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Toluene-Induced Locomotor Activity is Blocked by 6-Hydroxydopamine Lesions of the Nucleus Accumbens and the mGluR2/3 Agonist LY379268

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The abuse of volatile inhalants remains a prominent, yet poorly understood, form of substance abuse among youth. Nevertheless, the identification of a mechanism underlying the reinforcing properties of inhalants has been hampered by the lack of a clearly identifiable neural substrate upon which these chemicals act. One ingredient that is common to many abused inhalants is toluene, an organic solvent that is self-administered by nonhuman primates and rodents. Most drugs of abuse have been found to elicit forward locomotion in rats, an effect owing to the activation of mesoaccumbal dopamine (DA) pathways. Thus, the present study was undertaken using two different approaches to determine whether toluene-induced locomotor hyperactivity is also ultimately dependent upon DA neurotransmission in the mesolimbic nucleus accumbens (NAC). Here we report on the effects of 6-hydroxydopamine (6-OHDA) lesions of the NAC or pretreatment with the metabotropic mGlu2/3 receptor agonist LY379268 on toluene-induced locomotor activity. Both procedures, which are known to alter neurotransmission within the NAC, significantly attenuated toluene's locomotor stimulatory effects. These results provide strong support for a central mechanism of action of inhalants, which in the past has been more typically attributed to general nonspecific mechanisms throughout the brain. Moreover, as with other drugs of abuse, the NAC may be the final common pathway subserving toluene's abuse liability.

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INTRODUCTION

The incidence of inhalant abuse is exceeded at present only by alcohol, cigarette and marijuana use (NIDA Research Report, 1999). As with other drugs of abuse, inhalants can produce a variety of symptoms including euphoria, hallucinations, dependence, and psychosis (Flanagan and Ives, 1994; Balster, 1998). In fact, inhalants produce in humans a level for pleasant feelings equivalent to that produced by methamphetamine but more than that produced by either alcohol or nicotine (Kono *et al*, 2001). Toluene, a prototypical solvent found in many substances inhaled for their psychotropic effects, has also been shown to support self-administration in monkeys (Weiss *et al*, 1979), produce a conditioned place preference in mice (Funada *et al*, 2002), and to enhance intracranial self-stimulation (ICSS) at lower concentrations in the rat (Yavich and Zvartau, 1994). These positive findings in animals confirm the observation that toluene-containing inhalants possess abuse liability in humans.

A common property of drugs of abuse is to increase dopaminergic neurotransmission in mesolimbic terminal regions, such as the nucleus accumbens (NAC) (DiChiara and Imperato, 1988; Koob, 1992). This effect is thought to underlie not only the locomotor stimulatory effects of these substances, but to play a pivotal role in the reinforcing and abuse liability properties of drugs of abuse. In fact, the correlation between increased extracellular levels of dopamine (DA) in the NAC, and the reinforcing/locomotor stimulant effects of such drugs as nicotine, cocaine, amphetamine, and PCP has been well established through the use of 6-hydroxydopamine (6-OHDA) lesions of the NAC. Although the cellular site(s) and mechanism(s) mediating the central actions of abused inhalants containing toluene have not been completely identified, there is substantial neurobehavioral and electrophysiological data to support a toluene–DA interaction (von Euler, 1994). For

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example, toluene both *in vivo* and *in vitro* increases rat ventral tegmental DA neuronal excitability (Riegel and French, 1999a; French and Riegel, 2001). Futhermore, the administration of toluene to freely behaving animals produces increased levels of extracellular DA in the striatum (Stengard *et al*, 1994). If toluene-induced locomotor activity is also mediated through increased mesolimbic dopaminergic neurotransmission, then it is reasonable to expect that 6-OHDA lesions of the NAC would abolish this effect.

The recent identification of selective ligands for metabotropic glutamate receptor (mGluRs) subtypes also provides an alternative opportunity to test for a toluene-DA interaction. In contrast to neurochemical lesions, this pharmacological approach relies upon the activation of mGlu receptors localized to presynaptic terminals in the NAC. When stimulated under high agonist availability mGlu2/3 receptors mediate a negative feedback onto neuronal signaling (Ohishi *et al*, 1994; Shigemoto *et al*, 1997). mGlu2/3 receptors are densely expressed in the NAC, and when stimulated regulate both glutamatergic and nonglutamatergic (ie GABA, DA, serotonin)-mediated neurotransmission (Testa *et al*, 1994; Cartmell and Schoepp, 2000; Schoepp, 2001). For example, pretreatment with LY379268, a nanomolar potent and systemically active mGlu2/3-receptor agonist possessing 10 000-fold selectivity over other mGluRs, attenuated the DA-dependent hyperactivity evoked by amphetamine and PCP (Moghaddam and Adams, 1998; Cartmell *et al*, 2000a; Clark *et al*, 2002). Since LY379268 may be a unique pharmacological agent for disrupting neurotransmission in the NAC, we examined the effects of this selective mGluR2/3 agonist on the locomotor stimulant effects of toluene.

