SUPPORTING INFORMATION

Animals.

We used male Swiss mice (35-40g), bred in the conventional animal facility of the University of Bordeaux, France. After birth, male pups stayed with their mothers until postnatal day 5 (P5) where they underwent a dopaminergic lesion by intra-ventricular injection of 6-hydroxydopamine (6-OHDA) (see below). The experimental protocols have been validated by the local ethics committee under number 13126C. The animals were kept in the normal dark-light cycle (light from 6 a.m. to 6 p.m.), with food and water *ad libitum*.

Neonatal 6-OHDA lesion (ADHD model).

Intracerebroventricular injection of 6-OHDA was performed at P5 in an adapted platform fixed to a stereotaxic instrument (David Kopf instrument, Tujunga, CA, United States) according to previously published protocols (1-3). The animals are randomized into 2 groups, the 6-OHDA and sham groups. Briefly, male pups were received an injection of the norepinephrine uptake blocker desipramine hydrochloride (20 mg/kg, s.c.; Sigma-Aldrich, Merck, Darmstadt, Germany). After 30 min, pups were anesthetized by hypothermia induced by placing pups on ice for 1 min. Then, they received 25 mg of 6-OHDA hydrobromide (Sigma-Aldrich) dissolved in 3 ml of ascorbic acid 0.1% (only vehicle for sham mice) in the right lateral ventricle (0.6 mm lateral to the medial sagittal suture, 2 mm rostral to the lambda and 1.3 mm in depth from the skull). Injections were performed at 1.5 ml/min using a 30G needle (Carpule, Bayer, Osaka, Japan) connected to a 25 µl Hamilton syringe. After the injection, the pups were warmed up at 37°C, and returned to their mothers until weaning.

Inflammatory pain.

Central sensitization to inflammatory pain was carried out at P40, by the injection of 20 µl of complete Freund's adjuvant (CFA) in the right posterior hind paw. Freund's adjuvant is a lipid mixture that causes an immune response (4, 5).

Methylphenidate injections.

Methylphenidate (MpH 3.0 or 5.0 mg/kg) or vehicle was administered intraperitoneally (i.e. 10 ml/kg in 0.9% NaCl solution) 45 minutes before all behavioral tests.

Ibuprofen injections.

Ibuprofen (No-Dol® fen, SANOFI, Morocco) or vehicle was administered intraperitoneally (6-8) (100 mg/kg in saline solution) 30 minutes prior to von Frey or Open Field testing.

Behavioral tests.

Von Frey Test. The von Frey test measures paw withdrawal responses to a range of mechanical stimuli (9). The mice are accustomed to the experimental context (the chamber and the experimental device) for three consecutive days before the test, for 15 min / d. The mice are placed individually in a cage (12 x 12 x 10 cm), the floor of which is equipped with a mesh metal

net (5 x 5 mm squares). A series of Von Frey monofilaments ranging from 0.02 to 8.0 g, with logarithmically increasing bending forces, were used to deliver mechanical pressure to the right hind paw according to the ascending method (9). Each filament is applied for 2-3 seconds, seven times in a row. The pain response is characterized by a sharp withdrawal of the paw. If the animal does not respond to at least 4 out of 7 stimulations, the next force filament is tested.

Hot/Cold plate test. The Hot/cold plate test consists in evaluating the degree of thermal sensitivity (10). The device (Bioseb, Model LE 7406) consists of a metal heating plate (20 cm in diameter) surmounted by a transparent polyester cylinder (20 cm x 25 cm). The mice are placed on the plate adjusted to a constant temperature (cold 5 ° C or heat 55 ± 1°C) for a maximum of 30 seconds. Latency at the first leg withdrawal was recorded as a sign of nociception.

Hargreaves test. To assess thermal hyperalgesia, the reactivity to brief nociceptive thermal stimulation can also be measured using a plantar test (Plantar Analgesia Meter, Bioseb, France). This technique involves applying a beam of radiant heat under the animal paw and measuring the time required for removal (11). Like for the von Frey test, the animal was placed in the transparent box and allowed to explore freely for 10 minutes. The thermal stimulus is applied under the hind paw, when the animal stops exploring. The intensity of the stimulus is adjusted to generate baseline average latencies of 10 to 12 seconds. If the animal does not respond after 20 seconds the heat beam is stopped to avoid damaging the tissue.

Open field (OF) test. The OF test was used to assess the general locomotor activity. It consisted of a square black box made of Plexiglas (50 × 50 × 50 cm high), equipped with the video-based Ethovision video tracking System (Noldus Information Technology, Wageningen, The Netherlands). Each animal was placed in the box for ten minutes while behavior was recorded according to published protocols (3). The distance traveled in the box was measured, as well as the velocity and the amount of time spent in movement. The box was cleaned with 70% ethanol between each test.

Elevated Plus Maze (EPM) test. The EPM measures the anxiety induced by open spaces and height. The apparatus comprised two opposing open arms (50 × 5 cm) and two closed arms (50 × 5 × 15 cm), which joined at a square central area (5 × 5 cm) to form a plus sign. The entire apparatus was elevated to a height of 45 cm above the floor. All mice were placed in the center of the maze and were allowed to run freely around the maze for 5 minutes with their behavior being recorded using a CCD video tracking system above the maze. After each test, the mice were returned to their home cage, and the plus-maze was cleaned with a water-moistened paper towel and dried after each mouse. For this test, the time spent and the number of entries into each arm were analyzed automatically using EthoVision software (Noldus Information Technology).

Five-Choice Serial Reaction Time Task (5-CSRTT) test. In the beginning, all animals had restricted access to food and their body weights were adjusted to 85% of their free-feeding weights. Animals had free access to water at all times except during training sessions. Mice were trained in operant chamber (24×20×15 cm) placed inside ventilated sound-attenuating cubicles (Med Associates Inc., St. Albans, VT, USA). The chamber consisted of a curved wall containing nine round apertures equipped with infrared detectors and bright white led (1.7 W) at the rear. Four of the nine apertures were blocked with a metal plate, thus allowing five functioning apertures equally spaced 2.5 cm apart. A magazine was located centrally in the opposite wall, equipped with an infrared detector and connected to a pellet dispenser delivering 25 mg food pellets into

a food tray covered by a hinged door (López Morenas, SL, Spain). Each chamber was automatically controlled by Packwin software (Panlab S.P., Cornella, Barcelona, Spain) and data were collected via a computer. Pre-training and training procedures were identical to previous studies (2, 12). For the pre-training phase, mice were placed in the chambers for 15 min with the house-light off. During this time, the pellet dispenser containing 15 food pellets was open in order to habituate mice eating the reinforcer in the magazine. The training consisted of a 20-min daily session for 5 days a week over a period of 20 weeks. The behavioral training was carried out during the light phase.

In vivo electrophysiology.

