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Effects of d- and l-Amphetamine on Dorsal and Ventral Hypothalamic Self-Stimulation in Three Inbred Strains of Mice

PIERRE CAZALA

Laboratoire de Psychophysiologie, Université de Bordeaux I, Avenue des Facultés
33405 Talence, France

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CAZALA, P. *Effects of d- and l-amphetamine on dorsal and ventral hypothalamic self-stimulation in three inbred strains of mice.* PHARMAC. BIOCHEM. BEHAV. 5(5) 505–510, 1976. – The effects of intraperitoneal injections of increasing doses of d- and l-amphetamine on self-stimulation behaviour in dorsal and ventral hypothalamic areas, were studied in BALB/c Orl., DBA/2 Orl, and C57BL/6 Orl inbred mice. Both isomers improved and disrupted self-stimulation as a function of the doses injected. However, the improvements obtained with d-amphetamine were higher than those obtained with l-amphetamine. In contrast the l isomer generally provoked the highest disruptions. In addition, the three strains presented various sensitivities to d-amphetamine, which improved self-stimulation first in BALB/c (0.25 and 0.50 mg/kg), then in DBA/2 (0.50 and 1.0 mg/kg) and finally in C57BL/6 (2.0, 4.0 and 8.0 mg/kg). The dorsal hypothalamic self-stimulation system presented a greater sensitivity to d-amphetamine than the ventral system; while the two reward systems reacted identically to l-amphetamine. The differential effects observed are set into relationship with neurochemical data concerning the central catecholaminergic mechanisms.

Self-stimulation Hypothalamus d- and l-amphetamine Mouse BALB/c DBA/2 C57BL/6

THE INTRACRANIAL self-stimulation behaviour (ICSS) of mice implanted in the lateral hypothalamus (LH) is strain dependant: BALB/c strain in which ICSS is always more intense than in DBA/2 and C57BL/6, presents the lowest ICSS thresholds, and the lowest frequency of seizures [6,8].

In addition, in the same strains, ICSS performances in dorsal LH are always higher than in ventral region. Finally, dorsal LH presents the lowest ICSS thresholds and the weakest sensitivity to seizures [6,7].

These behavioural differences probably correspond to neuroanatomical and, perhaps, neurochemical differences. Indeed, on the one hand, correlations have already been observed in genetic, between behavioural and neurochemical data [23,26], on the other, LH presents a very heterogeneous distribution of the catecholaminergic pathways [22,39], which seem to play a great role in ICSS behaviour [20].

To test hypothesis of neurochemical differences, we have studied in a first experiment the effects of d- and l-amphetamine on dorsal and ventral hypothalamic ICSS in BALB/c, DBA/2 and C57BL/6 mice. It is well known, in the rat, that amphetamine which acts both on release and uptake of cerebral catecholamines [5,33] lowers the ICSS thresholds [36] and improves the performances [35]. The degree of improvement is however dependent on the isomer used [4, 16, 28, 29, 35]. This phenomenon could be attributed to a differential action of the two isomers on both noradrenergic and dopaminergic mechanisms.

MATERIALS AND METHOD

Animals

One hundred and sixteen DBA/2 Orl (N=40), BALB/C Orl (N=40) and C57BL/6 Orl (N=36) male mice were tested. The animals were operated upon when nine weeks old.

Surgery

Under deep anesthesia by sodium thiopental (DBA/2 130 mg/kg, BALB/c 90 mg/kg, C57BL/6 70 mg/kg) the animals were stereotaxically implanted with a bipolar electrode made by tightly twisting two strands of 0.09 mm platinum wire.

For each strain, the animals were splitted in two groups. In the first, the electrode was implanted into dorsal LH; in the other it was implanted into ventral LH. The stereotaxic coordinates used have been previously indicated [6].

Apparatus and Experimental Procedure

The material used had already been described [6,7]. We used four identical cages, each having two levers on the

same side. These levers could either be partly or completely separated by a removable partition's device.

One of the levers was linked to the stimulator. Pressing on it triggered a central electrical stimulation of 0.2 sec (100 HZ, sinewave); the other lever, not linked to the stimulator indicated the operant behavior of the animal.

After acquisition and stabilization of ICSS performances using low level intensities, ICSS thresholds were determined using 1 μA steps [7].

The performances were stabilized for all animals at a similar level by multiplying the dorsal thresholds values by 1.10 and the ventral thresholds values by 1.25 [7] (Table 1).