Given both the obvious popularity and potential detrimental effects attributed to inhalants, the identification of the neuronal substrate underlying the abuse potential of these compounds remains a critical step in understanding how these classes of drugs influence brain function. Here we focus on identifying a neural substrate mediating one of the behavioral effects of the abused inhalant toluene. The studies described herein employ a well-established neurobehavioral correlate for enhanced mesolimbic neurotransmission, namely locomotor activity (Koob, 1992). Based on their clearly recognized ability to precipitate DA-dependent and -independent locomotor activity, amphetamine and scopolamine were selected to serve as positive and negative controls, respectively (Joyce and Koob, 1981; Riegel and French, 1999c). To moderate a potential interference from other biogenic amines and further confer a degree of selectivity for DA, test subjects also received pretreatments of the noradrenergic blocker desipramine, whose relative binding affinities are described elsewhere (Frazer, 2001). The results of this study specifiy a central mechanism of action for a commonly abused solvent, which appears ultimately to be dependent upon DA neurotransmission in the NAC.

MATERIALS AND METHODS

All experiments were conducted on 240–300 g male Sprague–Dawley rats (Harlan Sprague Dawley, Inc.) according to protocols approved by the University of Arizona Institutional Animal Care and Use Committee. In all instances, the animals were maintained in a central animal facility under controlled lighting (12 h light/dark schedule) and temperature, with free access to food and water. All animals were pharmacologically naive and were randomly assigned to the various experimental groups. For several days prior to any drug treatment all rats were acclimated to the activity cages for a total of 5 h. On the day of drug challenge each rat was again exposed to the cage for 60 min immediately preceding drug injection. Following injection, no entry into the testing room occurred until the 3-h evaluation period was complete. Gross behavioral observations were made but not quantified. Statistical comparisons of the grouped data were made using Student's *t*-test, while drug treatment interactions were analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test. A P value of <0.05 was considered statistically significant. Data are presented as the mean $\pm$ -standard error of the mean (SEM).

6-OHDA Experiments

The surgical preparation and neuroanatomical coordinates used to inject rats with 6-OHDA or vehicle into the NAC are described in detail elsewhere (French and Vantini, 1984). To confer a degree of selectivity for the uptake of 6-OHDA into DA terminals and to minimize monoamine oxidase (MAO)-mediated degradation of the neurotoxin each animal was pretreated with pargyline (50 mg/kg, i.p.) and desipramine (25 mg/kg, i.p.) (Sigma Chemicals, St Louis, MO, USA) 30 min prior to bilateral 6-OHDA injections (4 μg/μl free base in a vehicle solution containing 0.2 mg/ml ascorbic acid in 165 mM NaCl) (Frazer 2001; French and Vantini, 1984). Animals comprising the sham group were treated in an identical manner but received bilateral NAC injections of the vehicle solution. A 30-gauge stainless-steel cannula was used to inject 2 μl of 6-OHDA or vehicle at a rate of 1 μl/3 min. At the end of the infusion the cannula remained in place for an additional 3 min. Locomotor activity measurements were begun 14 days following surgery. Horizontal activity was measured in photocell-equipped cages as previously described (Riegel and French, 1999c). Photocell beam interruptions were recorded every 10 min for a total of 3 h. A more thorough analysis of the dose–response relation for toluene has been previously published by our laboratory (Riegel and French, 1999c). Based on these earlier studies, the dose of toluene used here (600 mg/kg, i.p.) was selected for its ability to produce adequate locomotor stimulation in the absence of an interfering ataxia that begins to be expressed at higher doses.

In the saline and 6-OHDA-injected animals, systemic challenges with amphetamine, scopolamine, toluene, and an olive oil vehicle were given every other day. Specifically, three treatment groups were tested (naive, 6-OHDA lesioned and sham lesioned; $n=8$/group). Animals from the three groups received amphetamine (1.5 mg/kg, i.p.) on day 15, toluene (600 mg/kg, i.p.) on day 17, scopolamine (1.25 mg/kg, i.p.) on day 19, and olive oil vehicle (1.5 ml, i.p.) on day 20 postsurgery.

