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Opposite Effects of Intraventricular Serotonin and Bufotenin on Rat Startle Responses

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GEYER, M. A., J. D. WARBRITTON, D. B. MENKES, J. A. ZOOK AND A. J. MANDELL. *Opposite effects of intraventricular serotonin and bufotenin on rat startle responses.* PHARMAC. BIOCHEM. BEHAV. 3(4) 687–691, 1975. – Rat startle responses to air puffs were monitored in a stabilimeter during the intraventricular infusion of various doses of 5-hydroxytryptamine (5–HT) or the putative hallucinogenic congener of 5–HT, 5-hydroxy-N, N-dimethyltryptamine (5-HDMT) or saline. Qualitatively opposite effects were observed, with 5-HT producing a dose-dependent decrease in responsivity and 5-HDMT increasing the magnitude of startle responses. No specific effects of either compound could be demonstrated on the presumably separable processes of sensitization and habituation. The results are discussed in the context of a central serotonergic system which facilitates behavioral inhibition and which is antagonized by indoleamine hallucinogens.

Serotonin 5-Hydroxytryptamine Startle responses Habituation Bufotenin Hallucinogens Intraventricular infusions Response inhibition

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ATTENTION to the functional roles of brain 5-hydroxytryptamine (5-HT) has begun to focus on the involvement of serotonergic neuronal systems in behavioral reactivity. Manipulations of central 5-HT neurons such as the following have consistently produced alterations in the responsivity of animals to sensory stimuli: synthesis inhibition by parachlorophenylalanine (PCPA) [4, 5, 6]; gross lesions of the serotonergic cell bodies [8,20]; nerve terminal destruction by 5,6- or 5,7-dihydroxytryptamine [2,11]; electrical stimulation of the raphe nuclei [24]. Furthermore, it has been suggested often that indoleamine hallucinogens act in part by interfering with normal serotonergic functioning [1, 3, 22]. That these agents have such profound effects upon sensory experience gives impetus to the hypothesis that some brain serotonergic systems serve to dampen or inhibit behavioral responsivity to the perceptual environment.

The rat startle response is a useful behavioral dependent variable with which to assess such phenomena as reactivity, sensitization, and habituation [8]. With it, we have now confirmed the hypothesis that behavioral responsivity, as reflected in the rat startle response to air puff, is reduced by the direct administration of 5-HT into the brain. To circumvent the blood-brain barrier for 5-HT and to minimize the complications attendant to rapid intracranial injections or immobilization, we used the intraventricular infusion method in unrestrained rats to elevate brain 5-HT levels gradually. We also compared the effects of infused 5-HT with the effect of infused 5-hydroxy-N,N-dimethyltryptamine (5-HDMT, bufotenin), the dimethylated hallucinogenic congener of serotonin [13,26] and found that bufotenin infusions increase the magnitude of rat startle response.

EXPERIMENT 1

Method

Animals. Twenty male Sprague-Dawley rats (Carworth, 320 to 400 g) were housed individually in a temperature-regulated animal room on a 12 hr/12 hr light/dark cycle. Food and water were available ad lib.

Apparatus. The stabilimeter (cf. Connor *et al.* [6]) consisted of a Plexiglas animal chamber (21.5 X 8 X 9 cm) spring-mounted on counterbalanced arms (50 cm each) inside a sound-proofed, ventilated box (72 X 78 X 48 cm). The Plexiglas chamber had a wire mesh floor, and four plastic rods (3/16 in.) spaced lengthwise across the top allowed for infusion tubing and administration of the air puff stimuli from above. Weights were added to the chamber to compensate for each rat's daily weight. Movements of the animal chamber were detected by a spring-mounted phosbronze contact on the distal end of the cantilevered arm which slid against a linear resistor (5 K ohm). The potentiometric signals generated by deflections of the chamber were amplified and rectified through a capacitance circuit before being displayed on a 10-in. strip-chart recorder (Beckman). The stabilimeter was calibrated with a 500 g weight. Stan-

dardization involved knocking various weights off the top of the chamber and monitoring the recorded deflections. Consistent linearity was achieved, and standard calibrations were made each day to preclude instrumental variation.

Air puffs were delivered simultaneously through two vertical tubes (1/4 in.) 3 cm above the top of the chamber. Air pressure was regulated (20 lb/sq. in.) and pulsed (300 msec) through a timer-activated solenoid valve. Air puffs were used instead of auditory stimuli because of the potential variability induced by ear damage during stereotaxic surgery.

