

# Abused Inhalants Enhance GABA-Mediated Synaptic Inhibition

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Abused inhalants are widely used, especially among school-age children and teenagers, and are 'gateway' drugs leading to the abuse of alcohol and other addictive substances. In spite of this widespread use, little is known about the effects produced by inhalants on the central nervous system. The similarity in behavioral effects produced by inhalants and inhaled anesthetics, together with their common chemical features, prompted this study of inhalant actions on a well-characterized anesthetic target, GABA synapses. Whole-cell patch clamp recordings were conducted on CA1 pyramidal neurons in rat hippocampal brain slices to measure effects on resting membrane properties, action potential discharge, and GABA-mediated inhibitory responses. Toluene, 1,1,1-trichloroethane, and trichloroethylene depressed CA1 excitability in a concentration-dependent and reversible manner. This depression appeared to involve enhanced GABA-mediated inhibition, evident in its reversal by a GABA receptor antagonist. Consistent with this, the abused inhalants increased inhibitory postsynaptic potentials produced using minimal stimulation of stratum radiatum inputs to CA1 neurons, in the presence of CNQX and APV to block excitatory synaptic responses and GGP to block GABA<sub>B</sub> responses. The enhanced inhibition appeared to come about by a presynaptic action on GABA nerve terminals, because spontaneous inhibitory postsynaptic current (IPSC) frequency was increased with no change in the amplitude of postsynaptic currents, both in the presence and absence of tetrodotoxin used to block interneuron action potentials and cadmium used to block calcium influx into nerve terminals. The toluene-induced increase in mIPSC frequency was blocked by dantrolene or ryanodine, indicating that the abused inhalant acted to increase the release of calcium from intracellular nerve terminal stores. This presynaptic action produced by abused inhalants is shared by inhaled anesthetics and would contribute to the altered behavioral effects produced by both classes of drugs, and could be especially important in the context of a disruption of learning and memory by abused inhalants.

*Neuropsychopharmacology* (2009) **34**, 2296–2304; doi:10.1038/npp.2009.57; published online 3 June 2009

**Keywords:** hippocampus; synapse; calcium; dantrolene; ryanodine; endoplasmic reticulum

## INTRODUCTION

Over the past 10 years, solvent abuse has increased among grade-school children, adolescents, and some adults. The Monitoring the Future Survey (2000) found that 8th graders reported a high rate of current (4.5 percent), past year (10.2 percent), and lifetime (17.9 percent) inhalant abuse. The National Household Survey on Drug Abuse (2001) indicates that abuse of inhalants outpaced both crack cocaine and heroin use among young adults. In addition, abused inhalants are a major 'gateway' drug—leading to abuse of alcohol and other drugs. Recent data from the National Survey on Drug Use (2006) reported that over 783 000 initiates aged 12 or more have tried inhalants. Despite this widespread usage, there is little known about the effects of inhaled solvents on the nervous system (Balster, 1998; Del Re *et al*, 2006; Lubman *et al*, 2008).

Abused inhalant solvents could share similar actions with inhalational anesthetics, because both types of chemicals are halogenated or are aromatic hydrocarbons, and because volatile anesthetics also have a history of abuse potential (Balster, 1998; Beckstead *et al*, 2000; Bowen *et al*, 1999a). Also like anesthetics, abused inhalants exhibit nonselective actions on a number of neurotransmitter- and voltage-gated ion channels (Bale *et al*, 2005; Cruz *et al*, 2000; Del Re *et al*, 2006; Shafer *et al*, 2005; Smothers and Woodward, 2007). Synaptic sites of action play a major role for inhalational anesthetic effects on the central nervous system (Pittson *et al*, 2004; Pocock and Richards, 1991). Anesthetics enhance inhibitory synaptic responses (Nicoll *et al*, 1975; Nishikawa and MacIver, 2000, 2001). Early research indicated that amino-acid transmitters in hippocampal cortex were altered by abused inhalant exposure in animals (Bjornaes and Naalsund, 1988; Briving *et al*, 1986) and more recent work demonstrates effects on isolated GABA and glutamate receptors (Bale *et al*, 2005; Bowen *et al*, 1999b; Cruz *et al*, 2000), and chronic exposure alters GABA synapses (Liu *et al*, 2007); and these effects could alter inhibitory synaptic transmission. Toluene can directly excite ventral tegmental area dopaminergic neurons recorded in brain slices (Riegel *et al*, 2007). Toluene also

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Received 5 March 2009; revised 13 April 2009; accepted 1 May 2009

stimulated nondopaminergic cells *in vitro* (Riegel *et al*, 2007), but not *in vivo* (Riegel and French, 1999), suggesting additional depressant effects to enhance GABAergic inhibition could be involved (Riegel *et al*, 2004; Riegel *et al*, 2007).

A previous study using field potential recordings in hippocampal slices found that toluene could inhibit synaptically evoked CA1 neuron population spikes (Ikeuchi and Hirai, 1994), suggesting that enhanced inhibition could occur. CA1 neuron GABA-mediated synaptic inhibition is enhanced during anesthesia *in vivo* (Ma and Leung, 2006; MacIver *et al*, 1996; Nicoll *et al*, 1975; Pearce *et al*, 1989) and abused inhalants could produce similar alterations, because abused inhalants can produce anesthesia and inhaled anesthetics produce some behavioral changes similar to abused inhalants in mice (Balster, 1998). This study compared effects produced by abused inhalants on GABA-mediated inhibitory synapses, using whole-cell patch clamp recordings from CA1 pyramidal cells in rat hippocampal brain slices. Three abused solvents were compared, two halogenated hydrocarbons (1,1,1-trichloroethane and trichloroethylene), as well as the aromatic agent, toluene, providing representation of inhalants with distinct chemical profiles.

