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Repeated stress increases locomotor response to amphetamine

Jean-Paul Herman, Louis Stinus, and Michel Le Moal

Laboratoire de Psychobiologie des Comportements Adaptatifs, INSERM U259, Domaine de Carreire, Rue Camille Saint-Saens, F-33077 Bordeaux, France

Abstract. Adult male rats submitted to mild, 20 min electric foot shock sessions for 10 days displayed an enhanced locomotor response to 0.75 mg/kg (+)amphetamine 24 h after the last shock session, when compared to non-stressed controls. This effect was still present in rats specifically deprived of their forebrain noradrenergic innervation, suggesting the involvement of a dopaminergic mechanism. Cortical and limbic dopamine turnover which increased immediately after acute and repeated foot shocks returned to normal 24 h later, at the time of the pharmacological testing. This fact indicates that a permanent modification of the basal DA activity is not responsible for the above effect of stress. Locomotor hyperactivity produced by 0.6 mg/kg apomorphine was enhanced in experimental animals, while hypoactivity resulting from the injection of 0.05 mg/kg apomorphine was similar in control and shocked rats. This latter result suggests the existence of an increased postsynaptic DA sensitivity as a result of repeated stress.

Key words: Stress – Amphetamine – Locomotor activity – Dopamine – Rat

Previous biochemical measurements have shown that acute stress induces an activation of the mesocorticolimbic dopaminergic (DA) system (Fadda et al. 1978; Lavielle et al. 1978; Herman et al. 1982), while the nigrostriatal DA neurons seem to be unresponsive in these conditions. Although this biochemical activation disappears rapidly after the cessation of stressful stimuli (Herman et al. in preparation) it is possible that some latent modifications exist in the functioning of these neurons or in some neural system connected to them. This hypothesis is supported by previous work (Herman et al. 1982) showing that the simple presentation of stimuli previously associated with stress can elicit the biochemical activation of the mesocortical DA neurons. Furthermore Antelman and Chiodo (1983) showed that the repeated exposure of rats to different stressors increases the stereotypy response to (+)amphetamine. The aim of the present study was to determine whether some long-term modification could be revealed within the mesocorticolimbic DA system itself following repeated stress achieved by daily exposure of rats to electric foot shocks. In order to demonstrate a possible modification in the functioning of the mesocorticolimbic DA system, (+)amphetamine was used at a low dose inducing locomotor hyperactivity which corresponds to the activation of these neurons (Kelly et al. 1977). The existence of postsynaptic receptor modifications has also been tested with a direct DA agonist, apomorphine.

Offprint requests to: J.-P. Herman

Materials and methods

Animals. Male Wistar rats (Iffa-Credo, Lyon) weighing 230–250 g at the beginning of the experiment were used. The animals were housed in plastic cages in groups of four with food and water freely available. The animal room was air-conditioned and maintained at 22° C with light on between 8.00 and 20.00 h.

Shock procedure. Rats submitted to electric foot shocks were placed in a Skinner box (Campden Instruments) housed in a sound attenuating cubicle and equipped with fan, light and a speaker connected to a white noise generator. Scrambled electric shocks (1.5 mA, 2 s, one per min) were delivered for 20 min by a constant current generator (Campden Instruments) through the stainless steel grid floor of the chamber. Control animals were placed in a separate chamber where no electric shocks were delivered. Shocks were administered between 12.00 and 18.00 h. For chronic shocks this procedure was repeated daily for 10 days. The pharmacological tests were done on the day following the last session.

Pharmacological tests. Locomotor activity was measured between 10.00 and 18.00 h in a circular corridor (10 cm wide, 140 cm long with walls 50 cm high) equipped with four photocells. Care was taken to do the successive tests for the same animals at the same time of the day. The animals were habituated for 1 h in the corridor in order to obtain a low baseline activity, then their spontaneous activity was measured for 90 min following SC injection of isotonic saline. Finally (+)amphetamine sulfate was injected at a dose of 0.75 mg/kg in 2 ml/kg saline SC and locomotor activity was measured for an additional 90 min. The same procedure was followed for the evaluation of the action of apomorphine (0.6 mg/kg in 2 ml/kg SC), except that the spontaneous activity following saline injection was measured on separate groups, apomorphine being administered directly after the habituation period.