At 24 h after the last drug challenge, the animals were sacrificed (day 21) and the NAC was removed bilaterally and stored frozen at -80°C until the later determination of

DA content using HPLC techniques as described elsewhere (Imam and Ali, 2001).

LY379268 Experiments

Single daily i.p. injections of 1.5, 3, or 6 mg/kg of the mGluR2/3 agonist LY379268 were made over the course of 8, 9, and 12 days, respectively, in order to initiate a tolerance to the motor-depressant effects associated with LY379268 as described elsewhere (Cartmell *et al*, 2000b). The number of vehicle injections given to the control animals was matched to the same number of injections that were given to the 6 mg/kg LY379268 group. On days 9, 10, and 13, each group of animals again received 1.5, 3, or 6 mg/kg of LY379268, respectively, and 30 min later they were challenged with toluene (600 mg/kg, i.p.).

Drugs

The doses of the challenge drugs were selected for their demonstrated optimal effects on forward locomotion (French and Vantini, 1984; Riegel and French, 1999c). Toluene (99.8% purity) and its olive oil vehicle solution were prepared as described elsewhere (Riegel and French, 1999c). The final volume for all toluene injections was 1.5 ml. Dextro-amphetamine sulfate (Smith Kline and French Labs, Philadelphia, USA) and scopolamine HCl (Research Biochemicals International, MA, USA) were dissolved in saline, and their doses were calculated based upon weight of the salt. LY379268, which was kindly provided by Lilly Research Laboratories, Eli Lilly Company, was dissolved in water.

RESULTS

6-OHDA Experiments

Previously published studies demonstrate that the DA-dependent pattern of locomotor activity associated with injections of amphetamine is highly sensitive to 6-OHDA lesions of the NAC. In contrast, the pattern of locomotor activity produced by scopolamine has also been extensively examined, but it appears unrelated to DA neurotransmission and thus insensitive to 6-OHDA lesions (Joyce and Koob, 1981; French and Vantini, 1984). On this basis, the test compounds amphetamine and scopolamine served as positive and negative controls, respectively. The locomotor stimulatory effects of toluene, amphetamine, scopolamine, and vehicle over the 180 min following injection are shown in Figures 1 and 2. The locomotor activity produced in response to injections of toluene in the present study was comparable to that seen in a previously published study (Riegel and French, 1999c). When compared to vehicle, injections of amphetamine, toluene, and scopolamine produced significant increases in activity (compare inset graphs of Figure 2b with Figures 1a and b, 2a). Although the amount of locomotor activity produced by amphetamine is clearly greater than that produced by toluene, the effects of both agents were significantly reduced in the 6-OHDA-lesioned animals compared to the sham-lesioned group (amphetamine by 66.5%, $P<0.01$, and toluene by 55%, $P<0.02$). The responses of the naive and sham-lesioned

