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Subacute exposure to low concentrations of toluene affects dopamine-mediated locomotor activity in the rat

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Summary

The effects of low concentrations of toluene (40—80 ppm, 3 days, 6 h/day) were investigated on spontaneous and on apomorphine-induced locomotor activity in the rat, and were correlated to effects on *S*(-)[*N*-propyl-^{3}H(*N*)]-propylnorapomorphine ([^{3}H]NPA) binding in rat neostriatal membranes, on membrane fluidity, membrane leakage, and calcium levels in synaptosomes from the frontoparietal cortex, the neostriatum and the subcortical limbic area, and on serum hormone levels. Toluene exposure (80 ppm, post-exposure delay 18 h) alone did not affect locomotor activity, but attenuated apomorphine-induced (0.05 mg/kg, s.c.) suppression of rearing, and potentiated apomorphine-induced (1 mg/kg, s.c.) increases in locomotion and rearing. Toluene exposure increased the K_D value of [^{3}H]NPA binding without affecting the B_{max}. All these effects were absent at 40 ppm of toluene or at a post-exposure delay of 42 h. Toluene exposure (80 ppm, post-exposure delay of 18 h) did not affect the serum levels of prolactin, TSH, corticosterone, or aldosterone, or synaptosomal membrane fluidity and calcium levels, whereas membrane leakage was increased in the neostriatum. The present study indicates that the reduction of D-2 receptor affinity by short-term, low-dose toluene exposure is accompanied by a reduced D-2 autoreceptor function and an enhanced postsynaptic D-2 receptor function.

Key words: Apomorphine; Dopamine receptor; Membrane fluidity; Calcium; Prolactin; Basal ganglia

Introduction

The organic solvent toluene is widely used in industry. The threshold value for work exposure is set to around 100 ppm in many countries. In man, exposure to

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toluene in this dose range has been reported to induce behavioural changes in some [1,2] but not in all studies [3,4]. In the rat, chronic exposure to 80 ppm of toluene did not affect spontaneous locomotor activity or passive avoidance behaviour [5]. However, following intraperitoneal administration of toluene (200—600 mg/kg acutely, and 100—200 mg/kg for 14 days) marked behavioural effects were observed in the rat, which correlated to changes in monoamine metabolites [6,7]. Several studies have shown that short-term exposure to low concentrations of toluene (80—1000 ppm) can affect the levels and utilization of catecholamines, such as noradrenaline and dopamine [8—16]. The changes in catecholamine turnover may be related to changes in monoamine receptor binding [17—21]. Of the receptors hitherto tested, the dopamine D-2 receptors appear to be especially vulnerable to toluene exposure [20,21]. The effects of toluene on the D-2 receptors have been suggested to be due to changes in membrane fluidity, since toluene added in vitro increases membrane fluidity [22,23] and causes a similar reduction in D-2 receptor affinity as in vivo [20,21]. Furthermore, both subacute (up to 1000 ppm) and chronic exposure to toluene (80 ppm) has been shown to increase serum prolactin levels in the male rat [5,8,9]. This increase may be due to a presumed decrease in hypophyseal D-2 receptor efficacy that could lead to an attenuated inhibition of prolactin release by dopamine from the tubero-infundibular dopamine system.

In the present study, we have investigated the effects of low concentrations of toluene (40—80 ppm) on spontaneous and on apomorphine-induced locomotor activity in the rat, to further investigate whether the changes in D-2 receptor characteristics can be of behavioural relevance. The effects of various toluene concentrations and post-exposure delays were studied not only at the behavioural levels but also on dopamine D-2 receptor binding in rat neostriatal membranes, in order to evaluate a possible relationship. The present study also examined the effects of toluene on membrane fluidity, membrane leakage, and calcium levels in synaptosomes, and on serum hormone levels, in order to understand the mechanisms involved in the toluene-induced alterations in dopamine transmission in the basal ganglia.

Materials and methods

Inhalation exposure to toluene

Male specific pathogen-free Sprague—Dawley rats (300—350 g body wt; Alab, Sollentuna, Sweden) were kept under regular lighting (lights on at 0600 h and off at 2000 h) and temperature conditions (23°C). The rats had free access to food pellets and tap water. They were exposed for 3 days by inhalation of 40 ppm or 80 ppm toluene (from 0900 h to 1500 h) in stainless steel inhalators. The control group was exposed to circulating air only. During exposure all groups were kept in wire-bottomed stainless steel cages with free access to water but with food removed. The vapour concentration of toluene in the inhalator was monitored continuously by an infra-red analyser (Miran 1A, Wilks Scientific Corporation, U.S.A.) at a wavelength of 13.7 nm. The standard deviation of the exposure levels was less than 10% as calculated from the graphic recordings during all exposure periods. The rats were killed or tested for locomotor activity 18 h, 42 h, or 114 h after the last exposure.

