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5HT Blockade and the Stimulant Effects of D- and L-Amphetamine: No Interaction in Self-Stimulation of Prefrontal Cortex, Hypothalamus, or Dorsal Tegmentum. Unexpected Lethality in Hippocampal Sites

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FRANKLIN, K. B. J. AND A. ROBERTSON. *5HT blockade and the stimulant effects of d- and l-amphetamine: No interaction in self-stimulation of prefrontal cortex, hypothalamus, or dorsal tegmentum. Unexpected lethality in hippocampal sites.* PHARMAC. BIOCHEM. BEHAV. 13(3) 365–370, 1980.—We investigated the role of serotonin in the differential stimulatory effects of D- and L-amphetamine (1.0 mg/kg) on locomotor activity and on self-stimulation (SS) in rats. The serotonin antagonist methysergide (12.5 mg/kg) had no effects alone on activity or on SS. Methysergide enhanced the strong locomotor stimulatory effects of D-amphetamine and the weaker effects of L-amphetamine. D-amphetamine facilitated SS more strongly than L- in hypothalamic, dorsal tegmental, hippocampal and medial prefrontal cortical sites, but the effect of D-amphetamine was much weaker in prefrontal cortex than in other sites. Methysergide did not alter the effects of D- or L-amphetamine in any site except the hippocampus. Here, methysergide plus L-amphetamine suppressed SS; methysergide plus D-amphetamine suppressed SS in some rats and greatly increased it in others. When SS was facilitated by D-amphetamine plus methysergide, the combination was lethal. The possibility that lethality was due to adrenal crisis is discussed.

Amphetamine isomers Dorsal tegmentum Hippocampus Lateral hypothalamus Methysergide Prefrontal cortex Serotonin Self-stimulation

THERE is now a large literature supporting the view that self-stimulation (SS) and its facilitation by amphetamine depends on the release of dopamine (DA) and/or noradrenaline (NA) in the brain [8]. It is also widely known that D-amphetamine is more potent than L-amphetamine at facilitating SS at some sites, but is equipotent or only slightly more potent at other brain sites [11,22]. The reason for this difference is not, however, known. It was originally suggested that the isomers are equipotent when electrodes are in a dopaminergic region and that D-amphetamine is more potent than L-amphetamine when electrodes are in a noradrenergic region [22]. This was based on the observation that D- is more potent than L- at releasing NA but that the two isomers are equipotent at releasing DA [35]. This finding came into question with later experiments showing that D-amphetamine is more potent than L-amphetamine in releasing DA but equipotent in releasing NA [7,10]. At present, therefore, it is unclear just what role the catecholamines play in producing the D-L potency differences. But amphetamine also releases serotonin (5HT), with the D-isomer reportedly twice as potent as the L-isomer in this release [13]. It has recently been suggested that alteration of 5HT transmission might mediate some of the locomotor activity and stereotyped behaviors caused by high doses of amphetamine [16, 32, 41]. However, the role of 5HT in the effects of the amphetamine isomers on SS has not been directly investigated, although there is some evidence that 5HT may play a role in SS of some brain sites. 5HT has been hypothesized to inhibit SS of the LH-MFB [23, 25, 37] and ventral midbrain [24]. In contrast, 5HT may be excitatory in SS of forebrain sites such as the hippocampus [37], caudate-putamen [23] or subfornical organ [27]. Evidence concerning the role of 5HT in SS of the raphe nuclei is contradictory, some investigators suggesting an excitatory [18,36] and some an inhibitory role [31].

