acute vehicle treatment).

§Values represent the mean ± SEM (sham; $n = 14$; lesion: $n = 7$) change in water intake (in milliliters).

¶$p < 0.05$, the indicated acute treatment is significantly different from vehicle control, paired t-test.

and also analysis of covariance (ANCOVA) (covariate: shocks received following acute treatment with chlordiazepoxide vehicle). Similarly, the effects of amygdaloid lesions on the response to acute phenobarbital or acute carbamazepine challenges were determined using one-way ANCOVA (main effect: lesion/sham treatment; covariate: shocks received following vehicle treatment). The frequency of occurrence of defensive burying in sham-treated vs. amygdaloid-lesioned subjects was compared using χ^2 for proportions. Finally, data on the latency to onset and duration of burying behavior in sham-treated vs. amygdaloid-lesioned subjects were compared using t-tests for unpaired values. Posthoc comparisons were made using the least significant difference (LSD) test. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance (31).

RESULTS

Baseline CSD Conflict Behavior

Baseline (i.e., prelesion) CSD performance was characterized by a stable number of shocks accepted (32 ± 4; values represent the mean ± SEM number of shocks accepted per session and were derived from 21 subjects averaged across 2 weeks of CSD sessions preceding lesion or sham treatment) and a stable volume of water consumed (10.1 ± 0.5 ml) per session. It should be noted that the number of tube contacts during the shock component (30–40 per session) was insignificant when compared to the number of tube contacts during the unpunished component (2,500–3,000 per session). Thus, the volume of water consumed accurately reflects unpunished responding in the CSD.

The top panel of Fig. 1 illustrates the effects of lesions of the central nucleus of the amygdala on punished responding in the CSD conflict paradigm. Baseline (i.e., prelesion) performance was comparable in the two groups (sham: 32 ± 5; lesion: 32 ± 5; values represent mean ± SEM for 2 weeks prior to lesion/sham treatment). Lesions of the central nucleus of the amygdala did not alter feeding habits, body weights, or general activity levels relative to sham-treated control subjects. Over the course of 6 weeks of CSD testing, sham-treated subjects exhibited a tendency for a decrease in the number of shocks received. In contrast, lesions of the central nucleus of the amygdala resulted in a dramatic increase in punished responding. This increase in punished responding was apparent on the first week of CSD testing and persisted for 6 weeks of CSD testing. Statistically, there was a significant main effect for lesion/sham treatment, $F(1, 19) = 14.79$, $p < 0.05$, a significant main effect for test weeks, $F(6, 114) = 7.23$, $p < 0.05$, and a significant lesion/sham × test week interaction, $F(6, 114) = 4.24$, $p < 0.05$. Posthoc LSD tests revealed that amygdaloid-lesioned subjects accepted significantly more shocks than did sham-treated controls at all test weeks after lesion/sham treatment. Although Fig. 1 depicts data for only 6 weeks following lesion or sham treatment, it should be noted that amygdaloid-lesioned subjects continued to accept more shocks than sham-treated controls for up to 20 weeks (Table 1).

The bottom panel of Fig. 1 depicts the effects of amygdaloid central nucleus lesions on water intake in the CSD conflict paradigm. Baseline (i.e., prelesion) performance was comparable in the two groups [sham: 9.9 ± 0.6 (mean ± SEM) ml/session; lesion: 10.6 ± 0.8 ml/session]. Sham treatment did not affect water intake in the CSD paradigm; amygdaloid lesions reduced water intake slightly, although this effect was not significant. Statistically, the main effect for lesion/sham was not significant, $F(1, 19) = 0.03$, NS; the main effect for test weeks also was not significant, $F(6, 114) = 2.55$, NS, as was the lesion/sham × test week interaction, $F(6, 114) = 1.57$, NS.

