Cellular and Molecular Neurobiology (2019) 39:435–449
https://doi.org/10.1007/s10571-019-00660-0

ORIGINAL RESEARCH

Cyclohexane Inhalation Produces Long-Lasting Alterations in the Hippocampal Integrity and Reward-Seeking Behavior in the Adult Mouse

Tania Campos-Ordonez[1,2] · David Zarate-Lopez[1,2] · Nereida Ibarra-Castaneda[1] · Jonathan Buritica[3] · Oscar Gonzalez-Perez[1]

Received: 12 October 2018 / Accepted: 5 February 2019 / Published online: 15 February 2019

Abstract

Cyclohexane (CHX) is an organic solvent commonly used as a drug-of-abuse. This drug increases the oxidative stress and glial reactivity in the hippocampus, which suggests that this brain region is vulnerable to CHX effects. This study aimed to establish the behavioral changes and the pathological alterations that occur in the Cornu Ammonis 3 (CA3) and Dentate Gyrus (DG) after a long-lasting exposure to CHX. We exposed CD1 mice to a recreational-like dose of CHX (~ 30,000 ppm) for 30 days and explored its consequences in motor skills, reward-seeking behavior, and the CA3 and DG hippocampal subfields. Twenty-four hours after the last administration of CHX, we found a significant decrease in the number of c-Fos+ cells in the hippocampal CA3 and DG regions. This event coincided with an increased in NMDAR1 expression and apoptotic cells in the CA3 region. At day 13th without CHX, we found a persistent reduction in the number of c-Fos+ and TUNEL+ cells in DG. At both time points, the CHX-exposed mice showed a strong overexpression of neuropeptide Y (NPY) in the CA3 stratum lucidum and the hippocampal hilus. In parallel, we used an operant-based task to assess motor performance and operant conditioning learning. The behavioral analysis indicated that CHX did not modify the acquisition of operant conditioning tasks, but affected some motor skills and increased the reward-seeking behavior. Altogether, this evidence reveals that CHX exposure provokes long-lasting changes in the hippocampal subfields, induces motor impairments and increases the motivation-guided behavior. These findings can help understand the deleterious effect of CHX into the adult hippocampus and unveil its potential to trigger addiction-like behaviors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10571-019-00660-0) contains supplementary material, which is available to authorized users.

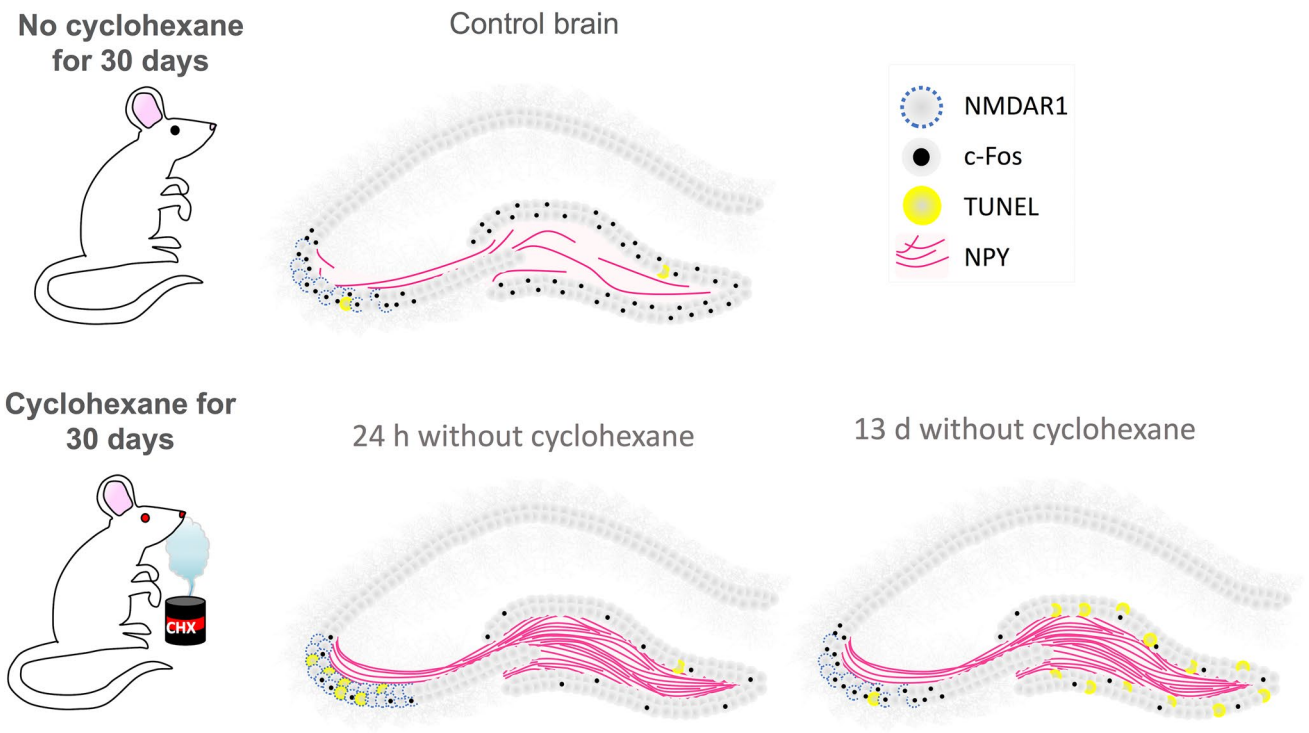
✉ Oscar Gonzalez-Perez
osglez@ucol.mx

[1] Laboratory of Neuroscience, School of Psychology, University of Colima, Av. Universidad 333, 28040 Colima, Colonia, Mexico

[2] Physiological Sciences PhD Program, School of Medicine, University of Colima, Av. Universidad 333, 28040 Colima, Colonia, Mexico

[3] Centro de Estudios e Investigaciones en Comportamiento, University of Guadalajara, St. Francisco de Quevedo 180, 44130 Guadalajara, Jalisco, Mexico

Graphical Abstract



Keywords Apoptosis · NMDAR1 · Neuropeptide Y · Hippocampus · Excitotoxicity · Inhalant abuse · Solvent · Reward motivation · Motor skills

Introduction

Cyclohexane (CHX, hexahydrobenzene) is a lipophilic hydrocarbon used as a "safe" replacement of other organic solvents (*n*-hexane, xylene, and benzene) in several industrial products, such as adhesives, paint thinner, and gasoline. These volatile products are not only inhaled during occupational or accidental exposure, but also they are intentionally consumed as drugs of abuse. Experimental exposure to hydrocarbons produces oxidative stress and impairs the glutamatergic neurotransmission in the postnatal hippocampus (Fifel et al. 2014; Furlong et al. 2016). This synaptic glutamate dysfunction may contribute to excitotoxicity that, in turn, triggers a neurodegenerative process (Rodriguez et al. 2009) and behavioral impairment (Schmelzeis and Mittleman 1996; Gourley et al. 2010). In the inhalant use disorder, high doses of hydrocarbons produce euphoria, lethargy, psychomotor retardation, muscle weakness, tremor, stupor or coma (American Psychiatric Association 2013). In contrast, occupational or accidental inhalation of CHX may provoke dizziness, limb weakness, motor variations and verbal memory impairment (Campos-Ordonez and Gonzalez-Perez 2016). However, the persistent alterations on motor control and cognitive functions after chronic CHX exposure are still poorly identified.

In rodents, a 30-day CHX exposure induces astrogliosis, microglial activation and overexpression of AP endonuclease-1 protein (APE1) in the CA1 and CA3 hippocampal regions (Campos-Ordonez et al. 2015). The CA3 region is very susceptible to neuro-degeneration and seizures (Cherubini and Miles 2015). An excessive release of glutamate in this region produces excitotoxicity that contributes to the degeneration of CA3 pyramidal neurons (Medvedeva et al. 2017). Therefore, the CA3 region appears to be a primary target of organic solvents, including CHX. The dentate gyrus is a hippocampal subfield that is essential for maintaining synaptic plasticity and memory (Jedlicka et al. 2018). Granule neurons of the dorsal dentate gyrus are also susceptible to neurodegenerative signals (Fuster-Matanzo et al. 2011). This region contains a diverse group of interneurons that use γ-aminobutyric acid (GABA) as their primary neurotransmitter. Interestingly, the glutamatergic and GABAergic neurons seem to be differentially affected by some drugs, such as ethanol or inhalants (Bale et al. 2005; Tiwari et al. 2014). This evidence raises the possibility that CHX may have differential effects on diverse neuronal subpopulations

and provoke either transitory or persistent alterations on the hippocampal integrity during the withdrawal period. In this study, we analyzed the effects of CHX on the expression of c-Fos, NMDA receptor 1 (NMDAR1), neuropeptide Y (NPY), and apoptosis in the adult hippocampus. Besides, we studied whether CHX altered motivation and motor skills. Our findings indicate that CHX exposure produces persistent changes in neuronal homeostasis, causes behavioral abnormalities and differentially affects the hippocampal subfields in the adult brain.

Materials and Methods

Animals and Housing

CD1 male mice (P60) were distributed in four groups (two groups per time point): the control group (non-exposed to CHX) and the experimental group exposed to ~ 30,000 ppm of CHX. All animals were housed under standard biotery conditions, i.e., polycarbonate cages (28 × 12 × 15 cm), air-conditioned room at 24 ± 1 °C and relative humidity between 40 and 60%. All experimental procedures followed the legal regulation for laboratory animals' care (Mexican Official Norm [NOM] 062-ZOO-1999), approved and supervised by the Committee of Animal Care and Use of the University of Colima.

CHX Exposure

We used an animal model that mimics the human condition of solvent-abuse episodes, i.e., a short exposure period with a high dose of solvents (Bowen et al. 2006). All the animals were placed into a polycarbonate chamber (41 × 27.5 × 12 cm^3) at 25 °C that had four cylinders (11.5 cm length) for drug delivery inserted at every corner (Campos-Ordonez et al. 2015). The CHX group was exposed approximately to 30,000 ppm of CHX (99.5% PRA grade, Sigma-Aldrich, St. Louis, MO) for 30 min twice a day (with a 6-h interval) per 30 days. We used the following equation for calculating the parts per million of CHX: ppm = (mg/m^3) × (molar volume/molecular weight), where mg = the total amount of CHX delivered (1.6 mg; density = 0.78); m^3 = volume of the chamber (0.0157 m^3); molar volume = L/mol at 25 °C; CHX M.W. = 84.16 g mol^{-1}. Thus, the total amount of CHX delivered per session was 29,606 ppm (IQR 29, 606–28,681) and the time course of this delivery is shown in Supplementary Table 1. All the experiments started at 8:30 AM and 3:30 PM. Relative humidity and temperature were monitored and kept constant throughout the experiment. After the exposure, all the mice were transferred to a recovery cage for 15 min before being returned to their home cages. The control group was handled and placed under similar experimental conditions but without CHX.