The action of a low dose of apomorphine (0.05 mg/kg SC) was also tested. In this case the drug was injected immediately prior to the placement of the animals in the corridor in order to be able to evaluate the locomotor hypoactivity evoked by this "pre-synaptic" dose of apo-

morphine (Seeman 1980) against a high baseline activity, and the locomotor activity was recorded only for 50 min.

Surgical procedure. Lesion of the ascending noradrenergic pathway of the rats was achieved by the injection of the neurotoxin 6-hydroxydopamine (4 μg in 1 μl isotonic saline containing 0.1 mg/ml ascorbic acid) bilaterally at two different vertical levels on each side (5.7 mm posterior to the bregma, 1.5 mm lateral from the midline, 7 and 8.2 mm below the skull surface), at the level of the superior cerebellar peduncle. The animals were pre-treated 30 min before surgery with 50 mg/kg IP pargyline.

Biochemical measurement. Animals were sacrificed by decapitation and the brain structures dissected out as described earlier (Herman et al. 1982). The activity of the DA neurons was evaluated by two alternative approaches: taking dihydrophenylacetic acid (DOPAC) level as an index of neuronal activity (Roth et al. 1976) and measuring the turnover of the transmitter after the inhibition of its synthesis (Neff 1972). Catecholamines and DOPAC were measured by a radioenzymatic assay (Fekete et al. 1978). For turnover measurement animals were injected with 250 mg/kg IP DL-*a*-methyl-*p*-tyrosine methyl ester (AMPT) 90 min before sacrifice. In that case DOPAC was also measured on the animals treated with saline instead of AMPT and representing zero minute controls.

Statistical analysis. Results were analysed by mean of ANOVA followed by Newman-Keuls test for multiple comparison between means.

Results

Effects of stress on amphetamine action. The action of amphetamine on locomotor activity was tested on control rats and on rats exposed to foot shocks at three different times: before the shock procedure, 24 h after the first foot shock session and 24 h after the tenth session, the same animals being exposed repeatedly to shocks and tested at these times. No significant difference was detected between the control and shocked groups in spontaneous locomotion at any times (Fig. 1A.). On the other hand the evolution of the locomotor response to amphetamine was significantly different in the shocked and the control animals [Fig. 1B.; three-way ANOVA, Shock × Drug × Time interaction: $F(2, 44) = 3.70$, $P < 0.05$]. Although the locomotor activation evoked by amphetamine increased slightly upon the subsequent injections in the control animals (Fig. 1B.), this increase between the subsequent tests did not reached the level of significance. In contrast, the response to amphetamine in the shocked animals was significantly augmented over the sessions as shown on the Fig. 1B. This increase in response when compared to the locomotor response in the previous test session was already apparent after one shock session, with an even more marked augmentation following ten shock sessions. Therefore this latter experimental design was used for all the subsequent experiments in order to obtain a more reliable and marked effect.

Effect of the lesion of the ascending noradrenergic pathway. In order to test whether the augmentation of the action of amphetamine following repeated stress could be ascribed to

