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Behavioral and Corticosterone Effects in Conditioned Taste Aversion Following Hippocampal Lesions

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SMOTHERMAN, W.P., G. BURT, D. P. KIMBLE, G. STICKROD, R. BREMILLER AND S. LEVINE. *Behavioral and corticosterone effects in conditioned taste aversion following hippocampal lesions.* PHYSIOL BEHAV. 27(4) 569–574, 1981. —Hippocampal-lesioned rats engaged in more drinking bouts during the retention of a conditioned taste aversion, but failed to show the elevation of plasma cortocosterone levels seen in the neocortical-lesioned and unoperated control animals, thus showing a disassociation of behavioral and hormonal responses to the retention situation. The presence or absence of anomalous sympathetic innervation in the hippocampal-lesioned rats was demonstrated to be without any behavioral significance.

Hippocampus Conditioned taste aversion Corticosterone Anomalous sympathetic innervation

FOLLOWING lesions of the anterior hippocampus or medial septal nucleus there is an extensive neural growth into the residual hippocampal tissue [6, 12, 16, 17, 26].

This anomalous sympathetic innervation (ASI) results from the collateral sprouting of axons whose cell bodies are in the superior cervical ganglion of the sympathetic nervous system and can be completely eliminated by removal of the superior cervical ganglia [12,16]. The possible behavioral consequences of ASI have been a topic of intensive investigation in one of our laboratories (DPK) over the past three years. To date, our efforts have not shown any behavioral effects attributable to this novel innervation pattern [12, 13, 14]. However, it remains a possiblity that any effects of this innervation might only occur under conditions of stress or arousal in which activation of the sympathetic nervous system is more likely. One behavioral situation in which such sympathetic discharge might occur is during the acquisition and retention of a conditioned taste aversion. This situation can be considered "stressful" in that plasma titers of corticosterone are elevated in normal rats after they have sampled a substance which contains a taste previously paired with lithium chloride (LiCl) induced intestinal distress [1, 23, 27]. However, more recent studies have shown that behavioral and pituitary-adrenal measures of conditioned taste aversion do not always co-vary [25]. Also, in another recently completed study [24], rats with bilateral lesions of the dorsal hippocampus showed normal suppression of drinking of a sweetened milk solution in a conditioned taste aversion paradigm. Interestingly, however, they *failed* to show the typical plasma corticosterone elevation observed in intact control rats.

In reviewing previous work in this area, two points appeared to stand out: (1) Although there are ample data to suggest that hippocampal lesions do not produce any significant effects on the *acquisition* of conditioned taste aversion [2, 19, 20, 27], there is considerable evidence that such lesions do affect the behavior of animals during the extinction of conditioned taste aversion [13,19]. (2) There is growing evidence that hippocampal-lesioned animals do not show the normal elevations in adrenal steroids that are displayed by unoperated animals during the conflict situation of extinction. Thus, two converging lines of evidence argue for an examination of the possible effects of ASI on retention of a conditioned taste aversion.

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We anticipated an effect of the hippocampal lesion during the test for retention of the taste aversion, and we anticipated that the sympathetic neurons from which the ASI develops would be activated in such a "stress" situation. Therefore, if there is a behavioral situation in which the collateral sprouting from these neurons is going to affect behavior, the retention of a conditioned taste aversion is a likely prospect.

METHOD

Animals

The subjects were 30 male rats derived from the Sprague-Dawley strain maintained by Charles River Co., Wilmington, MA (CD, random bred). They were housed in pairs on a 12 hr diurnal cycle, with the dark cycle from 7:00 a.m. until 7:00 p.m. Food and water were available ad lib unless otherwise noted. The animals were approximately 90 days of age at the time of first surgery. Prior to the present experiment, all subjects had been tested for spontaneous alternation tendencies and on acquisition of several spatial maze problems [14].

Surgery

Fifteen animals underwent bilateral cervical ganglionectomy 7–14 days prior to any subsequent brain lesion. This operation eliminates the anomalous sympathetic innervation which would otherwise be produced. The remaining 15 animals underwent a sham operation of the neck in which the carotid artery was visualized bilaterally but nothing removed. The surgical procedures involved in the ganglionectomy have been published previously [12].