Behavioral Analysis

Observational Assessment

Throughout the drug-exposure sessions, we documented animal behaviors with a checklist aimed to recognize some motor deficits that are commonly observed during the recreational use of inhalants in humans (Neiman et al. 2000; Lubman et al. 2008; Dingwall et al. 2011), such as hyperactivity, ataxia, immobility and seizures. Seizure-like behavior is a reliable indicator of the severity of inhalant intoxications (Neiman et al. 2000). Thus, the frequency of forelimb clonus with rearing (seizure-like behavior) and the latency period of each seizure were graphed in a colorimetry raster plot.

Operant Procedure

Hippocampal damage provokes alterations in the motivation-reward system (Schmelzeis and Mittleman 1996; Gourley et al. 2010), motor incoordination and hyperactivity (Rivadeneyra-Domínguez and Rodríguez-Landa 2016). To determine whether CHX may disrupt motor performance and motivation, we evaluated the reward-seeking behavior by using a progressive ratio (PR) schedule. After CHX exposure, the animals were individually housed and put under a food restriction regime to induce weight loss and keep it stable at ~ 85% of their previous weight. Twenty-four hours after the last CHX exposure, we evaluated the PR schedule for ten consecutive days (Fig. 2a). Briefly, the operant procedures were performed in Lafayette chambers (Mod. 80,003), internal chamber dimension: 18.5 cm length, 18 cm width and 14.5 cm height. The lever was placed 5 cm above the cage floor, and a force of 0.026 N was required to register a response. As a behavioral reward, the liquid dispenser provided 0.02 mL of 20% sucrose solution. Data were acquired using and the ABET II Lafayette software.

In the acquisition phase (a 30-min session), a drop of sweetened water was dispensed every 30 s and when the experimental animal pressed the lever. In the maintenance phase (a 60-min session), the animals only had access to fluid if they pushed the lever. This method is called continuous reinforcement schedule (CRF), an operant paradigm to assess the effort an animal is willing to invest in obtaining a reward. In the next ten sessions (45-min each), the animals received a reward only if they pressed the lever twice more than the previous one. That is, the effort to get a reward followed an arithmetic progression with common difference of 2 (1, 3, 5, 7, etc.). These data were computed and used to calculate the breakpoint, i.e., the maximum number of lever presses a mouse is willing to do for a sweet drop.

Since the breakpoint may be affected by motor skill and motivation, we used the mathematical principles of reinforcement (MPR), using the last three PR2 sessions to calculate behavioral performance. Briefly, we computed the responding latency (Post-Reinforcement Pause-PRP) and the total trial duration in an Excel's macro kindly provided by Bradshaw and Killeen (Bradshaw and Killeen 2012). Thus, we estimated the running rate and the global rate with the following equations:

For the running rate:

$$R_{RUN,\,i} = \frac{1}{\delta(1 + T_{TOT,\,i-1}/\mathrm{a})} \quad (1)$$

where $\boldsymbol{T_{TOT,\,I-1}}$ is the total duration of the previous trial, '$\boldsymbol{\delta}$' is motor skill, and '$\boldsymbol{a}$' is motivation.

For the global rate:

$$R_{OVERALL,\,i} = \frac{N_i}{T_0 + kT_{TOT,i-1} + N_i\delta(1 + T_{TOT,\,i-1}/\mathrm{a})} \quad (2)$$

where $\mathbf{N_i}$ is the ratio requirement in the present trial, $\mathbf{T_0}$ is the postprandial pause due to reward consumption, and $\boldsymbol{k}$ is the slope of the linear function in post-reinforcement pause.

In this case, the motor skill (δ) was defined as the minimum latency to press the lever. Motivation (*a*) was defined as the total time that an animal is willing to invest to get a reward. Therefore, if the motor skill is poor, the calculated rates decrease, whereas if the motivation is strong the activation time is high.

Tissue Processing

Mice were anesthetized with sodium pentobarbital (50 mg/kg body weight) before killing. Then, transcardial perfusion was done with a 0.9% NaCl solution at 37 °C followed by 4% paraformaldehyde in 0.1 M phosphate buffer and overnight post-fixation at 4 °C in the same fixative solution. To analyze the complete organization of CA3 and dentate gyrus (DG) cells in the dorsal hippocampus, 30-µm-thick coronal sections were cut with a vibratome from + 1.34 mm to − 2.54 mm (anterior-posterior coordinates relative to Bregma) (Paxinos and Franklin 2001). For sampling purposes, we serial collected sections at 200-µm intervals per brain.

Immunohistochemistry

We used three immunohistochemical markers: (1) *N*-methyl-D-aspartate receptor 1 (NMDAR1) that has a role in the excitotoxicity process (Rodriguez et al. 2009); (2) NPY protein that is activated in the presence of brain tissue damage (Colmers and El Bahh 2003); and (3) c-Fos protein that indicates neuronal activity levels (Part 2015) (Table 1). For each immunostaining used in this study, the brain sections from the control and the experimental group were processed simultaneously to minimize the possibility of some staining variability. Briefly, the brain sections were rinsed (3 times for 10 min) in 0.1 M phosphate-buffered saline (PBS). Peroxidase inactivation was done by immersing the brain sections in 3% hydrogen peroxide (H_2O_2) for 30 min followed by three 10-min rinses with 0.1 M PBS. We incubated the sections in 0.1 M PBS + 0.1% Triton + 10% fetal bovine serum for 1 h at room temperature. Sections were then incubated with rabbit IgG anti-c-Fos protein (dilution 1:800, Cell Signaling technology, cat. 2250S, RRID: AB_2247211), rabbit IgG anti-NMDAR1 (dilution 1:500, Millipore cat. AB9864R, RRID: AB_10807557) or rabbit IgG anti-NPY (dilution 1:5000, Cell Signaling technology, cat. 11976, RRID: AB_2716286). All primary antibodies were incubated overnight at 4 °C in blocking solution + 0.1% Triton-X. The following day, tissue sections were rinsed three times with 0.1 M PBS and incubated in darkness with

Table 1 Antibodies used for the histological analysis

Antibody/immunogen	Host/dilution	Source/catalog #/research resource identifier (RRID)	Epitope[a]/peptide sequence[b]
c-Fos/Fos proto-oncogene	Rabbit (monoclonal) 1:800	Cell signaling technology/2250S/ AB_2247211	Synthetic peptide corresponding to the sequence of human c-Fos/ (*KRIRRERNKMAAAKSRNRRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAH*)
NMDAR1/N-Methyl-D-Aspartate Receptor Channel, Subunit Zeta-1	Rabbit (monoclonal) 1:500	Merck /AB9864R /AB_10807557	a.a. 909–938[Rattus sp.]/(*LQNQKDTVLPRRAIERE EGQLQLCSRHRES*)
NPY/36-Residue synthetic peptide NPY	Rabbit (monoclonal) 1:5000	Cell signaling technology/11976/ AB_2716286	Residues near the amino terminus of human NPY/(*YPSKPDNPGEDAPAED MARYYSALRHYINLITRQRY*)

[a]Molecular site recognized by the antibody: amino acid (a.a.) and animal species

[b]a.a. sequence

a biotinylated secondary antibody (goat anti-rabbit biotin; AbD Serotec, Cat. 401008, RRID: AB_609700), dilution 1:200 dissolved in blocking solution for 60 min at room temperature. The brain sections were then rinsed three times in 0.1 M PBS and incubated in darkness with the avidin–biotin complex (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA, cat. No. PK-6101, RRID: AB_2336820) for 45 min at room temperature. After three rinses in 0.1 M PBS, the sections were revealed for 1 min at room temperature using 0.03% DAB solution (Sigma-Aldrich, No. 261890) + 0.05% nickel ammonium sulfate (Sigma-Aldrich 574988). Brain sections were mounted on glass slides, air-dried and sealed with resin (DPX Mountant; Aldrich, No. 06522).

TUNEL Method

TUNEL method allows visualizing DNA fragmentation, a process that is part of the process of programmed cell death. To analyze apoptotic DNA fragmentation, we used the protocol of the Situ Cell Death Detection Kit, TMR red (Roche, Ref. 1256792910, Lot 11269100) as described previously (Gonzalez-Perez et al. 2018). Briefly, brain sections were permeabilized in distilled water + 0.1% Triton + Sodium citrate for 15 min at 2–8 °C, followed by two rinses (5 min each) in 0.1 M PBS at room temperature and incubated for 10 min at 20 °C in buffer stock solution (Tris-HCl pH 7.5 + 10% fetal bovine serum). Samples were then washed twice with 0.1 M PBS for 5 min. To label the single and double-stranded DNA breaks, we incubated the samples for 60 min at 37 °C in darkness in the TUNEL reaction buffer that contains the enzyme solution and a label solution. Samples were rinsed threefold for 5 min, mounted, air-dried and covered with fluoroshield mounting medium with diamidino-2-phenylindole (DAPI; Aldrich, Cat. F6057).

Quantification

The number of c-Fos+ and TUNEL+ cells in the dorsal hippocampus was quantified in seven 30-µm sections that were randomly selected and collected at 200-µm intervals from + 1.34 mm and − 2.54 mm (coordinates to relative to Bregma). For the c-Fos analysis, we used a 400x magnification (field area = 0.15 mm^2), and the CA3 and DG regions of the hippocampus were analyzed. We only quantified the labeled cells that were in the same focal plane. In all cases, the focal plane was set at the middle of the section using the computerized Z-position of the microscope. All pictures and histological analyses were done with a Zeiss Axio-Observer D1 microscope (Göttingen, Germany) and Axio-Vision 4.8.1 acquisition Software (Göttingen, Germany). To analyze the number of TUNEL+ expressing cells, a series of photographs were taken under the × 40 objective (field area = 0.15 mm^2) with a Zeiss LSM 700 confocal microscope (Göttingen, Germany) and the ZEN 2012 acquisition software.

