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Discriminative control of behavior by electrical stimulation of the dorsal raphe nucleus: generalization to lysergic acid diethylamide (LSD)

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Much research on the mechanism of action of drugs in the brain has made the assumption that the sites where many drugs act are at neuronal synapses. There is considerable evidence that serotonin (5-hydroxytryptamine; 5-HT) is one of several neurotransmitters at synapses in the brain[3]. A number of drugs have been reported to affect serotonin, *e.g.* by altering its concentration, metabolism, distribution, or pharmacological effect, and this has resulted in theories that the mechanism of action of these drugs is some kind of interaction with serotonin. Two such drugs are D-lysergic acid diethylamide (LSD) and morphine. Since LSD resembles serotonin in chemical structure, the initial discovery that LSD was a potent antagonist of 5-HT in isolated smooth muscle preparations[5,16] stimulated much research on the relationship of LSD's mental effects to serotonin. Although a wide variety of experimental approaches have been used, the mechanism of action of LSD is still unknown. Besides evidence that LSD may act by antagonizing 5-HT, there is also evidence to support the theory that LSD mimics 5-HT in the brain[12].

The evidence that morphine might produce its analgesic and other effects by interacting with 5-HT comes principally from data showing that procedures which decrease the concentration of 5-HT in the brain often result in a decreased morphine effect[10,15,17]. In addition, electrical brain stimulation (ESB) in the region of the dorsal raphe nucleus, which is comprised primarily of serotonin-containing neurons, produces analgesia and this analgesia is also antagonized by treatments which deplete 5-HT[2,11].

The present experiments sought to determine whether electrical stimulation of the dorsal raphe nucleus can serve as a discriminative stimulus in the rat. If it can, then we thought that a comparison of the stimulus effects of ESB with the stimulus effects of certain pharmacological agents might be an informative approach to the investigation of how the actions of these agents are related to serotonin systems.

Bipolar electrodes were constructed of twisted, stainless steel wires, 200 μm in diameter, insulated with teflon except at the cut cross-sections of their tips. These were chronically implanted in the dorsal raphe nucleus (DRN) of each of 10 male Sprague–Dawley rats. Electrode placement was verified by histological examination at the con-

clusion of the experiment. Following recovery from surgery, the subjects were reduced to 80% of their free feeding weights and trained, by successive approximations, to press both levers of a standard operant test chamber (Lehigh Valley Electronics). Reinforcement was sweetened milk. After bar pressing was established, discrimination training was begun. Depression of one of the 2 levers resulted in reinforcement in the presence of brain stimulation and responses on the opposite lever were reinforced in the absence of brain stimulation. For 5 animals, the right lever was correct during electrical stimulation and the left was correct in its absence; these conditions were reversed for the remaining 5 animals. The 2 conditions were presented in a double alternation sequence, *i.e.*, 2 days of stimulation were followed by 2 days of non-stimulation. In the non-stimulation condition, animals were connected to the stimulator cable and handled in the same manner as they were for stimulation, except that the circuit between the stimulator and the animal was switched open. A Nuclear-Chicago stimulator provided constant current ESB, which began 2 min before the rat was placed in the operant chamber. Stimulation, consisting of biphasic rectangular pulse pairs delivered at a frequency of 20/sec, was continuously monitored on a Tektronix oscilloscope. The pulse pair was composed of two 50-μsec pulses of opposite sign separated by 100 μsec. Current intensity was adjusted for each animal to a point just below that where a depression of response rate was apparent. These ranged from 300 μA to 600 μA. Discrimination training began with 4 preliminary training sessions of 15 min duration during which all responses on the correct lever were reinforced (FR-1). Subsequent sessions were also of 15 min duration, but began with a short period during which no responses were reinforced. The ratio of responding on the 2 bars during the unreinforced period was the index of discrimination. Initially, this unreinforced period was 1.5 min. During the next 4 weeks, the schedule of reinforcement was gradually increased to FR-3 and the initial unreinforced period was increased to 2 min.

The results obtained when stimulation and non-stimulation of the dorsal raphe nucleus served as discriminative stimuli are shown in Fig. 1. A high percentage of responses on the stimulation-correct bar during stimulation sessions and a low percentage of responses on the same bar during non-stimulation sessions is evident beginning with the first session block. The degree of discrimination gradually increased with continued training. For the 10 session blocks, the difference between responding during stimulation and non-stimulation is highly significant ($P < 0.01$, Wilcoxon's signed ranks test, two-tailed). The effectiveness of electrical stimulation of the dorsal raphe in controlling discriminated responding is comparable to the strongest stimulus control by drugs in a similar training procedure[7,8]. Although there are several reports that electrical stimulation of certain brain sites can function as discriminative stimuli[4, 14], the present results are the first report that stimulation of the raphe nuclei can function as a stimulus.