Single unit in vivo recording of spinal nociceptive neurons. The animal is anesthetized by intraperitoneal injection of urethane 20% at 1 g/kg administered in a single intra-peritoneal injection. The depth of anesthesia is evaluated by the absence of nociceptive reflexes after pinching the paw. The animal vital temperature is kept constant at 37.5°C ± 0.5 using a homeothermic blanket. The animal is placed in a stereotaxic frame (M2e, Montreuil, France) and the spine is held stable using forceps on either side of the area of interest. The spinal cord is exposed by a laminectomy at the level of the T13 and L1 vertebrae. An 8-10 MΩ tungsten electrode, 75 µm in diameter is inserted with a micromanipulator (WPI, Hitchin, UK) into the ipsilateral dorsal horn of the right hind paw. Neuron activity is observed by touching the paw. After a search for neurons responding to painful (pinching) and non-painful (brush) stimuli applied to the receptive field on the ipsilateral hind paw, we assessed the unit activity of wide dynamic range (WDR) neurons. The criterion for the selection of a WDR neuron was the presence of a A-fiber-evoked response (0–80 ms) followed by a C-fiber-evoked response (80–300 ms) to electrical stimulation of the paw with bipolar electrodes connected to a stimulator and placed in the center of the WDR receptive field (13) (see **Fig.S2**).

After the identification of this cell type, a series of von Frey monofilaments with increasing bending forces ranging from 0.02 to 8.0 g was used to deliver mechanical pressure to the right hind paw. Each filament is applied three times for 5 seconds. The response to each mechanical stimulus is measured as the number of action potentials per stimulation (14). The recorded signals are filtered in bandwidth between 300 and 8000 Hz, and amplified x 10000 (Dam-80, Plexon, Dallas, USA). The data is acquired on a computer via a CED 1401 interface and analyzed by Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Right after we have recorded the basal activity of the WDR neuron in the 1st series of Von Frey, MpH (3.0 or 5.0 mg/kg) or vehicle (NaCl 0.9%) was injected intraperitoneally, then the mechanical stimulation was applied in the receptive field of the right hind paw every 10 min and the recording continues for 90 min.

Single unit in vivo recording of anterior cingulate cortex (ACC) neurons. At P40, the animal is anesthetized by intraperitoneal injection of urethane 20% at 1 g/kg. The depth of anesthesia is evaluated and the animal body temperature is maintained as described above. After fixing the mouse in the stereotaxic frame, a craniotomy of about 1 mm in diameter is made under the binocular magnifier with a pneumatic bur. The skull is exposed, an 8-10 MΩ tungsten electrode, 75 µm in diameter, is inserted into the left side of the anterior cingulate cortex ([AP] +0.7, [ML] -0.3 and [DV] -1.5 mm) according to the stereotaxic coordinates of the Paxinos and Franklin Atlas (2001). Once the discharge of a neuron is identified, the spontaneous unit activity is recorded for

45 min. Next, we record the evoked activity by mechanical stimuli applied to the right hind paw. Signal filtering and amplification is performed as described above.

Single unit* in vivo *recording of WDR neurons upon ACC manipulation. We stimulated or inhibited the activity of the ACC (left side) and assessed the response of spinal WDR neurons to mechanical stimulation of the right hind paw.

Electrical stimulation of ACC. a bipolar stimulation electrode (0.15 mm OD; model MS308/SPC; PlasticsOne, Roanoke, USA) is placed in the ACC ([AP] +0.7, [ML] -0.3 and [DV] -1.5 mm). Electrical stimulation at ACC is performed with rectangular pulses (pulse duration: 100 µs; frequency: 100 Hz; intensity: 10, 20 and 30V) of 1s duration. The total stimulation period is 10s. We first assessed the basal activity of WDR neurons in response to mechanical stimulation before ACC stimulation, just after the end of pulses at the different intensities tested and the recovery time of WDR neuron electrical activity.

Pharmacological inhibition of ACC. For intra-ACC infusion, 1 µl of muscimol (1 µg/µl) or saline solution was administered within 90 s using a glass micropipette attached to a pump. We evaluated the basal WDR neuron activity of in response to mechanical stimulation before the pharmacological injection into the ACC and every 15 min after muscimol injection for 1.5 hours.

Intracerebral micro-injection of virus.

We injected AAV5.CamkII.ChR2/ArchT3.0.eGFP into the ACC at P21 on sham and 6-OHDA animals. The animals were anesthetized by inhalation of isoflurane (3% in an induction chamber then 2% under mask). The depth of anesthesia was evaluated by the absence of nociceptive reflexes after pinching the paw. Before the surgery and at the end of the procedure, the animals received an injection of buprenorphine analgesic at 0.1 mg / kg subcutaneously to avoid post-surgical pain. Before and after any surgical procedure, the equipment was cleaned and disinfected. The mice received an application of an ophthalmic ointment (Ocry-gel, Laboratoire TVM, Lempdes, France) to avoid ocular dehydration. The skin of the skull was shaved and betadine was applied to the skin of the skull to prevent wound infections. An injection of 1% lidocaine (100 µl) was made under the scalp. The skin was cut with a sterile scalpel. A craniotomy of approximately 1 mm in diameter was made under the binocular magnifying glass with a pneumatic bur. A glass capillary was then descended into the ACC. The injection was carried out according to the stereotaxic coordinates defined by a pilot study (n = 15). Once in the area of interest, the virus was ejected from the capillary using a minipump delivering a volume of 50 nl/min. The total volume injected was 500 nl of the initial virus solution (AAV5.CamKII.ChR2.eGFP: $1x10^{13}$ ng/ml; AAV5.CamKII. ArchT3.0.eGFP: $3x10^{12}$ ng/ml; AAV5.CamKII.eGFP: $1x10^{13}$ ng/ml) in the left ACC ([AP] +1.0 mm bregma, [ML] -0.3 mm, [DV] -0.9 mm). After the injection, the needle was held in place for an additional 5 min to facilitate the spread of the virus and then the wound was sutured. The animals were warmed up by placing the cage on a heating blanket at 37°C until waking up. They were kept under surveillance for 2 hours and then brought back to the housing facility. Several groups were tested to determine the time-course of the virus expression. For behavioral and electrophysiology experiments, the mice were used after four weeks allowing maximum expression of the virus.

Implantation of optical fibers.

The purpose of this procedure was to implant the optical fibers in the ACC or posterior insula (PI) at P50 on sham and 6-OHDA animals. Four weeks after the injection of the virus, the animals were anesthetized and fixed in the stereotaxic frame as described above. A craniotomy was performed by drilling a hole. Then, the simple fiberglass cannula, 1.7 or 4 mm long and with a diameter of 220 mm (MFC_220/250-0.66_1.7 mm_RM3_FLT, doric lenses, Quebec, Canada) was implanted in the left ACC or the left PI, respectively. The stereotaxic coordinates of ACC and PI were: (ACC/PI: [AP] +0.7/+0.3 mm bregma, [ML] -0.3/-3.8 mm, [DV] -1.7/-4 mm). The cannula was implanted in the skull with a layer of cyanoacrylate glue followed by dental cement until 1.5 or 3.8 mm of optical fiber was inserted into the brain, respectively. The mice were warmed up until waking up, and kept under surveillance. After the surgery, the mice benefited from 7 days of recovery before starting the experimental protocol. During this period, the animals were daily weighed and handled in order to detect any postoperative incident.

