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Behavioral and neurochemical effects induced by subchronic exposure to 40 ppm toluene in rats

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Abstract

Chronic toluene inhalation at concentrations above occupational exposure limits (e.g., 100 ppm; NIOSH) has been repeatedly shown to induce neurotoxic effects. In contrast, although few clinical and experimental data are available on the effects of toluene exposure at concentrations below occupational exposure standards, some of these data may support adverse effects of long-term exposure to low toluene concentrations. To test this hypothesis, we investigated the neurobehavioral and neurochemical effects of 40 ppm inhaled toluene in a rat model of 16-week subchronic exposure, examining locomotor and rearing activities; adaptation/sensitization to narcosis produced by acute exposure to toluene at high concentration; and tyrosine hydroxylase and tryptophan hydroxylase activities, and dopamine (DA) and serotonin (5-HT) turnovers in the caudate–putamen, nucleus accumbens, hippocampus, prefrontal cortex, and cerebellum. Our results mainly show that subchronic exposure to 40 ppm toluene significantly resulted in a sensitization to toluene-induced narcosis, a decrease in rearing activity, and alterations in DA and 5-HT transmissions. This demonstrates that subchronic toluene exposure at a low concentration may lead to adverse changes in neurobehavioral and neurochemical functioning, and further questions in a public health perspective the actual neurotoxic potential of toluene and other organic compounds, because deficits in functioning are generally viewed as precursors of more serious adverse effects.

Keywords: Toluene; Low concentration; Subchronic exposure; Behavior; Dopamine; Serotonin; Male; Female; Rat

1. Introduction

Chronic toluene inhalation at concentrations above occupational exposure limits (e.g., 100 ppm; NIOSH, 2000) has been repeatedly shown to induce neurotoxic effects. These effects results inter alia in cognitive, motor, and balance function disorders in humans (for review, see Ostergaard, 2000), and both behavioral (Forkman et al., 1991; Korsak et al., 1992) and neurochemical (Bjornaes and Naaslund, 1988; Ladefoged et al., 1991) disturbances in animals.

In contrast, only few clinical and experimental data are available on the effects of chronic toluene exposure at concentrations below occupational exposure standards. Yet, some of these data may support adverse effects of low toluene concentrations on the central nervous system. For instance, significant correlations have been demonstrated between chronic toluene exposure at doses lower than 50 ppm and the occurrence of subjective or neurasthenic symptoms (Yin et al., 1987; Orbaek and Nise, 1989; Ukai et al., 1993), which are thought to reflect early neurotoxic effects (Arlien-Soborg, 1992). As well, an investigation in toluene-exposed workers has shown that significant neurobehavioral impairments may occur at toluene concentrations as low as 88 ppm (Foo et al., 1990). In rodents, subchronic exposures to toluene at 80 ppm from 6 days to 4 weeks produce neurobehavioral and neurochemical disorders (von Euler et al., 1989, 1991, 1993, 1994, 2000; Hillefors-Berglund et al., 1995). In addition, a recent study has shown that repeated exposures to toluene at low levels led to a sensitization process (i.e., to a progressive and persistent increase of the neurobehavioral responses to toluene) (Rogers et al., 1999). These latter data points out the importance of considering not

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only exposure level but also exposure duration in order to estimate the actual toxicity of toluene.

Therefore, the aim of this study was to determine the behavioral and neurochemical effects of 40 ppm inhaled toluene (a dose that has been shown to produce no adverse effects in a 4-week exposure paradigm; Hillefors-Berglund et al., 1995) in a rat model of 16-week subchronic exposure. Locomotor and rearing activities, adaptation/sensitization to narcosis induced by high acute toluene concentration, as well as tyrosine hydroxylase and tryptophan hydroxylase activities and dopamine (DA) and serotonin (5-HT) turnovers in the caudate–putamen, nucleus accumbens, hippocampus, prefrontal cortex, and cerebellum (which are neurotransmitters and brain structures that may be related to the behavioral functions above) were investigated in both male and female animals.

2. Materials and methods

2.1. Animals

All animal use protocols were in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). Male ($n = 12$) and female ($n = 12$) Sprague–Dawley rats (IFFA Credo, France), weighing 220–225 g (22 and 24 weeks, respectively) at the beginning of the experiment, were used. Rats were divided randomly to serve as toluene-exposed rats (six males, six females) and control rats (six males, six females). Then, they were housed socially in Perspex home cages at a constant temperature of 21 ± 0.5 °C, under a reversed cycle with lights on from 8 p.m. to 8 a.m., with free access to food and water.