TABLE 1

CURRENT INTENSITIES AND SELF-STIMULATION RATES DURING THE PREINJECTION PERIOD

Mouse strain	Group	Intensity (μA)	Presses (20 min.)
BALB/c	Dorsal	11 ± 1*	525 ± 66*
	Ventral	18 ± 2	554 ± 76
DBA/2	Dorsal	13 ± 1	611 ± 85
	Ventral	20 ± 2	565 ± 74
C57BL/6	Dorsal	17 ± 1	543 ± 52
	Ventral	26 ± 2	494 ± 55

*mean ± SEM.

Every other day, the animals self-stimulated. Twenty minutes after the beginning of each session, they received an intraperitoneal injection of either d-, or l-amphetamine, or isotonic NaCl. Soon after the injection the animals were replaced into the ICSS cage for a two hour period.

The doses of d- and l-amphetamine injected were identical. However, for each strain injections were interrupted as soon as motor disturbance was observed. This occurred for BALB/c at 1.0 mg/kg; for DBA/2 at 2.0 mg/kg and for C57BL/6 at 8.0 mg/kg.

Histological Control

At the end of the experiment the animals were sacrificed and their brains fixed into a 10% Formalin solution. Frontal frozen sections of 40 μm were performed and stained using a $-1°/_{oo}$ thionin solution.

Statistical Test

The Student's *t*-test was used (paired test)

RESULTS

Among the 116 implanted mice, 106 presented ICSS behaviour. Effects of d-amphetamine were examined in 48 mice (16 BALB/c, 16 DBA/2, 16 C56BL/6); those of l-amphetamine were studied in 36 animals (12 BALB/c, 12 DBA/2, 12 C57BL/6). The other animals were used as controls; their stable performances are not indicated on the graphs.

Effects of d-amphetamine

The first effect of d-amphetamine (Fig. 1) was a generally brief but statistically significant improvement of ICSS performances: at 0.25 ($p<0.01$) and 0.50 mg/kg

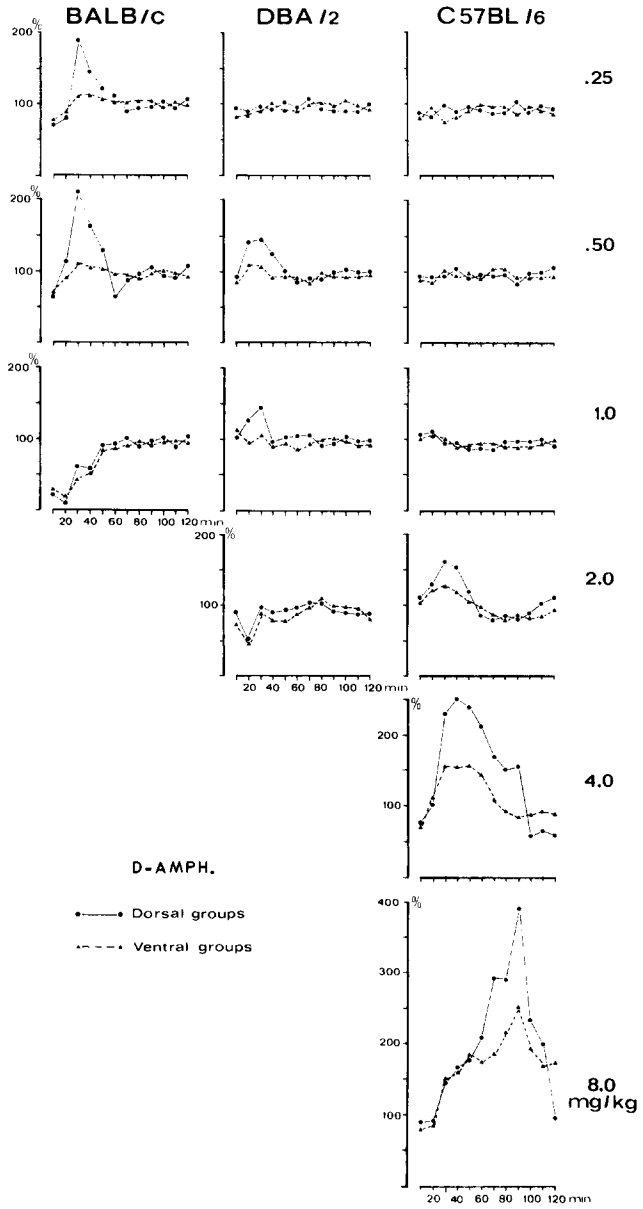

FIG. 1. Effects of injection of increasing doses of d-amphetamine on dorsal and ventral hypothalamic self-stimulation in BALB/c, DBA/2 and C57BL/6 mice strains. Abscissae: the time in min. Ordinate: the mean of lever pressing in 10 min expressed in percentage of the pre-injection scores.