Optogenetic manipulations.

In all optogenetic protocols, the light was delivered for 3 minutes. In the case of activation, there were two 'off' sessions and one 'on' (off-on-off) session. During the 'on' session blue light (473 nm) was emitted at a frequency of 2 Hz (5 ms pulse), with an intensity of 15 mW/mm^2 at the tip of the optical fiber. In the case of inhibition, there were two 'off' sessions and one 'on' (off-on-off) session. During the 'on' session, yellow light (575 nm) was emitted continuously with an intensity of 10 mW/mm^2 according to published protocols (15-17). The hind paw withdrawal threshold was measured four times during the 'off' and 'on' sessions and averages were calculated.

After the evaluation of nociception by behavioral tests, we recorded the unitary activity of WDR spinal neurons upon optogenetic manipulation of the left (contralateral) ACC or PI as described above for electrophysiology experiments. After identifying a WDR neuron, we recorded its activity in response to mechanical stimulation of the animal hind paw before ('off'), during ('on') and after ('off') the optogenetic stimulation of ACC or PI. The recovery was assessed by recording WDR activity every 2 min for 15 min after stopping the optogenetic stimulation. We limited our recordings to one neuron per animal because the ACC manipulation can modify the basal activity of other spinal neurons. Thus, the number of registered neurons and animals used was identical.

Immunohistochemistry.

Tissue preparation. Mice were anesthetized and perfused through the ascending aorta with cold fixative containing 4% paraformaldehyde diluted in phosphate buffer (PB, 0.1 M). The brain and spinal cord were rapidly dissected and postfixed in the same fixative for 2 hours. Tissues were then incubated in phosphate-buffered saline (PBS, pH 7.4) containing 12% sucrose and sodium azide at 4°C overnight. The brains were frozen and cut at 18µm in a cryostat (Leica, Wetzlar, Germany). Sections were collected in cell wells and the subsequent steps were carried out on free floating sections. Sections were rinsed in PBS, and incubated in PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich) (PBS-BSA) for 30 minutes at room temperature. Sections were then incubated with primary antibodies.

GFP immunostaining. Sections were incubated overnight at 4°C with anti-GFP IgG goat antisera (1:500, ab104139, Abcam) in PBS-BSA, washed in 1× PBS and incubated for 2 hours at room temperature with Alexa Fluor 488, anti-goat secondary antibody (1:500; Thermo Fisher Scientific, Waltham, USA) in PBS-BSA.

TH fiber immunostaining. Sections were incubated overnight at 4°C in PBS-BSA with sheep anti-TH primary antibody (1/500, RD System [Bio Techne] AF7566-SP). For double-labeling experiments, sections were co-incubated with rabbit anti-dopamine beta hydroxylase (DBH) primary antibody (1/1000, Merck Sigma HPA070789), rat anti-dopamine active transporter (DAT) primary antibody (1/1000, Merck Sigma MAB369), rabbit anti-Aldh1a1 primary antibody (1/500, abcam ab227964), or rabbit anti-VIP primary antibody (1/500, abcam ab22736). They were then rinsed 3 times 10 minutes in PBS containing 0.05% Tween 20 (PBT). Alexa 488-conjugated donkey anti-sheep (1:500 in PBS-BSA; Thermo Fisher Scientific) was then applied for 2 hours at room temperature. Alexa 564-conjugated donkey anti-rabbit/rat (1:500 in PBS-BSA; Thermo Fisher Scientific) was added for double-labeling experiments.

Co-detection of synaptic markers. Mouse anti-synaptophysin primary antibody (clone SY38; 1:500; Merck) was diluted together with rabbit anti-homer 1 (1:500; Synaptic System, Göttingen, Germany) or rabbit anti-gephyrin (1:200; Thermo Fisher Scientific) in PBS + BSA (1%) + Triton (0.3%) (Sigma-Aldrich). Sections were incubated in this solution overnight at 4°C. Sections were then washed in 1× PBS and incubated for 2 hours at room temperature with Alexa Fluor 568 and Alexa Fluor 488, anti-rabbit secondary antibody (1:500; Thermo Fisher Scientific) in PBS + BSA (1%).

Microscope observation. For all immunohistochemistry experiments, sections were rinsed in PBS, mounted on glass slides, air dried, covered with an anti-fading agent (Fluoroshield. Sigma-Aldrich). Specificity of the antibodies was determined by the manufacturer and in our hands by omitting the primary antibodies. Sections were viewed with a confocal microscope (Leica SPE, Mannheim, Germany).

Quantification. Images to be compared were collected during the same session using identical scanning settings. They were then imported into "ImageJ" free software (version 1.42q) (NIH, Bethesda, MA) for quantitative analysis mice (4 sections per mouse, 4 mice per condition) according to previously published protocols (18-20). Background was subtracted by thresholding. The mean gray level corresponding to fluorescence intensity, and the immunolabeled area, were measured in sham and 6-OHDA (20). Results were expressed as a percentage of the intensity, or immunolabeled area, in sham animals. The extent of colocalization between synaptophysin and inhibitory (gephyrin) or excitatory (homer 1) post-synaptic markers was quantitatively assessed in the entire fields acquired in the dorsal horn of the spinal cord using the JACoP plugin of Image J. Results were expressed as a percentage of gephyrin or homer 1 total labeling that is seen in the area. Statistical analysis was performed with student t-test.

Western blot

Spinal cords from sham and 6-OHDA treated mice (n=3 in each group) were homogenized using a Teflon-glass homogenizer in ice-cold buffer containing (sucrose 0.32 M, HEPES 10 mM, pH 7.4) and a protease inhibitor mixture (1:1,000; Calbiochem). Homogenate was spun at 1,000 × g for 10 min at 4 °C. The resulting supernatant was centrifuged at 12,000 g for 20 minutes at +4°C. The

supernatants which are cytoplasmic fractions, were collected and quantified by the Lowry method (Bio-Rad DC Protein Assay) and then stored at -80°C their use.

