Behavioural Brain Research, 34 (1989) 111–116
Elsevier
BBR 00942

Evidence that apomorphine and (+)-amphetamine produce different types of circling in rats

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(Received 6 June 1988)
(Revised version received 5 January 1989)
(Accepted 6 January 1989)

Key words: Apomorphine; (+)-Amphetamine; Circling; Stepping; Rat

Apomorphine and (+)-amphetamine are known to produce circling in naive rats. Frame by frame analysis of videotape recordings of the behaviour of Wistar rats treated with a subcutaneous injection of apomorphine (1.1 mg/kg; $n = 8$) or (+)-amphetamine (0.5 and 1.0 mg/kg; $n = 8$ and $n = 8$) was used to study this behaviour in more detail. In line with previously reported studies, apomorphine was found to change the functioning of hindlimb stepping. In contrast, (+)-amphetamine was found to change the functioning of forelimb stepping. These data imply that apomorphine and (+)-amphetamine produce their drug-specific circling via different substrates within the brain.

INTRODUCTION

In 1872 apomorphine had already been reported to produce circling in dogs: this circling was associated with paralysis of the hindlimbs, but not of the forelimbs[32]. In rats too, apomorphine elicits circling[12,19,20,35–37]. Again, this circling is closely coupled to the occurrence of a progressive dysfunctioning of hindlimb stepping: in fact, hindlimb stepping ultimately ceases at the peak of the drug's action, whereas forelimb stepping continues throughout the drug's action[36]. It has been argued that such apomorphine-induced effects are due to (hyper)-stimulation of postsynaptic dopamine receptors of nigrostriatal dopamine neurons, resulting in a progressive dysfunctioning of the output-stations of the nigrostriatal dopamine neurons[18,34,36]. (+)-Amphetamine too is known to elicit circling in rats[12,19]. Nevertheless, (+)-amphetamine exerts an action on dopamine neurons that significantly differs from that of apomorphine. In contrast to apomorphine which stimulates D_2 receptors and, to a lesser degree, D_1 receptors[22,30,38], (+)-amphetamine, among other things, enhances dopamine release and blocks its re-uptake[13,16,24,31,42]. Moreover, apomorphine and (+)-amphetamine differentially affect presynaptic and postsynaptic dopamine receptors[2,7,17,42]. And, finally, apomorphine and (+)-amphetamine differentially affect nigrostriatal and mesolimbic dopamine neurons[1,6,21,23,25,28,29,33,41]. Nevertheless, it is generally accepted that both agents elicit circling via influencing the same neuronal elements, i.e. the synapses of the nigrostriatal dopamine neurons[12]. During the course of a

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detailed study, now in progress in the laboratory of one of the authors (I.G.) on (+)-amphetamine-induced behaviour in laboratory rats, as a result of the morphogenesis-findings, which will be published elsewhere in detail, we decided to score (+)-amphetamine-induced changes in hindlimb and forelimb stepping during circling behaviour and to compare them with those induced by apomorphine.

MATERIALS AND METHODS

Male Wistar rats (Central Animal Laboratory, Nijmegen) weighing 200 ± 20 g were used. Animals were grouped and housed with free food and water in a colony room and lights on from 07.00 to 19.00 h, and were tested during the light portion of the cycle. First, the experiments of Szechtman and co-workers were replicated by using an analogous experimental set-up and paradigm[36]. Thus, immediately after a subcutaneous (s.c.) neck injection of apomorphine hydrochloride dissolved in distilled water ($n = 8$), each rat was placed individually onto the surface of a flat glass table (160 × 160 cm and 95 cm high). The behaviour displayed during the following 45 min was recorded on videotape by means of a closed circuit TV, and studied with the help of frame by frame analysis. In pilot experiments it was found that our strain of rats was slightly more sensitive to apomorphine than that of Szechtman and co-workers who used 1.25 mg/kg s.c. apomorphine. For that purpose we selected a dose of apomorphine (1.1 mg/kg s.c.) which elicited effects similar to those described by Szechtman et al.[36]. Second, analogous experiments were performed with 8 Wistar rats treated with an s.c. neck injection of 0.5 mg/kg (+)-amphetamine sulfate dissolved in distilled water. Third, the latter experiments were repeated with 8 rats treated with 1.0 mg/kg (+)-amphetamine. Finally, 8 rats treated with an s.c. injection of distilled water, viz. the solvent of the chosen drugs, were included in control experiments. For the purpose of the present study, we merely analysed drug-induced changes in hindlimb and forelimb stepping during circling behaviour.

