

Xylene Impairs Neuronal Development by Dysregulating Calcium Homeostasis and Neuronal Activity in Developing Hippocampal Neurons

Yunkyung Eom and Sung Hoon Lee*

College of Pharmacy, Chung-Ang University, Seoul 06974, Republic of Korea

Abstract

Xylene is an aromatic hydrocarbon widely used as a solvent and emitted from numerous commercial products in industrial or indoor settings. Epidemiological studies of occupational exposure indicate that xylene primarily targets the central nervous system, producing neurobehavioral impairments and other neurological disorders. Both environmental and occupational exposure to volatile organic compounds, therefore, raise concerns about neurodevelopmental risk; however, the specific neurotoxicity of xylene in developing neurons remains poorly characterized. Here, we investigated the effects of xylene (2 or 5 mM) on developing mouse hippocampal neurons, with a focus on Ca^{2+} homeostasis, presynaptic function, and electrophysiological activity. We assessed Ca^{2+} homeostasis with selective inhibitors of voltage-gated Ca^{2+} channels and organelle-specific Ca^{2+} indicators. Presynaptic activity was evaluated in transgenic mouse pups expressing a genetically encoded pH sensor within the synaptic vesicle lumen. Xylene suppressed cytosolic Ca^{2+} transients by inhibiting P/Q-type Ca^{2+} channels, thereby reducing Ca^{2+} uptake into the endoplasmic reticulum. It also decreased Ca^{2+} influx at both presynaptic and postsynaptic sites, impairing synaptic vesicle exocytosis and endocytosis. Electrophysiological and morphological analyses further showed reduced spontaneous firing and hindered synaptic maturation. Collectively, these findings provide mechanistic insight into the neurotoxic actions of xylene and underscore its potential hazard to brain development and function.

Key Words: Xylene, Hippocampal neurons, Ca^{2+} homeostasis, Organelle, Neuronal activity, Synaptic development

INTRODUCTION

Xylene (dimethylbenzene, commonly called xylol) is an aromatic hydrocarbon used extensively as a solvent in laboratories, industry, and numerous commercial formulations, including clearing agents and paints. A constituent of gasoline and aviation fuel, xylene is emitted into the atmosphere by industrial activity and vehicle exhaust. Because many consumer products—such as interior paints, furnishings, and air purifiers—contain xylene, indoor concentrations are typically higher than outdoor levels (Wang *et al.*, 2007). Reported concentrations reach 14 ppm in ambient air and up to 100 ppm in industrial workplaces where xylene is manufactured or applied (Langman, 1994). Although concentrations in groundwater and soil are less well documented, <6% of environmental xylene is estimated to enter drinking water. As a volatile organic compound (VOC), xylene readily evaporates and undergoes photodegradation in air, with a half-life of 8-14 h. In contrast,

when released into water or soil—often through leaks from petroleum storage tanks—it degrades far more slowly, with a half-life of 25-287 days, owing to limited volatilization and slower abiotic breakdown (United States. Agency for Toxic Substances and Disease Registry, Research Triangle Institute, and Sciences International Inc., 1995).

Humans are primarily exposed to xylene by inhalation, which may occur through smoking, evaporation of xylene-containing household products, or the use of solvents in the workplace. Xylene exposure also arises from ingestion of contaminated food or drinking water and from dermal contact with polluted materials. Both acute and chronic exposure to xylene pose health risks (Kandyala *et al.*, 2010), and the central nervous system is particularly vulnerable to its toxic effects. Xylene is lipophilic and highly soluble in blood, so it is readily absorbed and distributed to the brain. Exposure produces neurobehavioral impairment and symptoms of sick-building syndrome, including headache, cognitive impairment, dizziness, and

Open Access <https://doi.org/10.4062/biomolther.2025.038>

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received Mar 9, 2025 Revised Jun 3, 2025 Accepted Jun 26, 2025

Published Online Aug 31, 2025

***Corresponding Author**

E-mail: sunghoonlee@cau.ac.kr

Tel: +82-2-820-5675, Fax: +82-2-820-5114

depression (Kishi *et al.*, 1993). Epidemiological studies have shown that technicians and other workers in xylene-contaminated environments are chronically exposed to elevated levels of the solvent, resulting in neurological symptoms (Guo *et al.*, 2004; Yu *et al.*, 2004). The Occupational Safety and Health Administration sets the permissible exposure limit for xylene at 100 ppm as an 8-h time-weighted average (Kandyala *et al.*, 2010). Xylene-induced neurotoxicity has been attributed to the disruption of neuronal membrane lipids and proteins essential for proper neuronal function (Savolainen and Pfaffli, 1980). Altered brain neurotransmitter levels have been reported after both acute and subchronic exposure to xylene (Honma *et al.*, 1983; Wang *et al.*, 1999). Low-level exposure is associated with neurophysiological dysfunction, whereas high-level exposure can lead to severe neurological disorders such as peripheral neuropathy, cognitive impairment, and memory loss (Kandyala *et al.*, 2010). Furthermore, epidemiological studies suggest that maternal occupational exposure increases the risk of neurodevelopmental disorders in offspring, manifesting as cognitive dysfunction and attention-deficit phenotypes (Till *et al.*, 2001; Laslo-Baker *et al.*, 2004; Pelé *et al.*, 2013). However, these studies are limited, and the underlying toxic mechanisms remain unclear, partly because it is difficult to separate direct systemic effects from indirect ones. Consequently, *in vitro* experiments have been proposed to elucidate the neurotoxic effects of xylene.

Ca²⁺ signaling is essential for neuronal development and differentiation because it regulates synaptic activity, neurotransmitter specification, and the activation of transcription factors required for maturation (Rosenberg and Spitzer, 2011). It also induces conformational changes in Ca²⁺-sensitive proteins and activates Ca²⁺-dependent kinases, thereby driving cytoskeletal remodeling that underlies axonal and dendritic growth (McKinney *et al.*, 2022). Both spontaneous and activity-dependent Ca²⁺ transients shape synaptic network formation during brain development. Intracellular Ca²⁺ homeostasis is tightly controlled by membrane and organellar Ca²⁺ channels, transporters, and pumps. Ca²⁺ influx through voltage-gated Ca²⁺ channels (VGCCs), followed by Ca²⁺ release from the endoplasmic reticulum (ER), rapidly raises cytosolic Ca²⁺ to 1–3 μ M, whereas mitochondria buffer these transients by sequestering Ca²⁺ (Panda *et al.*, 2021). Disrupted Ca²⁺ signaling caused by dysfunctional Ca²⁺-permeable channels or transporters has been implicated in neurodevelopmental disorders, including autism spectrum disorders and infantile epilepsy.

Among VGCCs, P/Q-type Ca²⁺ channels are indispensable for synaptic transmission in hippocampal neurons (Nimmervoll *et al.*, 2013). They reside closer to the active zone than other VGCCs (Wu *et al.*, 1999) and predominate in mediating neurotransmission in both immature and mature neurons (Ishikawa *et al.*, 2005; Nakamura *et al.*, 2015). P/Q-type channels govern synaptic vesicle (SV) exocytosis through interactions with the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex, a protein assembly critical for SV fusion (Nanou and Catterall, 2018). Aberrant P/Q-type channel function is strongly associated with neurodevelopmental disorders such as autism spectrum disorders and intellectual disability (Nanou and Catterall, 2018; Kessi *et al.*, 2021). Consistently, mice lacking Cav2.1, which encodes the P/Q-type channel, display ataxia and seizures during development, accompanied by altered spontaneous firing and reduced neurotransmitter release (Mark *et al.*, 2011).

Taken together, these findings raise the possibility that xylene impairs the developing brain by inhibiting P/Q-type Ca²⁺ channels. In the present study, we therefore assessed the effects of xylene on Ca²⁺ channel activity in developing hippocampal neurons using live-cell imaging.

MATERIALS AND METHODS

Animals

All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee of Chung-Ang University (approved protocol number: 2020-00049) and the National Institutes of Health Guidelines for Laboratory Animal Care. C57BL/6J mouse pups (postnatal day 1 [P1], 1.4–1.6 g) were purchased from Nara-Biotec (Seoul, Korea) and used for hippocampal neuron cultures. Tg(Thy1-spH)21Vnmu transgenic mice (synaptobluorin [spH] 21; strain #014651, Jackson Laboratory; gift of Dr. Venkatesh N. Murthy) were used to measure hippocampal presynaptic activity. The transgenic animals were maintained as breeding pairs. All mice were maintained on a 12 h light/dark cycle at 15–25°C with ad libitum access to food and water. Genotyping was performed to identify transgenic pups (P1), which were subsequently used for hippocampal neuron cultures. A total of 20 C57BL/6J pups (10 males and 10 females) were used to investigate VGCC subtypes. An additional four pups (two males and two females) were used for cell-viability measurements, four pups (two males and two females) for multi-electrode array (MEA) recordings, six pups (three males and three females) to study presynaptic and postsynaptic development, and 20 pups (10 males and 10 females) to examine ER, mitochondrial, presynaptic, and postsynaptic Ca²⁺ influx. Moreover, six spH 21 pups (three males and three females) were allocated to presynaptic-activity measurements. Sex was determined by genital inspection at collection, and one to three pups of each sex were used per culture.

