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Effects of Amygdala Lesions on Taste Aversions Produced by Amphetamine and LiCl

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GRUPP, L. A., M. A. LINSEMAN AND H. CAPPELL. *Effects of amygdala lesions on the taste aversions produced by amphetamine and LiCl.* PHARMAC. BIOCHEM. BEHAV. 4(5) 541–544, 1976. – Rats which sustained bilateral damage to the amygdala were treated with one of two diversely acting agents (either d-amphetamine sulphate 4 mg/kg, or lithium chloride 0.24 M) in a taste aversion paradigm. Both groups of animals showed an attenuation of the aversion on the first test day after the initial pairing with the drug thus demonstrating that this effect of the lesion was not UCS specific. The implication of these findings for the hypothesis concerning the role of the amygdala in taste aversion conditioning is discussed.

Amphetamine Lithium chloride Taste aversion Amygdala lesions

WHEN the ingestion of a solution with a distinctive taste is followed by a state of general malaise induced by the injection of a toxin (e.g., lithium chloride [7] or by exposure to X-irradiation [6], animals quickly learn to avoid consuming that solution. However, it has also been demonstrated that such conditioning can be brought about when psychoactive drugs such as amphetamine or morphine at dose levels normally self administered by animals are used as the unconditioned stimulus [4,5]. It is clear then, that while all these unconditioned stimuli (UCS) may differ widely in their systemic effects, they nevertheless have similar functional properties. In recent years a number of studies have been devoted to the understanding of the neural basis of this gustatory conditioning phenomenon and a wide variety of structures including the dorsal hippocampus [2], lateral hypothalamus [9], ventromedial hypothalamus [10] and cortex [3] have been implicated.

In a rather detailed study of the role of the basolateral amygdala in taste aversion conditioning, Nachman and Ashe [8] have suggested that the impairment that results from bilateral lesions to this structure is a result of the loss of an "ability to recognize and respond to the significance or meaning of stimuli" [8] p. 640). Such a contention implies that regardless of the nature of the particular UCS used, damage to the amygdala should result in a decreased tendency to learn an aversion. A number of studies, however, support the notion that the choice of the UCS itself is an important variable in determining certain features of taste aversion conditioning. For example, Berger *et al.* [1] studied the effects of area postrema damage on the aversion produced by two diversely acting agents (methylscopolamine and amphetamine) and found that the lesion was effective in attenuating the aversion produced by the former agent but ineffective in the case of the latter. Similarly, Cappell *et al.* [5] in a study of the effects of chronic pre-exposure to the UCS on the subsequent formation of a taste aversion found that while the previous exposure to amphetamine was effective in attenuating the conditioning by both itself and by morphine, prior exposure to morphine did not reduce the aversion produced by amphetamine. Thus the effectiveness of the preexposure manipulation appeared to be dependent on the particular UCS involved.

In light of these findings support for the hypothesis of Nachman and Ashe could come from the demonstration that their results using the sickness inducing agent lithium chloride as the UCS could also be had using an agent with very different unconditioned effects. The following experiment was carried out in an attempt to examine this possibility.

METHOD

Animals

The animals were 98 naive male Wistar rats weighing approximately 300-350 g at the beginning of the experiment. The animals were individually housed with food and water available ad lib prior to surgery. A 12 hr light-dark cycle was in effect throughout.

Surgical Procedure

Stereotaxic surgery was performed on all animals while anaesthetized with pentobarbital sodium (50 mg/kg). For the animals receiving bilateral amygdala lesions, a nichrome wire electrode 250 μ in dia. and totally insulated except for 0.5 mm at the tip was positioned at the coordinates 2.0–2.5 mm posterior to bregma, 4.0 5.0 mm lateral to either side of the midsagittal suture and 9.0 mm below the

cortical surface. Anodal DC current of 2 mA was passed for 20 sec between the tip of the electrode and the indifferent attached to the animal's ear. The animals which served as controls were treated identically to the experimental animals except that no current was passed (sham operated).