One to two weeks after the ganglionectomy or sham neck operation, 12 animals underwent an operation which produced small lesions to the anterior tip of the hippocampus. Six underwent bilateral lesions of the neopallium overlying the hippocampal formation. The remaining 12 animals served as non-brain-lesioned controls. All brain operations were performed under visual control in one stage. Lesions were performed by aspiration. The hippocampal lesions were intended to sever the fimbria-fornix, but leave substantial amounts of the hippocampal formation (hippocampus proper, dentate gyrus) intact to serve as "target tissue" for subsequent ASI in those animals not receiving prior ganglionectomies. All operations were done under anesthesia induced by sodium pentobarbital (Nembutal) in doses of 50–55 mg/kg, IP. Atropine sulfate (0.04 mg/animal) was also administered to reduce mouth and throat secretions. There were no losses due to either surgical procedure or to illness, and all 30 subjects completed all phases of the experiment.

Half of the animals in each surgical group (six hippocampal-lesioned rats, six non-brain lesioned rats, and three neopallial-lesioned rats) were then randomly assigned to the experimental group to receive LiCl injections, and the other half was assigned to the group to receive isotonic saline injections.

Behavioral Procedure

Taste aversion conditioning procedures were similar to those used in several previously published reports [22,23]. To summarize, the animals were taken from their home cage and placed in a drinking box (26.7 cm×24.1 cm×30.5 cm). Animals were tested in squads of six. In the drinking box they were presented with a sucrose solution (20% by weight) daily for three consecutive days. For the next three days the milk solution which would serve as the CS was presented for 15 min each day. This milk solution was prepared by adding 50 g of sugar to 384 ml of evaporated milk (Carnation Brand) diluted 3.5:1 with tap water. This extensive pre-exposure procedure insured that rats began to drink immediately upon presentation of the CS, thereby producing a constant CS-UCS interval across animals [25].

Drinking was monitored with a contact-type drinkometer. Lick produced resistance changes were amplified and covereted into relay operations. These switch closures were then taken to electromechanical counters and to an Esterline-Angus multi-pen event recorder geared to record at the rate of 7.5 sec per minor chart division. On the day of the third CS presentation, animals in the LiCl condition were removed from the drinking box and taken to an adjacent room where they received an injection of 0.15 M LiCl (7.5 ml/kg) IP 30 min following the beginning of the drinking session. Animals in the SAL condition were injected in a similar fashion with the volumetric equivalent of isotonic saline.

All animals were given 24 hr to recover from the injection. After this time all animals were placed on food and water deprivation. This deprivation regimen was continued for the next 48 hr. A test for retention of the taste aversion took place at this time. Animals were again placed in the drinking boxes (testing again took place in squads of six animals) where they remained for 15 min. Animals were then removed from the drinking box and returned to their home cage where they remained for 15 min. At this time they were taken individually to an adjacent room and quickly anesthetized with ether. A blood sample (0.8 ml) was collected in a heparinized syringe by cardiac puncture [7,25]. This technique allows for the determination of existing levels of corticosterone [7]. Whole blood was centrifuged at 2,000 rpm for 20 min, the plasma extracted and frozen until assay. Plasma levels of corticosterone were determined by the fluorometric micromethod of Glick, von Redlich and Levine [9].

Histology

Following the last experiment, the rats were sacrificed by decapitation and their unperfused brains quickly frozen in a blast of compressed CO_2. Sections were cut at 16 microns using a cryostat at −20°C, and processed for monoamine fluorescence according to the method of de la Torre and Surgeon [8]. Alternate sections were fixed and stained with thionin in order to determine the extent of the lesion [4]. Sections were examined from the brains of all twelve hippocampal-lesioned rats, (six with and six without ganglionectomies) and from three cortically-lesioned rats.

Anomalous Sympathetic Innervation

No ingrowth of anomalous fibers was observed in any of the rats that received ganglionectomies, nor in the animals with neopallial lesions only. Anomalous fibers were found in all animals who had hippocampal lesions, but no ganglionectomy. The distribution of fibers was variable. In two animals the ingrowth occurred only on one side, while in the other four animals a bilateral distribution was observed. When the fibers were presented they were always found in the septal (rostral) portion of the area dentata. Localization in the temporal (caudal) area of the structure was less consistent. Fibers were seen in this area in only three animals and in each