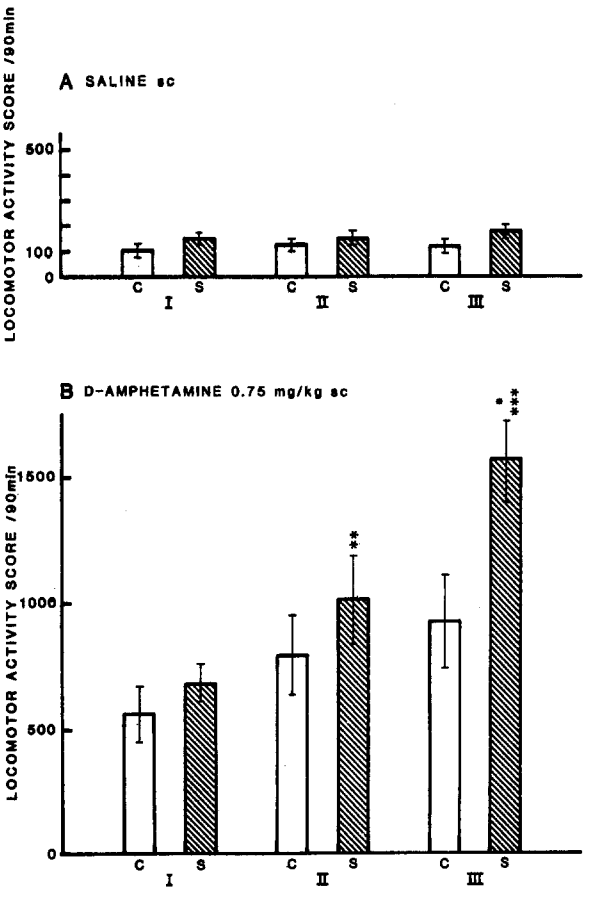

Fig. 1. Locomotor activity of shocked and control rats following the injection of saline **(A)** and 0.75 mg/kg (+)amphetamine **(B)** at three different times. Values on this and the subsequent figures represent mean ± SEM, $n = 12$. *Empty bars:* control group. *Shaded bars:* shocked group. *I.* Locomotor activity test done before the shock procedure; *II.* Locomotor activity test done 24 h after the first shock session; *III.* Locomotor activity test done 24 h after the tenth shock session; * $P < 0.005$, ** $P < 0.01$ compared to the precedent amphetamine score of the same group; *** $P < 0.005$ compared to the amphetamine score of the control group on the same day

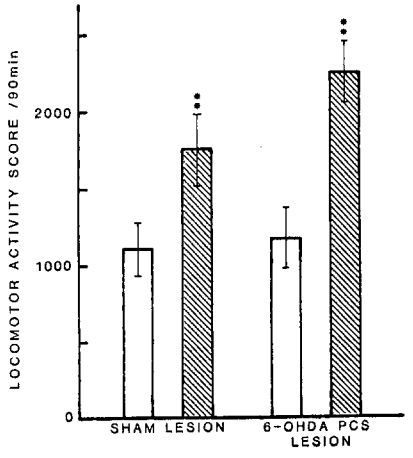

Fig. 2. Effect of the 6-hydroxydopamine lesion of ascending noradrenergic pathway on locomotor response to amphetamine in control and chronically shocked rats, 24 h after the last shock session, $n = 12$. *Empty bars:* control groups. *Shaded bars:* shocked groups. ** $P < 0.01$ shocked group compared to the respective control group

Table 1. Effect of 6-hydroxydopamine lesion of ascending noradrenergic pathway on central catecholamine levels. Values expressed in pg/mg tissue, mean ± SEM, $n = 8$. ND: non detectable

	Frontal cortex		Nucleus accumbens		Hypothalamus	
	Control	Lesion	Control	Lesion	Control	Lesion
NE	280.8 ± 18.2	5.4 ± 1.4***	346.3 ± 41.1	ND	1,919.8 ± 76.0	219.9 ± 20.3***
DA	170.7 ± 14.9	204.6 ± 21.5	7,800.0 ± 910.0	7,890.0 ± 360.0	428.0 ± 48.4	548.9 ± 33.8

*** $P < 0.001$

an interaction with the noradrenergic system, the effect of repeated foot shocks was tested in rats whose noradrenergic pathway has been lesioned 4 weeks before. As shown on Table 1 the lesion effectively destroyed the noradrenergic terminals of different forebrain areas while leaving their DA terminals virtually intact. However the shock-induced enhancement of the amphetamine response was present even in the noradrenaline-depleted animals, as shown in Fig. 2 [two-way ANOVA, Shock effect: $F(1, 44) = 18.90$, $P < 0.001$, Shock × Lesion interaction $F(1, 44) = 1.16$, n.s.].