## MATERIALS AND METHODS

Brain slice preparation methods have previously been described in detail (Bieda and MacIver, 2004). In short, standard transverse hippocampal slices (450  $\mu$ m) from adolescent Sprague–Dawley rats (P33–36) were prepared using a vibratome. All procedures conform to Society for Neuroscience and NIH guidelines and were approved by the Stanford University Animal Use Committee.

## Electrophysiology

Standard visualized slice procedures were used ([31]). All recordings were from CA1 pyramidal neurons in stratum pyramidale. Pyramidal neurons exhibited accommodating action potential trains in response to depolarizing current injection, but varied in resting membrane potential and threshold current required to produce spiking; there was no spontaneous spiking evident. Input resistances varied from 200 to 550 M $\Omega$ . There were no obvious differences between cells in response to toluene: every neuron displayed only a small decrease in spiking and input resistance. Throughout current-clamp experiments, sets of current steps were applied repetitively at fixed intervals (typically, 3 steps/set: 1 hyperpolarizing, one at zero pA, and 1 depolarizing; 1 set/min). The depolarizing current step was adjusted to produce seven action potentials during the depolarization in control conditions for each neuron. In both current-clamp and voltage-clamp experiments cells were allowed to remain at rest, tonic currents were not applied to adjust resting potentials. All experiments were conducted at room temperature (22–23°C) using a submersion chamber and perfusion vessels, valves and >95% of connectors and tubing were Teflon to minimize drug loss or binding. Using continuous perfusion of ACSF at 3 ml/min complete bath replacement took <30 s, as measured by dye exchange. Each slice was used for only a single experiment, and each

animal provided 1–3 slices—limited by the long duration of each experiment (1–3 h). The following external (ACSF) was used (in mM): 124 NaCl, 3.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 MgSO<sub>4</sub>, 2 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 10 glucose; it was bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub> to reach pH 7.4. To prevent loss of the abused inhalant from the perfusate, a known volume of inhalant was added to pre-gassed ACSF and stored in Teflon perfusion vessels. Preliminary experiments showed that this prevented loss of volatile agents (less than 5% in 5 h) measured using high pressure liquid chromatography (HPLC; see below). A similar degree of loss was evident from samples taken next to brain slices in the experimental chamber used for recording neuronal responses.

We used standard whole-cell methods (pipette resistance 4–6 M $\Omega$ ). For all current-clamp recordings a KGluconate-based internal solution was used (in mM): 100 KGluconate, 10 EGTA, 5 MgCl<sub>2</sub>, 40 HEPES, 2 Na<sub>2</sub>ATP, 0.3 NaGTP (pH 7.2 with KOH). For voltage-clamp experiments, a KCl internal-based solution with QX-314 (1–5 mM) to block sodium currents was used, and, in experiments on evoked IPSCs, the concentration of MgCl<sub>2</sub> was changed to 3 mM. KCl internal was 100 KCl, 10 EGTA, 5 MgCl<sub>2</sub>, 40 HEPES, 2 Na<sub>2</sub>ATP, 0.3 NaGTP, 1 QX-314 (pH 7.2 with KOH).

Slice viability was tested using population spike responses and only slices exhibiting response amplitudes >10 mV were used for subsequent experiments. For recording of population spikes from CA1 pyramidal cells, an ACSF filled pipette (2 to 4 M $\Omega$ ) was placed at the boarder of stratum pyramidale and stratum oriens. To stimulate synaptic inputs, a bipolar tungsten electrode was placed in stratum radiatum, as previously described (Bieda *et al*, 2009). Recordings were established for >15 min before recording baseline data, and preparations showing unstable response properties (>5% variability) were not used.