It has been reported that blocking 5HT transmission does increase the facilitatory effects of DL-amphetamine on SS of the LH [30]. It is not clear, however, whether 5HT might be involved in the effects of both D- and L-amphetamine and, if so, whether this might be site-specific. The present study was designed to clarify the role of 5HT in SS of midbrain, diencephalic and forebrain sites and to investigate the contribution of 5HT to the differences in the effects of D- and L-amphetamine on SS. To this end, we examined the effects of the 5HT antagonist, methysergide, on SS and its modulation by D- and L-amphetamine at sites in the midbrain tegmentum, lateral hypothalamus, hippocampus and prefrontal cortex. Additionally, we examined the effects of methysergide on the locomotor stimulation produced by D- and

L-amphetamine to compare the efficacy of the methysergide treatment on locomotor activity with its efficacy on ICSS.

METHOD

Drugs

D- and L-amphetamine (1.0 mg/ml) were dissolved in isotonic saline. Methysergide bimaleate was dissolved in 10 N HCl, buffered with NaOH and diluted with distilled water to a concentration of 12.5 mg/ml. The pH was between 6.0 and 6.5.

Locomotor Tests

Twenty-five male albino rats, weighing from 198 g to 282 g, were tested for locomotor activity in a Plexiglas box (32×32×32 cm) with a tilt floor which was activated by the rat crossing the center line of the floor. Interruptions of a photocell beam directed across the middle of the test chamber at right angles to the axis of tilt provided a measure of movement in another horizontal plane. The two scores were combined to arrive at a total activity count.

Rats were given two 20 to 30 min sessions of adaptation to the apparatus and handling before testing began. For the drug tests, rats were randomly divided into 6 groups (n=6). Each rat received 2 injections. A first injection of methysergide or its acid vehicle followed 2 min later by saline, D-amphetamine or L-amphetamine. Each rat was placed in the activity cage immediately after the second injection and activity was recorded for the following 60 min.

Self-Stimulation Tests

Animals and surgery. Thirty-two male albino rats, weighing from 250 g to 290 g at the time of surgery, were used. Electrodes (Plastic Products, 0.005 in. in diameter at the tips) were implanted under Nembutal anaesthesia (60 mg/kg) into one of the following sites in the brain: medial prefrontal cortex (PFC; n=7); the lateral hypothalamus (LH; n=7); the dorsal tegmentum (DT; n=9); and the hippocampus (HPC; n=9).

Apparatus and pre-training. Following a one-week recovery period from the surgery, rats were trained to bar-press for electrical stimulation in a test chamber (30×30×30 cm) with a lever at one end, 6 cm above the floor. During training, each depression of the lever delivered a 0.2 sec train of monophasic rectangular pulses, each 0.2 msec in duration, delivered at 100 Hz. Stimulation current was monitored on an oscilloscope.

When rats had learned to bar-press for stimulation, the current was adjusted to twice that of the threshold for responding, and remained at that level for the duration of the experiment. Except for those with HPC electrodes, rats were trained to respond on a random interval 10 sec schedule of reinforcement in daily 90 min sessions. Because rats with HPC electrodes had very low response rates which declined over a session they were trained in 60 min sessions on VI 5 sec. The number of bar-presses was automatically recorded every 5 min on a Med Associates Printer. Drug tests were begun after a minimum of 5 sessions on this regimen, when responding had stabilized.

Testing procedure. Rats were tested during 90 min sessions, once every three days. Responding in the first 25 min of the session was used to calculate baseline responding, the drug or vehicle was injected IP between the 25th and 30th min of the session. Responding was then recorded for the following 60 min. The exception to this was that rats with HPC electrodes were tested for 60 min rather than 90 min sessions. In these rats, drugs were administered before each test session began. These animals received control sessions without drug on days between drug tests, in order to calculate baseline response rates.

