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Short communication

When injected into the fimbria-fornix/cingular bundle, not in the raphe, 5,7-dihydroxytryptamine prevents amphetamine-induced hyperlocomotion

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Abstract

The locomotor effects of acute amphetamine treatment (1 mg/kg, i.p.) were assessed in Long–Evans rats after 5,7-dihydroxytryptamine (5,7-DHT) injections into the fimbria-fornix/cingular bundle (FiFx/CB; 4 μg/side), or the dorsal and median raphe (Raphe; 10 μg). In control rats, amphetamine induced a significant increase of home-cage activity for about 2 h. This effect was similar in Raphe rats, but was absent in FiFx/CB rats. The raphe lesions reduced serotonin concentrations by 50% in the dorsal hippocampus, 75% in the ventral hippocampus and 58% in the fronto-parietal cortex. After FiFx/CB lesions, the reduction amounted 50, 61 and only 25%, in each of these regions, respectively. In the fronto-partietal cortex, dopamine concentration was significantly decreased in Raphe (− 27%) and FiFx/CB rats (− 65%). The results suggest that a serotonergic denervation of the hippocampus by injections of 5,7-DHT into the FiFx/CB pathways hampers the stimulating effects of amphetamine on locomotor activity. This effect might be related to the reduced dopaminergic tone in the fronto-parietal cortex.

Keywords: Amphetamine; Cingular bundle; 5,7-dihydroxytryptamine; Dopamine; Fimbria–fornix; Hippocampus; Locomotor activity; Raphe; Serotonin

Electrolytic or excitotoxic lesions of the raphe nuclei induce locomotor hyperactivity in rodents [3,12,13]. Beside other cells and fibers, these nuclei contain the cell bodies of neurons belonging to the ascending serotonergic projection system [11]. Whether damage to the serotonergic neurons in the raphe accounts for the hyperactivity was, and probably still is, in debate. When the serotonergic neurotoxin 5,7-dyhydroxytryptamine (5,7-DHT) is injected into the cerebral ventricles, a technique inducing a general serotonergic depletion in the brain, there are reports showing the activity level to be increased [15,19], decreased [17,20], or unchanged [22]. When 5,7-DHT is injected directly into the median raphe, the serotonergic lesion seems to have no effect on activity [3,18]. When the toxin is injected directly into the fimbria-fornix, a major pathway of hippocampal afferents and efferents, there seems to be an increase of nocturnal activity [27]. Williams and Azmitia [27] even reported that the level of nocturnal activity and that of hippocampal serotonin depletion were negatively correlated, an observation also made by Jacobs et al. [12] after electrolytic lesions of the raphe. Such data suggest that the serotonergic innervation of the hippocampus could play some role in the modulation of locomotor activity. That hippocampal function can be linked in some respects to locomotor activity is in line with various findings. Indeed, more

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or less extensive lesions of the hippocampus itself, or of the fimbria-fornix fibers induce hyperlocomotion [4,25]. Wilkinson et al. [26] propose this hyperactivity to result from the disruption of a mechanism involving hippocampal outputs that exert an inhibitory influence on dopaminergic neurons in the nucleus accumbens. Although very simplistic, this view is compatible with experiments showing hippocampal or fimbria-fornix lesions to potentiate amphetamine-induced locomotion [8,29]. Whether the serotonergic innervation of the hippocampus plays a role in this regulation seems also controversial. Balse et al. [4] recently reported that grafts rich in serotonergic neurons placed into the hippocampus denervated by an aspiration of the fimbria-fornix/cingular bundle (FiFx/CB) pathways reduced the lesion-induced potentiation of the effects of amphetamine on activity. Lipska et al. [17] found the locomotor reactivity towards amphetamine to be decreased after intracerebroventricular injections of 5,7-DHT. Earlier, Asin and Fibiger [3] found that 5,7-DHT injected into the median raphe produced a weak but significant potentiation of amphetamine-induced hyperlocomotion. In the present study, we injected 5,7-DHT into the FiFx/CB or the raphe in order to make a direct comparison between the effects of these two lesions on spontaneous and amphetamine-induced locomotor activity. After completion of behavioural testing, the lesion-induced effects were verified neurochemically.