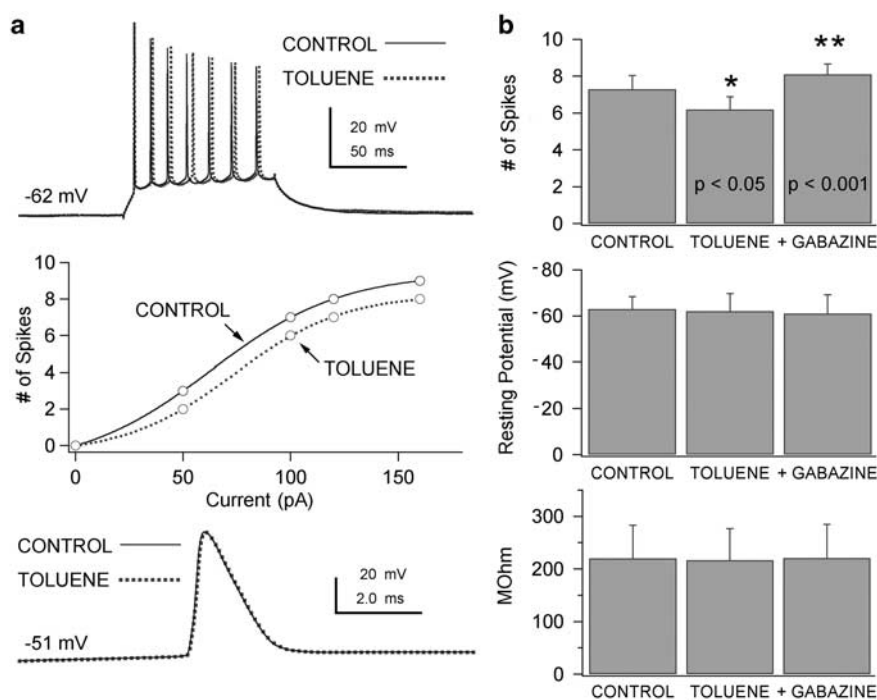
## Compounds and Concentration Analysis

All compounds were reagent grade or better from Sigma/RBI (St Louis, MO), except for toluene, trichloroethane, and trichloroethylene (all >99.99%), which were obtained from Aldrich (Milwaukee, WI). All solutions were made fresh daily using HPLC grade ‘OmniSolv’ water obtained from EMD–Merck (Gibbstown, NJ).

Due to the volatile nature of the abused inhalants, it was essential to measure bath concentrations to determine the extent of loss of drug from the perfusion vessels, tubing, and brain slice chamber. Chemical analysis was performed on ACSF samples (1.5 ml) taken from the perfusate and run on an HP 1050 HPLC using a 40/60% (water/acetonitrile) carrier solvent on a 25 cm spherisorb column. Major detection peaks for concentration analysis were found at 225 and 240 nm wavelengths with a retention time of approximately 8.5 min.

## Data Collection and Analysis

Whole-cell and synaptically evoked responses were collected and analyzed online using Igor Pro 6.0 (Wavemetrics, Oswego, OR) and graphed on computer monitors to ensure that consistent baselines were established for each preparation. A two-tailed Student’s *t*-test or repeated measures ANOVA with Tukey test were used to evaluate statistical



**Figure 1** Toluene depressed excitability of CA1 pyramidal neurons, but produced very little effect on resting membrane responses. (a) Direct current evoked discharge frequency, was slowed by toluene (950  $\mu$ M; top recordings), but there was no apparent change in spike threshold, action potential rise time, amplitude, or decay time (bottom recordings). (b) Group data based on measures from 10 pyramidal neurons show a small, but significant (ANOVA;  $*p < 0.05$  and  $**p < 0.01$ , both compared to pre-drug control), depression of discharge produced by toluene. This depression was reversed by a GABA antagonist, gabazine, indicating a possible involvement of enhanced inhibition in the effect produced by toluene (top graph). Neither toluene nor gabazine had any apparent effect on resting membrane potential but membrane resistance followed the same trends as spike discharge, however, only the gabazine effect was significant (compared to toluene;  $p < 0.1$ ; bottom graphs;  $p < 0.05$  for normalized data, not shown).

significance ( $p < 0.05$  or less; GraphPad Prism software; Prism Inc., San Diego, CA, USA).

## RESULTS

### Abused Inhalants Produce Little Effect on Postsynaptic Excitability

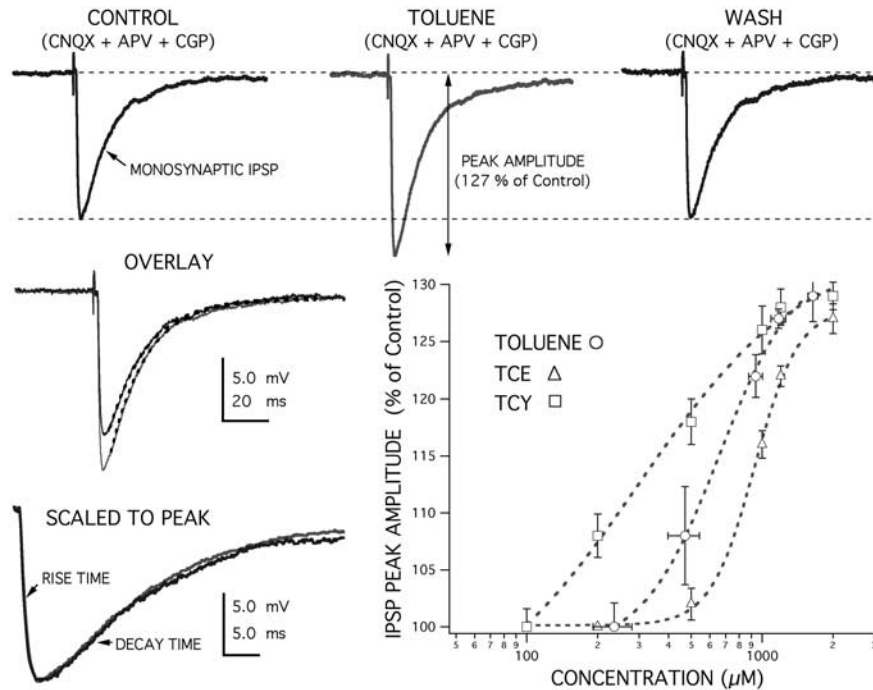
Even at high concentrations, toluene produced only a small depression of CA1 neuron excitability (Figure 1), measured as an ability to generate action potentials in response to depolarizing current injection. There was no effect apparent on action potential peak amplitude, rise time, half-width, or decay phase. At a concentration of 1 mM, toluene depressed action potential discharge from seven spikes in control to  $6.2 \pm 0.7$  (mean  $\pm$  SD) spikes in the presence of the inhalant (Figure 1a). Although this depression was statistically significant ( $p < 0.05$ ;  $n = 10$  experiments, each from different slices, from 8 rats), only a minor degree of inhibition was evident. The depression of spike discharge was not accompanied by a change in the resting membrane potential of CA1 neurons, although a small, statistically insignificant, decrease in membrane resistance was observed.

