Pharmacology Biochemistry & Behavior, Vol. 18, pp. 695–699, 1983. © Ankho International. Printed in the U.S.A.

The Influence of Amphetamine on Preference for Lateral Hypothalamic Versus Prefrontal Cortex or Ventral Tegmental Area Self-Stimulation

TIMOTHY H. HAND AND K. B. J. FRANKLIN

McGill University, Department of Psychology, 1205 Dr. Penfield Avenue, Montreal, Quebec H3A 1B1, Canada

Received 12 March 1982

HAND, T. H. AND K. B. J. FRANKLIN. *The influence of amphetamine on preference for lateral hypothalamic versus prefrontal cortex or ventral tegmental area of self-stimulation.* PHARMACOL BIOCHEM BEHAV 18(5) 695–699, 1983.—Rats were trained to bar-press for intermittent reinforcement on a concurrent schedule offering self-stimulation (SS) at the animal's choice of one of two different brain loci. On the concurrent schedule, the relative reward value of the two reinforcers is evaluated by the way the subject divides its session time responding for these reinforcers, thus yielding a rate-free measure of reward in addition to response rate data. In animals with electrodes in the lateral hypothalamus (LH) and prefrontal cortex (PFC), amphetamine dose-dependently increased response rates as well as the proportion of time allotted to LH stimulation, demonstrating that the reward value of LH stimulation was increased relative to PFC stimulation. This finding supports the hypothesis that DA systems modulate the rewarding value of LH but not PFC SS, and it suggests that differing neural mechanisms underlie these two behaviors. In animals with LH/ventral tegmental area (VTA) implants, amphetamine had no effect on preference, although it produced an overall increase in rate. This suggests that the drug elevates the rewarding value of LH and VTA stimulation to a similar degree, and that the two regions may have a common DA-related reward substrate. Finally, it was found that when the two reinforcers were equally preferred (50% session time allotted towards each reinforcer), response rates for the two rewards were not necessarily equal. This confirms that SS response rate is not a simple function of reward magnitude.

Concurrent schedule Lateral hypothalamus Ventral tegmental area Prefrontal cortex Amphetamine Dopamine Incentive Reward Self-stimulation Response rate Rate-free measure Preference

IT is well known that amphetamine enhances self-stimulation (SS) responding [26]. However, this facilitatory effect is highly dependent upon the site of stimulation. At the lateral hypothalamus (LH) and ventral tegmental area (VTA) the effect is very strong [1, 9, 12, 15, 22, 29], and the amphetamine dose-response curve for these two regions is nearly identical [1]. Facilitation of SS is comparatively weak at the PFC [9, 12, 19, 20], and of intermediate strength at various other SS sites [9, 12, 15, 16, 18, 20]. This suggests that amphetamine potentiates the rewarding effect of LH and VTA stimulation more than that of PFC stimulation. However, most studies of amphetamine effects on SS have used response rate on a continuous reinforcement schedule as the dependent variable, despite the shortcomings of this measure as an index of reward [13,28]. SS thresholds have also been used to evaluate amphetamine effects on LH reward [7, 22, 27, 29]. While there is agreement that amphetamine decreases LH SS thresholds, the threshold measure itself may be distorted by behavioral perseveration induced by amphetamine [17]. Moreover, amphetamine effects on PFC SS have been evaluated only with response rate [9, 12, 19, 20]. Thus it is not clear whether the site-dependency of amphetamine's enhancement of SS reflects genuine variations in the drug's influence on brain stimulation reward, or reflects differences in the subject's capacity to respond for stimulation at the various sites.