Densitometry (Relative Optical Density)

To determine changes in the expression of NMDAR1 and neuropeptide Y, we did a densitometry analysis and calculated the relative optical density as described previously (Gonzalez-Perez et al. 2018). Briefly, at least 5–7 hippocampal sections from each mouse ($n = 4$–6 mice per group) were randomly selected and immunostained as described above. To analyze the NMDAR1 expression, 50 images per group were taken using the × 20 objective (field area = 0.63 mm^2) in the hippocampal CA3 region. To evaluate the NPY expression in the CA3 region, 54 images per group were taken with the × 40 objective (field area = 0.15 mm^2), whereas, in the DG region, 150 images per group were obtained. Pictures were taken with an inverted microscope (Zeiss Axio-Observer D1, Göttingen, Germany) and analyzed with the software ImageJ 1.51J8 (National Institute of Health). For imaging processing, we took a series of pictures that were converted to 8-bit images to define better the tone of pixels in each image. Then, we carefully delineated the region of interest to avoid undesired brain regions in the analysis. We measured the area integrated density and the mean gray value for each brain section. In all cases, the appropriate negative controls (sections without the proper primary antibody) were included and analyzed with the same procedure. To determine the marker expression normalized, we used the following formula: The corrected marker expression = integrated density − (selected area × mean intensity of background staining) (Gonzalez-Perez et al. 2018). A blinded researcher to the group assignment did all the morphological analyses.

Statistical Analysis

Data are expressed as the median and interquartile range (IQR Q3–Q1). The cell quantification was plotted using boxplot graphs to obtain a visual representation and the exact distribution of data. The Mann–Whitney "U" test was used to determine statistically significant differences between the groups. In all cases, the level of confidence of statistical significance was set at 95% ($P \leq 0.05$). SPSS 21 software was utilized to analyze histological data. The R version 3.5.1 statistical package was used for the behavioral analysis.

Results

Organic solvents produce several behavioral changes that may include inhibitory (sedation, ataxia, anesthesia) or excitatory behaviors (hyperactivity, trismus, convulsions)

(Neiman et al. 2000; Bowen et al. 2006; Lubman et al. 2008; Dingwall et al. 2011). Throughout the intoxication period (60 sessions in 30 days), we observed that all mice displayed motor alterations, such as hyperactivity (running, jumping, circling), ataxia (uncoordinated movements) and immobility. We found that these motor alterations used to precede the seizure-like behavior that began with abnormal whisker movements, followed by head nodding, dorsal kyphosis posture, forelimb clonus and culminated with bilateral forelimb clonus (Minjarez et al. 2017) (Fig. 1a). Therefore, we quantified the frequency of forelimb clonus with rearing and the latency period of seizures and graphed them on a colorimetry raster plot (Fig. 1b). We observed that the CHX exposure consistently induced a seizure-like behavior in all animals, but the latency of these events varied among them. These findings indicate that CHX promotes excitatory behaviors that suggest strong neuronal excitability.

CHX Exposure Modifies the Number of c-Fos-Expressing Cells in CA3 and DG

The immediate early gene *c-fos* is considered a molecular marker of neural activity (Chung 2015; Perrin-Terrin et al. 2016). To determinate whether a long-lasting CHX exposure produced a persistent alteration in the neuronal activity in the adult hippocampus, we quantified the number of c-Fos-expressing cells in the CA3 and DG regions. Twenty-four hours after the last CHX exposure, we found a significant decrease in the number of c-Fos+ cells in the CA3 area between the CHX group (5.35 cells, IQR 6.83–4.38; $n=4$ mice) with respect to the control group (16.42 cells, IQR 22.76–11.96, $n=5$ mice, $P=0.014$, $U=0$, Fig. 2a–c). In the DG area (Fig. 2d–f), the CHX group showed a significant decrease in the number of c-Fos+ cells (4.7 cells, IQR 5.63–4.11; $n=4$ mice) when compared to the control group (9.52 cells, IQR 10.80–9.44; $n=5$ mice; $P=0.014$, $U=0$). Thirteen days after the last CHX exposure, the CHX inhalation group did not show a decrease in the number of c-Fos+ cells in the CA3 area (8.21 cells, IQR 8.31–3.94; $n=5$ mice) with respect to the control group (8.85 cells, IQR 11.81–6.67; $n=4$ mice; $P=0.413$, $U=6$; Fig. 2g–i). Interestingly, the CHX group showed a significant decrease in the number of c-Fos+ cells in the dentate gyrus (4.50 cells, IQR 4.87–1.78; $n=5$ mice) as compared to the control group (8.73 cells, IQR 10.88–5.92; $n=5$ mice; $P=0.050$, $U=2$, Fig. 2j–l). These data suggest that a CHX exposure

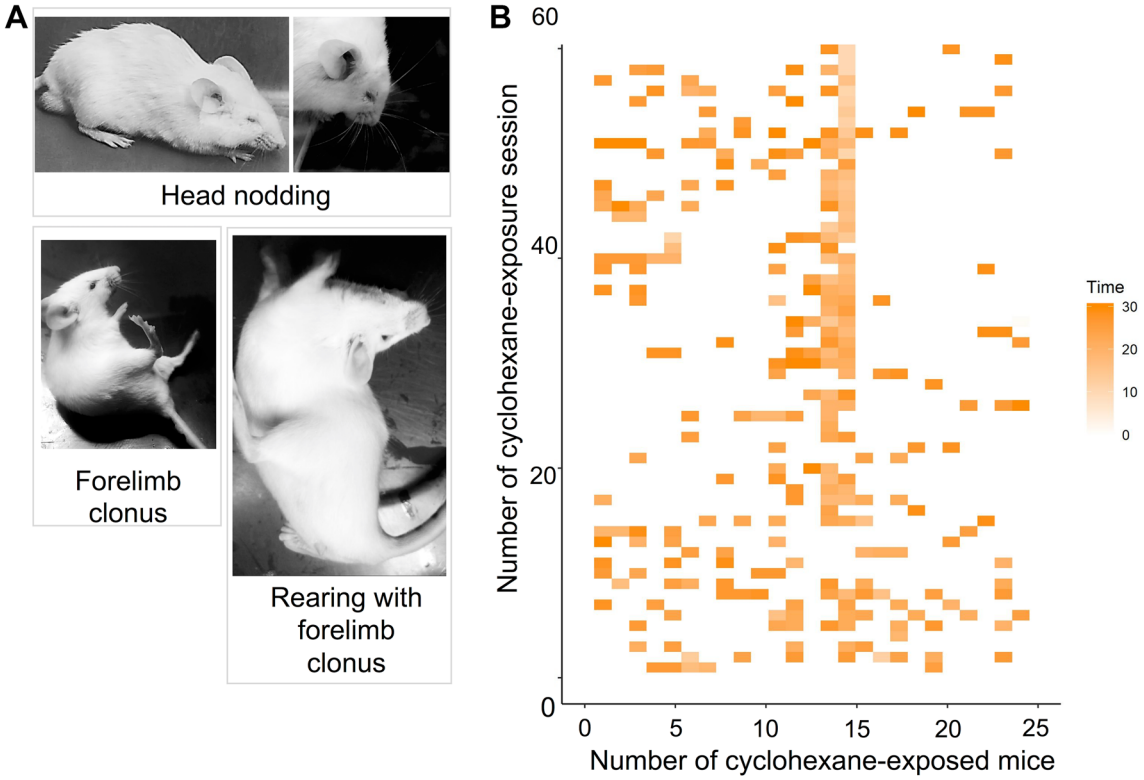


Fig. 1 **a** Representative body postures observed during seizures. **b** Analysis of seizure-like behavior in the group exposed to CHX. The *X*-axis shows each animal exposed to CHX and the *Y*-axis shows the incidence of seizures in each one of the 60 sessions. One bar represents a single seizure per subject, and the color of the bar indicates the latency in which the seizure occurred. We did not observed seizures before the 10th min

