Electrical Stimulation of the Amygdala as a Conditioned Stimulus in a Bait-Shyness Paradigm

Abstract. *Animals receiving low-intensity electrical stimulation of the basolateral nucleus of the amygdala while drinking plain tap water were injected with toxic doses of lithium chloride to examine whether brain stimulation can serve as a conditioned stimulus in a bait-shyness paradigm. Subjects receiving this pairing greatly reduced their water intake in a retention test, in a similar manner to a group in which saccharin was paired with poisoning. Pairing lithium chloride with stimulation of the amygdala had no effect on subsequent water intake in the absence of brain stimulation. This effect appears to be locus specific, as caudate stimulation could not serve as a conditioned stimulus.*

Electrical stimulation of the brain (ESB) is known to have perceptual properties that can influence behavior. In addition to serving as a reinforcing stimulus for which animals will work repeatedly (*1*), ESB can serve as a conditioned stimulus (CS) in classical conditioning (*2*) and as a discriminative stimulus in instrumental learning (*3*). In classical conditioning, stimulation at a wide variety of cortical and subcortical sites can serve as an effective CS (*4*). There have now been several refinements of this technique, including the demonstration that conditioning can occur when stimulation at one cortical site serving as the CS repeatedly precedes the unconditioned stimulus (US) produced by direct electrical stimulation of the motor cortex (*5*). In perhaps the ultimate extension of this procedure, classical conditioning has been shown at sites in the lateral geniculate nucleus with ESB at two intensities serving as both the CS and US (*6*). As such, the use of ESB has important implications for the study of neural mechanisms of conditioning.

A conditioning paradigm that has received a great deal of attention recently is bait shyness, also referred to as taste aversion (*7*). Bait shyness can be produced by following the ingestion of a novel flavored solution with the injection of a toxin that produces visceral discomfort. Animals reexposed to the taste of this solution will diminish their intake greatly, thus expressing the aversion. Attempts to define the neural mechanisms of conditioned taste aversion have examined the disruptive effects of lesions and electrical stimulation at a number of subcortical loci (*8*). These studies suggest an important role for the amygdala, because lesions of the basolateral nuclei impair the acquisition of taste aversion (*9*) as does seizure-inducing electrical stimulation (*10*). To our knowl-