Hippocampal neuron cultures and treatment

Hippocampal cultures were established as previously described (Kim *et al.*, 2023b). Pups were deeply anesthetized with isoflurane and rapidly decapitated. Hippocampi were dissected from P1 mouse brains under an optical microscope (C-PSN, Nikon, Tokyo, Japan). Hippocampal tissue was dissociated into single cells and seeded (60,000 cells per well) onto poly-D-lysine-coated plates or coverslips (Marienfeld, Lauda-Königshofen, Germany). Neurons were cultured in Neurobasal medium (Thermo Fisher Scientific, MA, USA) supplemented with 2% (v/v) B27, 1% (v/v) Glutamax, and 1% penicillin/streptomycin (all Thermo Fisher Scientific). Neurons were incubated at 37°C in 5% CO₂. Xylene was applied transiently to developing neurons on day *in vitro* (DIV) 1 at the indicated concentrations and was replaced with fresh medium the following day. Vehicle (Veh) controls received dimethyl sulfoxide (DMSO) at volumes equivalent to those of xylene. Treated samples were anonymized before analysis.

Plasmid transfection

ER-GCaMP6-150 (Addgene plasmid #86918) was a gift from Timothy Ryan. pCAG mito-GCaMP5G (Addgene plasmid #105009) and pCAG-vGlut1-GCaMP5G (Addgene plasmid #105008) were gifts from Franck Polleux. pCAGGS-PSD95-

GCaMP2 (Addgene plasmid #18931) was a gift from Karel Svoboda. Enhanced green fluorescent protein (eGFP; Addgene plasmid #13031) was a gift from Doug Golenbock. Transfections were performed in DIV 5 neurons using Lipofectamine LTX (Thermo Fisher Scientific) per the manufacturer's protocol. After 4 h, the transfection medium was replaced with fresh medium to minimize neuronal toxicity.

MTT assay

Cell viability was evaluated with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Neurons were exposed to 100 μ M MTT for 1 h at 37°C. Formazan crystals were solubilized in DMSO, and absorbance was recorded at 540 nm using a microplate reader (BioTek Instruments, VT, USA). Absorbance values were normalized to the mean of the Veh control.

Live-cell imaging

Intracellular Ca^{2+} imaging was carried out in mature neurons (DIV 15-18) loaded with Fluo-4 AM (fluo-4 acetoxymethyl ester, Thermo Fisher Scientific). Cells were incubated in Neurobasal medium containing 5 μ M Fluo-4 AM and F127 for 30 min at 37°C, then for an additional 30 min at 15-25°C in a bath solution (119 mM NaCl, 2 mM CaCl_2 , 2 mM MgCl_2 , 2.5 mM KCl, 30 mM glucose, and 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4). Neurons were subsequently transferred to a fresh bath solution, and Ca^{2+} imaging was performed at 15-25°C. To evaluate the contribution of VGCCs, an initial train of 40 action potentials (APs) at 20 Hz was delivered to Fluo-4 AM-loaded neurons. The VGCC inhibitors ω -agatoxin IVA (500 nM, Tocris, MN, USA), ω -conotoxin GVIA (1 μ M, Tocris), and nifedipine (5 μ M, Abcam, Cambridge, UK) were then added to the bath and incubated for 10 min. The same stimulation protocol (40 APs at 20 Hz) was repeated. Inhibitor concentrations were selected according to published studies (Chameau *et al.*, 1999; Giansante *et al.*, 2020). Veh (DMSO) was applied at the corresponding volume in control experiments. ER, mitochondrial, and postsynaptic Ca^{2+} imaging were performed in standard bath solution, whereas presynaptic Ca^{2+} imaging required bath solution supplemented with 10 μ M CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and 50 μ M APV (($\pm$)-2-amino-5-phosphonopentanoic acid) to suppress postsynaptic activity by blocking AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (N-methyl-D-aspartate) receptors, respectively. Presynaptic imaging was performed in hippocampal neurons (DIV 28) from spH-21 transgenic mice once fluorescence signals were robust.

Presynaptic activity was recorded at 15-25°C in a bath solution supplemented with CNQX and APV. Field stimulation consisted of a 1 ms, 20 mA pulse delivered through platinum electrodes driven by a pulse stimulator (Model 2100, A-M Systems, WA, USA). Fluorescence images were acquired with an electron-multiplying charge-coupled-device camera (iXON-L-888, Andor Technology, Belfast, UK) mounted on an inverted microscope (Ti2, Nikon) equipped with a 60 $\times$ oil-immersion objective (numerical aperture 1.4). A mercury lamp (C-HGFI, Nikon) provided illumination, and a fluorescein isothiocyanate filter set selected excitation and emission wavelengths. Images were captured at 2 Hz with 200 ms exposure, controlled by NIS software (Nikon). Photobleaching was excluded by monitoring fluorescence for $\geq$ 5 s in the absence of stimulation

before each trial.

Image analysis

Images were analyzed using NIS-Elements software (Nikon). For intracellular Ca^{2+} imaging, square regions of interest (ROIs; 10 $\times$ 10 μ m) were placed over neuronal somata. Organelle Ca^{2+} signals were quantified in ROIs drawn around individual puncta. For presynaptic imaging, 1.5 $\times$ 1.5 μ m ROIs were positioned over single synaptic boutons. Baseline fluorescence (F_0) was the mean intensity during the first 10 s of recording. Fluorescence change ($\Delta F = F - F_0$) was expressed as $(\Delta F/F_0) \times 100$. An increase was deemed significant when the mean of three consecutive frames exceeded F_0 . Peak fluorescence was quantified after AP stimulation. The contribution of each VGCC subtype was calculated from the percentage decrease in peak amplitude after inhibitor application. The decay rate ($\text{Rate}_{\text{decay}}$) of spH-21 fluorescence was obtained from the 4-10 s interval after stimulation. No sex-dependent differences were detected in presynaptic Ca^{2+} signals or SV exocytosis and endocytosis (Kim *et al.*, 2023a).

MEA

Spontaneous neuronal activity and network dynamics were evaluated with a 24-well MEA system (Axion Biosystems, GA, USA). Each well contained 16 poly(3,4-ethylenedioxythiophene) electrodes (30 μ m diameter) spaced 350 μ m apart. Cultured hippocampal neurons (100,000 cells) were seeded on poly-D-lysine-coated MEA plates. Recordings were obtained on DIV 14 for 15 min after a 15 min stabilization period. Recording chambers were maintained at 37°C and 5% CO_2 . Signals were sampled at 12.5 kHz using a 200-3000 Hz band-pass filter and analyzed in AxIS software (ver. 3.6.2, Axion Biosystems) to quantify burst firing (more than five spikes with an inter-spike interval [ISI] <100 ms) and network bursts (>25 spikes with an ISI <50 ms).

Immunocytochemistry

Neurons were fixed in 4% paraformaldehyde for 15 min at 15-25°C and permeabilized with 0.1% Triton X-100 in Dulbecco's phosphate-buffered saline (DPBS) for 10 min at 15-25°C. Coverslips were then incubated overnight at 4°C with primary antibodies against synaptotagmin 1 (Syt, 1:500, Thermo Fisher Scientific), synaptophysin (Syn, 1:500, Thermo Fisher Scientific), glutamate receptor 1 (GluR1, 1:100, Thermo Fisher

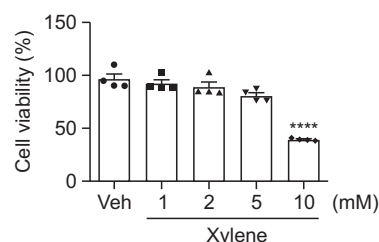


Fig. 1. Cellular toxicity of xylene in cultured developing hippocampal neurons. Neurons were incubated with the indicated concentrations of xylene, and cellular viability was assessed with the MTT assay. Veh denotes DMSO applied at the same volume as xylene. $n=4$ independent cultures. Bars indicate mean $\pm$ SEM. **** $p<0.0001$ vs. Veh by 1-way ANOVA, followed by Tukey *post hoc* test.

Scientific), and NMDA receptor 1 (NR1, 1:100, Thermo Fisher Scientific) in blocking solution (1% bovine serum albumin in DPBS). After three DPBS washes, Alexa 594-conjugated secondary antibodies (Abcam) were applied for 1 h at 15–25°C. Coverslips were mounted with gel-mount solution (Biomed, CA, USA), and images were acquired on a confocal microscope (Zeiss, Oberkochen, Germany). Dendritic spine subtypes were classified according to the head-to-neck ratio and spine-head width (Grossman *et al.*, 2006).

Statistical analysis

Graphs were generated in Prism 8 (GraphPad Software,

CA, USA) or Igor Pro (WaveMetrics, OR, USA); all statistical tests were performed in Prism 8. Data are expressed as mean $\pm$ standard error of the mean from at least three independent cultures, with each dot representing one culture. One- or two-way ANOVA (analysis of variance) followed by Tukey's *post hoc* test was used for multiple comparisons. A *p*-value <0.05 was considered statistically significant.