Recently it has been shown [20] that site-to-site variation in SS reward value can be directly assessed by allowing the animal to choose the site of stimulation on a Findley [8] concurrent schedule. This schedule allows the subject to sample either of two reinforcers, both of which are available on the same response lever. An unrewarded response on a separate lever changes the currently available reinforcer for the alternate reinforcer. The animal divides its time responding for these reinforcers in direct proportion to their relative rewarding value [2]. Robertson, Laferriere and Franklin [20] used this method to show that in animals with LH-PFC electrode placements, increasing LH current causes an increase in session time allotted to LH stimulation, while increasing PFC current had no significant effect on PFC preference. In the present study, we have confirmed the results of Robertson *et al.*, and have used the concurrent schedule to

determine the effect of amphetamine on the relative reward value of LH and PFC stimulation, and on that of LH and VTA stimulation.

METHOD

Animals, Surgery and Histology

The subjects were adult male hooded rats (Long-Evans) implanted under Nembutal anaesthesia (60 mg/kg, IP) with two bipolar electrodes aimed at the LH and PFC or at the LH and VTA. Stereotaxic coordinates from bregma were 4.5 mm anterior, 0.7 mm lateral and 3.5 mm below the skull surface for the PFC, −0.9, 1.4 and −8.8 for the LH, and −2.7, 0.7 and −8.8 for the VTA. The incisor bar was set at 5 mm above the interaural line. At the end of the experiment, rats were killed with chloral hydrate and perfused intracardially with 0.9% saline followed by 10% Formalin. The brains were removed and thionin-stained frozen 30 μ sections were prepared for histological examination. All placements were confirmed with the aid of a light microscope.

Apparatus and Pre-Training

Ten days after surgery, the subjects were trained to self-stimulate in a conventional Skinner box in a sound-attenuated chamber. It contained a retractable lever (Lehigh Valley) at one end 8 cm above the grid floor. Each lever press was followed by a 100 Hz, 0.2 sec train of 0.15 msec rectangular pulses. Current was monitored on a Tektronix oscilloscope measuring across a 100 ohm resistor in series with the rat. When the subjects were self-stimulating reliably, they were introduced to a multiple schedule which alternated 5 minutes of stimulation at one site with 5 minutes of stimulation at the other, both available on the same retractable lever. These 5 minute intervals were separated by a 4 sec period during which the lever retracted. For LH/PFC animals, stimulation of either site was delivered on a random interval (RI) 3.3 sec schedule during training and subsequent testing. The LH/VTA animals were trained and tested on a RI 5 sec schedule. One hour sessions were given each day. A 1.5 watt light located 3 cm above the retractable lever signalled at which site stimulation was available (on=PFC or VTA, off=LH).

When responding at both sites became stable, a nonretractable changeover lever (Lehigh Valley) was introduced, 5 cm to the left of the retractable response lever. A press of this changeover lever terminated the availability of stimulation at the current SS site and reinstated it at the other site after the 4 sec timeout. During the timeout the changeover lever was inoperative. Subjects were shaped to operate the lever by putting one site on extinction, thereby forcing the animal to either wait 5 minutes for reinforcement or press the changeover lever to reinstate reinforcement availability. The "extinction site" was alternated every other day to prevent the animal from associating the 1.5 watt light with the presence or absence of reward, rather than with stimulation site.

After the use of the changeover lever was learned, to a criterion of 75% or more session time allotted to the rewarded site, currents were found for each animal at which 45–55% of the session time was spent responding for LH stimulation; in other words, the two reinforcers were equally preferred. This was successful with all but one subject (see Table 1). These currents were retained as baseline throughout all experiments. Subjects were retested on them following each experimental treatment.

TABLE 1

RELATIONSHIP BETWEEN CURRENT, SITE PREFERENCE AND RESPONSE RATES AT BASELINE

	Subject	Stimulation Current (μA)	Preference (% Session Time)	Responses per min
LH/PFC stimulation	1	250/100	50/50	17.3/18.4
	2	300/175	45/55	25.9/6.4
	3	275/75	47/53	52.7/24.2
	4	110/250	70/30	2.4/14.5
LH–VTA stimulation	5	435/480	50/50	2.1/34.6
	6	270/530	46/54	3.6/17.1
	7	430/390	49/51	4.6/29.5

Current Intensity Tests

After three consecutive days of stable, approximately equal time allotments between sites were observed on the baseline currents, subjects with LH/PFC placements underwent current intensity tests. Intensities were manipulated in daily one hour sessions in the following order: (1) LH current increased 50 μA above baseline; PFC current maintained at baseline, (2) LH current increased 100 μA; PFC current at baseline, (3) LH current decreased 50 μA; PFC current at baseline, and (4) LH current at baseline; PFC current increased 100 μA. These four treatments were given on alternate days. Days between treatments were reserved for the reestablishment of baseline time preferences using the baseline currents.