Amygdaloid Central Nucleus Lesions and Anxiolytic Drug Challenges

Figure 2 illustrates the effects of acute challenges with chlordiazepoxide on CSD behavior in sham-treated and amygdaloid-lesioned subjects. Data in the left panels represent the absolute change in shocks received (top panel) and water in-

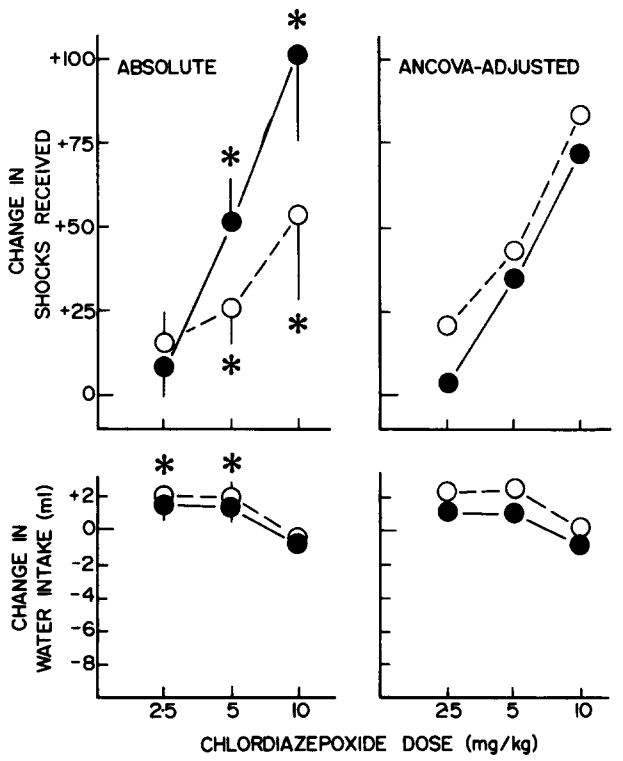

FIG. 2. Acute chlordiazepoxide treatment effects in amygdaloid-lesioned (central nucleus) and sham-treated subjects. Plotted are the change in shocks received (top panel) and water intake (lower panel) produced by acute challenges with chlordiazepoxide in sham-treated (open symbols; $n = 14$) and amygdaloid-lesioned (filled symbols; $n = 7$) subjects. Left panels depict absolute mean ± SEM change from acute vehicle challenges; right panels depict ANCOVA-based adjusted means (covariate = acute vehicle data) for the change in shocks received and water intake. *The indicated dose is significantly different from the acute vehicle in that treatment condition, $p < 0.05$, paired t-test.

take (bottom panel). Data in the right panels represent ANCOVA-based adjusted means for the change in shocks received and the change in water intake. As can be seen in the top panels, acute treatment with chlordiazepoxide resulted in a robust and dose-dependent increase in shocks received. When the data are expressed as the absolute change in shocks received (left panel), there was a tendency for a greater response in amygdaloid-lesioned subjects, although the main effect for lesion/sham treatment was not significant, $F(1, 19) = 1.01$, NS. This difference is largely the result of the elevated baseline observed in lesioned subjects and the relationship between baseline shocks received and the absolute change in shocks received (for 5 mg/kg chlordiazepoxide, Pearson's correlation coefficient values were $r = 0.78$ for sham-treated subjects, $r = 0.88$ for amygdaloid-lesioned subjects, and $r = 0.79$ when the data from both groups were combined, $p < 0.05$), as evidenced by the fact that ANCOVA-adjusted means for the change in shocks received (right panel) revealed no significant difference between amygdaloid-lesioned and sham-treated subjects, $F(1, 18) = 0.71$, NS. Thus, the apparent enhanced response to 5 and 10 mg/kg chlordiazepoxide in amygdaloid-lesioned subjects is likely an artifact of the elevated baselines of amygdaloid-lesioned subjects.