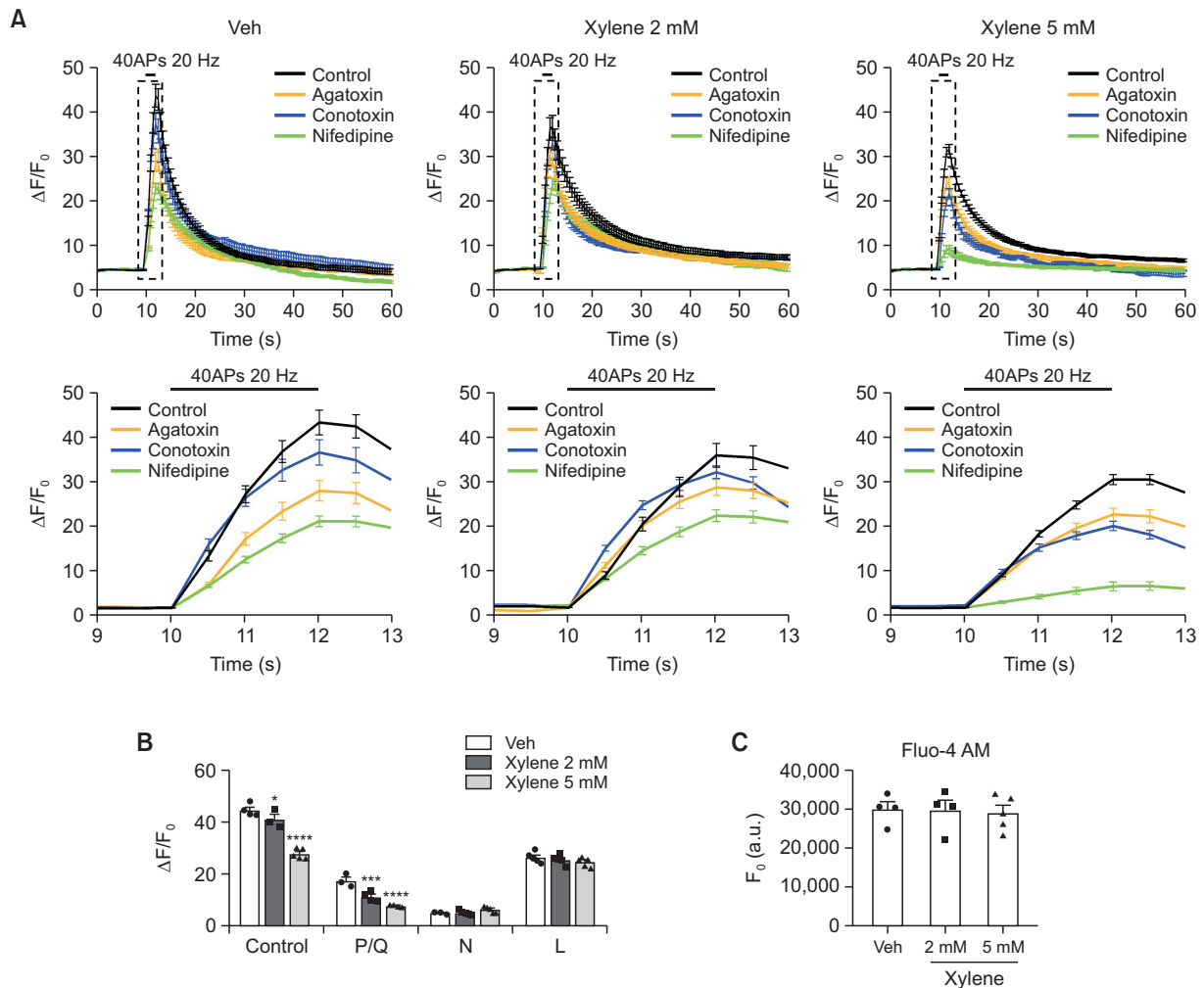


Fig. 2. Xylene reduces somatic Ca^{2+} entry by inhibiting P/Q-type Ca^{2+} channels in hippocampal neurons. Somatic Ca^{2+} dynamics were measured with Fluo-4 AM in xylene-treated hippocampal neurons. Fluo-4 AM-loaded neurons were incubated with agatoxin (P/Q-type Ca^{2+} channel inhibitor), conotoxin (N-type Ca^{2+} channel inhibitor), or nifedipine (L-type Ca^{2+} channel inhibitor), followed by field stimulation (40 APs at 20 Hz). Action potentials were evoked by field stimulation, which delivered current across the neuronal culture. Action potentials were confirmed by a stimulus-locked increase in Fluo-4 AM fluorescence. Fluorescence returned to baseline after stimulation ceased. Veh denotes DMSO applied at the same volume as xylene. Control indicates DMSO applied at the volume used for the channel inhibitors. (A) Top: averaged, normalized traces of Fluo-4 AM fluorescence intensity in the soma. Bottom: magnification of the dashed region. Black, orange, blue, and green represent control, agatoxin, conotoxin, and nifedipine, respectively. (B) The contribution of each VGCC was quantified as the peak Fluo-4 AM response elicited by stimulation in the presence of its inhibitor (Veh: $n=4$ for control, $n=3$ for agatoxin, $n=3$ for conotoxin, $n=5$ for nifedipine; xylene 2 mM: $n=3$ for control, $n=4$ for agatoxin, $n=5$ for conotoxin, $n=5$ for nifedipine; xylene 5 mM: $n=5$ for control, $n=4$ for agatoxin, $n=5$ for conotoxin, $n=5$ for nifedipine). Bars indicate mean $\pm$ SEM. * $p<0.05$, *** $p<0.001$, **** $p<0.0001$ vs. Veh by two-way ANOVA, followed by Tukey's *post hoc* test. (C) Fluo-4 AM fluorescence intensity in the soma of xylene-exposed neurons ($n=4$ independent cultures for Veh; $n=4$ for xylene 2 mM; $n=5$ for xylene 5 mM). Bars indicate mean $\pm$ SEM.

RESULTS

Xylene suppressed cytosolic Ca^{2+} transients by inhibiting P/Q-type Ca^{2+} channels in hippocampal neurons

Xylene concentrations neurotoxic to developing hippo-

campal neurons were first determined with the MTT assay, which revealed no significant cytotoxicity except at 10 mM. Consequently, 2 mM and 5 mM were selected for subsequent experiments (Fig. 1). Live-cell Ca^{2+} imaging with Fluo-4 AM was performed to examine xylene-evoked changes in cyto-