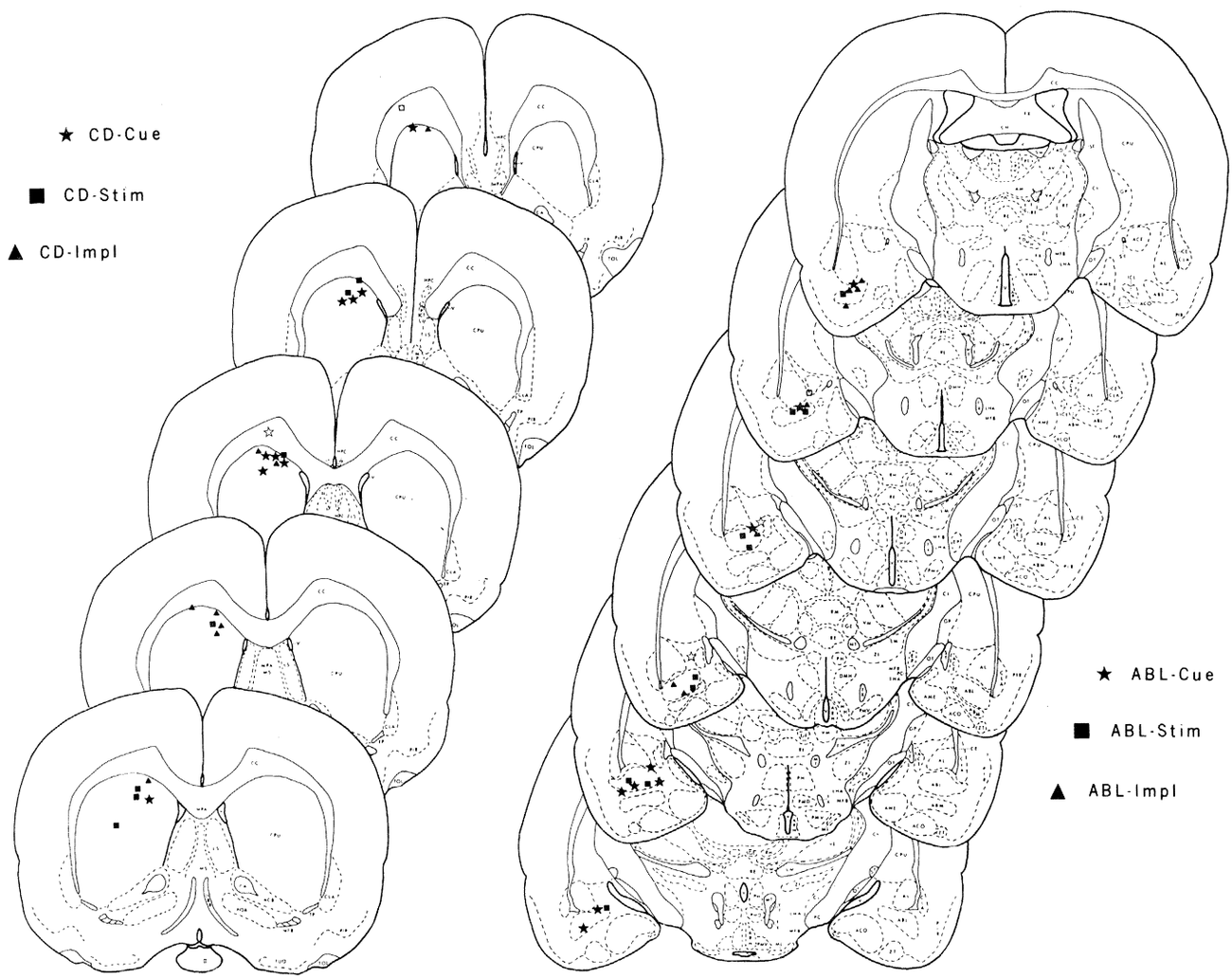

Fig. 1. Electrode placements from animals in the caudate (*CD*) and amygdala (*ABL*) groups depicted on coronal sections.

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edge, there have been no attempts to use the CS properties of ESB to identify neural systems involved in taste aversion. We now provide further evidence for the involvement of the amygdala in taste aversion by demonstrating that unilateral, low-intensity stimulation of the basolateral nucleus can substitute for taste as the CS in this paradigm. Garcia and his co-workers (*11*) have emphasized the selective relationship between olfactory-gustatory stimuli and food aversion. In this context, our data acquire added significance in that the amygdala receives input from both the anterior olfactory nucleus (*12*) and the pontine taste area (*13*).

Groups of male Wistar rats had bipolar electrodes permanently implanted under stereotaxic control into either the basolateral nucleus of the amygdala (ABL) or the head of the caudate nucleus (CD). An additional experimental group did not undergo surgery. Stereotaxic coordinates, with the mouth bar set at −4.2 mm below the interaural line, were ABL, 0.8 mm posterior to bregma, 4.8 mm lateral to the sagittal suture, 8.6 mm ventral to the dorsal surface of the skull; CD, 1.0 mm anterior to bregma, 2.5 mm lateral, 5 mm ventral. The CD placements were selected because we have successfully used stimulation at this site with comparable values to impair retention of a step-down passive avoidance response (*14*). Including CD groups would therefore provide a suitable control for the locus-specific effects of amygdala stimulation. After recovering from surgery, all animals were habituated to a deprivation schedule on which they were permitted to drink water for 10 minutes per day. Throughout the experiment, food was continuously available. All testing was conducted in five Plexiglas chambers (22 by 30 by 45 cm) with water or saccharin solutions dispensed from graduated cylinders fitted with curved ball-tip drinking spouts which protruded through one wall of the test chamber 5.0 cm above the floor. Each chamber was housed in a sound-attenuating box.