2.2. Exposure to toluene

Male and female toluene-exposed animals (2×6) were housed separately within a common Perspex environmental chamber (L × l × h: $0.70 \times 0.50 \times 0.45$ m; surface: 0.35 m^2; volume: 0.16 m^3). Rats were exposed to toluene for 16 weeks on the basis of 104 h/week as follows: from Sunday 9 p.m. to Tuesday 9 p.m. (48 h), and from Wednesday 9 a.m. to Friday 5 p.m. (56 h). This exposure protocol was chosen to mimic toluene indoor air pollution that may be present at home and/or at work (Sick Building Syndrome). Male and female control animals (2×6) were housed separately within a common Perspex environmental chamber (see above) and exposed to standard clean air conditions using an exposure protocol similar to that used for toluene-exposed animals. Between the twice-weekly exposures to either toluene or standard clean air, the animals were handled and moved from their environmental chamber to their home cages.

Toluene was introduced in the experimental chamber using a low-pressure air source, an airflow regulator set at a flow rate of 1 l/min, and a solvent vaporizer set to deliver a constant concentration of 40 ppm toluene. Measurement of toluene concentration was performed twice a day, using an automatic calibrated pump (ref. accuro; Draeger, Strasbourg, France) and high-precision analytical colorimetric tubes (ref. 5/b; Draeger). During control experiments, standard clean air was introduced in the exposure chamber using a low-pressure air source and an airflow regulator set at a flow rate of 1 l/min. Temperature within the chamber was maintained at 21 ± 0.5 °C; sodalime and silica gel canisters were used to maintain carbon dioxide and humidity below 30 ppm and 75%, respectively. In both control and toluene-exposed animals, behavioral and neurochemical investigations were performed, respectively, on Days 1 and 2 after toluene or clean air exposure was completed.

2.3. Behavioral studies

2.3.1. Assessment of locomotor and rearing activity

Locomotor and rearing activities were quantified using an experimental setting of four individual cages (L × l × h: $0.30 \times 0.20 \times 0.17$ m; surface: 0.06 m^2; volume: 0.01 m^3), equipped with two couples of parallel horizontal infrared beams (Imetronic, Pessac, France) placed 3 and 11 cm above the floor of each activity cage in order to detect locomotor (horizontal) activity and rearing (vertical) activity, respectively. Beam interruptions were identified via an electrical interface, and accumulated and recorded over 1-min intervals using a PC computer. Test sessions lasted for 2 h.

2.3.2. Adaptation/sensitization to narcosis induced by acute toluene at high concentration

Experiments were conducted in a Perspex environmental chamber (L × l × h: $0.70 \times 0.45 \times 0.35$ m; surface: 0.315 m^2; volume: 0.11 m^3) equipped with a Perspex cylinder of 0.3 m diameter and 0.5 m length. Rats were installed in individual compartments inside the cylinder and then rotated at a constant rate of 1 rpm, corresponding approximately to 1 m/min. Toluene was admitted in the experimental chamber up to a dose just sufficient to induce loss of righting reflex, using a low-pressure air source, a solvent vaporizer, and an airflow regulator set at a constant flow rate of 15 l/min. The narcotic effects of toluene were evaluated using a four-stage scale, whose criteria describe progressive degrees of narcosis: Stages 1 and 2 correspond to the time the animal rolls over on the floor and rights immediately (< 3 s) for the first and the third time, respectively; Stage 3 corresponds to the time the animal rolls over on the floor and rights in a delayed manner (>5 s); and Stage 4 corresponds to loss of righting reflex, which is well known as a reliable criterion of narcosis induced by inert gases, gaseous anesthetics, and volatile substances such as solvents (Miller et al., 1973; Abraini et al., 1998, 1999).

Measurements of toluene concentration corresponding to Stages 1–4 were made using an automatic calibrated pump connected to the experimental chamber (ref. accuro; Draeger) and high-precision analytical colorimetric tubes (ref.