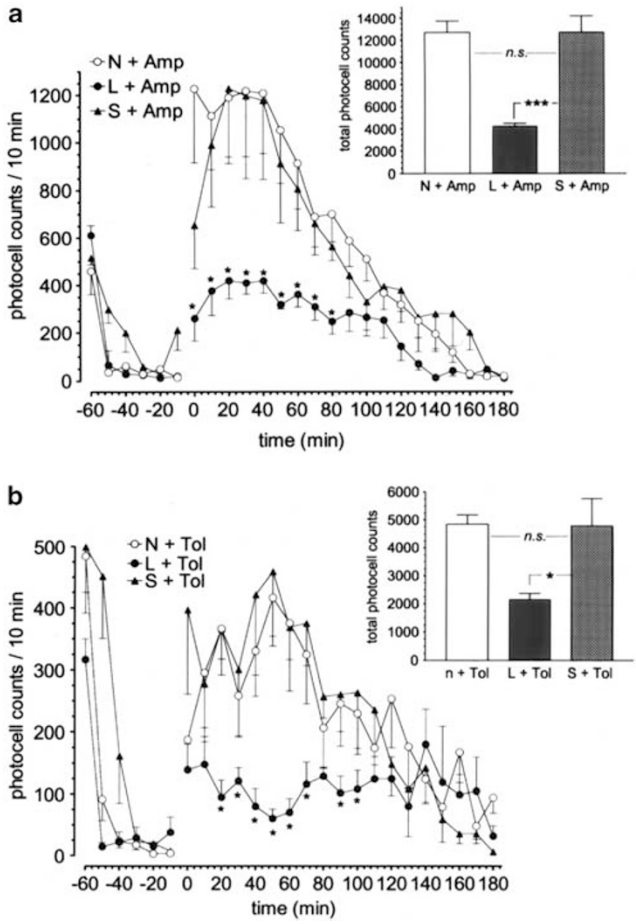

Figure 1 Acute locomotor stimulant properties of (a) amphetamine (Amp, 1.5 mg/kg, i.p.) and (b) toluene (Tol, 600 mg/kg, i.p.) in naive (N)-, sham (S)-, and 6-OHDA-lesioned (L) animals. Injections were given at time '0' following a 60 min acclimation period. For the time courses, data represent the mean photocell counts ± SEM at each 10 min interval. Note the different scaling on the *y*-axis of (a) and (b). Significant differences between treatment groups were determined by a repeated measure analysis of variance followed by *post hoc* testing using Dunnett's multiple comparison test. In both Tol and Amp treatments significant differences were found between the (L) and (S) groups, $*P<0.01$ (Student's *t*-test). Inset graphs depict the mean total photocell counts over the entire 3 h recording period following Amp and Tol. $***P<0.001$, $*P<0.05$, NS—nonsignificant (Student's *t*-test).

animals to the test drugs were indistinguishable from each other ($P=0.92$) (Figures 1 and 2). An examination of the time courses of drug action also revealed that amphetamine- and toluene-induced activity peaked between 20–60 min postinjection. In contrast to the stimulatory effects of toluene and amphetamine, the magnitude and time course of the stimulatory effects evoked by either scopolamine or vehicle were not altered by 6-OHDA lesions ($P>0.05$ when compared to sham controls) (Figure 2a, b), a result consistent with previously published reports (Joyce and Koob, 1981; French and Vantini, 1984). All treatment groups demonstrated adequate habituation to the test cages as evidenced by the rapid decline in activity during the 1 h of acclimation prior to drug treatment.

The subsequent neurochemical analysis (Figure 3) of the NAC found DA levels to be significantly reduced (~90%; $P<0.001$, Student's *t*-test) in the 6-OHDA-treated animals.

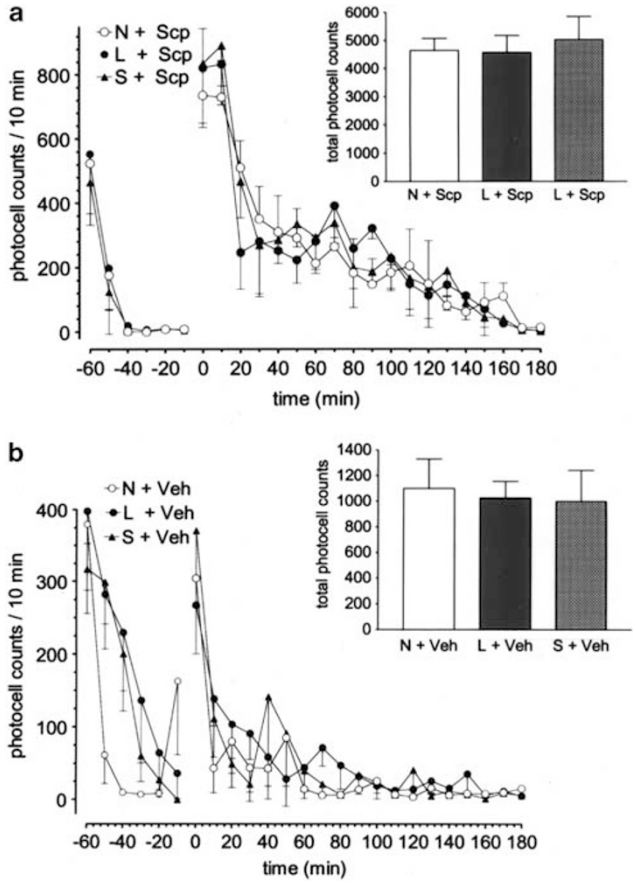

Figure 2 Effects of scopolamine (a) (Scp, 1.25 mg/kg, i.p.) and vehicle (b) (Veh, 600 mg/kg, i.p.) on locomotor activity in naive (N)-, sham (S)-, and 6-OHDA-lesioned (L) animals. Data represent the mean photocell counts ± SEM at each 10 min interval. Injections were given at time '0' following a 60 min acclimation period. Note the different scaling on the *y*-axis of (a) and (b). Inset graphs depict the mean total photocell counts over the 3 h recording period following scopolamine or vehicle for each group. Differences between groups were nonsignificant ($P > 0.05$, Student's *t*-test).

In contrast, DA concentrations in the naive group (884 ± 24 ng/100 mg wet weight) and sham-lesion group (873 ± 44 ng/100 mg) were nearly identical ($P > 0.05$) (Figure 3).