Behavioural measurements

Measurements of locomotor activity were conducted as described in detail elsewhere [24]. Single animals were placed in Macrolon cages (25 × 40 × 30 cm) located on top of Electronic Motility Meters (Motron Mark 24Fc IR, Motron Products, Sweden). Horizontal movements were detected by 48 photosensors placed in the floor of the activity meters. Control animals were always run concurrently and a balanced design was used.

D-2 agonist binding sites labelled by [^{3}H]NPA

Rats were decapitated at 0900—1000 h with a guillotine (Harvard Apparatus Ltd., Edenbridge, U.K.), and the brain was rapidly removed and placed on ice. The brain was partly cut in the midline and the neostriatum was removed with sharp forceps. The tissue was weighed, put in polypropylene vials, and sonicated for 30 s (Soniprep 150, MSE Ltd., Crawley, U.K.) in ice-cold 15 mM Tris—HCl buffer (pH 7.6) containing 0.01% L(+)-ascorbic acid and 1 mM EDTA. Tris buffer was added and the membranes were centrifuged for 10 min at 45 000 *g* (Sorvall RC-5B, DuPont Instruments, U.S.A.). The supernatant was discarded and the membrane pellet was resuspended by sonication. In order to remove endogenous ligands, the membranes were incubated for 30 min at 37°C, followed by another centrifugation for 10 min at 45 000 *g*.

This crude membrane preparation (final concentration approx. 0.2 mg protein/ml) was resuspended in 0.5 ml Tris—HCl buffer. Saturation curves with 10 concentrations (0.05—2 nM) were performed by incubation with *S*[−][*N*-propyl-^{3}H(*N*)]-propylnorapomorphine ([^{3}H]NPA, 2.0 TBq/ mmol) for 30 min at 25°C [20,21,25,26]. Non-specific binding was defined as the binding in the presence of 1 μM LY 171555 (Eli Lilly and Company, U.S.A.). The incubations were stopped by washing the membranes 3 times with 5 ml ice-cold Tris—HCl buffer over Whatman GF/B filter and reduced pressure (Millipore Corp., Bedford, MA, U.S.A.). The radioactivity content of the filters was detected by liquid scintillation spectrometry (Beckman LS 1800, Irvine, CA, U.S.A.). The protein content was determined with BSA as standard according to Lowry and co-workers [27].

Preparation of synaptosomes

The frontoparietal cortex was cut with scissors (range, 150—200 mg) and the neostriatum (50—70 mg) was dissected out as described above. The two brain halves were put together again and cut with a razor coronally at the posterior border of the olfactory tubercles and at a level 3 mm rostral of this level. Subcortical limbic areas (60—80 mg) were dissected out by two 45° cuts with a scissor from the lateral olfactory tract to the midline and a horizontal cut ventral to the anterior commissure. The tissue from 4 rats were pooled together, frozen slowly to −70°C in 10 vols. of 0.32 M sucrose [28]. After quick-thawing, the tissue was homogenized at 4°C and centrifuged (1500 *g*, 10 min) to give a post-nuclear supernatant, which was layered over 1.2 M sucrose and centrifuged for 25 min (250 000 *g*). The interphase band was removed and layered over 0.8 M sucrose and centifuged again for 25 min at 250 000 *g* [28,29]. The purified synaptosomes were resuspended in Hepes buffer (pH 7.4) at 0.15 g-equivalent/ml corresponding to a final concentration of 120—140

μg protein/ml. The Hepes buffer was composed of 125 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 5 mM $NaHCO_3$, 6 mM glucose, 1 mM $CaCl_2$, 25 mM Hepes, and adjusted with NaOH to pH 7.4.

Determinations of membrane fluidity

Synaptosomal membrane order was evaluated by fluorescence polarization studies using two probes. 1,6-Diphenyl-1,3,5 hexatriene (DPH) is a non-polar lipophilic molecule capable of penetrating into and through inner lipid-rich membrane layers [30]. 1-[4(Trimethylamine)phenyl]-1,3,5 hexatriene (TMA-DPH) is a related compound with a polar-constituent that causes the molecule to be aligned at the outer surface of limiting membranes with the polar head at the hydrophilic surface, while the non-polar body penetrates the lipid interior [31,32].

Synaptosomes prepared as described above were incubated at 37°C in the presence of TMA-DPH (30 min) or DPH (15 min) added in tetrahydrofuran (Fisher Scientific, Pittsburgh, PA, U.S.A.). The final concentrations of the probes were 10 μM. The synaptosomes were re-precipitated (13 000 × *g*, 2 min) using a microfuge (235B, Fisher Scientific), resuspended in Hepes buffer and allowed to equilibrate for 10 min at 37°C prior to measuring fluorescence intensity and polarization in a water-jacketed cuvette holder maintained at 37°C (Aminco SPF-500 spectrofluorometer, American Instrument Co., Urbana, SPF, U.S.A.). An excitation wavelength of 360 nm (bandwidth 10 nm) was used with a determination of emission at 430 nm (bandwidth 10 nM). Corrections for light scattering (membrane suspension minus probe) and for fluorescence in the ambient medium (after pelleting membranes) were made.