The raw data for the LH and PFC response rates from the current intensity tests were square root transformed, as variation in these rates increased as LH current increased.

Amphetamine Tests

Immediately before the test session, LH/PFC animals received dextro-amphetamine sulphate (Smith, Kline and French (Canada), Ltd.), in a volume of 1 ml/kg, IP. Doses were 0.3, 0.8 and 1.2 mg/kg, given in that order. Animals with LH/VTA placements received doses of 0.3, 0.8 and 2.0 mg/kg, in that order. The 0.9% NaCl solvent was used for a control injection. Treatment days were alternated with baseline reestablishment days. Currents used throughout these tests were those previously found to result in equal time allotments between sites.

RESULTS

Current, Site Preference and Response Rates at Baseline

Table 1 shows the currents used for each animal during testing with their respective LH preferences and response rates at baseline with vehicle injection. These data are of interest because they show that when the two sites are equally preferred, neither the stimulation currents nor the response rates for the two sites are necessarily equivalent.

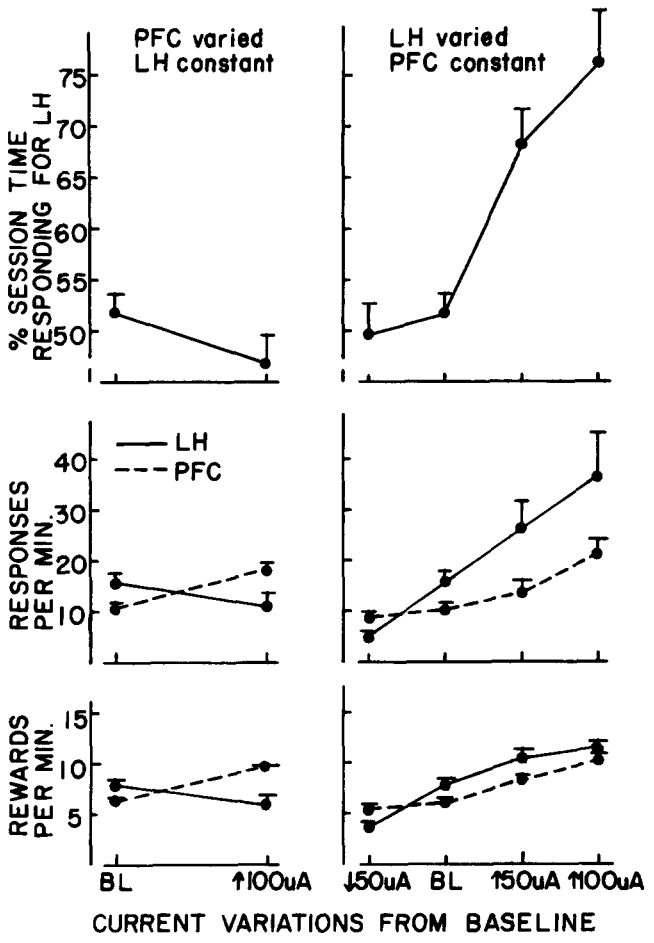

FIG. 1. Effects of LH or PFC current intensity manipulations on time allotment and response rate. The left portion of the figure shows the results of increasing PFC current by 100 μA, leaving LH current constant. In the right portion, LH current is varied and PFC current is constant. The top panel shows % session time spent responding for LH stimulation. The middle panel shows mean response rates. The bottom panel shows mean reinforcement rates.