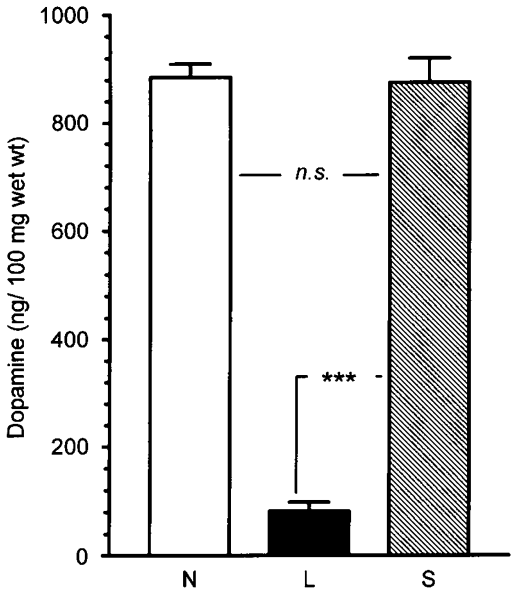

Figure 3 DA levels in the NAC of naive (N)-, sham (S)-, and 6-OHDA-lesioned (L) animals. Data represent the mean tissue concentrations (ng/ 100 mg wet weight ± SEM). Significant differences in DA levels between treatment groups were determined by the Student's *t*-test, ***$P < 0.001$, NS—nonsignificant.

LY379268 Experiments

An extensive body of literature demonstrates that the acute administration of LY379268 is associated with a nonspecific motor-depressant effect, to which animals develop a rapid tolerance with repeated administration (Cartmell *et al*, 1999, 2000b). Consistent with these reports, we also found that the initial injections of LY379268 used here produced a reduction in spontaneous activity compared to the vehicle groups receiving water. In order to overcome these motor-depressant effects, the animals ($n = 6$/group) were given repeated daily injections of LY379268 in a manner similar to previously published studies, and then 30 min later injected (i.p.) again with 1.5 ml (i.p.) olive oil vehicle (Cartmell *et al*, 1999, 2000a, b). Using this procedure we found that over the course of these daily pretreatments with LY379268 (8-12 days) the locomotor-depressant effects of LY diminished, eventually becoming indistinguishable from activity levels of the animals receiving only daily vehicle pretreatments followed by olive oil ($P > 0.05$). This result concurs with that reported by Cartmell *et al* (1999, 2000b). Specifically, the number of injections needed to reduce the locomotor-depressant effects of LY379268 was directly proportional to the dose administered. That is, for animals receiving 1.5, 3, or 6 mg/kg LY379268, the reductions in spontaneous activity were no longer evident by the 8th, 9th, and 12th day of treatment, respectively. With the elimination of the nonspecific motor effects of LY the animals were then challenged with toluene. As seen in Figure 4, toluene-induced locomotor activity was reduced in the LY-treated animals in a dose-dependent fashion ($P < 0.001$, $F = 19.1$, df 4,25). Specifically, the locomotor response to toluene in animals pretreated with 1.5, 3.0, or 6.0 mg/kg LY379268 was 68.5% ($P < 0.01$), 43.6 % ($P < 0.001$), and 29.0 % ($P < 0.001$) of the activity elicited by toluene in animals receiving repeated daily vehicle injections, respectively (Figure 4).

DISCUSSION

6-OHDA Studies and Toluene

The present results strongly suggest that the locomotor stimulatory effect of toluene is mediated via a specific site of action rather than by a nonspecific generalized effect throughout the CNS. Moreover, our findings in 6-OHDA-lesioned animals indicate that toluene-induced locomotor activity is ultimately dependent upon intact dopaminergic neurotransmission in the NAC. Similar inhibitory effects of 6-OHDA lesions have also been reported for amphetamine, cocaine, and PCP, forming the basis for the conclusion that many drugs of abuse act through a mesolimbic-DA *dependent* mechanism (Joyce and Koob, 1981; Wise and Bozarth, 1987). In contrast, the locomotor activation