RESULTS

Since pilot experiments showed that changes in stepping under both apomorphine and (+)-amphetamine were especially prominent when the rats were circling, we limited the analysis of stepping to periods during which the rats made body-turns of 45 degrees or more. Solvent-treated rats displayed highly characteristic forelimb and hindlimb stepping during this type of circling. The forelimb contralateral to the direction of turning (the outer forelimb) was no longer placed in front and ahead of the forefoot ipsilateral to the direction of turning (the inner forelimb), thus shifting the animal's weight forward, but landed alongside the inner forefoot almost touching it. This so-called 'closing' step was then followed by a sideward or open step of the inner forelimb. The sequential occurrence of a closing and an open step was called a 'doublet' (Fig. 1A). Both apomorphine and (+)-amphetamine increased the number of such doublets of the forelimbs as illustrated by the means ± S.E.M.: 102 ± 18 (solvent); 512 ± 30 (1.1 apomorphine: $P < 0.01$ vs controls); 291 ± 55 (0.5 mg (+)-amphetamine: $P < 0.05$ vs controls); 346 ± 33 (1.0 mg (+)-amphetamine; $P < 0.01$ vs controls (two-tailed Mann–Whitney U-test)). These drug-induced increases were simply due to the fact that both drugs enhanced the number of body-turns made. In contrast to solvent- and apomorphine-treated rats, however, (+)-amphetamine-treated rats displayed, in addition, a qualitatively different pattern of forelimb stepping. Instead of placing the outer forefoot alongside the inner forefoot, they sometimes placed their outer forefoot across the inner forefoot (Fig. 1B). Since the number of these so-called 'crossing steps' is associated with the number of doublets made, we expressed the number of these crossing steps as percentage of the total number of forelimb doublets. As shown in Fig. 2, (+)-amphetamine dose-dependently increased this percentage of crossing steps of the forelimbs, whereas neither the solvent nor apomorphine had any effect in that respect.

When solvent-treated rats made body-turns of 45 degrees or more, the hindlimb stepping was not

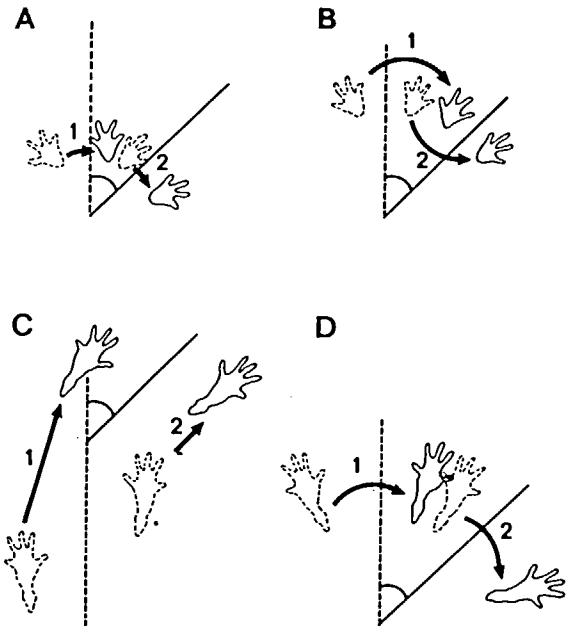

Fig. 1. Patterns of stepping during turning. In all examples the rat is turning clockwise. Angle of turning 45°. The figure shows original position of longitudinal axis of trunk (interrupted straight lines and final position (solid straight line). In all examples the outer leg in relation to the direction of turning (left leg) steps first, and the inner leg (right leg) steps second. Initial location of foot (interrupted line), and final location (solid line), are represented. Arrows and numerals represent direction and order of stepping. A: forelegs; a doublet consisting of a closing (1), and open (2) step (typical under apomorphine and solvent). B: forelegs; a doublet consisting of a crossing (1), and open (2) step (typical under amphetamine). C: hindlegs; a pair of forward steps (typical under amphetamine and solvent). D: hindlegs; a doublet consisting of a closing (1) and open (2) step (typical under apomorphine).