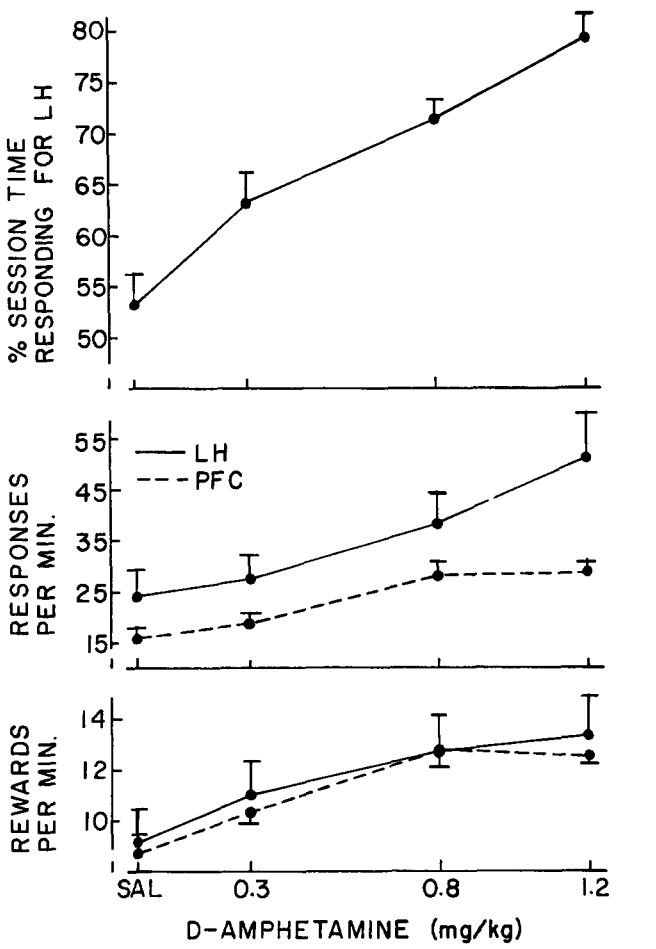

FIG. 2. Effects of amphetamine on time allotment and response rate in LH/PFC animals. The top panel shows % session time spent responding for LH stimulation. The middle panel shows mean response rates. The bottom panel shows mean reinforcement rates.

Current Intensity Effects, LH vs. PFC

Figure 1 shows that increasing or decreasing LH current intensity correspondingly increased or decreased time preference for LH stimulation relative to PFC stimulation, $F(4,12)=11.36$, $p=0.0005$. Post-hoc multiple comparisons showed that, although a 100 μA or even 50 μA increase in LH current intensity greatly increased LH time preference ($p<0.01$, $p<0.05$, respectively, Newman-Keuls), a 100 μA increase in PFC current failed to increase preference for PFC stimulation (NS, Newman-Keuls). There was no significant fluctuation in baseline preferences taken between each current intensity test, $F(3,9)=1.21$, NS. LH response rates increased significantly as a function of current delivered to that site, $F(4,12)=3.53$, $p=0.0399$. Increased LH current intensity appeared to have some effect on PFC response rates also, $F(4,12)=3.25$, $p=0.0506$, but increased PFC current intensity failed to modulate LH response rates in a similar manner (see Fig. 1). There was a significant site by treatment interaction, $F(4,12)=3.79$, $p=0.0323$ in the response rates during current intensity testing, confirming that LH response rates climbed more rapidly than PFC response rates as LH current was increased (see Fig. 1).

The lack of a site by dose interaction in reinforcement frequencies, $F(4,12)=2.80$, NS, shows that these frequencies did not tend to diverge as a function of current level (bottom panel, Fig. 1).

