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Behavioral Effects of Serotonin or a Blocking Agent Applied to the Septum of the Rat[1]

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PERSIP, G. L. AND L. W. HAMILTON. *Behavioral effects of serotonin or a blocking agent applied to the septum of the rat.* PHARMAC. BIOCHEM. BEHAV. 1(2) 139–147, 1973.–Previous data indicating the presence of a cholinergic system functional in the inhibition of responding to nonrewarding stimuli have emphasized the importance of the integrity of the anteromedial septal areas (AMS) and its hippocampal projections. The relationship of this system to the ascending serotonergic fibers of the medial forebrain bundle, which terminate in the AMS, was investigated. The direct application of serotonin or a blocking agent to the AMS via a cannula preceded testing of exploratory habituation, T-maze position habit reversal, and flinch-jump thresholds to electrical shock. The data indicated that the AMS-serotonin system may function by desensitizing an organism to various types of stimuli, perhaps by facilitating an AMS-hippocampal inhibitory system.

Serotonin Cinnanserin Inhibition Shock thresholds Septal area Chemical stimulation

A NUMBER of recent studies have provided evidence that the behavioral dysfunctions following septal lesions do not represent the disruption of a unitary function shared by the various nuclear groups comprising this region of the limbic forebrain. Both behavioral and anatomical evidence support the notion a functional differentiation between the anteromedial septal region (AMS) and the more lateral and posterior groups of the septal area. In general, lesions of the AMS produce behavioral deficits which can be characterized as a reduction in behavioral plasticity (cf., [8]). For example, rats with lesions involving the AMS show impaired performance on DRL schedules and position-habit reversal [1, 8, 10, 20], discrimination reversal and spatial alternation [17, 30, 32, 41], FI schedules [9, 31] and go-no-go types of discrimination and extinction [41, 42].

Raisman [28] has pointed out that the whole of the hippocampal projection passes through the septal region. Hence, lesions of the AMS result not only in degeneration of fibers taking origin in the septal nuclei but also of fibers of passage from the hippocampus. This suggests that at least some of the behavioral deficits resulting from AMS lesions may be due to the destruction of hippocampal efferents, rather than to a specific disruption of an AMS-hippocampal inhibitory projection system.

Evidence that the behavioral effects noted above are mediated by a septo-hippocampal circuit is the observation that both AMS lesions and systemic injections of anticholinergic drugs attenuate hippocampal theta [8, 35]. The effects of AMS lesions may, therefore, be related to the interruption of a central cholinergic inhibitory mechanism which either terminates or courses through the AMS region [13, 19]. The similarity of behavioral dysfunctions produced by AMS lesions and those resulting from blockade of central cholinergic pathways has led to the hypothesis that the role played by the AMS-hippocampal system in the processes of behavioral inhibition is, at least in part, mediated via cholinergic pathways (cf., [2, 3, 4]). Hamilton, McCleary and Grossman [21] provided some experimental support for this hypothesis with their findings that microinjections of atropine into the septal area of cats mimicked septal lesions on a variety of behavioral tasks.

The attempts to correlate AMS-hippocampal circuitry and septal cholinergic pathways [24, 33] with processes relating to behavioral inhibition are made difficult by the current lack of data regarding the potential functions of other biogenic amines in these regions of the limbic system. Of particular interest to the present authors has been the accumulating evidence implicating serotonergic forebrain systems in basic processes of behavior.

Through selective brain lesions, Heller, Harvey, and Moore [22] found that significant reductions in brain 5-HT occurred only when lesions were restricted to areas which

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[2] Requests for reprints should be mailed to Leonard W. Hamilton.

either contribute fibers to the medial forebrain bundle (e.g., ventral midbrain tegmental area of Tsai, dorsomedial tegmentum) or transect fibers of the medial forebrain bundle (e.g., septal area). The highest reductions in brain 5-HT were found following lesions of the medial forebrain bundle itself.

Studies of the ascending serotonergic system based upon electrical stimulation or lesions of the raphe nuclei have not proven too successful in elucidating the role of serotonin in the forebrain. In view of these difficulties, the present investigation was undertaken to explore the effects of the direct application of serotonin or a blocker of serotonin into the AMS. Behavioral tasks were chosen to determine the effects of these treatments upon the responses of rats to novel nonrewarding stimuli, rewarding stimuli, and aversive stimuli.

Studies of central 5-HT mechanisms have suffered from the lack of specific antagonists (rather than synthesis inhibitors, such as p-chlorophenylalanine) of serotonin receptor sites. Researchers at the Squibb Institute for Medical Research (New Brunswick, N. J.) have recently developed a compound which appears to be a potent antagonist of tryptaminoceptive receptor sites. Unlike most other serotonin antagonists available, which also show varying degrees of anticatecholaminergic, antihistaminergic, anticholinergic, etc., properties, this compound has been found to be a highly potent and specific antagonist of serotonin on such peripheral tissues as the rat's uterus, bronchioles of anesthetized dogs, blood pressure of phenobarbitalized dogs, and several other preparations [29]. Studies on central effects of the drug [14] indicated a rather limited range of pharmacologic activity, but this is not untypical for many compounds administered systemically. The compound, called cinnanserin, is listed as SQ 10, 643 and has a chemical name of 2'-(3-dimethylaminopropylthio) cinnamanilida hydrochloride.