The bottom panel of Fig. 2 illustrates the effects of acute challenges with chlordiazepoxide on the change in water intake in these amygdaloid-lesioned and sham-treated rats. The left panel depicts the data expressed as the absolute change from baseline; the right panel depicts ANCOVA-based adjusted means for the change in water intake. As can be seen, the lower doses of chlordiazepoxide (2.5 and 5 mg/kg) increased water intake, whereas the higher dose (10 mg/kg) tended to decrease water intake. This was supported by a significant main effect for chlordiazepoxide dose, $F(2, 38) = 6.03$, $p < 0.05$, for ANOVA; $F(2, 36) = 5.71$, $p < 0.05$, for ANCOVA. ANOVA revealed that there was no significant effect for lesion status, $F(1, 19) < 1.0$, NS. ANCOVA-based adjusted mean scores for the change in water intake were lower in lesioned subjects at all doses of chlordiazepoxide; however, the main effect for lesion status was not significant, $F(1, 18) = 2.43$, NS. There was no lesion status × chlordiazepoxide dose interaction with either ANOVA, $F(2, 38) < 1.0$, NS, or ANCOVA, $F(2, 36) < 1.0$, NS.

Table 1 illustrates the effects of acute challenges with single doses of phenobarbital or carbamazepine. Both drugs produced an increase in punished responding in sham-treated rats and in rats with lesions of the central nucleus of the amygdala. As was observed with chlordiazepoxide, examination of the absolute change in shocks received suggests that amygdaloid-lesioned rats actually were more responsive to the acute drug challenges than were sham-treated controls. However, as with the chlordiazepoxide challenges, normalizing for the differences in baselines between amygdaloid-lesioned and sham-treated subjects with ANCOVA resulted in adjusted means for the change in shocks received that were not significantly different between amygdaloid-lesioned and sham-treated subjects [for phenobarbital, $F(1, 18) < 1.0$, NS; for carbamazepine, $F(1, 18) < 1.0$, NS]. Water intake was increased slightly by acute challenges with carbamazepine and, to a greater extent, phenobarbital. The increase in water intake following these acute challenges did not differ between sham-treated and amygdaloid-lesioned subjects.

Defensive Burying Behavior

The results of defensive burying testing in these sham-treated and amygdaloid-lesioned subjects are depicted in Table 2. As can be seen, lesions of the central nucleus of the amygdala did not significantly alter the frequency of occurrence of burying when compared to sham-treated controls. Amygdaloid-lesioned subjects did exhibit a significantly greater latency to onset of defensive burying relative to sham-treated controls, $t(6) = 2.67$, $p < 0.05$. Finally, there was a tendency for amygdaloid-lesioned subjects to spend less time engaged in defensive burying behavior; this difference, however, was not significant, $t(9) = 1.23$, NS.

TABLE 2

EFFECTS OF CENTRAL AMYGDALOID LESIONS OR SHAM TREATMENT ON DEFENSIVE BURYING BEHAVIOR

Treatment	Frequency of Occurrence	Latency to Initiation (seconds)	Duration of Burying (seconds)
Sham	64% (9/14)	168 ± 42	37 ± 21
Lesion	71% (5/7)	442 ± 94*	7 ± 4

*$p < 0.05$, unpaired t-test.