Effects of the repeated stress on dopamine turnover. The increased amphetamine effect could reflect an increased turnover of the DA terminals of the mesocorticolimbic system at the time of the pharmacological testing. To test this hypothesis we evaluated the DA activity in these terminals by measuring DOPAC level as well as dopamine turnover 24 h after the tenth stress session. We also compared the acute DOPAC augmentation induced by the first and the tenth shock session in order to be sure that the dopaminergic activation was still present following repeated exposures of the animals to this stress.

As shown in Fig. 3 by the augmented levels of DOPAC, acute stress effectively elicited an increase of dopamine turnover in the terminals of the mesocorticolimbic system of rats sacrificed immediately after the administration of electric shocks, and this elevation of DOPAC could also be detected immediately following the 10th foot shock session [two-way ANOVA, Shock effect: $F(1, 24) = 39.63$ for the accumbens nucleus, $F(1, 24) = 60.58$ for the amygdala, $F(1, 24) = 29.10$ for the medial frontal cortex, $P < 0.001$ in all three cases]. However the magnitude of this shock-induced elevation of DOPAC concentration was diminished in the accumbens nucleus and the medial frontal cortex and this decrease reached the level of statistical significance in the latter case [Shock × Time interaction: $F(1, 24) = 4.98$, $P < 0.05$]. On the contrary 24 h after the tenth shock session – at the time of pharmacological testing – DA activity of shocked animals returned to control level as judged both from the normal DOPAC levels (Fig. 3.) and from the identical dopamine depletion in the stressed and non-stressed groups following AMPT (39% and 38% depletion respectively in the nucleus accumbens, 36% and 38% in the amygdala, 69% and 70% in the medial frontal cortex, the initial dopamine concentrations being identical in all three regions for the two groups).

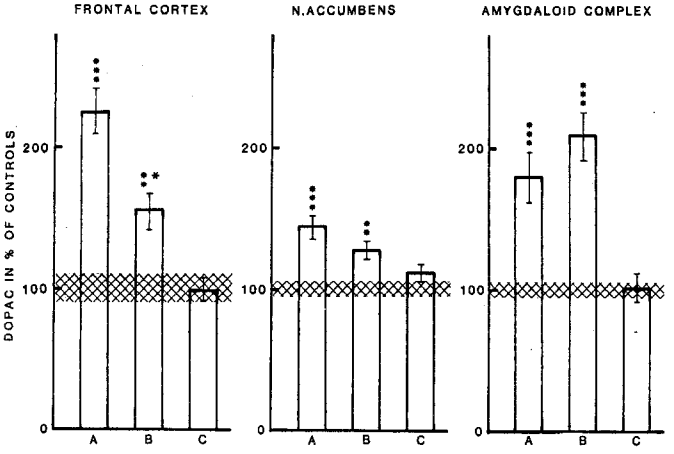

Fig. 3. DOPAC levels in terminal regions of the mesocorticolimbic pathway expressed as % of control values (Control values ± SEM being represented by the horizontal shaded bars), $n = 8$. *A.* Animals sacrificed immediately after one shock session; *B.* Animals sacrificed immediately after the tenth shock session; *C.* Animals sacrificed 24 h after the tenth shock session. *$P < 0.01$ *B* compared to *A*; **$P < 0.01$, ***$P < 0.005$ compared to control level. *Control levels:* Frontal cortex: 21.1 ± 2.1 pg/mg tissue, nucleus accumbens: 1,160.5 ± 60 pg/mg tissue, amygdaloid complex: 34.2 ± 2.1 pg/mg tissue

