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# Differential Effects of Amphetamine Isomers on SN Self-Stimulation: Evidence for DA Neuron Subtypes

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FRANKLIN, K. B. J. AND F. J. VACCARINO. *Differential effects of amphetamine isomers on SN self-stimulation: Evidence for DA neuron subtypes.* PHARMACOL BIOCHEM BEHAV 18(5) 747–751, 1983.—The present experiment investigated the effects of varying doses of D- and L-amphetamine on intracranial self-stimulation (ICSS) in the medial or lateral substantia nigra (SN). It was found that the effects of D- and L-amphetamine on ICSS in the SN differ in these two sites. In the medial SN, there were no significant differences between the effects of D- and L-amphetamine on ICSS at any of the doses tested. Both isomers moderately facilitated ICSS with the peak effect at 0.8 to 2.0 mg/kg. By contrast, in the lateral SN, D-amphetamine produced a strong dose-dependent facilitation of ICSS which peaked at 2 mg/kg while L-amphetamine was ineffective below 7 mg/kg. Above 7 mg/kg L-amphetamine increased ICSS rates. The present experiments suggest that the medial and lateral SN are functionally different with respect to ICSS. The possibility that the present medial-lateral SN differences are mediated by two different types of dopamine cells is discussed. In addition, the effects of D- and L-amphetamine on ICSS in the lateral hypothalamus are discussed in light of the present findings.

Dopamine D-Amphetamine L-Amphetamine Self-stimulation Reward

D- AND L-AMPHETAMINE both facilitate self-stimulation (ICSS) but their relative potency differs from one ICSS site to another. In most lateral hypothalamic (LH) sites, dorsal tegmental and dorsal pontine sites D-amphetamine is considerably more potent than L-amphetamine but in the substantia nigra (SN), nucleus accumbens and some lateral hypothalamic sites the isomers are equipotent [4, 16, 18, 21, 23, 25, 26, 29]. Such differences in the effects of D- and L-amphetamine were originally interpreted as revealing differences in the relative role of noradrenaline (NA) and dopamine (DA) in ICSS at these sites [26]. This interpretation was based on evidence that D-amphetamine was more potent than L-amphetamine in inhibiting NA reuptake while the two isomers were equipotent in inhibiting DA uptake [11]. Since ICSS of the SN was thought to depend on DA while ICSS of the LH and dorsal pons were thought to involve NA the behavioral and biochemical effects of the amphetamine isomers seemed consistent.

More recent biochemical investigations have clearly disconfirmed the earlier work and revealed precisely the opposite relationship. D-amphetamine is 2–5 times more potent than L-amphetamine in releasing or inhibiting uptake of DA and the isomers have similar potencies in their effects on NA [13, 19, 20, 22, 27, 29, 31]. Moreover, there is now strong evidence that NA is not directly involved in ICSS or its facilitation by D-amphetamine and that DA plays the major role in these effects [5, 6, 7, 8, 9, 10, 12, 14, 15, 32].

Very recently it has been found that D- and L-amphetamine have different potency ratios in reducing the firing rate of DA cells in the substantia nigra and ventral tegmental area [2] indicating that DA cells vary in their sensitivity to the two isomers. This suggests that variations in the behavioral effects of D- and L-amphetamine might be due to involvement of different subgroups of DA neurons. In line with this idea we have previously found that D- and L-amphetamine had similar, weak effects on ICSS of the medial SN pars compacta while in lateral SN sites D-amphetamine strongly facilitated ICSS and L-amphetamine had little effect [30]. However, these findings were obtained with only a single dose (1.0 mg/kg) of the amphetamine isomers and did not establish the relative potencies of the isomers in the two sites. The present experiment was carried out to replicate our previous finding and to provide dose response data for the two isomers.

## METHOD

### *Subjects*

Subjects were 48 male hooded rats obtained from Canadian Breeding Farms. They were housed in 14×25×47 cm plastic cages on a 12-hr diurnal cycle. Food (Purina Rat Lab Chow) and water were available ad lib throughout the experiment. The rats weighed 250–350 g at the time of electrode implantation.