All procedures involving animals were conducted according to international laws and policies.

The study used 18 Long–Evans male rats (CERJ, France) aged of about 90 days at the time of surgery. They were housed individually, with food and water ad libitum, in transparent cages ($42 \times 26 \times 15$ cm) under a 12.00:12.00 h dark–light cycle (lights on at 07:00 h) and controlled temperature (21°C). Under sodium pentobarbital (65 mg/kg, i.p., Sanofi, France) anaesthesia, rats were subjected to a bilateral injection of 8 μg of 5,7-DHT (in 0.64 μl of saline, 4 μg/side; Sigma, St Louis, USA) in the fimbria-fornix/cingular bundle (Group FiFx/CB, $n = 6$), or to an injection of 10 μg of 5,7-DHT (in 1μl) in the median and dorsal raphe (Group Raphe, $n = 6$). Saline contained 20 mg/ml ascorbic acid. Rats with injection of vehicle were used as controls (Group Sham, $n = 6$; three with vehicle in the fimbria-fornix/cingular bundle and three with vehicle in the median and dorsal raphe). Injections were performed stereotaxically through a 1 μl-Hamilton syringe at the following coordinates (in mm from Lambda [21]) : $A = +5.7$, $L = \pm 0.9$, $V = -4.0$ for the fimbria-fornix (0.2 μl/site), $A = +5.7$, $L = \pm 0.4$, $V = -2.2$ for the cingular bundle (0.12 μl/site), $A = -7.8$, $L = 0.0$, $V = -8.2$ for the median raphe, and $A = +7.8$, $L = 0.0$, $V = -6.0$ for the dorsal raphe (0.5 μl/site). The incisor bar was at 3.0 mm below the interaural line. After each injection, the needle was left in situ for 6 min. All rats were pretreated with desipramine (25 mg/kg, i.p., in saline; Sigma), 20 min before anaesthesia [5]. On uneven days, locomotor activity of the rats was determined in their home cages as previously reported [15]. On days 3 and 5, all rats were given an i.p. injection of saline (1 ml/kg) 15 min before recording was started (11:00–14:00 h). On days 7 and 9, activity scores were recorded for 3 h after an injection of D-amphetamine sulfate (1 mg/1 ml saline per kg, i.p.; Sigma) 9–11 minutes before recording was started. Four days after activity testing, all rats were again injected with 1 mg/kg amphetamine in order to allow two naive experimenters to check for stereotypies (each rat was observed for 20 minutes post-injection). The next day, all rats were sacrificed by microwave irradiation (2.0 s; 6.3 kW; Sairem, Villeurbanne, France) and their brain processed as described elsewhere [15]. Concentration of dopamine (DA), noradrenaline (NA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were measured using high performance liquid chromatography (HPLC) with electrochemical detection as previously described [15]. All data were analysed by an analysis of variance (ANOVA) followed, where appropriate, by 2×2 comparisons based on the Newman Keuls multiple range test.

Only the main neurochemical and behavioural results will be presented. The concentration of 5-HT and 5-HIAA was reduced significantly in both lesion groups in the dorsal (by about 50 and 60%, respectively) and ventral hippocampus (by about 70 and 60%, respectively, on the average: $F = 17.58$, at least; 2/15 df; $P < 0.001$), and was not altered in the striatum. In the fronto-parietal and the entorhinal cortex, the serotonergic markers were significantly reduced (by about 60%) only in Raphe rats ($F = 5.63$, at least; df 2/15; $P < 0.05$). There was a weak reduction of NA concentration in the dorsal hippocampus of FiFx/CB rats (-17%: $F = 3.88$; df 2/15; $P < 0.05$). The concentration of dopamine was reduced significantly ($F = 24.43$; 2/15 df; $P < 0.001$) in the fronto-parietal cortex of Raphe (-27%; $P < 0.01$) and FiFx/CB rats (-65%, $P < 0.001$).