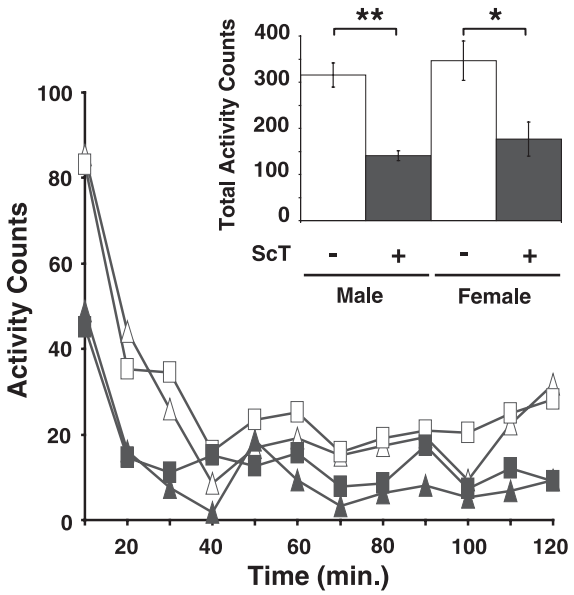

Fig. 1. Effect of subchronic toluene exposure at 40 ppm on vertical locomotor activity (rearing) recorded during a 120-min testing period in control male (△) and female (□) rats and toluene-exposed male (▲) and female (■) animals. Inset shows total activity count ± S.E.M. for the entire testing period. ScT = subchronic toluene exposure ($n = 6$, $*P < .05$, $**P < .01$).

100/a; Draeger). Temperature and carbon dioxide were maintained as described above.

2.4. Neurochemical studies

2.4.1. Dissection, tissues preparation, and concentrations assays

Briefly, 24 h after the behavioral experiments were performed, the rats were injected intraperitoneally with NSD_{1015} (3-hydroxybenzyl hydrazine dihydrochloride, 50 mg/kg; Aldrich, Strasbourg, France) in 0.9% saline to complete L-DOPA and L-amino acid decarboxylase blockade. Twenty minutes later, rats were killed by decapitation and brains were rapidly removed, frozen, and cut in frontal sections of 500 μm thick. Discrete brain regions, including the caudate–putamen, nucleus accumbens, hippocampus, prefrontal cortex, and cerebellum, were punched out, according to the method of Palkovits and Brownstein (1988), using a hollow needle and placed in 100 μl of 0.4 M perchloric acid containing 2.7 mM ethylenediaminetetraacetic acid (EDTA). After disruption by ultrasound, homogenates were centrifuged (4000 × g, 15 min) at 4 °C. Tissue concentrations of DA and 5-HT, and of their respective precursors (dihydroxyphenylalanine, DOPA; 5-hydroxytryptophan, 5-HTP) and main metabolites (3,4-dihydroxyphenylacetic acid, DOPAC; 5-hydroxyindolacetic acid, 5-HIAA), were analyzed directly in the supernatant by high-performance liquid chromatography with electrochemical detection (Cransac et al., 1996).

2.4.2. Estimation of tyrosine hydroxylase and tryptophan hydroxylase activities and of DA and 5-HT turnovers

Given that DOPA and 5-HTP accumulations in the brain are linear during the 20-min period after the injection of NSD_{1015} (Cottet-Emard et al., 1997), DOPA and 5-HTP accumulation reflects tyrosine hydroxylase and tryptophan hydroxylase activities, respectively (Lachuer et al., 1992; Poncet et al., 1997). Enzyme rates were expressed as picomole of L-DOPA and 5-HTP formed per 20 min per structure. DA and 5-HT turnovers were evaluated by calculating the ratio of DA/DOPAC and 5-HT/5-HIAA, respectively.

2.5. Data presentation and statistical analysis

Results were expressed as mean ± S.E.M., and analyzed using nonparametric statistics. Between-group comparisons of locomotor and rearing activities were carried out using the Kruskall–Wallis analysis of variance; following a significant H value, a post-hoc comparison was made using the Mann–Witney U test. Between-group comparisons of effective