To determine whether the depression of action potential discharge was due to an inhalant-induced enhancement of synaptic inhibition, the ability of a GABA receptor antagonist to reverse the depression was studied. The GABA receptor antagonist, gabazine (SR95531) at a saturating concentration (10  $\mu$ M) completely reversed the

inhalant-induced depression of discharge ( $8.1 \pm 0.6$  spikes), indicating that an increase in GABA-mediated inhibition could account for this depression. Gabazine also reversed the small change in membrane resistance that was observed, but the difference was only significant at the  $p < 0.05$  level when gabazine data were compared to the normalized resistance in the presence of toluene (Figure 1b). Gabazine alone had no effect on action potential amplitude, rise time, duration, or on resting membrane potential.

### Abused Inhalants Enhanced GABA-Mediated Synaptic Inhibition

To study the effects of abused inhalants on GABA-mediated inhibition, isolated, monosynaptic inhibitory postsynaptic potentials (IPSPs) were evoked with stimulating electrodes placed in stratum radiatum. Glutamate and GABA-B-mediated synaptic responses were blocked using receptor antagonists—CNQX (18  $\mu$ M and APV 100  $\mu$ M) and CGP (1.0  $\mu$ M), respectively. Stimulus location, within stratum radiatum, as well as intensities and polarity were adjusted to produce minimal, stable response amplitudes; typically about 1.5 times threshold for the smallest amplitude responses seen. Using this approach, IPSPs exhibited amplitudes from 5 to 15 mV and failure rates of approximately 20%. The abused inhalants increased IPSP amplitudes, but did not appear to alter the rise time, decay time, duration, or failure rate of these synaptic responses (Figure 2).

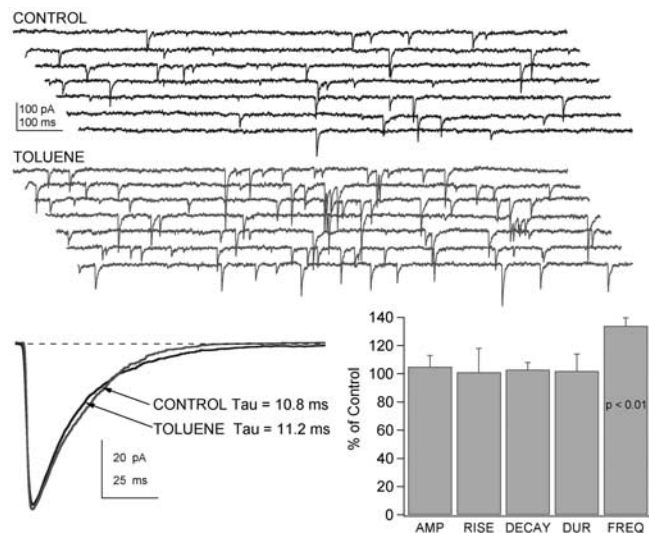


**Figure 2** Toluene enhanced GABA-mediated inhibition measured using monosynaptically evoked IPSPs recorded from CA1 pyramidal neurons (top recordings) and this effect recovered following washout of the inhalant. IPSP response amplitude was measured from prestimulus baseline to peak negativity, as indicated by the arrows. An increased IPSP amplitude was clearly evident in the overlay of control and toluene recordings (left, middle). The dashed lines in the overlay plot are fits to a single exponential, used to measure IPSP decay times (see results). Neither the rise time nor decay time of IPSPs was altered by toluene, as shown in the expanded overlay of recordings (left, bottom) in which the control response was scaled to the same peak amplitude as the toluene IPSP. The toluene effect on GABA-mediated IPSPs was concentration dependent and was also evident for other abused inhalants (trichloroethane—TCE and trichloroethylene—TCY). Each point in the concentration-effect graph (lower right) represents the mean  $\pm$  SD for at least five measures. The dashed lines are fits to the Hill equation using a least squares approach. All of the abused inhalants appeared to be equally efficacious, but TCY was approximately three times more potent than either toluene or TCE.