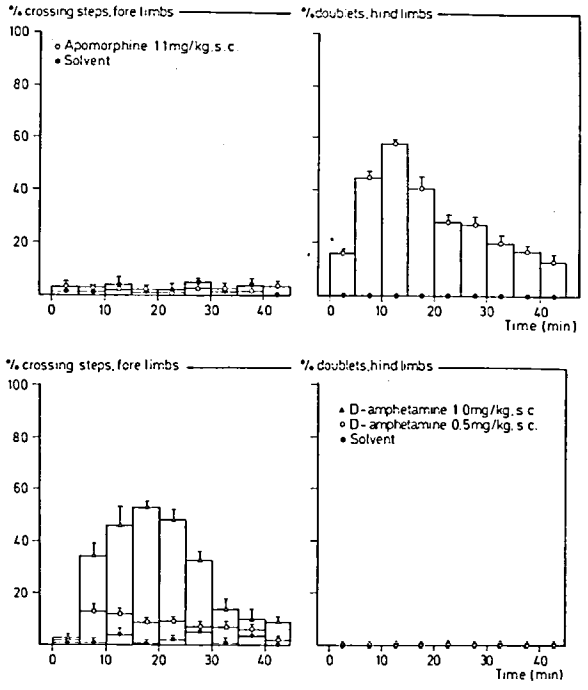

Fig. 2. Changing in the functioning of forelimb and hindlimb stepping produced by apomorphine (upper panel) and (+)-amphetamine (lower panel). The number of crossing steps of the forelimbs is expressed as percentage of the total number of forelimb doublets. The number of doublets of the hindlimbs is expressed as percentage of the total number of hindlimb steps.

significantly different from that shown during forward walking. The outer hindlimb was always placed in front and ahead of the inside hindlimb, even when the rate of forward progression diminished greatly (Fig. 1C). (+)-Amphetamine-treated rats too showed this pattern of hindlimb stepping as long as they turned. In contrast to solvent- and (+)-amphetamine-treated rats, however, apomorphine-treated rats displayed, in addition, a qualitative different pattern of hindlimb stepping. Instead of placing the outer hindlimb in front and ahead of the inner hindlimb, they sometimes placed their outside hindlimb alongside the inside hindlimb, touching or almost touching it. This closing step of the outer hindlimb was immediately followed by a sidewards or open step of the inside hindlimb, thus producing doublets of the hindlimbs. It was apomorphine, in contrast to the solvent and (+)-amphetamine, that elicited such doublets (Fig. 1D). Since the number of doublets is associated with the number of steps made, we expressed the number of doublets as percentage of the total number of hindlimb steps. As shown in Fig. 2, only apomorphine elicited these doublets of the hindlimbs. The performance of these hindlimb doublets was followed by a number of additional changes in hindlimb stepping. These are described elsewhere in detail[36].

DISCUSSION

The present data show that the effect of (+)-amphetamine is primarily evident in the form of forelimb stepping in contrast to the effect of