Fluorescence anisotropy (r) was determined by the formula $r = I_{VV} - I_{VH}/[I_{VV} + 2(I_{VH})]$. I_{VV} is the fluorescence intensity with excitation and emitted light polarized vertically and I_{VH} is the intensity obtained with a vertical orientation of the exciting polarizer with the emitted fluorescence passing through a horizontal polarizer. Total fluorescence intensity $F = I_{VV} + 2(I_{VH})$. Relative microviscosity is proportional to r_0/r_{-1}, where r_0 is the maximal limiting anisotropy of the probe; 0.362 for DPH [33] and 0.39 for TMA-DPH [31]. A correction factor (G) for instrument asymmetry was also made using $G = I_{HV}/I_{HH}$ where I_{HV} is fluorescence intensity with horizontal excitation light and emitted light read vertically, and I_{HH} is the corresponding value with the entire light path horizontally aligned. This compensates for the sensitivity of the detection system toward vertically and horizontally polarized light. All values of I_{VH} were multiplied by G in the calculation of r. All corrections made amounted to less than 6% of the original unmodified readings.

Intrasynaptosomal levels of free calcium

Synaptosomes were prepared as described above and were incubated at 37°C in the presence of fura-2 dissolved in DMSO for 10 min [34]. They were then diluted 10 times in Hepes buffer and incubated for another 5 min. The final concentration of fura-2 was 5 μM. The synaptosomes were centrifuged for 8 min at 3000 *g* and the pellet was resuspended in Hepes buffer.

For each assay, 0.5 ml of synaptosomes was rapidly centrifuged (2 min, 13 000 *g*) and the resulting pellet was resuspended in 1 ml Hepes buffer at 37°C. The buffer was as described above without $NaHCO_3$ and NaH_2PO_4 to prevent the precipita-

tion of calcium at elevated pH (required during the determination of minimal fluorescence). The tube was rinsed with another 1 ml of Hepes buffer and the total 2 ml sample was placed in a quartz cuvette at 37°C and left to equilibrate for 10 min. Excitation of fura-2 was at 340 and 380 nm (bandwidth 3 nm) and emission determinations were made at 510 nm (bandwidth 20 nm). Corrections for light scattering (membrane suspension minus probe) and fluorescence in the ambient medium (after pelleting membranes) were made.

Extrasynaptosomal fura-2 was quenched by 4 μM $MnCl_2$ [35]. The ratio (R) between the fluorescence excitation at 340 and 380 nm was used. $[Ca^{2+}]_i$ was calculated using the formula $[Ca^{2+}]_i = K_d(R - R_{min})/(R_{max} - R) \times [S_{f2}/S_{b2}]$ where K_d is the dissociation constant of the fura-2-Ca^{2+} complex, taken to be 224 nM [35], R_{min} is the ratio in the presence of excess amounts of EGTA (10 mM) and R_{max} the ratio in excess amounts of calcium (18 mM). S_{f2} and S_{b2} denote fluorescence of fura-2 at zero calcium concentration and full calcium saturation, respectively, at an excitation wavelength of 380 nm.

Calcium leakage through the plasma membrane

Dye leakage into the extracellular compartment was calculated after the addition of $MnCl_2$ to a final concentration of 4 μM. The resulting depression in emitted fluorescence when excitation was at 340 nm, was expressed as a percentage of the difference between the corresponding value prior to $MnCl_2$ addition and the value at zero calcium conditions (EGTA and 0.1% sodium dodecyl sulfate being present).

Serum hormone levels

Serum levels of prolactin, thyroid stimulating hormones (TSH), corticosterone, and aldosterone were determined by radioimmunoassay. Following the decapitation of the 64 rats taken to synaptosomal preparation, trunk blood was collected in precooled plastic test tubes, allowed to clot at 4°C and subsequently centrifuged at 3000 g at 4°C for 20 min. The sera were stored at -20°C prior to radioimmunoassay [36].

Statistical analysis

Student's two-tailed, unpaired t-test was used for the comparison between air and toluene-exposed rats.