Histology

At the conclusion of the experiment all of the experimental animals and a number of the control animals were anaesthetized and perfused intracardially with isotonic saline followed by 10% Formalin. Their brains were removed, stored in formalin and then frozen sectioned at 50μ, mounted and stained with cresyl violet. The sections were examined to determine the locus and extent of the lesion.

Testing Procedure

For 3 days following surgery all animals were given wet mash in addition to the ad lib food and water. Beginning on the fourth postoperative day the wet mash and the ad lib water were discontinued and access to fluid was restricted to a 15 min drinking period given at 24 hr intervals. During this period the animals were removed from their home cages, weighed, and placed in a drinking cage which differed from their home cage only in that a 100 ml Richter tube was attached to the front. No food was available in the drinking cage. At the end of the 15 min period, the amount of fluid consumed was recorded and the animals were replaced in their home cages.

From the fourth to the ninth postoperative day, the Richter tubes were filled with room temperature tap water; this period served to adapt the animals to the drinking cage and to drinking from the tubes. On the tenth postoperative day [8] both the amygdala lesioned animals and the sham operated animals were each randomly divided into 3 groups one of which was to receive a series of 4 IP injections of a 4 mg/kg dose of d-amphetamine sulphate (1 ml per 100 g); the second, a series of 4 IP injections of a 0.24 M solution of lithium chloride (12.5 ml per kg); and the third, a series of 4 IP injections of isotonic saline (1 ml per 100 g). Six groups were thus formed: (a) Group LA – rats with amygdala lesions injected with amphetamine (N = 21) (b) Group LL – rats with amygdala lesions injected with lithium chloride (N = 21) (c) Group LS – rats with amygdala lesions injected with saline (N = 20) (d) Group SA – sham rats injected with amphetamine (N = 13) (e) Group SL – sham rats injected with lithium chloride (N = 13) (f) Group SS – sham rats injected with saline (N = 10). All injections occurred on the tenth, thirteenth and sixteenth postoperative days and were given 5 min after the animal had been replaced in his home cage (i.e. 20 min after the beginning of the drinking period). It was on these 3 days as well as on the nineteenth day that a 0.1% saccharin solution was substituted for the normal tap water during the daily 15 min drinking period. Thus each group was given four 15 min exposures to the saccharin solution, the first three of which were paired with drug or saline injections. Each of these exposures or test days was separated by 2 days on which access to normal tap water was given.

RESULTS AND DISCUSSION

Histological examination revealed that 19 of the 21 animals in Group LA, 14 of the 21 in Group LL and 14 of the 20 in Group LS sustained extensive bilateral damage to the basolateral and/or medial nuclei of the amygdala without notably encroaching on neighbouring structures. The data reported here are based on the results obtained only from these animals. Figure 1 provides representative examples of the lesions.

