EVIDENCE OF A COMPLETE INDEPENDENCE OF THE NEUROBIOLOGICAL SUBSTRATES FOR THE INDUCTION AND EXPRESSION OF BEHAVIORAL SENSITIZATION TO AMPHETAMINE

M. CADOR,* Y. BJIOU and L. STINUS

Unité INSERM 259, rue Camille Saint-Saëns, Domaine de Carrière, Université de Bordeaux II, 33077 Bordeaux Cedex, France

Abstract—The repeated administration of amphetamine in rats produces behavioral sensitization which is characterized either by a progressive enhancement of the locomotor activity induced by the drug or by an enduring behavioral hypersensitivity to the drug after the cessation of the treatment. Some authors have suggested that the action of amphetamine at the level of the nucleus accumbens is responsible for the expression of behavioral sensitization, whereas the action of amphetamine at the level of the dopamine cell bodies in the ventral tegmental area induces some changes responsible for the initiation of the phenomenon. The present study fully tested this hypothesis. In two separate experiments, the effects of different doses of amphetamine repeatedly administered in the ventral tegmental area or in the nucleus accumbens were tested on the later behavioral reactivity to the administration of amphetamine in the nucleus accumbens. Independent groups of rats received five repeated administrations (one injection every other day) of different doses of amphetamine either in the ventral tegmental area (0, 1, 2.5, 5 μg/0.5 μl per side) or in the nucleus accumbens (0, 1, 3, 10 μg/1 μl per side). Two days following the last intracerebral amphetamine injection, each group received a phosphate buffer solution challenge directly into the nucleus accumbens followed two days later by an amphetamine challenge (1 μg/1 μl per side) in the nucleus accumbens and two days later by a peripheral challenge with amphetamine (0.5 mg/kg, s.c.). Locomotor responses were recorded following each injection. Results showed that injections of amphetamine into the nucleus accumbens induced a dose-dependent increase in locomotor activity which remained identical with the repetition of the injections. No difference between the different intra-accum-bens pretreated groups was observed following the diverse phosphate-buffered saline solution and amphetamine challenges. In contrast, intra-ventral tegmental area administration of amphetamine did not produce any modification of locomotor activity. However, whereas no difference between the differently pretreated groups was observed following phosphate-buffered saline administration into the nucleus accumbens, a potentiation of the locomotor response to a challenge dose of amphetamine into the nucleus accumbens was observed which was dependent on the dose of amphetamine pretreatment into the ventral tegmental area. Similar potentiation was observed following peripheral challenge with amphetamine. Finally, cross-sensitization was observed when a challenge dose of cocaine (10 μg/1 μl per side) was injected into the nucleus accumbens, as well as when a peripheral challenge dose of morphine (2.5 mg/kg, s.c.) was administered to the ventral tegmental area-pretreated groups.

Altogether, these results demonstrate that an amphetamine action solely at the level of the dopamine cell bodies in the ventral tegmental area is necessary and sufficient to promote changes subserving behavioral sensitization, which can be later revealed by an amphetamine action at the level of the dopamine terminals in the nucleus accumbens. On the contrary, the sole amphetamine action at the level of the nucleus accumbens is not sufficient to promote these changes but is necessary to allow their expression. These findings argue for a complete dissociation for the neuroanatomical substrates which mediate the induction and the expression of the behavioral sensitization to amphetamine.