Results

Apomorphine-induced locomotor activity

The rats exposed to 80 ppm toluene for 3 days, 6 h/day, and left without exposure for 18 h did not show changes in locomotion, motility, or rearing examined during the habituation phase (30 min) in the locomotor cage, compared with air-exposed rats (Fig. 1, Table I). However, when the rats were injected subcutaneously with 0.05 mg/kg of apomorphine, a dose which produced a suppression of locomotor activity (Fig. 2), the suppression in rearing was significantly less in the toluene-treated group

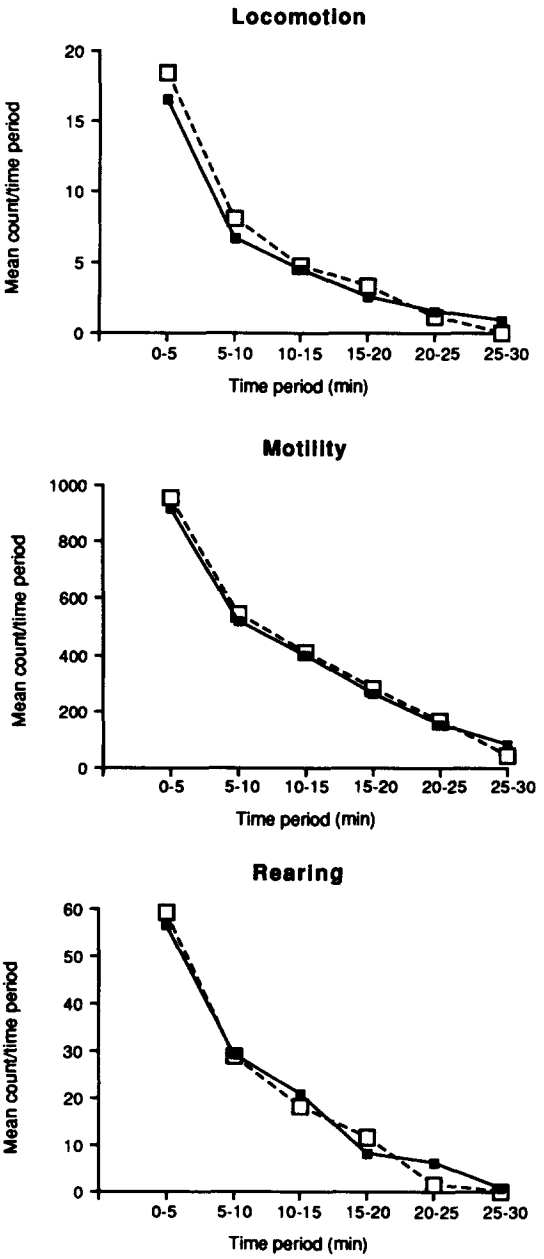

Fig. 1. Effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) on the time-course of spontaneous locomotor activity in the male rat during the habituation phase in the locomotor cage. Air exposure (□), toluene-exposure (■). Data represent mean values of 12 rats in each group. For the integrated response, see Table I.

TABLE I

EFFECTS OF SUBACUTE TOLUENE EXPOSURE ON BASAL AND APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY IN THE RAT

Effects of inhalation to low concentrations of toluene (40 ppm and 80 ppm, 3 days, 6 h/day, and left without exposure 18 h, 48 h or 114 h before killing) on locomotor activity in rats, some of which were injected with apomorphine (0.05 and 1 mg/kg, s.c.) at the start of the recording. Means ± S.E.M. are shown. Data represent the total counts during 30 min for the experiments with 0.05 mg/kg apomorphine, and during 50 min for experiments with 1 mg/kg apomorphine. For time-course displays, see Figs. 1—3.

Exposure	Post-exposure delay (h)	Apomorphine (mg/kg)	*n*	Locomotion	Motility	Rearing
Air	18	0	12	71.1 ± 4.7	4780 ± 230	240 ± 26
Toluene (80 ppm)	18	0	12	65.7 ± 4.9	4660 ± 336	246 ± 37
Air	18	0.05	12	14.3 ± 1.5	1040 ± 85	61.0 ± 8.0
Toluene (80 ppm)	18	0.05	12	17.0 ± 1.6^{T}	1240 ± 92^{T}	79.5 ± 8.0*
Air	42	0.05	12	14.5 ± 1.0	1122 ± 56	44.8 ± 5.2
Toluene (80 ppm)	42	0.05	12	14.3 ± 1.0	1057 ± 63	42.3 ± 4.9
Air	18	1	12	15.8 ± 5.3	1570 ± 210	2.8 ± 1.0
Toluene (80 ppm)	18	1	12	39.7 ± 8.6*	2120 ± 330^{T}	17.2 ± 4.8**
Air	42	1	6	3.7 ± 1.9	1995 ± 160	12.0 ± 9.8
Toluene (80 ppm)	42	1	6	9.8 ± 6.7	2440 ± 470	15.2 ± 11.6
Air	114	1	18	23.5 ± 9.2	3060 ± 380	22.2 ± 16.4
Toluene (80 ppm)	114	1	18	27.2 ± 7.8	3090 ± 230	14.7 ± 5.3
Air	18	1	12	30.4 ± 19.4	2079 ± 530	68.8 ± 35.7
Toluene (40 ppm)	18	1	12	17.4 ± 5.0	1917 ± 308	76.8 ± 45.6

$^{T}P < 0.1$, $*P < 0.05$ according to Student's two-tailed, unpaired *t*-test.

compared with the air-group ($P < 0.05$), and the same trend was observed for locomotion and motility (Fig. 2, Table I). When the post-exposure delay was increased to 42 h these changes disappeared (Table I).