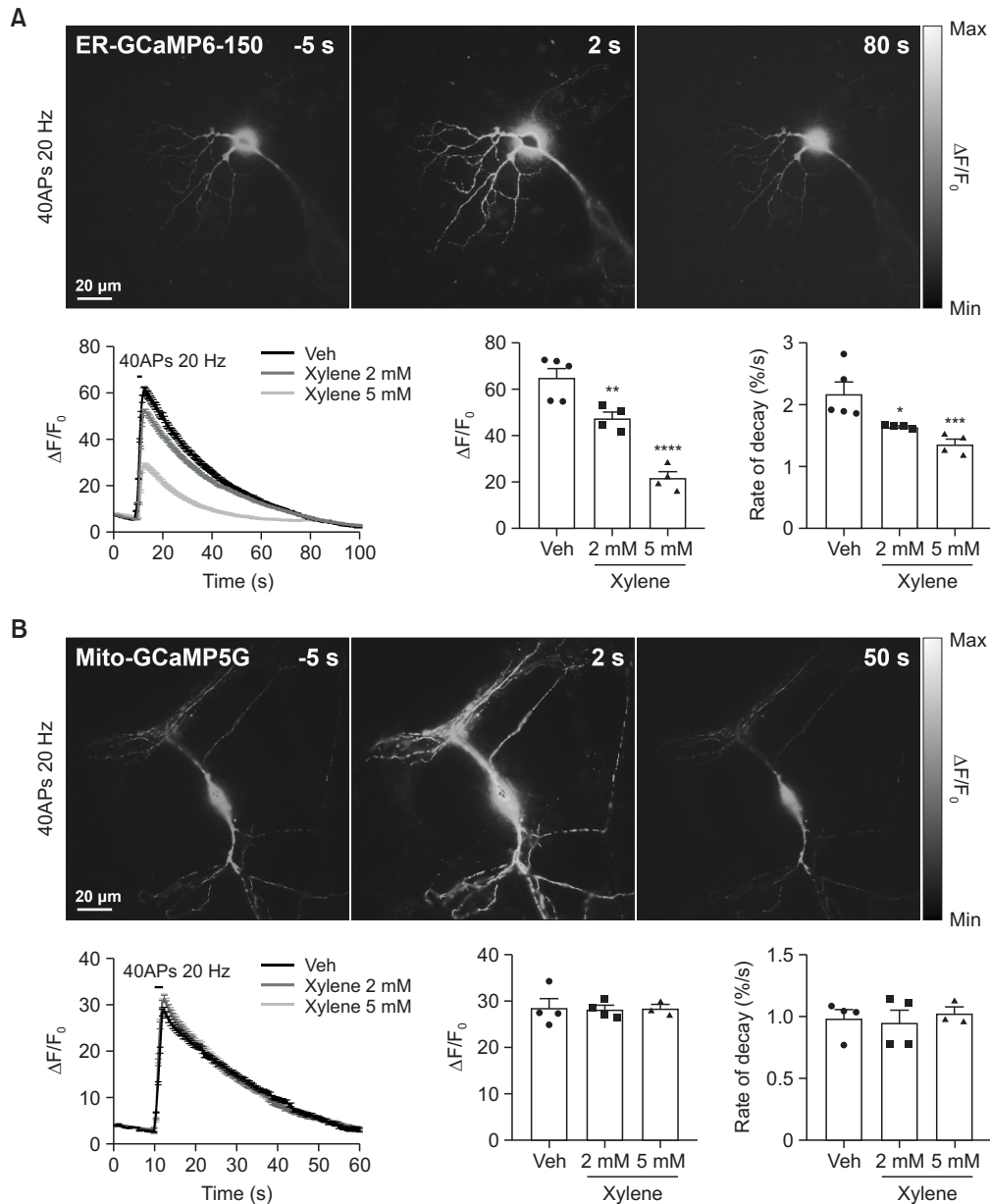


Fig. 3. Effects of xylene on ER and mitochondrial Ca^{2+} uptake in hippocampal neurons. ER or mitochondrial Ca^{2+} dynamics were measured in neurons transfected with ER-GCaMP6-150 or mito-GCaMP5G, respectively. Field stimulation (40 APs at 20 Hz) was applied to each culture. (A) Top: representative pseudocolor time-lapse images of ER-GCaMP6-150-expressing neurons during electrical stimulation. Bottom: averaged, normalized traces of ER-GCaMP6-150-expressing neurons (left). Right: peak amplitude and post-stimulus decay rate of ER-GCaMP6-150. $n=5$ independent cultures (Veh), 4 (2 mM xylene), and 4 (5 mM xylene). Bars represent mean $\pm$ SEM. (B) Top: pseudocolor time-lapse images of mito-GCaMP5G-expressing neurons. Bottom: averaged, normalized traces of mito-GCaMP5G-expressing neurons (left). Right: peak amplitude and post-stimulus decay rate of mito-GCaMP5G. $n=4$ independent cultures (Veh), 4 (2 mM xylene), and 3 (5 mM xylene). Bars represent mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. Veh (one-way ANOVA with Tukey *post hoc* test). (C) Averaged fluorescence intensity of ER-GCaMP5G-expressing ROIs. $n=5$ independent cultures (Veh), 4 (2 mM xylene), and 4 (5 mM xylene). Bars represent mean $\pm$ SEM. (D) Averaged fluorescence intensity of mito-GCaMP5G-expressing ROIs. $n=4$ independent cultures (Veh), 4 (2 mM xylene), and 3 (5 mM xylene). Bars represent mean $\pm$ SEM.

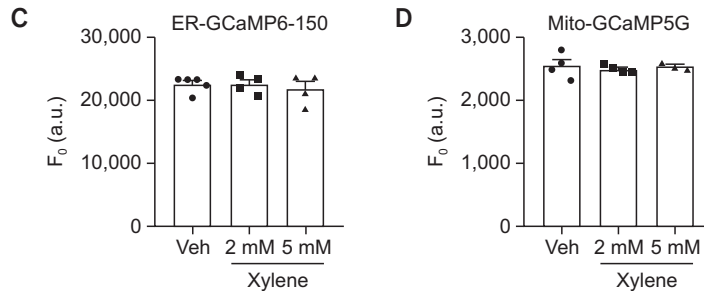


Fig. 3. Continued.

solic Ca^{2+} influx. P/Q-, N-, and L-type Ca^{2+} channels are the principal VGCCs that mediate neuronal Ca^{2+} entry (Catterall, 2011). Their individual contributions were assessed with agatoxin, conotoxin, and nifedipine, selective blockers of P/Q-, N-, and L-type channels, respectively (Chameau *et al.*, 1999; Giansante *et al.*, 2020). Electrical stimulation (40 APs at 20 Hz) transiently increased Fluo-4 AM fluorescence in somata (black trace, Fig. 2A). In Veh-treated neurons, each inhibitor significantly attenuated this response; nifedipine produced the largest reduction (green trace, Fig. 2A), indicating that L-type channels contribute most to somatic Ca^{2+} influx (Fig. 2B). Xylene exposure markedly diminished total Ca^{2+} transients and selectively suppressed P/Q-type channel activity, whereas N- and L-type channels remained unaffected (Fig. 2A, 2B). Resting Ca^{2+} levels (F_0) were comparable across groups (Fig. 2C). These findings demonstrate that xylene reduces Ca^{2+} influx by specifically inhibiting P/Q-type Ca^{2+} channels in hippocampal neurons.

Effects of xylene on Ca^{2+} homeostasis in the ER and mitochondria

We next examined ER and mitochondrial Ca^{2+} dynamics in xylene-treated neurons using ER-GCaMP6-150 and mito-GCaMP5G, which selectively report Ca^{2+} changes in the ER and mitochondria, respectively (Kwon *et al.*, 2016; de Juan-Sanz *et al.*, 2017). In ER-GCaMP6-150-transfected neurons, fluorescence rose rapidly during electrical stimulation (40 APs at 20 Hz) and declined slowly after stimulation ceased (Fig. 3A, top). Most ER-GCaMP6-150 puncta brightened, indicating Ca^{2+} uptake into the ER, consistent with previous observations (de Juan-Sanz *et al.*, 2017). Xylene attenuated the stimulus-evoked rise in ER-GCaMP6-150 fluorescence and lowered its peak amplitude (Fig. 3A, bottom). Moreover, xylene markedly slowed the decay of ER-GCaMP6-150 fluorescence, implying impaired clearance of intraluminal Ca^{2+} . Similar responses were observed in mito-GCaMP5G-expressing neurons: fluorescence increased rapidly and then decreased slowly during and after stimulation, reflecting mitochondrial Ca^{2+} uptake and release (Fig. 3B, top). In contrast to the ER signal, the peak amplitude of mito-GCaMP5G fluorescence did not differ among groups (Fig. 3B, bottom). Basal Ca^{2+} levels in both organelles were also unchanged by xylene (Fig. 3C, 3D). Collectively, these results demonstrate that xylene diminishes ER Ca^{2+} influx without affecting mitochondrial Ca^{2+} handling.

Xylene reduced Ca^{2+} influx at presynaptic and postsynaptic terminals of hippocampal neurons

Because P/Q-type Ca^{2+} channels govern presynaptic and postsynaptic Ca^{2+} entry essential for synaptic transmission, we assessed whether xylene alters these fluxes. The small size of hippocampal boutons precludes capacitance measurements; therefore, neurons were transfected with vGlut1-GCaMP5G and PSD95-GCaMP2, which selectively monitor Ca^{2+} signals at presynaptic and postsynaptic sites, respectively (Mao *et al.*, 2008; Kwon *et al.*, 2016). In neurons expressing vGlut1-GCaMP5G, fluorescence increased during electrical stimulation (Fig. 4A, top). Xylene significantly blunted this increase and reduced the peak amplitude (Fig. 4A, bottom). A parallel reduction was observed for PSD95-GCaMP2 fluorescence (Fig. 4B, top and bottom). Resting Ca^{2+} concentrations at both sites remained unchanged (Fig. 4C, 4D). Thus, xylene suppresses activity-evoked Ca^{2+} influx at presynaptic and postsynaptic terminals.

Xylene impaired SV exocytosis and endocytosis at the presynaptic terminal

Presynaptic Ca^{2+} entry regulates SV exocytosis and endocytosis (Klingauf *et al.*, 1998; Schneggenburger and Neher, 2000). P/Q-type channels couple neuronal excitation to SV exocytosis (Ishikawa *et al.*, 2005) and modulate SV endocytosis (Xue *et al.*, 2012). To determine whether xylene-induced reduction of presynaptic Ca^{2+} entry compromises SV cycling, we monitored SV dynamics in hippocampal neurons from spH21 transgenic mice, which express the pH-sensitive probe spH within the SV lumen (Kim *et al.*, 2023b; Li *et al.*, 2005). In control neurons, spH fluorescence rose rapidly during stimulation and decayed slowly thereafter, reflecting SV exocytosis and subsequent endocytosis (Fig. 5A). Xylene significantly diminished the stimulus-evoked rise in spH fluorescence and delayed its decay (Fig. 5B). Consequently, xylene disrupts both SV exocytosis and endocytosis by limiting presynaptic Ca^{2+} influx in hippocampal neurons.