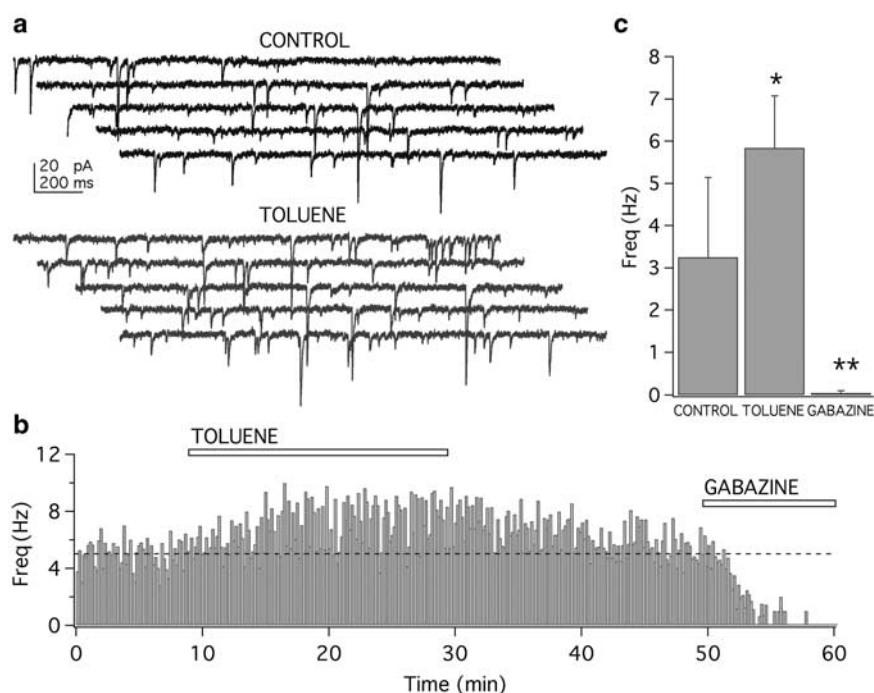
Toluene (TOL), trichloroethane (TCE), and trichloroethylene (TCY) appeared to be equally efficacious at increasing GABA-mediated inhibition, enhancing IPSP amplitudes to  $129 \pm 2.3$ ,  $128 \pm 1.6$ , and  $127 \pm 0.9\%$  of control responses, respectively. The inhalants differed considerably, however, in potencies with a rank order of  $TCY > TOL > TCE$  over a twofold range of concentrations. The concentration producing a half maximal effect ( $EC_{50}$ ) was determined from Hill equations, fitting the data with a least squares approach. The TCY  $EC_{50}$  was  $360 \mu M$ , and  $EC_{50}$  for TOL and TCE were  $628 \mu M$  and  $895 \mu M$ , respectively. Hill coefficients also varied considerably: 1.2 for TCE, 2.7 for TOL, and 4.7 for TCY.

### Abused Inhalants Enhanced GABA-Mediated Synaptic Inhibition by a Presynaptic Action

To determine whether the enhanced GABA-mediated inhibition resulted from pre- or postsynaptic mechanisms of action, spontaneous inhibitory postsynaptic currents (IPSCs) were recorded from voltage-clamped CA1 neurons (Figure 3). In control conditions, IPSC amplitudes ranged from 2 pA, just detectable above noise, to 650 pA with an average amplitude of  $75.8 \pm 14.2$  pA seen across 23 720 events recorded from 10 CA1 neurons. Frequency varied considerably, even within each cell, but averaged



**Figure 3** Toluene enhanced GABA inhibition by a presynaptic mechanism, evident in spontaneous IPSC recordings from CA1 pyramidal neurons. Recordings on top show 1.0 s long consecutive traces in control or after exposure to  $950 \mu M$  toluene. Unlike inhaled anesthetics, toluene did not appear to increase the decay time constants of these inhibitory currents, even for larger amplitude IPSCs (lower left). Toluene produced a marked increase in the frequency of spontaneous IPSCs (ANOVA  $p < 0.01$ ), with no significant change in current rise time, amplitude, decay time, or duration (lower right grouped data from 10 experiments).



**Figure 4** Toluene appeared to act directly at GABA nerve terminals, because an increase in miniature IPSC frequency was evident in the presence of tetrodotoxin used to block action potentials in the inhibitory interneurons. (a) Consecutive 5 s long recordings of miniature IPSCs for control and in the presence of toluene. (b) Rate meter plot showing the time course of toluene-induced increase in the frequency of IPSCs and recovery following washout of the abused inhalant. The GABA receptor antagonist, gabazine was applied at the end of this experiment to demonstrate that these synaptic currents were all GABA dependent. (c), Summary data from 10 experiments showing that toluene produced a significant increase in IPSC frequency (mean  $\pm$  SD, \* $p$  < 0.05; \*\* $p$  < 0.001).

$4.3 \pm 1.7$  Hz. IPSC rise time ( $1.58 \pm 0.21$  ms), decay time ( $16.9 \pm 5.2$  ms), and duration ( $24.6 \pm 7.8$  ms) measurements were quite consistent across cells. These IPSCs were completely blocked with  $20 \mu\text{M}$  gabazine (not shown), indicating that they were GABA-mediated synaptic currents.

All three abused inhalants significantly increased IPSC frequency: TOL to  $134 \pm 5.6\%$  of control, TCE to  $130 \pm 6.3\%$  and TCY to  $123 \pm 7.1\%$  of control, for concentrations of  $940 \mu\text{M}$  TOL, and  $800 \mu\text{M}$  of both TCE and TCY (Figure 3). These frequency increases were not accompanied by any measurable effect on holding currents needed to maintain the resting membrane potential at the original control values for the CA1 neurons. No significant effects on IPSC amplitude, rise time, decay time, or duration were observed in the presence of any abused inhalant. Thus, the abused inhalant-induced increase in inhibition appears to come about by a presynaptic action to increase the release of GABA from inhibitory nerve terminals.