In humans, the repeated use of psychostimulants such as amphetamine may culminate in paranoid psychotic episodes and, in individuals with previous history of amphetamine abuse, psychotic episodes can be reinstated when they are re-exposed to the drug or to stress after months or even years of drug abstinence. In animals such as rodents, this phenomenon has been modeled and called behavioral sensitization or reverse tolerance, which is characterized by a progressive enhancement of the behavioral activation induced by the drug which can culminate in the appearance of stereotypy which are behavioral characteristics of much higher doses. It has been shown to be an enduring phenomenon, since a hypersensitivity (up to one year) to the locomotor activity effect of amphetamine as well as many other dopaminergic-acting drugs persists long after the cessation of the repeated administration.
This long-lasting behavioral sensitization has been correlated most often with a neurochemical sensitization of the dopaminergic (DA) system innervating the nucleus accumbens (NACC), which is also mainly responsible for the acute effect of the drug. Using in vivo methods, an increased release of DA in the NACC has been demonstrated following an amphetamine challenge in amphetamine pretreated rats compared to controls (but see Ref. 37). According to these results, the DA transmission of the NACC has been proposed to mediate the expression of behavioral sensitization. However, the implication of the NACC in the induction of the phenomenon has been questioned. On the one hand, it has been reported that the administration of amphetamine in the NACC whereas increasing locomotor activity does not appear to produce behavioral sensitization when repeatedly administered. However, this finding has been questioned recently by Hooks and colleagues, who found a progressive increase of the locomotor activity induced by the repeated intra-NACC injection of cocaine. On the other hand, since systemic administration of amphetamine also produces DA release at the level of DA cell bodies of the ventral tegmental area (VTA), the effect of repeated amphetamine administration in the VTA has been tested. Surprisingly, whereas amphetamine injected at this level did not induce behavioral activation, an increased response to peripheral challenge of amphetamine, morphine or cocaine was observed in intra-VTA amphetamine pretreated rats. These results have led some authors to favor a role for the DA cell bodies in the initiation of the phenomenon, whereas the DA terminals in the NACC would appear to be mostly responsible for its expression. However, since a combination of local and peripheral administrations of the drugs has been used in the above reported studies, a complete dissociation of the neurochemical substrates responsible for the induction and the expression of behavioral sensitization is not certain and may explain some contradictory findings in the literature.

Thus, in the present study, the involvement of the DA cell bodies of the VTA alone and of the DA terminals of the NACC alone in behavioral sensitization was assessed. In order to do this, a comparison of the effect of different doses of amphetamine administered repeatedly (one injection every other day for five days) in the VTA (0, 1, 2.5, 5 μg/0.5 μl) or in the NACC (0, 1, 3, 10 μg/1 μl) to a later pharmacological challenge directly into the NACC (1 μg/1 μl) or at the periphery (0.5 mg/kg) was performed. Using this approach, either the DA cell bodies of the VTA alone or the DA terminals of the NACC alone were exposed to amphetamine and thus allowed evaluation of the implication of the respective action of amphetamine at these two levels in behavioral sensitization.

**Experimental procedures**

**Animal housing and surgery**

Male Wistar rats (Iffa-Credo, France), weighing 200–220 g upon arrival, were singly housed in an animal room maintained at 22°C with a 12:12 h light:dark cycle (light on at 6.00 a.m.). Food and water were available ad libitum. Surgery was performed three days after arrival. Subjects were anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed in a stereotaxic frame with incisor bars at 5 mm above the interaural line. Bilateral stainless steel guide cannulae (22-gauge, 10 mm length) were implanted 2.5 mm above the final injection site. In Experiment I, guide cannulae were bilaterally positioned to aim at the NACC (AP +3.4 from bregma, L ±1.7, V −5.2 from skull), whereas in Experiment II, for each rat, cannulae were aimed both at the NACC (same coordinates as in Experiment I) and at the VTA (AP −2.9 from bregma, L +1.7, V −4 from skull). Guide cannulae were secured in place with stainless steel screws attached to the skull and cranialplast dental cement. Following surgery, removable stylets were inserted in the guide cannulae to prevent clogging. Animals were left undisturbed for 12 days in the animal section to recover from surgery.

**Apparatus**

General motor activity was measured in Plexiglas cages (20 × 25 × 36 cm) equipped with two parallel horizontal infrared beams positioned 2 cm above the floor and spaced evenly along the longitudinal axis. The activity cages were linked to a computer which recorded photoresistance beam breaks. Activity boxes were kept in a normally illuminated room and white noise was continuously present.