Xylene reduced spontaneous neuronal firing and network activity

Electrophysiological activity was measured with the MEA, which records spontaneous, synchronized neuronal firing and overall network activity, to further investigate the neurotoxic effects of xylene. A heatmap illustrated firing frequencies at individual MEA electrodes and revealed reduced firing rates in xylene-exposed neurons (Fig. 6A). Neuronal activity increased during differentiation in the Veh group, whereas xy-

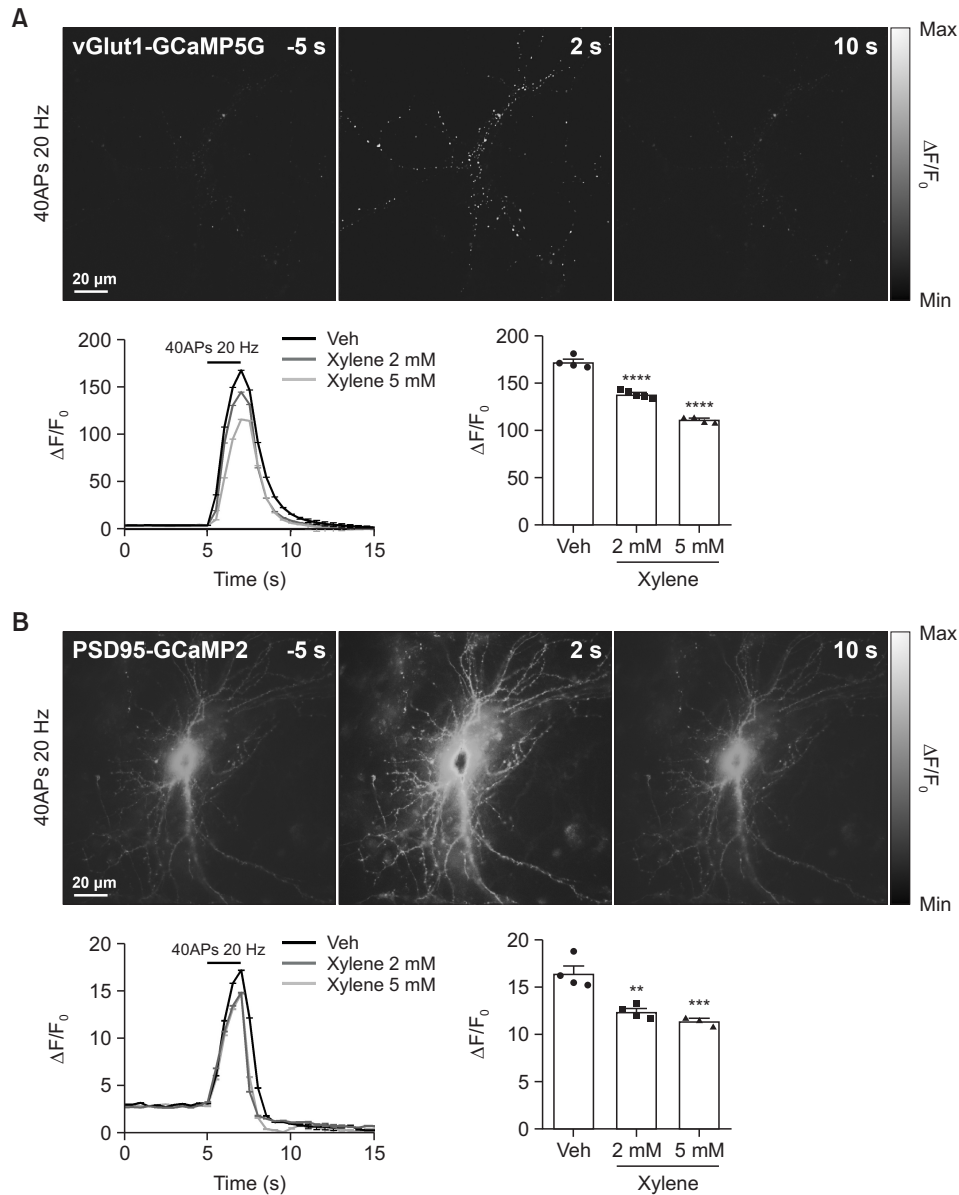


Fig. 4. Effects of xylene on Ca^{2+} influx at presynaptic and postsynaptic terminals. Presynaptic and postsynaptic Ca^{2+} influx were measured in neurons transfected with vGlut1-GCaMP5G and PSD95-GCaMP2, respectively. Electrical stimulation consisted of 40 APs delivered at 20 Hz. (A) Top: pseudocolor time-lapse images of vGlut1-GCaMP5G-expressing neurons during electrical stimulation. Bottom: normalized traces (left) and peak-amplitude quantification (right). $n=4$ independent cultures (Veh), 5 (2 mM xylene), and 4 (5 mM xylene). Bars represent mean $\pm$ SEM. (B) Top: representative pseudocolor time-lapse images of PSD95-GCaMP2-expressing neurons during electrical stimulation. Bottom: averaged traces (left) and peak-amplitude quantification (right). $n=4$ independent cultures (Veh), 4 (2 mM xylene), and 3 (5 mM xylene). Bars represent mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. Veh (one-way ANOVA with Tukey *post hoc* test). (C) Fluorescence-intensity quantification of vGlut1-GCaMP5G-expressing ROIs. $n=4$ independent cultures (Veh), 5 (2 mM xylene), and 4 (5 mM xylene). Bars represent mean $\pm$ SEM. (D) Fluorescence-intensity quantification of PSD95-GCaMP2-expressing ROIs. $n=4$ independent cultures (Veh), 4 (2 mM xylene), and 3 (5 mM xylene). Bars represent mean $\pm$ SEM.

lene suppressed neuronal activity, including single and burst firing rates as well as network bursts (Fig. 6B, 6C). However, ISIs within bursts were comparable among groups (Fig. 6C).

Xylene repressed synaptic development in developing neurons

To assess neurodevelopmental toxicity, we stained presynaptic and postsynaptic markers in eGFP-expressing neurons.

Immature neurons treated with xylene (DIV 5) were transfected with eGFP, cultured for 2 weeks, and subsequently stained for presynaptic and postsynaptic markers. eGFP-positive axonal processes labeled with presynaptic markers exhibited fewer Syt and Syp puncta after xylene exposure (Supplementary Fig. 1A, 1B). eGFP-expressing dendritic processes stained with postsynaptic markers displayed spine morphology, and xylene decreased dendritic GluR1 and NR1 puncta

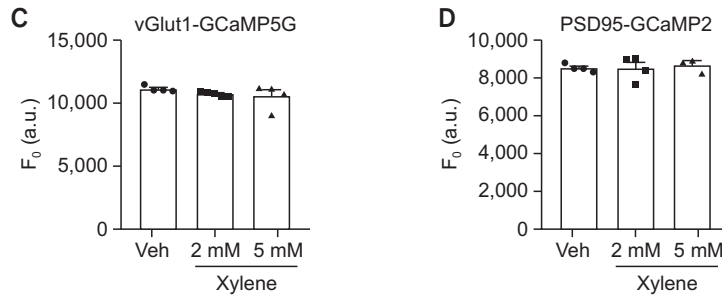


Fig. 4. Continued.

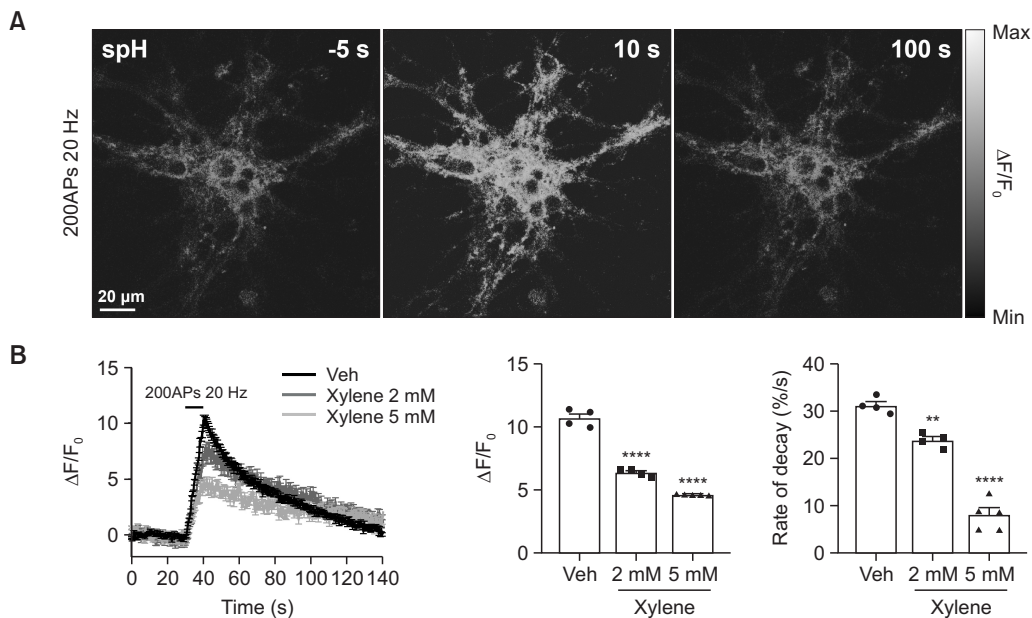


Fig. 5. Impairment of SV exocytosis and endocytosis in xylene-exposed neurons. (A) Representative pseudocolor time-lapse images of neurons from spH 21 transgenic mice during electrical stimulation (200 APs at 20 Hz). (B) Left: averaged whole-trace recordings from spH 21-expressing ROIs. Right: quantification of the peak amplitude elicited by electrical stimulation and the post-stimulus fluorescence decay rate. $n=4$ independent cultures for Veh, $n=4$ for xylene 2 mM, and $n=5$ for xylene 5 mM. Bars indicate mean $\pm$ SEM. ** $p<0.01$ and **** $p<0.0001$ vs. Veh by one-way ANOVA followed by Tukey's *post hoc* test.