On the day of conditioning, the animals were assigned to six treatment groups on the basis of electrode placement and water intake. The first two groups were designated ABL-Cue and CD-Cue. As with all the groups with implants, they were presented with unflavored tap water during the 10-minute conditioning session. Intracranial stimulation (60-Hz sine wave) was delivered during the 10-minute drinking session in repeated trains that were on for 3 seconds and off for 6 seconds throughout

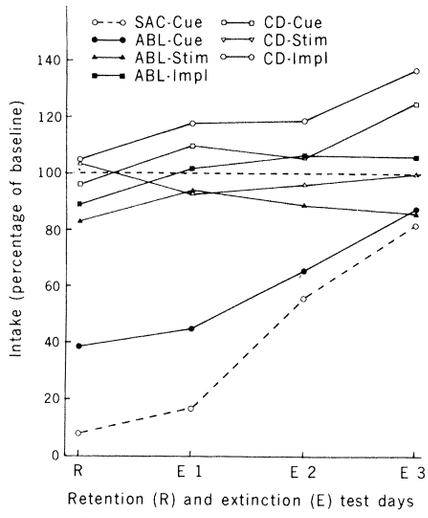

Fig. 2. Fluid intake at 48-hour intervals following toxic injections of lithium chloride (*SAC-Cue, ABL-Impl, CD-Impl*) or isotonic saline (*ABL-Stim, CD-Stim*), paired with saccharin (*SAC*) or electrical stimulation of the basolateral nucleus of the amygdala or caudate nucleus.

the session. The current was set initially at 10 μA, and if this intensity inhibited drinking in the first minute of the session, it was reduced to a level at which drinking resumed. The resulting mean current intensities (root mean square) were 3 μA in the ABL groups and 8 μA in the CD groups. Ten minutes after water was withdrawn, each subject in the ABL-Cue and CD-Cue groups was injected intraperitoneally (20 ml per kilogram of body weight) with 0.15*M* lithium chloride. Should electrical stimulation of the ABL serve as a CS in a taste aversion paradigm, those animals receiving ABL stimulation while drinking water prior to poisoning with LiCl, would reduce their water intake significantly on subsequent retest with ABL stimulation present during the drinking session. Unoperated animals were allowed to drink a 0.2 percent (weight to volume) saccharin solution for 10 minutes prior to the injection of LiCl. This experimental group (SAC-Cue) was incorporated to provide a baseline against which to compare the effect of ESB as a CS in the bait-shyness paradigm. As a control for the possible aversive effects of prolonged ESB, animals with ABL or CD placements were stimulated during the 10-minute drinking session but injected with isomolar saline (20 ml/kg) instead of LiCl. These groups were designated ABL-Stim and CD-Stim. As a final control for the effects of electrode implantation, animals with ABL electrodes (ABL-Impl) or CD electrodes (CD-Impl) did not receive ESB during the drinking session but were injected with LiCl, 10 minutes later. Water alone was presented to all groups 24 hours after the conditioning trial and on successive alternate days to ensure that no general drinking deficit had been produced by the LiCl injections. Fluid intake was retested 48 hours after training, under the same condition as prevailed for each group during training. Three extinction tests were run on days 4, 6, and 8 after training.

Upon completion of testing, the animals were killed, and their brains were removed for histological confirmation of the electrode placements. An analysis of the electrode placements from 56 animals with implants, by two independent observers, confirmed that 51 of the placements were correctly located in either the ABL or CD (Fig. 1). The data from these 51 subjects were included in the statistical analysis.

Only two groups showed a marked reduction in fluid intake during the 48-hour retention test (Fig. 2). Animals in the SAC-Cue group reduced their intake of saccharin by 92.5 percent, and the water intake in the ABL-Cue group was reduced by 61.5 percent of the intake before injection of LiCl. Water intake for both groups was unchanged 24 hours after conditioning with mean values of 12.6 ml and 15.5 ml for ABL-Cue and SAC-Cue groups, respectively (preconditioning levels: 13.8 and 13.1 ml). Stimulation of the CD prior to LiCl injections had no effect on subsequent water intake, nor did electrode implantation in either ABL or CD, or simulation of these structures in the absence of LiCl treatment. These data were analyzed by a repeated-measures analysis of variance, which showed significant main effects for treatments [F (6, 51) = 10.64, $P < .01$], test days [F (3, 153) = 15.58, $P < .01$], and the interaction term [F (18, 153) = 3.19, $P < .01$]. Additional one-way analyses of variance for treatment effects were applied to each test day; significant effects were found for the retention test and the first two extinction tests. Duncan's multiple range test confirmed that the intake scores for animals tested with saccharin (SAC-Cue) or those given ABL stimulation while drinking water, prior to LiCl treatment (ABL-Cue), were significantly lower than the five remaining control groups ($P < .05$). Although both groups drank significantly less than controls, the ABL-Cue group consumed significantly more than the SAC-Cue group ($P < .05$), which suggests that the taste was a more salient CS. In the first extinction test, the SAC-Cue and ABL-Cue groups again differed significantly ($P < .05$) from the