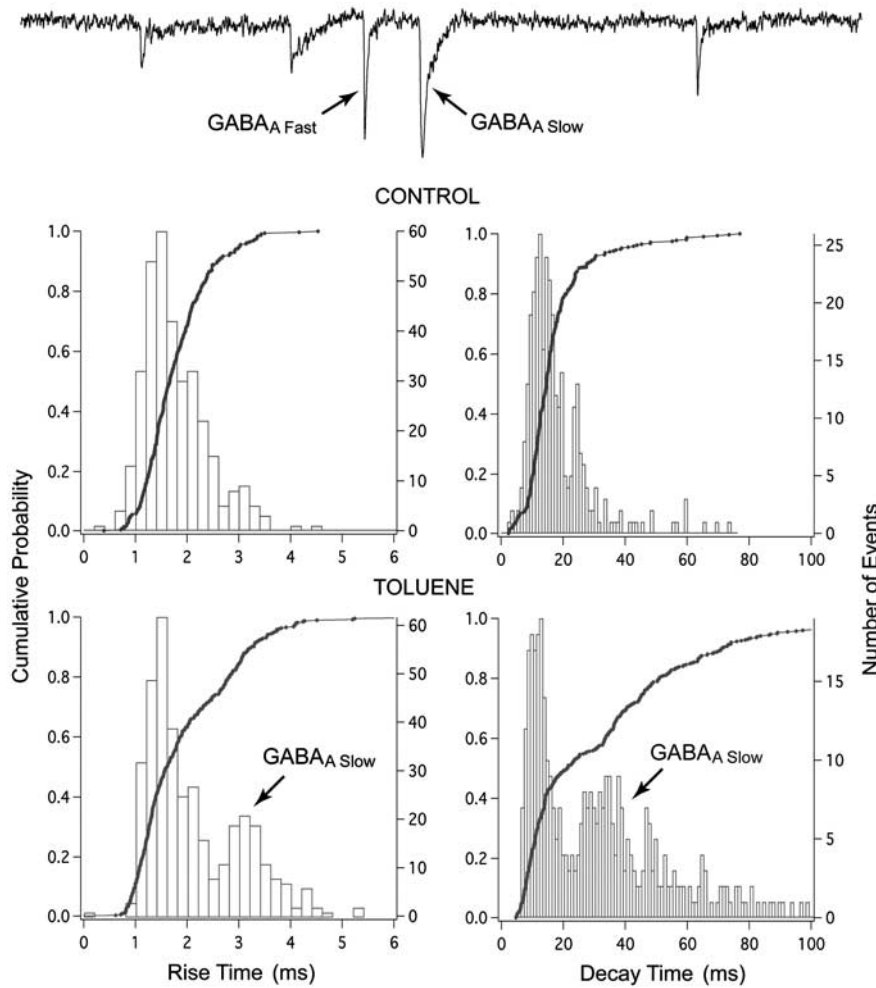
### Abused Inhalants Act Directly on GABA Nerve Terminals

The increased IPSC frequency produced by the inhalants could have come about by increased discharge of action potentials in inhibitory interneurons, or by a direct effect on interneuron nerve terminals. To test whether either or both of these effects contribute to the increased IPSC frequency, miniature IPSCs (mIPSCs) were recorded in the presence of TTX ( $1.0 \mu\text{M}$ ) to block sodium channels, and, hence, abolish action potential discharge of inhibitory neurons. If the inhalant-induced increase in mIPSC frequency still

occurred, then a direct action on GABA nerve terminals would be implicated.

In control conditions, mIPSC amplitudes were much smaller than IPSCs, averaging  $27.4 \pm 5.3$  pA. Rise times and decay times were essentially the same as seen for control conditions in the absence of TTX, however, mIPSC frequency was reduced to  $3.25 \pm 1.89$  Hz across the 9189 events recorded from 10 CA1 neurons studied. In the neuron shown in Figure 4b, toluene ( $940 \mu\text{M}$ ) produced a near doubling in mIPSC frequency (from 5.5 to 8.3 Hz), that reached steady state within 10 min and recovered following removal of toluene within 20 min. All of these synaptic currents were blocked by  $20 \mu\text{M}$  gabazine applied at 50 min. Grouped data for all 10 experiments are shown in Figure 4c and the increased frequency of mIPSCs was statistically significant ( $p$  < 0.01), as was the gabazine block ( $p$  < 0.001), both compared to control frequencies measured before application of drug.

For the majority of experiments (7 of 10), mIPSC amplitude, rise time, decay time, and duration were not altered by the inhalant. In the remaining three experiments, an apparent toluene-induced increase in rise time (from  $1.78 \pm 0.63$  to  $2.55 \pm 0.98$  ms), decay time (from  $17.11 \pm 11.23$  to  $29.87 \pm 28.65$  ms), and duration was seen in the initial analysis. On closer inspection, it was evident that these cells had prominent GABA<sub>A</sub> Slow mIPSCs mixed in amongst the typical GABA<sub>A</sub> Fast responses seen during control recordings (Figure 5). Toluene appeared to have a selective effect to enhance the frequency of GABA<sub>A</sub> Slow mIPSCs (to 120% of control) in these cells and this produced an apparent increase in decay time and duration, because more slow



**Figure 5** Toluene appeared to selectively enhance the frequency of GABA<sub>A</sub> Slow synaptic currents. In some experiments (3 of 10), two kinds of miniature IPSC kinetics were evident: fast and slow (recording on top). In these experiments, toluene selectively enhanced the proportion of slow IPSCs, evident in the graphs of rise time and decay shown below. GABA<sub>A</sub> Fast IPSCs typically exhibit rise times less than 2.0 ms and decay times less than 20 ms. GABA<sub>A</sub> Slow IPSCs, in contrast, exhibit rise times of 3.0 ms and decay times over 20 ms (often 30 to 60 ms). Toluene skewed the distribution of IPSC rise and decay times in favor of GABA<sub>A</sub> Slow IPSCs (bottom graphs), indicating a selective effect on nerve terminals that give rise to these slower synaptic currents. For kinetic analysis, all mIPSCs in the 3 neurons during 30 s recordings in control (356 events) and drug conditions (428 events) were used. For rise times, 0.2 ms bins were used and for decay times, 1.0 ms bins were used.