### *Apparatus*

ICSS was tested in a 30×30 cm plywood cage with one Plexiglas wall through which the rat was observed. A lever was situated on the wall opposite the Plexiglas wall, 6.5 cm

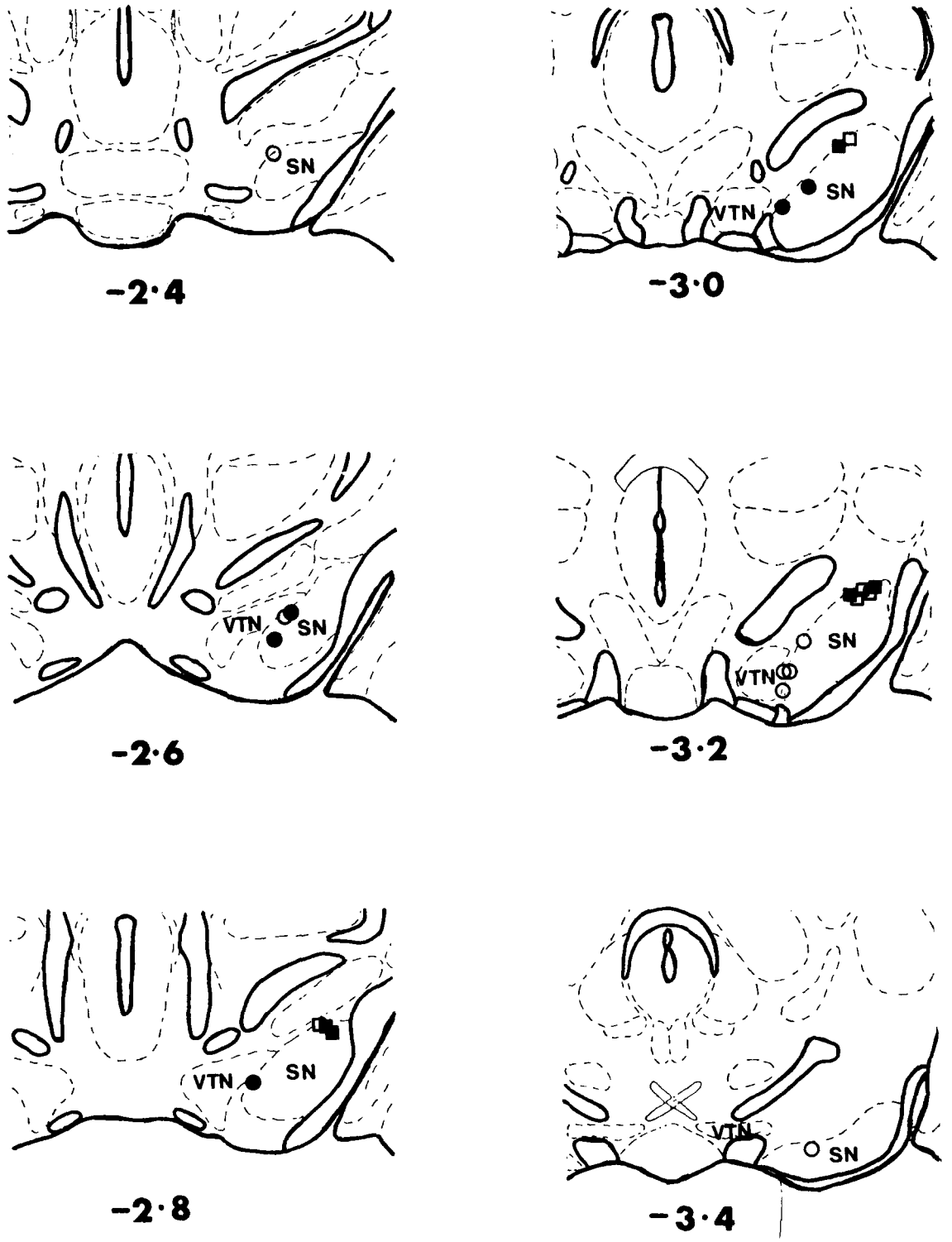


FIG. 1. Self-stimulation sites in the medial SN (circles) and lateral SN (squares). Shaded symbols represent sites in rats which received L-amphetamine. Open symbols represent sites in rats which received D-amphetamine.

above the floor of the cage, and could be depressed with a force of approximately 16 g. Lever presses delivered 0.2 sec trains of 0.2 msec, 100 Hz monophasic pulses to the rat's implanted electrode via a lead and a mercury commutator. Stimulation current was monitored on an oscilliscope. A 0.1 $\mu$F capacitor was connected in series with the rat to prevent electrode polarization.