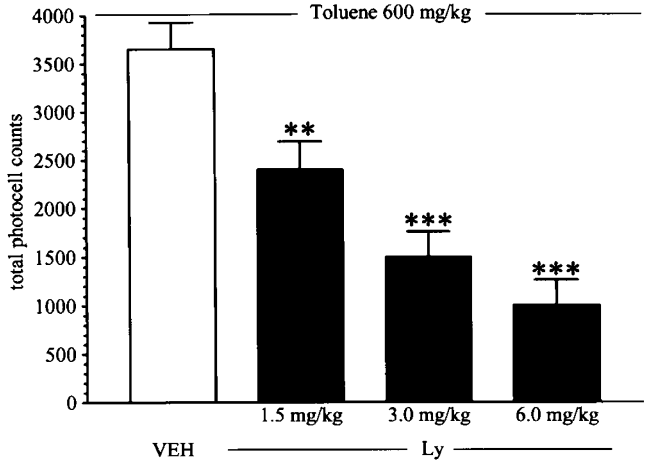

Figure 4 Toluene-induced locomotor activity is reduced in a dose-dependent fashion in rats behaviorally tolerant to the motor-depressant effects of LY379268. At 24 h after the last injection of LY379268 (days 9, 10, and 13), rats received an injection of vehicle (VEH), 1.5, 3.0, or 6.0 mg/kg LY379268 followed 30 min later by toluene (600 mg/kg, i.p.). All pretreatment injections were preceded by a 60 min acclimation period. $^{**}P<0.01$ and $^{***}P<0.001$ indicate statistically significant differences between treatment groups and vehicle (Student's *t*-test).

produced by scopolamine, a muscarinic receptor antagonist, is unaltered by 6-OHDA lesions and considered, therefore, not to be mediated through DA neurotransmission within the NAC (Joyce and Koob, 1981; French and Vantini, 1984). The blockade of 6-OHDA destruction of noradrenergic terminals supports a predominant role of DA in toluene-induced hyperactivity. This dependence on DA neurotransmission for the behavioral stimulatory effect of toluene would also concur with previous reports showing that a selective DA receptor antagonist can block toluene-induced hyperactivity (Riegel and French, 1999c). Therefore, it would appear that the locomotor activation elicited by toluene, like that of other psychomotor stimulants, is mediated through DAergic mechanisms within the mesolimbic NAC. Notably, it is the activation of this system, which is also thought to play a prominent role in the motivational aspects of drug reward (Koob, 1992; Kalivas, 1993). Nonetheless, the central question of how toluene might increase DA neurotransmission in the NAC remains to be answered. Although explanations are not immediately forthcoming from the present data, there are several speculative mechanisms both inside and outside the NAC which are pertinent. Westerink and Vijverberg (2002) recently demonstrated a toluene-induced increase in the frequency of vesicular catecholamine release from PC12 cells. Intriguingly, this effect was dependent upon calcium influx via voltage-gated calcium channels (VGCC). By analogy, toluene could conceivably activate VGCC in the NAC to release directly DA. Alternatively, the toluene-PC12 cell effect may reflect a membrane depolarization (Westerink and Vijverberg, 2002). This second possibility is interesting given that (1) at present there exists no data supporting a toluene-related impulse-*independent*, amphetamine-like mechanism of action and (2) *in vivo* toluene-associated release of neurotransmitter is reported as TTX sensitive, and thus likely impulse *dependent* (Stengard and O'Connor, 1994; Westerink and Vijverberg, 2002).

At the behavioral level, volatile inhalants like toluene share considerable overlap with other CNS depressants such as barbiturates, benzodiazepines, and ethanol (EtOH), and so may possess similar mechanisms of action (Hinman, 1987; Bowen *et al*, 1999). The administration of these substances in rats has been shown to stimulate DA release (DiChiara and Imperato, 1988; Stengard *et al*, 1994; Yim *et al*, 1998, 2002). At the systems level, the increased release of DA associated with the administration of toluene and the CNS-depressant EtOH has been temporally associated *in vivo* with an increase in the number of impulses originating within the VTA, the site of origin of the mesolimbic DA pathway (Gessa *et al*, 1985; Riegel and French, 1999a, b). At the cellular level both substances stimulate DA neuronal activity—an effect that has been attributed to disinhibitory mechanisms as well as the direct activation of VTA DA neurons (Brodie *et al*, 1990, 1999). Taken together, these noticeable similarities would appear to support the general conclusions that the abused inhalant toluene may trigger the release of DA in the NAC via impulse-*dependent* mechanisms initiated upstream at the level of the cell bodies in the VTA.

Given the recognized correlation between drugs of abuse, locomotor hyperactivity and DA release in the NAC, the present results were anticipated. Thus, the data presented here would also be consistent with a number of other observations showing toluene-evoked (1000–2000 ppm) release of DA, GABA, and acetylcholine in motor pathways, cortical structures, and the cerebellum (Stengard *et al*, 1993; Stengard and O'Connor, 1994). Nonetheless, two microdialysis studies did not find a similar toluene-related (3000 ppm and 800 mg/kg, i.p.) increase of DA in the striatum or NAC, respectively (Kondo *et al*, 1995; Gerasimov *et al*, 2002). While these reports may appear contradictory, they may more likely underscore several important conditions underlying the toluene-induced release of DA.