(Supplementary Fig. 1C, 1D). Quantitative analysis confirmed that xylene inhibited synaptic development (Supplementary Fig. 1E). Although the total number of dendritic spines was similar across groups, xylene reduced the proportion of mushroom spines and increased the proportion of thin spines along eGFP-expressing dendrites (Supplementary Fig. 1F). Collectively, xylene disrupted Ca^{2+} homeostasis, neuronal activity, and synaptic maturation in hippocampal neurons.

DISCUSSION

Xylene is highly lipophilic and readily accumulates in—and damages—lipid membranes. The brain's high lipid content renders it particularly vulnerable, and xylene exposure primarily causes neurological symptoms such as reduced responsiveness, anesthesia, memory deficits, depression, dizziness, headache, and nausea (Fang *et al.*, 1996; Kandyala *et al.*,

2010). Accidental ingestion of high doses may even induce persistent coma in humans (Recchia *et al.*, 1985). Regions with high, chronic xylene exposure underscore the need for dedicated experimental studies. However, the prevalent co-existence of xylene with other VOCs complicates its risk assessment. Therefore, single-agent exposure experiments and epidemiological studies at high xylene levels are essential for accurate molecular toxicity profiling (Niaz *et al.*, 2015). Laboratory-grade xylene comprises 20% o-, 44% m-, and 20% p-xylene, and each isomer exerts similar detrimental effects (Condie *et al.*, 1988). Although individual isomers are employed in certain industries, humans are generally exposed to the mixed solvent (Macholz, 1982). Xylene exposure elevates malondialdehyde and superoxide dismutase levels while reducing glutathione peroxidase activity (Tang and Xu, 2005). Consequently, xylene may damage the brain by amplifying oxidative stress, to which neural tissue is particularly susceptible.

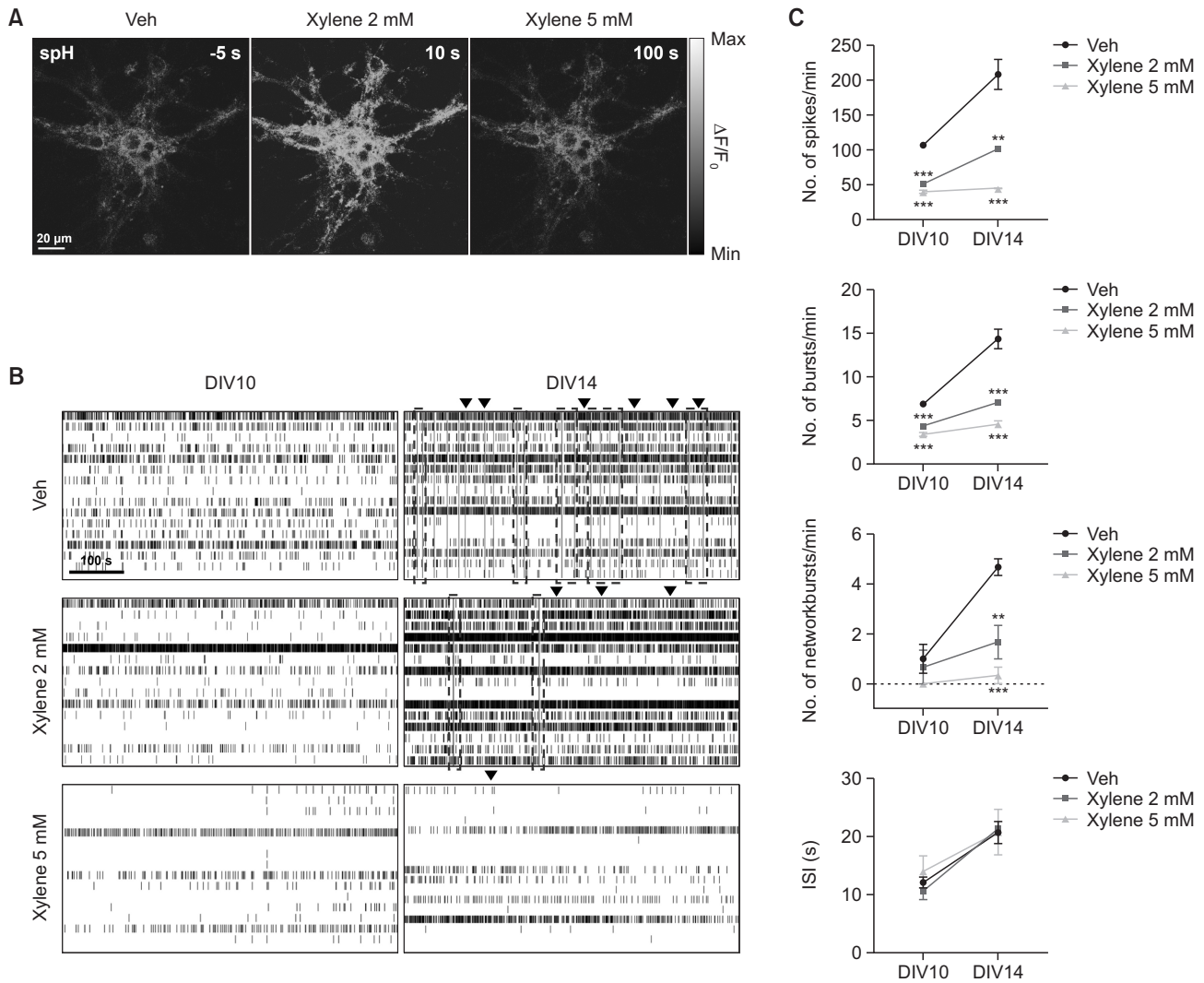


Fig. 6. Reduction of spontaneous and network activities in xylene-exposed neurons. (A) Representative color-coded heat map of MEA recordings from neurons (DIV 10). Each circle represents an active electrode within a 4×4 array. Firing frequency is color coded (red/white, >10 Hz; blue, <1 Hz). (B) Raster plot of individual electrodes indicating neuronal spikes (black), burst firing (arrowhead), and network bursts (dashed box). (C) Quantification of firing rate, burst rate, network burst frequency, and ISI. n=4 independent cultures for Veh, n=4 for xylene 2 mM, and n=5 for xylene 5 mM. Bars indicate mean ± SEM. ***p*<0.01 and ****p*<0.001 vs. Veh by two-way ANOVA followed by Tukey's *post hoc* test.

P/Q-type Ca^{2+} channels govern neuronal activity and shape APs through physical association with voltage-gated K^{+} channels (Womack and Khodakhah, 2004; Berkefeld *et al.*, 2006). Using selective VGCC inhibitors, we demonstrated that xylene depresses P/Q-type Ca^{2+} channel activity. Given that organellar Ca^{2+} modulates synaptic activity by regulating presynaptic Ca^{2+} influx (Kwon *et al.*, 2016; de Juan-Sanz *et al.*, 2017), we found, via organelle-specific indicators, that xylene impairs organellar Ca^{2+} influx and inhibits SV exocytosis and endocytosis at presynaptic terminals. Resting Ca^{2+} levels in neuronal organelles and compartments—including the ER, mitochondria, soma, and presynaptic and postsynaptic terminals—remained normal after xylene exposure. Moreover, xylene selectively reduced P/Q-type channel activity without affecting N- or L-type Ca^{2+} channels. Collectively, these findings suggest that xylene targets Ca^{2+} channels directly rather than disrupting the

plasma membrane. Our data, therefore, link xylene-induced Ca^{2+} dysregulation to impaired synaptic activity in hippocampal neurons. Ca^{2+} transients in neural progenitors and radial glia drive embryonic proliferation, migration, and postnatal astrogliogenesis (McKinney *et al.*, 2022); xylene may, therefore, be neurotoxic to additional cell types in the developing brain.