*Surgery*

A twisted bipolar electrode (Plastic Products, Roanoke, VA) 127 $\mu$m in diameter was implanted under sodium pentobarbitol anaesthesia (Nembutal 50 mg/kg). Electrode tips were aimed at the SN pars compacta (SNC) of the right hemisphere of each animal. Coordinates [24] for the medial group were 3.2 mm posterior to bregma, 1.9 mm lateral to the midline suture and 8.6 mm ventral to the dorsal surface of the skull. For the lateral group they were 3.2 mm posterior, 2.6 mm lateral and 8.0 mm ventral.

*ICSS Procedure*

*Training.* Five to 7 days following surgery rats were placed in the ICSS testing box and shaped to self-stimulate. When self-stimulation began, rats were given 5–10 additional sessions to stablize their performance. On the last stabilization session the minimum current needed for each rat to maintain ICSS (lower threshold) was determined by successively decreasing current levels by 25 $\mu$A, at 5 min intervals, until the rat stopped responding for 5 min. Ten daily shaping attempts were made on rats failing to self-stimulate.

*Testing.* The day after the last ICSS session (Day 1) a rate-intensity function was determined for each rat by measuring the rate of ICSS at current levels of 35, 70, 140, 280 and 560 $\mu$A above threshold. Each current level was tested for 10 min. When current was changed rats were allowed to respond for 2 min at the new current levels before testing resumed. The order of testing of current levels was randomized.

Half the rats in the medial and lateral groups were to be given D-amphetamine (0.1–7.0 mg/kg) and the other half L-amphetamine (0.3–9.0 mg/kg). D- and L-amphetamine sulphate (doses expressed as the salt) were dissolved in a 0.9% saline solution and administered IP in a volume of 1 ml/kg. Drugs were tested in ascending doses beginning at the lowest dose. Forty-eight hours separated drugs tests for doses up to 2 mg/kg and 72 hours separated drug tests for doses above 2 mg/kg.

On drug test days each rat was allowed to self-stimulate for 30 min, during which time bar-pressing rates were recorded. Based on the rate-intensity data current was set at 140 $\mu$A above threshold to allow for both increases and decreases in ICSS rates. The rats then received an injection of either D- or L-amphetamine (depending on their drug grouping). Rats were then allowed to self-stimulate for a further 30 min and the rate of ICSS was recorded. On non-drug test days rats were given a 45 min ICSS session, drug free.

When testing was complete rats were killed under sodium pentobarbital anaesthesia and thionin stained sections of the brains were prepared.

## RESULTS

*Histology*

Figure 1 shows the distribution of electrode tips in self-stimulating rats. All rats which self-stimulated (n=22) had electrodes with tips in the target region of the SN pars compacta. One medial and one lateral rat had electrodes in the SN pars reticulata and did not self-stimulate. All other rats had electrodes dorsal to the substantia nigra. Of the medial self-stimulators, 7 rats received D-amphetamine and 5 received L-amphetamine. Of the lateral self-stimulators, 5 rats received D-amphetamine and 5 received L-amphetamine.