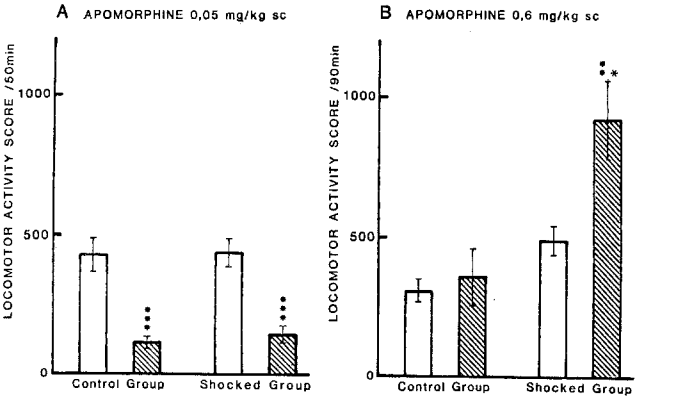

Fig. 4. Locomotor activity of stressed and non-stressed rat following the injection of 0.05 mg/kg (**A**) or 0.6 mg/kg apomorphine (**B**). Amorphine and saline were injected 24 h after the tenth shock session, $n = 5$. *Empty bars:* saline injection. *Shaded bars:* apomorphine injection. *$P < 0.01$ compared to score of control animals following apomorphine injection; **$P < 0.01$, ***$P < 0.001$ apomorphine group compared to saline group

Action of apomorphine following repeated shocks. In order to test whether a modified DA receptor sensitivity could explain the enhancement of the response to amphetamine, locomotor activity was measured following an injection of apomorphine 24 h after the tenth foot shock session. Two different doses of apomorphine were used: 0.05 mg/kg to test a modification of dopamine autoreceptors and 0.6 mg/kg to test post-synaptic dopaminergic receptor sensitivity (Seeman 1980).

In accordance with the preferential action on autoreceptors, 0.05 mg/kg apomorphine decreased the locomotor activity of the animals [Fig. 4A., two-way ANOVA, Drug effect: $F(1, 16 = 49.97)$, $P < 0.001$], and this decrease was similar in the control and shocked groups. On the other hand, the increase of the locomotor activity following the injection of 0.6 mg/kg apomorphine was significantly greater in the shocked group [Fig. 4B., two-way ANOVA, Drug effect: $F(1, 16) = 9.37$, $P < 0.01$, Drug × Shock interaction $F(1, 16) = 9.13$, $P < 0.01$].

Discussion

In the present study we have examined whether repeated stress could induce long-term modifications in the function of the mesocorticolimbic DA system. Our results show that (1) electric foot shocks increase the responsiveness of rats to the locomotor action of a low dose of *d*-amphetamine, and this phenomenon can be detected not only 24 h after a series of 10 shock session but also, although to a lesser extent, 24 h after a single shock session; (2) this effect is not related to the activation of central noradrenergic neurons nor to a change in basal DA activity or a modification of the sensitivity of the pre-synaptic dopamine receptors but (3) can be at least partly explained by an increase in the sensitivity of post-synaptic dopamine receptors. It has been shown that stress is able to enhance the stereotypy-inducing action of *d*-amphetamine (Antelman and Chiodo 1983; MacLennan and Maier 1983). Our results extend these data by showing that not only stereotypy but also locomotion, which may be considered as mediated primarily by the limbic DA system (Kelly et al. 1977), can be enhanced by stress.

The increase of the response to amphetamine seen after the tenth session could simply reflect an effect built up progressively during the time elapsed since the exposure to the first stress session, independently of the subsequent repetition of stress. In fact, a single exposure to footshock followed by a 15 day treatment-free interval before amphetamine elicits the same senzitization as measured on amphetamine-induced sniffing as that seen 24 h after the last session of a 15 day stress exposure (Antelman and Chiodo 1983). In accordance with these results, if the above-mentioned hypothesis was true we would expect in our experiments to observe an equivalent sensitization 24 h after the first and the tenth shock session. The fact that the augmentation of the amphetamine response seen 24 h after the tenth stress session was, on the contrary, significantly greater than that seen 1 day after a single session (Fig. 1) suggests, therefore, that in addition to an eventual time effect the repetition of stress also has a role in our case.

