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# Toluene decreases Purkinje cell output by enhancing inhibitory synaptic transmission in the cerebellar cortex

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## HIGHLIGHTS

- The abused solvent toluene decreases Purkinje cell action potential output.
- Toluene increases the frequency and amplitude of GABAergic IPSCs.
- Toluene does not affect action potential output from molecular layer interneurons.
- Toluene-evoked motor impairments may be due in part to cerebellar effects.

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## ABSTRACT

Toluene belongs to a class of psychoactive drugs known as inhalants. Found in common household products such as adhesives, paint products, and aerosols, toluene is inhaled for its intoxicating and euphoric properties. Additionally, exposure to toluene disrupts motor behaviors in a manner consistent with impairments to cerebellar function. Previous work has suggested a role of GABA in mediating toluene's neurobehavioral effects, but how this manifests in the cerebellar cortex is not yet understood. In the present study, we examined the effects of toluene on cerebellar Purkinje cell action potential output and inhibitory synaptic transmission onto Purkinje cells using patch clamp electrophysiology in acute rat cerebellar slices. Toluene (1 mM) reduced the frequency of Purkinje cell action potential output without affecting input resistance. Furthermore, toluene dose-dependently enhanced inhibitory synaptic transmission onto Purkinje cells, increasing the amplitude and frequency of inhibitory postsynaptic currents; no change in the frequency of action potentials from molecular layer interneurons was noted. The observed decreases in Purkinje cell action potential output could contribute to toluene-evoked impairments in cerebellar and motor functions.



## 1. Introduction

The recreational use of volatile solvents for the purpose of intoxication is increasing, particularly among middle school-aged children [26]. The most commonly abused solvent is toluene, which is found in a variety of household products such as adhesives, paint products, and aerosols. Inhalation of toluene vapors produces an intoxicated state characterized by alterations in sensory, motor, and cognitive functioning [19]. Despite the prevalence of use, neurobiological mechanisms underlying the effects of toluene remain incompletely understood.

Toluene-induced impairments in motor control include incoordination, loss of balance, nystagmus, and intention tremor, in both human and rodent studies [5,10,15,30]. These impairments are consistent with cerebellar dysfunction, and notably, structural abnormalities in the cerebellum are observed following prolonged toluene exposure [22,37]. The rapid onset of motor impairments during toluene inhalation suggests that toluene may directly alter the physiology of cerebellar neurons or synapses as well, as noted elsewhere in the brain [2,9,20]. In fact, acute toluene exposure has been shown to increase extracellular γ-aminobutyric acid (GABA) levels in the cerebellar cortex during inhalation [32]. Because Purkinje cells are the sole output of the cerebellar cortex, the action potential output of these cells, and the synaptic control of this output, may be a useful readout of toluene-evoked changes in gross cerebellar physiology. Here we have studied the effects of toluene on action potential output and inhibitory synaptic inputs for young adolescent rat Purkinje cells in acute in vitro cerebellar slices, as a means to provide some insight

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into toluene-evoked impairments in cerebellar-dependent motor functions.

## 2. Materials and methods

### 2.1. Animals

Male and female Long-Evans rats (postnatal day (PND) 12–30) arriving with their dams at PND 10–14 (Charles River Laboratories, Saint-Constant, QC) were used. Rats were weaned at PND 21, pair housed, and maintained under standard conditions (12:12 photoperiod with lights on at 0800 h local time; constant temperature of 21 °C; food and water available ad libitum). All experiments were approved by the Wilfrid Laurier University Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

### 2.2. Solutions

Chemicals were purchased from VWR (Mississauga, ON), except for ethylene glycol tetraacetic acid (EGTA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), K-gluconate, CsCl, and picrotoxin, which were obtained from SIGMA (Oakville, ON) and isoflurane (Baxter Healthcare Corporation, Deerfield, IL). All compressed gases were purchased from Praxair Canada Inc. (Kitchener, ON). Artificial cerebrospinal fluid (aCSF) was used as the cutting solution, and consisted of, in mM: 125 NaCl, 3.25 KCl, 1.5 $CaCl_2$, 1.5 $MgCl_2$, 25 $NaHCO_3$, and 25 glucose. The bath solution consisted of aCSF plus 0.02% polyoxyethylene (20) sorbitan monooleate (TWEEN), unless otherwise specified. aCSF was bubbled with carbogen gas (95% oxygen, 5% carbon dioxide). For current clamp experiments, pipettes were filled with, in mM: 135 K-gluconate, 5 KCl, 7 NaCl, 0.3 $MgCl_2$, 0.1 EGTA, and 10 HEPES. For voltage clamp experiments, pipettes were filled with, in mM: 140 CsCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 5 EGTA, and 10 HEPES. Potassium hydroxide was added to both intracellular solutions to achieve a final pH of 7.3.