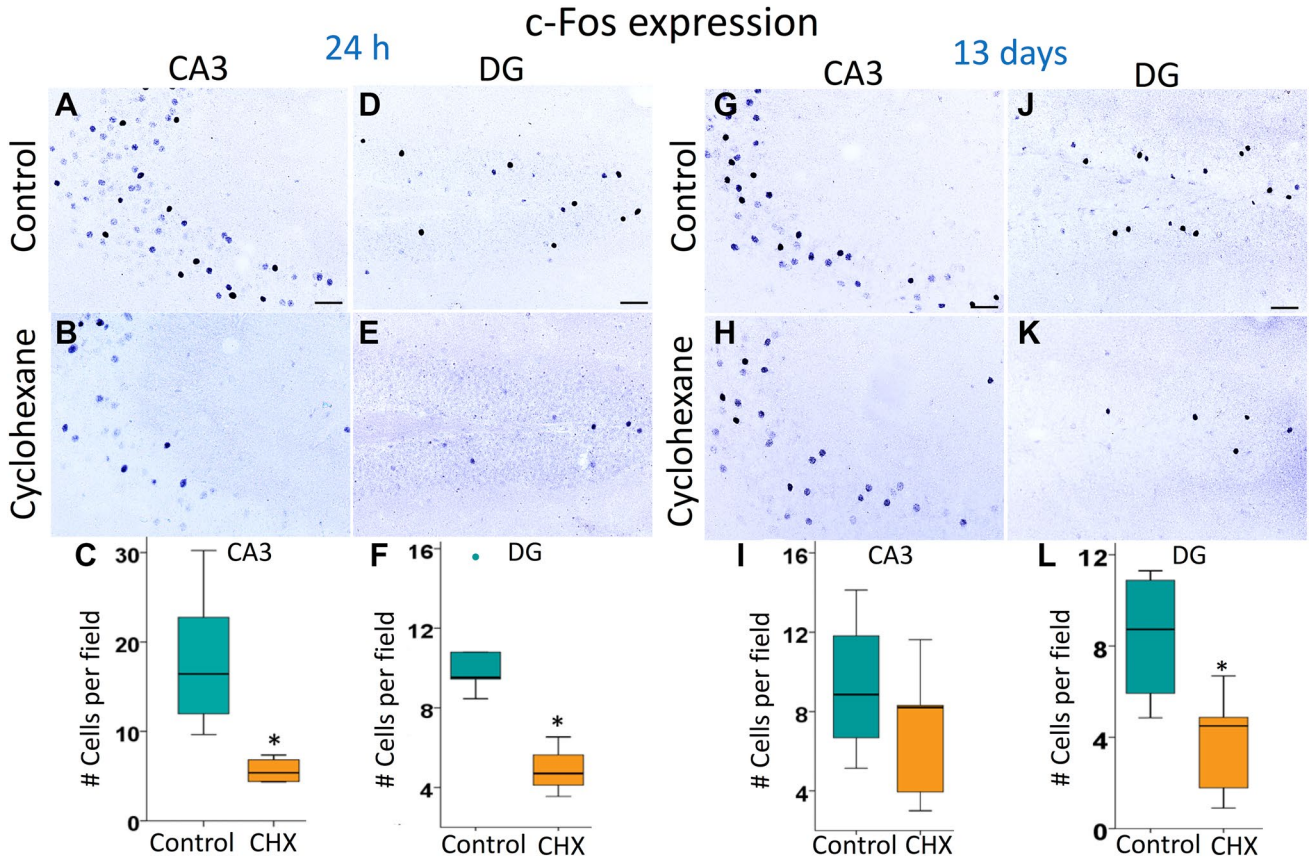


Fig. 2 CHX inhalation reduces the number of c-Fos-expressing cells in CA3 and DG. 24 h after the last CHX exposure, the number of c-Fos+ cells decreased significantly in the CA3 and DG regions of the CHX group (**a**–**f**). Thirteen days after the last CHX exposure, the number of c-Fos+ cells decreased in the DG granule neurons of CHX-exposed animals as compared to controls **(j–l)**. In the CA3 region, we did not find statistically significant differences **(g–i)**. All data are expressed as the median and interquartile range (IQR Q3−Q1); $n=5$ mice per group. *CA3 Cornu Ammonis, DG* Dentate gyrus, *CHX* CHX. Circles represent outliers. Asterisks $P \leq 0.05$; Mann–Whitney "U" test. Objective = ×40 (field area = 0.15 mm^2). Bars = 20 µm

induces a short-term reduction in the c-Fos expression in both hippocampal sub-regions, but this alteration is only persistent in the dentate gyrus as observed at day 13th after CHX removal.

CHX Exposure Increases the Number of TUNEL+ Cells in CA3 and DG

To establish whether CA3 and DG neurons have different susceptibility to CHX exposure and whether these cells entered apoptosis, we analyzed the number of TUNEL-positive cells in the hippocampal CA3 and DG areas. After 24 h, the last CHX exposure, the number of TUNEL-positive cells in the CA3 region of the CHX group (0.66 cells, IQR 0.76–0.58; $n=5$ mice) was significantly higher than the control group (0.16 cells, IQR 0.26–0.14; $P=0.036$, $U=2.5$; Fig. 3a, b). However, at day 13th from the last CHX exposure, the number of TUNEL-positive cells in the CHX group (0.26 cells, IQR 0.29–0.21; $n=4$ mice) was not statistically different with respect to the control group (0.20 cells, IQR 0.32–0.11, $n=4$ mice; $P=0.56$, $U=6$, Fig. 3c, d). Conversely, 24 h after CHX removal, we did not find statically significant differences in the number of TUNEL+ cells in the DG of the CHX group (0.44 cells, IQR 0.47–0.29; $n=6$ mice) vs. the control group (0.40 cells, IQR 0.44–0.22, $n=5$ mice; $P=0.714$, $U=13$, Fig. 3a, b). However, at day 13th without CHX, the drug-exposed group showed a significant increase in the number of TUNEL+ cells (0.32 cells, IQR 0.45–0.24; $n=4$ mice) with respect to the control group (0.065 cells, IQR 0.08–0.02; $n=4$ mice, $P=0.021$, $U=0$; Fig. 3c, d). Altogether, these findings suggest that hippocampal subfields have a differential apoptosis susceptibility upon the CHX exposure.

CHX Exposure Increases the NPY Expression in CA3 Stratum Lucidum and Hilus

Neuropeptide Y has been associated with a neuroprotective role against glutamate, intracellular Ca^{2+} levels influx, and

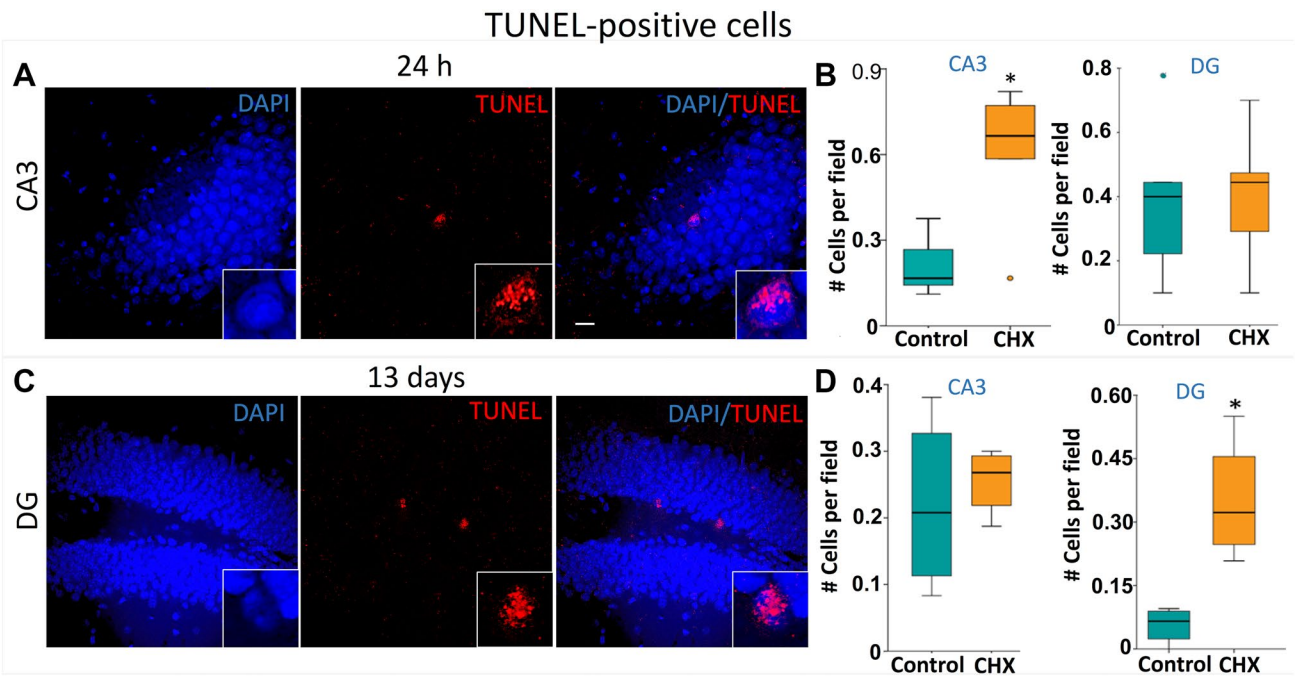


Fig. 3 Analysis of TUNEL-positive cells in CA3 pyramidal neurons and DG granule cells. **a** Confocal image of a representative TUNEL+ cell in the CA3 at 24 h. **b** After 24 h without the drug, the CHX group showed a significant increase in the number of TUNEL-positive cells in CA3 neurons as compared to controls. **c** Confocal image of a representative TUNEL+ cell in the DG at day 13th without CHX. **d** At day 13th without the drug, the experimental group showed a significant increase in the number of TUNEL-positive cell in the DG as compared to controls. Data are expressed as the median and interquartile range (IQR Q3−Q1); $n=5$–6 mice per group. *CHX* CHX. Circles = outliers. Asterisks $P<0.05$; Mann–Whitney "U" test. Objective ×40 (field area = 0.15 mm^2). Bar = 20 µm

oxidative stress alterations by acting as a modulator of cell-death pathways (Duarte-Neves et al. 2016). To investigate possible changes in NPY expression levels in the hippocampus, we determined the expression of NPY+ in the CA3 stratum lucidum and the hippocampal hilus. Twenty-four hours after the last CHX administration, we found a strong overexpression of NPY in the CA3 stratum lucidum region (Fig. 4a–c) of the CHX group (72.96 densitometry units, IQR 87.87–24.95; $n=6$) as compared to controls (5.03 densitometry units, IQR 9.83–2.28; $P=0.004$; $U=0$; $n=6$). Strikingly, at day 13th from the last drug exposure (Fig. 4d, f), the CHX group remained showing a high expression of NPY (33.79 densitometry units, IQR 35.92–18.60) as compared to controls (0.55 densitometry units, IQR 1.23–0.14, $P=0.021$; $U=0$). We also observed a high expression of NPY in the hippocampal hilus, a region adjacent to the evident border of the dentate gyrus. Therefore, we decided to quantify the expression of NPY 24 h after the last CHX exposure. Our data indicated that the CHX group showed a significant increase in the NPY expression (66.77 densitometry units, IQR 73.58–13.71) in comparison with the control group (2.61 densitometry units, IQR 7.09–2.08; $P=0.010$; U = 2; Fig. 4a–c). As observed in the striatum lucidum, the NPY overexpression in the hippocampal hilus persisted until day 13: the CHX group (31.41 densitometry units, IQR 44.42–15.03; $n=4$ mice) vs. the control group (0.29 densitometry units, IQR 3.69–0.13, $n=4$ mice; $P=0.043$, $U=1$; Fig. 4d–f). This evidence indicates that CHX activates a long-lasting NPY overexpression in the CA3 stratum lucidum and the hippocampal hilus.

CHX Exposure Increases the NMDAR1 Expression in CA3 Region

NPY strongly regulates glutamate release and receptor expression (Vezzani et al. 1999). Therefore, we measured the expression of NMDAR1 in the hippocampal CA3 region after CHX exposure. Twenty-four hours after CHX withdrawal, we found that the CHX-exposed group showed a statistically significant increase in the expression of NMDAR1 (13.69 densitometry units, IQR 14.94–13.55) as compared to the control group (6.33 densitometry units, IQR 6.79–5.58; $P=0.009$; $U=0$; $n=5$; Fig. 5a–c). However, at day 13th from the CHX withdrawal, we did not find statically significant differences in the NMDAR1 expression between the CHX group (7.55 densitometry units, IQR 11.60–4.94; $n=5$) and the control group (6.79 densitometry units, IQR 7.88–4.62; $n=4$; $P=0.624$, $U=8$, Fig. 5d). Thus, we concluded that CHX produces a transient change in the NMDAR1 expression in the CA3 hippocampal region.