Amphetamine Effects, LH vs. PFC

The effects of d-amphetamine on time preference for LH stimulation were highly significant, $F(3,9)=14.07$, $p=0.001$ (see Fig. 2). Dramatic increases in preference for LH stimulation over PFC stimulation were observed upon administration of either the 0.8 mg/kg dose or the 1.2 mg/kg dose ($p<0.01$, Newman-Keuls). Baseline preferences taken between amphetamine treatments showed no significant fluctuations, $F(3,9)=0.29$, NS. Amphetamine also dose-dependently increased LH response rates, $F(3,9)=4.30$, $p=0.0385$, and PFC response rates, $F(3,9)=4.79$, $p=0.0292$ (see Fig. 2). Cumulative records showed that amphetamine's

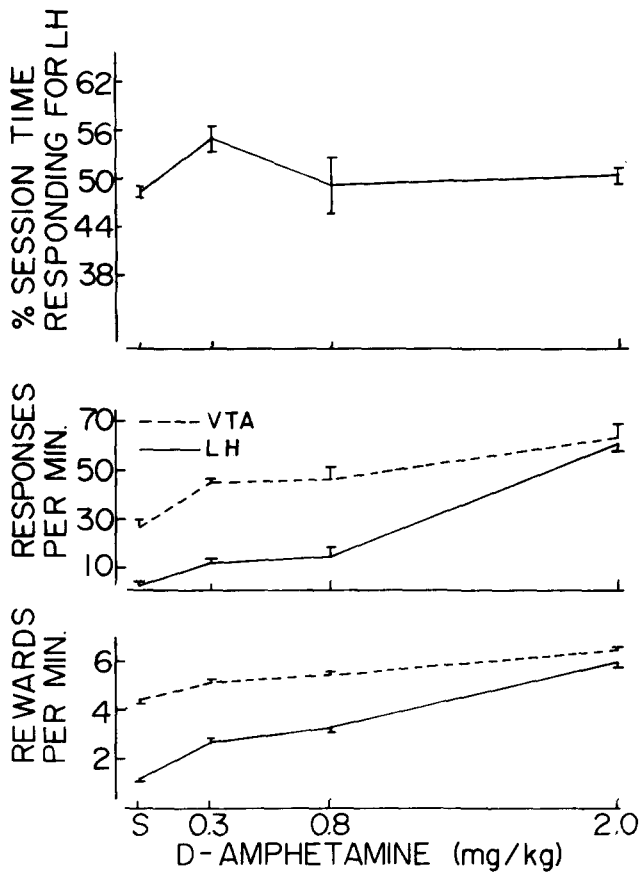

FIG. 3. Effects of amphetamine on time allotment and response rate in LH/VTA animals. The top panel shows % session time spent responding for LH stimulation. The middle panel shows mean response rates. The bottom panel shows reinforcement rates.

peak effect on rate usually preceded the shift in preference toward the LH.

Analysis of reinforcement frequencies revealed no site by dose interaction, $F(3,9)=0.18$, NS, indicating no preferential increase of these frequencies at one site as dose increased (bottom panel, Fig. 2).

Amphetamine Effects, LH vs. VTA

As Fig. 3 shows, amphetamine had no effect on preference for LH stimulation, $F(3,6)=0.93$, NS, despite an overall facilitation of response rate, $F(3,6)=13.71$, $p=0.0043$. However, for response rate there was a significant site by dose interaction, $F(3,6)=21.43$, $p=0.0013$, which suggests a preferential increase in rate at one site (LH, see Fig. 3, middle panel). No site by dose interaction in reinforcement frequencies was observed, $F(3,6)=2.79$, NS (bottom panel, Fig. 3).

DISCUSSION

The most unexpected finding of these studies was the lack of correspondence between site preference as revealed by time allotment (usually equal between sites) and response rate ratios (see Table 1, columns 3 and 4 respectively). Allotment of session time between two different rewards directly reflects their relative reinforcing value [2]; if this is true, response rate ratios between sites are certainly poor indicators of relative reward. There are numerous problems associated with the use of rate as an index of SS reward (see also [13,28]), and these problems indicate the need for a measure of reward which is well correlated with reinforcing effectiveness, yet not confounded by the effects of brain stimulation or drug treatments on sensory or motor capacity. Among the theoretical implications of this finding is that while SS response rate may be partially under the control of incentive-related factors, it is also controlled by other as yet unknown variables which appear to be site-dependent.