Procedures. One week after the rats arrived, stainless steel cannulae were stereotaxically implanted in their right lateral ventricles under sodium pentobarbital anesthesia (Nembutal®, 40 mg/kg IP). The cannulae were mounted with skull screws and dental cement (DeGroot: AP + 5.4, V + 8.4, L −1.6). Details of surgery and the infusion apparatus and procedure are described elsewhere [14].

Ten days later the animals were assigned to two groups that were matched for body weight. Each group was tested every other day for a total of 8 sessions, 3 sham sessions followed by alternating infusion and sham sessions. An animal was weighed every time and infusion tubing was attached to his cannula before he was placed in the stabilimeter. The infusion pump was started after the outer door was closed. A session consisted of a 20 min infusion or sham period followed by 40 air puff stimuli during which the infusion was continued at a constant rate of 20 μl/hr. Sham sessions were identical to infusion sessions except that no infusion was given, the tubing being connected to the cannula, but without a needle. A 15-sec inter-stimulus interval was used to maximize intrasession habituation and to minimize habituation over days [7].

The mean startle response magnitude for the last two of the three baseline sham sessions was designated the mean baseline response, against which all subsequent experimental responses were normalized. Normalization was advisable because variability between animals is typically substantial relative to variability within each subject's behavior ([8], Geyer, unpublished observations]. Accordingly, three dependent variables, normalized for each animal, were defined as follows:

$$\text{NIR} = \frac{\text{Infusion Response Magnitude}}{\text{Mean Baseline Response}}$$

$$\text{NSR} = \frac{\text{Sham Response Magnitude}}{\text{Mean Baseline Response}}$$

$$\text{NRDIF} = \text{NIR-NSR} = \frac{\text{Infusion Response Magn. - Sham Resp. Magn.}}{\text{Mean Baseline Response}}$$

Note that each of these varies with Days (3, each derived from one sham session and one infusion session) and Trials within each session (8 blocks of 5 stimuli each).

After the first three sham sessions the animals were divided into 5 groups which were approximately matched with respect to startle response magnitude and body weight. Each animal was infused for 30 min at 20 μl/hr with either 0.9 percent saline or 1.0, 2.5, 6.25, or 15.625 μg/μl 5-HT (expressed as the free base; serotonin creatinine sulfate, Sigma) dissolved in 0.9 percent saline and adjusted to pH 6.5 with 0.5 N NaOH. Each animal received the same treatment for all three infusion sessions, each being preceded by a sham session. After the last infusion the animals were infused with 10 μl of Evans Blue dye and sacrificed for histological confirmation of cannula placement and ventricular flow. Since all animals showed consistent cannula placements with dye perfusing the lateral ventricles, no animals were excluded.

Results

Intraventricular infusions of 5-HT in the dose range from 1.0 to 15.625 μg/μl significantly reduced the startle response. The overall analysis consisted of a three-way mixed analysis of variance (ANOVA) with three orthogonal factors: Pharmacology; Days (3); and Trials (8 blocks of 5). As defined above, the dependent variables of interest are the normalized infusion response (NIR), the normalized sham response (NSR), and the normalized response difference (NRDIF).

Both NIR and NSR varied significantly with Trials, showing a substantial decrease in response magnitude over successive trials (NIR: $F(7,105) = p<0.001$; NSR: $F(7,105)$, $p<0.001$; Fig. 1). To test for the extent of linear decay, we defined a new variable, Trial Trend, to equal

$$7X_1 + 5X_2 + 3X_3 + X_4 - X_5 - 3X_6 - 5X_7 - 7X_8,$$

where for each animal on each day X_i is the mean NIR value for the ith Trial block. Trial Trend was analyzed in a two-way mixed ANOVA and was found statistically invariant with respect to Pharmacology, Days, and their interaction, which showed that the rate of linear decay was not significantly controlled by either of those factors. However, the mean of Trial Trend, which should be zero under the null hypothesis of zero linear decay, was 3.37 for NIR (z score = 5.02, $p \ll 0.001$), which showed that a reliable decay of response magnitude occurs in a linear fashion over successive trials.

The effect of serotonin infusions was assessed by a specific comparison of the saline controls with all four doses by use of the variable NRDIF. This comparison showed that 5-HT significantly reduced responsivity relative to saline controls, $F(1,15) = 5.67$, $p<0.05$. Serotonin did not significantly affect NSR or NIR, nor did it interact with either Days or Trials, which suggests little effect upon habituation per se and no carry-over between sessions.