five control groups but not from each other. In the second extinction test, only the SAC-Cue group was significantly different from controls ($P < .05$), and no significant differences were observed between groups ($P > .05$) on the third extinction trial.

Taken together, these data show that electrical stimulation of the ABL, but not CD, can serve as a CS in a bait-shyness paradigm. This conclusion is supported by the following findings. (i) The ABL-Cue group suppressed its drinking in the retention test in a manner similar to that of the SAC-Cue group; (ii) the suppression of drinking was maintained through the first extinction test but returned to baseline on succeeding extinction tests; (iii) the pairing of ABL stimulation with LiCl illness did not affect drinking on the tests with water and no ESB given after the injection; (iv) stimulation of ABL without the LiCl illness (ABL-Stim group) had no significant effect on water intake during retention and extinction tests; (v) electrode implantation (ABL-Impl group) had no significant effect on subsequent water intake, even though this group was injected with LiCl; and (vi) none of the CD groups, including the animals stimulated in the CD prior to the LiCl illness, showed a significant reduction in water intake throughout the experiment.

The locus-specific effect noted is important because it distinguishes the taste-aversion paradigm from other forms of classical conditioning, such as the rabbit's nictitating-membrane response, in which the effectiveness of ESB as a CS is not related to locus of stimulation (*15*). This finding suggests a unique relationship between the amygdala and taste aversion, one that may be related to the importance of this structure in the integration of visceral and gustatory signals (*13, 16*). As such, these data further emphasize the importance of using the CS properties of ESB in the analysis of neural substrates of conditioning.

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References and Notes

1. J. Olds and P. Milner, *J. Comp. Physiol. Psychol.* **47**, 419 (1954); E. T. Rolls, *The Brain and Reward* (Pergamon, Oxford, 1975).
2. R. W. Doty, *Ann. Rev. Psychol.* **20**, 289 (1969).
3. R. M. Stutz, *J. Comp. Physiol. Psychol.* **65**, 79 (1968).
4. R. W. Doty, L. T. Rutledge, R. M. Larsen, *J. Neurophysiol.* **19**, 401 (1956); H. C. Neilson, J. M. Knight, P. B. Porter, *J. Comp. Physiol. Psychol.* **55**, 168 (1962).
5. R. W. Doty, and C. Giurgea, in *Brain Mechanism and Learning*, A. Fessard *et al.*, Eds. (Blackwell, London, 1961), p. 702.
6. F. B. Colavita and F. Szeligo, *Physiol. Behav.* **6**, 41 (1971).
7. J. Garcia, D. J. Kimeldorf, R. A. Koelling, *Science* **122**, 157 (1955); L. M. Barker, M. R. Best, M. Domjan, Eds., *Learning Mechanisms in Food Selection* (Baylor Univ. Press, Waco, Tex., 1977).
8. B. McGowan, W. Hankins, J. Garcia, *Behav. Biol.* **7**, 841 (1972); S. R. Roth, M. Schwartz, P. Teitelbaum, *J. Comp. Physiol. Psychol.* **83**, 184 (1973); R. P. Kesner and R. F. Berman, *Physiol. Behav.* **18**, 763 (1977).
9. E. T. Rolls and B. J. Rolls, *J. Comp. Physiol. Psychol.* **83**, 248 (1973).
10. J. B. Arthur, *Behav. Biol.* **13**, 369 (1975).
11. J. Garcia, W. G. Hankins, K. W. Rusiniak, *Science* **185**, 824 (1974).
12. L. E. White, Jr., *Anat. Rec.* **152**, 465 (1965).
13. R. Norgren, *J. Comp. Neurol.* **166**, 17 (1976).
14. H. C. Fibiger and A. G. Phillips, *Brain Res.* **116**, 23 (1976); A. G. Phillips and F. G. LePiane, *Soc. Neurosci. Abstr. No. 747* (1977).
15. R. B. Hupka, *Physiol. Behav.* **5**, 1355 (1970); J. W. Moore *et al.*, *ibid.* **10**, 581 (1973).
16. B. E. Eleftheriou, *Adv. Behav. Biol.* **2**, 1 (1972).
17. Supported by a grant from the National Research Council of Canada. A.G.P. is a Canada Council Killam Senior Research Scholar.