EXPERIMENT 1: EFFECTS OF SEROTONIN AND CINNANSERIN SEPTAL APPLICATIONS OF THE RESPONSES OF RATS TO A NOVEL ENVIRONMENT

Animals and Surgical Procedures

Male albino rats (n=90) of the CFN strain (Carworth, N.Y.) constituted the basic sample of animals for all experiments described in this report. In the event of deaths or inappropriately positioned cannulae, additional animals of the same strain and weight were substituted in order to maintain an equal number of animals for the Treatment groups of Experiment 1. The animals were individually housed in a colony which was kept under constant illumination. For Experiment 1, all animals were allowed free access to Purina food pellets and water.

The double-walled cannulae consisted of an outer 23-G needle which was permanently implanted in the brain, and a removable 27-G inner needle. The drugs to be administered were ground to a uniformly fine powder which could be tapped into the inner cannula. One tap of the inner needle into the powdered drugs was calculated to provide approximately 5μg of the drug.

All animals were anesthetized with 0.30 cc/100 g body weight of Equi-Thesin (Jensen-Salsbery Co.). The cannulae were stereotactically lowered into the brain such that the tip of the cannula was directed to the midline and directly above the anteromedial septal nucleus (AMS). The stereotaxic placement of the cannulae was 8.2 mm anterior to the ear bars, directly on the midline, and 1.0 mm above the stereotaxic zero plane [7]. The cannulae were angled between 12° and 15° in order to avoid damage to the superior sagittal sinus. Following surgery the animals were returned to their home cages and allowed from 5-7 days for recovery. All animals weighed between 170-200 g at the beginning of Experiment 1.

Histological Evaluation

At the conclusion of the experimental series, all animals were sacrificed with an overdose of Equi-Thesin and perfused intracardially with isotonic saline solution followed by 10% formol saline. The brains of the animals were removed from the skulls and, following a storage period in formol saline, representative 60-μ frozen sections through the site of maximal penetration of the cannulae were made. The sections were viewed through a microprojector and compared with the corresponding figures in the DeGroot [7] atlas (See Fig. 1).

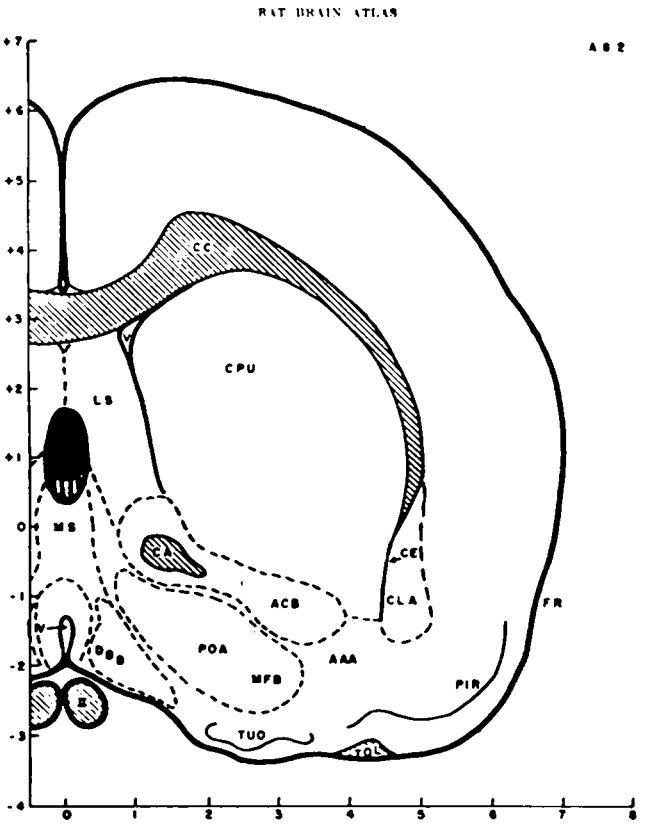

FIG. 1. Reconstruction of the area of maximal cannula penetration of the anteromedial septum for the 90 rats used in the experiments, projected onto the DeGroot [7] atlas plate 8.2 mm anterior to the ear bars. The black area represents 80% of the placements. The striped area immediately below the black area represents the remaining 20%. Symbols: MS = medial septum (anterior part), LS = lateral septum, DBB = diagonal band of Broca (rostral extension of the horizontal limb).

Experimental Procedures

Animals. The 90 animals with cannulae implanted into the AMS were unsystematically assigned to one of the following groups: operated controls (CON), animals to receive serotonin creatinine sulphate injections (Group 5-HT), and animals to receive injections of cinnanserin (Group CIN). An additional group of 30 unoperated male CFN rats, weighing the same as the above groups at the time of testing, showed no significant differences in the response measures (see below) from those of the operated control animals. These findings were accepted as justification for the use of operated animals as controls in the present experiment.

Drug-injection procedure. Prior to the administration of any drugs the inner cannulae were removed and thoroughly cleansed with ethyl alcohol, allowed to dry, and tested to assure that no blockage of the needle had taken place as a result of accumulated and solidified tissue fluids. For the rats in Group 5-HT, 40 μg of serotonin creatinine sulphate (Sigma Chemical Company) were tapped into the inner needle which was then replaced into the animal's brain 30 min prior to the experimental test period. For Group CIN, 40 μg of the antiserotonin drug cinnanserin was administered 30 min prior to testing.