Diverse methods have been developed to monitor SV exocytosis and endocytosis, including electron microscopy coupled with optogenetic stimulation, capacitance measurements, and quantum dots (Kononenko and Haucke, 2015). Although they require genetic manipulation that may be toxic to organelles, pHluorin-fused SV proteins are widely used to measure presynaptic activity in living hippocampal neurons. Neurons from spH21 transgenic animals endogenously express pHluorin within SV lumens, enabling observation of presynaptic activity without additional transfection. Presynaptic

activity is essential for neurotransmission and synaptic network function (Citri and Malenka, 2008). In the present study, xylene-treated neurons exhibited impaired SV exocytosis and endocytosis attributable to P/Q-type Ca^{2+} channel inhibition, accompanied by markedly reduced spontaneous firing and synaptic network activity. Such reduced activity may further compromise neuronal development, as spontaneous firing is required for synaptic maturation and proper circuit formation (Luhmann *et al.*, 2016).

The environmental relevance of the present xylene exposure paradigm constitutes a limitation of this work. Animal or cellular models may not fully capture occupational or environmental exposure in humans. Humans are chronically exposed to xylene as part of mixed VOCs at lower doses and through multiple routes; the precise dose in pregnant women, infants, or workers, however, remains unclear. Epidemiological studies have shown that workers chronically exposed to 14 ppm xylene developed musculoskeletal problems linked to central nervous system dysfunction (Uchida *et al.*, 1993). Moreover, spontaneous abortion has been reported among female laboratory workers chronically exposed to xylene (Taskinen *et al.*, 1994). Variability in blood xylene concentrations caused by exercise or smoking (Langman, 1994) and inter-individual differences in sensitivity (Kandyala *et al.*, 2010) further complicate risk assessment. Nevertheless, developmental exposure to xylene induces skeletal deformities, delayed growth, and reduced body weight in fetuses (Niaz *et al.*, 2015). Chronic prenatal or postnatal exposure to VOCs has also been associated with neurodevelopmental delay, reduced birth weight, and lower cognitive scores in children (Chang *et al.*, 2017, 2018; Nakaoka *et al.*, 2021). Pregnant women often spend more time indoors in preparation for childbirth (Nethery *et al.*, 2009), and the ubiquity of indoor VOCs may, therefore, result in higher solvent concentrations in mothers and their unborn children than in the general adult population (Amiri and Turner-Henson, 2017). These observations highlight the need to monitor exposure to organic solvents during pregnancy and early childhood and to elucidate the detailed neurotoxic mechanisms involved. Although the doses used in the present study exceed typical human exposure, high-dose, short-term paradigms remain valuable for hazard identification (Borgert *et al.*, 2021). Our functional analyses in living neurons offer mechanistic insight into xylene-induced neurotoxicity and support its classification as a neurodevelopmental hazard. Future studies employing lower, chronic doses of xylene alone or in combination with other VOCs are required to determine its long-term impact on the brain and its environmental relevance.

Although the neurotoxic effects of xylene have been established in biochemical assays, the underlying mechanisms in developing neurons, particularly those governing neuronal activity, have remained incompletely understood. Here, we show that xylene suppresses Ca^{2+} influx through P/Q-type Ca^{2+} channels, thereby reducing Ca^{2+} entry into the ER and at synaptic terminals. Disrupted Ca^{2+} homeostasis, in turn, inhibits SV exocytosis and endocytosis and dampens neuronal activity (Supplementary Fig. 2). Given that dysregulation of P/Q-type Ca^{2+} channels and altered synaptic activity are closely related to neurodevelopmental disorders (Ullman *et al.*, 2018), our findings offer a plausible mechanistic link between VOC exposure and increased risk of such disorders.

CONFLICT OF INTEREST

The authors report no relevant financial or nonfinancial interests.

ACKNOWLEDGMENTS

We thank Dr. Timothy Ryan (ER-GCaMP6-150), Franck Polleux (mito-GCaMP5G, vGlut1-GCaMP5G), Karel Svoboda (PSD95-GCaMP2), and Doug Golenbock (eGFP) for providing plasmids.

AUTHOR CONTRIBUTIONS

YE: Data curation, formal analysis, investigation. SHL: Conceptualization, investigation, supervision, writing – original draft, writing – review & editing.

REFERENCES

- Amiri, A. and Turner-Henson, A. (2017) The roles of formaldehyde exposure and oxidative stress in fetal growth in the second trimester. *J. Obstet. Gynecol. Neonatal. Nurs.* **46**, 51-62.
- Berkefeld, H., Sailer, C. A., Bildl, W., Rohde, V., Thumfart, J. O., Eble, S., Klugbauer, N., Reisinger, E., Bischofberger, J., Oliver, D., Knaus, H. G., Schulte, U. and Fakler, B. (2006) BKCa-Cav channel complexes mediate rapid and localized Ca^{2+} -activated K^{+} signaling. *Science* **314**, 615-620.
- Borgert, C. J., Fuentes, C. and Burgoon, L. D. (2021) Principles of dose-setting in toxicology studies: the importance of kinetics for ensuring human safety. *Arch. Toxicol.* **95**, 3651-3664.
- Catterall, W. A. (2011) Voltage-gated calcium channels. *Cold Spring Harb. Perspect. Biol.* **3**, a003947.
- Chameau, P., Lucas, P., Melliti, K., Bournaud, R. and Shimahara, T. (1999) Development of multiple calcium channel types in cultured mouse hippocampal neurons. *Neuroscience* **90**, 383-388.
- Chang, M., Lee, D., Park, H., Ha, M., Hong, Y. C., Kim, Y., Kim, B. N., Kim, Y., Lim, Y. H. and Ha, E. H. (2018) Prenatal TVOCs exposure negatively influences postnatal neurobehavioral development. *Sci. Total Environ.* **618**, 977-981.
- Chang, M., Park, H., Ha, M., Hong, Y. C., Lim, Y. H., Kim, Y., Kim, Y. J., Lee, D. and Ha, E. H. (2017) The effect of prenatal TVOC exposure on birth and infantile weight: the Mothers and Children's Environmental Health study. *Pediatr. Res.* **82**, 423-428.
- Citri, A. and Malenka, R. C. (2008) Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology* **33**, 18-41.
- Condie, L. W., Hill, J. R. and Borzelleca, J. F. (1988) Oral toxicology studies with xylene isomers and mixed xylenes. *Drug Chem. Toxicol.* **11**, 329-354.
- de Juan-Sanz, J., Holt, G. T., Schreiter, E. R., de Juan, F., Kim, D. S. and Ryan, T. A. (2017) Axonal endoplasmic reticulum Ca^{2+} content controls release probability in CNS nerve terminals. *Neuron* **93**, 867-881.e866.
- Fang, Z. X., Sonner, J., Laster, M. J., Ionescu, P., Kandel, L., Koblin, D. D., Eger, E. I. and Halsey, M. J. (1996) Anesthetic and convulsant properties of aromatic compounds and cycloalkanes: Implications for mechanisms of narcosis. *Anesth. Analg.* **83**, 1097-1104.
- Giansante, G., Marte, A., Romei, A., Prestigio, C., Onofri, F., Benfenati, F., Baldelli, P. and Valente, P. (2020) Presynaptic L-type Ca^{2+} channels increase glutamate release probability and excitatory strength in the hippocampus during chronic neuroinflammation. *J. Neurosci.* **40**, 6825-6841.
- Grossman, A. W., Elisseeu, N. M., McKinney, B. C. and Greenough, W. T. (2006) Hippocampal pyramidal cells in adult Fmr1 knockout mice