For assessment of the dose-dependency of the response reduction produced by 5-HT, a regression of NRDIF was performed on the normalized logarithms of 5-HT dose. The utilization of log effective dose is common [15]; the effective dose is defined here to be (D + S)/S, where D is the μg of 5-HT infused in the 20-min pre-responding period, and S is the estimated μg of unbound 5-HT in the uninfused rat brain. The value of S (0.375 μg) was calculated from the mean of several estimates of whole brain unbound 5-HT cited by Erspamer [12], and is not critical to the validity of the regression analysis. The mean NRDIF value for each animal (mean of 3 Day blocks) was negatively correlated with $\log_e [(D + S)/S]$ ($r = -0.535$, $N = 20$; $p<0.02$). Figure 2 illustrates the group means and the regression line calculated to best predict NRDIF, $F(1,18) = 7.02$, $p<0.02$. These results indicate a statistically reliable, dose-dependent effect of infused 5-HT on the startle response in rats.

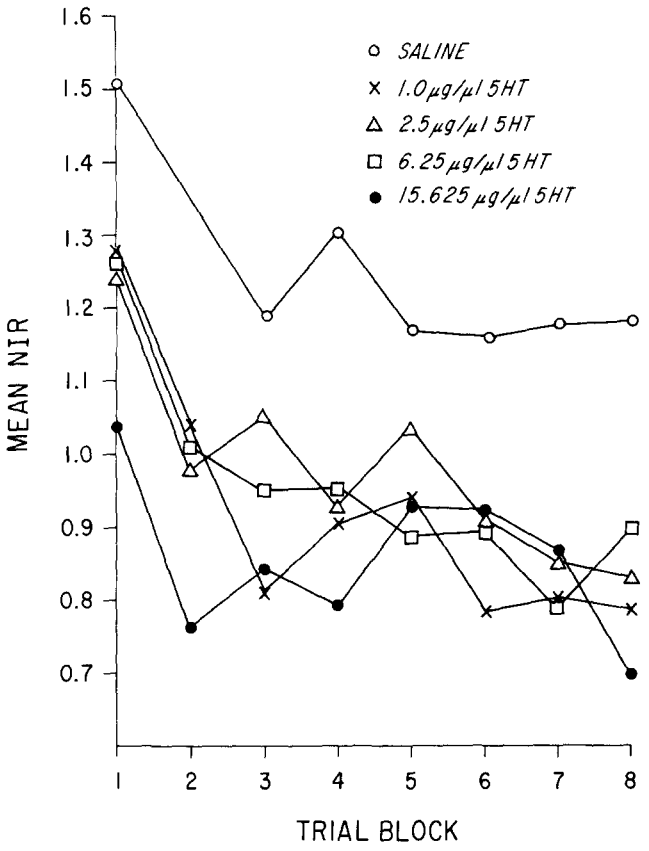

FIG. 1. Plot of normalized infusion response (NIR) versus trial block for each of the five 5-HT dose levels. Each point represents the mean of 12 trial block responses to the air puff stimulus (4 animals X 3 Days). Illustrated here are the effects of trial block, $F(7,105) = 21.51$, $p<0.001$, and trial trend, a variable designed to assess the extent of linear decay of NIR with trial block ($z = 5.02$, $p\ll0.001$).

