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Behavioral Assessment of Norepinephrine and Serotonin Function and Interaction in the Hippocampal Formation

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GAGE, F. H. AND J. E. SPRINGER. *Behavioral assessment of norepinephrine and serotonin function and interaction in the hippocampal formation.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 815–821, 1981.—Norepinephrine (NE) and serotonin (5-HT) were injected either into the dorsal or ventral hippocampal formation of rats in doses ranging from 0.005 μg/μl to 5.0 μg/μl. Behavioral reactivity was assessed by recording latency to paw lick when placed on a hot plate and magnitude of force displaced in a vertical direction to a footshock. In addition open field activity was measured. NE injections resulted in a dose-dependent increase in behavioral reactivity to the hot plate and footshock; 5-HT injections resulted in a dose-dependent decrease in behavioral reactivity to hot plate and footshock. Both NE and 5-HT injections resulted in a dose-dependent increase in open field activity. NE injections were more effective in increasing reactivity when injected into the dorsal hippocampus while 5-HT injections were more effective in decreasing behavioral reactivity when injected into the ventral hippocampus. Both NE and 5-HT were most effective in increasing open field behavior, however, when injected into the dorsal hippocampus. When NE and 5-HT were injected simultaneously they resulted in no change in behavioral reactivity as compared to saline injections. Simultaneous injections of NE and 5-HT neither enhanced nor antagonized the increase in open field activity of each amine injected alone. The results are discussed in terms of the functional significance of NE and 5-HT in the hippocampus, their modes of action and significance for understanding dorsal-ventral hippocampal differences.

Hippocampal formation Norepinephrine Serotonin Behavioral reactivity

EVIDENCE from animal experiments and clinical investigations has suggested than an antagonistic relationship exists between norepinephrine (NE) and serotonin (5-HT) [5, 22, 23]. Many of the experiments which have suggested the antagonistic relationship were aimed at elucidating the neurochemical substrates of arousal and emotional behavior. The hippocampal formation has been suggested to play a central role in integrating internal and external stimuli associated with arousal and emotional behavior [17].

On the basis of converging autoradiographic, histofluorescent, microiontophoretic and biochemical assay techniques [19, 24, 29], NE and 5-HT have been implicated as putative neurotransmitters within the hippocampal formation. This monoaminergic innervation appears to arise entirely from neurons whose cell bodies originate in the dorsal and medial raphe and locus coeruleus nuclei. Axons leaving these pontine nuclear complexes ascend in the tegmental tracts to join the medial forebrain bundle which then projects to the rostral septum. From the septum, both serotonergic and noradrenergic axons have been visualized to turn dorsally and caudally to enter the hippocampus via the fornix/fimbrial and cingulum bundle pathways [8, 16, 19]. Both NE and 5-HT neurons innervating the hippocampal formation have a very distinct and overlapping distribution. Specifically, both amines have their densest innervation along the ventral border of the dentate granule cells which extends the entire length of the area dentata [19]. This overlap aids in establishing this area as a unique site to investigate NE and 5-HT interaction.

The hippocampal formation, however, is not a homogeneous structure. Among the major anatomical demarcations in the hippocampal formation (HF) are the hippocampus proper, the dentate gyrus, the subicular area and the fimbria-fornix. Investigations of the HF have also revealed a functional organization along the anterior-dorsal and posterior-ventral axis extending from the septal pole of the HF to the entorhinal cortex. In this context the differences along the anterior-dorsal and posterior-ventral axis appear more as differences along a continuum rather than distinct localized differences. This continuum will be referred to as the dorsal-ventral axis throughout this paper. Behavioral [14, 15, 26], anatomical [18, 25, 27] and electrophysiological [4,6] studies all substantiate differentiation along the dorsal-ventral axis. This difference is further supported by neurochemical data, indicating clear differences in concentration [11], kinetic constants [10], and patterns of

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innervation or norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation [20].

The present study was designed to answer the following questions: (1) Do intrahippocampal injections of NE and/or 5-HT affect behavioral responses to external stimuli associated with arousal and emotional behaviors? (2) Do 5-HT and NE change behavioral responses in the same direction? (3) Do NE and 5-HT act antagonistically? (4) Do NE and 5-HT exhibit the same behavioral effect when injected in the dorsal and ventral hippocampal formation?

METHOD

Subjects

Twenty-four Sprague Dawley rats weighing 250–300 g were housed in individual cages with access to ample food and water.