Drugs of abuse that stimulate motor (nigrostriatal) pathways generally also stimulate reward (mesolimbic) pathways, albeit at lower concentrations. Moreover, the reinforcement produced by such psychoactive substances is related to *changes* in drug concentration in the brain rather than to steady-state blood levels (Balster and Schuster, 1973). Rapid changes in brain concentrations are also reflected as having greater reinforcement efficacy and, in fact, with *increasing* exposure durations, drug reinforcement responses show a change in response rates similar to a *decrease* in unit dose (Balster and Schuster, 1973). As the behavioral effects of a drug must ultimately result from some alteration in neuronal activity, it is particularly interesting that abuse-relevant concentrations of toluene in the blood ($\sim$300 μM) are very efficacious at stimulating VTA DA neuronal activity both *in vivo* and*in vitro*: an effect principally observed during transient (seconds to minutes) 'pulses' of the solvent (Riegel and French, 1999a, 2002). As the 'pulse' length is extended beyond $\sim$12–15 min VTA DA neurons both *in vivo* and *in vitro* rapidly inactivate through an apparent depolarization block mechanism (Riegel and French, 1999a, 2002). Thus, the absence of change in striatal or accumbal extracellular DA (Kondo *et al*, 1995; Gerasimov *et al*, 2002) could result from a blood/brain concentration of toluene at a level that is actually reducing DA neuronal activity, possibly through a depolarization inactivation

mechanism. Coincidently single i.p. injections of drugs such as ethanol also produce substantial, albeit transient, increases in DA release. However, accumbal DA concentrations rapidly return to basal levels despite the continued presence of elevated brain concentrations of ethanol (Yim *et al*, 2000). A similar cell body–terminal relation may explain in part why primates will self-administer *single* 15 s 'pulses' of toluene (3000 ppm) (Weiss *et al*, 1979), but changes in accumbal DA release in rats are not apparent during continuous exposure to the same concentration of solvent (Gerasimov *et al*, 2002). Such mechanisms may also explain why human abusers do not continuously (>15 min) inhale for long periods, but rather prefer to titer their doses by repeatedly 'huffing' very-high-exposure concentrations for only seconds to minutes (Flanagan and Ives, 1994).

Experimental Protocol and Blood Toluene Concentrations (BTC)

Toluene dose–effect relations with inhalation exposure is complex. 'Behaviorally relevant' BTCs can only be described adequately by examining two variables, exposure concentration and duration (Miyagawa *et al*, 1984). For instance, similar BTCs of $\sim 800\,\mu M$ can be generated by exposure to 11 500 ppm for 16 min or 1780 ppm for 100 min (Rees *et al*, 1985; Riegel and French, 1999a). It should be pointed out, however, that inhalation exposure does produce highly variable BTC in animals exposed to the same concentrations over the same period of exposure (Rees *et al*, 1985). Moreover, toluene vapors are irritating to the eyes at concentrations as low as 100 ppm (Andersen *et al*, 1983). For these reasons i.p. injections were used in the present study. This route of administration is also routinely used in animals to study drugs commonly abused by humans either by inhalational or oral ingestion (eg cannabinoids, nicotine, cocaine and amphetamines) (Miyagawa *et al*, 1984). In a study by Wada (1999) where rats were administered the identical 600 mg/kg i.p. of toluene, as done here, BTC were measured at $\sim 790\,\mu M$, a value consistent with a number of behaviorally relevant reports addressing the rewarding properties of abused inhalants (Table 1).

LY379268 Studies and Toluene

The present finding that LY379268, a selective mGlu2/3 agonist, attenuated toluene-induced activity further suggests that mGlu receptors (mGluRs) may be recruited subsequent to the toluene-induced increase in DA neurotransmission (Cartmell and Schoepp, 2000; Xi *et al*, 2002). Typically inactive, mGluRs function as autoreceptors during elevated neuronal activity to attenuate DA, GABA, glutamate, and acetylcholine release (Cartmell and Schoepp, 2000). Given the widespread influence of mGluRs and the list of ion channel and G-protein coupled receptors affected by toluene, further research is necessary to clarify the cellular sequence of events underlying any toluene-induced behavior (Bale *et al*, 2002; Cruz *et al*, 1988; Beckstead *et al*, 2000; Tsuga *et al*, 1999, 2002). Nevertheless, the above observations at the systems level are consistent with the fact that LY379268 similarly reduces the DA-dependent behavioral effects of PCP and to a lesser extent those of amphetamine (Moghaddam and Adams, 1998; Cartmell *et al*, 1999, 2000a; Clark *et al*, 2002). Although it is unclear where in the brain LY379268 may act to block the locomotor stimulatory effects of toluene, the modulation of excitatory glutamatergic afferents from prefrontal cortex (PFC) to