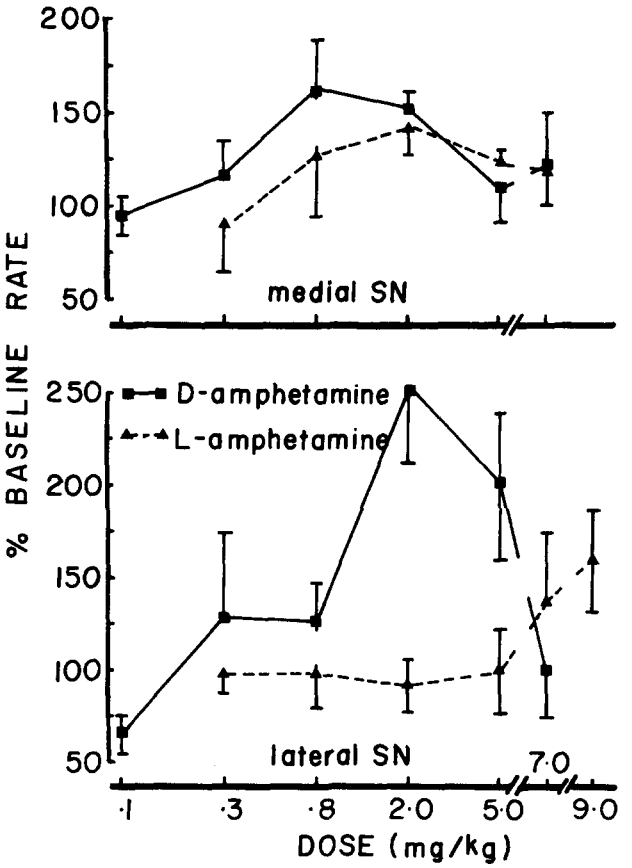


FIG. 2. Dose response curves for D- and L-amphetamine on self-stimulation of the medial and lateral substantia nigra.

*ICSS*

Results from the rate intensity function obtained during the first phase of ICSS testing showed no significant group effect or group × current interaction, $F(1,20)=0.89$, $p=0.3564$, and $F(4,80)=1.13$, $p=0.3502$. Animals in both the medial and lateral groups increased their response rates as current was increased. Rates of responding of rats in the medial and lateral groups did not differ significantly at any of the current levels tested, $F(1,20)=0.89$, NS.

ICSS response rates under D- and L-amphetamine were expressed as a percentage of the pre-drug rate. A 2×2×5 analysis of variance comparing group × drug × dose was carried out on the percentage of pre-drug responding. It should be noted that since the highest and lowest doses for D- and L-amphetamine were different, the doses used in this analysis were only the doses common to both drugs, i.e., 0.3, 0.8, 2, 5 and 7 mg/kg.

There was found to be a significant group × drug × dose

interaction, $F(4,80)=2.98$, $p=0.0241$. As can be seen in Fig. 2, in the lateral group, D-amphetamine increased ICSS rates above baseline with a peak effect at 2 mg/kg, $T=0$, $p<0.05$ (Wilcoxon 1-tail test), which dose-dependently decreased and reached baseline levels at 7 mg/kg. L-amphetamine, in the lateral group, had no effect until 7 mg/kg where it was facilitatory (see Fig. 2). In the medial group 0.8 mg/kg D-amphetamine and 2 mg/kg L-amphetamine produced peak effects and significantly increased responding above baseline, $T=1$, $T=0$, $p$'s$<0.05$. Within group comparisons in the medial group showed that there were no significant differences between the effects of the isomers at any of the doses. Within drug comparisons showed that at 2 mg/kg ICSS rates for the lateral group were enhanced significantly more by D-amphetamine, than were ICSS rates for the medical group, $t(10)=2.3$, $p<0.05$.

The effects of 0.1 mg/kg D-amphetamine and 9 mg/kg L-amphetamine were analyzed separately. In the lateral group, 0.1 mg/kg D-amphetamine significantly depressed responding below baseline, $T=0$, $p<0.05$. Nine mg/kg L-amphetamine significantly increased responding above baseline in the lateral group, ($T=0$, $p<0.05$).

To test if baseline responding in medial and lateral rats differed, pre-drug rates during 2 mg/kg testing were compared. The mean bar pressing rates for medial and lateral groups were $1044\pm244$ and $1010\pm137$, respectively. A $t$-test comparing these two means showed no significant differences, $t(20)=0.12$, NS.

## DISCUSSION

In self-stimulation of the lateral SN, D-amphetamine produced a strong dose-dependent facilitation of ICSS which peaked at 2 mg/kg while D-amphetamine was ineffective below 7 mg/kg. Above 7 mg/kg L-amphetamine also increased ICSS. By contrast, in medial SN sites there was no significant difference between the effects of D- and L-amphetamine on ICSS. Both isomers moderately facilitated ICSS with the peak effect at 0.8 to 2.0 mg/kg. In both medial and lateral SN sites once peak rates were reached responding fell off at higher doses of amphetamine. The apparent depression of lateral SN ICSS produced by 0.1 mg/kg D-amphetamine may be an artifact of comparing pre- and post-injection responding under an ineffective dose of amphetamine. During training 3/6 lateral SN rats responded more slowly in the second 30 min than the first 30 min.