Surgical Procedures

Rats were injected with 0.2 cc/kg of atropine sulfate followed by an anesthetic dose (1 cc/kg) of a mixture of ketamine and chloral hydrate. The animal was placed in a stereotaxic instrument and its scalp incised and retracted. Two holes were trephined in the skull in order to implant bilateral cannulae (Plastic Products Company) in either the dorsal or the ventral hippocampus. The coordinates for the cannulae placements were taken from bregma with a five degree elevation of the skull. For dorsal placements the coordinates were: AP—2.6, ML—2.8, DV—2.8, and for the ventral placements: AP—5.0, ML—5.0, DV—6.2 [21].

Behavioral Apparatus

Behavioral reactivity was measured by three methods. One was the latency for a rat to lick its hind paw when placed on a Technilab hotplate apparatus. The surface of the hot plate was kept at 53 degrees centigrade during testing. The rat was removed from the apparatus when either a hind paw lick was observed or a 60 sec time limit had elapsed.

The second measure of behavioral reactivity was response to footshock and was measured in an apparatus described elsewhere [9]. Briefly, it consists of a balanced testing box with a Grass FT 10 force transducer and a Grass model 5 polygraph. The testing box and the transducer are housed in a sound attenuated room. The floor and walls of the box are wired for shock with a Grason-Stadler 700 shock generator. The testing box is supported by a steel frame which is on top of the transducer. The transducer produces a voltage output proportional to the downward deflection of the testing box. The pen on the polygraph records the amount of downward force that is exerted by the rat. This pen deflection is then converted into a ratio equal to the animal's weight-induced deflection plus response of the animal in the box divided by the deflection created by the animal's weight.

The third measure was taken from an open field arena to assess general ambulatory activity. The dimensions of the arena were 117 cm×117 cm×50 cm with 25 squares, 23.5×23.5 cm, marked off on the floor in white tape. Data recorded from the open field were the number of lines crossed in a 2 min period.

General Procedure

Groups were designated by dorsal (DH) or ventral (VH) hippocampal cannula placements and then further divided depending upon whether the subject was to receive NE or 5-HT. The animals received one injection and were tested once a day with a two day interval. Five concentrations of the amines were used: 0.005, 0.05, 0.5, 2.5, and 5.0 μg/μl. The volume injected was 2 μls at a rate of 1 μl/min. The sequence in which the concentrations were administered was randomized with a saline injection between each dose. After these five doses were administered, a final dose of 5.0 μg/μl each of NE and 5-HT combined was injected. The amines were dissolved in a 0.9% saline and 0.02% ascorbic acid solution. Following the injection, a 5 min habituation period was allowed. In the shock chamber the animal received 3 repetitions of 2 shock intensities, 0.4 and 1.0 ma each. The mean response of each shock intensity was used to determine the animal's reactivity. The shock duration was 0.3 sec with a 30 sec intershock interval. In the second test the animal was placed on the hot plate apparatus which was kept at 53°C. When hind paw licking was observed, or 60 sec cut-off time, the animals were removed and the time spent on the hot plate recorded. The third test was ambulatory activity in an open field. The order of the three behavioral tests was randomized to avoid order effects.

Histology

After the final testing day, the animals were anesthetized and perfused with 0.9% saline followed by a 10% Formalin solution. The brains were removed and placed in 10% Formalin for 2 weeks, sectioned at 40 μ, stained with thionin and mounted on slides for reconstruction of the cannula placements.

Several animals were injected with 2 μl of methylene blue 5 min before decapitation to obtain an estimate of the extent of the spread of the amine in vivo. In addition to the spread of the amines, the specificity of the uptake of the injected amines was determined by histofluorometric evaluation of the dentate hilus, using a modification of the method developed by de la Torre [3]. Procedurally, animals were injected unilaterally with 2 μl of 0.5 μg/μl of NE into the HF and 2 μl of saline in the contralateral HF. Photomicrographs were taken of the dentate hilus of the HF to obtain an estimate of the magnitude of NE in this area, of maximal NE terminal density, and the extent to which NE was taken up into terminals.

Statistical Evaluation

Analysis of the behavioral data was performed using a one-way analysis of variance with repeated measures. When appropriate, further evaluation was performed using a Scheffé (post hoc) analysis [30].

RESULTS

One animal from each group receiving 5-HT injections was removed due to respiratory complications or inappropriately placed cannulae, thus leaving the total number of subjects at 5 for both 5-HT groups. Two animals were removed from each group receiving NE injections for the same reasons, bringing the total number of subjects to 4 for both the NE groups.