Table 1

End point	Blood toluene concentration	
In vivo		
Self administration (human)	$\sim 330\,\mu M$[a,b]	Garriott *et al* (1981)[a]
Conditioned place preference (mice)	$<220\,\mu M$[c,b]	Funada *et al* (2002)
↑Frequency ICSS	$>1090\,\mu M$[c,b,d]	Yavich and Zvartau (1994)
Onset CNS excitation	$294\,\mu M$[a]	Kishi *et al* (1988)[a]
↑Locomotor activity, no ataxia	$<792\,\mu M$[c,b]	Riegel and French (1999c)
↑Locomotor activity+ ↑ataxia	$>800\,\mu M$[c,b]	Kondo *et al* (1995)
↑VTA DA cell firing	$5–436\,\mu M$[a,b]	Riegel and French (1999c)[e]
Complete DpB	$>868\,\mu M$[a,b]	Riegel and French (1999a)[a]
↑DA(microdialysis striatum)	$349\,\mu M$[c,b]	Stengard *et al* (1994)
→DA (micro dialysis NAC)	$>800\,\mu M$[c,b]	Kondo *et al* (1995)
	700–1090 μM at 20–120 min[c,b]	Gerasimov *et al* (2002)
	700–1090 μM at 20–120 min[c,b]	Gerasimov *et al* (2002)
↑DA (micro dialysis PFC)		
In vitro		
↑VTA DA cell firing	$20–800\,\mu M$[a]	Riegel and French (2002)
Complete DpB	$>828\,\mu M$[a]	Riegel and French (2002)

The data provided in the table were selected for their relevance to the role of midbrain dopamine neurons and mesolimbic structures in toluene-induced behaviors. Unless indicated, all studies were performed in rats.

Relevant toluene concentrations in blood perfusate were determined from the following sources:

[a]Published literature values or calculated from raw data in published reports.

[b]Indicates conversion of μg/ml or μl/ml to μM (Arlien-Soborg, 1991).

[c]Similar/identical exposure concentration and duration data reported by Kishi *et al* (1988) or Rees *et al* (1985).

[d]Using the 60 min exposure to 3600 ppm of Yavich and Zvartau (1994).

[e]Identical toluene doses reported by Wada (1999).

↑, ↓, → refers to increases, decreases, and no change respectively; DpB refers to depolarization block.

VTA DA neurons should be examined (Wang and French, 1993; Karreman *et al*, 1996). Another possibility includes an LY379268–NAC interaction, which could in a sense pharmacologically replicate our chemical lesions induced by 6-OHDA. Interestingly, Hu *et al* (1999) reported that direct injections of the group II mGluR agonist DCG-4 into the NAC reduced extracellular levels of DA in that structure. Moreover, the application of the nonselective mGluR agonist *t*-ACPD directly into the NAC blocked PFC- or VTA-electrical stimulation-induced increases in NAC DA (Taber and Fibiger, 1995). Also, the superfusion of selective mGluR3 agonists such as L-AP4 onto accumbal slices is reported to inhibit DA release (Manzoni *et al*, 1997). While an LY379268–PFC mechanism cannot be ruled out, it appears unlikely. Data from a related report indicates that the neurochemical changes produced in the PFC following systemic injections of LY379268 could not be mimicked when LY379268 was applied locally (Cartmell *et al*, 2001). Thus, multiple lines of evidence support the toluene-related stimulation of DA neurotransmission in the NAC.

In summary, these experiments provide the first data associating the locomotor-stimulant effects of toluene with increased DA release specific to the mesolimbic NAC. This finding provides an important indication that inhalants may target the same neuronal substrate activated by most drugs of abuse. This connection may be particularly important for a better understanding of the abuse potential of inhalants containing toluene and the identification of the functional consequences of toluene abuse. These results are also consistent with the effects of other commonly abused drugs, which consistently show a striking specificity for primary mesolimbic DA reward fibers. Moreover, the attenuation of toluene's locomotor effects by a selective mGluR2/3 agonist may provide insight into the development of novel therapeutic interventions for the treatment of inhalant addiction.

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