When the toluene-exposed (80 ppm, 18 h post-exposure delay) rats were subcutaneously injected with 1 mg/kg of apomorphine, significant increases in locomotion and rearing were observed as well as a trend for an increase in motility in these rats, compared with air-exposed rats (Fig. 3, Table I).

When the post-exposure delay was increased to 42 h and 114 h, this enhanced response to apomorphine disappeared (Table I). Similarly, an exposure of 40 ppm of toluene for 3 days, 6 h/day, and a post-exposure delay of 18 h did not affect the behavioural response to 1 mg/kg of apomorphine (Table I).

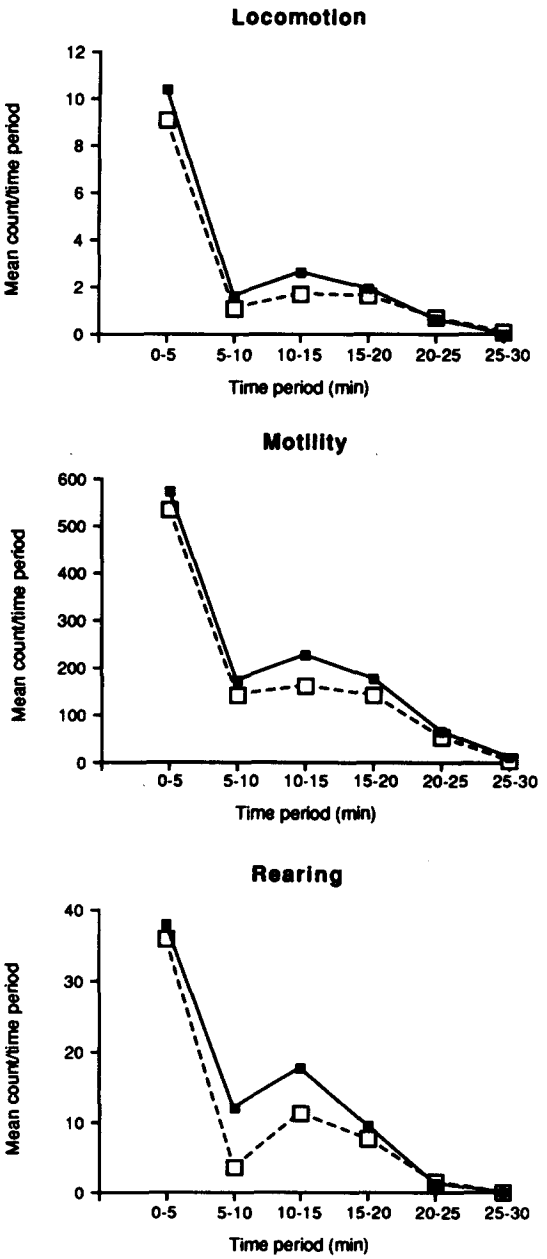

Fig. 2. Effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) on the time-course of apomorphine-induced inhibition (0.05 mg/kg, s.c.) of locomotor activity in the male rat. Air exposure (□), toluene-exposure (■). Apomorphine was injected immediately before putting the rat into the locomotor cage and the start of the recording. Data represent mean values of 12 rats in each group. For the integrated response, see Table I.