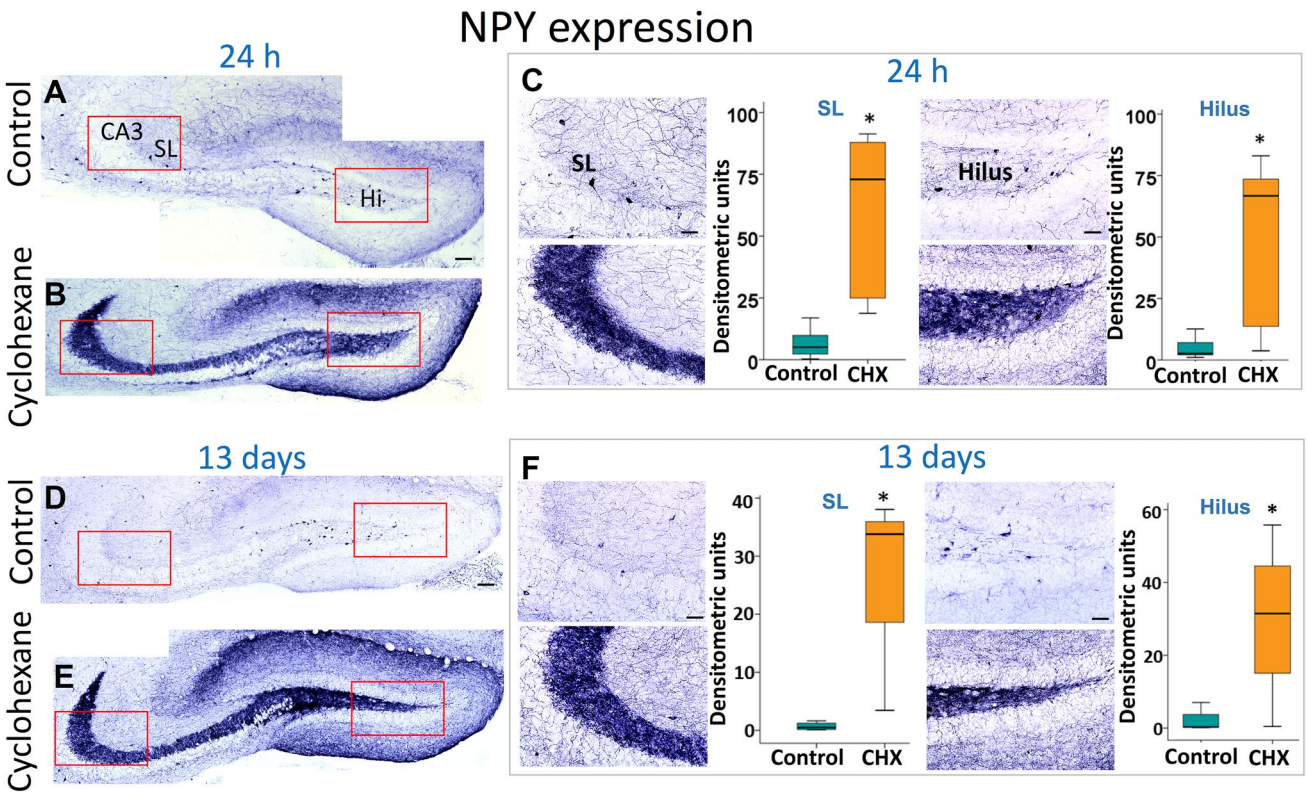


Fig. 4 NPY expression in the CA3 stratum lucidum and hilus. **a**–**c** 24 h after the last exposure to CHX, we found a significant increase in NPY expression in the CA3 stratum lucidum and hilus as compared to controls. **d**–**f** This NPY expression persisted in the same brain regions of the CHX group at day 13th. Data are expressed as the median and interquartile range (IQR Q3–Q1); $n = 5$–6 mice per group. *CA3 Cornu Ammonis* 3, *SL* stratum lucidum, *CHX* CHX. Asterisks $P < 0.05$; Mann–Whitney "U" test. Objective = ×40 (field area = 0.15 mm^2). Bars = 20 µm

Summary statistics of these data are shown in the supplementary material (Table 2 and 3).

CHX Inhalation Reduces Motor Skills and Increases Motivation to Obtain a Positive Reinforcement

The adult hippocampus is an essential region that regulates decision-making tasks (Fuchs et al. 2004; Barry and Trevor 2005; McHugh et al. 2008), spatial navigation (Gonzalez-Perez et al. 2018) and motivation-guided behaviors (Schmelzeis and Mittleman 1996; Gourley et al. 2010). Besides, hippocampal lesions produce motor incoordination and hyperactivity (Rivadeneyra-Domínguez and Rodríguez-Landa 2016). Our data indicate that CHX provokes motor alterations that precede a seizure-like behavior and produces significant cellular changes in the hippocampus. To determine whether these histological changes had a functional correlate, we assessed the learning of a reward-seeking task (Fig. 6a). In the acquisition phase (Fig. 6b), we did not find statistically significant differences between the CHX group (72 responses, IQR 121–55) and control mice (71 responses, IQR 180–46; $P = 0.93$, $U = 48.5$, $d = 0.11$). Similar findings were found in the maintenance phase (Fig. 6c): the CHX group (108 responses IQR 121–96) versus the control group (133 responses IQR 182–84; $P = 0.31$, $U = 64$, $d = 0.38$). These findings suggest that CHX did not affect the learning of a reward-seeking task.

To determine whether CHX may disrupt motor performance and motivation, we used a PR schedule. Our data analysis indicates that the animal's breakpoint did not show statistically significant differences between both groups: $F(1, 18) = 1.51$, $P = 0.23$, $\eta^2 = 0.07$, or among all individual sessions: $F(9, 162) = 1.67$, $P = 1$, $\eta^2 = 0.08$ (Fig. 6d). Therefore, we then used the MPR model (Bradshaw and Killeen 2012) to determine whether the CHX exposure may affect motivation or motor skills. Our data indicated that the control group showed a higher running rate at low PR values (Fig. 6e) and more lever presses in the global rate when compared to the CHX group (Fig. 6f). We then examined the interval time between each lever press (inter-response time) (Fig. 6g) and found that the control group had shorter inter-response time (3.89 s, IQR 4.45–3.33) than the CHX group (7.54 s, IQR 8.86–6.24; $P = 0.005$, $U = 14$, $d = 0\,0.77$). The analysis of the activation time (Fig. 6h) indicated that

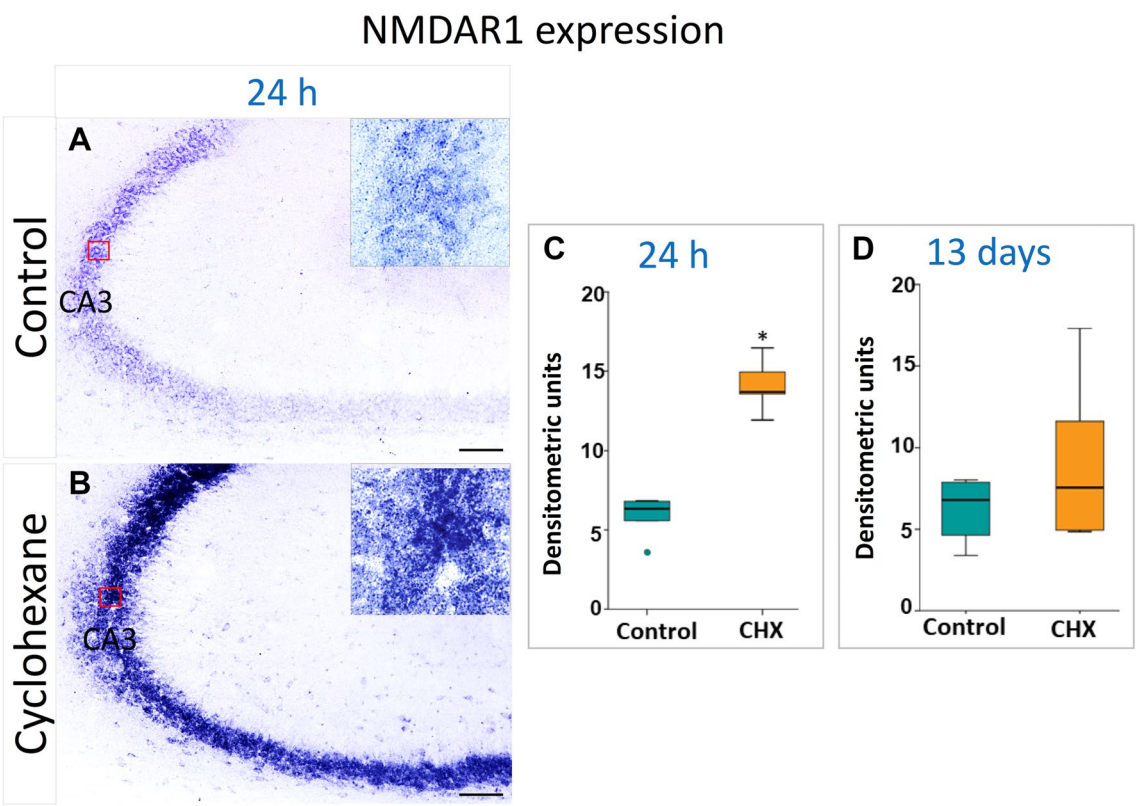


Fig. 5 NMDAR1 immunohistochemistry of the hippocampal CA3 region. NMDAR1 expression in the CA3 pyramidal neurons of controls (**a**) and the CHX group (**b**). Inset: higher magnifications of NMDAR1 receptors that surround neuronal bodies. (**c**) 24 h after CHX exposure, we observed high NMDAR1 expression in the CA3 region, which significantly decreased on day 13 (**d**). Data are expressed as the median and interquartile range (IQR Q3−Q1); $n=4$–5 mice per group. *CHX* CHX. Circles = outliers. Asterisks $P<0.05$; Mann–Whitney "U" test. Objective ×40 (field area = 0.15 mm^2). Bars = 20 µm

the CHX group had longer activation time (242.1 s, IQR 319.1–165) than the control group (99.5 s, IQR 125.7–73.3; $P=0.04$, $U=23$, $d=0.82$). These findings indicate that CHX increases a motivation-guided behavior and decreases some motor skills.

Discussion

To assess whether CHX altered the hippocampal cell homeostasis during the withdrawal period, we exposed mice to a high dose of CHX for 30 days. Our findings indicated that CHX exposure had different effects on pyramidal and granule neurons of the hippocampus. Twenty-four hours after the last CHX exposure, we found a decrease in the c-Fos expression in pyramidal and granule neurons. This event coincided with an increase in the expression of NMDAR1 and TUNEL-positive cells in the CA3 region. At day 13th after CHX exposure, we found a persistent reduction in c-Fos+ cells and more TUNEL+ cells in the DG. Interestingly, CHX exposure produced a sustained NPY overexpression in the CA3 stratum lucidum and the hilus at both time points analyzed (Fig. 7). Altogether, this evidence reveals that CHX exposure provokes long-lasting changes in neuronal homeostasis that differentially affects the hippocampal subfields. Remarkably, these changes coexist with significant motor impairment and higher reward-seeking behavior.