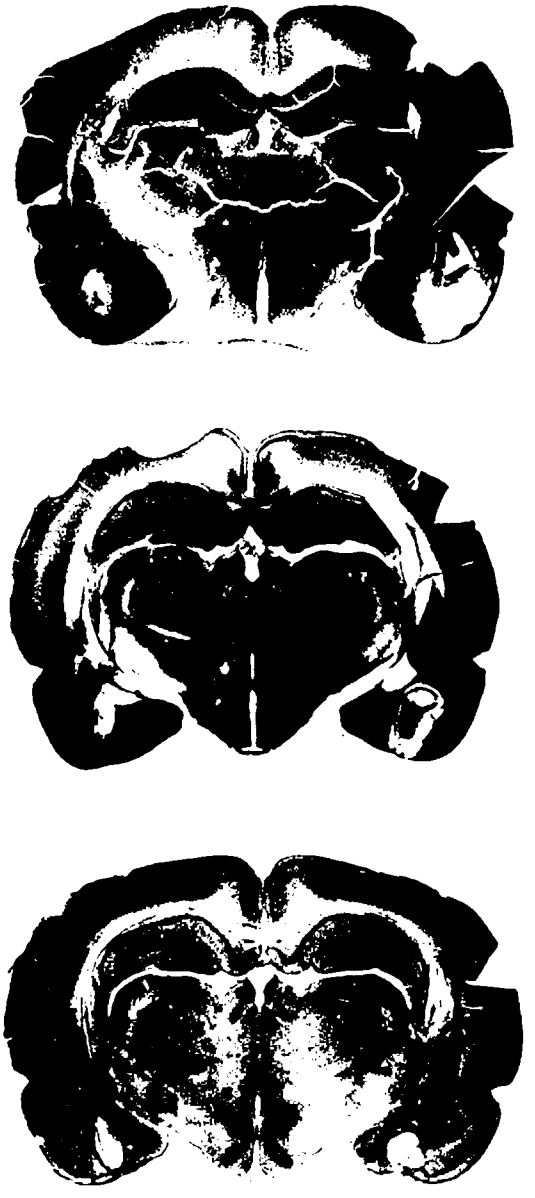

FIG. 1. Examples of the amygdala lesion from Group LA (A) Group LL (B) and Group LS (C). Most of the animals in all three lesion groups had sustained similar damage.

An initial statistical analysis revealed that Group LA drank significantly more saccharin on the first test day than did the SA control group. However, since a similar difference was not found in the comparison between the LS and LL groups and their sham operated controls, it is therefore attributable to sampling error and not to any effect of the lesion per se on saccharin intake. In order to compensate for this, an analysis of covariance was carried