The diurnal and nocturnal spontaneous activity levels were modified by neither lesion (data not illustrated). The activity scores recorded after control or amphetamine injections (Fig. 1) were analysed separately for each hour. During the first hour, there were significant lesion ($F = 3.79$; 2/15 df; $P < 0.05$), drug ($F = 13.77$; 3/45 df; $P < 0.001$), and lesion × drug interaction ($F = 2.64$; 6/45 df; $P < 0.05$) effects. The lesion effect reflected overall activity scores which were significantly lower in FiFx/CB rats than in Raphe rats ($P < 0.05$). The comparison between FiFx/CB and Sham rats only yielded a tendency ($P < 0.07$). The drug effect reflected activity scores significantly higher after the amphetamine injections as compared to saline injections ($P < 0.01$ at least). The interaction can be inter-

preted as follows: whereas Raphe rats reacted to amphetamine as the Sham rats, FiFx/CB rats failed to show a significant reaction to amphetamine.

During the second hour after the injections, there was only a significant drug ($F = 16.36$; 3/45 df; $P < 0.001$) effect. This drug effect was due to overall activity scores which were significantly higher after amphetamine injections as compared to the scores recorded after saline injections ($P < 0.01$ at least). Finally, when all mean values were compared, we again found the activity of the Sham rats to be significantly increased by each amphetamine injection as compared to either saline injection ($P < 0.01$, at least). Again, FiFx/CB rats failed to significantly respond to the drug.

During the third hour, neither of the aforementioned effects was significant. When the rats were observed for stereotypies, we could not observe any clear-cut manifestation of stereotypies (e.g. snout contact, head jerk, gnawing...; data not illustrated) (Fig. 1).

The injection of 5,7-DHT into the septohippocampal pathways induced a depletion of serotonergic markers in the hippocampus, but not in the other structures analysed. When injected into the raphe region, 5,7-DHT depleted serotonergic markers in the hippocampus, fronto-parietal and entorhinal cortices, but not in the striatum. It also reduced the concentration of DA in the fronto-parietal cortex. This effect was more pronounced in FiFx/CB rats (-65%) than in Raphe rats (-27%). Whereas Raphe rats responded to amphetamine by hyperactivity, FiFx/CB rats did not.