The acute intoxication induced by several drugs and some hydrocarbons produces hippocampal impairment that generates seizures (Ramos Silva-Filho et al. 1992; Neiman et al. 2000; Bowen et al. 2006; Van Hooste 2017). In our study, we observed that the mice exposed to CHX exhibited seizure-like behaviors that rapidly disappeared after CHX removal. In this regard, the abuse of other hydrocarbons has been associated with neuronal activity dysfunction, high glutamate levels, excitotoxicity and neuronal death (Filley et al. 2004; Lee et al. 2005; Kanter 2007; Ramcharan et al. 2014; Tormoehlen et al. 2014). These alterations were observed in both acute and chronic exposure, and recent evidence indicates that pathological changes in the GluN2A and GluN2B glutamate-receptor subunits can persist in the hippocampus for a long time (Furlong et al. 2016). In our study, we consistently observed that CHX-exposed animals presented seizure-like behaviors during the drug exposure,

which suggests that CHX may be triggering neuronal excitotoxicity in glutamatergic brain regions.

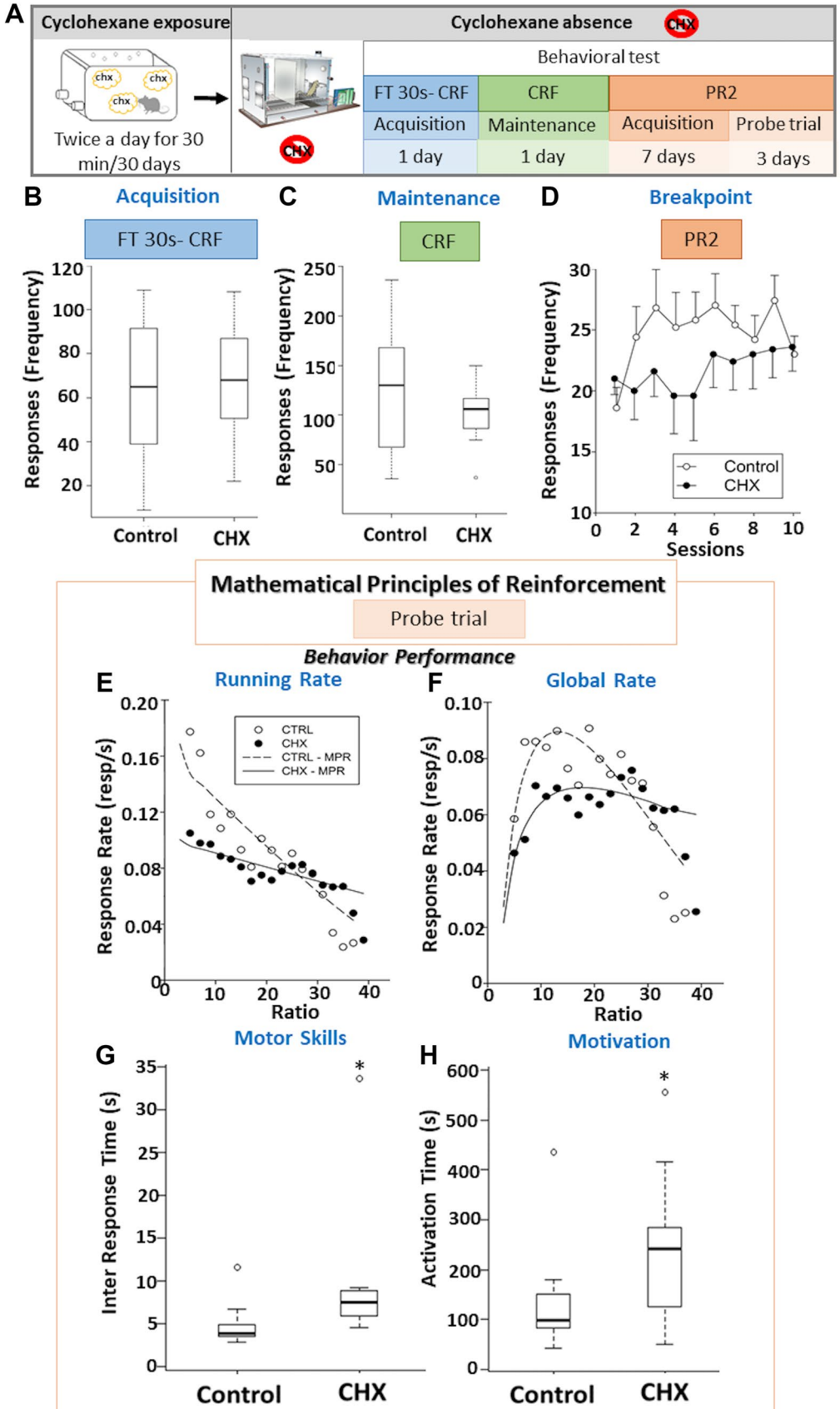
Our data indicate that the exposure to a high dose of CHX generates a transitory reduction in c-Fos protein in CA3 pyramidal neurons for 24 h. Conversely, in the granule cells of DG, a persistent decrease in c-Fos protein was observed at 24 h and 13 days after CHX, which suggests that the neuronal activity of pyramidal and granule neurons is differentially affected by CHX withdrawal. Interestingly, the control group analyzed 24 h after the sham exposure to CHX showed more c-Fos-expressing cells than the control group killed 13 days after the last sham exposure. Because c-Fos expression is noticeably modified by animal handling (Asanuma and Ogawa 1994; Kubik et al. 2007), the c-Fos differences found between the control groups could be due to the time elapsed between the sham manipulation and the animals' killing. c-Fos expression is also altered by drug-abuse conditions and convulsive agents (Wang et al. 1999; Mohammadi et al. 2009). These abnormalities coincide with neural disturbances that include excitotoxicity, oxidative stress and cell death (Ness et al. 2008; Mohammadi et al. 2009). The c-Fos protein seems to play an essential role in regulating neuronal survival and death in excitotoxic lesions (Zhang et al. 2002). In this regard, mutant mice with a conditional deletion of c-fos in CA2 and CA3 neurons show more seizures and higher cell death after administrating kainic acid (KA, an analog of glutamic acid) than controls (Zhang et al. 2002). Interestingly, under hypoxic conditions, the hippocampal c-Fos expression is only found in ischemia-resistant neurons, but it is not found in neurons that cleaved caspase-3 (Ness et al. 2008). In consequence, a downregulation of c-Fos expression increases the apoptosis rate in the CA3 region after KA-induced excitotoxicity (Mohammadi et al. 2009). Therefore, we hypothesize that low expression of c-Fos in the hippocampus of CHX-exposed animals may be indicating an increase in the neuronal vulnerability to excitotoxicity.

Toluene and 1,1,1,-trichloroethane (TCE) are organic solvents that alter glutamatergic transmission (Bowen et al. 2006; Furlong et al. 2016). Repeated exposure to toluene increases the density of NR1 receptors in the hippocampus (Bowen et al. 2006). Remarkably, alterations in GluN2A and GluN2B receptor subunits remain in the hippocampus for at least 10 weeks after the last toluene exposure (Furlong et al. 2016). In our study, we found that the increase in NMDAR1 expression after CHX withdrawal only lasts for 24 h. Thus, the CHX exposure appears to have a transitory effect on NMDAR1, which may indicate transient hippocampal excitotoxicity (Rodriguez et al. 2009). However, further research is needed to establish whether CHX can affect the metabotropic receptors of glutamate in the long term.

Neuropeptide Y is a 36-amino-acid peptide that is contained by GABA neurons (Colmers and El Bahh 2003) and has a neuroprotective role against hippocampal damage by regulating Bcl-2/Bax proteins (Gonçalves et al. 2012b). In our study, the animals exposed to CHX showed a persistent overexpression of NPY in the CA3 stratum lucidum and the hilus. This kind of expression pattern has been identified as "aberrant" in models of Alzheimer's disease (Palop et al. 2007; Krezymon et al. 2013), epilepsy and seizures (Goodman and Sloviter 1993; Chafetz et al. 1995; Vezzani et al. 1999; Jing et al. 2009). Interestingly, NPY is an efficient neuromodulator that can modify the synaptic plasticity and glutamatergic neurotransmission in the adult hippocampus (Acuna-Goycolea et al. 2005; El Bahh et al. 2005; Benarroch 2009; Kovac and Walker 2013). Therefore, the NPY overexpression found after CHX withdrawal might be implicated in the inhibition of neural excitability and neuroprotection as that observed with other drugs of abuse, such as, nicotine (Slawecki et al. 2005), methamphetamine (Gonçalves et al. 2012a), cocaine-induced seizures (Goodman and Sloviter 1993) or ethanol abstinence (Bison and Crews 2003).

Decision-making tasks (Fuchs et al. 2004; Barry and Trevor 2005; McHugh et al. 2008), spatial navigation (Gonzalez-Perez et al. 2018) and motivation-guided behaviors (Schmelzeis and Mittleman 1996; Gourley et al. 2010) require the integration of multisensorial information in the adult hippocampus. Our data indicate that CHX reduces motor skills and promotes reward-seeking behavior. This evidence suggests that motor impairment induced by CHX is partially counteracted with higher motivation, which may explain the absence of statistical differences in the breakpoint analysis. However, this motor deterioration may represent the first step to develop long-lasting motor impairments as those observed with other hydrocarbons that increase the risk of the development of Parkinson's disease (Reis et al. 2016). These behavioral changes can be explained by a disruption in the balance between the glutamatergic and GABAergic neurotransmission in the hippocampal circuitry. The administration of NMDA or muscimol in the hippocampus provokes motor alterations, which suggest that glutamate and GABA neurotransmission regulate the sensorimotor processing in the adult hippocampus (Bast and Feldon 2003) as demonstrated in other animal models (Gonzalez-Perez et al. 2018). However, the effects of CHX on motricity and reward-motivated behaviors should be analyzed in-depth to determine whether this solvent can produce irreversible motor impairment and trigger addiction-like behavior.