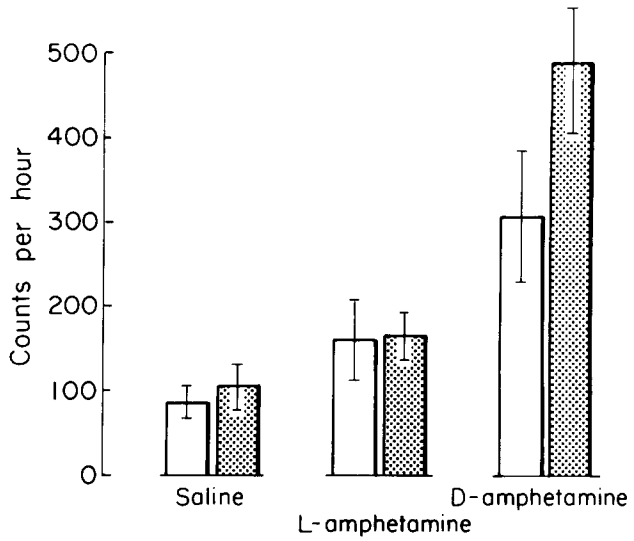

FIG. 1. Locomotor activity in counts per hour after saline, L-amphetamine or D-amphetamine in combination with methysergide (shaded bars) or its acid vehicle (open bars).

All drugs were administered in a volume of 1.0 ml/kg. First, in three consecutive drug sessions, rats received D-amphetamine 1 mg/kg, L-amphetamine 1 mg/kg and saline in counterbalanced orders. One week later, in three more consecutive drug sessions, most rats received methysergide 12.5 mg/kg 2 to 3 min before D-amphetamine, L-amphetamine and saline, again in counterbalanced order. Five rats with PFC and 4 rats with DT electrodes received this series of drugs 2 rather than 1 week later. In the interim week, these rats received 3 doses of 10 mg/kg naloxone combined with D- or L-amphetamine or saline. None of these treatments or the methysergide amphetamine treatment altered intersession baseline rats. Naloxone effects will be reported elsewhere.

Data analysis. For rats with PFC, DT or LH electrode ICSS, response rates for the 60 min under a drug condition were expressed as a percentage of the baseline rates in the 25 min preceding drug administration. The results for the PFC, DT and LH groups were subjected to repeated measures design ANOVA followed by Newman-Keuls tests for the comparisons of interest. For rats with HPC electrodes, there was excessive heterogeneity of variance, and the effects of the methysergide plus D-amphetamine condition were bimodal. These results were therefore analysed by non-parametric methods. Locomotor activity scores were also analysed non-parametrically.

Histology. Upon completion of the behavioral tests, rats were killed with an overdose of Nembutal and perfused with Formal-saline. Brain sections of 60–80 μ thickness were cut using a freezing microtome. Selected sections were then mounted in distilled water and were used as negatives for photographic prints. Additionally, some sections from rats with HPC placements were stained with Luxol fast blue and neutral red for light microscopy.