These dose-response curves are consistent with our previous finding that at 1.0 mg/kg D- and L-amphetamine had little effect on ICSS rates in the medial SN but showed a large D-L difference in the lateral SN [30]. The dose-response data for our medial SN groups are similar to those previously reported for ICSS in the SN [18, 21, 26]. For the lateral SN sites, the dose response curve for D-amphetamine resembles those reported for D-amphetamine on ICSS of the LH [18,26] where D-amphetamine is a very powerful stimulant of ICSS and at 1–2 mg/kg rates are increased more than 200%. However the effect of L-amphetamine on ICSS of the lateral SN does not resemble its effect in LH ICSS. In the LH, L-amphetamine, while less potent than D-amphetamine, does facilitate ICSS at doses as low as 1.0 mg/kg. In the lateral SN 9.0 mg/kg L-amphetamine was required to produce a significant facilitation of ICSS.

Taken together the pattern of D- and L-amphetamine effects does not fit with earlier interpretations [21] which have already been questioned on other grounds (see introductory section) but our present results for SN ICSS sites do bear a remarkable resemblence to recent electrophysiological data. Browder, German and Shore [2] recorded the amphetamine induced depression of the firing rate of DA cells which is believed to reflect inhibitory feedback from increased DA output [3]. They found that DA cells identified by established criteria could be divided into different types on the basis of their response to D- and L-amphetamine. Both D- and L-amphetamine equally depressed the firing rate of DA neurons in the VTA and dorsal half of the SN pars compacta though VTA neurons were more sensitive to both isomers. Neurons in the ventral SN pars compacta were sensitive to D-amphetamine but were very insensitive to L-amphetamine (0.25 to 7.0 mg/kg). Though Browder *et al.* [2] divide the SN dorsoventrally while our distinction is for the medial-lateral plane, there is an obvious parallel between the characteristics of SN ICSS and DA cell firing under amphetamine. Our electrodes are too large to localize accurately within the dorso-ventral division of the SN. However it seems reasonable to suppose that our medial electrodes might be stimulating predominantly dorsal SN pars compacta and VTA cells while the lateral electrodes simulate mainly the ventral SN pars compacta DA cells.

If the D-L differences in the SN can be explained by the involvement of different groups of DA cells the question arises as to the explanation of the relative effectiveness of amphetamine isomers on LH ICSS. One plausible hypothesis is that the LH effects represent the summed or averaged effect of the different classes of DA neurons. Indeed a simple mean of the dose-response curves given by Browder *et al.* ([2], p. 337) yields curves in which both D- and L-amphetamine have an effect in the 0.25 to 2.0 mg/kg dose range with D-amphetamine about twice as potent as L-amphetamine. These composite curves resemble the ICSS results for the LH but are quite different from those for the SN. The notion that LH ICSS characteristics represent an average of several patterns of response to amphetamine is consistent with findings that the amphetamine sensitivity of LH ICSS differs from rat to rat [4,29].

The fact that amphetamine effects on ICSS vary systematically with the position of the stimulating electrode shows that there is an important relationship between the reward substrate and the DA neurons. When a self stimulating animal is given amphetamine, DA release will be increased in many regions of the brain but the consequence of this depends on the particular sites of the ICSS electrode. This implies that the interaction between DA release and brain stimulation reward occurs at different places for different ICSS sites. Thus at least part of the putative reward system must be topographically organized and in register with the DA system. Since there is strong evidence that DA neurons are not the reward substrate directly stimulated by ICSS electrodes [1,17], the interaction between DA release and brain stimulation reward probably takes place at DA terminal regions rather than at the electrode sites. This would be consistent with the fact that amphetamine is effective on ICSS at sites remote from the DA projections. Finally the notion that there is topographic correspondence between DA projections and the ICSS substrate is also supported by recent evidence that while LH and VTA ICSS can be facilitated by microinjections of DA into the striatum and nucleus accumbens the effective sites are restricted to localized regions which are different for different ICSS sites [28].

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