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Locomotor Activity in Relation to Dopamine and Noradrenaline in the Nucleus accumbens, Septal and Frontal Areas: A 6-Hydroxydopamine Study

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Abstract. Locomotor activity was automatically recorded in a circular corridor in rats treated with 6-hydroxydopamine in the ventral tegmental area (VTA), septum and frontal cortex. Control and experimental groups showed similar hyperlocomotor responses in the novel apparatus lasting 3 h. Circadian changes are described. VTA lesions resulted in increased dark activity and a large response to apomorphine compared to other lesion and control groups. Septal lesions did not affect locomotion. The frontal group showed a small increase of locomotion after apomorphine treatment that might reflect increased receptor sensitivity in cortical or subcortical areas. Together with correlations between motor activity and cortical levels of dopamine and noradrenaline these results are interpreted to support a role for dopamine, noradrenaline and the frontal cortex in modulating locomotion which is primarily mediated by VTA-accumbens-dopamine activity.

Introduction

Numerous studies have shown that locomotion in rodents increases after stimulation of dopamine (DA) receptors in the nucleus accumbens. Nonetheless three types of question about the catecholaminergic control of locomotion still require investigation: to what extent (1) does DA-mediated motor activity depend on circumstances (e.g. time of day, novelty of apparatus), (2) does noradrenergic (NA) activity affect DA-mediated locomotion, and (3) does catecholaminergic activity in other brain regions contribute to locomotor changes?

Mediation of locomotion by DA has been shown in two ways. Firstly, motor activity increased (×2–3) after 6-hydroxydopamine lesion (6-OHDA; 2–3 µg) of the ventral tegmental-DA-A 10 area (VTA) [Le Moal et al., 1975; Galey et al., 1977; Tassin et al., 1978; Dunnett et al., 1984] and increased markedly (×8–20) after further treatment with moderate subcutaneous doses of apomorphine (100 µg) [Koob et al., 1981; Andrews et al., 1982; Kelley and Stinus, 1985]. Secondly intra-accumbens application of DA [Anden and Jackson, 1975] and DA agonists [Costall et al., 1976; Cools, 1977], particularly after 6-OHDA lesion of the n. accumbens [Kelly and Iversen, 1975], resulted in hyperlocomotion.

An increase of locomotion after VTA damage is most marked by night. By day no change [Joyce, 1980], hypolocomotion [Brundin et al., 1985] and a 2-fold increase have been recorded [Galey et al., 1977]. There is no report of changed activity levels in response to novelty after VTA lesion, but specific investigation of day versus night or novelty-induced versus habituated motor activity after lesion of structures other than the VTA are lacking. Whereas, with the exception of the running wheel, qualitatively similar results are usually obtained with automatic and observational recording methods [Campbell and Baldessarini, 1982], variations in the time of day and habituation to test apparatus may account for the quantitative differences between studies.

Reports of hypolocomotion after VTA 6-OHDA treatment may reflect the use of different doses [Nakamura and Nakamura, 1976; Koob et al., 1981; Carey, 1983] or damage to different VTA projections [Robbins and Everitt, 1982]. This brings us to the related ques-

Table I. Levels of DA, DOPAC and NA in prefrontal cortex, septum, and n. accumbens

6-OHDA treatment	Prefrontal cortex			Septum			N. accumbens		
	DA	DOPAC	NA	DA	DOPAC	NA	DA	DOPAC	NA
Control	103 (19)	36 (5)	246 (15)	550 (68)	163 (20)	1,012 (104)	5,784 (532)	1,781 (164)	550 (63)
VTA	5*** (2)	7*** (2)	147*** (11)	55*** (12)	13*** (3)	498*** (49)	584*** (213)	171*** (78)	611 (180)
Septum	56* (10)	47 (12)	254 (19)	117*** (18)	60*** (5)	600** (86)	6,770 (590)	2,182 (217)	574 (47)
Frontal cortex	16*** (3)	26 (3)	174** (14)	374 (40)	174 (40)	767 (96)	6,270 (908)	2,120 (175)	581 (95)

The number of rats in each group was 10 ng/g wet wt. SEM in parentheses. Student's t test, with respect to controls: *$p < 0.05$; **$p < 0.02$; ***$p < 0.001$.

tions (2) and (3). Of the possible projection areas likely to influence accumbens-DA function the prefrontal cortex and septum are two likely candidates.