Apparatus and testing conditions. The apparatus was identical to that described by Feigley and Hamilton [13] and consisted of clear Plexiglas testing chambers, each wall measuring 20 cm high X 21 cm wide. The floor of each chamber was made of wire mesh (1.3 cm openings). Protruding from, and centered in, each of the front and back walls were single black Plexiglas tubes, 12 cm long and 5 cm in dia. The outer end of each tube was blocked by a clear Plexiglas partition through which light (when it was available) was presented. Interruption of an infrared light beam located 1 cm down the length of the tubes operated electronic equipment for recording the frequency and cumulative duration of head poking responses. Under certain conditions (indicated below) interruption of the photobeam also operated a 28-V Dialco cartridge type pilot lamp which was clipped to the end of one tube. Each of the three testing chambers was enclosed in a sound attenuating compartment which was painted black and measured 60 X 35 X 35 cm. Fans, enclosed to prevent the penetration of light, provided ventilation and further masked environmental noise. Light sensitive paper was found to be unexposed after having been placed in the chamber for a length of time equal to a testing session, thus verifying that light from external sources could not enter the apparatus.

The 30 animals in each group were subdivided such that half of each group was tested under one of two conditions–an experimental condition or a control condition. For the animals in the experimental condition interruption of the photobeam in one tube (designated as the light tube) resulted in light onset; interruption of the beam in the other tube (the no-light tube) had no effect. For animals in the control condition interruption of the photobeam in neither tube resulted in light onset, though for purposes of statistical analyses, one tube was always designated as the light tube and the other as the no-light tube. It should be noted that the control condition, therefore, refers simply to the absence of response contingent light presentation and should not be confused with the abbreviation CON which refers only to the fact that these animals received no drugs in the experiment. Thus, as was the case for the 5-HT and CIN groups, half of the rats in the CON group were exposed to the experimental test condition and the other half to the control test condition.

On the day of testing each animal was placed into the apparatus for a 30-min session. The frequency and total duration of beam interruptions (i.e., nose pokes) for each tube were recorded at the end of each 5-min interval throughout the session. From these data the following response measures were obtained for each of the six 5-min intervals (trials): (a) number of pokes into the light tube; (b) number of pokes into the no-light tube; (c) mean duration of head pokes into the light tube; and, (d) mean duration of head pokes into the no-light tube.

Results

Response frequency. For the four response measures separate analyses of variance (ANOVA) were performed comparing the CON, 5-HT, and CIN groups for each tube under each condition. The data for these analyses are presented in the four panels of Figs. 2 and 3. In cases where the overall ANOVA showed significant differences, two-sample post-hoc comparisons (0.05 alpha level) were carried out using Fisher's test [12] for the least significant difference (LSD test). Essentially, all ANOVA consisted of modification of the Winer Case 11 split plot design [40] with one factorial variable in the plots (drug groups) and one subplot variable (5 min trials). Applications of the LSD test following these ANOVAs utilized a hybrid error term, which represented the addition of the respective error terms for the plot and subplot variables. It was felt that this provided a more conservative application of the LSD test for the ANOVA technique used.

Observation of the frequency data for the control condition (Panels A and B of Fig. 2) showed a decline in the rate of responding for all groups throughout the session. For the light and no-light tubes (a mock variable under this condition) this decrease in response rate was highly significant ($F=24.77$ and 24.16, respectively, $df=5/210$ and $p<0.001$ in both cases). A significant Groups effect was also evident with the CIN and 5-HT animals having a lower response rate than the CON group both in the light and no-light tubes ($F=6.34$ and 7.65, respectively, $df=2/42$ and $p<0.01$ for both tubes.) The Groups X Trials interaction was not significant for the light tube, but marginally significant in the no-light tube ($F=2.41$, $df=10/210$, $p<0.05$). Inspection of the data for the no-light tube (Panel B) revealed that this effect was probably the result of a greater amount of variability in the data of the CON animals for this tube. Hence, it appears likely that all groups in the control condition exhibited similar rates of decline throughout the session, though the 5-HT and CIN animals maintained a significantly lower overall level of responding than the CON animals. Application of the Fisher LSD test to the data for the light and no-light tubes under the control condition generated critical difference values of 2.23 and 1.80, respectively. These data lead to the conclusion that under conditions in which no response contingent light stimulation was available, the application of serotonin or cinnanserin to the AMS resulted and in lower levels of exploratory responding than that emitted by animals under non-drug conditions (i.e., CON group).

In the experimental condition, the response contingent presentation of light in the light tube (Panel C) produced a temporary increase in the rate of responding of rats in the

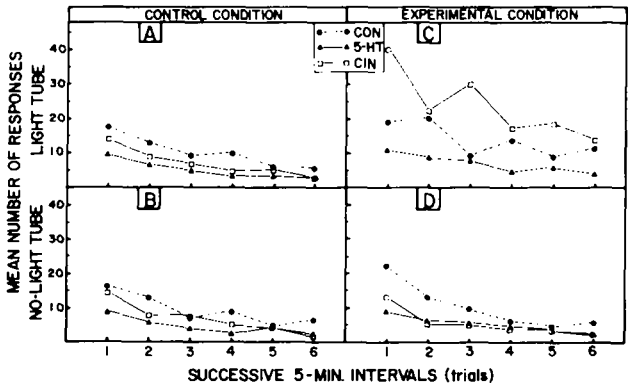

FIG. 2. Response frequency measures showing the effects of serotonin and cinnanserin septal applications on nose-poke responding, with (experimental condition) or without (control condition) response-contingent light presentation.