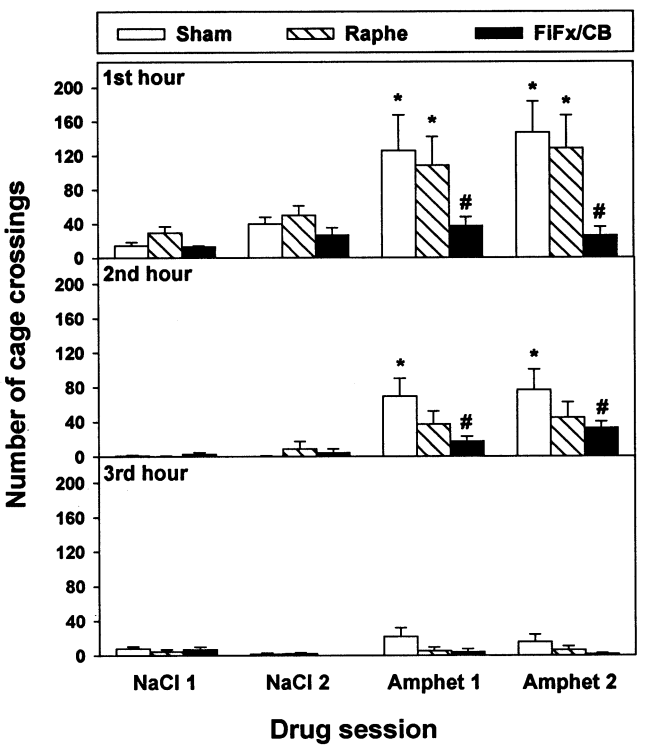

Fig. 1. Locomotor activity shown as the average (+SEM) number of cage crossings during the first (top), second (middle) and third (bottom) hours after an injection of saline (1 ml/kg, i.p.) on days 3 (NaCl 1) and 5 (NaCl 2) of the experiment, or an injection of amphetamine (1mg in 1 ml/kg, i.p.) on days 7 (Amphet 1) and 9 (Amphet 2) of the experiment (see methods). Statistical analysis: * significantly different from NaCl 1 and NaCl 2, $P < 0.05$, respectively; # significantly different from Sham and Raphe within the same drug session, $P < 0.05$.

A striking finding in this experiment is the depleted DA concentration in the fronto-parietal cortex of FiFx/CB rats and, although less pronounced, of Raphe rats. Unfortunately, with the exception of the study by Murtha and Pappas [20], in all previous studies which used injections of 5,7-DHT into the fimbria-fornix, cingular bundle or raphe, the determination of markers of cortical DAergic functions has not been performed [3,27]. In the study by Murtha and Pappas [20], the concentration of DA was not affected by the lesion in the anterior cortex, but was reduced in the posterior cortex. However, due to high variability in the control rats, this reduction was not significant. It is also noteworthy that intracerebroventricular injections of 5,7-DHT produced a DA receptor subsensitivity in the prefrontal cortex of rats [2].

Two non-exclusive possibilities might account for the decrease of the dopaminergic marker in the fronto-parietal cortex. In the absence of a DA re-uptake inhibitor treatment (e.g. nomifensine), 5,7-DHT can damage dopaminergic neurons. The present experiment did not use such a protection. Thus, one plausible explanation could be that 5,7-DHT has diffused to the fronto-parietal cortex (in FiFx/CB rats) or to dopaminergic nuclei within the mesencephalon such as A9, A10 (in raphe rats), and has damaged dopaminergic neurons or fibers. If so, the serotonergic markers should have been damaged to an extent comparable to that of the dopaminergic marker, or at least sufficient to induce a significant depletion of 5-HT concentration in the fronto-parietal cortex. This was the case in Raphe rats. However, in FiFX/CB rats, there was an about 25% reduction of 5-HT and 5-HIAA concentrations in the fronto-parietal cortex, and neither of these changes was significant. If 5,7-DHT had damaged dopaminergic neurons in the mesencephalon of Raphe rats, a region providing the cortex with dopaminergic afferents, a reduction of dopaminergic markers should also be observed in structures such as the hippocampus, the striatum or the entorhinal cortex which all are targets of mesencephalic neurons [16]. Our neurochemical data show that this is not the case. Therefore, it might be considered that the reduced DA concentration observed in the fronto-parietal cortex could be part of a physiological consequence of the serotonergic hippocampal denervation. Although the mechanism involved in such changes remains to be elucidated, our results in FiFx/CB rats would be compatible with a serotonergic control of a hippocampal influence on the dopaminergic tone in the fronto-pari-

etal cortex. This possibility does not contradict the demonstration of hippocampo-cortical and subiculo-cortical projections [1,6], but requires further studies. There is also evidence that subicular 5-HT_{1B} heteroreceptors mediate 5-HT interactions with the mesolimbic dopaminergic system by a modulation of the glutamatergic hippocampo-accumbens pathways [6]. At the level of the fronto-parietal cortex, such a mechanism might involve a direct or indirect action on the dopaminergic terminals, as dopaminergic neurons are, to our knowledge, not found in the cortex. Concerning the presynaptic control of the cortical dopaminergic tone, most work has been carried out in prefrontal cortex preparations. It is therefore difficult to further progress on that question. In the prefrontal cortex, it is established that the dopaminergic tone can be increased by a direct presynaptic action of glutamate on AMPA receptors [24], but decreased by an indirect action of glutamate on NMDA [24] or AMPA/KA [9] receptors presumably located on GABAergic interneurons. Whether a similar mechanism is also possible in other cortical structures and how it could be linked to the serotonergic innervation of the hippocampus is a question that remains open.