Nonspecific septal damage produces a hyperreactivity syndrome [Brady and Nauta, 1953] and increases basal levels of locomotion by 30% [Carey, 1982]. But it was recently reported that septal 6-OHDA treatment did not alter locomotion in novel apparatus or overnight [Taghzouti et al., 1985].

Similarly 6-OHDA lesion of frontal areas has been reported not to affect locomotion in an open field or activity cage [Joyce et al., 1983; Dunnett et al., 1984]. In these studies NA levels dropped by 70–80%. But there are earlier reports that, if NA terminals are protected, increased locomotion followed frontal 6-OHDA damage [Carter and Pycock, 1980; Pycock et al., 1980]. Although these results have not been directly confirmed the question of frontal and/or NA-DA influence on locomotion remains open, for (1) NA depletion affects frontal DA activity [Tassin et al., 1979; Herve et al., 1982] and motor activity [Fishman et al., 1983]; (2) frontal DA levels correlate negatively with locomotion [Tassin et al., 1978], and (3) frontal damage can modify accumbens-DA receptor function [Reibaud et al., 1984].

We report the effects of 6-OHDA damage in the VTA, septum and prefrontal areas on novel, habituated and circadian levels of locomotion. We also studied the effects of apomorphine treatment after lesion as an indicator of receptor sensitivity and post hoc correlations of DA and NA levels in different regions with locomotion.

Methods

Male Sprague-Dawley rats (260–320 g, IFFA Credo, Lyon, France) were housed individually on a 12-hour light-dark cycle (light 08.00–20.00) in a climate-controlled room. They had free access to food and water and were handled daily for 5 min for 2 weeks both before and after operation. Under chloral hydrate anesthesia (4%, 10 ml/kg) rats received a bilateral infusion of 6-OHDA (4 µg/µl calculated as base) or vehicle in 1 µl ascorbic acid saline (0.1 mg/ml) over 4 min to the VTA (+5 mm; 3.2 posterior to bregma, 0.5 lateral to sinus, 8.8 ventral from skull), the septum (1.5 A; 0.7 L; 5.8 V) and frontal cortex (3.3 and 3.8 A; 0.5 L; 4.0 V). The 30-gauge cannulae were left in place 3 min after infusion. Animals were pretreated with 20 mg/kg desmethylimipramine (Pertofran, Ciba Geigy, Switzerland); for reasons of toxicity 80% of the usual dose was used. There were 10 animals per group.

After 30 days animals were decapitated, the brains rapidly dissected on ice and samples stored at −87 °C until analysis. Samples from frontal cortex, septum, n. accumbens, anterior striatum and amygdala were dissected from each side of the brain [Koob et al., 1984]. NA, DA and its metabolite dihydroxyphenylacetic acid (DOPAC) were analyzed by high-performance liquid chromatography (Perkin-Elmer) with electrochemical detection (bioanalytical systems) using a pH 4, 0.1 *M* phosphate buffer, and a 5-µ RP C18 column [Oades et al., in submission]. Values presented are the average for the two sides of the brain; where DA depletion was less than 50%, animals were eliminated. The signal was nonlinear for values below 20 pg.

The locomotor test was conducted 21–25 days after operation; animals had been trained on a conditioned blocking task on days 12–18. The apparatus was a circular corridor (12 cm wide, 170 cm long) with 4 photocells [Le Moal et al., 1969]. Cumulative records were taken each hour for 27 h from 13.00 (3 h habituation to novelty followed by 24 h circadian activity with food and water present). Then animals received 0.2 mg/kg apomorphine (0.3 ml s.c.) and 9 consecutive 10-min cumulative records were taken.