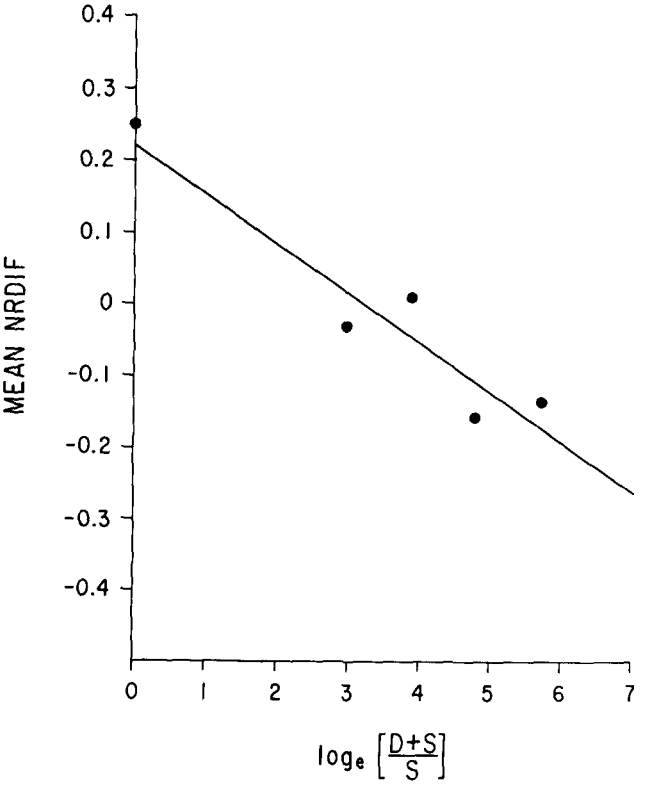

FIG. 2. Plot of normalized response difference (NRDIF) versus logarithm effective dose of 5-HT. Each point represents the mean of four animals' average NRDIF scores (see Results, Experiment 1). Also plotted is the least-squares regression line, NRDIF = $0.228 - 0.07 \log_e [D+S/S]$, which was found to predict NRDIF reliably, $F(1,18) = 7.20$, $p<0.02$. The Pearson product-moment correlation for the two variables was -0.535 ($p<0.02$).

The first response on each experimental day was analyzed and found to be invariant with respect to Pharmacology, Days, and their interaction. An index of sensitization as distinct from habituation was obtained by subtracting the first response from the mean of responses 2 through 10, defining a new variable, 5-HTDELTA (which was normalized with respect to each animal's baseline mean). This variable was also invariant with respect to Pharmacology, Days, and their interaction, which suggested no reliable change in sensitization due to infused 5-HT.

EXPERIMENT 2

Method

Fourteen rats (280 to 350 g) similar to those used in Experiment 1 were cannulated as described. All animals were tested in the stabilimeter with a simplified paradigm which consisted of a single sham session to establish a baseline from which NSR values were calculated, followed by a single infusion session. The animals were divided into two equal groups that were matched with respect to body weight and baseline startle amplitude. Two days later each rat was infused once with 0.9 percent saline or 1 μg/μl 5-HDMT (5-hydroxy-N,N-dimethyltryptamine, Aldrich) dissolved in 0.9 percent saline and adjusted to pH 6.5.

Results

As described in METHOD, the design consisted of two orthogonal factors: Pharmacology (5-HDMT versus Saline, between animals) and Trials (8 blocks of 5 trials each, within animals). The variables, as in Experiment 1 above, were NSR, NIR, and NRDIF, which were analyzed in a two-way mixed ANOVA. Both NSR and NIR were significantly influenced by Trials, showing linear decay similar to that observed in Experiment I (NSR: $F(7,84) = 4.32$, $p<0.01$; NIR: $F(7,84) = 6.66$, $p<0.01$). Intraventricular infusions of 5-HDMT significantly affected both NIR, $F(1,12) = 5.34$, $p<0.05$, and NRDIF $F(1,12) = 5.49$, $p<0.05$. Figure 3 illustrates this effect, which indicates that 5-HDMT increases the magnitude of startle responses in rats relative to saline-infused controls. Again, both the first response and the difference between the mean of responses 2 through 10 and first response (5-HDMTDELTA) were analyzed with respect to Pharmacology, with the baseline normalized as before; both were found to be insignificant.

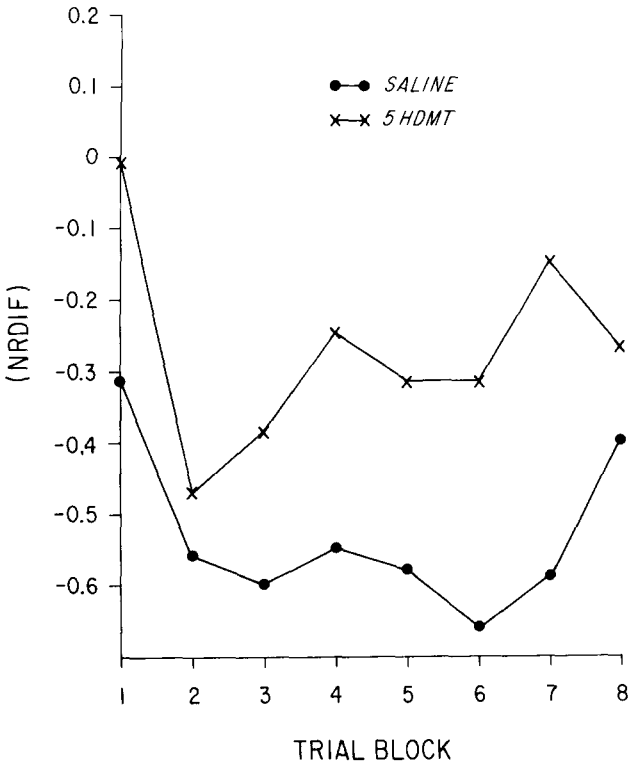

FIG. 3. Plot of normalized response difference (NRDIF) versus trial block for both the experimental (5-HDMT) and control (Saline) infusion groups in Experiment 2. Illustrated here is the significant elevation of responsivity produced by 5-HDMT infusions, $F(1,12) = 5.59$, $p<0.05$. Each point represents the mean response for 7 animals for each trial block.