Inappropriate cannulae placements were defined by the extension of the cannula tract through the hippocampus to penetrate the lateral ventricle ventral or lateral to the hippocampus. In cases where this was observed, there had been

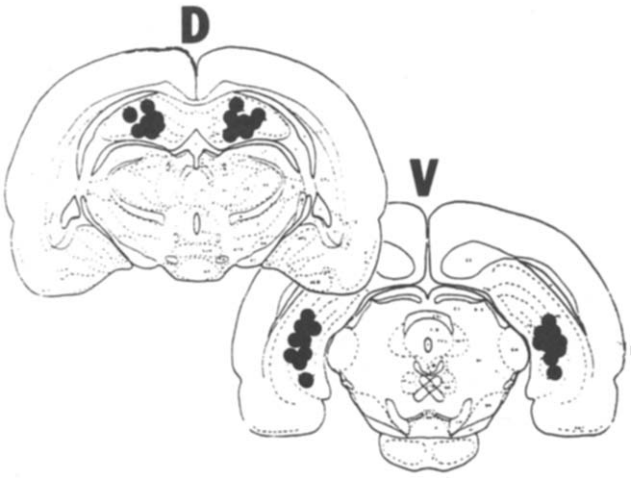

FIG. 1. Schematic representation of cannula tip placements in the dorsal (D) and ventral (V) hippocampus.

no observed change in behavior following injection of the amines or saline. These results provide further evidence that the behavioral changes were due to the direct effect of the amines on the HF, rather than being due to diffusion outside of the HF.

Anatomical Results

Figure 1 is a representation of the bilateral cannula placements. No systematic behavioral differences were observed from the variability of cannulae placements in the HF. The extent of spread was approximately 1 mm in the anterior-posterior dimension, 1.5 mm in the medial-lateral dimension, and 2 mm in the dorsal-ventral dimension.

The placements restricted the spread of the injected solution such that it was contained within either the dorsal or ventral hippocampus.

Histofluorometric evaluation of the dentate hilus of the hippocampal formation after unilateral injections of 2 μl of 0.5 μg/μl NE revealed a substantial increase in fluorescence. All slides were exposed for photomicrography for the same time, 2 min, so that comparisons could be made. The NE injected side revealed not only an overall increase in background luminescence but an increase in terminal density and terminal luminescence in the subgranular layer, an area normally high in NE fluorescence.

Figure 2 contains photomicrographs of dentate hilus following injections of either NE (left) or saline (right).

Behavioral Results

Dose-related differences occurred between the dorsal and ventral hippocampal groups following NE injections such that the DH group had a greater reactivity to noxious stimuli and an increase in ambulatory activity in the open field. Dose-related differences also occurred between the two groups following 5-HT injections. Both 5-HT groups showed a decrease in reactivity to noxious stimuli but the VH group required a smaller dose than the DH group. An increase in open field activity was observed in both groups following 5-HT injections with the DH group increasing activity at a lower dose compared to the VH group.

The behavior of each group was analyzed independently of the other groups. An analysis of variance showed that there were no significant differences between saline days on hot plate scores, (DH-NE; $F(6,21)=2.21$, $p>0.05$; VH-NE; $F(6,21)=2.31$, $p>0.05$; DH-5HT; $F(6,28)=2.14$, $p>0.05$; VH-5HT, $F(6,28)=2.36$, $p>0.05$), on response to footshock, (DH-NE, $F(6,21)=2.44$, $p>0.05$; VH-NE, $F(6,21)=2.19$, $p>0.05$, DH-5HT, $F(6,28)=2.30$, $p>0.05$; VH-5HT, $F(6,28)=2.16$, $p>0.05$), or on ambulatory activity, (DH-NE; $F(6,21)=2.17$, $p>0.05$; VH-NE, $F(6,21)=2.33$, $p>0.05$; DH-5HT, $F(6,28)=2.25$, $p>0.05$; VH-5HT, $F(6,28)=2.19$, $p>0.05$).

With no differences in any group in response to saline injections, the responses were combined across saline days, and this score was used to test behavioral reactivity after each drug injection.

Hot Plate

Following NE injections, the DH group showed a significant decrease in paw lick latency: $F(6,21)=9.69$, $p<0.01$, whereas the VH group showed no change: $F(6,21)=2.31$, $p>0.05$. Figure 3 represents the results of the NE injections of both the DH group and VH group.

Post hoc analysis revealed that a decrease in paw lick latency occurred at the 0.5, 2.5 and 5.0 μg/μl doses.

After injections of serotonin, a significant increase in paw lick latency occurred in both the DH and VH groups: $F(6,28)=6.33$, $p<0.01$ and $F(6,28)=5.34$, $p<0.01$, respectively. Figure 4 shows the paw lick latencies of both groups.