Our data indicate that CHX targets the CA3 and DG regions and, upon CHX removal, the molecular response of these neurons is entirely different. This evidence might indicate a differential susceptibility of some hippocampal neurons and regions to this drug. The hippocampal formation contains local circuits that include two major neuronal subtypes: pyramidal neurons that release glutamate and local interneurons that release GABA neurotransmitter


A
Cyclohexane exposure
Cyclohexane absence
CHX
chx
Twice a day for 30 min/30 days
Behavioral test
FT 30s- CRF
CRF
PR2
Acquisition
Maintenance
Acquisition
Probe trial
1 day
1 day
7 days
3 days
B
Acquisition
FT 30s- CRF
Responses (Frequency)
Control
CHX
C
Maintenance
CRF
Responses (Frequency)
Control
CHX
D
Breakpoint
PR2
Responses (Frequency)
Control
CHX
Sessions
Mathematical Principles of Reinforcement
Probe trial
Behavior Performance
E
Running Rate
CTRL
CHX
CTRL - MPR
CHX - MPR
Response Rate (resp/s)
Ratio
F
Global Rate
Response Rate (resp/s)
Ratio
G
Motor Skills
Inter Response Time (s)
Control
CHX
H
Motivation
Activation Time (s)
Control
CHX

◂**Fig. 6** **a** Experimental design of the operant-based task used for assessing motor performance and motivation-guided behavior. The plots show the frequency of lever presses recorded during the acquisition (**b**) and maintenance session (**c**), and the maximum number of lever presses that mice were willing to do for the reward (animals' breakpoint) (**d**). These three analyses did not show statistically significant differences between both groups. **e, f** Analysis of behavioral performance. The control group displayed a high activity level that decays rapidly as compared with CHX-exposed mice (**e**). However, control animals were more proficient during the first 3/4 of the assay (**f**). These results indicate that the CHX-exposed animals were slower but more persistent than controls in obtaining the reward. **g** In the analysis of the minimum inter-response time, the control group showed shorter intervals than the CHX group, suggesting that CHX induces motor impairment. Interestingly, the analysis of the activation time (**h**) indicates that control mice showed less activity than the drug-exposed group, suggesting that CHX increases motivation-guided behavior. *PR* progressive ratio, *CRF* continuous schedules of reinforcement, *FT* fixed time

(Booker and Vida 2018). Glutamatergic and GABAergic neurons are differentially affected by other drugs (Bale et al. 2005; Tiwari et al. 2014). Therefore, our findings also suggest that CHX can produce deleterious effects in diverse hippocampal regions, but further research is needed to confirm this assumption.

Some evidence obtained with cocaine or stimulants indicates the existence of sex-related toxicity that appears to produce an imbalance in the glutamate/GABAergic neurotransmission between sexes (Maguire and Mody 2009; Buccelli et al. 2016; Del Río et al. 2018). However, these changes have not been reported in models of solvent inhalation and our experimental design cannot confirm or discard these intersex differences. Thus, future studies that analyze a possible allosteric regulation mediated by sex hormones is still required. In summary, the present results suggest that CHX produces a long-lasting disruption in the hippocampal integrity and triggers neuroprotective mechanisms into the brain (Fig. 7). Understanding these alterations is a relevant step in the design of novel strategies to counteract the brain damage that occurs after organic solvent intoxication.

Author Contributions TCO conception and design of experiments, data collection, analysis, interpretation and manuscript writing. DZL data collection, analysis, and interpretation. NIC data collection, analysis and interpretation. JB design of behavioral experiments, data collection and analysis, and financial support. OGP design and conception of the study, data interpretation, manuscript writing, financial support and final approval of the manuscript.

Funding This work was supported by grants from the Consejo Nacional de Ciencia y Tecnologia (CONACyT No. PN 2015-01-465 and INFR-280414) and the Programa para el Desarrollo Profesional Docente PRODEP (213544) for OGP, and the CONACyT Fellowship Grants for TCO and DZL (No. 736339 and No. 738774). JB was supported by a Grant from CONACyT INFR-281265.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

Ethical Approval All applicable international, national, and institutional guidelines for the care and use of animals were followed.

References

Acuna-Goycolea C, Tamamaki N, Yanagawa Y, Obata K, van den Pol AN (2005) Mechanisms of neuropeptide Y, peptide YY, and pancreatic polypeptide inhibition of identified green fluorescent protein-expressing GABA neurons in the hypothalamic neuroendocrine arcuate nucleus. J Neurosci 25(32):7406–7419. https://doi.org/10.1523/jneurosci.1008-05.2005

Asanuma M, Ogawa N (1994) Pitfalls in assessment of c-fos mRNA expression in the brain: effects of animal handling. Rev Neurosci 5(2):171–178. https://doi.org/10.1515/REVNEURO.1994.5.2.171

Association AP (2013) Diagnostic and statistical manual of mental disorder: DSM-5. APA, Washintong, DC

Bale AS, Tu Y, Carpenter-Hyland EP, Chandler LJ, Woodward JJ (2005) Alterations in glutamatergic and gabaergic ion channel

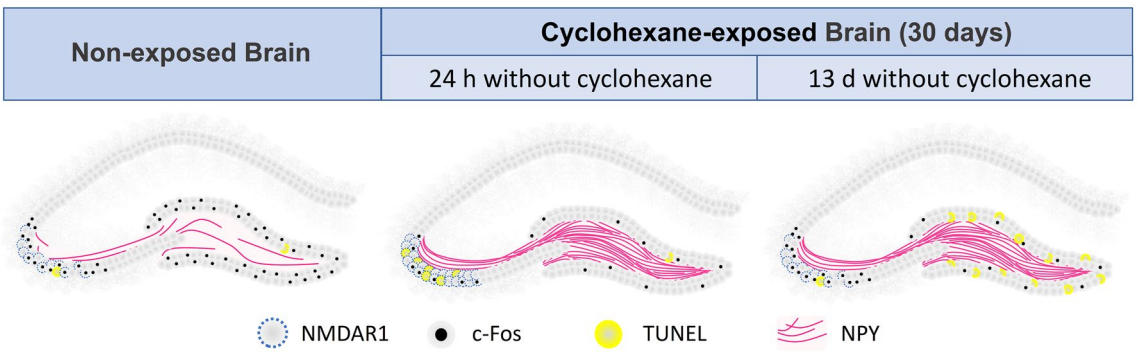


Fig. 7 Hippocampal drawings that summarize the main findings observed after CHX withdrawal

activity in hippocampal neurons following exposure to the abused inhalant toluene. Neuroscience 130(1):197–206. https://doi.org/10.1016/j.neuroscience.2004.08.040

Barry JE, Trevor WR (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481. https://doi.org/10.1038/nn1579

Bast T, Feldon J (2003) Hippocampal modulation of sensorimotor processes. Prog Neurobiol 70(4):319–345. https://doi.org/10.1016/S0301-0082(03)00112-6

Benarroch EE (2009) Neuropeptide Y: its multiple effects in the CNS and potential clinical significance. Neurology 72(11):1016

Bison S, Crews F (2003) Alcohol withdrawal increases neuropeptide Y immunoreactivity in rat brain. Alcoholism 27(7):1173–1183. https://doi.org/10.1097/01.ALC.0000075827.74538.FE

Booker SA, Vida I (2018) Morphological diversity and connectivity of hippocampal interneurons. Cell Tissue Res 373(3):619–641. https://doi.org/10.1007/s00441-018-2882-2

Bowen SE, Batis JC, Paez-Martinez N, Cruz SL (2006) The last decade of solvent research in animal models of abuse: mechanistic and behavioral studies. Neurotoxicol Teratol 28(6):636–647

Bradshaw CM, Killeen PR (2012) A theory of behaviour on progressive ratio schedules, with applications in behavioural pharmacology. Psychopharmacology 222(4):549–564. https://doi.org/10.1007/s00213-012-2771-4

Buccelli C, Della Casa E, Paternoster M, Niola M, Pieri M (2016) Gender differences in drug abuse in the forensic toxicological approach. Forensic Sci Int 265:89–95. https://doi.org/10.1016/j.forsciint.2016.01.014

Campos-Ordonez T, Gonzalez-Perez O (2016) CHX, a potential drug of abuse with pernicious effects for the brain. Front Pharmacol. https://doi.org/10.3389/fphar.2015.00291

Campos-Ordonez T, Zarate-Lopez D, Galvez-Contreras AY, Moy-Lopez N, Guzman-Muniz J, Gonzalez-Perez O (2015) CHX produces behavioral deficits associated with astrogliosis and microglial reactivity in the adult hippocampus mouse brain. Cell Mol Neurobiol 35(4):503–512. https://doi.org/10.1007/s10571-014-0146-6

Chafetz RS, Nahm WK, Noebels JL (1995) Aberrant expression of neuropeptide Y in hippocampal mossy fibers in the absence of local cell injury following the onset of spike-wave synchronization. Mol Brain Res 31(1):111–121. https://doi.org/10.1016/0169-328X(95)00041-P

Cherubini E, Miles R (2015) The CA3 region of the hippocampus: how is it? What is it for? How does it do it? Front Cell Neurosci 9:19. https://doi.org/10.3389/fncel.2015.00019

Chung L (2015) A brief introduction to the transduction of neural activity into fos signal. Dev Reprod 19(2):61–67. https://doi.org/10.12717/dr.2015.19.2.061

Colmers WF, Bahh E, B (2003) Neuropeptide Y and epilepsy. Epilepsy Curr 3(2):53–58. https://doi.org/10.1046/j.1535-7597.2003.03208.x

Del Río JP, Alliende MI, Molina N, Serrano FG, Molina S, Vigil P (2018) Steroid hormones and their action in women's brains: the importance of hormonal balance. Front Pub Health 6:141–141. https://doi.org/10.3389/fpubh.2018.00141

Dingwall KM, Maruff P, Fredrickson A, Cairney S (2011) Cognitive recovery during and after treatment for volatile solvent abuse. Drug Alcohol Depend 118(2–3):180–185

Duarte-Neves J, Pereira de Almeida L, Cavadas C (2016) Neuropeptide Y (NPY) as a therapeutic target for neurodegenerative diseases. Neurobiol Dis 95:210–224. https://doi.org/10.1016/j.nbd.2016.07.022

El Bahh B, Balosso S, Hamilton T, Herzog H, Beck-Sickinger AG, Sperk G et al (2005) The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y2 and not Y5 receptors. Eur J Neurosci 22(6):1417–1430. https://doi.org/10.1111/j.1460-9568.2005.04338.x

Fifel K, Bennis M, Ba-M'hamed S (2014) Effects of acute and chronic inhalation of paint thinner in mice: behavioral and immunohistochemical study. Metab Brain Dis 29(2):471–482