($p<0.001$) in BALB/c; at 0.50 ($p<0.02$) and 1.0 mg/kg ($p = 0.05$) in DBA/2; finally at 2.0 ($p<0.02$), 4.0 ($p<0.001$) and 8.0 mg/kg ($p<0.001$) in C57BL/6.

With 1.0 mg/kg in BALB/c and 2.0 mg/kg in DBA/2 d-amphetamine depressed ICSS performances. This effect was not observed in C57BL/6 which exhibited only some stereotypy at 4.0 and 8.0 mg/kg.

These improvements were only observed in the dorsal LH with BALB/c and DBA/2 strains. Likewise, in C57BL/6 an improvement was firstly observed in the dorsal hypothalamic area (2.0 mg/kg). Then, at 4.0 and 8.0 mg/kg an enhancement appeared in ventral hypothalamic area, but it

was always lower than the dorsal improvement. (The statistical comparison of the gains in dorsal and ventral groups for the points which represented the maximum enhancement, indicated for 4.0 mg/kg, $p<0.01$, and for 8.0 mg/kg, $p<0.02$).

Effects of l-Amphetamine

Contrary to d-amphetamine, the l isomer little improved ICSS performances (Fig. 2).

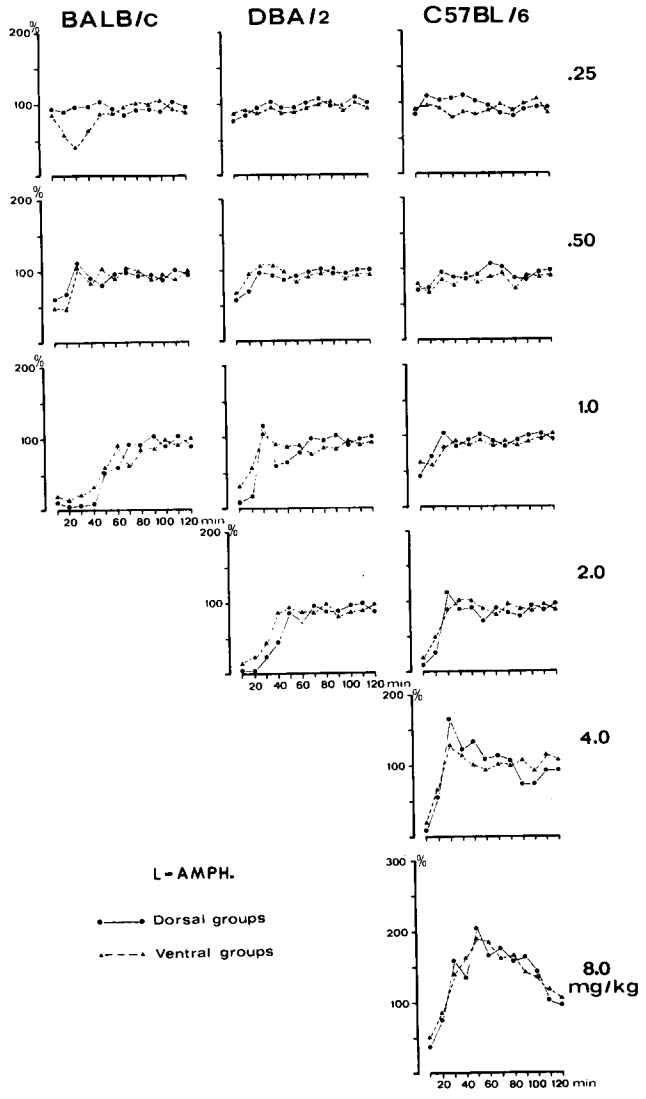

FIG. 2. Effects of injection of increasing doses of l-amphetamine on dorsal and ventral hypothalamic self-stimulation in BALB/c, DBA/2 and C57BL/6 mice strains. Abscissae: the time in min. Ordinate: the mean of lever pressing in 10 min expressed in percentage of the pre-injection scores.

No statistically significant improvement was observed in BALB/c and DBA/2 strains, whatever the stimulated area may be. Only C57BL/6 showed at 4.0 and 8.0 mg/kg an identical enhancement in the two hypothalamic areas.