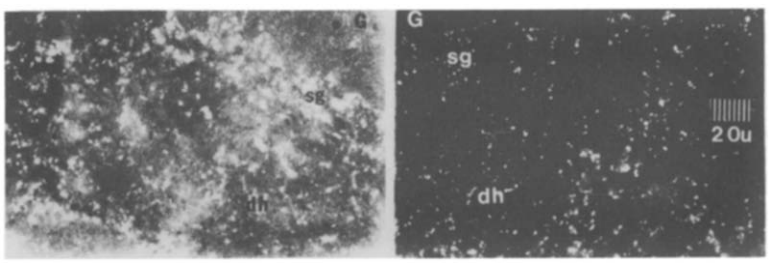

FIG. 2. Photomicrographs of histofluorometrically processed hippocampus of one animal with a unilateal intrahippocampal injection of 2 μl of 0.05 μg/μl NE (left) and 2 μl of Saline (right). G=Granule cell layer, sg=subgrandular, dh=dentate hilus.

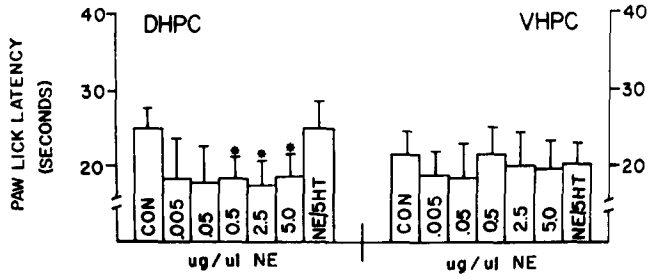

FIG. 3. Paw lick latency of rats receiving injections in dorsal hippocampus (DHPC) or ventral hippocampus (VHPC) with either saline (CON), a range of NE concentrations, or a combined injection of 5 $\mu g/\mu l$ of NE and 5-HT.

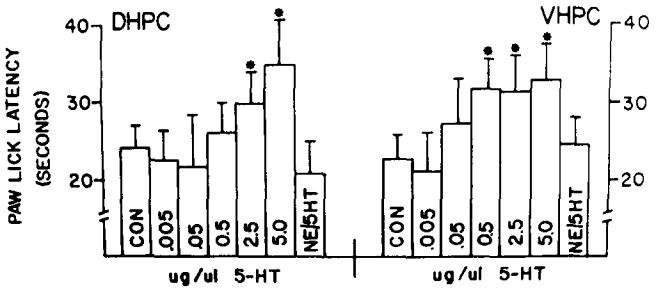

FIG. 4. Paw lick latency of rats receiving injections in dorsal hippocampus (DHPC) and ventral hippocampus (VHPC) with either saline (CON), a range of 5-HT concentrations, or a combined injection of 5 $\mu g/\mu l$ of NE and 5-HT.

Post hoc analysis revealed that an increase in paw lick latency occurred at the 2.5 and 5.0 $\mu g/\mu l$ doses in the DH group, and the 0.5, 2.5, and 5.0 $\mu g/\mu l$ doses in the VH group.

Scheffé tests revealed that following the injection of the combined amines (5.0 $\mu g/\mu l$) of NE and 5-HT, the latency scores were not different from control but were different from the injection of NE or 5-HT alone at the same concentration (see Figs. 3 and 4).

Footshock

Following NE injections there were no differences in reactivity to footshock at the 0.4 mA intensity for the DH group or VH group: $F(6,21)=2.25$, $p>0.05$ and $F(6,21)=2.18$, $p>0.05$, respectively. At the 1.0 mA intensity, there was a significant increase in reactivity to footshock in the DH group, $F(6,21)=3.7$, $p<0.025$, but not in the VH group, $F(6,21)=2.34$, $p>0.05$. Figures 5 and 6 represent the DH group and VH group, respectively, and the reactivity to footshock of each group.

Post hoc analysis demonstrated that only the 2.5 and 5.0 $\mu g/\mu l$ doses of NE were effective in increasing the reactivity to footshock of the DH group.

Following 5-HT injections there were no differences in reactivity to footshock at the 0.4 mA intensity for either the DH group, $F(6,28)=2.15$, $p>0.05$, or the VH group,

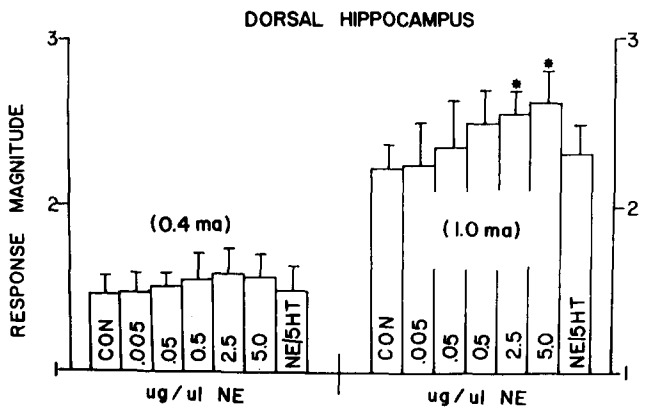

FIG. 5. Response magnitude to footshock of 0.4 ma and 1.0 ma in arbitrary units following injections of saline (CON), a range of NE concentrations, or combined injection of 5 $\mu g/\mu l$ of NE and 5-HT into the dorsal hippocampus.