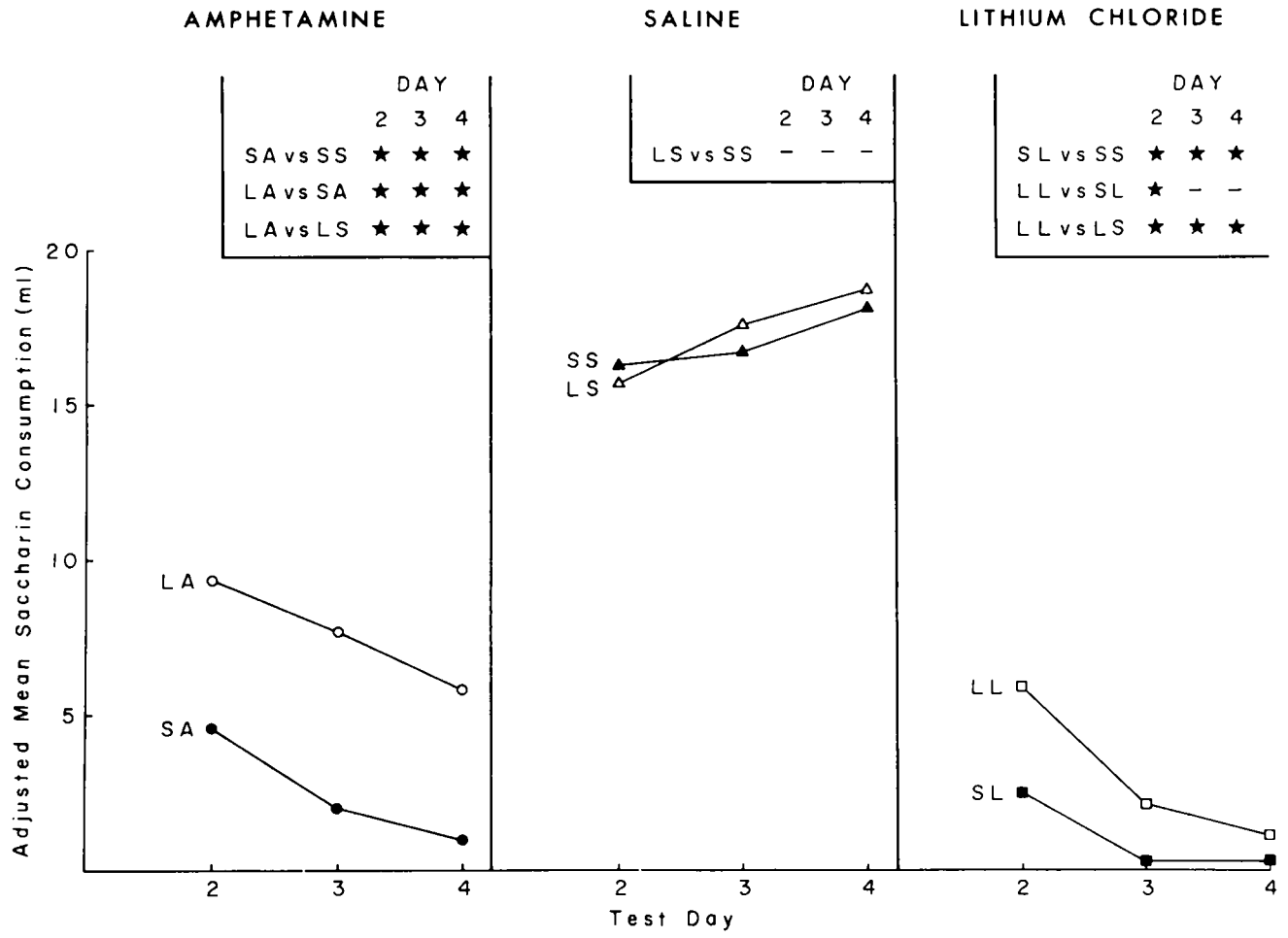

FIG. 2. Relative amount of saccharin consumed for the 6 groups across the 3 test days. Inserts give the various statistical comparisons done between groups and the stars indicate those that attained statistical significance ($p<0.01$).

out using the scores of test Day 1 as the covariate. This analysis revealed a significant effect of the lesion, $F(1,76) = 19.57$, $p<0.001$, a significant drug effect, $F(2,76) = 173.26$, $p<0.001$, a significant effect of the conditioning trials, $F(2,153) = 15.81$, $p<0.001$, and a significant interaction between drug and conditioning trials, $F(4,153) = 15.76$, $p<0.001$. The nature of the lesion effect was further analyzed by making multiple comparisons on the adjusted means for the final 3 test days using the Tukey test. Figure 2 shows the adjusted mean saccharin intake on the test days for all six groups and the various group comparisons which attained statistical significance.

It is clear from Fig. 2 that both the amphetamine treated and lithium chloride treated animals drank significantly less saccharin ($p<0.01$) than the saline treated animals across all 3 test days. This was true for both the lesioned and sham operated groups (comparisons SA vs SS and LA vs LS for the amphetamine animals and SL vs SS and LL vs LS for the lithium chloride treated animals) and demonstrates the ability of both drugs at the doses used, to produce a gustatory aversion. The effect of the amygdala lesion in both drug conditions was to significantly attenuate the resulting aversion. Thus on test Day 2 both the LA and LL groups drank significantly more ($p<0.01$) of the saccharin solution than did their unlesioned counterparts, groups SA and SL respectively. However, only for the LA group did the attenuation persist for the subsequent 2 test days. This could, in part, be due to a difference in the relative strength of the 2 drug doses used and not to an interaction between a particular drug and the lesion. For this reason, no inferences about the differences between the two drug groups on test Days 3 and 4 can be made.

The aim of this study was to determine whether the attenuation in the lithium chloride induced taste aversion brought about by lesions to the amygdala would also occur when a UCS with different systemic effects was used. A positive answer to this question was considered basic to the hypothesis of Nachman and Ashe [8] concerning the role of the amygdala in taste aversion conditioning in view of the findings that under some circumstances, the nature of the UCS can be an important variable in determining the strength of an aversion. The present findings which demonstrate an attenuation by amygdala lesions of an amphetamine induced aversion as well as a lithium chloride induced aversion tend to support this hypothesis by indicating that regardless of the particular effects any one UCS may have, the ability of that substance to produce a taste aversion is, in part, dependent on the normal functioning of the amygdala.

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