apomorphine which is primarily evident in the form of hindlimb stepping. Apomorphine has been reported to produce a dysfunctioning of hindlimb stepping in dogs[32], rabbits[15] and rats[36] (also present study). Detailed kinematic analysis is necessary before it can be decided whether indeed apomorphine acts exclusively on the hindquarter movement and amphetamine exclusively on the forequarter movement, or whether both drugs affect the movements of the whole animal, but the effect is less discernible under apomorphine in the forequarters, and under amphetamine in the hindquarters. The latter is most likely, since Ziegler and Schechtman have already found that both drugs act differently on hindleg stepping in unilateral[43] lesioned rats. Anyhow, the present data imply that the effects under discussion are mediated via different substrates within the brain, viz. a suggestion that fits in with earlier reported data[43]. As mentioned, apomorphine and (+)-amphetamine differentially affect nigrostriatal and mesolimbic dopamine neurons. In naive rats intracranial apomorphine injections are only behaviourally effective when injected into the neostriatum, i.e. the principal target organ of the nigrostriatal dopamine neurons, but not when injected into the nucleus accumbens, i.e. the principal target organ of the mesolimbic dopamine neurons[3–5,11,27]. In contrast, intracranial (+)-amphetamine injections are highly effective when injected into the nucleus accumbens, but not when injected into the neostriatum[5,26,27,39]. Finally, it was reported that nigrostriatal and mesolimbic neurons are differentially involved in the control of hindlimb and forelimb movements. In naive rats, dopaminergic antagonists are reported to produce deficiencies in hindlimbs following injections into the dorsomedial part of the neostriatum, but not following such injections into the nucleus accumbens[1,8–10,21]. On the other hand, haloperidol, a dopaminergic antagonist with an additional α-noradrenolytic effect, produces deficiencies in forelimbs following injections into the nucleus accumbens, but not following such injections into the neostriatum[4,8,10,14,40] In view of these data, we suggest that the effect of apomorphine which is observed in hindlimb stepping is due to (hyper)stimulation of dopamine receptors of nigrostriatal dopamine neurons, and the effect of (+)-amphetamine which is reflected in forelimb stepping is due to drug-induced changes in the activity within the synapses of mesolimbic dopaminergic and/or noradrenergic neurons. Apomorphine has been found to produce a progressive dysfunctioning of the output-stations of nigrostriatal dopamine neurons[18,36]. Since deficiencies reflected in hindlimbs are typical for dysfunctioning output-stations of the nigrastriatal dopamine neurons[9], it is suggested that the apomorphine-induced effects in the hindlimbs are due to the drug-induced progressive dysfunctioning of the output-stations of the nigrostriatal dopamine neurons. Analogously, it is attractive to postulate that (+)-amphetamine-induced deficiencies in the forelimbs are due to the drug-induced progressive dysfunctioning of the output-stations of the mesolimbic neurons which terminate within the nucleus accumbens. The latter suggestion opens the perspective that (+)-amphetamine can be used as a tool to study behavioural deficits typical for a progressive dysfunctioning of the output-stations of mesolimbic neurons. Future research is required to provide evidence in that respect.

As a final remark, it is important to note that systemic administration of high doses of (+)-amphetamine are known to affect both forelimbs and hindlimbs. Since the nucleus accumbens innervates, among others, dopaminergic cells giving rise to the nigrostriatal fibres, it is not unlikely that high, but not low, doses of (+)-amphetamine transsynaptically alter the dopaminergic activity in the neostriatum as well.

ACKNOWLEDGEMENTS

The present study was partly supported by the Israel Institute for Psychobiology, Charles E. Smith Family Foundation, Grant 6-87-8.

REFERENCES

1 Anden, N.E. and Johnels, B.O., Effect of application of apomorphine to the corpus striatum and to the nucleus accumbens on the reserpine-induced rigidity in rats, *Brain Res.*, 133 (1977) 386–389.
2 Braestrup, C., Biochemical differentiation of ampheta-

mine vs methylphenidate and nomifensine in rats, *J. Pharm. Pharmacol.*, 29 (1977) 463.

3 Cools, A.R., Mesolimbic dopamine and its control of locomotor activity in rats: differences in pharmacology and light/dark periodicity between the olfactory tubercle and the nucleus accumbens, *Psychopharmacologia*, 88 (1986) 460–466.

4 Costall, B., Naylor, R.J. and Neumeyer, J.L., Differences in the nature of the stereotyped behaviour induced by aporphine derivatives in the rat and in their actions in extrapyramidal and mesolimbic brain areas, *Eur. J. Pharmacol.*, 31 (1975) 1–16.

5 Costall, B., Fortune, D.H., Hui, Siu-Chun, G. and Neylor, R.J., Neuroleptic antangonism of the motor inhibitory effects of apomorphine within the nucleus accumbens: drug interaction at presynaptic receptors, *Eur. J. Pharmacol.*, 63 (1980) 347–358.

6 Demarest, K.T., Lawson-Wendling, K.L. and Moore, K.E., d-amphetamine and γ-butyrolactone alteration of dopamine synthesis in the terminals of nigrostriatal and mesolimbic neurons, *Biochem. Pharmacol.*, 32 (4) (1983) 691–697.