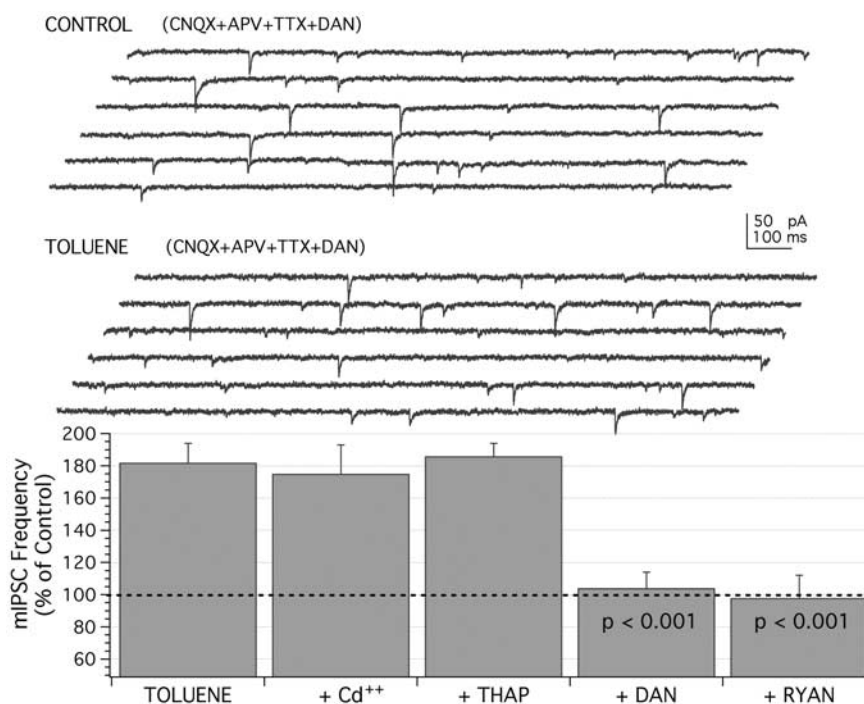
events were contributing to the analysis in the presence of inhalant.

Previous studies have shown that volatile anesthetics increase mIPSC frequency by acting on intracellular calcium stores in GABA nerve terminals (Doze and MacIver, 1998; Yamashita *et al*, 2001). To determine whether a similar mechanism contributed to the toluene-induced increase in mIPSC frequency, the effects of blocking calcium influx into nerve terminals was studied. The toluene-induced increase in mIPSC frequency persisted in the presence of 500  $\mu$ M Cd<sup>++</sup>, used to block calcium entry into nerve terminals, thus, toluene appeared to share an action on intracellular stores with volatile anesthetics. Nerve terminals appear to have at least two intracellular calcium storage compartments: a thapsigargin-sensitive store that is associated with IP<sub>3</sub> receptor-mediated regulation and a caffeine-sensitive store that is associated with ryanodine receptor-mediated regulation. Thapsigargin, ryanodine, or dantrolene, when applied alone, appeared not to alter

mIPSC frequency, except for a transient increase (2–5 min) following dantrolene exposure. Pretreatment of preparations with thapsigargin did not alter the toluene-induced increase in mIPSC frequency (Figure 6), however, pretreatment with either dantrolene (a calcium store depletion agent) or ryanodine (a caffeine store calcium channel blocker) blocked the effect produced by toluene.

## DISCUSSION

Abused inhalants depressed CA1 pyramidal neurons by increasing inhibition at GABA synapses. All three inhalants appeared to increase inhibition over the concentration range thought to occur *in vivo* (Balster, 1987; Beckstead *et al*, 2000). This increased inhibition was evident in recordings of monosynaptic IPSPs and occurred with no apparent effect on resting membrane potential or action potential threshold produced by the inhalants. The



**Figure 6** Toluene increased IPSC frequency by releasing calcium from intracellular stores, because the effect persisted in the presence of Cd<sup>++</sup>, used to block calcium entry through nerve terminal membrane channels, but was blocked when calcium stores were depleted, or when ryanodine receptor/channels were blocked. Recordings on the top show mIPSCs in the presence of CNQX and APV used to block glutamate synaptic currents, TTX used to block presynaptic action potentials, and dantrolene (DAN) used to deplete intracellular calcium stores. Toluene no longer produced an increase in mIPSC frequency when calcium stores were depleted. The bar graph on the bottom compares the effect produced by toluene on mIPSC frequency in control conditions, the lack of effect produced by Cd<sup>++</sup> block, and lack of effect seen with thapsigargin (THAP), used to block calcium release from IP<sub>3</sub> sensitive stores. Both dantrolene and ryanodine (RYAN) blocked the toluene-induced increase in mIPSC frequency indicating a selective effect on caffeine sensitive calcium stores. Each bar represents the mean  $\pm$  SD for at least five determinations of the toluene-induced effect from separate experiments. Statistical comparisons were done using ANOVA, with Cd, THAP, DAN, and RYAN data compared to the control (toluene-induced) response.