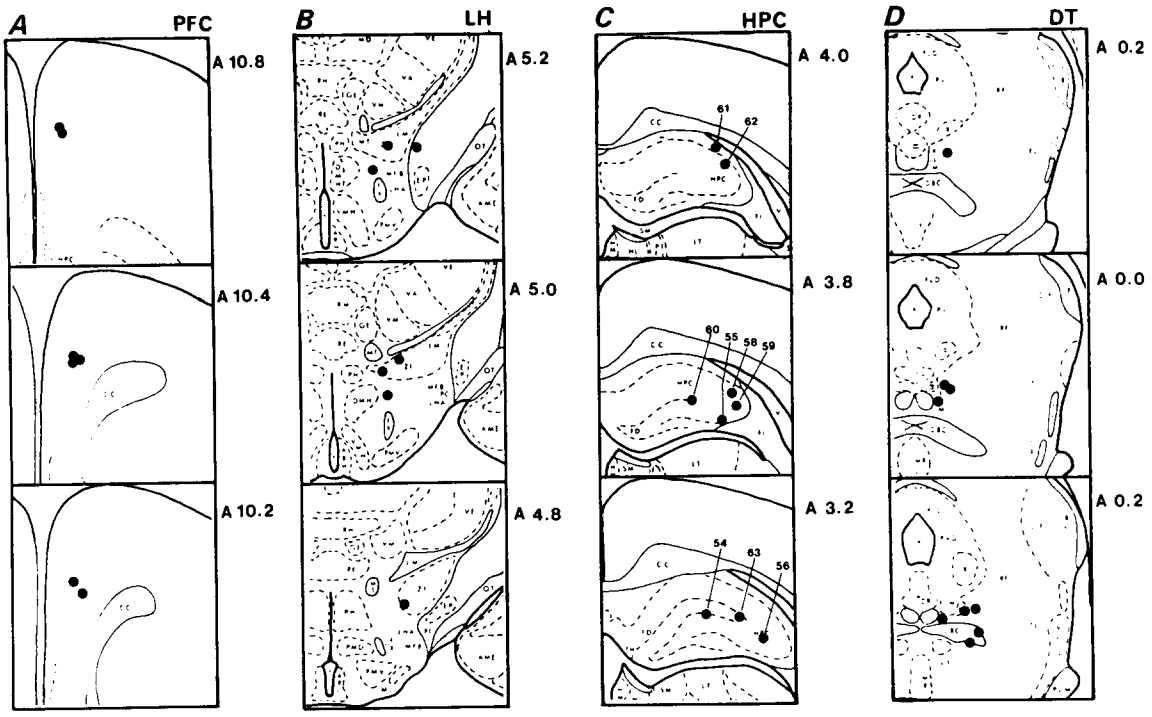

FIG. 2. Self-stimulation sites on planes of the atlas of Pellegrino and Cushman for subjects self-stimulating in *A*. prefrontal cortex; *B*. hypothalamus; *C*. hippocampus, and *D*. dorsal tegmentum. Numbers labelling sites in panel *C* are the subject numbers referred to in Fig. 4 and the text.

RESULTS

Locomotor Activity

As can be seen in Fig. 1, D-amphetamine greatly increased locomotor activity from a mean of 85.3 counts per hour after saline injection to 316.3 (Mann Whitney U=0, $p=0.001$) while activity after methysergide was not significantly different from the saline control (U=13.5, NS). However, when methysergide was combined with D-amphetamine activity increased to 482 counts per hour, significantly above the activity produced by amphetamine alone (U=4, $p=0.013$). L-amphetamine insignificantly increased activity over saline control levels to 161 counts per hour (U=10, NS). Methysergide combined with L-amphetamine increased activity to 165.5 (± 25.5) counts per hour which was significantly greater than the level under saline (U=6, $p=0.32$) or methysergide alone (U=7, $p=0.047$) but not significantly different from L-amphetamine alone (U=16, NS).

Self-Stimulation

In rats self-stimulating in the LH, DT and PFC (see Fig. 2A, B and D respectively for electrode sites) methysergide was without effect on ICSS or on its facilitation by amphetamine. Figure 3 shows the results for these groups. In the hypothalamic SS group there was a significant effect of amphetamine, $F(2,12)=29$; $p<0.001$, no effect of methysergide, $F(1,6)=0.22$, NS, and no interaction, $F(2,12)=1.19$, NS. Further analysis of the amphetamine effect confirmed that both D- and L-amphetamine significantly increased responding ($p<0.05$) and D-amphetamine produced a larger increase than L-amphetamine ($p<0.01$). Dorsal tegmentum SS was similarly affected. Amphetamine increased responding $F(2,16)=23.75$; $p<0.001$, D- being more effective than L-amphetamine ($p<0.01$), while methysergide did not alter these effects, $F(1,8)=0.39$; $F(2,16)=1.04$.

ICSS of the PFC was much less affected by amphetamine than the other sites. The maximum increase was only to 115% of baseline compared to 210% for DT rats and 250% for LH and 170% for HPC rats, $\chi^2(3)=10.31$, $p<0.02$, Kruskal-Wallis. Nevertheless there was a significant effect of amphetamine, $F(2,12)=17.7$; $p<0.001$, although only D-amphetamine increased response rates ($p<0.01$). Methysergide again had no effect and did not interact with methysergide, $F(1,6)=1.61$; $F(2,12)=0.71$, NS.