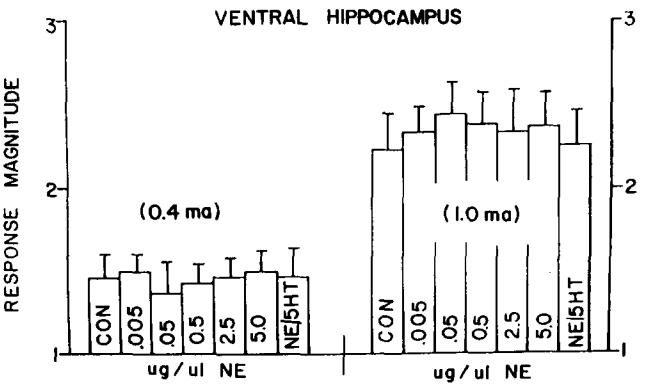

FIG. 6. Response magnitude to footshock of 0.4 ma and 1.0 ma in arbitrary units following injections of saline (CON), a range of NE concentrations, or a combined injection of 5 $\mu g/\mu l$ of NE and 5-HT into the ventral hippocampus.

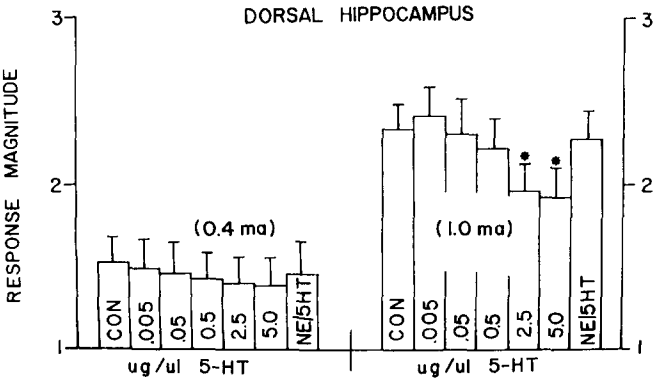

FIG. 7. Response magnitude to footshock of 0.4 ma and 1.0 ma in arbitrary units following injections of saline (CON), a range of 5-HT concentrations, or a combined injection of 5 $\mu g/\mu l$ of NE and 5-HT into the dorsal hippocampus.

TABLE 1

MEAN NUMBER OF LINES CROSSED IN A TWO MINUTE PERIOD FOLLOWING INJECTIONS OF NOREPINEPHRINE INTO THE DORSAL (N=4) OR VENTRAL (N=4) HIPPOCAMPUS

	Concentration of NE ($\mu g/\mu l$)						
Group	Con	0.005	0.05	0.5	2.5	5.0	5.0 (NE+5-HT)
Dorsal	26.3±3.2	20.3±6.3	67±32.1	42.5± 7.3†	43.1±10.1*	39.7± 9.6*	38.2±8.1*
Ventral	26.4±4.2	19.5±5.4	32± 7.4	28.6±10.1	32.6±12.8	38.4±14.1	37.9±8.1*

*$p<0.05$.
†$p<0.01$.

TABLE 2

MEAN NUMBER OF LINES CROSSED IN A TWO MINUTE PERIOD FOLLOWING INJECTIONS OF SEROTONIN INTO THE DORSAL (N=5) OR VENTRAL (N=5) HIPPOCAMPUS

	Concentration of 5-HT ($\mu g/\mu l$)						
Group	Con	0.005	0.05	0.5	2.5	5.0	5.0 (NE+5-HT)
Dorsal	24.3±3.6	22.6±6.1	46.2±16.0*	48 ±13.5†	39.8± 3.6†	42.2± 9.5†	41.6±14.2†
Ventral	22.6±3.0	24.8±4.4	20.2± 5.6	28.6±12.3	46.2±18.2*	54 ±15.9†	39.4± 8.7*

*$p<0.05$.
†$p<0.01$.

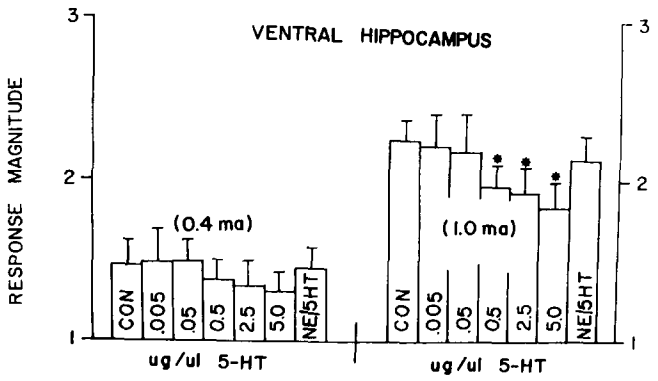

FIG. 8. Response magnitude to footshock of 0.4 ma and 1.0 ma in arbitrary units following injections of saline (CON), a range of 5-HT concentrations, or a combined injection of 5 $\mu g/\mu l$ of NE and 5-HT into the dorsal hippocampus.