Dopaminergic activity returned to control level 24 h after the foot shock procedure, i.e., at the time of the behavioral test, suggesting that a permanent modification of basal DA activity is not responsible for the increased responsiveness to amphetamine. It is interesting to note the decrease in the biochemical response (elevation of DOPAC levels) in the limbic and cortical areas upon repeated stress, which is correlated with a decrease of the vocalization and locomotor responses to successive shocks (unpublished observation) related perhaps to a stress-induced analgesia (Bodnar et al. 1980).

The increased effect of 0.6 mg/kg apomorphine seems to indicate an increased sensitivity of the postsynaptic dopaminergic receptors. A diminution of the behaviorally competing stereotypic response to apomorphine in the shocked animals is not likely to explain this enhanced effect of apomorphine, as the control and shocked groups displayed upon direct observations a similar degree of stereotyped sniffing. On the other hand, an increased sensitivity of not the receptors but of the processes distal to the receptor itself cannot be excluded on the basis of the present results, and should be examined in further experiments.

Two further questions arise concerning the cellular mechanisms and the neuronal circuitry involved in the phenomenon described above. With respect to the cellular mechanisms, it could be hypothetized that the repeated stimulation of the DA systems is able to evoke a long-lasting increase in their reactivity to amphetamine. In the present work the stimulation of the mesocortical DA neurons was achieved by the exposure to electric shocks which augmented the locomotor response to amphetamine. In addition, at least two more phenomenona can be cited for the illustration of this hypothesis. One is the "reverse tolerance" to amphetamine, i.e., the increased responsiveness in locomotor and stereotyped responses (Segal and Mandell. 1974) to subsequent amphetamine administration, the other being the increased responses to amphetamine following repeated stimulation of the mesocorticolimbic DA neurons either by local administration of substance P (Stinus et al. 1978) or by intracranial self-stimulating electrodes (Antelman and Chiodo 1983). It is possible that a single mechanism underlies these various findings, including our results; then, in addition to the postsynaptic modification demonstrated here, a presynaptic modification may also be involved in the explanation of these results, as it has been shown that the establishment of reverse tolerance to amphetamine following its repeated administration is accompanied by a subsensitivity of presynaptic dopamine receptors (Muller and Seeman 1979; Antelman and Chiodo 1983) and – possibly as a consequence – an enhancement of the dopamine releasing action of this drug in vitro (Robinson et al. 1982). Furthermore, the comparison of pharmacological and biochemical results indicates that even when no difference can be seen in basal neuronal activity as shown by the control level of dopamine turnover 24 h after the last stress session, some latent modifications can in fact be present and be revealed by pharmacological stimulation of the neurons (amphetamine, Fig. 1), a conclusion which may have important implications for the study of the central DA systems.

As to the neuronal circuitry playing a role in the establishment of the effect of stress, one possibility may involve the substance P pathway innervating the region of the A9 and A10 DA neurons (Cuello and Kanazawa 1978). In fact, it has been shown that (1) foot shocks evoke a massive release of substance P in the ventral tegmental area where the A10 neurons are located (Lisoprawski et al. 1981), (2) the neutralization of the action of substance P through the local administration of substance P antibodies inhibits the activation of the DA system by stress (Bannon et al. 1983), (3) the repeated local administration of substance P in the A10 area, stimulating the DA neurons, evokes a subsequent enhancement of the locomotor response to amphetamine (Stinus et al. 1978). This latter

effect may be simulated by the shock procedure used here which – according to the above mentioned results – would repeatedly evoke a local substance P release and thereby increase the activity of the A10 DA neurons.

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