Rats self-stimulating in the hippocampus (Fig. 2C for electrode sites) behaved differently from the other groups. They lever pressed at a lower rate than the other rats and could not be induced to respond faster by increasing the stimulating current. In spite of the very low rate hippocampal self-stimulation was very reliable. As can be seen in Fig. 3 their response to amphetamine resembled that of the PFC group in that D-amphetamine significantly increased responding (Wilcoxon T=2, $p<0.01$) but L-amphetamine did not (T=13, NS). Methysergide itself did not significantly alter response rates but when methysergide was combined with L-amphetamine responding was significantly depressed, relative to the rate under saline (T=0, $p<0.01$) or L-amphetamine alone (T=5, $p<0.05$).

The combination of methysergide with D-amphetamine produced more dramatic results. Figure 3 suggests that methysergide simply exaggerated the facilitation produced by D-amphetamine but the individual results depicted in Fig. 4 show that in 4/9 rats methysergide abolished or reversed the facilitory effect of amphetamine while in another 4 rats large increases in responding occurred. Furthermore, at the

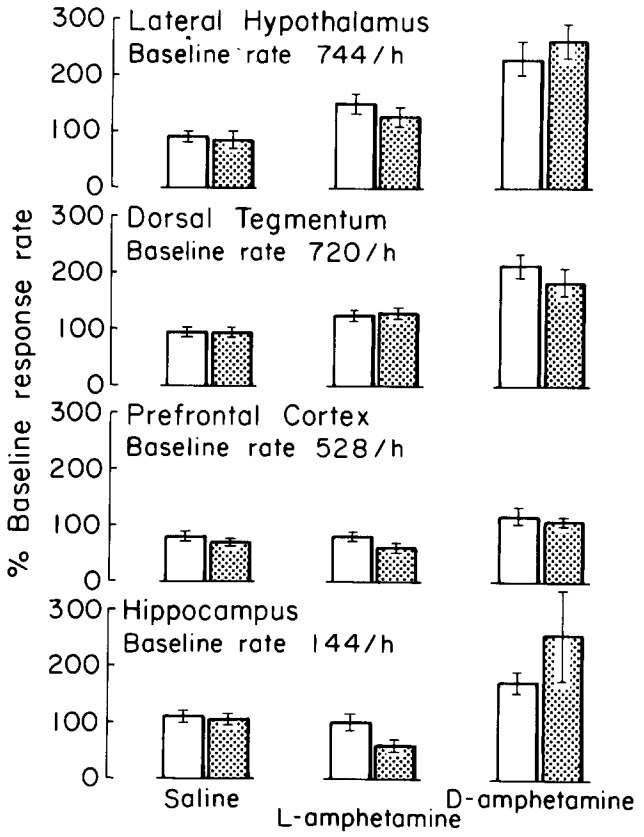

FIG. 3. Changes in VI self-stimulation rate after saline, L-amphetamine or D-amphetamine in combination with methysergide (shaded bars) or its acid vehicle (open bars).

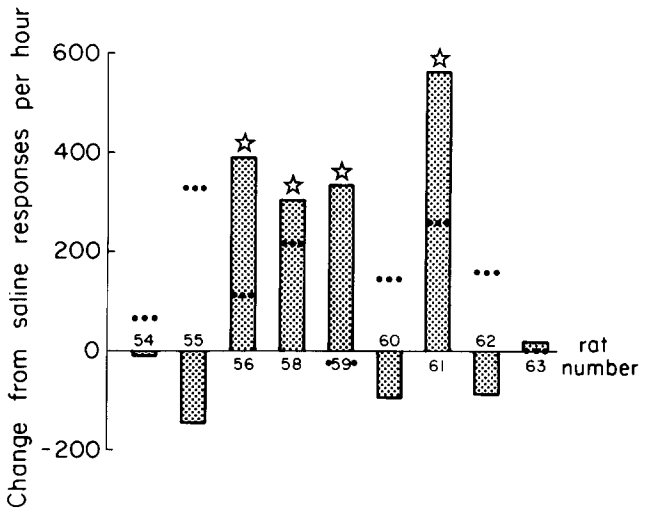

FIG. 4. Change in hippocampal self-stimulation rates of individual rats treated with methysergide plus D-amphetamine (shaded bars) and with D-amphetamine alone (dotted line). Starred bars indicate rats which showed hyperactivity and collapse.