increased inhibition appeared to come about by a presynaptic mechanism, because it was associated with an increase in spontaneous IPSC frequency with no effect on postsynaptic current amplitudes. The increased IPSC frequency appeared to involve actions directly on GABA nerve terminals, because the effect persisted after blockade of action potentials secondary to blocking sodium channels with tetrodotoxin. Similarly, the toluene effect persisted after blocking calcium influx into nerve terminals, indicating that the increased mIPSC frequency involved a toluene-induced release of calcium from intracellular stores. The increase in mIPSC frequency was blocked by pretreatment with either dantrolene or ryanodine, but not by thapsigargin, indicating that toluene caused a release of calcium from a caffeine-sensitive store, but not from an IP<sub>3</sub>-regulated store. In this respect, the abused inhalants appear to act via a mechanism that is similar to that used by inhaled anesthetics.

Enhanced GABA-mediated inhibition is a common effect produced by inhaled anesthetics, such as halothane and isoflurane (Banks and Pearce, 1999; Bieda *et al*, 2009; Franks, 2008; Jones and Harrison, 1993; [32]; Tanelian *et al*, 1993). As this is a prevalent effect seen in hippocampal neurons, it has been proposed to play a role in anesthetic-induced memory loss (Simon *et al*, 2001; Tanelian *et al*, 1993). Indeed, volatile anesthetics have been shown to block long-term potentiation (MacIver *et al*, 1989) and long-term

depression at CA1 neuron synapses (Ishizeki *et al*, 2008; Simon *et al*, 2001), as do other anesthetics (Cheng *et al*, 2006). The selective enhancement of GABA<sub>A</sub> slow mIPSCs observed could be particularly important in this regard because these synaptic currents have the ideal time course to modulate NMDA responses (Banks *et al*, 2000), which are critical for synaptic plasticity in several major synaptic pathways. These forms of synaptic plasticity are widely thought to contribute to hippocampal-dependent learning and memory, as well as to some long-term mechanisms of drug addiction (Hyman *et al*, 2006). Thus, the enhanced GABA-mediated inhibition seen in this study would provide a mechanism for the disruption of learning and memory that is produced by abused inhalants (Bowen *et al*, 2006). It should be noted that good evidence exists for additional effects of abused solvents that could also impair learning mechanisms, in particular actions on NMDA receptors and other neurotransmitter systems, and brain regions, would be very likely to contribute (Beckstead *et al*, 2000; Riegel *et al*, 2007; Smothers and Woodward, 2007; Woodward *et al*, 2004).

Although anesthetics and abused inhalants both increase GABA-mediated inhibition, important differences in the mechanism of action were seen. Anesthetics enhance inhibition by at least three distinct actions: (1) a presynaptic effect similar to that seen here (ie, an increased frequency of

IPSCs; Banks and Pearce, 1999; Yamashita *et al*, 2001); (2) a postsynaptic effect to prolong synaptic inhibition (Mody *et al*, 1991; Nicoll *et al*, 1975; Pittson *et al*, 2004); and (3) a postsynaptic effect to enhance tonic (extrasynaptic) GABA-gated currents (Bieda and MacIver, 2004; Bieda *et al*, 2009; Caraiscos *et al*, 2004). Abused inhalants, in contrast, shared only the first (presynaptic) effect with inhaled anesthetics, and neither of the postsynaptic actions were evident. This enhanced presynaptic release of GABA has also been observed for ethanol (Weiner and Valenzuela, 2006). Prolongation of IPSCs, such as an increase in decay time or duration, was not produced. Neither was an increase in tonic, extrasynaptic currents evident in either current-clamp or voltage-clamp experiments. Perhaps it is only the presynaptic effects of the anesthetics and abused inhalants that are needed to disrupt learning and memory, whereas the postsynaptic actions contribute to sedation and loss of consciousness produced by the inhaled anesthetics. The relatively weak anesthetic properties of abused inhalants are consistent with the lack of postsynaptic actions seen in this study, of course it might only reflect a more limited degree of enhanced inhibition too, regardless of pre- or postsynaptic sites of action.

In summary, abused inhalants increased synaptic inhibition in hippocampal neurons and this likely contributes to learning deficits associated with solvent abuse.

## ACKNOWLEDGEMENTS

I thank Marin MacDonald and Patricia Turnquist for technical assistance with brain slice preparation and electrophysiology. I thank Eric Hu for technical assistance with HPLC analysis and Professor James R Trudell for HPLC assistance, guidance and advice about the use and chemical properties of the abused inhalants throughout this project. Dr JJ Woodward and Dr RA Pearce provided helpful comments and suggestions on portions of this work.

Research support was provided by NIH: NIDA R01DA017884, and I am thankful for the encouragement and guidance provided by Dr Charles Sharp and Dr Jerry Frankenheim at NIDA.

## DISCLOSURE/CONFLICT OF INTEREST

The author declares that no financial support or compensation has been received from any individual or corporate entity and there are no personal financial holdings that could be perceived as constituting a real or potential conflict of interest in this work.

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