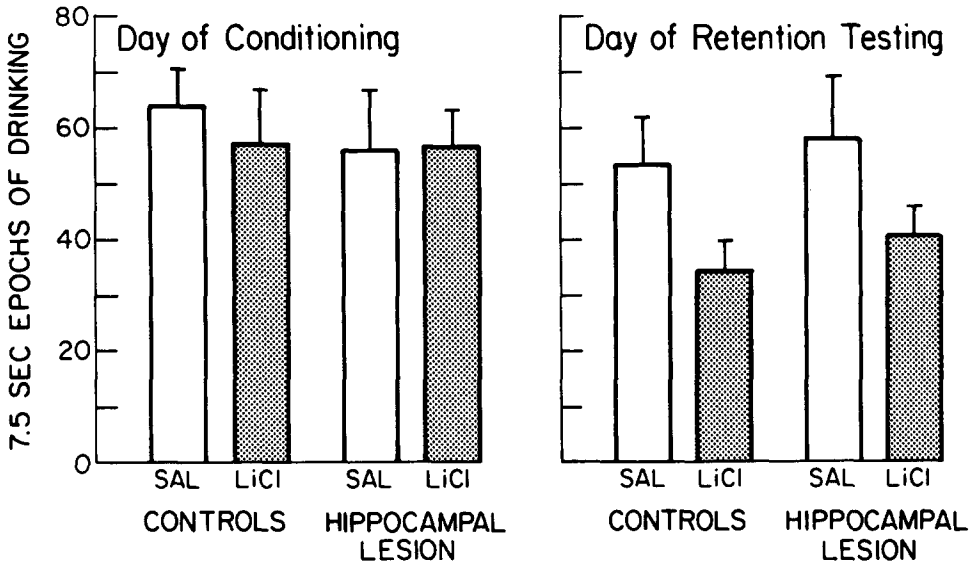

FIG. 1. Drinking epochs on the day of conditioning (left) and on the day of retention testing (right). Bars represent means±S.E.M.

case the distribution was unilateral. An ingrowth of fibers into CA3 of Ammon's Horn was also observed; the pattern of fibers was similar to that described for the area dentata with respect to their distribution along the septo-temporal axis.

The results are consistent with the conclusion reported by Loy *et al.* [17] who suggest that the pattern of ingrowth varies depending upon the position and extent of damage to the fimbria and/or fornix. In the present study, for example, when anomalous fibers were found to invade the more temporal regions of the structure, damage to the lateral fimbria was also present. In the two cases where a unilateral invasion of fibers was seen, it appeared that the dorso-medial and lateral fimbria sustained little if any damage on that side where no ingrowth occurred.

Examination of the thionin-stained sections revealed that the hipprocampal lesions were confined to the anterior quarter of the hippocampus. In most animals hippocampal damage was not present posterior to about level Å 3430 in the König and Klippel atlas of the rat brain [15]. The most posterior lesion was in one animal in which damage was present as far back as level Å 2870. The hippocampal lesions across both groups (with and without superior cervical ganglionectomy) did not show any systematic differences in either lesion size or placement (nor were there any behavioral differences between these groups). The neopallial lesions were designed to be somewhat more extensive in the brain lesioned control rats, and in these animals the cortical damage extended from about level Å 1050 to about Å 1760. Some slight scraping of the dorsal surface of the hippocampus occurred in all neopallial-lesioned rats. Damage was confined to the alveus and a small number of the CA_1 neurons.

RESULTS

Taste Aversion

Drinking data were analyzed in two ways. First, the number of *epochs* (7.5 sec intervals where drinking took place) were compared. Epochs were described in this fashion so that data could be easily transcribed from the minor chart divisions on the paper of the event recorder. Second, the number of *bouts* (defined as an epoch of drinking separated by one or more non-drinking epochs) were also compared. While the epochs measure is a more traditional measure of the amount of drinking that occurred within the 15 min session, the bouts measure is more closely related to the sequence of behaviors as initiated and terminated by the animal and thus yields information about the pattern of drinking, i.e., the stimulus sampling/exposure which is controlled by the animal within the 15 min session. Preliminary inspection of drinking and plasma corticosterone data indicated that there was no effect of superior cervical ganglionectomy. Therefore, a single hippocampal-lesioned group was formed (n=12). Further comparisons indicated that the unoperated control and neopallial-lesioned groups did not differ in their drinking before conditioning, on the day of conditioning or on the retention test day. These groups were therefore collapsed into a single control group (n=18). All subsequent comparisons were therefore made between the hippocampal-lesion and control groups.

Separate two-factor (Surgical treatment; hippocampal-lesion vs control groups) and (Injection: LiCl or Saline) ANOVAs were used to compare drinking epochs and drinking bouts on the day of conditioning. As seen on the left side of both Fig. 1 and Fig. 2 the surgical groups did not differ in their drinking on the day of conditioning (epochs p's>0.25; bouts p's>0.25).

The two factor ANOVA used to compare epochs of drinking on the day of retention testing indicated the significant main effect of Injection, $F(1,26)=6.7$, $p<0.05$; Fig. 1, right side. The taste aversion conditioning was quite clear. The LiCl injected animals engaged in significantly fewer epochs of drinking. Hippocampal lesion was without effect on this more traditional measure of drinking.