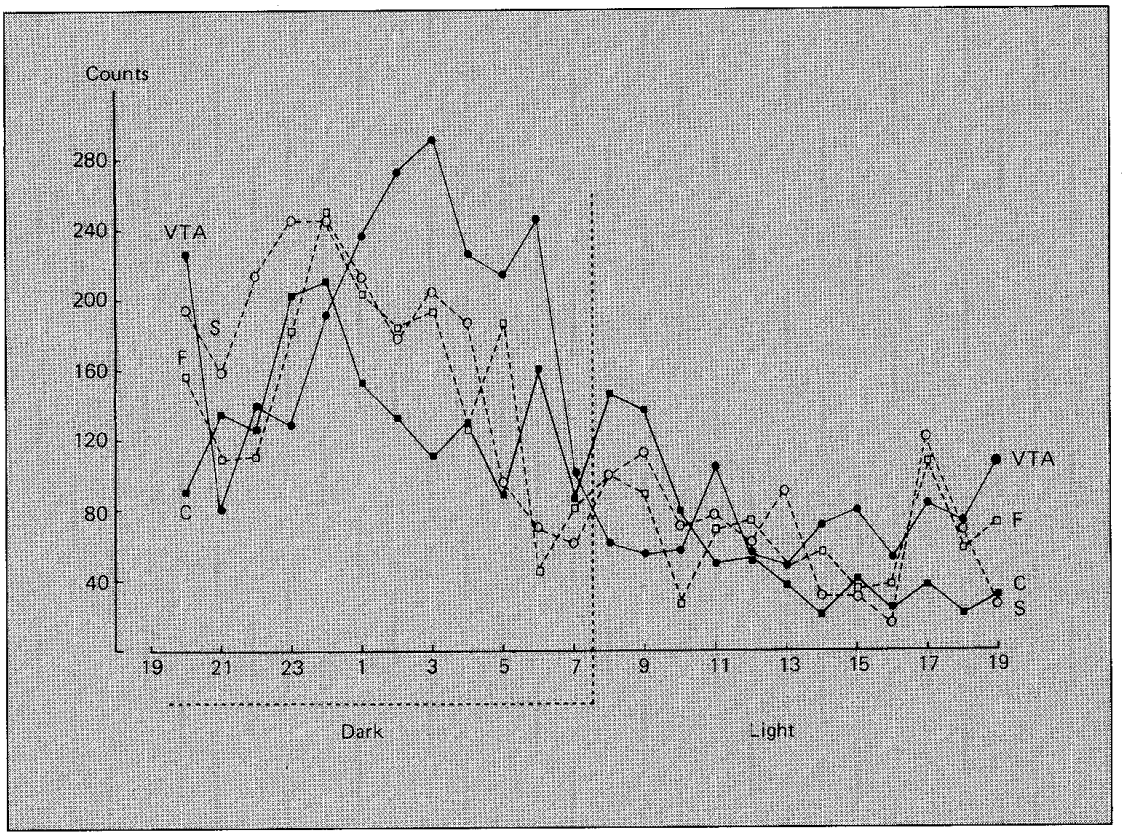

Fig. 1. Cumulative photocell counts per hour of locomotion in a circular corridor over 12 h light and 12 h dark for rats treated with vehicle (C = control) or 6-OHDA in frontal cortex (F), septum (S) and VTA.

Results

In the area of the lesion DA and DOPAC were severely depleted in frontal and septal groups (85 and 79%, table I). These values are comparable with previous reports [Joyce et al., 1983; Taghzouti et al., 1985]. The VTA lesion depleted DA and DOPAC in all projection regions (90–95%). NA levels were mildly depleted in lesioned areas. This was probably a result of incomplete protection of NA terminals.

Locomotor levels in the light were similar for all groups (fig. 1). These levels were 43% of those shown in the dark (control and frontal groups) but slightly less for septal and VTA groups (table II). In the dark only the VTA group showed significantly more activity (t: 1.83; $p < 0.05$). Peak activity occurred 3 h later in this group but lasted longer than in controls in all lesion groups (fig. 1).

Hyperactivity in the novel apparatus was similar for all 4 groups (table III). After 3 h this level was still 3 times the average for the whole light period. The VTA and frontal groups took slightly longer to habituate (cf. peak at 17.000 h, fig. 1). An apparent overresponsiveness in the VTA group was not significant (t: 1.56).