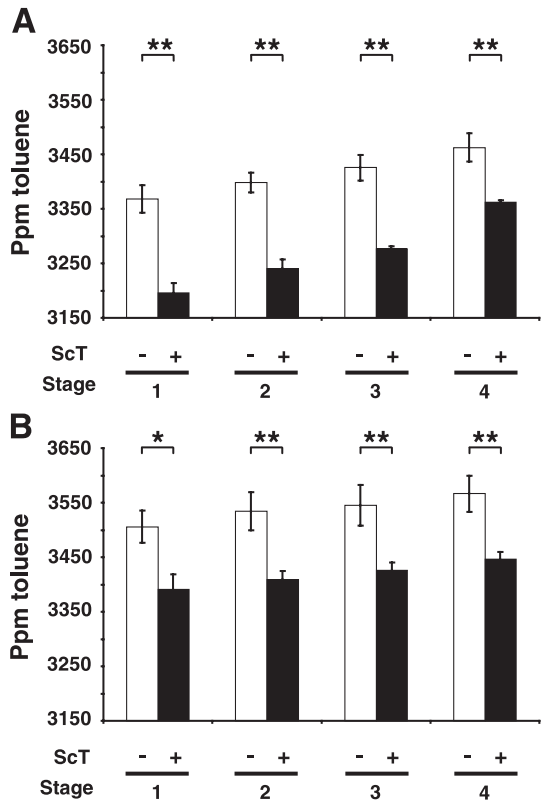

Fig. 2. Effect of subchronic toluene exposure at 40 ppm on toluene-induced narcosis in male (A) and female (B) animals. Toluene-induced narcosis occurred at a significant lower concentration in toluene-treated rats than in control animals in both males and females, indicating a sensitization process. In addition, in both control and toluene-exposed rats, it should be noted that narcosis occurred at a significant lower toluene concentration in male than in female animals (symbols of statistical significance were omitted for clarity of presentation). ScT = subchronic toluene exposure. Results are expressed as mean ± S.E.M. ($n = 6$, $*P < .05$, $**P < .02$).

toluene concentration inducing loss of righting reflex and neurotransmitter indices were made using the Mann–Witney *U* test.

3. Results

3.1. Behavioral studies

3.1.1. Locomotor activity

Exposure to toluene did not significantly alter basal locomotor activity in male (323.67 ± 18.76) and female (624.33 ± 43.11) rats compared to control males (378.5 ± 51.42; $H = 2572$, n.s.) and females (774 ± 70.23; $H = 2269$, n.s.). However, locomotor activity was significantly higher in female rats than in male rats in both control ($H = 1450$, $P < .0001$; $U = 0$, $P < .01$) and toluene-exposed animals ($H = 1475$, $P < .0001$; $U = 0$, $P < .005$).

3.1.2. Rearing activity

As illustrated in Fig. 1, toluene exposure significantly decreased rearing activity in both male ($H = 2984.5$, $P < .0001$; $U = 30$, $P < .01$) and female rats ($H = 3040$, $P < .0001$; $U = 26$, $P < .05$), as compared to control animals. In contrast to locomotor activity, no significant difference was found between male and female rats in both control ($H = 2340.5$, n.s.) and toluene-exposed animals ($H = 1369$, $P < .05$; $U = 10$, n.s.).

3.1.3. Adaptation/sensitization to narcosis induced by acute toluene at high concentration

Toluene-induced narcosis occurred at significant lower concentrations in toluene-treated rats than in control animals in both males (Stages 1–4: $U = 0$, $P < .02$; Fig. 2A) and females (Stage 1: $U = 4$, $P < .05$; Stages 2–4: $U = 2$, $P < .02$; Fig. 2B). This result may reflect a sensitization process. In addition, in both control and toluene-exposed rats, it should be noted that narcosis occurred at a significant lower toluene concentration in males than in females (Stages 1–4: $0 < U < 2$, $P < .02$). This indicates a differential gender sensitivity to toluene.

3.2. Neurochemical studies

3.2.1. DA neurotransmission

As it can be seen in Table 1, toluene exposure led to a significant increase of the DA/DOPAC ratio in the putamen of both male ($U = 2$, $P < .02$) and female ($U = 6$, $P < .05$) rats exposed to toluene, and to a further significant decrease in DOPAC concentration in the putamen of male rats ($U = 3$, $P < .02$), as compared to control rats. It should be noted that in both the putamen and the nucleus accumbens, the DA/DOPAC ratio was significantly higher in female rats than in male rats in both control and toluene-exposed rats ($5 < U < 6$, $P < .05$); this may explain the differences in motor activity between male and female rats in both control and toluene-exposed animals. No significant change in DA or DOPAC was seen in the hippocampus, prefrontal cortex, and cerebellum.