Interestingly enough, however, the two factor ANOVA

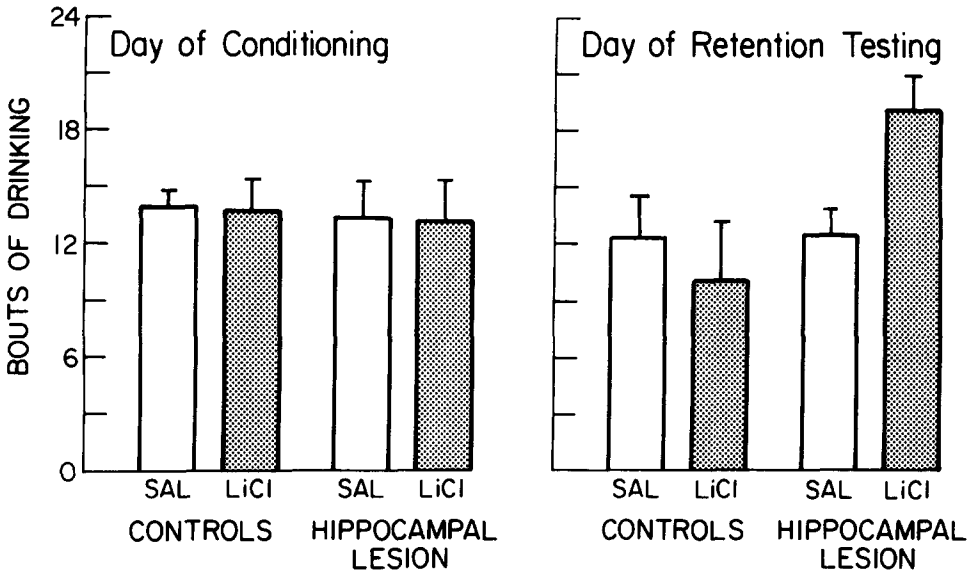

FIG. 2. Drinking bouts on the day of conditioning (left) and on the day of retention testing (right). Bars represent means±S.E.M.

that compared bouts of drinking on the day of retention testing yielded a different pattern of results. The main effects of Surgical Treatment, $F(1,26)=28.5$, $p<0.01$, and Injection, $F(1,26)=13.4$, $p<0.01$ were significant as was the interaction of Surgical Treatment by Injection, $F(1,26)=5.3$, $p<0.05$, Fig. 2, right side. Inspection of Fig. 2 indicates that control subjects injected with LiCl showed a reduction in the number of drinking bouts compared with saline-injected controls while the opposite pattern was shown by the hippocampal-lesioned group where LiCl injection resulted in an increase in bouts of drinking.

Plasma Corticosterone

Prior work has demonstrated that under similar experimental conditions, hippocampal-lesioned, neopallial-lesioned and unoperated animals do not differ in the basal resting levels of corticosterone, and, in addition, they do not differ in response to LiCl challenge [24]. A two factor ANOVA used to compare plasma cortocosterone levels on the day of retention testing showed the significant interaction of Surgical Treatment by Injection, $F(1,26)=4.3$, $p<0.05$, Fig. 3. Post hoc comparisons of individual treatment means indicated that controls injected on the day of conditioning with LiCl showed corticosterone levels which were significantly elevated over levels of animals injected with saline, $t=2.2$, $df=16$, $p<0.025$. Such was not the case for hippocampal-lesioned animals. LiCl and saline injected animals with hippocampal lesions did *not* show different plasma corticosterone elevation after re-exposure to milk, $t=0.3$, $df=9$, $p>0.25$, Fig. 3. While all the corticosterone values were elevated (compared to basal levels collected under similar conditions [24]) due in part to the food and water deprivation and rehydration with the sugar solution [10], the hippocampal-lesioned animals did not show the elevation in plasma cortocosterone seen in control animals, despite the fact that both groups showed normal acquisition of the aversion to the milk paired with LiCl injections.