In the frontal group motor activity correlated negatively with DA levels in frontal cortex and n. accumbens

Table II. Total locomotion counts by day and night

Treatment	Light	Dark	Light/light and dark, %
Control	665 (114)	1,603 (205)	43 (5.2)
VTA	838 (147)	2,344* (349)	36 (3.2)
Septum	780 (118)	2,063 (225)	38.5 (5.0)
Frontal cortex	763 (127)	1,822 (203)	43.8 (6.6)

Mean photocell counts with SEM in parentheses.
* $p < 0.05$.

Table III. Influence of novelty on locomotion

	1st h	3rd h	3rd/1st h %
Control	817 (127)	148 (47)	17.9 (5.4)
VTA	826 (72)	285 (94)	32.7 (8.6)
Septum	858 (65)	191 (47)	23.9 (6.4)
Frontal cortex	892 (74)	181 (66)	18.1 (5.7)

Mean photocell counts with SEM in parentheses.

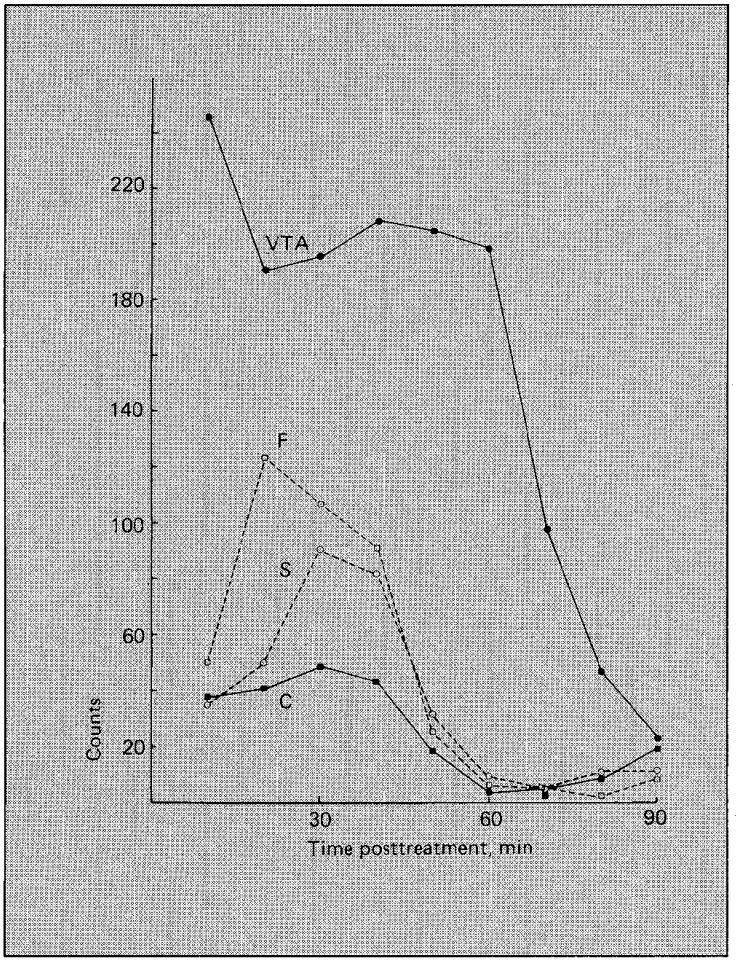

Fig. 2. Cumulative photocell counts per 10 min in a circular corridor over 90 min after apomorphine injection (0.2 mg/kg) for rats treated with vehicle (C = control, ■) or 6-OHDA in frontal cortex (F, □), septum (S, ○) or VTA (●). * $p < 0.05$; ** $p < 0.01$.

($r_s = -0.61$; $p < 0.05$, Spearman rank correlation) but not with NA levels. The correlation was positive for DA utilization in frontal cortex ($r_s = 0.66$; $p < 0.05$). There were no correlations with DA or NA in septum, amygdala or anterior striatum. For the septal group correlations for locomotion were positive with DA and DOPAC in the septum ($r_s = 0.85, 0.76$; $p < 0.01$), but negative with NA in the frontal cortex ($r_s = -0.73$; $p < 0.05$). In the VTA group frontal DA and NA levels were negatively correlated with increased motor activity ($r_s = -0.69, -0.6$; $p < 0.05$). There were no correlations in other brain areas.