$F(6,28)=2.37$, $p>0.05$. At the 1.0 mA level, both groups showed a decrease in reactivity to footshock, DH; $F(6,28)=7.3$, $p<0.01$, and VH; $F(6,28)=9.4$, $p<0.01$. Figures 7 and 8 represent the scores of the DH and VH groups, respectively.

Post hoc analysis revealed that only the 2.5 and 5.0 $\mu g/\mu l$ doses were effective in the DH group, whereas the 0.5, 2.5, and 5.0 $\mu g/\mu l$ doses were effective in the VH group. Scheffé analysis also demonstrated that the injection of the amines simultaneously was not different from the saline injections.

Open Field Activity

After NE injections, the DH groups showed an increase in lines crossed, $F(6,21)=5.54$, $p<0.025$, with no change in the number of lines crossed by the VH group, $F(6,21)=2.25$, $p>0.05$. Table 1 shows the number of lines crossed for each group at each dose level.

Post hoc analysis revealed that the DH group showed an increase in lines crossed at the 0.5, 2.5 and 5.0 $\mu g/\mu l$ doses, while the VH group showed no change.

Following 5-HT injections both the DH and VH groups showed an increase in lines crossed, $F(6,28)=4.62$, $p<0.02$, and $F(6,28)=5.78$, $p<0.01$, respectively. Table 2 gives the scores of both groups at each dose level.

Scheffé analysis revealed that for the DH group the 0.5, 2.5, and 5.0 $\mu g/\mu l$ doses were effective whereas in the VH group only the 2.5 and 5.0 $\mu g/\mu l$ doses showed any effect.

Post hoc analysis also demonstrated that the combination of both amines was effective in increasing the number of lines crossed in both groups.

DISCUSSION

Both NE and 5-HT had significant though opposite effects when injected into the hippocampus. Norepinephrine injections resulted in a dose-dependent increase in behavioral reactivity to shock and heat while serotonin injection resulted in a dose-dependent decrease in behavioral reactivity to shock and heat. The subjects were more sensitive to NE injected into the dorsal hippocampus than when NE was injected into the ventral hippocampus. In contrast the subjects were more sensitive to 5-HT injection in the ventral hippocampus than in the dorsal hippocampus. When both NE and 5-HT were injected simultaneously into either the dorsal or ventral hippocampus, the effects of the

injection of either amine alone were antagonized when measured by the subjects' response to shock or heat. Both NE and 5-HT increased the subjects' activity in an open field in dose-dependent manner.

For this behavioral test, both NE and 5-HT effected the greatest change when injected into the dorsal hippocampus. When injected simultaneously, NE and 5-HT did not either antagonize each other as they had for the previous behavioral measures, or enhance each other's effects on open field activity.

In contrast to the results presented in this paper, a recent paper has indicated that no effect of either serotonin or noradrenaline was observed when these amines were injected into the dorsal hippocampus, though similar behaviors were measured: heat induced tail-flick, response to pin-prick and occurrences of spontaneous behaviors [13]. One reason the above cited study may have revealed no behavioral change is because the dose range was 50–200 $\mu g/\mu l$ and our experimental dose range was 0.005–5.0 $\mu g/\mu l$. The higher dose levels could have saturated the hippocampus inducing non-specific effects which may have antagonized the primary effects of the amines. In the only other paper reporting the effect of biogenic amine injection into the hippocampal formation on behavioral reactivity in the rat, Geyer reported a significant decrease in startle response following 3 μl of a 1 $\mu g/\mu l$ into the dorsal hippocampus [12]. These results support the suggestion that the ineffectiveness of the previous study was due to the high dose levels.

Convincing evidence for an antagonistic role of serotonin and catecholamines has been previously obtained, with increases in catecholamines being associated with behavioral arousal and behavioral states of excitation [22,23] and increases in serotonin associated with decreases in pain sensitivity [2]. Our present results provide direct confirmation of these reports. Of additional importance, however, is that, for the two behavioral responses to aversive stimuli, heat and shock, NE and 5-HT acted antagonistically, while both NE and 5-HT injections increased open field behavior. These results emphasize the importance of using multiple measures to define the functional roles of NE and 5-HT. Not only do these amines appear to have different functions depending on the measure of function applied, but they appear to have different functions depending on the neuroanatomical region into which they are injected [2].