time that responding increased (30–45 min into the session), the 4 rats became extremely hyperactive, running around the test chamber and jumping continually against the lid of the chamber. When the rats were removed from the test box, they appeared to be hyperthermic (rectal temperature in 2 rats was about 40°C). Hyperactivity continued for about 15 min, followed by lethargy and collapse. Two of these rats (56 and 61) later died. Rat 56 died 1–1/2 hours after drug administration. Its heart was found to be stopped when breathing ceased. Rat 61 was cooled for 3 hr in an attempt to lower its body temperature, appeared to be revived, but died overnight. Rats 58 and 59 were treated with chlorpromazine (0.5–1 mg IP) 10–20 min after the onset of hyperactivity and both rats survived. During the test or post-test period the rats' frantic activity was not accompanied by seizures although 3/4 rats normally did have a grand mal seizure during each self-stimulation period.

There was no correlation between the nature of the response to D-amphetamine plus methysergide and the baseline response rate, the response to D- or L-amphetamine alone, or the response to methysergide alone. There was a significant association between the nature of the response to D-amphetamine plus methysergide and the position of the electrode within the hippocampus. Four of 6 rats with electrodes in the CA fields showed the hyperactivity while none of the 3 rats with electrodes in the dentate gyrus did so ($\chi^2=5.2$; $p<0.05$).

DISCUSSION

Methysergide, like other treatments which reduce brain 5HT activity [4, 16, 17] potentiated the locomotor stimulant effect of amphetamine. In agreement with Lucki and Harvey [16] the powerful stimulant effect of 1.0 mg/kg D-amphetamine was greatly increased while the insignificant effect of 1.0 mg/kg L-amphetamine became significant after methysergide.

Except that D-amphetamine was always more potent than L-amphetamine, the results for self-stimulation did not parallel those for locomotor activity. The effectiveness of amphetamine varied from site to site. Methysergide did not alter SS or its facilitation by amphetamine except in the hippocampus where it made L-amphetamine depress SS and D-amphetamine depress SS in some animals and markedly facilitate it in others. These results are inconsistent with a recent report [30] that 5HT antagonists potentiate amphetamine effects on Ss and with a number of reports suggesting that 5HT depletion facilitates SS [23, 24, 31]. The discrepancy cannot be due to ineffectiveness of our drug treatment. Doses of methysergide similar to those used here have been shown to block central 5HT receptors [28] and to be effective in reducing the suppressant effects of punishment [34]. Moreover, in our first experiment the same methysergide treatment was very effective in potentiating the locomotor stimulation of D-amphetamine.

On the other hand, the effects on SS of interfering with 5HT do seem to be variable. PCPA induced depletion has been reported to facilitate, and depress, hypothalamic SS [23, 24, 33]. There have been reports both that PCPA enhances and depresses SS of the dorsal or median raphe [18, 31, 36] and, recently, that median raphe SS was both depressed by methysergide and later reinstated by PCPA after SS had spontaneously ceased [14]. The present study differed from previous reports in that SS was maintained on a variable interval schedule. Other studies have used continuous reinforcement (CRF) which is not always a reliable indicator of incentive [12,26]. One factor which influences response rate on CRF is that brain stimulation reward often