CON group, a very marked increase in responding of rats in the CIN group, and a small increase in response frequency for the rats in the 5-HT group. Highly significant Groups effect ($F=28.62$, $df=2/42$, $p<0.001$) and Trials effect ($F=18.18$, $df=5/210$, $p<0.001$) were obtained. The magnitude of these differences on response frequency was reflected in the ANOVA by a significant Groups X Trials interaction ($F=5.86$, $df=10/210$, $p<0.001$). The frequency of responding in the no-light tube (Panel D) was also influenced by the response contingent light presentation in the light tube, but the groups differed in the direction of this influence. Whereas the animals in the CON group showed higher levels of responding in comparison with CON animals under the control condition, the animals treated with CIN dropped to frequency levels significantly lower than that of CON animals. For the experimental condition the critical difference values for the LSD test were 3.22 (light tube) and 2.26 (no-light tube). Hence, for animals in the CIN group the only major change in response frequency occurred in the tube which was associated with response contingent light presentation. The animals treated with 5-HT responded with low frequency levels in all tubes and conditions of the experiment, the slight increase in responding in the light tube under the experimental condition being statistically nonsignificant. Control animals exhibited increased frequencies of responding in both light and no-light tubes under the experimental condition when compared with the frequency measures under the control condition. In all cases, with the exception of the light tube in the experimental condidtion, the CON animals were significantly higher than animals in the 5-HT and CIN groups.

Response Duration. The mean response duration data are presented in the four panels of Fig. 3. Under the control condition (Panels A and B) the average durations per response of the CON animals increased throughout the session to values exceeding 4.0 sec, whereas the rats in the 5-HT group maintained a consistent response duration of approximately 1.5 sec in both the light and no-light tubes. The animals treated with CIN displayed a constant duration of responding in the light tube, but exhibited a tendency to increase their response durations in the no-light tube. This increase, however, did not approach the levels attained by the CON group. The overall ANOVAs for the light and no-light tubes indicated a nonsignificant trials effect. The Groups effect was significant for the light tube ($F=10.20$, $df=2/42$, $p<0.001$) as well as for the no-light tube ($F=6.38$, $df=2.42$, $p<0.005$). The LSD critical difference values for the light and no-light tubes were 0.57 and 0.88 respectively. Application of these critical values to the data shown in Fig. 3 demonstrate that in the case of the light tube, the CIN and 5-HT groups perform identically throughout the session and, by the third trial, differ significantly from the CON group. In the no-light tube, the tendency for increased response duration of the CIN animals resulted in significant differences between all three groups. As both tubes were identical under the control condition, this difference in response duration in the no-light tube for rats treated with CIN is not readily explainable. Inspection of the data for individual animals in group CIN, however, revealed that two of the animals exhibited increasingly prolonged response durations (e.g. 8–10 sec) towards the end of the session. It is likely that these atypical responses were responsible for elevating the group means in the case of the no-light tube.

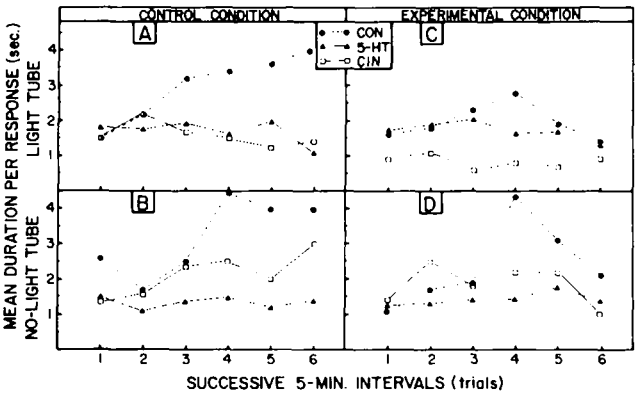

FIG. 3. Response duration measures showing the effects of serotonin and cinnanserin septal applications on nose-poke responding, with (experimental condition) or without (control condition) response-contingent light presentation.

In Panel C it is evident that the response contingent presentation of light resulted in a decline in the response durations of the CON group to levels approximating the values shown by animals treated with 5-HT (1.5–2.5 sec). The ANOVA for these data revealed a nonsignificant Trials effect, but a highly significant Groups effect ($F=12.13$, $df=2/42$, $p<0.001$). The LSD critical difference value of 0.37 indicated that this Groups effect was due to a significant drop in the response duration data of the CIN group to a level of approximately 0.75 sec. A significant Group effect ($F=5.41$, $df=2/42$, $p<0.01$) was also obtained for the no-light tube (Panel D). Unlike the Group effect for the light tube, however, the LSD critical difference value of 0.49 showed that this difference for the no-light tube was principally due to the high degree of variability of the CON group in the middle trials of the session. By the end of the last trial in both tubes all three groups were responding at approximately the same levels of response duration.