Subchronic toluene exposure further led to a significant reduction of DOPA accumulation in the hippocampus of male rats but not of female rats, as compared to controls ($U = 0$, $P < .02$); this resulted in a significant difference in DOPA accumulation between male and female rats exposed to toluene ($U = 0$, $P < .02$; Table 1). No significant difference in DOPA accumulation was found between control and

Table 1
Effect of subchronic exposure to 40 ppm toluene on dopaminergic neurotransmission of male and female rats in various brain regions

Brain structure	*Gender*	*ScT*	*DOPA acc.*	*DA*	*DOPAC*	*Ratio DA/DOPAC*
PUTAMEN	Males	-	36,8 ± 2,6	527,5 ± 13,6	52,2 ± 4,2 **	6,6 ± 0,5 ** *
		+	35,7 ± 1,8	552,6 ± 40,6	38,2 ± 2,9	8,6 ± 0,4
	Females	-	36,3 ± 2,6	528,4 ± 33,4	39,3 ± 3,2	8,6 ± 0,5 * *
		+	37,4 ± 1,7	568,4 ± 46,1	33,8 ± 3,9	13,3 ± 1,6
NUCLEUS ACCUMBENS	Males	-	24,8 ± 1,6	211,5 ± 16,3	38,6 ± 4,2 **	5,7 ± 0,5 * *
		+	25,2 ± 1,3	211,0 ± 16,0	30,6 ± 4,6	7,3 ± 0,5
	Females	-	21,2 ± 1,4	186,3 ± 15,8	26,0 ± 2,9	7,5 ± 0,7 *
		+	24,8 ± 1,2	217 ± 15,2	24,6 ± 2,5	9,0 ± 0,6
HIPPOCAMPUS	Males	-	2,6 ± 0,2 **	0,03 ± 0,0	0,03 ± 0,0	1,0 ± 0,0
		+	0,03 ± 0,0	0,03 ± 0,0	0,03 ± 0,0	1,0 ± 0,0
	Females	-	1,3 ± 0,6 **	0,03 ± 0,0	0,03 ± 0,0	1,0 ± 0,0
		+	2,2 ± 0,1	0,03 ± 0,0	0,03 ± 0,0	1,0 ± 0,0
PREFRONTAL CORTEX	Males	-	3,8 ± 0,5	2,1 ± 1,4	0,03 ± 0,0	69,1 ± 45,6
		+	2,9 ± 0,7	0,03 ± 0,0	0,03 ± 0,0	1,0 ± 0,0
	Females	-	3,4 ± 0,4	2,6 ± 1,2	0,03 ± 0,0	86,1 ± 39,0
		+	3,3 ± 0,2	4,5 ± 1,5	0,03 ± 0,0	151,0 ± 49,0
VERMIS	Males	-	4,8 ± 1,7	6,0 ± 2,0	0,03 ± 0,0	201,6 ± 65,2
		+	7,6 ± 0,8	9,5 ± 1,3	0,03 ± 0,0	316,5 ± 42,8
	Females	-	4,8 ± 1,7	11,7 ± 2,5	0,03 ± 0,0	390,7 ± 82,6
		+	6,3 ± 2,2	9,4 ± 0,7	0,03 ± 0,0	313,5 ± 24,9

ScT = subchronic toluene exposure; DOPA acc = dihydroxyphenylalanine accumulation; DA = dopamine; DOPAC = 3,4-hydroxyphenylacetic acid.
Results are expressed as mean ± S.E.M. ($n = 6$ per condition, * $P < .05$, ** $P < .02$).

Table 2
Effect of subchronic exposure to 40 ppm toluene on serotoninergic neurotransmission of male and female rats in various brain regions