In the three strains l-amphetamine had a more important disturbing effect than d-amphetamine. We showed specially in DBA/2 strain (2.0 mg/kg) that 30 min after d-amphetamine injection, ICSS performances were returned to pre-injection level in the two groups of implantations; whereas 30 min after the injection of l-amphetamine the performances did not exceeded 50% of the pre-injection level (Decline of ICSS performances was still statistically significant in the dorsal group: $p<0.01$, and in the ventral group: $p<0.02$). In C57BL/6 a brief disruption appeared immediately after the injection (2.0, 4.0, 8.0 mg/kg). This impairment was not observed with d-amphetamine.

Histological Control

As in our previous experiment [6] dorsal and ventral areas of implantation appear identical in the three strains. Electrode tips are situated dorso-laterally to the fornix in the dorsal groups (Fig. 3 A) and ventro-laterally to the fornix in the ventral groups (Fig. 3 B).

DISCUSSION

If it is now accepted that brain catecholamines are involved in the central action of amphetamine [5, 18, 33] the respective effects of the two isomers on dopamine (DA) and noradrenaline (NA) are very controversial.

In initial biochemical data, d-amphetamine was reported to act more strongly than l isomer on release [1] and inhibition of uptake [13,37] of NA; whereas d- and l-amphetamine had an identical inhibitive effect on the uptake of DA. These results had been used to explain some observations obtained in ICSS [4, 16, 28, 29].

Unfortunately, these first biochemical data were quickly nullified [17, 21, 38]. The d- and l-amphetamine were equipotent on NA uptake inhibition, whereas d-amphetamine was 4 times more potent than l isomer to block DA uptake. Finally, Chiueh and Moore [9,10] showed that d-amphetamine caused a greater release of DA than of NA. These results seemed to be confirmed by some behavioural and electrophysiological data, which indicated on the one hand that d-amphetamine seemed to act through the intermediary of DA [2, 3, 12, 31], and on the other, that NA was more involved in the action of l than in that of d-amphetamine [3,34].

Our results seem to confirm that the central action of the two isomers is dependant to different neurochemical controls: (1) The rare improvements of performances obtained with l-amphetamine in C57BL/6 are, especially in the dorsal LH area, clearly lower to those obtained with an identical dose of d-amphetamine; which confirms the observations of previous authors on ICSS in the rat[4, 16, 28, 29, 35]. (2) In addition, in C57BL/6, l-amphetamine improves identically, ICSS in the two LH areas, whereas d-amphetamine always preferentially stimulates the dorsal hypothalamic ICSS system in the three strains.

The dorsal improvements obtained, in BALB/c and DBA/2, with the lowest doses of d-amphetamine suggest a specific action of this isomer on certain ICSS mechanisms without effect on the general activity of the animal. This hypothesis already formulated by other authors [4,35] seems to be confirmed by the results obtained with C57BL/6. This strain which presents, at first a preferential effect of d-amphetamine on dorsal ICSS (2.0 mg/kg), presents then an improvement of ventral ICSS. This ventral improvement may be probably the product of the solicitation of mechanisms different of those involved in ICSS,