Twenty five micrograms of the extracted proteins were subjected to SDS-PAGE electrophoresis (4–20% Mini-PROTEAN TGX Gel Bio-rad, Marnes-la-Coquette, France). Gels were then transferred onto nitrocellulose membrane during 1 hr at 100V. After blocking 1 hr in 5% milk in Tris-saline - 0.05% tween 20 (TBST), the membranes were incubated overnight at 4°C with different primary antibodies, rabbit anti-CaMKIIα antibody (Biotechne, NB100-1983; 1/200) or rabbit anti-ERK antibody (Biotechne, 1/10000), rabbit anti-pERK (Biotechne; 1/2000) CREB (Biotechne, 1/1000) or rabbit anti-pCREB (Biotechne; 1/2000). Equal loading was confirmed by probing with β-actin (Santa Cruz Biotechnology, 1/3000) monoclonal antibody.

Immunoreactivity was detected using polyclonal goat anti-mouse or anti rabbit immunoglobulins-HRP at 1:1000 in TBST 0.5% milk (DakoCytomation). The density of bands was quantitated by densitometry using a Syngene machine (ChemiGenius 2XE model, Synoptics Ltd, Cambridge, UK). Immunoblots were semiquantified by normalizing the band density to that of actin (CaMKII), or non-phosphorylated protein (pERK and pCREB) bands. The images were analysed using image J software.

Statistics.

Statistical analyzes were performed using SigmaPlot 11.0 software (SigmaStat, Systat Software Inc, San Jose, CA, USA). The graphs were made by using GraphPad Software Prism 6. The results are expressed as a mean ± SEM, with consideration of $p < 0.05$ as the threshold of statistical significance. The t-Student test was used for simple two-sample comparisons (spontaneous ACC activity, immunolabeling). ANOVA Two-way repeated measures ANOVA was used for multiple comparisons (ACC evoked activity). Two-way ANOVA followed by the post-hoc Tukey test was performed to assess ADHD behavior (with CFA) and pain (without CFA). A factor analysis of variance ANOVA with two factors with repeated measures, followed by a multiple comparison by the post-hoc Tukey test was used to analyze the performances of 5-CSRTT, pain behavior (with CFA) and the analyzes of in vivo electrophysiology in the spinal cord. For optogenetic manipulations, the t-student paired test is used to analyze pain behavior and two-way repeated measures ANOVA for electrophysiological analyzes in the spinal cord.

SUPPLEMENTARY BIBLIOGRAPHY

1. Sontag TA, Tucha O, Walitza S, & Lange KW (2010) Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. *Atten Defic Hyperact Disord* 2(1):1-20.
2. Bouchatta O, Manouze H, Ba-M'Hamed S, Landry M, & Bennis M (2020) Neonatal 6-OHDA Lesion Model in Mouse Induces Cognitive Dysfunctions of Attention-Deficit/Hyperactivity Disorder (ADHD) During Young Age. *Frontiers In Behavioral Neuroscience* 14:27.
3. Bouchatta O*, et al.* (2018) Neonatal 6-OHDA lesion model in mouse induces Attention-Deficit/ Hyperactivity Disorder (ADHD)-like behaviour. *Scientific Reports* 8(1):15349.
4. Abboud C*, et al.* (2020) Animal models of pain: diversity and benefits. *J Neurosci Methods*:108997.
5. Bortalanza LB*, et al.* (2002) Anti-allodynic action of the tormentic acid, a triterpene isolated from plant, against neuropathic and inflammatory persistent pain in mice. *Eur J Pharmacol* 453(2-3):203-208.
6. Foley PL, Kendall LV, & Turner PV (2019) Clinical Management of Pain in Rodents. *Comp Med* 69(6):468-489.
7. Salama RAM, El Gayar NH, Georgy SS, & Hamza M (2016) Equivalent intraperitoneal doses of ibuprofen supplemented in drinking water or in diet: a behavioral and biochemical assay using antinociceptive and thromboxane inhibitory dose-response curves in mice. *PeerJ* 4:e2239.
8. Thompson JM*, et al.* (2019) Front and hind paw differential analgesic effects of amitriptyline, gabapentin, ibuprofen, and URB937 on mechanical and cold sensitivity in cisplatin-induced neuropathy. *Mol Pain* 15:1744806919874192.
9. Deuis JR, Dvorakova LS, & Vetter I (2017) Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* 10:284.
10. Shi M, Qi WJ, Gao G, Wang JY, & Luo F (2010) Increased thermal and mechanical nociceptive thresholds in rats with depressive-like behaviors. *Brain Res* 1353:225-233.
11. O'Brien DE, Brenner DS, Gutmann DH, & Gereau RWt (2013) Assessment of pain and itch behavior in a mouse model of neurofibromatosis type 1. *The journal of pain* 14(6):628-637.
12. Humby T, Wilkinson L, & Dawson G (2005) Assaying aspects of attention and impulse control in mice using the 5-choice serial reaction time task. *Curr Protoc Neurosci* Chapter 8:Unit 8 5H.
13. Fossat P*, et al.* (2010) Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. *J Neurosci* 30(3):1073-1085.
14. Senapati AK, Huntington PJ, & Peng YB (2005) Spinal dorsal horn neuron response to mechanical stimuli is decreased by electrical stimulation of the primary motor cortex. *Brain Res* 1036(1-2):173-179.
15. Kang SJ*, et al.* (2015) Bidirectional modulation of hyperalgesia via the specific control of excitatory and inhibitory neuronal activity in the ACC. *Mol Brain* 8(1):81.
16. Madisen L*, et al.* (2012) A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nat Neurosci* 15(5):793-802.
17. Zhang F*, et al.* (2007) Multimodal fast optical interrogation of neural circuitry. *Nature* 446(7136):633-639.
18. Landry M, Bouali-Benazzouz R, El Mestikawy S, Ravassard P, & Nagy F (2004) Expression of vesicular glutamate transporters in rat lumbar spinal cord, with a note on dorsal root ganglia. *J Comp Neurol* 468(3):380-394.
19. Papon MA*, et al.* (2020) Spinal Inhibition of GABAB Receptors by the Extracellular Matrix Protein Fibulin-2 in Neuropathic Rats. *Front Cell Neurosci* 14:214.
20. Zaky A, Bouali-Benazzouz R, Favereaux A, Tell G, & Landry M (2018) APE1/Ref-1 redox function contributes to inflammatory pain sensitization. *Exp Neurol* 307:1-11.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary legend for Figure 4.

A. There was a significant increase of ACC neuron activity in 6-OHDA mice as compared to sham (6.00 ± 0.88 *vs* 2.30 ± 0.74; t=3.70, p=0.002).

B. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,9)}$=63.70, p=0.0001), peripheral stimulation ($F_{(4,36)}$=58.53; p=0.0001) and interaction 6-OHDA x peripheral stimulation ($F_{(4,36)}$=10.58, p=0.0001) on ACC discharges. There was a significant increase of the ACC neuron discharge in 6-OHDA mice in response to innocuous (1.4g: 12.30 ± 2.33 *vs* 2.20 ± 1.98; q=2.75, p<0.05) and noxious peripheral stimuli (pressure: 67.20 ± 4.69 *vs* 30.40 ± 3.56; q=10.00, p=0.0001) as compared to sham mice.