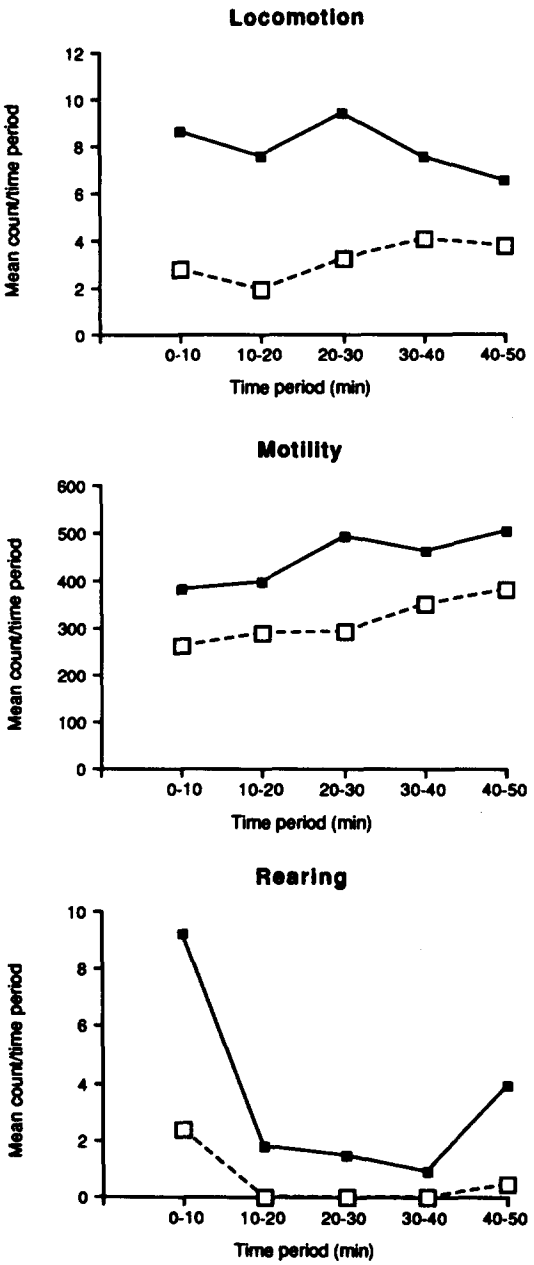

Fig. 3. Effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) on the time-course of apomorphine-induced activation (1 mg/kg, s.c.) of locomotor activity in the male rat. Air exposure (□), toluene-exposure (■). Apomorphine was injected immediately before placing the rat in the locomotor cage and the start of the recording. The data represent mean values of 12 rats in each group. For the integrated response, see Table I.

TABLE II

EFFECTS OF SUBACUTE TOLUENE EXPOSURE ON [^{3}H]NPA BINDING IN RAT NEOSTRIATAL MEMBRANES

The effects of inhalation of low concentrations of toluene (40 ppm and 80 ppm, 3 days, 6 h/day, and left without exposure 18 h or 48 h before killing) were analyzed on the binding characteristics of *S*[-][*N*-propyl-^{3}H(*N*)]-propylnorapomorphine ([^{3}H]NPA) in membranes from the rat neostriatum. Mean ± S.E.M. are shown.

Exposure	Post-exposure delay (h)	*n*	K_D (pM)	B_{max} (fmol/mg protein)
Air	18	4	134 ± 25	143 ± 5
40 ppm	18	4	171 ± 21	149 ± 4
Air	18	18	133 ± 10[a]	136 ± 12[a]
80 ppm	18	16	197 ± 23[a,*]	167 ± 28[a]
Air	42	4	92 ± 9	139 ± 9
80 ppm	42	4	96 ± 9	140 ± 5

*$P < 0.05$ according to Student's two-tailed, unpaired *t*-test; [a]pooled data from earlier studies [20,21].

Dopamine D-2 agonist binding

Following a 3-day exposure, 6 h/day, to 80 ppm of toluene and a post-exposure delay of 18 h, the K_D value of [^{3}H]NPA binding was increased by 50% in membrane preparations from rat neostriatum, whereas the number of binding sites was not significantly affected (Table II). At 40 ppm of toluene the changes in the K_D

TABLE III

EFFECTS OF SUBACUTE TOLUENE EXPOSURE ON MEMBRANE FLUIDITY IN SYNAPTOSOMAL MEMBRANES FROM RAT BRAIN AREAS

The effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) were analyzed on the anisotropy (*r*) of TMA-DPH and DPH in synaptosomal membranes from rat brain areas. Data represent means ± S.E.M.; $n = 8$ in each group. There are no significant differences between air and toluene treated groups according to Student's two-tailed, unpaired *t*-test.

Area	Treatment	Anisotropy (*r*)	
		TMA-DPH	DPH
Frontoparietal cortex	Control	0.291 ± 0.012	0.244 ± 0.007
	Toluene (80 ppm)	0.309 ± 0.007	0.242 ± 0.003
Neostriatum	Control	0.300 ± 0.010	0.271 ± 0.009
	Toluene (80 ppm)	0.307 ± 0.004	0.254 ± 0.004
Subcortical limbic area	Control	0.304 ± 0.006	0.248 ± 0.004
	Toluene (80 ppm)	0.307 ± 0.003	0.254 ± 0.006

value were not significant. Striatal [^{3}H]NPA binding was not modified after a post-exposure delay of 42 h following a 80 ppm exposure to toluene (Table II).

Membrane fluidity

Exposure to 80 ppm of toluene for 3 days, 6 h/day (post-exposure delay of 18 h) did not produce any changes in anisotropy levels of TMA-DPH or DPH in synaptosomes from the frontoparietal cortex, neostriatum and subcortical limbic area (Table III).

Intracellular calcium levels

No differences in calcium levels were seen between air-exposed groups and rats treated with 80 ppm of toluene for 3 days, 6 h/day (post-exposure delay of 18 h) in synaptosomes from the frontoparietal cortex, the neostriatum and the subcortical limbic area (Table IV).

Membrane leakage

Rats treated with 80 ppm of toluene for 3 days, 6 h/day (post-exposure delay of 18 h) showed an increase in fura-2 leakage in synaptosomes from the neostriatum but not in those from the frontoparietal cortex and subcortical limbic area (Table IV).