Discussion

The performance of the control animals in the present study was consistent with the findings of Feigley and

Hamilton [13]. When tested under the control condition, response frequency declined as a function of exposure time, whereas response duration increased. In the experimental condition, the response contingent light presentation was accompanied by an increase in frequency of responding and a decrease in response duration.

The effects of injecting the serotonergic blocking agent, cinnanserin, into the septum were similar in many respects to the effects of systemic cholinergic blockade or lesions of the AMS [13]. Each of these manipulations was accompanied by a decrease in response duration under the control condition and an increase in the response frequency associated with response contingent light presentation. The effects of cinnanserin application were, however, more specific in that the increase in response frequency was restricted to the light tube of the experimental condition–the rate of responding was lower than normal under all other conditions.

The injection of serotonin decreased both the rate and duration of responding under all conditions, and the response characteristics were unchanged by response-contingent light presentation.

Our interpretation of these data agrees with the suggestion of Feigley and Hamilton [13] that frequency and response duration measures of exploratory responding may well be under separate neural controls. The traditional definition of habituation which emphasizes the gradual dimunition of responding to stimuli which are nonbiologically significant (cf., [3]) does not provide a clear-cut definition to explain the effects of the treatments used in this experiment on habituation. For example, in all conditions of this study animals treated with 5-HT exhibit low frequencies of responding in comparison with CON animals. This would suggest that AMS stimulation with serotonin facilitates habituation. However, the response duration measure would indicate that, in comparison with CON animals, serotonin produces an impairment of the processes of habituation. It is also difficult to understand how cinnanserin produces essentially the same results as serotonin under all conditions with the sole exception of the response measures for the tube which offers response contingent light presentation. In this latter case, cinnanserin produces a heightened impairment of habituation for both response measures. Tapp [36] has suggested that an important variable controlling the amount of exploratory responding is the amount of feedback stimulation provided by the exploratory behavior. On the basis of the frequency data, one might speculate that serotonin in the AMS serves to reduce the organisms sensitivity to stimuli and, consequently, to diminish the influence of response contingent stimulation on exploratory activity. Cinnanserin, on the other hand, may increase the organisms sensitivity to stimuli by blocking the effects of endogenous serotonin at the AMS level. This increased sensitivity would not be general but specifically directed toward those stimuli which are produced by the organisms responses.

EXPERIMENT 2: ACQUISITION AND REVERSAL OF A T-MAZE POSITION HABIT

Animals

The animals consisted of 45 male albino rats which were selected from the animals used in Experiment 1. The rats weighed between 200 and 250 g at the beginning of Experiment 2.

Apparatus

The apparatus consisted of a T-maze constructed of plywood and painted a neutral gray. The dimensions were as follows: stem = 30 cm long X 30 cm high X 12 cm wide; arms = 45 cm long X 30 cm high X 12 cm wide. The start box was defined by a clear Plexiglas guillotine door located in the middle of the stem. Similar doors, located 5 cm into each of the arms, prevented retracing after the animal had entered one of the arms. The three doors opened and closed as a unit. Metal water receptacles were located at the end of each arm. A small lamp in the testing room provided a uniform and dim illumination throughout the apparatus.

Procedures

Deprivation schedule. The total daily water intake of the rats during pretraining and subsequent training was limited to the amount of water consumed in the maze plus a 30 min period of free access to water in the home cage. The latter period began 20 min after the experimental session. Since each experimental session lasted about 10 min, the animals were approximately 23 hr water deprived at the time of testing.

Training. On the first and second day of the experiment (pretraining), 2.5 ml of water were placed into the receptacles and the rats were allowed free exploration of the maze. An animal was given up to 15 min to consume the water on each pretraining day and was then returned to the home cage. Whether or not the water was consumed, no animal was removed from the chamber until at least 5 min had elapsed nor allowed to remain beyond 15 min. The entire apparatus was cleansed with a damp cloth containing a dilute solution of vinegar following the completion of testing each animal to reduce olfactory cues.

On the third day training of the position-habit was begun. For the first trial, 0.5 ml of water was placed into each receptacle. The rat was placed into the start box and 20 sec later the door was raised. To prevent retracing after the animal had completely entered one of the arms, the doors were closed and the rat was allowed to drink the water. The next trial was initiated by returning the rat to the start box for a 20 sec intertrial interval, replenishing the water reward, and raising the doors. Position-habit training to the initially preferred side (i.e., the side to which the rat went on the first trial) was continued for 10 trials per day until the following criteria were met: at least 9/10 correct responses with latencies less than 5 sec for three consecutive days, and 4 of the first 5 trials correct with latencies less than 5 sec on the third criterion day. Reversal of the position-habit was begun on the day after the animal had reached the acquisition criteria, and reversal training continued daily until the subject had reached the same criteria as required for acquisition training. It was felt that the stringency of this reversal criteria would be more likely to demonstrate differences in reversal performance as a result of the drug treatments. The minimum number of days required to reach the reversal criterion following the completion of acquisition training was therefore three days.