has a punishing component and rats will learn to self-stimulate and escape from stimulation of the same site [3,26]. The aversion seems to build up more slowly than the reward produced by brain stimulation [3,26] and accumulates over successive trains of brain stimulation [29]. Thus, in SS on CRF it is often observed that rats emit a rapid series of lever presses and then withdraw from the lever—presumably because the series of brain stimulations has raised the aversion component to an unacceptable level. The presence of an aversive component to brain stimulation rewards, the magnitude of which varies from site to site [1, 2, 19], suggests an explanation for the variability of results with 5HT blockade. Panksepp, Gandelmann and Trowill [20] have shown that the anti-anxiety drug chlordiazepoxide increased self-stimulation at sites where escape behavior was also obtained but decreased self-stimulation at sites where consistent escape behavior was not obtained. It is well established that 5HT blockade has the same effect as anti-anxiety drugs in reducing the suppressive effect of punishment [9, 34, 39]. Thus, PCPA and 5HT receptor antagonists might be expected to increase self-stimulation when that stimulation has an aversive component [39]. In the present experiments the spacing of the stimulation of a VI schedule and the short pulse trains (0.2 sec) would eliminate the accumulation of the aversive component and thus blocking 5HT would not be expected to alter SS.

In hippocampal SS D-amphetamine was facilitatory. Although the undrugged response rate was extremely low (mean 153 responses/hour), D-amphetamine increased it by about 100% (295 responses/hour)—a proportionate increase in line with increases at other subcortical sites. L-amphetamine did not, however, alter SS of the HPC, but when combined with methysergide, depressed SS. When D-amphetamine was combined with methysergide, in some rats SS was depressed or unchanged, but in others the facilitatory effect of D-amphetamine was exaggerated. These rats later became seriously ill. Because methysergide plus amphetamine was lethal in rats which increased SS, it is difficult to interpret the significance of these observations for SS. Since the drug combination is lethal only in SS of the HPC, the lethality is clearly not due to the drugs themselves.

The cause of death in these animals was not established. One possibility is acute adrenocortical insufficiency. Because HPC response rates were low animals collected fewer rewards than the VI schedule permitted (max. rewards 720/hr) so that increases in response rates resulted in more brain stimulation. Hippocampal stimulation is known to inhibit ACTH release under stressful conditions [6,15]. In the present experiment, the stressor effect of amphetamine would be potentiated by methysergide increasing its locomotor stimulant effect and by exaggerating its cardiovascular effects via blockade of 5HT inhibitory projections to sympathetic preganglionic neurons [5]. Therefore, these drugs together might well be lethal in an animal whose ACTH production is depressed by repeated hippocampal stimulation. The symptoms of adrenal crisis are similar to those observed—stress is followed by severe fever, then lethargy, deepening into somnolence. Death results from blood pressure falling and pulse failing as hypovolemic vascular shock ensues [40]. This and other possible causes of death are currently being investigated.

It is clear that hippocampal stimulation in the presence of these drugs causes profound physiological disturbance. It is not surprising therefore that most animals avoid SS during the period of peak action—indeed, all rats reduced SS when methysergide was combined with the weaker stimulant L-amphetamine. Rather, it is puzzling that some rats did increase SS and precipitated a physiological crisis. The only factor which was correlated with increased HPC SS was electrode location within the HPC but this in itself does not suggest an explanation. It is possible that methysergide prevented the aversive effects of HPC stimulation from suppressing responding but it is difficult to see why methysergide should have this effect at some HPC sites and not others.

To conclude, our results suggest that 5HT plays no direct role in SS of cortical, diencephalic and midbrain SS sites, and that the differential facilitatory effects of amphetamine isomers on SS cannot be explained by their effects on 5HT transmission. The results also demonstrate that the neural substrate of hippocampal SS is not functionally homogeneous and that 5HT may be involved in the effects of amphetamine on some hippocampal SS sites. Finally, the unexpected lethality of SS in D-amphetamine-methysergide treated animals indicates that more attention should be given to the autonomic effect of self-stimulation and its modulation by drugs.

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