7 Di Chiara, G., Corsini, G.U., Mereu, G.P., Tissari, A. and Gessa, G.L., Self-inhibitory dopamine receptors: their role in the biochemical and behavioural effects of low doses of apomorphine. In P.J. Roberts, G.N. Woodruff and L.L. Iversen (Eds.), *Advances in Biochemical Psychopharmacology, Vol. 9*, Raven, New York, 1978, 275 pp.

8 Ellenbroek, B., Schwarz, M., Sontag, K.H., Jaspers, R. and Cools, A.R., Muscular rigidity and delineation of a dopamine-specific neostriatal subregion: tonic EMG activity in rats, *Brain Res.*, 345 (1985) 132–140.

9 Ellenbroek, B., Schwarz, M., Sontag, K.H. and Cools, A.R., The importance of the striato-nigro-collicular pathway in expression of haloperidol-induced tonic electromyographic activity, *Neurosci. Lett.*, 54 (1985) 189–194.

10 Ellenbroek, B., Van den Hoven, J. and Cools, A.R., The nucleus accumbens and forelimb muscular rigidity in rats, *Exp. Brain Res.*, 72 (1988) 299–304.

11 Ernst, A.M. and Smelik, P.G., Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats, *Experientia*, 22 (1966) 837.

12 Glick, S.D., Jerussi, T.P., Cox, R.D. and Fleisher, L.N., Pre- and postsynaptic actions of apomorphine: differentiation by rotatory effects in normal rats, *Arch. Int. Pharmacodyn.*, 225 (1977) 303–307.

13 Glowinski, J. and Axelrod, J., Effects of drugs on the uptake release and metabolism of [^{3}H]norepinephrine in the rat brain, *J. Pharmacol. Exp. Ther.*, 149 (1965) 43.

14 Grabowski-Anden, M. and Anden, N.E., Stimulation of postsynaptic DA2 receptors produces jerks in reserpine-treated rats, *J. Pharm. Pharmacol.*, 35 (1983) 543–545.

15 Harnack, E., Über die Wirkungen des Apomorphins am Saugetier und am Forsch, *Arch. Exp. Pathol. Pharmacol.*, 2 (1874) 254–306.

16 Hyttel, J., Inhibition of [^{3}H]dopamine accumulation in rat striatal synaptosomes by psychotropic drugs, *Biochem. Pharmacol.*, 27 (1978) 1063.

17 Iversen, S.D., Kelly, P.H., Miller, R.J. and Seviour, P., Amphetamine and apomorphine responses in the rat after lesion of mesolimbic or striatal dopamine neurones, *Br. J. Pharmacol.*, 54 (1975) 244.

18 Jaspers, R.M.A. and Cools, A.R., Behavioural correlates of a progressive dysfunctioning of the caudate nucleus: effects of apomorphine, *Behav. Brain Res.*, 27 (1988) 193–204.

19 Jerussi, T.P. and Glick, S.D., Amphetamine-induced rotation in rats without lesions, *Neuropharmacologia*, 13 (1974) 283–286.

20 Jerussi, T.P. and Glick, S.D., Apomorphine-induced rotation in normal rats and interaction with unilateral caudate lesions, *Psychopharmacologia*, 40 (1975) 329–334.

21 Johnels, B., Reserpine-induced rigidity in rats: drug effects on muscle tone from corpus striatum and nucleus accumbens, *Pharmacol. Biochem. Behav.*, 19 (1983) 463–470.

22 Kebabian, J.W. and Calne, D.B., Multiple receptors for dopamine, *Nature (Lond.)*, 277 (1979) 93–96.

23 Kelly, P.H., Seviour, P.W. and Iversen, S.D., Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, *Brain Res.*, 94 (1975) 507–522.

24 Moore, K.E., Amphetamines. Biochemical and behavioral actions in animals. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), *Handbook of Psychopharmacology*, Plenum, New York, 1978, 41.

25 Nielsen, J.A., Chapin, D.S. and Moore, K.E., Differential effects of d-amphetamine, β-phenylethyl amine, cocaine and methylphenidate on the rate of dopamine synthesis in terminals of nigrostriatal and mesolimbic neurons and on the efflux of dopamine metabolites into cerebroventricular perfusates of rats, *Life Sci.* 33 (1983) 1899–1907.