Brain structure	*Gender*	*ScT*	*5-HTP acc*		*5-HT*	*5-HIAA*	*Ratio 5-HT/5-HIAA*
PUTAMEN	Males	-	1,5 ± 0,1		0,03 ± 0,0	38,4 ± 3,8	0,0 ± 0,0
		+	1,1 ± 0,2		0,03 ± 0,0	33,2 ± 4,8	0,0 ± 0,0
	Females	-	1,4 ± 0,3		0,03 ± 0,0	38,3 ± 6,3	0,0 ± 0,0
		+	1,5 ± 0,4		0,03 ± 0,0	31,1 ± 4,5	0,0 ± 0,0
NUCLEUS ACCUMBENS	Males	-	1,9 ± 0,3		0,03 ± 0,0	36,4 ± 5,3	0,0 ± 0,0
		+	2,0 ± 0,3		0,03 ± 0,0	33,4 ± 4,8	0,0 ± 0,0
	Females	-	2,2 ± 0,2		0,03 ± 0,0	38,4 ± 3,7	0,0 ± 0,0
		+	2,8 ± 0,3		0,03 ± 0,0	40,0 ± 4,2	0,0 ± 0,0
HIPPOCAMPUS	Males	-	1,2 ± 0,6		1,9 ± 0,7	16,8 ± 7,8	33,7 ± 22,8
		+	0,03 ± 0,0		1,6 ± 1,0	31,2 ± 2,6	0,0 ± 0,0
	Females	-	0,03 ± 0,0	*	1,4 ± 0,6	22,4 ± 7,5	0,4 ± 0,2
		+	2,4 ± 0,6		0,5 ± 0,5	11,1 ± 7,0	0,7 ± 0,2
PREFRONTAL CORTEX	Males	-	0,8 ± 0,4		0,03 ± 0,0	35,8 ± 12,4	0,0 ± 0,0
		+	0,03 ± 0,0		0,03 ± 0,0	25,9 ± 2,3	0,0 ± 0,0
	Females	-	1,0 ± 0,4	*	0,03 ± 0,0	13,4 ± 6,0	0,5 ± 0,2
		+	1,3 ± 0,3		0,03 ± 0,0	26,9 ± 1,7	0,0 ± 0,0
VERMIS	Males	-	2,2 ± 0,7		10,5 ± 4,5	21,1 ± 3,1	0,5 ± 0,3
		+	3,0 ± 0,5		0,03 ± 0,0	20,0 ± 1,4	0,0 ± 0,0
	Females	-	1,5 ± 0,7		0,03 ± 0,0	17,8 ± 2,7	0,0 ± 0,0
		+	1,5 ± 0,7		0,03 ± 0,0	23,6 ± 0,8	0,0 ± 0,0

ScT = subchronic toluene exposure; 5-HTP acc = 5-hydroxytryptophan accumulation; 5-HT = serotonin; 5-HIAA = 5-hydroxyindolacetic acid.
Results are expressed as mean ± S.E.M. ($n = 6$ per condition, * $P < .05$).

toluene-exposed rats in the caudate–putamen, nucleus accumbens, prefrontal cortex, and cerebellum.

3.2.2. 5-HT neurotransmission

As compared to controls, subchronic exposure to toluene tended to increase 5-HTP accumulation in the hippocampus and the prefrontal cortex of females exposed to toluene, while it tended to decrease it frankly in toluene-exposed males (it should be noted that these toluene-induced decreases in 5-HTP in male rats did not reach statistical significance because of a high variability that may reflect individual susceptibility). This resulted in a significant difference in 5-HTP accumulation between toluene-exposed males and females ($2 < U < 6$, $P < .05$; Table 2). No significant difference was seen in 5-HTP accumulation in the putamen, nucleus accumbens, and cerebellum. No significant change was found in 5-HT or 5-HIAA concentration and turnover.

4. Discussion

The major finding of this study is that subchronic exposure (16 weeks, 104 h/week) to a very low concentration of toluene (40 ppm) may lead to significant alterations in behavioral and neurochemical functionings in male and female rats. Behavioral investigations were only made once, on Day 1 after toluene exposure was completed, in order to avoid learning effects, while neurochemical investigations were made on Day 2.

At the behavioral level, as compared to control rats, subchronic exposure to toluene at low concentration led to a significant decrease in rearing activity and to a significant increase in sensitivity to toluene-induced narcosis, but failed to alter locomotor activity. This lack of effect of subchronic exposure to toluene on locomotion is in good agreement with previous findings that have reported no effect on spontaneous locomotor activity following an acute, subchronic, or chronic exposure to 80 ppm toluene (von Euler et al., 1988, 1991, 1993, 1994). In contrast, our results clearly show some discrepancies with studies that have reported no change in rearing activity in rats exposed to a 4-week exposure to 80 ppm toluene (von Euler et al., 1993, 1994). However, this discrepancy may be due to difference in exposure protocol because the average experimental exposure to toluene was 14 ppm (80 ppm × 6/24 h × 5/7 days = 80 ppm × 30/168 h) in the study by von Euler et al. (1993, 1994) and 24 ppm (40 ppm × 104/168 h) in our study. Difference in testing protocol between both studies may also account for this discrepancy because rearing activity was tested only on Day 17 postexposure in the work of von Euler et al. (1993, 1994) and on Day 1 postexposure in the present study.