Drug administration. At the start of the experiment the rats were randomly assigned to one of three groups of 15 animals each. One group contained 15 operated animals which were to receive no drugs throughout the study (Group CON). The other two groups were to be adminis-

tered either 40 μg of serotonin (Group 5-HT) or 40 μg of cinnanserin (Group CIN) via the AMS cannulas, 30 min prior to an experimental session. No animal received any drugs during acquisition training. Drug administration to the 5-HT and CIN groups began on the first day of reversal training and continued until the reversal criteria had been attained. The inner cannulas of the CON animals were also removed and replaced in the brains (without drugs) 30 min prior to testing.

Results

Acquisition. Four of the animals in the CIN group were dropped from the study as they had not attained the reversal criterion at the end of 28 days. Therefore, all data were analyzed by ANOVA for unweighted means [40]. Providing the overall ANOVA reached statistical significance, posthoc comparisons were made by application of the Fisher LSD test. Analysis of the number of daily sessions required to reach the acquisition criteria revealed no significant differences between the groups ($F<1$). As no drugs had been administered prior to the first reversal day of training, this result was to be expected.

Reversal. Analysis of the number of sessions required to reach the reversal criteria revealed highly significant differences among the groups ($F=114.36$, $df=3/38$, $p<0.001$). The Fisher LSD test generated a critical difference value of 1.81. Inspection of the group means indicated that the rats injected with CIN were significantly impaired on the reversal of the position habit in comparison with the CON animals, whereas the rats treated with 5-HT were significantly facilitated. (Mean number of days to reversal were 6.5, 4.5, and 14.2 for Groups CON, 5-HT and CIN, respectively.) The marked difference between the 5-HT and CIN rats in attaining the reversal criterion was not attributable to any differences in latencies between these groups, but rather to the tendency of CIN rats to make frequent errors.

A savings score was computed for each animal by subtracting the number of sessions required to reach the reversal criteria from the number required to reach the acquisition criteria. Analysis of these data revealed a significant groups difference ($F=73.43$, $df=2/38$, $p<0.001$) with the 5-HT and CON groups demonstrating positive savings scores, and the CIN group negative savings. The Fisher LSD test produced a critical difference value of 2.45 sessions. Comparisons of the mean savings scores showed a significant impairment in reversal learning of the rats in group CIN when compared with rats from either the CON or 5-HT groups. Although the 5-HT group exhibited a greater savings score than did the CON animals, the difference did not approach statistical significance. These data are presented in Fig. 4.

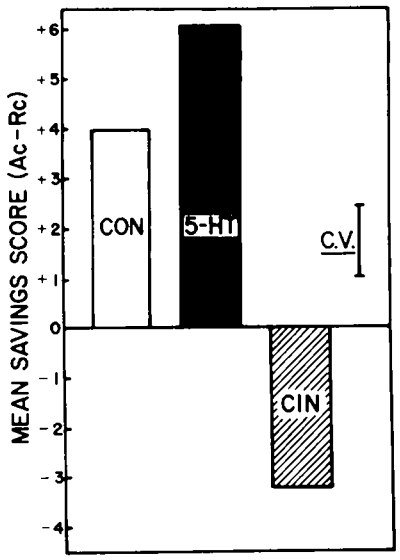

FIG. 4. Mean savings scores (sessions to acquisition criterion minus Sessions to reversal criterion) of control (CON), serotonin 5-HT), and cinnanserin (CIN) rats on a T-maze position-habit reversal task. CV. = critical difference value of Fisher's LSD test (alpha = 0.05).

Discussion

In terms of the overall analysis of these data, it may be concluded that the stimulation of AMS serotonin receptor sites facilitates the learning of a T-maze position-habit reversal task, whereas the inhibition of these tryptaminoceptive sites with cinnanserin results in a marked impairment of performance on this task. These findings are of particular interest in that the impairment in the learning of this task is similar to the deficit produced by lesions of the AMS [20]. Whether these results are due to a loss of response inhibition to the nonreward side in reversal learning [27] or are more directly related to alterations in the general reactivity to the stimuli presented by the non-reward side [25, 26] cannot be ascertained by the present data. However, in terms of understanding the potential function(s) of the AMS serotonin circuit, it is interesting that such impairment in the learning of this task can be attained by AMS serotonin blockade, but (at least in the cat), not by cholinergic septal blockade [21]. Possible species differences and differences in the apparatus used by Hamilton, *et al.* [21] for their study of the effects of cholinergic septal blockade in the cat on position-habit reversal, makes it desirable for research to be performed in which the rat AMS is treated with anticholinergic drugs under the same conditions as utilized in the present experiment. However, in terms of the data presented in Experiment 1, it is at least plausible to consider the AMS serotonin system as being of importance in facilitating the organisms reactivity to rewarding stimuli, perhaps as a consequence of increasing the degree of response inhibition to nonrewarding stimuli which are present at the same time. Certainly the lowered frequency of responding to the presentation of light in the former experiment is consonant with such a viewpoint.

EXPERIMENT 3: EFFECTS OF SEROTONIN AND CINNANSERIN ON SHOCK THRESHOLDS

Animals

A group of 35 rats with AMS cannulae selected from those employed in Experiment 1 and weighed between 200 and 250 g at the time of testing. The rats were divided into three treatment groups. A group of operated control animals (CON) consisted of 13 animals, eight of which were assigned to this group due to the fact that their inner cannulae had become blocked with solidified tissue fluids at

the time of testing and therefore could not be used for AMS drug administration. The remaining 22 rats were equally divided into two groups, one of which received 40 μg of serotonin (5-HT). For the CIN and 5-HT rats, the drugs were administered 30 min prior to the testing session.