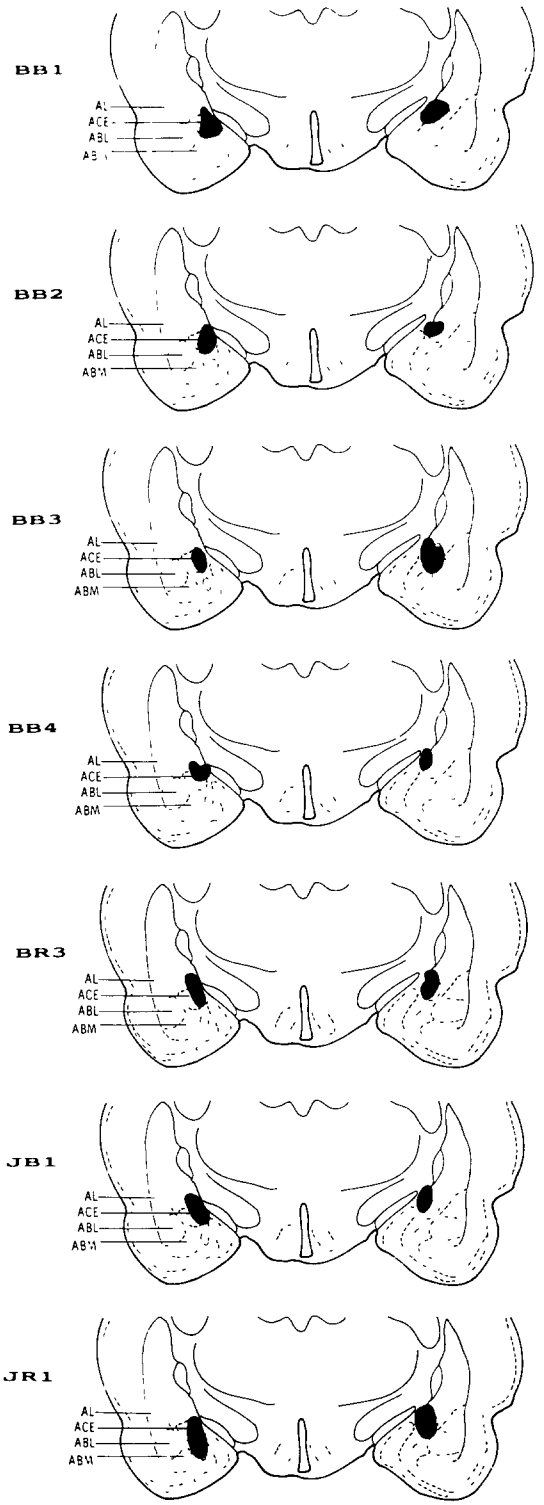

FIG. 3. Reconstruction from histological examination of the extent of lesion in each of the seven animals included in the lesion group of the present study. AL, lateral amygdala; ACE, central amygdala; ABL, basolateral amgydala; ABM, basomedial amygdala.

Histology

Figure 3 depicts the extent of the lesions in each of the seven subjects that displayed bilateral lesions of the central nucleus of the amygdala, and were therefore included in the present study. Figure 4 depicts a representative photomicrograph from an animal with a bilateral lesion of the central nucleus of the amygdala and a sham-treated animal.

DISCUSSION

In the present experiment, lesions of the central nucleus of the amygdala resulted in anxiolytic-like effects in the CSD conflict paradigm, as measured by an increase in number of shocks accepted when compared to sham-treated controls. This finding is consistent with previous work in other animal models for anxiety. For example, Shibata et al. (30) reported that lesions of the central nucleus of the amygdala produced significant and long-lasting increases in punished responding in the Geller–Seifter conflict paradigm, and Yamashita et al. (39) reported that lesions of the central nucleus of the amygdala led to a significant increase in punished responding. Grishkat et al. (13) also reported an increase in punished responding in rats with lesions of the central, basolateral, and medial nuclei of the amygdala in the Vogel acute conflict task. Hitchcock and Davis (16) reported that lesions of the central amygdaloid nucleus decreased the potentiated startle reflex. In addition, Rosen and Davis (26) reported that low-level electrical stimulation of the amygdala enhances the potentiated startle reflex, although this effect also was observed when the neighboring intercalated or medial nuclei were stimulated. Together, these findings suggest that the central nucleus of the amygdala plays an important role in maintaining a tonic level of anxiety-like behavior.

The defensive burying paradigm has been used extensively as a model for the study of anxiety and antianxiety agents (4,7,35). Relative to sham-treated controls, amygdaloid-lesioned subjects did not exhibit a lower frequency of occurrence of burying behavior. This finding is somewhat surprising because many anxiolytic treatments (i.e., barbiturates, benzodiazepines, buspirone) do reduce the frequency of occurrence of defensive burying behavior (4,33,35). Moreover, Treit and Pesold (34) reported that lesions of the septal nucleus in rats cause dramatic anxiolytic-like effects, reported as complete suppression of the frequency of occurrence and/or duration of defensive burying. In the present study, lesions of the central nucleus of the amygdala did significantly increase the latency to initiation of burying in those subjects that exhibited burying. Amygdaloid lesions also reduced the duration of burying behavior, although this effect was not statistically significant. These findings are consistent with the effects of anxiolytic-like treatments reported previously (4,33,34). Thus, lesions of the central nucleus of the amygdala produced anxiolytic-like effects on some, but not all, measures of anxiety-like behavior in this animal model.