- exhibit an immature-appearing profile of dendritic spines. *Brain Res.* **1084**, 158-164.
- Guo, H., Lee, S. C., Chan, L. Y. and Li, W. M. (2004) Risk assessment of exposure to volatile organic compounds in different indoor environments. *Environ. Res.* **94**, 57-66.
- Honma, T., Sudo, A., Miyagawa, M., Sato, M. and Hasegawa, H. (1983) Significant changes in the amounts of neurotransmitter and related substances in rat brain induced by subacute exposure to low levels of toluene and xylene. *Ind. Health* **21**, 143-151.
- Ishikawa, T., Kaneko, M., Shin, H.S. and Takahashi, T. (2005) Presynaptic N-type and P/Q-type Ca²⁺ channels mediating synaptic transmission at the calyx of Held of mice. *J. Physiol.* **568**, 199-209.
- Kandyala, R., Raghavendra, S. P. and Rajasekharan, S. T. (2010) Xylene: an overview of its health hazards and preventive measures. *J. Oral Maxillofac. Pathol.* **14**, 1-5.
- Kessi, M., Chen, B., Peng, J., Yan, F., Yang, L. and Yin, F. (2021) Calcium channelopathies and intellectual disability: a systematic review. *Orphanet. J. Rare Dis.* **16**, 219.
- Kim, S. R., Eom, Y. and Lee, S. H. (2023a) Comprehensive analysis of sex differences in the function and ultrastructure of hippocampal presynaptic terminals. *Neurochem. Int.* **169**, 105570.
- Kim, Y. K., Eom, Y., Yoon, H., Lee, Y. and Lee, S. H. (2023b) Benzo[a]pyrene represses synaptic vesicle exocytosis by inhibiting P/Q-type calcium channels in hippocampal neurons. *Ecotox. Environ. Safe* **263**, 115301.
- Kishi, R., Harabuchi, I., Katakura, Y., Ikeda, T. and Miyake, H. (1993) Neurobehavioral effects of chronic occupational exposure to organic-solvents among Japanese industrial painters. *Environ. Res.* **62**, 303-313.
- Klingauf, J., Kavalali, E. T. and Tsien, R. W. (1998) Kinetics and regulation of fast endocytosis at hippocampal synapses. *Nature* **394**, 581-585.
- Kononenko, N. L. and Haucke, V. (2015) Molecular mechanisms of presynaptic membrane retrieval and synaptic vesicle reformation. *Neuron* **85**, 484-496.
- Kwon, S. K., Sando, R., Lewis, T. L., Hirabayashi, Y., Maximov, A. and Polleux, F. (2016) LKB1 regulates mitochondria-dependent presynaptic calcium clearance and neurotransmitter release properties at excitatory synapses along cortical axons. *PLoS Biol.* **14**, e1002516.
- Langman, J. M. (1994) Xylene - its toxicity, measurement of exposure levels, absorption, metabolism and clearance. *Pathology* **26**, 301-309.
- Laslo-Baker, D., Barrera, M., Knittel-Keren, D., Kozer, E., Wolpin, J., Khattak, S., Hackman, R., Rovet, J. and Koren, G. (2004) Child neurodevelopmental outcome and maternal occupational exposure to solvents. *Arch. Pediat. Adol. Med.* **158**, 956-961.
- Li, Z. Y., Burrone, J., Tyler, W. J., Hartman, K. N., Albeanu, D. F. and Murthy, V. N. (2005) Synaptic vesicle recycling studied in transgenic mice expressing synaptophluorin. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6131-6136.
- Luhmann, H. J., Sinning, A., Yang, J. W., Reyes-Puerta, V., Stüttgen, M. C., Kirischuk, S. and Kilb, W. (2016) Spontaneous neuronal activity in developing neocortical networks: from single cells to large-scale interactions. *Front. Neural. Circuit.* **10**, 40.
- Macholz, R. M. (1982) The Handbook of Environmental Chemistry. Wiley, New York.
- Mao, T., O'Connor, D. H., Scheuss, V., Nakai, J. and Svoboda, K. (2008) Characterization and subcellular targeting of GCaMP-type genetically-encoded calcium indicators. *PLoS One* **3**, e1796.
- Mark, M. D., Maejima, T., Kuckelsberg, D., Yoo, J. W., Hyde, R. A., Shah, V., Gutierrez, D., Moreno, R. L., Kruse, W., Noebels, J. L. and Herlitze, S. (2011) Delayed postnatal loss of P/Q-type calcium channels recapitulates the absence epilepsy, dyskinesia, and ataxia phenotypes of genomic mutations. *J. Neurosci.* **31**, 4311-4326.
- McKinney, A. A., Petrova, R. and Panagiotakos, G. (2022) Calcium and activity-dependent signaling in the developing cerebral cortex. *Development* **149**, dev198853.
- Nakamura, Y., Harada, H., Kamasawa, N., Matsui, K., Rothman, J. S., Shigemoto, R., Silver, R. A., DiGregorio, D. A. and Takahashi, T. (2015) Nanoscale distribution of presynaptic Ca(2+) channels and its impact on vesicular release during development. *Neuron* **85**, 145-158.
- Nakaoka, H., Hisada, A., Matsuzawa, D., Yamamoto, M., Mori, C.; Japan Environment and Children's Study (JECS) Group; Kamijima, M., Yamazaki, S., Ohya, Y., Kishi, R., Yaegashi, N., Hashimoto, K., Mori, C., Ito, S., Yamagata, Z., Inadera, H., Nakayama, T., Iso, H., Shima, M., Kurozawa, Y., Suganuma, N., Kusuhaara, K. and Kato, T. (2021) Associations between prenatal exposure to volatile organic compounds and neurodevelopment in 12-month-old children: The Japan Environment and Children's Study (JECS). *Sci. Total Environ.* **794**, 148643.
- Nanou, E. and Catterall, W. A. (2018) Calcium channels, synaptic plasticity, and neuropsychiatric disease. *Neuron* **98**, 466-481.
- Nethery, E., Brauer, M. and Janssen, P. (2009) Time-activity patterns of pregnant women and changes during the course of pregnancy. *J. Expo. Sci. Environ. Epidemiol.* **19**, 317-324.
- Niaz, K., Bahadar, H., Maqbool, F. and Abdollahi, M. (2015) A review of environmental and occupational exposure to xylene and its health concerns. *EXCLI J.* **14**, 1167-1186.
- Nimmervoll, B., Flucher, B. E. and Obermair, G. J. (2013) Dominance of P/Q-type calcium channels in depolarization-induced presynaptic FM dye release in cultured hippocampal neurons. *Neuroscience* **253**, 330-340.
- Panda, S., Behera, S., Alam, M. F. and Syed, G. H. (2021) Endoplasmic reticulum & mitochondrial calcium homeostasis: the interplay with viruses. *Mitochondrion* **58**, 227-242.
- Pelé, F., Muckle, G., Costet, N., Garlandezec, R., Monfort, C., Multigner, L., Rouget, F. and Cordier, S. (2013) Occupational solvent exposure during pregnancy and child behaviour at age 2. *Occup. Environ. Med.* **70**, 114-119.
- Recchia, G., Perbellini, L., Prati, G. F., Dean, P. and Ancona, G. (1985) Coma caused by probably accidental ingestion of xylene: treatment with hemoperfusion using activated charcoal. *Med. Lav.* **76**, 67-73.
- Rosenberg, S. S. and Spitzer, N. C. (2011) Calcium signaling in neuronal development. *Cold Spring Harb. Perspect. Biol.* **3**, a004259.
- Savolainen, H. and Pfaffil, P. (1980) Dose-dependent neurochemical changes during short-term inhalation exposure to m-xylene. *Arch. Toxicol.* **45**, 117-122.
- Schneggenburger, R. and Neher, E. (2000) Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature* **406**, 889-893.
- Tang, D. and Xu, L. (2005) Study on lipid peroxidation in workers exposed to benzene, toluene and xylene. *Chinese Occup. Med.* **2**, 016.
- Taskinen, H., Kyyronen, P., Hemminki, K., Hoikkala, M., Lajunen, K. and Lindbohm, M. L. (1994) Laboratory work and pregnancy outcome. *J. Occup. Med.* **36**, 311-319.
- Till, C., Koren, G. and Rovet, J. F. (2001) Prenatal exposure to organic solvents and child neurobehavioral performance. *Neurotoxicol. Teratol.* **23**, 235-245.
- Uchida, Y., Nakatsuka, H., Ukai, H., Watanabe, T., Liu, Y. T., Huang, M. Y., Wang, Y. L., Zhu, F. Z., Yin, H. and Ikeda, M. (1993) Symptoms and signs in workers exposed predominantly to xylenes. *Int. Arch. Occup. Environ. Health* **64**, 597-605.
- Ullman, J. C., Yang, J., Sullivan, M., Bendor, J., Levy, J., Pham, E., Silm, K., Seifkar, H., Sohal, V. S., Nicoll, R. A. and Edwards, R. H. (2018) A mouse model of autism implicates endosome pH in the regulation of presynaptic calcium entry. *Nat. Commun.* **9**, 330.
- United States. Agency for Toxic Substances and Disease Registry, Research Triangle Institute, and Sciences International Inc. (1995) Toxicological profile for xylene. U.S. Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, Ga. xvii, 270, 276, 273 p.
- Wang, S. B., Ang, H. M. and Tade, M. O. (2007) Volatile organic compounds in indoor environment and photocatalytic oxidation: State of the art. *Environ. Int.* **33**, 694-705.
- Wang, X. L., Jin, X. P., Fu, H., Chen, Z. Q., Da, C. D., Huang, X. F. and Ding, B. Q. (1999) Xylene-induced effects on brain neurotransmitters, behavior and fos protein in rats. *Biomed. Environ. Sci.* **12**, 116-124.
- Womack, M. D. and Khodakhah, K. (2004) Dendritic control of spontaneous bursting in cerebellar Purkinje cells. *J. Neurosci.* **24**, 3511-3521.

- Wu, L. G., Westenbroek, R. E., Borst, J. G. G., Catterall, W. A. and Sakmann, B. (1999) Calcium channel types with distinct presynaptic localization couple differentially to transmitter release in single calyx-type synapses. *J. Neurosci.* **19**, 726-736.
- Xue, L., Zhang, Z., McNeil, B. D., Luo, F., Wu, X. S., Sheng, J., Shin, W. and Wu, L. G. (2012) Voltage-dependent calcium channels at the plasma membrane, but not vesicular channels, couple exocytosis to endocytosis. *Cell Rep.* **1**, 632-638.
- Yu, I. T. S., Lee, N. L., Zhang, X. H., Chen, W. O., Lam, Y. T. and Wong, T. W. (2004) Occupational exposure to mixtures of organic solvents increases the risk of neurological symptoms among printing workers in Hong Kong. *J. Occup. Environ. Med.* **46**, 323-330.