Non-selective lesions of the fimbria-fornix or the hippocampus induce locomotor hyperactivity and potentiate the locomotor response to amphetamine [22,26]. In a recent experiment using rats with extensive lesions of the septohippocampal pathways, intrahippocampal grafts rich in serotonergic neurons were found to abolish the lesion-induced potentiation of the locomotor response to amphetamine [4]. The conclusion was that the serotonergic innervation of the hippocampus could modulate the locomotor responsiveness to amphetamine. From these results, we expected that a 5,7-DHT-induced lesion of the serotonergic innervation of the hippocampus would enhance the locomotor response to amphetamine. Although such an expectation is in line with the weak effects of amphetamine reported by Asin and Fibiger [3] in rats with 5,7-DHT injected into the median raphe, it contradicts the study by Lipska et al. [17] who used intracerebroventricular injections of 5,7-DHT, and our present findings: rats given 5,7-DHT into the raphe responded normally to amphetamine, whilst those with 5,7-DHT injected into the septohippocampal pathways did not. To account for the discrepancy of the latter observation with the proposal made by Balse et al. [4], one may highlight a major difference in the type of lesions used in the respective studies. In the Balse et al. study, the septohippocampal pathways were aspirated, whereby cholinergic, noradrenergic and serotonergic hippocampal afferents were damaged [10], but also cortical territories and hippocampal efferents. The latter include those which are supposed to exert an indirect inhibitory control over the dopaminergic tone in the nucleus accumbens [26,28]. In the present study, only part of the serotonergic hippocampal afferents of FiFx/CB rats were affected and cortical structures were virtually intact.

The attenuated responsiveness to amphetamine found in FiFx/CB rats cannot be ascribed without further qualification to the reduced serotonergic innervation of the hippocampus. Indeed, such a reduction was also observed in Raphe rats. If one assumes that the reduction of the cortical dopaminergic tone is not a direct effect of 5,7-DHT and may account for the abolished locomotor responsiveness to amphetamine, it seems that there may be a threshold-level under which the concentration of DA in the fronto-parietal cortex must fall before the locomotor response towards amphetamine begins to be attenuated. This account would be in line with the observation that the DA concentration was reduced by only 27% in the Raphe rats against 65% in the FiFx/CB rats. We are not aware about studies which, investigated the effects of DA depletion in the fronto-parietal cortex on locomotor reactivity to amphetamine injections. When DA is depleted in the prefrontal cortex with local injections of 6-hydroxydopamine, there is no change in spontaneous activity and amphetamine-induced hyperactivity is increased [23] or unchanged [14]. Conversely, when DA is depleted in the nucleus accumbens, the response to amphetamine is attenuated [7,14]. Interestingly, in rats given 6-hydroxydopamine in the nucleus accumbens, there is also evidence for reduced dopaminergic activity in the medial prefrontal cortex [7]. Unfortunately, our dissection of the striatal region did not distinguish the caudate/putamen region from the nucleus accumbens, and thus did not allow to check for possible changes in the nucleus accumbens.

In conclusion, we have shown that a serotonergic denervation of the hippocampus by injections of 5,7-DHT into the fimbria-fornix/cingular bundle abolishes or attenuates the stimulating effects of amphetamine on locomotor activity. Such an effect, which might be related to the reduced dopaminergic tone in the fronto-parietal cortex by a mechanism that remains to be elucidated, does not appear after injections of 5,7-DHT into the mesencephalic raphe.

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