Apparatus

The apparatus consisted of a square shock-box with adjacent walls constructed of Plexiglas and aluminum. Each wall was 66.04 cm high X 50.80 cm long, thereby providing a floor area of 1016 cm. The floor was composed of stainless steel rods, each 0.032 cm in dia. and spaced 0.127 cm apart. A Grayson-Stadler Shock Generator (Model E-1064-G5) served as a variable shock source.

Procedures

At the time of testing each rat was placed in the shock box and allowed 5 min to explore the chamber. Four series of alternating ascending and descending shock intensities were then administered according to the method of limits technique [18]. The first series for all rats was of ascending shock intensities. The values of shock used were 0.00, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 1.00, 1.30, and 1.60 mA, with each shock in a series being given for a 1 sec duration. The time between each shock in a series was 30 sec, and the time between each of the four series was 2 min.

The recording of shock thresholds followed the basic procedure and definitions of Evans [11]. A flinch response was recorded whenever the rat showed a crouching or startle type of behavior following shock onset. For a flinch response the rat's paws did not leave the grid floor and the response was seldom accompanied by signs of agitation (e.g. vocalization, defecation, urination). A jump response was recorded whenever shock onset resulted in the rat removing 2 or more paws from the grid floor. It was generally accompanied by vocalization and, at higher shock intensities, running around the chamber floor, attempts to jump out of the shock box, and defecation and urination were common. Ascending series were terminated when the rat made jump responses for two consecutive shock values. Descending series never began at higher shock levels than the values obtained for jump responses on the preceding ascending series. Flinch and jump threshold values were determined by averaging the values for the four series for each rat.

Results

Due to the unequal sample sizes an unweighted means ANOVA was used for data analysis, followed by application of the Fisher LSD test when the overall ANOVA proved statistically significant. The ANOVA for the flinch threshold data indicated a highly significant difference between the groups ($F=186.65$, $df=2/32$, $p<0.001$). The Fisher LSD test generated a critical difference value of 0.032. Comparison of the group means showed the CIN group to be significantly lower and the 5-HT group to be significantly higher, than the CON animals in flinch shock thresholds. The ANOVA for the jump threshold data also revealed highly significant group differences ($F=210.11$, $df=2/32$, $p<0.001$). Application of the LSD critical value of 0.063 to the group means showed that the jump threshold for the CIN group to be significantly lower and the 5-HT group to be significantly higher than the CON group. The mean flinch and jump shock thresholds for the groups are presented in FIg. 5.

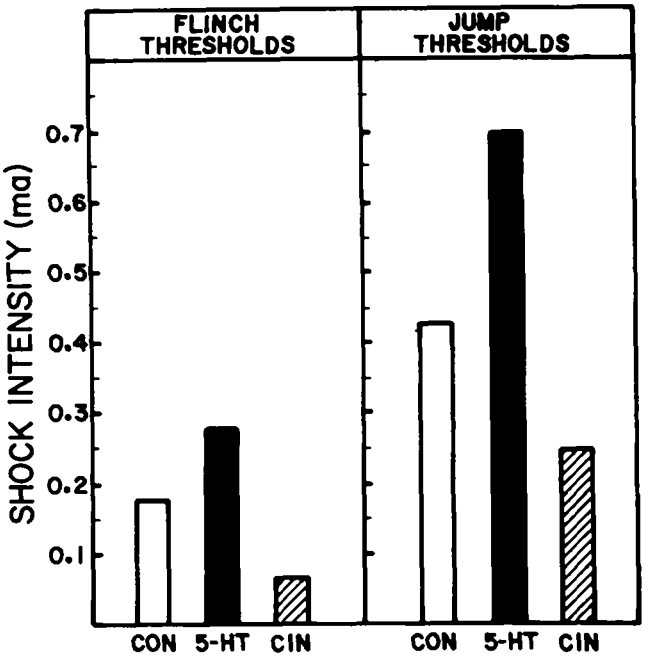

FIG. 5. Bar graphs showing mean flinch and jump shock thresholds as a function of serotonin (5-HT) or cinnanserin (CIN) septal applications as compared with animals receiving no drugs (CON). Critical difference values for the Fisher LSD test were 0.032 for flinch thresholds and 0.063 for jump thresholds (alpha = 0.05).