Serum hormone levels

The air-exposed groups and rats treated with 80 ppm of toluene for 3 days, 6 h/day (post-exposure delay of 18 h) did not differ in their serum levels of prolactin, TSH, corticosterone, or aldosterone (Table V).

TABLE IV

EFFECTS OF SUBACUTE TOLUENE EXPOSURE ON CALCIUM LEVELS AND FURA-2 LEAKAGE IN SYNAPTOSOMES FROM RAT BRAIN AREAS

The effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) were analyzed on intracellular calcium levels (above) and fura-2 leakage (below) in synaptosomes from the frontoparietal cortex, neostriatum, and subcortical limbic area of the rat. Data represent mean ± S.E.M.; n = 4—6 in each group.

Area	Treatment	Calcium levels (nM)	Fura-2 leakage (%)
Frontoparietal cortex	Control	295 ± 50	28 ± 7
	Toluene (80 ppm)	285 ± 40	34 ± 3
Neostriatum	Control	285 ± 120	24 ± 2
	Toluene (80 ppm)	170 ± 70	49 ± 3**
Subcortical limbic area	Control	80 ± 20	41 ± 3
	Toluene (80 ppm)	75 ± 15	52 ± 6

**$P < 0.01$ according to Student's two-tailed, unpaired t-test.

TABLE V

EFFECTS OF SUBACUTE TOLUENE EXPOSURE ON SERUM HORMONE LEVELS IN THE RAT

The effects of inhalation of low concentrations of toluene (80 ppm, 3 days, 6 h/day, and left without exposure 18 h before killing) were analyzed on the serum levels of prolactin, TSH, corticosterone, and aldosterone. Data show means ± S.E.M. calculated from 32 rats in each group. There are no significant differences between air and toluene-treated groups according to Student's two-tailed, unpaired *t*-test.

Exposure	Prolactin (μg/l)	TSH (μg/l)	Corticosterone (nmol/l)	Aldosterone (pmol/l)
Control	25.4 ± 3.3	3.14 ± 0.23	67.0 ± 17.4	593 ± 100
Toluene	25.6 ± 3.8	3.64 ± 0.24	78.4 ± 16.7	584 ± 86

Discussion

The major finding of this paper is the demonstration that short-term, low-dose inhalation exposure to toluene affects locomotor behaviours induced both by dopamine autoreceptor and postsynaptic dopamine receptor stimulation. This finding provides a functional correlate to the previously described actions of toluene on dopamine metabolism [8—10,15,16] and the reduction in the affinity of dopamine D-2 receptors [20,21], as is also seen following chronic styrene exposure [37]. It also demonstrates that disturbances of brain function may become apparent only after a specific challenge of the neuronal system affected, since short-term, low-dose toluene exposure alone did not affect spontaneous locomotor activity, in agreement with previous results following acute [38] and chronic toluene exposure [5].

The attenuated potency of a low dose of apomorphine (0.05 mg/kg), which is believed to act mainly via presynaptic dopamine D-2 receptors [39], can be explained by a reduced sensitivity of presynaptic D-2 receptors following toluene exposure. This possibility is supported by the increase in the K_D value of the D-2 agonist binding sites induced by toluene [20,21]. Interestingly, this change in presynaptic D-2 function does not reveal itself as a change in spontaneous locomotor activity, and dopamine turnover at this low concentration of toluene is actually reduced [10], suggesting complex actions of toluene on dopamine neurons. Furthermore, apomorphine at a postsynaptic dose (1 mg/kg) clearly enhanced the increase in locomotor activity in toluene-treated rats, which may contribute to the compensatory reduction of dopamine turnover. The control K_D values were rather low in the experiment using a 42 h post-exposure delay. This may be due to slight differences in animal age, time of the year, handling conditions, or binding assay conditions, since the experiments were run on different occasions. These variations in control values between different sets of experiments were also seen in the behavioural experiments, underlining the importance of always comparing the effects of a treatment with a proper control group.

The potentiation of the behavioral response to a high dose of apomorphine is difficult to explain by the observed decrease in the affinity of the D-2 receptor. It is possible that toluene affects the D-1 receptor, which may also be activated by a high dose of apomorphine, in such a way that it overrides a possible inhibitory influence of the D-2 receptor efficacy. Another possibility is that a small increase in the number of D-2 receptors following toluene exposure [20] is more important than the decrease in D-2 receptor affinity at a high dose of apomorphine. It is also possible that the reduced affinity of the D-2 receptor corresponds to an increased transduction over the D-2 receptor in the same way as GTP decreases the affinity of the D-2 agonist binding but enhances its G-protein-mediated signal transduction. A similar paradoxical alteration in dopamine transmission is also observed after adrenalectomy associated with increases in dopamine-dependent behaviours [40] and increases in the K_D value of the D-2 agonist binding sites [41]. Furthermore, it should also be considered that the enhancement of dopamine-elicited behavioural responses at the behavioural level may reflect the ability of toluene to affect neuron systems, and perhaps also glia [42—45], beyond the D-2 receptor. This enhanced postsynaptic response may partly underly the toluene-induced arousal and euphoric sensation contributing to its abuse.