DISCUSSION

The results of Experiment 1 demonstrate that the central administration of 5-HT reduces the responsivity of rats to startle stimuli in a dose-dependent fashion. No specific effects of 5-HT on the presumably separate processes of sensitization and habituation [18] could be demonstrated statistically. The work of Davis and Sheard [8] has suggested that serotonergic systems influence sensitization rather than habituation, while the opposite conclusion was reached by both Carlton and Advokat [5] and Connor *et al.* [6] from experiments in which PCPA was used. Our findings are consistent with either of these possibilities as well as the many other similar observations of heightened reactivity or sensory responsivity after reductions in brain 5-HT [3, 4, 11, 20, 21].

In some reports large increases in brain 5-HT after precursor loading with tryptophan or 5-hydroxytryptophan and/or inhibition of monoamine oxidase have resulted in hyperactivity and hyper-reactivity in rats [16]. Conversely, our finding of a 5-HT dose-dependent decrease in responsivity would suggest the hypothesis that a functional increase in brain 5-HT leads to an inhibition of responses to sensory stimuli. This result is compatible with Persip and Hamilton's report [23] that depositions of 5-HT into the septal nuclei of rats increased habituation of exploratory activity, facilitated acquisition of the reversal of a T-maze position habit, and elevated both flinch and jump shock thresholds. All these effects were interpreted as reflecting an increase in behavioral inhibition. Furthermore, the implantation of the 5-HT antagonist cinnanserin produced qualitatively opposite results. Persip and Hamilton's work suggests that our intraventricular infusions of 5-HT may have their effect on startle responding by activating a septal-hippocampal serotonergic system. Both of these regions are adjacent to the lateral ventricle and show increased 5-HT after intraventricular infusions (Geyer, unpublished histofluorescence observations). These structures and related parts of the limbic system have also been implicated in a variety of inhibitory functions which are known to be modifiable by cholinergic manipulations [17]. Further work incorporating histofluorescence confirmation of the distribution of regionally infused 5-HT may help clarify the relevance of the septal nuclei and hippocampus to the processes underlying behavioral inhibition. The current method of intraventricular infusions may find more widespread utility in dose-response and drug-interaction studies of serotonergic functioning. Further tests of startle behavior after infusions of other neurotransmitters are necessary to confirm the selectivity of this effect.

The intraventricular infusion of bufotenin markedly increased startle responses throughout the test session (Fig. 3). Although limited by the blood-brain barrier, bufotenin has been suggested to be a potent hallucinogen which may be formed endogenously [13,26]. The better known indoleamine hallucinogen, N,N-dimethyltryptamine (DMT) is readily absorbed into the brain and quite active in man [26]. In rats, DMT, like lysergic acid diethylamide (LSD) [9], has been shown to increase startle responses at low dose levels, although reduced responsivity was observed after higher doses [10].

Our results with bufotenin are similar to the effects of low doses of DMT or LSD and may therefore reflect a decrease in the firing rate of the serotonergic raphe cells, which are more sensitive to these drugs than are the cells post-synaptic to the raphe neurons [9, 10, 19]. Furthermore, low doses of LSD have little effect on startle responding in animals with raphe lesions [9]. Serotonin, on the other hand, is relatively less potent as an inhibitor of raphe cells and may well depress responsivity by directly inhibiting the cells post-synaptic to the raphe neurons [19]. Thus, although 5-HT and indoleamine hallucinogens have qualitatively similar effects when administered iontophoretically to post-synaptic neurons, our findings together with the work of Davis and Sheard [9,19] and Haigler and Aghajanian [19] suggest a functional antagonism between 5-HT and 5-HDMT. Additional evidence for an antagonistic relationship between serotonin and such hallucinogens as 5-HDMT can be derived from behavioral studies in immature chicks, which lack an effective blood-brain barrier [25], as well as from various reports of reduced turnover rates for brain 5-HT after the administration of hallucinogens (see [3]). Therefore, one would predict that 5-HT should reduce responsivity when infused directly into forebrain limbic structures such as the hippocampus (cf. Persip and Hamilton [23]), whereas 5-HDMT should increase startle responses when administered directly to the raphe neurons but decrease responding when infused into limbic structures. Similarly, 5-HDMT, like LSD, should not increase responsivity in raphe lesioned rats.

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