C. Two-way repeated measures ANOVA showed a significant effect of electrical stimulation intensity ($F_{(4,36)}$=87.22, p=0.0001), peripheral stimulation (von Frey) ($F_{(2,18)}$=397.70; p=0.0001) and interaction stimulation intensity x peripheral stimulation ($F_{(8,72)}$=7.56, p=0.0001) on DHNs discharges in sham mice. There was a significant increase of contralateral DHNs evoked activity in response to peripheral innocuous (1.4g) ([10V]: 6.80 ± 0.83; q=3.01, *p>0.05*; [20V]: 9.00 ± 0.68; q=4.13, *p<0.05*; [30V]: 12.20 ± 1.21; q=5.77, *p<0.01*) and noxious (6.0g) ([10V]: 45.90 ± 2.67; q=6.99, p=0.0001; [20V]: 51.30 ± 1.69; q=9.75, p=0.0001; [30V]: 65.70 ± 3.38; q=17.10, p=0.0001) mechanical stimuli as compared to their baseline discharges ([1.4g]: 0.90 ± 0.10; [6.0g]: 32.20 ± 2.49, respectively) in the sham group. In 6-OHDA mice, two-way repeated measures ANOVA showed a significant effect of electrical stimulation intensity ($F_{(4,36)}$=151.20, p=0.0001) and peripheral stimulation (von Frey) ($F_{(2,18)}$=195.60; p=0.0001) on DHNs discharges; whereas the interaction electrical stimulation intensity x peripheral stimulation ($F_{(8,72)}$=1.27, p=0.27) had no effect. There was a significant increase of contralateral DHNs evoked activity in response to peripheral innocuous (1.4g) ([10V]: 17.60 ± 1.15; q=3.03, *p>0.05*; [20V]: 32.20 ± 2.49; q=6.49, p<0.001; [30V]: 44.30 ± 3.16; q=9.35, p=0.0001) and noxious (6.0g) mechanical stimuli ([10V]: 70.60 ± 2.38; q=4.85, p<0.01; [20V]: 92.20 ± 3.99; q=4.97, p=0.0001; [30V]: 105.90 ± 6.12; q=13.21, p=0.0001) as compared to their baseline discharges ([1.4g]: 4.80 ± 0.66; [6.0g]: 50.10 ± 1.93, respectively) in the 6-OHDA group. DHNs activity returned to baseline activity within 2 min after the last electrical stimulation in sham mice ([1.4g]: 0.80 ± 0.13; q=0.05, p>0.05; [6.0g]: 34.80 ± 2.37; q=1.33; p>0.05), regardless the stimulus strength, while the potentiation of DHNS activity in 6-OHDA mice was maintained up to 2 minutes ([1.4g]: 43.20 ± 2.49; q=9.09, p=0.0001; [6.0g]: 102.50 ± 6.51; q=12.40; p=0.0001).

D. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(3,27)}$=142.00, p<0.0001) on DHNs discharges in response to innocuous (1.4g) peripheral stimulus; while intra-ACC infusion of muscimol ($F_{(3,27)}$=0.93; p=0.44) and the interaction 6-OHDA x muscimol ($F_{(9,81)}$=0.99, p=0.45) had no effect. Two-way repeated measures ANOVA showed a significant effect of 6-OHDA ($F_{(3,27)}$=208.20, p<0.0001) and muscimol ($F_{(3,27)}$=5.49; p=0.005) on DHNs discharges in response to noxious (6.0g) peripheral stimulation whereas the interaction 6-OHDA x muscimol ($F_{(9,81)}$=1.30, p=0.25) had no effect. There was a significant decrease of the electrical activity of contralateral DHNs activity evoked by innocuous and noxious peripheral mechanical stimuli in sham ([1.4g]: 0.60 ± 0.60; q=0.19, p>0.05; [6.0g]: 7.90 ± 1.16; q=3.85; p<0.05) and 6-OHDA groups [1.4g]: 3.00 ± 0.52; q=4.46, p<0.05; [6.0g]: 30.70 ± 2.60; q=3.94; p<0.05) 30

minutes after intra-ACC muscimol infusion as compared to their baseline levels (Pre; sham: [1.4g]: 0.70 ± 0.47; [6.0g]: 16.60 ± 1.98; 6-OHDA: [1.4g]: 5.40 ± 0.43; [6.0g]: 39.60 ± 2. 39). Intra-NaCl infusion had no effect on DHNs discharge in response to peripheral innocuous and noxious mechanical stimulation in sham ([1.4g]: 0.30 ± 0.30; q=0.00, p>0.05; [6.0g]: 15.30 ± 2.34; q=0.58, p>0.05) and 6-OHDA mice ([1.4g]: 5.80 ± 0.71; q=0.19, p>0.05; [6.0g]: 40.00 ± 2.44; q=0.49, p>0.05) 30 minutes after intra-ACC infusion as compared to their baseline levels (Pre; sham: [1.4g]: 0.30 ± 0.30; [6.0g]: 14.00 ± 1.81; 6-OHDA: [1.4g]: 5.90 ± 0.48; [6.0g]: 41.10 ± 2.42).

Supplementary legend for Figure 5.

B1. There was a significant effect of 473 nm light on withdrawal thresholds to mechanical ([Sham]: Before: 4.00 ± 0.65 g *vs* Opto: 1.95 ± 0.31 g; t=2.83, p=0.03; [6-OHDA]: Before: 2.10 ± 0.29 g *vs* Opto: 1.33 ± 0.12 g; t=3.72, p=0.008) and thermal stimuli ([Sham]: Before: 24.75 ± 3.15 s *vs* Opto: 14.75 ± 1.92 s; t=2.58, p=0.04; [6-OHDA]: Before: 17.13 ± 1.13 s *vs* Opto: 10.38 ± 1.15 s; t=3.49, p=0.01) in both groups. After the light was off (Recovery), changes in mechanical and thermal withdrawal thresholds of sham mice were maintained during 2 minutes ([von Frey]: 1.95 ± 0.31 g; t=3.31, p=0.02; [IR40]: 15.50 ± 2.01 s; t=2.49, p=0.04), and returned to the baseline values after 5 minutes ([von Frey]: 4.18 ± 0.74 g; t=0.19, p=0.85; [IR40]: 25.25 ± 4.21 s; t=0.08, p=0.94). In contrast, changes in mechanical and thermal withdrawal threshold of the 6-OHDA group were further amplified at 2 minutes after the illumination was off ([von Frey]: 0.50 ± 0.08 g; t=5.17, p=0.001; [IR40]: 6.75 ± 0.75 s; t=6.52, p=0.0003). After 5 minutes, thermal withdrawal latency returned to baseline levels ([IR40]: 16.75 ± 3.30 s; t=0.09, p=0.93), while the mechanical threshold was not fully restored ([von Frey]: 1.15 ± 0.20 g; t=2.48, p=0.04). **B2.** Two-way ANOVA showed no significant effect on the mechanical withdrawal threshold of lesion (6-OHDA) ($F_{(1,28)}$=1.23, p=0.28), light stimulation (Opto) ($F_{(1,28)}$=0.90; p=0.35) and interaction 6-OHDA x Opto ($F_{(1,28)}$=0.90, p=0.35). There was a significant effect on the thermal withdrawal latency of lesion (6-OHDA) ($F_{(1,28)}$=12.47, p=0.002), light stimulation (Opto) ($F_{(1,28)}$=4.21; p=0.04) and interaction 6-OHDA x Opto ($F_{(1,28)}$=8.98, p=0.006). Changes were greater in 6-OHDA conditions than in sham at 2 minutes recovery in response to thermal (-61.15 ± 2.30 % *vs* -34.61 ± 5.55 %; q=6.53, p=0.001), but not to mechanical (-45.53 ± 6.60 % *vs* -46.46 ± 6.21 %; q=0.16, p>0.05) stimulus.

C. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=92.26, p<0.0001 and $F_{(1,7)}$=91.88, p<0.0001), light stimulation (Opto) ($F_{(3,21)}$=25.66; p<0.0001 and $F_{(3,21)}$=28.33; p<0.0001) and interaction 6-OHDA x Opto ($F_{(3,21)}$=3.17, p=0.04 and $F_{(3,21)}$=3.46, p=0.03) on DHNs discharges in response to innocuous (1.4g) and noxious (6.0g) peripheral stimulation, respectively. There was a significant effect of laser light the activity of DHNs to innocuous (1.4g: [sham]: Before: 1.88 ± 0.40 *vs* Opto: 5.38 ± 0.50; q=4.51, p<0.05; [6-OHDA]: Before: 6.63 ± 0.68 *vs* Opto: 10.75 ± 0.75; q=5.32, p<0.01) and noxious stimuli (6.0 g: [sham]: Before: 50.13 ± 3.39 *vs* Opto: 67.50 ± 3.05; q=4.69, p<0.05; [6-OHDA]: Off: 71.00 ± 3.62 *vs* On: 90.25 ± 3.25; q=5.20, p<0.01) in both groups. At 2 minutes after the light is off, increased DHN activity remained unchanged in sham mice ([1.4g]: 5.38 ± 0.63; q=4.51, p<0.05; [6.0g]: 66.88 ± 4.93; q=4.53, p<0.05) and returned to the baseline levels before illumination after 5 minutes ([1.4g]: 2.00 ± 0.53; q=0.16, p>0.05; [6.0g]: 51.75 ± 3.27; q=0.44, p>0.05). In contrast, spinal neurons activity was further amplified in 6-OHDA mice at 2 minutes after the light was off ([1.4g]: 13.75 ± 1.46; q=9.19, p<0.0001; [6.0g]: 106.88 ± 3.31; q=9.69, p<0.0001). In addition, at 5 minutes post-illumination, spinal neurons activity was not completely restored and remained significantly

different from baseline levels ([1.4g]: 5.88 ± 0.69; q=0.97, p>0.05; [6.0g]: 70.50 ± 3.81; q=0.14, p>0.05) in the 6-OHDA mice.

D1. There was a significant effect of 575 nm light on withdrawal thresholds to mechanical ([sham]: Before: 4.25 ± 0.25 g *vs*, Opto: 5.50 ± 0.50 g; t=2.38, p=0.04; [6-OHDA]: Before: 1.93 ± 0.08 g *vs* Opto: 3.50 ± 0.33 g; t=4.48, p=0.003) and thermal stimuli ([sham]: Before: 24.63 ± 2.82 s *vs* Opto: 33.38 ± 1.68 s; t=2.76, p=0.03; [6-OHDA]: Before: 18.00 ± 1.72 s *vs* Opto: 27.75 ± 2.31 s; t=3.73, p=0.007) in both groups. When the light was turned off, changes in mechanical and thermal withdrawal thresholds in sham mice were maintained during 2 minutes ([von Frey]: 5.50 ± 0.50 g; t=2.38, p=0.04; [IR40]: 33.38 ± 1.93 s; t=2.49, p=0.04), and returned to the baseline values after 5 minutes ([von Frey]: 4.25 ± 0.59 g; t=0.00, p=0.99; [IR40]: 24.88 ± 2.98 s; t=0.06, p=0.95). In contrast, changes in mechanical and thermal threshold of the 6-OHDA group were further amplified at 2 minutes after the illumination was off ([von Frey]: 4.50 ± 0.33 g; t=8.06, p=0.0001; [IR40]: 35.38 ± 1.90 s; t=6.01, p=0.0005). After 5 minutes, this increase was fully abolished in the 6-OHDA group ([von Frey]: 1.50 ± 0.12 g; t=2.43, p>0.05; [IR40]: 15.75 ± 2.33 s; t=0.62, p=0.55). **D2.** Two-way ANOVA showed a significant effect on the mechanical threshold of lesion (6-OHDA) ($F_{(1,28)}$=38.78, p<0.0001), light stimulation (Opto) ($F_{(1,28)}$=4.78; p=0.04) and interaction 6-OHDA x Opto ($F_{(1,28)}$=4.78, p=0.04). There was a significant effect of lesion (6-OHDA) ($F_{(1,28)}$=8.59, p=0.007) on thermal latency, while light stimulation (Opto) $F_{(1,28)}$=3.99; p=0.06) and interaction 6-OHDA x Opto $F_{(1,28)}$=4.14, p=0.05) had no effect. Changes were greater in 6-OHDA mice than in sham mice at 2 minutes recovery in response to both mechanical (135.71 ± 17.50 % *vs* 29.17 ± 8.77 %; q=8.41, p<0.0001) and thermal (104.02 ± 12.36 % *vs* 45.43 ± 13.92 %; q=4.97, p<0.01) stimuli.

E. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=358.40, p<0.0001 and $F_{(1,7)}$=320.20, p<0.0001), light stimulation (Opto) ($F_{(3,21)}$=8.18; p=0.0009 and $F_{(3,21)}$=31.18; p<0.0001) and interaction 6-OHDA x Opto ($F_{(3,21)}$=11.80, p<0.0001 and $F_{(3,21)}$=2.72, p=0.07) on DHN discharges in response to innocuous (1.4g) and noxious (6.0g) peripheral stimulation, respectively. Optogenetic inhibition of the ACC – PI excitatory pathway in sham mice decreased DHN electrical activity in response to noxious stimuli ([6.0g]: Before: 69.50 ± 3.97 *vs* Opto: 50.50 ± 3.55; q=4.40, p<0.05), while no effects were detected upon innocuous stimulus ([1.4g]: Before: 0.63 ± 0.18 *vs* Opto: 0.63 ± 0.18; q=0.00, p>0.05). At 2 minutes after the light is off, reduced DHN activity upon noxious stimuli remained unchanged in sham mice ([1.4g]: 0.50 ± 0.19; q=0.77, p>0.05; [6.0g]: 51.13 ± 3.73; q=4.26, p<0.05) and returned to the baseline levels before illumination after 5 minutes ([1.4g]: 0.63 ± 0.18; q=0.00, p>0.05; [6.0g]: 71.13 ± 2.84; q=0.38, p>0.05). In 6-OHDA mice, optogenetic inhibition of the ACC – PI excitatory pathway also blocked the activity of spinal neurons in response to innocuous ([1.4g]: Before: 3.25 ± 0.71 *vs* Opto: 2.38 ± 0.18; q=5.42, p<0.01) and noxious ([6.0g]: Before: 111.25 ± 3.32 *vs* Opto: 87.88 ± 4.32; q=5.42, p<0.01) mechanical stimuli. In contrast to sham mice, DHN activity was further decreased at 2 minutes after the light is off ([1.4g]: 1.50 ± 0.19; q=10.84, p<0.0001; [6.0g]: 69.88 ± 3.12; q=9.59, p<0.0001) and returned to baseline levels after 5 minutes ([1.4g]: 3.25 ± 0.16; q=0.00, p>0.05; [6.0g]: 106.50 ± 4.74; q=1.10, p>0.05).

Fig. S1: Methylphenidate (Mph) has no effect on nociception in 6-OHDA mice.

A. Paw licking latency in hot plate (55°C). **B.** Paw licking latency in cold plate (5°C). **C.** Paw withdrawal threshold using von Frey filaments. We explored the capacity of ADHD medication to alter nociception in sham and 6-OHDA mice by injecting a single dose of methylphenidate (3.0 or 5.0mg/kg Mph). Two-way ANOVA indicated a significant effect of lesion (6-OHDA) ([heat]: $F_{(1,54)}$=47.80, p<0.0001; [cold]: $F_{(1,54)}$=53.70, p<0.0001 and [von Frey]: $F_{(1,54)}$=82.13, p<0.0001). The treatment (Mph) ([heat]: $F_{(2,54)}$=1.18, p=0.32; [cold]: $F_{(2,54)}$=0.02, p=0.98 and [von Frey]: $F_{(2,54)}$=0.34, p=0.72) and interaction 6-OHDA x Mph ([heat]: $F_{(2,54)}$=0.03, p=0.97; [cold]: $F_{(2,54)}$=0.36, p=0.70 and [von Frey]: $F_{(2,54)}$=0.12; p=0.89) had no effect on thermal and mechanical sensitivity. Neither 3.0 mg/kg nor 5.0 mg/kg Mph influenced thermal (3.0mg/kg: sham: [heat]: q=0.82, p>0.05; [cold]: q=0.42, p>0.05; 6-OHDA: [heat]: q=0.72, p>0.05; [cold]: q=0.26, p>0.05; 5.0mg/kg: sham: [heat]: q=1.36, p>0.05; [cold]: q=1.04, p>0.05; 6-OHDA: [heat]: q=1.71, p>0.05; [cold]: q=0.64, p>0.05) or mechanical sensitivity ([3.0mg/kg]: sham: q=0.70, p>0.05; 6-OHDA: q=0.00, p>0.05; [5.0mg/kg]: sham: q=0.70, p>0.05; 6-OHDA: q=0.93, p>0.05) of sham or 6-OHDA mice. All data are means ± SEM (10 mice per group), **p<0.01; ***p<0.001 in comparison with sham.

D-E. Paw licking latency in hot plate (55°C) under inflammatory pain conditions in sham (**D**) and 6-OHDA (**E**) groups. **F-G.** Paw licking latency in cold plate (5°C) under inflammatory pain conditions in sham (**F**) and 6-OHDA (**G**) groups. **H-I.** Paw withdrawal thresholds using Von Frey filaments under inflammatory pain conditions in sham (**H**) and 6-OHDA (**I**) groups. Two-way repeated measures ANOVA showed a significant effect of treatment (Mph) ([heat]: $F_{(5,45)}$=50.22, p<0.0001; [cold]: $F_{(5,45)}$=35.39, p<0.0001 and [von Frey]: $F_{(5,45)}$=52.97, p<0.0001), inflammation (CFA) ([heat]: $F_{(4,36)}$=10.18, p<0.0001; [cold]: $F_{(4,36)}$=4.91, p=0.003 and [von Frey]: $F_{(4,36)}$=18.64, p<0.0001) and interaction Mph x CFA ([heat]: $F_{(20,180)}$=2.06, p=0.007; [cold]: $F_{(20,180)}$=2.52, p=0.0007 and [von Frey]: $F_{(20,180)}$=2.54; p=0.0006) on thermal and mechanical sensitivity in sham mice. There was also a significant effect of treatment (Mph) ([heat]: $F_{(5,45)}$=62.78, p<0.0001; [cold]: $F_{(5,45)}$=71.75, p<0.0001 and [von Frey]: $F_{(5,45)}$=97.91, p<0.0001), inflammation (CFA) ([heat]: $F_{(4,36)}$=10.14, p<0.0001; [cold]: $F_{(4,36)}$=25.46, p<0.0001 and [von Frey]: $F_{(4,36)}$=24.28, p<0.0001) and interaction Mph x CFA ([heat]: $F_{(20,180)}$=2.90, p<0.0001; [cold]: $F_{(20,180)}$=4.07, p<0.0001 and [von Frey]: $F_{(20,180)}$=4.95; p<0.0001) on thermal and mechanical sensitivity in 6-OHDA mice. Again, neither 3.0 mg/kg nor 5.0 mg/kg of Mph influenced thermal (3.0mg/kg: sham: [heat]: q=1.80, p>0.05; [cold]: q=1.77, p>0.05; 6-OHDA: [heat]: q=0.75, p>0.05; [cold]: q=1.20, p>0.05; 5.0mg/kg: sham: [heat]: q=2.94, p>0.05; [cold]: q=2.57, p>0.05; 6-OHDA: [heat]: q=1.76, p>0.05; [cold]: q=2.55, p>0.05) or mechanical ([3.0mg/kg]: sham: q=0.81, p>0.05; 6-OHDA: q=0.72, p>0.05; [5.0mg/kg]: sham: q=1.83, p>0.05; 6-OHDA: q=1.20, p>0.05) thresholds at 4 days post-CFA in both groups. All data are means ± SEM (10 mice per group). [a]p<0.05; [b]p<0.01; [c]p<0.001 *vs* NaCl. [d]p<0.05; [e]p<0.01; [f]p<0.001 *vs* Pre-CFA.

Fig. S2. Ibuprofen decreases mechanical sensitivity but has no effect on locomotor activity.