Discussion

The application of serotonin to the AMS produced a marked elevation of both flinch and jump shock thresholds, whereas the blockade of the AMS tryptaminoceptive sites profoundly lowered both thresholds. If the flinch threshold can be considered to represent the first or absolute perception of pain, and the jump threshold to reflect the emotionally influenced reaction of the organism to painful stimuli [11], then it would appear that one of the possible functions of 5-HT at the level of the AMS is to desensitize the animal both to the perception of, and the emotional reactivity to, noxious stimulation. These data are consistent with the general findings of Tenen [37] who reported that after large systemic doses of the serotonin depletor, p-CPA, rats exhibited significant reductions in both flinch and jump shock thresholds as measured by the Evans [11] technique. Tenen (personal communication in [23]) also reported that p-CPA markedly antagonizes the analgesic effects of morphine and related analgesics in rats, as measured by the Evans technique. The importance of the AMS serotonin system in alterations of shock thresholds was studied by Lints and Harvey [25] who demonstrated that lesions of the MFB, septal area, or dorsomedial

tegmentum increased sensitivity to footshock in rats as measured by the Evans technique, and decreased brain concentrations of serotonin. It will be recalled that Fuxe [15] emphasized the importance of the rostral raphe nuclear groups in the dorsomedial tegmentum as a primary source of cell bodies for the ascending serotonin MFB circuit to the AMS and other limbic forebrain regions. The data of Lints and Harvey [25] suggest that this pathway from the dorsomedial tegmentum to the AMS, via the MFB, serves an important function in regulating the reactivity of the organism to painful stimuli. In another study, Lints and Harvey [26] showed that the increased sensitivity to painful stimuli induced by lesions of this circuit could be reversed by administration of the serotonin precursor, 5-hydroxytrytpophano.

In conjunction with the results of the present experiment, these data provide strong evidence for implicating the MFB serotonin efferents to the AMS as a part of a system which functions in the reduction of reactivity of an organism to painful stimuli. Further support for the involvement of an AMS-hippocampal inhibitory system in this capacity was the demonstration of Soulairac, Gottesmann, and Charpentier [34] that one of the effects of morphine-type analgesics is to inhibit the hippocampal EEG desynchronization which typically accompanies the presentation of a strong aversive stimulus. The data of Tenen, as reported by Koe and Weissman [23] would indicate that this effect of morphine analgesics on hippocampal activity is mediated by their effects on central serotonin stores.

GENERAL DISCUSSION

The results of the three experiments would indicate that the ascending serotonergic system which terminates in part in the AMS region of the septum, functions basically as a modulator of activity in the AMS-hippocampal inhibitory system. Under mild levels of stimulation involving nonbiologically significant stimuli, 5-HT appears to facilitate the processes of habituation. The dramatic disinhibitory effects of serotonergic blockade by cinnanserin in the light tube under the experimental condition in Experiment 1, indicates that unless response-contingent feedback is relatively high, the serotonin system maintains a tonic facilitory influence on the AMS-hippocampal system. That this tonic modulatory function is necessary for adequate behavioral responding in appetitive situations was demonstrated by the difficulties exhibited in learning the reversal of the T-maze position habit following CIN application. The increased sensitivity to shock exhibited by animals treated with CIN also demonstrated the importance of septal serotonin in attenuating the degree of emotional reactivity to aversive stimuli. These data suggest that the serotonin efferent fibers to the septum function in attenuating the responsivity of the organism to all types of incoming stimuli. This action could be effected by facilitation of the AMS-hippocampal inhibitory system, possibly through direct modulation of the spontaneous firing rates of cholinergic septal cells, although several other mechanisms are equally tenable.

Garattini and Valzelli [16] and Welsh [39] have described a remarkable uniformity in the pattern of distribution of serotonergic fibers throughout all classes of vertebrates. The highest concentrations of serotonin appear in the more primitive sensory motor integrating systems of the neuraxis, and the major pathways of 5-HT from the raphe system of the reticular formation are primarily to the phylogenetically older portions of the central nervous system.

Dahlstrom and Fuxe [5, 6] have shown histochemically that the 5-HT containing axons are probably unmyelinated and of the diameters of peripheral C fibers, which are known to have slow conduction velocities. The combination of a range of slow conduction velocities (estimated at between 0.7 and 2.5 meters/sec) and the relatively long distances these fibers must travel from their brainstem sites of origin to limbic sites of termination, would result in a temporal dispersion of a nerve volley conducted over a distance when the brainstem raphe system is stimulated. The arrival of the nerve impluse at regions such as the AMS would therefore be somewhat dispersed in time. Furthermore, Weight and Salmoiraghi [38] have demonstrated that the onset and duration of neuronal responses to electrophoretically administered 5-HT are considerably slower than the responses to acetylcholine. Thus, synaptic potentials induced on AMS neurons by the release of serotonin would be expected to be slower in onset and of longer duration than is the case for acetylcholine. The combination of the temporal dispersion of the nerve volleys from brain stem 5-HT neurons and their slow development of synaptic potentials of long duration provides a basis for a tonic effect of the ascending serotonergic system upon neurons in the limbic system. All of these points are consistent with the hypothesis that the ascending serotonergic portion of the medial forebrain bundle which projects to the AMS may provide a tonic facilitation of septohippocampal inhibitory circuits.

It should be noted that our usage of the terms stimulation and blockade refer to the presumed action of serotonin and cinnanserin upon serotonergic receptor sites. The neurophysiological effect of serotonin appears to be inhibitory in most instances. Cinnanserin would be expected to block this effect.

Finally, a word of caution is in order regarding the specificity of the effects. Because of the lack of previous experimentation in this area, we chose to examine a wide variety of behaviors rather than providing detailed anatomical and dose-response relationships for a single behavior. However, the high degree of consistency between our present results and the results of previous anatomical and neurochemical studies strongly suggests that our effects are primarily attributable to the stimulation or blockade of serotonergic receptors within the septum.

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