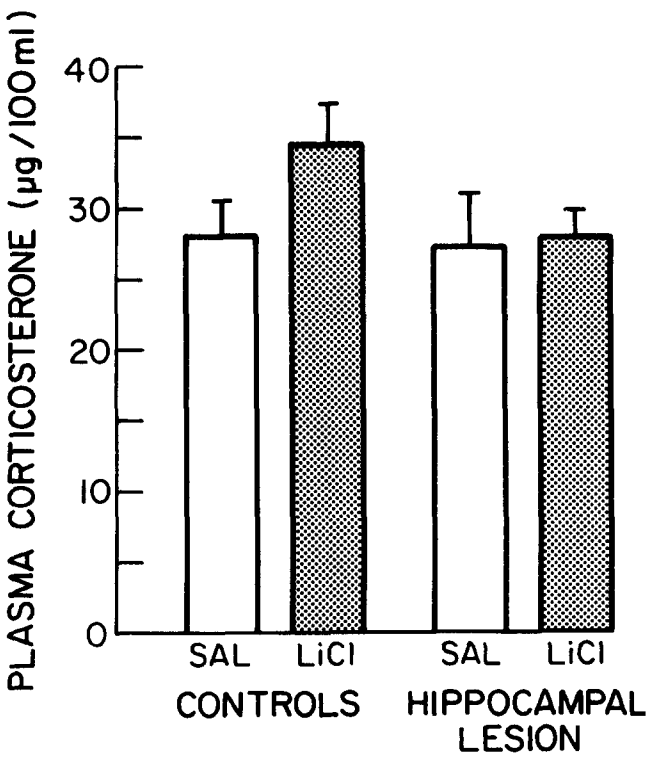

FIG. 3. Plasma levels of corticosterone (mg/100 ml) determined after the retention testing. Bars represent means±S.E.M.

DISCUSSION

While the presence or absence of ASI was without any discernable effect on the two measures of drinking or on conditioned changes in plasma corticosterone, hippocampal lesions *per se* did result in both significant behavioral and physiological effects during retention of the conditioned taste aversion. Throughout this paper we have used the phrase "retention test" to describe the procedure where animals are given a single exposure to the taste after conditioning. It should be noted that this same procedure results in extinction of conditioned taste aversion when the taste stimulus is presented repeatedly in the absence of LiCl.

These data are in contrast to reports which have failed to find significant effects of hippocampal lesions during the extinction of a taste aversion response [20,21]. Behaviorally, the hippocampal-lesioned rats drank about the same amount of the CS solution during the retention test, but they distributed their drinking in quite a different pattern than did controls. One way to view these drinking "bouts" or exposures to the CS, unaccompanied by the illness-causing UCS, is that they consitute extinction trials. The increased number of drinking bouts engaged in by the hippocampal-lesioned rats during the retention test can be considered functionally equivalent to an increased number of extinction trials, and thus perhaps might be expected to cause more rapid extinction among these animals. However, we know from previous work in our laboratory that extinction of a taste aversion is in fact *slower* in hippocampal-lesioned rats when evaluated across a two-week extinction period [13]. In fact, behavioral differences did not emerge in our earlier study until after a week of daily 21 min extinction trials.

The hippocampal lesion also resulted in an elimination of the elevation of plasma corticosterone levels seen in the control rats when sampling the CS during retention testing. The memory for the conditioned taste aversion would appear to be intact in the lesioned animals, as they do not extinguish more quickly than normal; in fact, they appear to do so more slowly [13]. Nor is it reasonable to suggest that there is some fundamental inability of hippocampal-lesioned rats to display elevated corticosterone levels in response to various stressful stimuli such as ether, surgical trauma or exposure to a novel environment, since most of the available evidence suggests the contrary [5,28]. It may not be unreasonable, however, to speculate that the necessary brain connections between these neurons responsible for the memory of the aversive consequences of the CS and the neurons responsible for the activation of the pituitary-adrenocortical response are disrupted by the hippocampal lesions. Presumably these latter neurons are those which secrete CRF (almost certainly located in the hypothalamus). In the continuing debate concerning hippocampal functions, the possibility that one of its roles is to modulate hypothalamic neurons concerned with the regulation of CRF, ACTH and adrenal steroid secretion should not be overlooked. This possibility has, of course, been suggested by several other writers [3, 5, 11, 18, 28]. It is unlikely that any such modulation will turn out to be simply "excitatory" or "inhibitory" but considerably more subtle and complex.

In summary, our results support three conclusions:

1. The extensive innervation of residual hippocampal tissue by axon sprouts from sympathetic neurons following anterior hippocampal lesions does not have observable behavioral consequences, even in situations which for normal rats can be classified as states of heightened arousal or stress as indexed by elevated plasma corticosterone levels.

2. Hippocampal lesions do not interfere with the acquisition of a conditioned taste aversion, but do result in abnormal extinction behavior in which rats with hippocampal lesions distribute their drinking behavior in significantly more "bouts," while consuming approximately the same amount of the CS as control rats.

3. Hippocampal lesions prevent the normal elevation of plasma corticosterone in the presence of the aversive CS as observed in retention testing.

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