Filley CM, Halliday W, Kleinschmidt-Demasters BK (2004) The effects of toluene on the central nervous system. J Neuropathol Exp Neurol 63(1):1–12. https://doi.org/10.1093/jnen/63.1.1

Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH et al (2004) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 30:296. https://doi.org/10.1038/sj.npp.1300579

Furlong TM, Duncan JR, Corbit LH, Rae CD, Rowlands BD, Maher AD et al (2016) Toluene inhalation in adolescent rats reduces flexible behaviour in adulthood and alters glutamatergic and GABAergic signalling. J Neurochem 139(5):806–822. https://doi.org/10.1111/jnc.13858

Fuster-Matanzo A, Llorens-Martín M, de Barreda EG, Ávila J, Hernández F (2011) Different susceptibility to neurodegeneration of dorsal and ventral hippocampal dentate gyrus: a study with transgenic mice overexpressing GSK3β. PLoS ONE 6(11):e27262. https://doi.org/10.1371/journal.pone.0027262

Gonçalves J, Baptista S, Olesen MV, Fontes-Ribeiro C, Malva JO, Woldbye DP et al (2012a) Methamphetamine-induced changes in the mice hippocampal neuropeptide Y system: implications for memory impairment. J Neurochem 123(6):1041–1053. https://doi.org/10.1111/jnc.12052. doi

Gonçalves J, Ribeiro CF, Malva JO, Silva AP (2012b) Protective role of neuropeptideY Y2 receptors in cell death and microglial response following methamphetamine injury. Eur J Neurosci 36(9):3173–3183. https://doi.org/10.1111/j.1460-9568.2012.08232.x

Gonzalez-Perez O, López-Virgen V, Ibarra-Castaneda N (2018) Permanent whisker removal reduces the density of c-Fos+ cells and the expression of calbindin protein, disrupts hippocampal neurogenesis and affects spatial-memory-related tasks. Front Cell Neurosci. https://doi.org/10.3389/fncel.2018.00132

Goodman JH, Sloviter RS (1993) Cocaine neurotoxicity and altered neuropeptide Y immunoreactivity in the rat hippocampus; a silver degeneration and immunocytochemical study. Brain Res 616(1):263–272. https://doi.org/10.1016/0006-8993(93)90217-B

Gourley SL, Lee AS, Howell JL, Pittenger C, Taylor JR (2010) Dissociable regulation of instrumental action within mouse prefrontal cortex. Eur J Neurosci 32(10):1726–1734. https://doi.org/10.1111/j.1460-9568.2010.07438.x

Jedlicka P, Muellerleile J, Schwarzacher SW (2018). Synaptic plasticity and excitation-inhibition balance in the dentate gyrus: insights from in vivo recordings in Neuroligin-1, Neuroligin-2, and collybistin knockouts. Neural Plast. https://doi.org/10.1155/2018/6015753

Jing M, Shingo T, Yasuhara T, Kondo A, Morimoto T, Wang F et al (2009) The combined therapy of intrahippocampal transplantation of adult neural stem cells and intraventricular erythropoietin-infusion ameliorates spontaneous recurrent seizures by suppression of abnormal mossy fiber sprouting. Brain Res 1295:203–217. https://doi.org/10.1016/j.brainres.2009.07.079

Kanter M (2007) Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. Neurochem Res 33(3):579. https://doi.org/10.1007/s11064-007-9481-z

Kovac S, Walker MC (2013) Neuropeptides in epilepsy. Neuropeptides 47(6):467–475. https://doi.org/10.1016/j.npep.2013.10.015

Krezymon A, Richetin K, Halley H, Roybon L, Lassalle J-M, Francès B et al (2013) Modifications of hippocampal circuits and early disruption of adult neurogenesis in the Tg2576 mouse model

of Alzheimer's disease. PLoS ONE 8(9):e76497. https://doi.org/10.1371/journal.pone.0076497
Kubik S, Miyashita T, Guzowski JF (2007) Using immediate-early genes to map hippocampal subregional functions. Learning Memory 14(11):758–770. https://doi.org/10.1101/lm.698107
Lee Y-F, Lo P-S, Wang Y-J, Hu A, Chen H-H (2005) Neonatal toluene exposure alters *N*-methyl-D-aspartate receptor subunit expression in the hippocampus and cerebellum in juvenile rats. Neuropharmacology 48(2):195–203. https://doi.org/10.1016/j.neuropharm.2004.09.008
Lubman DI, Yucel M, Lawrence AJ (2008) Inhalant abuse among adolescents: neurobiological considerations. Br J Pharmacol 154(2):316–326
Maguire J, Mody I (2009) Steroid hormone fluctuations and GABA(A) R plasticity. Psychoneuroendocrinology 34(Suppl 1):S84–S90. https://doi.org/10.1016/j.psyneuen.2009.06.019.
McHugh SB, Campbell TG, Taylor AM, Rawlins JNP, Bannerman DM (2008) A role for dorsal and ventral hippocampus in intertemporal choice cost-benefit decision making. Behav Neurosci 122(1):1–8. https://doi.org/10.1037/0735-7044.122.1.1
Medvedeva YV, Ji SG, Yin HZ, Weiss JH (2017) Differential vulnerability of CA1 versus CA3 pyramidal neurons after ischemia: possible relationship to sources of Zn^{2+} accumulation and its entry into and prolonged effects on mitochondria. J Neurosci 37(3):726–737. https://doi.org/10.1523/jneurosci.3270-16.2016
Minjarez B, Camarena HO, Haramati J, Rodriguez-Yanez Y, Mena-Munguia S, Buritica J et al (2017) Behavioral changes in models of chemoconvulsant-induced epilepsy: a review. Neurosci Biobehav Rev 83:373–380. https://doi.org/10.1016/j.neubiorev.2017.10.016
Mohammadi S, Pavlik A, Krajci D, Al-Sarraf H (2009) NMDA preconditioning and neuroprotection in vivo: Delayed onset of kainic acid-induced neurodegeneration and c-Fos attenuation in CA3a neurons. Brain Res 1256:162–172. https://doi.org/10.1016/j.brainres.2008.12.019
Neiman J, Haapaniemi HM, Hillbom M (2000) Neurological complications of drug abuse: pathophysiological mechanisms. Eur J Neurol 7(6):595–606. https://doi.org/10.1046/j.1468-1331.2000.00045.x
Ness JM, Harvey CR, Washington JD, Roth KA, Carroll SL, Zhang J (2008) Differential activation of c-Fos and caspase-3 in hippocampal neuron subpopulations following neonatal hypoxia-ischemia. J Neurosci Res 86(5):1115–1124. https://doi.org/10.1002/jnr.21573
Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N et al (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55(5):697–711. https://doi.org/10.1016/j.neuron.2007.07.025
Part A (2015) Using c-fos to study neuronal ensembles in corticostriatal circuitry of addiction. Brain Res 1628:157–173. https://doi.org/10.1016/j.brainres.2014.11.005
Paxinos G, Franklin K (2001) The mouse brain in stereotaxic coordinates. Academic Press, San Diego
Perrin-Terrin A-S, Jeton F, Pichon A, Frugière A, Richalet J-P, Bodineau L et al (2016) The c-FOS protein immunohistological detection: a useful tool as a marker of central pathways involved in specific physiological responses in vivo and ex vivo. J Vis Exp. https://doi.org/10.3791/53613
Ramcharan K, Ramesar A, Ramdath M, Teelucksingh J, Gosein M (2014) Encephalopathy and neuropathy due to glue, paint thinner, and gasoline sniffing in trinidad and tobago-MRI findings. Case Rep Neurol Med. https://doi.org/10.1155/2014/850109
Ramos Silva-Filho A, Nogueira Pires ML, Shiotsuki N (1992) Anticonvulsant and convulsant effects of organic solvents. Pharmacol Biochem Behav 41(1):79–82. https://doi.org/10.1016/0091-3057(92)90063-L
Reis J, Benbrick E, Bonneterre V, Spencer PS (2016) Parkinson's disease and solvents: Is there a causal link? Rev Neurol (Paris) 172(12):761–765. https://doi.org/10.1016/j.neurol.2016.09.012
Rivadeneyra-Domínguez E, Rodríguez-Landa JF (2016) Motor impairments induced by microinjection of linamarin in the dorsal hippocampus of Wistar rats. Neurología (English Edition) 31(8):516–522. https://doi.org/10.1016/j.nrleng.2014.10.005
Rodriguez MJ, Pugliese M, Mahy N (2009) Drug abuse, brain calcification and glutamate-induced neurodegeneration. Curr Drug Abuse Rev 2(1):99–112
Schmelzeis MC, Mittleman G (1996) The hippocampus and reward: effects of hippocampal lesions on progressive-ratio responding. Behav Neurosci 110(5):1049–1066
Slawecki CJ, Thorsell AK, Khoury AE, Mathé AA, Ehlers CL (2005) Increased CRF-like and NPY-like immunoreactivity in adult rats exposed to nicotine during adolescence: Relation to anxiety-like and depressive-like behavior. Neuropeptides 39(4):369–377. https://doi.org/10.1016/j.npep.2005.06.002
Tiwari V, Veeraiah P, Subramaniam V, Patel AB (2014) Differential effects of ethanol on regional glutamatergic and GABAergic neurotransmitter pathways in mouse brain. J Neurochem 128(5):628–640. https://doi.org/10.1111/jnc.12508
Tormoehlen LM, Tekulve KJ, Nañagas KA (2014) Hydrocarbon toxicity: A review. Clin Toxicol 52(5):479–489. https://doi.org/10.3109/15563650.2014.923904
Van Hooste WLC (2017) Myoclonic seizure prior to diagnosis of chronic toxic encephalopathy: a case report. J Med Case Rep 11:36. https://doi.org/10.1186/s13256-016-1188-9
Vezzani A, Sperk G, Colmers WF (1999) Neuropeptide Y: emerging evidence for a functional role in seizure modulation. Trends Neurosci 22(1):25–30. https://doi.org/10.1016/s0166-2236(98)01284-3
Wang XL, Jin XP, Fu H, Chen ZQ, Da CD, Huang XF et al (1999) Xylene-induced effects on brain neurotransmitters, behavior and fos protein in rats. Biomed Environ Sci 12(2):116–124
Zhang J, Zhang D, McQuade JS, Behbehani M, Tsien JZ, Xu M (2002) c-fos regulates neuronal excitability and survival. Nat Genet 30:416. https://doi.org/10.1038/ng859