Apomorphine treatment elicited a major increase of locomotion in the VTA group (t: 3.31; $p < 0.01$) and a minor increase in the frontal group (t: 1.98; $p < 0.05$). For the septal group motor activity was not significantly different from the control group. The VTA group took longer to return to normal levels of locomotion (fig. 2).

Discussion

The purpose of this study was 3-fold, firstly, to compare novelty-induced with habituated and day with night-time motor activity; secondly, to see if DA depletion after VTA, septal and frontal lesion affected locomotion differently in these conditions, and thirdly, to see if locomotor differences were related to changed levels of DA and NA.

On a 12-hour light/dark cycle control rats showed least activity in the last 6 h of light. Locomotion peaked 4–5 h after onset of darkness. There was a 10-fold difference between highest and lowest scores with individuals varying up to 100-fold in a given hour. When the apparatus was novel, activity was 4 times higher than the highest habituated level [supporting observations, Reinberg and Halberg, 1971; Tadokoro et al., 1981; Dunnett et al., 1984].

VTA 6-OHDA lesions depleted DA in all projection areas. NA levels were unchanged in the n. accumbens. The activity of these animals in the dark was higher than that of controls (146%) [like Pucilowski et al., 1983; Kelley and Stinus, 1985; contrast reports in introduction] and took slightly longer to habituate to novelty than in other groups. The very low levels of DA precluded correlations with activity. The level of NA in the frontal cortex, but not in other regions, correlated negatively with locomotion. A correlation between frontal NA and locomotion was also found after septal damage.

Frontal lesions resulted in nonsignificant increases of dark and novelty-induced locomotion. Motor activity levels correlated negatively with DA in frontal [like Tassin et al., 1978; contrast: Willis et al., 1983] and accumbens areas [like Tassin et al., 1982; Brundin et al., 1985].

Hyperlocomotion has not been recorded after 70% depletion of frontal NA levels [Joyce et al., 1983; Dunnett et al., 1984] but was reported when NA levels were normal [i.e. an imbalance of NA:DA; Pycock et al., 1980]. The NA/DA imbalance may not have been large enough to *significantly* affect locomotion in our frontal group where NA levels were depleted 30%. But in view of frontal NA correlations in other lesion groups (above) and accumbens DA correlations with motor activity in the frontal group [like Carter and Pycock, 1980], it seems likely that frontal areas, in particular a DA-NA imbalance, may exert an influence on accumbens DA-mediated locomotion. To test this hypothesis adequately, firstly, utilization rather than the level of NA and DA should be measured. Secondly, animals with normal DA and depleted NA should also be studied.

This study confirms the absence of circadian or novelty-induced locomotor changes after septal 6-OHDA treatment [Taghzouti et al., 1985] but did not find correlations with DA levels in the amygdala after any lesion [contrast: Brundin et al., 1985].

A hyperlocomotor response to apomorphine after VTA lesion probably represents DA supersensitivity in the n. accumbens [Koob et al., 1981; Carey, 1983; Brundin et al., 1985]. But to our knowledge the increased response of the frontal group to apomorphine has not been reported. In the absence of changed levels of DA and DOPAC in the n. accumbens this increased motor activity may reflect receptor changes in the frontal area or n. accumbens [Reibaud et al., 1984] and confirm the frontal contribution to DA-mediated motor behavior [Tassin et al., 1978].

We conclude, with regard to methodology, that the degree of novelty-induced locomotion has been widely underestimated and through sampling activity for too short a period this has led to quantitatively different results in the literature. Although short tests may be valuable for verifying hypotheses on drug interactions, the extent of DA-depleting lesions or examining specifically the effects of novelty, they are less useful for the study of the neurobiological bases of locomotion. With regard to central factors influencing locomotion we confirm the importance of DA activity in the VTA-accumbens projection, that septal DA activity is not important and suggest a modulatory role for frontal DA-NA interactions in the fine adjustment of locomotor activity.

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