Rearing activity, as compared to horizontal locomotor activity, is thought to include major cognitive processes related to spatial learning (Swanson et al., 1997). Because toluene frankly altered rearing activity, but not horizontal locomotor activity, it may be suggested that cognitive functioning would be more sensitive to toluene than strict motor functioning. This hypothesis may agree with (i) recent animal investigations that have shown that subchronic exposure to toluene (80 ppm, 4 weeks) may induce persistent deficits in a learning retention task (von Euler et al., 2000), and (ii) human occupational studies that have demonstrated alterations in short-term memory, visual scanning and cognitive ability, perceptual–motor speed, and a manual dexterity task involving strategic processes in toluene-exposed

workers (Foo et al., 1990; Boey et al., 1997; Chouaniere et al., 2002). Such a higher sensitivity of cognitive functions to toluene would agree with the hypothesis of phylogenic hierarchical organization, which states that the sensitivity of brain systems and functions to stressful situations is related to their phylogenic age (Himwich, 1951).

Long-term subchronic exposure to 40 ppm toluene further led to a significant increase in sensitivity to narcosis induced by high acute toluene concentration, as investigated by loss of righting reflex. This finding, which may reflect a sensitization process related to the addictive properties of toluene, indicates that long-term exposure to toluene at low concentration may favor toluene acute neurotoxicity. In that way, Rogers et al. (1999) have found that a 4-week exposure to 80 ppm toluene may result in significant persistent alterations of the operant behavior of rats.

At the neurochemical level, subchronic exposure to toluene led to significant alterations in both DA and 5-HT neurotransmissions. However, it should be emphasized that our results did not draw a general trend of toluene action in both male and female rats, which makes it difficult for these toluene-induced neurochemical changes to account directly for the behavioral disturbances described above. In other respect, the results of the present investigation are hardly comparable with those of previous data (Fuxe et al., 1982, 1987; Celani et al., 1983; Rea et al., 1984; Ladefoged et al., 1991), given that the exposure protocols used actually differ from one study to another. Nevertheless, probably the most consistent finding in our results is the increase of the DA/DOPAC ratio in the caudate–putamen of male and female rats exposed to toluene, as compared to control animals. This increase in DA turnover could be explained by either a greater DA release and/or synthesis, or a lower DA catabolism (i.e., DA to DOPAC degradation). As regards to the unchanged DA concentration and DOPA accumulation, it is likely that long-term subchronic exposure to toluene at low concentration may alter preferentially DA catabolism rather than DA release or synthesis. In support of this finding are the significant increase in striatal DOPAC concentration in toluene-exposed male rats and the similar trend in females, as compared to control rats.

Generally speaking, our results suggest that subchronic inhalation of toluene at low concentration is able to induce adverse effects in rats (von Euler et al., 2000). However, it should be noted that the experimental study design that we used did not allow us to determine whether or not the behavioral and neurochemical alterations that we observed were acute or persistent. Although this information appears essential to determine the actual neurotoxicity of toluene subchronic exposure, existing data are somewhat controversial. For instance, Hillefors-Berglund et al. (1995) have demonstrated that serum prolactin levels returned to normal value from Day 17 postexposure, indicating that toluene subchronic exposure is insufficient to produce truly persistent alterations. In the same way, von Euler et al. (1993, 1994) have shown that toluene subchronic exposure produced no adverse behavioral effect when investigated on Day 17 postexposure. However, in contrast, von Euler et al. (1994) have shown persistent behavioral effects of subchronic toluene exposure on apomorphine-induced locomotor activity, indicating that subchronic exposure to toluene may produce a persistent sensitisation process. Altogether, these data make it difficult to determine presently whether or not subchronic exposure to toluene at low concentration may have persistent adverse effects.

In conclusion, our data show that subchronic exposure to toluene at low concentration (40 ppm) may produce both behavioral and neurochemical impairments. Given that 40 ppm toluene during a 4-week exposure corresponds to the No Observed Adverse Effect Level (NOAEL; Hillefors-Berglund et al., 1995), our results point out the importance of considering both exposure level and exposure duration in order to evaluate the actual neurotoxicity of toluene and solvent at large. Without doubt, setting the definitive environmental standards of exposure to solvents in a public health perspective constitutes a major challenge that will require to perform further single and multiple solvent dose-dependent (dose–response studies) and time-dependent experimental neurotoxicity studies in both male and female animals.

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