**Drugs and microinfusions**

D-Amphetamine sulfate, cocaine hydrochloride and morphine sulfate were obtained from Coopération Pharmacéutique Française (Melun, France). All drugs were dissolved in phosphate-buffered saline (PBS). Intracerebral microinfusions were performed bilaterally by lowering injection cannulae (12.5 mm length) through the guide cannulae. These injection cannulae were connected with PE10 tubing to a Harvard apparatus compact infusion pump. The solution was infused in a volume of 0.5 μl (intra-VTA) or 1 μl (intra-NACC) by side and delivered over 53 or 106 s, respectively. One minute was allowed for diffusion away from the tips of the cannulae. Animals were freely moving during this phase. Peripheral administrations were performed subcutaneously in the neck of the animals.

**Design and procedure**

All experiments were conducted between 9:00 a.m. and 3:00 p.m. Animals were homogeneously assigned to independent groups depending on their responsiveness to the novelty of the activity cages during their first habituation period. For each injection day, animals were first placed in the activity cage for a 1-h habituation period and then injected with their respective drug treatment. Following the injection, each rat was immediately returned to its activity cage and locomotor activity was recorded for a period of either 2 or 3 h depending on the substance injected.

**Experiment I**

This experiment was divided into two phases: first, a pretreatment phase which consisted of five intra-NACC injections of amphetamine, one every other day (1 μg/1 μl, n = 7; 3 μg/1 μl, n = 7; 10 μg/1 μl, n = 7) or its solvent (PBS, 1 μl, n = 7). Two days after the last injection of amphetamine, a testing phase began which consisted (for the four groups of rats) of a PBS injection directly into the NACC, followed two days later by an intra-NACC injection of amphetamine (1 μg/1 μl per side), followed two days later by a peripheral injection of amphetamine (0.5 mg/kg).
**RESULTS**

Assessment of cannula placements

Animals in which the traces of the injection cannulae were located outside the VTA or the NACC and animals showing important gliosis were discarded from the experiments. Following histological control, six rats were removed from Experiment I, mostly due to important gliosis, and five rats from Experiment II, mostly due to cannula misplacements either in the VTA or the NACC. Representative histological sections as well as schematic representations of cannula placements in rats kept for the statistical analysis for both Experiments I and II are depicted in Fig. 1.
Experiment I: effect of repeated administration of amphetamine into the nucleus accumbens

Behavioral effect of amphetamine administered repeatedly in the nucleus accumbens. Figure 2 represents the total activity exhibited during the 60 min following each of the five consecutive injections (one every two days) of PBS or amphetamine (1, 3, 10 g/μl) within the NACC. The ANOVA indicated a main dose effect \[ F(3, 18) = 14.6, P < 0.0001 \]. Post hoc multiple comparisons revealed that the 3 μg- and 10 μg-injected groups were significantly different from the control group (N.K., \( P < 0.05 \) and \( P < 0.001 \), respectively). This dose-dependent effect, as well as the amplitude of the behavioral effect for each dose, was conserved across the repetition of the injections. The ANOVA indicated no dose \( \times \) injection interaction \[ F(12, 72) = 1.08, \text{n.s.} \].

Effect of a phosphate-buffered saline challenge in the nucleus accumbens in amphetamine-pretreated animals. The general motor activity exhibited by the differently pretreated groups following a PBS challenge (1 μl) directly into the NACC is depicted in Fig. 3A. The aim of this challenge injection was to test the behavioral reactivity of the animals and to see whether any conditioned activity to the procedure of...
Fig. 4. Effect of repeated administration of amphetamine into the VTA at different doses. Rats received one intra-VTA injection of PBS (1/μl) or amphetamine (1, 2.5 or 5 μg/0.5 μl) on days 1, 3, 5, 7 and 9 and their locomotor activity was recorded.