26 Pijnenburg, A.J.J., Honig, W.M.M. and Van Rossum, J.M., Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat, *Psychopharmacologia*, 41 (1975) 87–95.

27 Pijnenburg, A.J.J., Honig, W.M.M., Van der Heyden, J.A.M. and Van Rossum, J.A.M., Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur. J. Pharmacol.*, 35 (1976) 45–58.

28 Rebec, G.V. and Zimmerman, K., Opposite effects of d-amphetamine on spontaneous neuronal activity in the neostriatum and nucleus accumbens, *Brain Res.*, 201 (1980) 485–491.

29 Robbins, T.W. and Everitt, B.J., Functional studies of the central catecholamines, *Int. Rev. Neurobiol.*, 23 (1982) 303–364.

30 Schechter, M.D. and Greer, N.L., Evidence that the stimulus properties of apomorphine are mediated by both D_1 and D_2 receptor activation, *Life Sci.*, 40 (1987) 2461–2471.

31 Scheel-Krüger, J., Comparative studies of various amphetamine analogues demonstrating different inter-

actions with the metabolism of the catecholamines in the brain, *Eur. J. Pharmacol.*, 14 (1971) 47–59.

32 Siebert, V., Untersuchungen über die physiologische Wirkungen des Apomorphins. *Inaug. Dorpat 1871 und Wagner's Archiv für Heilkunde, XII*, 1971, S. 522 ff.

33 Solomon, P.R. and Staton, D.M., Differential effects of microinjections of d-amphetamine into the nucleus accumbens or the caudate putamen on the rat's ability to ignore an irrelevant stimulus, *Biol. Psychiat.*, 17 (1982) 743–756.

34 Szechtman, H., Ornstein, K., Hofstein, R., Teitelbaum, P. and Golani, I., Apomorphine induces behavioral regression: a sequence that is the opposite of neurological recovery. In E. Usdin, T.L. Sourkes and M.B.H. Youdim (Eds.) *Enzymes and Neurotransmitters in Mental Disease*, Wiley, London, 1980, pp. 511–517.

35 Szechtman, H., Peripheral sensory input directs apomorphine-induced circling in rats, *Brain Res.*, 264 (1983) 332–235.

36 Szechtman, H., Ornstein, K., Teitelbaum, P. and Golani, I., The morphogenesis of stereotyped behavior induced by the dopamine receptor agonist apomorphine in the laboratory rat, *Neuroscience*, 14 (1985) 783–798.

37 Szechtman, H. and Pisa, M., Postural asymmetry and lateralized rotation in normal rats administered apomorphine, *Pharmacol. Biochem. Behav.*, 25 (1986) 689–691.

38 Stoof, J.C. and Kebabian, J.W., Opposing roles for D_1 and D_2 dopamine receptors in efflux of cyclic AMP from rat neostriatum, *Nature (Lond.)*, 294 (1981) 366–369.

39 Taylor, J.R. and Robbins, T.W., Enhanced behavioural control by conditioned reinforces following microinjections of d-amphetamine into the nucleus accumbens, *Psychopharmacologia*, 84 (1984) 405–412.

40 Vrijmoed-de Vries, M.C., Tönissen, H. and Cools, A.R., The relationship between hindlimb disturbances, forelimb disturbances and catalepsy after increasing doses of muscimol injected into the striatal-pallidal complex, *Psychopharmacologia*, 92 (1987) 73–77.

41 Westerink, B.H.C., Effects of drugs on the formation of 3-methoxytyramine, a dopamine metabolite, in the substantia nigra, striatum, nucleus accumbens and tuberculum olfactorium of the rat, *J. Pharm. Pharmacol.*, 31 (1979) 94–99.

42 Zetterström, T., Sharp, T., Marsden, C.A., Ugerstedt, U., In vivo measurements of dopamine and its metabolites by intracerebral dialysis: changes after d-amphetamine, *J. Neurochem.*, 41 (1983) 1769.

43 Ziegler, M. and Szechtman, H., Differences in the behavioural profile of circling under amphetamine and apomorphine in rats with unilateral lesions of the substantia nigra. *Behav. Neurosci.*, 102 (1988) in press.