Many previous experiments (see [11]) have inferred increased LH SS reinforcement magnitude from dose-dependent facilitation of response rates by amphetamine. These studies have not ruled out drug-induced performance factors, nor have they overcome the difficulties posed by the use of rate as an index of reinforcement, as mentioned above. The time allotment measure used here is not sensitive to the factors which confound the rate measure. Under the conditions of this experiment, an increase in the time allotted to one reinforcer can only result from repeated rejections of the other reinforcer. If the subject has no preference or is indifferent to both reinforcers, then time allotted to each one will be nearly equal, even if the subject expresses its indifference by not using the change lever (see Method section). Time allotment between reinforcers is thus a direct measure of their relative rewarding value [2]. Moreover, the response required to exhibit a preference is minimal (1 response on the change lever per 5 minutes) and therefore any treatment which merely interferes with operant performance would have little effect on this measure.

The finding that increased LH current intensity increases preference for LH stimulation over PFC stimulation, while PFC current increases are without effect, replicates the finding of Robertson *et al.* [20]. Paradoxically, the LH current increase augmented both LH and PFC response rates. This was probably the result of priming following the LH to PFC changeovers. It has indeed been shown [10] that when two electrode placements are made in the same animal, responding for stimulation at one site can be primed with stimulation to the other electrode.

Amphetamine brought about the same behavioral effects as did the increased LH current in these animals. The drug dose-dependently increased the time allotted to LH SS while simultaneously increasing response rates at both the LH and PFC. This nonspecific rate increase may reflect a number of factors including priming or a general motor enhancement, but the increased time preference for LH stimulation over PFC stimulation shows that amphetamine made the former relatively more rewarding than the latter. It confirms that amphetamine augments the rewarding effect of LH stimulation, as has been inferred from the drug's effects on rate and SS thresholds [7, 22, 27, 29]. While it may be argued that amphetamine acts as a general monoamine agonist [14], its enhancement of SS responding has been shown to be DA-dependent and unrelated to its actions upon NA [3–5, 19] or serotonin [9] systems. Moreover, there is very little likelihood that amphetamine is decreasing PFC reward rather than increasing LH reward, as PFC SS is known to be insensitive to disruptions of DA function [21,25].

In animals with LH/VTA placements, amphetamine increased response rates at both sites and failed to disrupt baseline preferences. Since the LH/PFC experiment showed

that amphetamine increases specifically the rewarding value of LH stimulation over PFC stimulation, the lack of effect on preference as a measure of relative LH/VTA reward suggests that amphetamine causes a simultaneous increase in VTA and LH reward. These findings are consistent with the hypothesis [24] that the LH and VTA contain a common reward substrate. Although amphetamine appeared to exert a greater facilitatory effect on LH rate than on VTA rate, this was largely due to a relatively low LH rate at baseline currents. Thus the amphetamine effect on rate seems to be rate-dependent under this paradigm. However, the presence of a site × dose interaction necessitates caution in interpreting the rate effect in this manner.

The present results suggest the existence of 2 at least partially distinct SS reward systems. A medial forebrain bundle (MFB) system includes the LH and the VTA, and is uniformly responsive to amphetamine and to modulations in stimulation current intensity. A second SS system, of which the PFC is a part, is relatively insensitive to amphetamine or to current manipulations. The hypothesis of two distinct SS systems is supported by the lack of effect of massive MFB lesions on PFC SS [6] and by the finding of divergent refractory periods and poor summation of reward between MFB and PFC SS substrates [23]. The presence of at least, two, and possibly more, independent SS systems in the brain now seems more plausible foundation for further investigation than that of a signle, neurochemically homogenous reward system.

ACKNOWLEDGEMENTS

This study was supported by grant A6303 from the National Sciences and Engineering Research Council of Canada to K. B. J. Franklin. We thank Smith, Kline and French (Canada) Ltd. for their gift of d-amphetamine.

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