The injection has developed. The ANOVA did not reveal any main significant difference between the four groups [F(3, 18) = 0.43, n.s.] However, there was a group x time interaction [F(33, 198) = 1.68, P < 0.05]. Post hoc two-by-two ANOVA indicated that the time course of the response of the 3 μg and 10 μg pretreated groups was different from the control group [F(11, 99) = 2.38, P < 0.01; F(11, 99) = 3.33, P < 0.001, respectively]. The 3 μg- and 10 μg-pretreated groups responded more to the PBS injection during the first 10 min of the session.

Sensitization test with a challenge of amphetamine (1 μg) within the nucleus accumbens. In Fig. 3B the mean locomotor activity during the 60 min of recording of the four pretreated groups following the injection of a unique dose of amphetamine (1 μg/1 μl) within the NACC is represented. No difference existed between the different groups as revealed by the ANOVA [F(3, 18) = 0.26, n.s.] and no significant group x time interaction was detected [F(33, 198) = 0.51, n.s.].

Sensitization test with a peripheral challenge of amphetamine (0.5 mg/kg). As indicated in Fig. 3C, no difference could be seen in the behavioral response exhibited by the four groups in response to a peripheral challenge to amphetamine [F(3, 18) = 0.26, n.s.] and no significant group x time interaction was detected [F(69, 414) = 1.1, n.s.].

Experiment II: Effect of repeated amphetamine administration into the ventral tegmental area

Behavioral effect of amphetamine administered repeatedly in the ventral tegmental area. Figure 4 represents the total motor activity during 60 min demonstrated by the four groups of animals treated with different intra-VTA doses of amphetamine.

Intra-VTA injections of amphetamine did not induce any significant behavioral modification compared to PBS injections [F(3, 23) = 0.88, n.s.]. Furthermore, the amplitude of the response remained stable across injections for the four groups, as revealed by the lack of a dose x days interaction [F(12, 92) = 0.46, n.s.].

Effect of a challenge with phosphate-buffered saline in the nucleus accumbens of amphetamine-pretreated animals. The time course of the locomotor response of the different groups to a PBS challenge directly within the NACC is depicted in Fig. 5A. The ANOVA indicated no significant difference between the three amphetamine-pretreated groups and the saline-pretreated group [F(3, 23) = 0.13, n.s.], as well as no group x time interaction [F(33, 253) = 0.4, n.s.].

Sensitization test with a challenge of amphetamine (1 μg) within the nucleus accumbens. The behavioral response of the four pretreated groups to a challenge dose of amphetamine (1 μg) administered locally within the NACC is illustrated in Fig. 5B. It appears that there was an increased locomotor activity which depended on the dose of amphetamine used for the pretreatment. The global ANOVA indicated a significant main group effect [F(3, 23) = 6.13, P < 0.01]. Post hoc comparisons revealed a significant difference between the 5 μg pretreated group and the control group (N.K., P < 0.01). Furthermore, a significant group x time interaction was detected [F(33, 253) = 2.22, P < 0.001]. Two-by-two post hoc comparisons revealed that there was a significant group x time interaction when the three amphetamine-pretreated groups were compared to the saline-pretreated group [PBS/1 μg, F(11, 121) = 2.04, P < 0.05; PBS/2.5 μg, F(11, 110) = 3.36, P < 0.001; PBS/5 μg, F(11, 110) = 2.61, P < 0.01; see Fig. 5B].

Sensitization test with a peripheral challenge of amphetamine (0.5 mg/kg). When the four groups of animals received a peripheral administration of amphetamine (0.5 mg/kg), the same potentiation of the locomotor activity was observed in the intra-VTA amphetamine-pretreated groups compared to the saline-pretreated group [PBS/1 μg, F(11, 121) = 2.04, P < 0.05; PBS/2.5 μg, F(11, 110) = 3.36, P < 0.001; PBS/5 μg, F(11, 110) = 2.61, P < 0.01; see Fig. 5B].