The finding that electrical stimulation of the dorsal raphe nucleus has stimulus properties provides a new method for studying possible behavioral functions of serotonergic neurons and the relation of drug effects to this system. Therefore, experiments were undertaken to determine whether LSD or morphine would either antagonize or

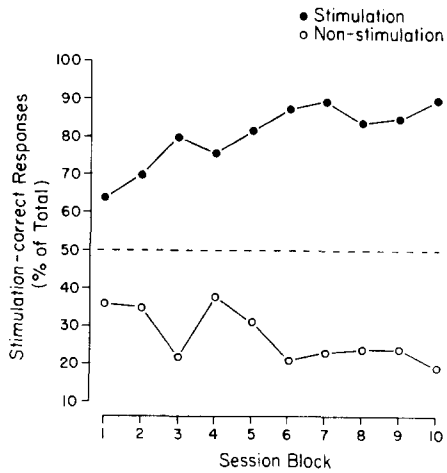

Fig. 1. Discriminated responding following stimulation or non-stimulation of the dorsal raphe nucleus. Each point is the mean of 2 determinations in each of 10 animals. On any given day, one-half of the subjects were stimulated and the remaining animals were not. Ordinate: number of responses on the stimulation-correct lever expressed as a percentage of total responses on both levers. Abscissa: successive blocks of 4 sessions each (2 stimulation sessions and 2 non-stimulation sessions).

mimic the stimulation cue. In sessions subsequent to those shown in Fig. 1, the same animals continued to receive stimulation and non-stimulation according to the same schedule. However, test sessions in which drugs were administered were interposed among the regular training sessions. Test sessions were sessions of 2 min duration, during which no responses were reinforced. Tests with stimulation always followed 2 days of non-stimulation and test sessions with non-stimulation followed 2 days of stimulation.

Fig. 2a shows the results obtained when various doses of morphine sulfate were administered in test sessions. With increasing doses, morphine decreased the percentage of stimulation-correct responding when the animals were being stimulated although, with the range of doses tested, they continued to make the majority of their responses on the stimulation-correct lever. In the absence of stimulation, morphine produced an increase in stimulation-correct responses, but responding was still appropriate for non-stimulation. (At 10 mg/kg of morphine, the percentage of stimulation-correct responses, both in the presence and absence of stimulation, is different from the respective 0 mg/kg at $P < 0.05$, Duncan's multiple range test.) Higher doses of morphine were not tested since 10 mg/kg is more than sufficient to produce effective and consistent stimulus properties and antinociception[6]. None of the doses of morphine tested altered the rate of bar pressing. These results suggest that the stimulus properties of morphine are different from both stimulation of the dorsal raphe nucleus and non-stimulation. This difference is in contrast to the similarities that have been observed between analgesia produced by ESB in the region of the dorsal raphe nucleus and that produced by morphine[2]. However, stimulation intensities needed to produce analgesia are far in excess of those used in the present study.

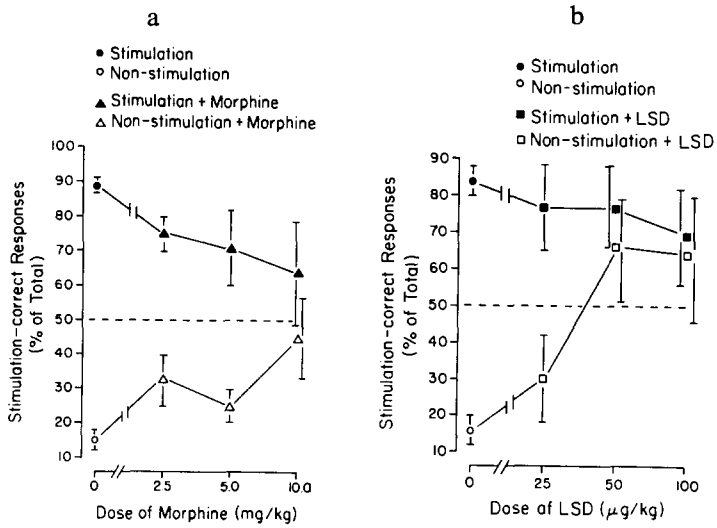

Fig. 2. Generalization of discriminated responding after stimulation or non-stimulation of the dorsal raphe nucleus to morphine (a) and LSD (b). Two minute test sessions, during which no responses were reinforced, were interposed among discrimination training sessions. Morphine was injected, i.p., 45 min before testing. LSD was injected, i.p., 5 min before testing. Each point is the mean of one determination in each of 7 animals in part (a) of the figure and one determination in 6 animals in part (b). Vertical lines represent ± S E.M. Ordinate: number of responses on the stimulation-correct lever expressed as a percentage of total responses on both levers. Abscissa: dose of morphine sulfate (a) or LSD tartrate (b) plotted on a log scale.

The results obtained when LSD tartrate was given to these animals are shown in Fig. 2b. It is apparent from these data that the stimulation cue generalized to LSD; *i.e.* LSD, in sufficient doses, produced responding appropriate to electrical stimulation (the percentage of stimulation-correct responses at 50 μg/kg and at 100 μg/kg each is different from 0 μg/kg at $P < 0.05$, Duncan's multiple range test). There was only a moderate, and statistically insignificant, alteration in responding during the stimulation condition. A decrease in the rate of responding produced by 100 μg/kg of LSD precluded the testing of a higher dose of LSD. These results indicate that electrical stimulation of the dorsal raphe nucleus and LSD have similar stimulus properties. Another common behavioral effect of LSD and stimulation of serotonergic neurons is their impairment of habituation to repetitive stimuli, which, as Aghajanian has noted[1], is a 'striking similarity'. Since electrical stimulation of raphe nuclei releases 5-HT in the forebrain[9,13], these results are consistent with the hypothesis that LSD produces at least some of its perceptual effects by mimicking the action of 5-HT in the brain.

In more general terms, the present results demonstrate that comparing the stimuli produced by ESB of certain brain areas with the stimulus properties of drugs is a viable technique. The technique is unique in that the comparison between a neurophysiological manipulation and a drug effect is direct. This is in contrast to more typical experiments in which the effects of the drug and neurophysiological manipulation are each measured independently and then compared.

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