Integrity of the central nucleus of the amygdala does not appear to be necessary for the expression of the anxiolytic-like effects of the antianxiety drug chlordiazepoxide. Consistent with other findings (13,38), acute challenges with the benzodiazepine chlordiazepoxide produced increases in punished responding in the CSD in both amygdaloid-lesioned rats and sham-treated controls. Indeed, when the present data are expressed as the absolute increase in shocks received over vehicle control it appears that amygdaloid-lesioned subjects were more affected by chlordiazepoxide than were sham-treated subjects. However, the magnitude of the chlordiazepoxide-induced increase in shocks received was not different in sham

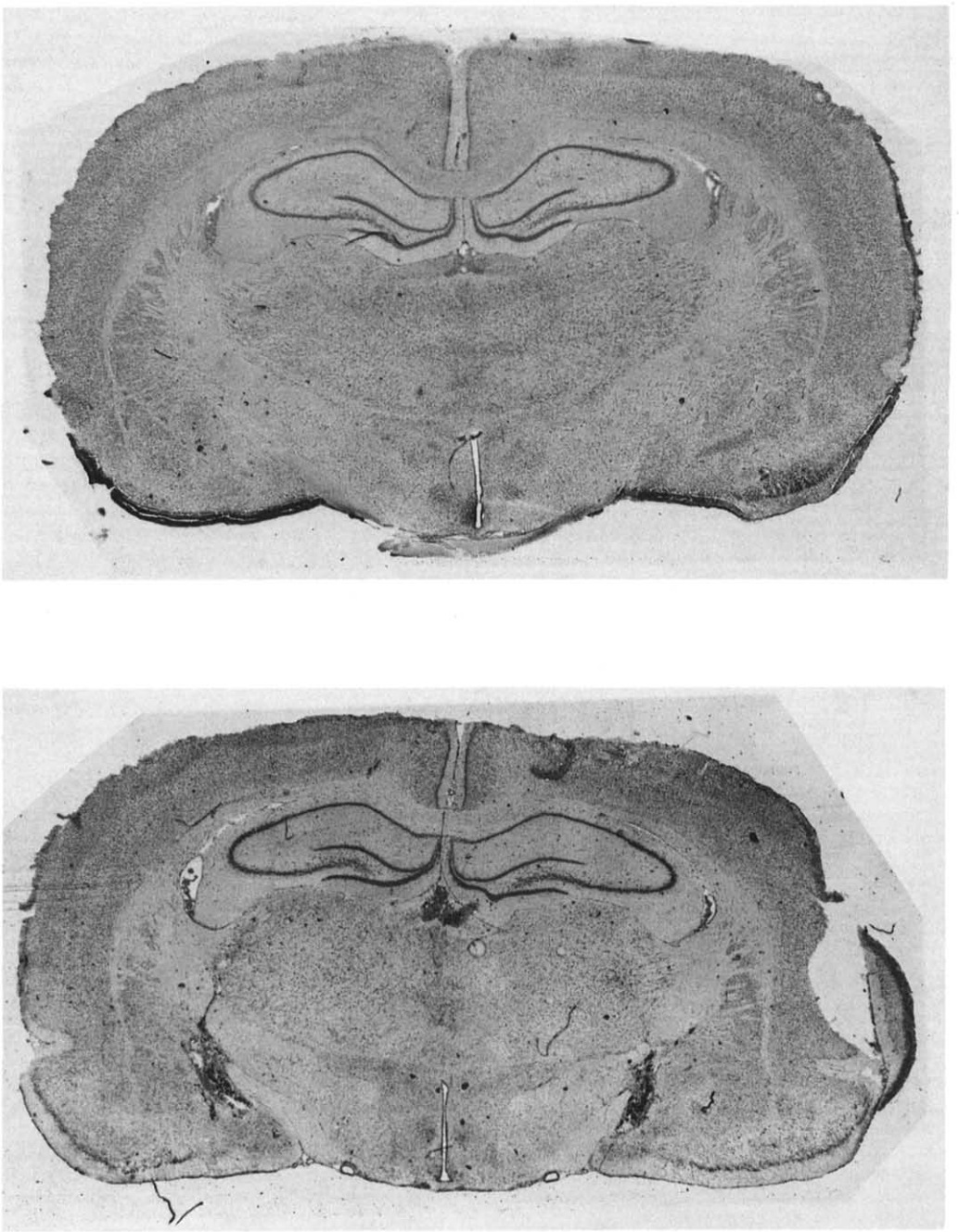

FIG. 4. Representative photomicrographs illustrating (top) a sham animal (subject AR1) and (bottom) an animal with a bilateral electrolytic lesion of the central amygdaloid nucleus (subject JB1). Histological sections were 40 μm in diameter. Dark areas indicate regions of greatest lesion-induced damage.

vs. lesioned subjects after ANCOVA-based adjustment to normalize for baseline differences in punished responding in these two groups.

Similar findings were obtained with the single doses of phenobarbital and carbamazepine, that is, acute challenges with both phenobarbital and carbamazepine resulted in an increase in punished responding in the CSD in both lesioned and sham-treated rats. When the data are expressed as the absolute increase in shocks received over vehicle, subjects with lesions of the central nucleus of the amygdala appeared to accept more shocks than did sham-treated controls. However, the ANCOVA-based adjusted means for the increase in shocks accepted were not greater in amygdaloid-lesioned subjects relative to sham-treated controls. Thus, chlordiazepoxide, carbamazepine, and phenobarbital are able to exert anticonflict effects despite lesions of the central amygdala nucleus, suggesting that the central nucleus of the amygdala is not the site of action for these drugs.

In contrast to the finding that lesions of the central nucleus of the amygdala do not alter the anxiolytic-like effects of