Cross-sensitization between intra ventral tegmental area amphetamine and cocaine injected into the nucleus accumbens. Two days following the sensitization test with peripheral amphetamine, all groups were challenged with a unique dose of cocaine (10 μg/μl) injected directly within the NACC. As shown in Fig. 6A, a cross-sensitization between cocaine and
intra-VTA amphetamine was evidenced. The global ANOVA indicated a significant group effect \( F(3, 20) = 30.44, \ P < 0.001 \). Post hoc comparisons between groups revealed that all amphetamine-pretreated groups showed a significantly greater locomotor response compared to the control group (N.K., PBS/1 µg, \( P < 0.01 \); PBS/2.5 µg, \( P < 0.01 \); PBS/5 µg, \( P < 0.01 \)). Moreover, a significant group \( \times \) time interaction was evidenced \( F(33, 250) = 5.48, \ P < 0.001 \); see Fig. 6A). Post hoc two-by-two ANOVAs indicated that the time course of the behavioral response of the different amphetamine-pretreated groups was significantly different from that of the control group [PBS/1 µg, \( F(11, 99) = 4.06, \ P < 0.001 \); PBS/2.5 µg, \( F(11, 110) = 0.21, \ P < 0.001 \); PBS/5 µg, \( F(11, 110) = 11.02, \ P < 0.001 \).

Cross-sensitization between intra-ventral tegmental area amphetamine and morphine administered peripherally. Finally, eight days following the intra-NACC cocaine challenge, all groups were subcutaneously injected with a challenge dose of morphine (2.5 mg/kg). There was evidence of cross-sensitization
VTA and NACC in amphetamine-induced behavioral sensitization

Fig. 6. Effects of cocaine and morphine challenges in rats pretreated with different doses of amphetamine (0, 1, 2.5 or 5 μg/0.5 μl) in the VTA. (A) Effect of a cocaine challenge (10 μg/μl) locally in the NACC. (B) Effect of a morphine challenge (2.5 mg/kg, s.c.) peripherally. **P < 0.01 compared to the PBS-pretreated group.

between intra-VTA amphetamine and morphine (see Fig. 6B). The global ANOVA showed a significant group effect \[F(3, 20) = 5.12, P < 0.01\]. Post hoc comparisons indicated that the 5 μg pretreated group was significantly different from the control group (N.K., P < 0.01). The global ANOVA also indicates a group × time interaction \[F(69, 460) = 2.91, P < 0.001\]. Post hoc ANOVA indicated a significant difference only for the 5 μg pretreated group \[F(23, 207) = 5.36, P < 0.001\].

DISCUSSION

The local approach used in the present study either to induce or to reveal sensitization allowed the demonstration of complete independence between the site for the induction and expression of behavioral sensitization to amphetamine. More specifically in Experiment I, amphetamine injected into the NACC dose-dependently increases locomotor activity without inducing behavioral sensitization, as indicated by
the stability of the behavioral effect across the five injections and the unchanged response to amphetamine injected into the NACC or at the periphery during the challenge tests. Experiment I, in which amphetamine was injected repeatedly in the VTA, offers a mirror image of Experiment I. Indeed, amphetamine injected directly into the VTA does not modify rat behavior but dose-dependently potentiates the locomotor response to a low dose of amphetamine injected either directly into the NACC or at the periphery. Furthermore, this behavioral sensitization may also be seen with cocaine injected into the NACC or morphine administered at the periphery.