### 2.3. Preparation of tissue slices

Rats were anesthetized with isoflurane. After loss of the righting reflex and ability to respond to nociceptive stimulation, rats were decapitated and the cerebellum was dissected out and placed in ice-cold aCSF for 60 s. The cerebellum was blocked in the parasagittal plane by removing the cerebellar hemispheres using a razor blade, and the vermis was mounted to a vibratome cutting tray with diisocyanates-containing glue. The vermis was sliced into 300 μm sections using a Pelco 102 vibratome sectioning system (Ted Pella, Inc., Redding, CA) in ice-cold aCSF. After slicing, the tissue was incubated at 34 °C in aCSF for 30–45 min before commencing electrophysiology.

### 2.4. Electrophysiology

Cerebellar slices were transferred to a recording chamber maintained at 34 °C via a TC-324B temperature controller (Warner Instruments, Hamden, CT), and perfused with aCSF at ~1 ml/min. Purkinje cells were identified by their large somata located between the granule and molecular layer [25], using a BX51 WI fixed-stage microscope (Olympus, Richmond Hill, ON) and a Q26005 infrared camera (QImaging, Surrey, BC) operated by Qcapture Pro 6 software (Qimaging). Molecular layer interneurons were identified by their smaller oval soma located in the inner one-third of the molecular layer (presumably basket cells). Pipettes were made from glass capillary tubes with microfilaments (OD 1.50 mm, ID 0.86 mm; A-M Systems, Sequim, WA) with a PC-10 two-stage puller (Narishige, East Meadow, NY), and had resistances of 3–4 MΩ.

Whole-cell and extracellular recordings were acquired at 25 or 50 kHz via an Axon Multiclamp 700B amplifier, controlled by the Multiclamp 700B commander, operating in either voltage or current clamp modes (Molecular Devices, Sunnyvale, CA), and low pass filtered at 10 kHz. Signals were digitized and sent to the host computer via an Axon Digidata 1440A (Molecular Devices), and recorded with pCLAMP 10 software (Molecular Devices). The timeline for all experiments was as follows: a 5 min baseline period, followed by wash-in of 0 or 1 mM toluene for 8 min, followed by an 8 min wash-out period. 1 mM toluene was used as physiologically based pharmacokinetic models have estimated toluene concentration in the brain to be ~1 mM during binge-like inhalation [4], which is in agreement with our own measurements of the brain concentration of toluene following inhalation of a binge-like (~5000 ppm) concentration of toluene vapor (Gmaz and McKay, unpublished observations).

### 2.5. Measurement of action potential output

Purkinje cells were maintained at −65 mV with hyperpolarizing current injections. During each phase of the experiment (baseline, wash-in of toluene, wash-out of toluene), Purkinje cells were stimulated with a family of 1 s duration current steps beginning from −300 pA (relative to the holding current) and increasing by 100 pA increments until a train of action potentials was observed. Input resistance was calculated as the slope of the line of the peak negative voltages evoked by each current step between −300 and 0 pA. Action potential amplitude, half-width, and afterhyperpolarization amplitude were calculated as the average of these parameters taken from each of the first 5 action potentials of the train; these measurements have been described in detail elsewhere [24]. Additionally, the frequency of the first 5 evoked action potentials (herein referred to as "initial frequency"), as well as the frequencies of all action potentials evoked during the 1 s long step (herein referred to as "mean frequency") was calculated. The new membrane potential in response to a given current injection was also recorded. Action potential output from molecular layer interneurons was recorded extracellularly using aCSF-filled pipettes.

### 2.6. Measurement of inhibitory post synaptic currents (IPSCs)

IPSC recordings were carried out in the absence of glutamatergic blockers as climbing fiber and mossy fiber afferents are not spontaneously active in the parasagittal slice preparation. The $GABA_A$ receptor antagonist picrotoxin (50 μM) was added to a subset of cells ($n = 3$) and confirmed that all recorded events were GABA receptor-mediated events. IPSCs were measured in cells voltage clamped at approximately −65 mV. Frequencies and amplitudes of IPSCs were measured using the event detection feature of Clampfit 10. For these experiments, we bath applied either 0, 0.1, 0.316, 1, or 3.16 mM of toluene.

### 2.7. Statistical analysis

Statistical analyses were completed with IBM SPSS Statistics v.19 (IBM, Armonk, NY) and consisted of independent samples *t*-tests.

## 3. Results

### 3.1. Toluene decreases Purkinje cell action potential output

Because toluene is hydrophobic and thus poorly soluble in water, it is typically dissolved into TWEEN-containing aCSF for bath application [29]. Thus we first tested the effects of TWEEN on Purkinje cell action potential output and found that there