benzodiazepines, evidence does exist to suggest that the lateral and/or basolateral nuclei of the amygdala are crucial in mediating the effects of benzodiazepines. Thomas et al. (32) and Niehoff and Kuhar (21) reported that the lateral and basolateral nuclei of the amygdala exhibit the greatest benzodiazepine receptor density. Thomas et al. (32) went on to demonstrate that chlordiazepoxide infused into the lateral amygdala resulted in a release of responding measured during the component of a conditioned emotional response task previously associated with an aversive stimulus. Hodges et al. (17) found that local infusion of the benzodiazepine antagonist Ro 15-1788 into the basolateral nucleus of the amygdala attenuated the anticonflict effects of a systemically administered benzodiazepine. Other researchers have also suggested a key role for the lateral and/or basolateral nuclei of the amygdala (23,24,27). Studies examining the effect of lesions of the lateral and basolateral nuclei on the response to anxiolytic treatments in the CSD and defensive burying paradigms are planned.

In the present study, the absolute change in shocks received following acute challenges with chlordiazepoxide, phenobarbital, or carbamazepine was greater in subjects with lesions of the central nucleus of the amygdala when compared to sham-treated controls. However, because ANCOVA-adjusted means for the change in shocks received did not differ this apparently greater responsiveness of lesioned subjects likely is the result of two phenomenon: a) elevated baselines of lesioned subjects relative to controls and b) a correlation between baseline and absolute change in shocks received. Frequently, differences in baselines are "corrected for" by expressing a drug-induced change as the percent of the baseline value. Although a similar conclusion (comparable sensitivity to chlordiazepoxide, phenobarbital, or carbamazepine in amygdaloid-lesioned and sham-treated subjects) would be made in this study using the percent of control approach, the use of percent of control to normalize for baseline differences may not always be correct because percent of control assumes that a correlation between baseline and drug-induced change exists. This is not always the case. Thus, it is likely that analysis using the ANCOVA adjustment most accurately reflects drug effects when baseline values differ.

In summary, lesions of the central amygdaloid nucleus produced "anxiolytic-like" effects in rats as measured by the CSD conflict paradigm and defensive burying paradigm. However, the anxiolytic-like effects of acute challenges with chlordiazepoxide, phenobarbital, and carbamazepine do not appear to be dependent upon central amygdaloid integrity.

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