According to many previous findings, amphetamine administered locally in the NACC in Experiment I increased locomotor activity in a dose-dependent manner. This effect has been shown to depend on amphetamine-induced DA release in this structure. The repeated injections of amphetamine in the NACC did not show either tolerance or sensitization of the locomotor response, as already reported. The different doses used in this experiment allowed us to show that the lack of sensitization to intra-NACC amphetamine is not due to any ceiling effect. Indeed, the 1 and 3 μg doses of amphetamine both induced hyperactivity, which remained stable across injections and which never reached the level of response of the rats injected with the 10 μg dose. Furthermore, when later tested with a unique low dose of amphetamine injected either in the NACC or at the periphery, the locomotor response induced was identical across groups, suggesting again that no modification of the sensitivity to intra-NACC amphetamine was promoted by previous intra-NACC amphetamine administration. The absence of differences between the pretreated groups when receiving a challenge injection with PBS indicated two things: first that the behavioral reactivity in the basal state of the different groups was not modified and second that no conditioned locomotor activity to the procedure of the injection and the environment had emerged. The possibility of the development of such conditioned hyperactivity was intended to be diminished by the use of 1-h habituation period before the amphetamine administration. Such procedure eliminates the strict association between the environment and the unconditioned effect of the drug, as is classically required in Pavlovian conditioning.

Thus, the repeated exposure of the NACC DA terminals to amphetamine, which is responsible for the hyperactivity produced by amphetamine, is not able to promote, by itself, the changes in the DA neurons which subserve behavioral sensitization.

This result is in agreement with previous reports showing the absence of sensitization to peripheral administration of amphetamine or morphine following intra-NACC administration of amphetamine, whereas it contrasts with the reported behavioral sensitization obtained with repeated cocaine administration into the NACC. One possible explanation would be the use of different DA acting drugs (amphetamine vs cocaine), especially since cocaine is a local anesthetic. This action may somehow distinguish it from amphetamine and permit the initiation of sensitization to cocaine in the NACC. Another explanation would be that the use of a higher dose of amphetamine for the intra-NACC challenge might have revealed a sensitization effect. However, this is unlikely since we did not observe sensitization during the repeated intra-NACC injections (within comparison), whereas Hooks and colleagues observed this effect with intra-NACC cocaine injections. In this context, it should be mentioned that other drugs like opiates, which induce hyperactivity when injected directly into the NACC, have also been shown to lack the capability to induce behavioral sensitization.

The results obtained in Experiment II are in agreement with and complement a very recent report by Perugini and Vezina, in which the authors showed that amphetamine (2.5 μg/μl) repeatedly injected into the VTA induced a potentiation of the locomotor effects of amphetamine injected into the NACC and which was dependent on the dose of the amphetamine challenge in the NACC. Altogether, these results demonstrate that the sole exposure of the DA cell bodies in the VTA to amphetamine initiates changes which ultimately lead the DA terminals which have never been exposed to amphetamine to respond more to it. Similarly, these terminal regions have never experienced amphetamine-induced DA release, since intra-VTA administration of amphetamine decreases rather than increases the level of DA release. This increased reactivity of the DA terminals of the NACC is dependent on the dose of amphetamine administered in the VTA, which suggest some pharmacologically-induced cellular changes at this level rather than non-specific effects. When PBS alone was injected into the NACC of VTA amphetamine-pretreated rats, no difference between the groups emerged, whereas two days later, following amphetamine injection into the NACC, clear differences appeared. This suggests that, in the basal state, the neural modifications which had taken place at the DA terminals are not expressed. The DA neurons need to be recruited in order to demonstrate a differential sensitivity. Interestingly, this effect can also be seen with other DA-activating agents, although the way they act on DA neurons is very different from amphetamine. Indeed, a cross-sensitization was found when cocaine was injected locally within the NACC or when morphine was administered peripherally. Amphetamine-induced DA release is impulse-independent, whereas the cocaine reuptake site blocking effect is impulse-dependent, suggesting that the two drugs act on different pools of DA.
When injected at the periphery, the action of morphine on DA release is also impulse-dependent, since it acts through an indirect release of DA neuron electrical activity. The cross-sensitization between these differently acting drugs suggests two possibilities. Either different mechanisms specific to each drug are altered by the amphetamine pretreatment (i.e. vesicular vs cytoplasmic pools of DA, reuptake system, DA firing) or, at one step or another, a common mechanism may explain the cross-sensitization between these different DA acting drugs. Many hypotheses have been proposed to explain sensitization at the level of the DA neurons, ranging from a redistribution in the intracellular localization of DA to subresponsivity of the autoreceptors accompanied by modifications of somatodendritic release of DA, both leading to an increase in DA neuron firing. A decrease in the reuptake mechanism following repeated administration of cocaine has also been reported. Although each of these mechanisms may explain the behavioral sensitization to a specific drug, by itself each cannot account for cross-sensitization between many pharmacological and environmental stimuli.