21 November 1977; revised 15 February 1978

Vasectomy Increases the Severity of Diet-Induced Atherosclerosis in *Macaca fascicularis*

Abstract. *Diet-induced atherosclerosis developed more extensively in vasectomized cynomolgus monkeys (*Macaca fascicularis*) than in sham-vasectomized control monkeys fed the same diet. The effect was most pronounced in the abdominal aortas, carotid arteries, distal segments of the coronary arteries, and intracranial cerebral arteries. Antibodies to sperm developed in all vasectomized monkeys, and complement and immunoglobulins were associated with atherosclerotic plaques in some of the vasectomized animals. The immunological response to sperm antigens that often accompanies vasectomy may exacerbate atherosclerosis.*

Atherosclerosis in rabbits can be exacerbated by experimentally inducing both serum sickness (immune complex disease) and hyperlipoproteinemia (*1*). This combination of repeated immunologic injury to arteries and a lipid-rich diet not only increases the extent of rabbit atherosclerosis, but also affects the qualitative characteristics of the plaques so that they more closely resemble atherosclerotic lesions in human beings (*2*). This phenomenon is not limited to rabbits; much more severe atherosclerosis also develops in baboons fed a lipid-rich diet and repeatedly immunized with foreign protein (*3*). Sharma and Geer (*4*) have reported the results of immunologic and morphologic studies of the lesions induced in the aortic intima of rabbits with experimentally induced serum sickness. They concluded that the injury and associated increased permeability of the endothelium were due to immune complex deposition, and that, by way of this mechanism, immunologic injury to arteries plays a role in the atherosclerotic process.

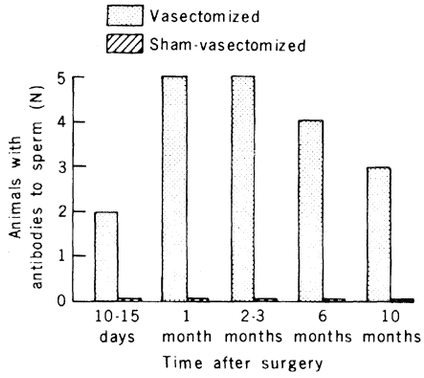

Fig. 1. Correlation between the number of vasectomized and sham-vasectomized monkeys in which antibodies to sperm developed and the time after surgery.

Multiple antigens are present in sperm, both on their surface and internally, and these can elicit production of autoantibodies. After animals are vasectomized, spermatozoa are confined to the epididymis and vas deferens. Presumably because these sperm lack a normal anatomical passage, they degenerate and release antigens that enter the circulation directly or are phagocytosed by macrophages. Sperm agglutination, sperm immobilization, and immunofluorescence provide the means to demonstrate circulating antibodies to sperm in about 50 percent of vasectomized men (*5–8*) and in vasectomized males of several other species (*9–11*).

Whether antibodies to sperm will develop in an individual after vasectomy may depend on genetically determined factors that affect immunologic responsiveness (*12*) or on different rates of sperm production. In vasectomized rhesus monkeys, high and sustained concentrations of antibody against sperm correlated significantly with high sperm counts before vasectomy (*13*); a similar finding has been reported in men (*14*). Another possibility is that antibodies to sperm develop in all vasectomized animals, but that routine methods measure only free antibodies and not

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