In a more general sense, the present results fit well within the recent conceptualization of a major role for NE and 5-HT in neuronal modulation. Woodward, *et al.* [31] have suggested that NE and perhaps 5-HT (personal communication) modulate the environment of the cells they contact such that, when specific inhibitory, gamma amino butyric acid (Gaba), or excitatory, Acetylcholine (Ach), transmitters contact a cell, their activity is enhanced in the presence of NE and diminished in the presence of 5-HT. In the present experiment an analogous situation is observed, by observations of increases in behavioral reactivity in the presence of NE and decreases in behavioral reactivity in the presence of 5-HT.

A mechanism underlying the NE-5-HT interaction in the HF was recently investigated [7]. This report demonstrated that exogeneous NE inhibited 3(H)-5-HT release from hippocampal slices by more than 70%. These authors suggested that their results reflected a postsynaptic receptor which was localized on serotonergic terminals in the hippocampus. In light of this suggestion the present results could be interpreted to suggest that the behavioral effects of exogenously applied NE are due either to the direct effects of NE or possibly the inhibition of 5-HT release and subsequently the inhibition of 5-HT's inhibiting response. This latter suggestion implies that the behavioral effects that we report are a result of either enhancement of serotonin activity by exogenous application of serotonin or inhibition of serotonin activity by exogenous NE application. In light of recent data supporting a primary role for B adrenergic receptors in hippocampal noradrenergic function [1], the above hypothesis could be tested more specifically using appropriate pharmacological agents.

Finally, our findings of differences for both NE and 5-HT in their effects on behavior, depending on whether the injections were in the dorsal or ventral hippocampus, are consistent with previous studies implicating such differences [4, 6, 11, 14, 15, 18, 20, 25, 26, 27]. An explanation of these differences is not obvious. However, in a recent kinetic analysis of 3(H)-5-HT and 3(H)-NE uptake in dorsal and ventral hippocampal synaptosomes, a greater number of nerve terminals which accumulate NE and 5-HT were present in the ventral hippocampus than in its dorsal counterpart [10]. For both NE and 5-HT, the lower dose required to elicit an increase in open field activity when injected into the dorsal hippocampus could reflect the more rapid removal of the injected compounds from postsynaptic sites in the ventral hippocampus, due to the greater number of terminals present.

This latter interpretation does not afford an explanation of differences in dorsal-ventral behavioral changes observed following heat and shock. However, in the same study evaluating uptake capacity of NE and 5-HT in dorsal and ventral HF, we found a difference in the Km for 5-HT between dorsal and ventral hippocampus and hypothesized that this was due to underlying differences between the functional characteristics of their respective cells of origin. This hypothesis is supported by the observations of Pasquier and Reinoso-Suarez [20] that the dorsalis raphe nucleus projects primarily to the dorsal hippocampal formation while the centralis superior nucleus projects to both the dorsal and ventral hippocampal formation. Thus both quantitative and qualitative differences may contribute to the functional differences observed between the dorsal and ventral hippocampal formation.

In summary, we have demonstrated: (1) NE and 5-HT have dose-dependent effects on two separate classes of behavioral response; (2) NE and 5-HT modulate behavioral reactivity to heat and shock in the opposite directions; (3) NE and 5-HT act antagonistically when injected simultaneously and the animals are tested for their response to shock or heat; (4) NE is more effective in modulating responses to shock and heat when injected in the dorsal hippocampus, whereas 5-HT is more effective in modulating response to shock and heat when injected into the ventral hippocampus. Both NE and 5-HT are more effective in modulating response to shock and heat when injected into the ventral hippocampus. Both NE and 5-HT are more effective in modulating open field behavior when injected into the dorsal hippocampus. Whether the antagonistic relationship between NE and 5-HT is mediated via the same or different receptors, or whether the NE effect is mediated through its presynaptic effect on 5-HT release remains to be determined. These results confirm an important functional role for the interaction of NE and 5-HT in the hippocampal formation.