The involvement of dopaminergic neurons of the substantia nigra on the effect obtained cannot be discarded. The diffusion of amphetamine from the VTA to the adjacent substantia nigra may be invoked in the effects obtained, since some DA neurons of the medial substantia nigra have been shown to project to the NACC. However, the possible role of DA neurons of the substantia nigra in the behavioral sensitization observed does not weaken the fact that DA cell bodies are necessary for the induction of the phenomenon, be they from the VTA or the substantia nigra.

Such total dissociation between the induction and expression of behavioral sensitization by amphetamine has been hypothesized on several occasions without being directly and unambiguously tested. Indeed, the first experiment conducted by Robinson and colleagues, showing that slices of striatum of rats previously treated with amphetamine were able to release more DA when exposed to K+, amphetamine or electrical stimulation, demonstrated that once sensitized, the DA cell bodies were no longer necessary to reveal this sensitized state. The same conclusions were drawn by other authors, who showed that amphetamine microinjected into the VTA potentiates the behavioral and the neurochemical responses to peripheral morphine, amphetamine and cocaine, although in these latter experiments a participation of morphine, amphetamine and cocaine action at the level of the DA cell body in the expression of the phenomenon could not be discarded as it is in our experiment. Thus, our data, in accord with previously reported studies, demonstrate that neural adaptations at the level of the DA terminals are responsible for the expression of amphetamine sensitization, although these neural adaptations are not promoted by the effect of amphetamine at the DA terminals but are rather the consequences of amphetamine action at the DA cell body level (and present results).

To further argue for the specific involvement of the VTA in the induction of behavioral sensitization to amphetamine, it has been shown that a pretreatment with peripheral as well as intra-VTA injections of the D$_1$ antagonist SCH23390 prevents the development of behavioral sensitization to peripheral administration of amphetamine. However, in this experiment the results were confounded by the fact that SCH23390 pretreatment also blocked the acute locomotor response to amphetamine. In a recent experiment, we found that the D$_1$ antagonist SCH23390 injected into the VTA dose-dependently blocked the induction of the behavioral sensitization induced by intra-VTA amphetamine, whereas D$_2$ antagonist did not show such an effect. Since the D$_2$ receptors which are present at quite low concentrations in the VTA are not localized on the DA cell bodies, their involvement in behavioral sensitization implicates VTA afferents. These afferent pathways have been shown to be of GABA, glutamatergic, cholinergic or peptidergic nature. So far, D$_1$ receptors have been visualized on GABA afferents arising from the striatum, although their presence on glutamate terminals has also been proposed. In agreement with a specific involvement of D$_1$ receptors of the mesencephalon in the initiation of behavioral sensitization, we also found an increase D$_1$ binding in the ventral mesencephalon in amphetamine-sensitized animals. However, the exact mechanism in the VTA through which amphetamine action and D$_1$ receptors interact to induce behavioral sensitization remains to be elucidated.

**CONCLUSION**

We have provided in the present set of experiments a clear-cut demonstration of the complete neuroanatomical dissociation between the neural substrates involved in the expression and induction of behavioral sensitization to amphetamine.

**REFERENCES**


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