The behavioural effects of toluene disappeared within 48 h after the last exposure, which corresponds to the recovery of D-2 receptor binding. Similarly, a lower dose of toluene, 40 ppm, is insufficient to affect either dopamine-mediated behaviors or D-2 receptor binding, as assayed 18 h after the last exposure. These findings indicate that a toluene exposure of 80 ppm for 3 days is close to the threshold limit for obtaining consistent, although reversible, effects of toluene.

The presently used low-dose toluene exposure did not affect membrane fluidity in the frontoparietal cortex, neostriatum, and subcortical limbic region, in agreement with the lack of effects following 500 ppm of toluene for 1 day up to 78 weeks [22] and of intraperitoneal injections (1 g/kg) of toluene [46], but in contrast with the increase in membrane fluidity seen after toluene treatment in vitro (2.5 mmol/l, 10 mmol/l) [22,23]. Thus, it seems as if changes in membrane fluidity [20,47] cannot fully explain the effects of toluene exposure in vivo on D-2 receptor binding even though the affinity of D-2 agonist binding sites are similarly reduced by in vivo and in vitro treatment [20,21], as is the modulation of D-2 agonist binding sites by neurotensin in vitro [21]. Furthermore, it should be recalled that the effects of toluene in vivo on the D-2 receptor can be inhibited by injections once daily with the endogenous ganglioside GM_1 [20] which stabilizes membrane fluidity [23].

The present toluene treatment did not affect the free levels of intrasynaptosomal calcium in the frontoparietal cortex, neostriatum, or subcortical limbic region in agreement with earlier studies showing effects after 4 weeks but not after 2 weeks of toluene exposure in vivo (500 ppm) [48]. These findings indicate that the changes in D-2 receptor function are unrelated to the toluene-induced changes in calcium-regulated protein phosphorylation [5,45,49]. These in vivo results differ from the increase in calcium levels seen after exposure to toluene in vitro (10 mmol/l) [23] which may be mediated by an inhibition of Ca^{++}/Mg^{++}-ATPase in the plasma membrane [22], perhaps through a toluene-induced increase in membrane fluidity. This possibility is supported by the fact that GM_1, which stabilizes membrane fluidity

[23] and activates Ca^{++}/Mg^{++}-ATPase [50], can prevent and reverse the toluene-induced increases in membrane fluidity and intrasynaptosomal calcium levels in vitro [23].

However, it cannot be excluded that the freezing procedures used in the present study diminished the possible effects by toluene, as synaptosomal integrity with regard to membrane leakage appeared to be rather high due to the freezing, even though the synaptosomes maintain their metabolic capacity relatively intact [28]. Toluene in vitro did not affect the low levels of membrane leakage in freshly prepared synaptosomes from whole brain [23]. Nevertheless, the present toluene exposure in vivo selectively increased the membrane leakage of synaptosomes from the neostriatum, indicating that this region is especially vulnerable to toluene exposure. A similar discrepancy between the results following low-dose in vivo exposure and in vitro exposure schedules are seen on the binding properties of the striatal neurotensin receptor which are unaffected following a 80 ppm short-term [51] and long-term exposure in vivo [5] but are changed by toluene treatment in vitro (20 mmol/l) [51]. Therefore, experiments using brief in vitro exposures may be more relevant for understanding the toxic effects of toluene observed under abuse conditions rather than those observed under working conditions.

The presently used exposure schedule of toluene did not affect the serum levels of prolactin, TSH, corticosterone, and aldosterone, as analyzed from as many as 32 control and 32 toluene-exposed rats. In an early study, a small delayed increase in serum corticosterone levels was observed at 500 ppm of toluene for 3 days [8]. Subsequent studies however, have shown a lack of effect on serum corticosterone levels after both short-term [9] and long-term toluene exposure [5]. The lack of effect on corticosterone and aldosterone levels in the present study, using a large number of rats, excludes the possible involvement of a general stress reaction to toluene as a possible mechanism whereby toluene at low concentrations affects brain function. The absence of effects on prolactin levels are interesting in view of the earlier detected dose-dependent increases after short-term exposures at 80—3000 ppm [9] which seems to reach significance at 1000 ppm [8]. Furthermore, chronic exposure to 80 ppm of toluene more than doubled the serum levels of prolactin [5]. Therefore, measurements of prolactin levels may still be a valuable and sensitive assay method to study human populations exposed to toluene vapour. Nevertheless, hormonal changes do not appear to contribute to the observed change in D-2 receptor binding and function.

In conclusion, the present study demonstrates that the previously observed effects of short-term, low-dose toluene exposure on striatal D-2 receptor binding are accompanied by changes in locomotor activity induced by pre- and postsynaptic dopamine receptor activation.

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