ACKNOWLEDGMENT

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REFERENCES

1. Crutcher, K. A. and J. N. Davis. Hippocampal and B-adrenergic receptors: Comparison of (3H) dihydroal prenolol and (3H) WB4101 binding with noradrenergic innervation in the rat. *Brain Res.* **182:** 107–117, 1980.
2. Davis, M., D. I. Astracheon and E. Kass. Excitatory and inhibitory effects of serotonin on sensorimotor reactivity measured with acoustic startle. *Science* **209:** 521–523, 1980.
3. de la Torre, J. C. and J. W. Surgeon. A methodological approach to rapid monoamine histofluorescence using a modified glyoxylic acid technique: The SPG method. *Histochemistry* **49:** 81–93, 1976.
4. Edinger, H., A. Siegel and R. Trojano. Single unit analysis of the hippocampal projections to the septum in the cat. *Expl Neurol.* **41:** 569–583, 1973.
5. Ellison, G. D. Behavior and the balance between norepinephrine and serotonin. *Acta neurobiol. exp.* **35:** 499–515, 1975.
6. Elul, R. Regional differences in the hippocampus of the cat. I. Specific discharge patterns of the dorsal and ventral hippocampus and their roles in generalized seizures. *Electroenceph. clin. Neurophysiol.* **16:** 470–488, 1964.
7. Frankhuyzen, A. L. and A. H. Mulder. Noradrenaline inhibits depolarization-induced 3H-serotonin release from slices of rat hippocampus. *Eur. J. Pharmac.* **63:** 179–182, 1980.
8. Fuxe, K., T. Hokfelt and U. Ungerstedt. Morphological and functional aspects of central monamine neurons. *Int. Rev. Neurobiol.* **13:** 93–126, 1970.
9. Gage, F. H., D. R. Armstrong and R. G. Thompson. Behavioral Kinetics: A method for deriving qualitative and quantitative changes in sensory responsiveness following septal nuclei damage. *Physiol. Behav.* **24:** 479–484, 1980.
10. Gage, F. H. and R. G. Thompson. Differential distribution of norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation. *Brain Res. Bull.* **5:** 771–773, 1980.
11. Gage, F. H., R. G. Thompson and J. J. Valdes. Endogenous norepinephrine and serotonin within the hippocampal formation during development and recovery from septal hyperreactivity. *Pharmac. Biochem. Behav.* **9:** 359–362, 1978.
12. Geyer, M. A. Functional heterogeneity within neurotransmitter systems. *Psychopharmac. Communs.* **1:** 675–685, 1975.
13. Glick, S. D. and L. A. Crane. Opiate-like and abstinence-like effects of intracerebral histamine administration in rats. *Nature* **273:** 547–549, 1978.
14. Grant, L. D. and L. E. Jarrard. Functional dissociation within the hippocampus. *Brain Res.* **10:** 392–401, 1968.
15. Johnson, C. T., D. S. Olton, F. H. Gage and P. G. Jenko. Damage to hippocampus and hippocampal connections: Effects on DRL and spontaneous alteration. *J. comp. physiol. Psychol.* **91:** 508–522, 1977.
16. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid flourescence method. *Acta physiol. scand. Suppl.* **412:** 1–48, 1974.
17. MacLean, P. D. An ongoing analysis of hippocampal inputs and outputs: Microelectrode and neuroanatomical findings in squirrel monkeys. In: *The Hippocampus, Vol 1*, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 179–211.
18. Meibach, R. C. and A. Siegel. Efferent connections of the septal area in the rat: An analysis utilizing retrograde and anterograde transport methods. *Brain Res.* **119:** 1–20, 1977.
19. Moore, R. Y. Monamine neurons innervating the hippocampal formation and septum: Organization and response to injury. In: *The Hippocampus, Vol. 1*, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 215–237.
20. Pasquier, D. A. and F. Reinoso-Suarez. Differential efferent connections of the brainstem to the hippocampus in the cat. *Brain Res.* **120:** 540–548, 1977.
21. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967
22. Samanin, R. and S. Garattini. The serotonergic system in the brain and its possible functional connections with aminergic systems. *Life Sci.* **17:** 1201–1210, 1975.
23. Schildkraut, J. and S. Kety. Biogenic amines and emotions. *Science* **156:** 21–30, 1967.
24. Segal, M. and F. E. Bloom. The action of norepinephrine in the rat hippocampus. I. Iontophoretic studies. *Brain Res.* **72:** 79–97, 1974.
25. Segal, M. and S. Landis. Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.* **87:** 1–15, 1974.
26. Siegel, A. and J. P. Flynn. Differential effects of electrical stimulation and lesions of the hippocampus and adjacent regions upon attack behavior in cats. *Brain Res.* **7:** 252–267, 1968.
27. Siegel, A. and J. P. Tassoni. Differential efferent projections of the lateral and medial septal nuclei to the hippocampus in the cat. *Brain Behav. Evolut.* **4:** 201–219, 1971.
28. Smith, R. F. Mediation of footshock sensitivity by serotonergic projection to hippocampus. *Pharmac. Biochem. Behav.* **10:** 381–388, 1979.
29. Storm-Mathisen, J. Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog. Neurobiol.* **8:** 119–181, 1977.
30. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.
31. Woodward, D. J., H. C. Moises, B. D. Waterhouse, B. J. Hoffer and R. Friedman. Modulatory action of norepinephrine in the central nervous system. *Fedn Proc.* **38:** 2109–2116, 1979.