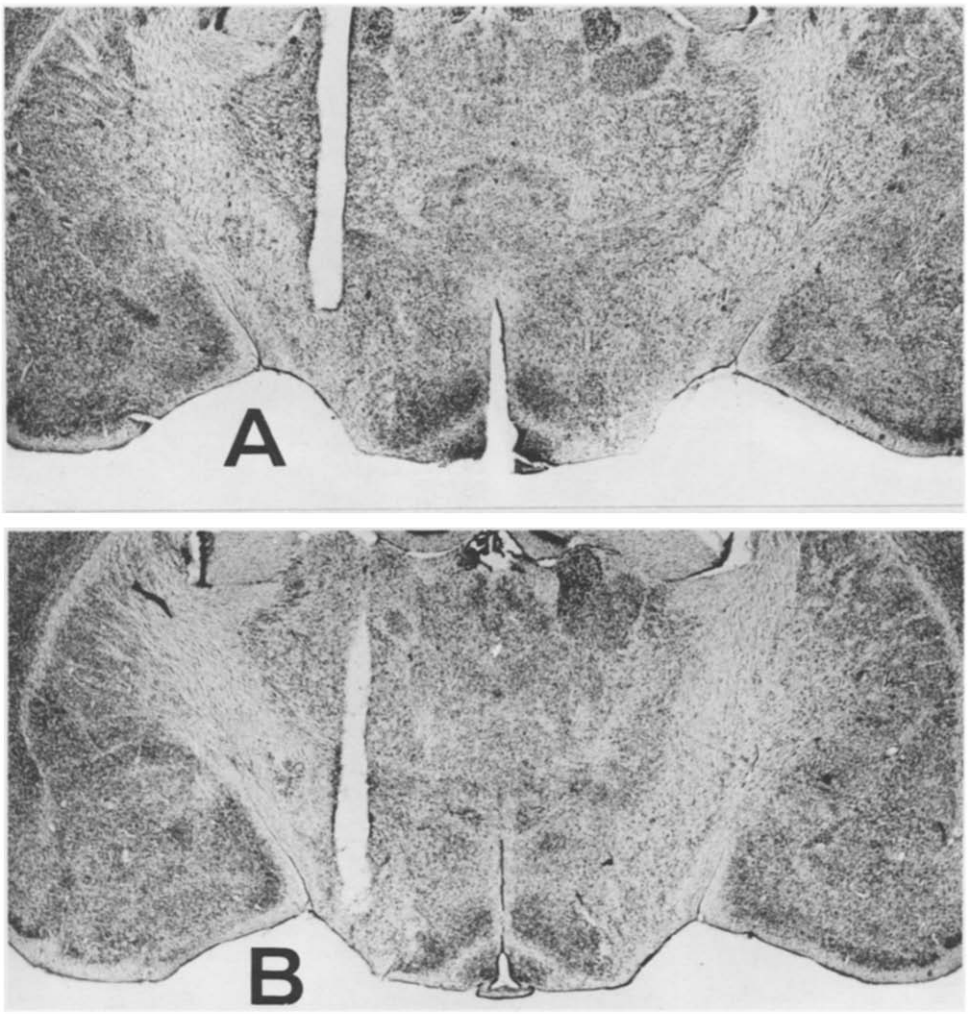

FIG.3. Photomicrographs of thionin stained sections (40 μm) through dorsal LH (3A) and ventral LH (3B) electrode tracts.

since it appears with the highest doses of amphetamine (4.0 and 8.0 mg/kg) which release stereotypies.

The specific action of d-amphetamine on the dorsal ICSS system can be explained if we consider that this isomer acts preferentially on DA. Histochemical fluroescence studies indicate that the pathways of the fibres of dopaminergic cell bodies A 9 (substantia nigra) and perhaps A 10 (mesocorticolimbic system) [22, 39] pass through the dorso lateral area of the hypothalamus. Lesions of the substantia nigra suppress ICSS in dorsal LH, whereas ventral ICSS is little disturbed [30].

Dorsal hypothalamic ICSS must then present an important dopaminergic componant on which probably d-amphetamine acts.

Among the three strains of mice studied, there are important differences of sensitivity to d-amphetamine. The BALB/c strain shows an earlier and more intense sensitivity than DBA/2; C57BL/6 presents the highest threshold of improvement. Thus, as already observed by several authors [25,27], genetic factors influence the effects of amphetamine. This change of sensitivity suggests that the central catecholaminergic regulations are different in these three strains. Now, the activity of tyrosine hydroxylase, which is involved in catecholamine biosynthesis, is twice as high in the brain of BALB/c as in the brain of DBA/2 [11]. If the literature does not give any information concerning C57BL/6, it is known however that in C57BL/10 which is genetically related, the enzymatic activity is slightly lower than in DBA/2 [11]. There seems therefore to exist a direct correlation between the enzyme activity of catecholamine biosynthesis, the ICSS performance levels [6], and the sensitivity of the three strains to amphetamine.

Finally, ICSS is disturbed in the three strains by the progressive increase of doses of d- and l-amphetamine. In DBA/2 and in BALB/c a decline of performances is observed, corresponding to a complete immobility of the animals starting a few minutes after the injection. C57BL/6 only shows sterotyped movements like those observed in the rat [15, 32, 40].

The important hypoactivity exhibited by BALB/c is difficult to explain. Some authors have already observed during studies on exploratory activity [14, 25, 27], that amphetamine does not provoque behavioural inhibition in all strains of mice; and that in a strain as predisposed as

BALB/c the influence of amphetamine on activity can fluctuate specially as a function of the doses injected [24] or of the injection procedure [25].

However, since the depressant effect of l-amphetamine is more important than that of d isomer, we can suppose that this effect is central in origin, since both isomers are equal in potency in the periphery [19,35].

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