A. A significant increase was found in the paw withdrawal threshold after Ibuprofen injection in sham ($F_{(1.20)}$=31.40, p<0.0001) and 6-OHDA mice ($F_{(1.20)}$=31.40, p<0.0001). The threshold of 6-OHDA mice remains lower than of sham mice in control (vehicle) conditions (1.13 ± 0.17 g *vs* 3.33 ± 0.42 g; q=5.87; p=0.0002) and after Ibuprofen injection (3.33 ± 0.41 g *vs* 5.33 ± 0.42 g; q=5.34;

p=0.016). Ibuprofen All data are means ± SEM (16 mice per group), ###p<0.001, #p<0.05 *vs* Sham, **p<0.01; ***p<0.001 *vs* vehicle.

B. Spontaneous locomotor activity for 10 min. **B1.** Distance traveled (cm). **B2**. Mobility mean time (s). **B3.** Speed of animal movement (cm/s). There was a significant effect of lesion (6-OHDA) ($F_{(1.20)}$=154.5, p<0.0001; $F_{(1.20)}$=52.66, p<0.0001; $F_{(1.20)}$=63.38, p<0.0001) but not of Ibuprofen ($F_{(1,20)}$=1.59, p=0.22; $F_{(1.20)}$=0.09, p=0.76; $F_{(1.20)}$=13.75, p=0.71) on distance traveled, mobility mean time and speed of animals movement. As compared to sham, 6-OHDA conditions incrased the distance traveled in both vehicle and Ibuprofen groups ([vehicle]: 239.5 ± 18.59 cm *vs* 464.3 ± 42.31; q=12.32, p<0.0001; [Ibuprofen]: 214.4 ± 19.86 cm *vs* 443.3 ± 17.14 cm; q=12.54, p<0.0001), mobility mean time ([vehicle]: 126.7 ± 19.58 s *vs* 221.7 ± 7.73 s; q=7.07; p<0.0001; [Ibuprofen]: 120.2 ± 14.15 s *vs* 220.0 ± 8.78 s; q=7.44, p<0.0001) and speed of animal movement ([vehicle]: 5.15 ± 0.49 cm/s *vs* 8.53 ± 0.54 cm/s; q=7.91, p<0.0001; [Ibuprofen]: 5.29 ± 0.25 cm/s *vs* 8.72 ± 0.36 cm/s; q=8.01, p<0.0001). All the data are means ± SEM, n = 6 mice per group, ###p<0.001 *vs* Sham.

Fig. S3. Methylphenidate (Mph) has no effect on electrical activity of wide-dynamic range (WDR) deep dorsal horn neurons (DHNs).

A. Example of the identification of DHNs as wide dynamic range (WDR) neurons by *in vivo* single unit recording in sham (left) and 6-OHDA (right) mice. Peripheral electrical stimulations elicited two distinct groups of action potentials corresponding to A (short latency) and C (long latency) fibers firing.

B-C. Single unit *in vivo* extracellular recordings of DHNs in response to peripheral mechanical stimulation (von Frey filament) before and after Mph treatment (5.0mg/kg i.p injection) under normal (**B**) (NaCl) and inflammatory pain (**C**) conditions (CFA).

Fig. S4. Dopaminergic but not noradrenergic fibers are altered by the neonatal 6-OHDA lesion.

A-B. Co-detection of TH and DAT (**A**), or TH and DBH (**B**) immunoreactivity in the ACC of adult sham (Aa-c, Ba-c) and 6-OHDA (Ad-f, Bd-f) mice. Arrowheads point to colocalization between markers.

C. Quantifications of TH colocalization with DAT (left) and DBH (right) indicate that the 6-OHDA lesion induces a loss of DAT-containing, dopaminergic fibers (n=6 sections in 4 mice; t-test, p<0.01), with no change in DBH-containing, noradrenergic fibers (n=6 sections in 4 mice; t-test, p>0.05). Bar: 50 µm. NS: non-significant.

Fig. S5. Specific sub-populations of dopaminergic fibers originating from the midbrain are altered by the neonatal 6-OHDA lesion.

A-B. Co-detection of TH and Aldh1a1 (**A**), or TH and VIP (**B**) immunoreactivity in the ACC of adult sham (Aa-c, Ba-c) and 6-OHDA (Ad-f, Bd-f) mice. Arrowheads point to colocalization between markers.

C. Quantifications of TH colocalization with Aldh1a1 (left) and VIP (right) indicate that the 6-OHDA lesion induces a loss of VIP-containing fibers (n=6 sections in 4 mice; t-test, **p<0.01), with no change in Aldh1a1-containing fibers (n=6 sections in 4 mice; t-test, p>0.05). Bar: 50 µm. NS: non-significant.

Fig. S6. Pain conditions influences ADHD-like symptoms.

A. Spontaneous locomotor activity for 10 min. **A1.** Video-tracking representative of locomotor activity in the open field. **A2.** Distance traveled (cm). **A3**. Mobility mean time (s). **A4.** Speed of animal movement (cm/s). There was a significant effect of lesion (6-OHDA) ($F_{(1.36)}$=239.10, p=0.0001; $F_{(1.36)}$=71.50, p=0.0001; $F_{(1.36)}$=118.30, p=0.0001) and inflammation (CFA) ($F_{(1,36)}$=28.87, p=0.0001; $F_{(1.36)}$=27.90, p=0.0001; $F_{(1.36)}$=27.74, p=0.0001) on distance traveled, mobility mean time and speed of animals movement. Interaction 6-OHDA x CFA ($F_{(1.36)}$=0.23, p=0.63; $F_{(1.36)}$=0.024, p=0.88; $F_{(1.36)}$=0.07, p=0.79) had no effect. CFA injection decreased the distance traveled in both groups ([sham]: 231.42 ± 27.79 cm *vs* 381.41 ± 30.84; q=4.89, p<0.01; [6-OHDA]: 690.71 ± 31.47 cm *vs* 870.12 ± 32.33 cm; q=5.85, p<0.01), mobility mean time ([sham]: 69.27 ± 10.18 s *vs* 114.93 ± 9.34 s; q=5.44; p<0.01; [6-OHDA]: 141.58 ± 6.47 s *vs* 184.64 ± 7.03 s; q=5.13, p<0.01) and speed of animal movement ([sham]: 2.24 ± 0.30 cm/s *vs* 4.61 ± 0.36 cm/s; q=5.53, p<0.01; [6-OHDA]: 7.02 ± 0.40 cm/s *vs* 9.17 ± 0.60 cm/s; q=5.00, p<0.01). All the data are means ± SEM, n = 10 mice per group, **p<0.01; ***p<0.001 *vs* NaCl; $^{\#\#\#}$p<0.001 *vs* Sham.

A5-7. Amplitude of changes in distance traveled (**A5**), mobility mean time (**A6**), and velocity (**A7**). CFA amplifies the difference in distance traveled (235.94 ± 38.56 % *vs* 134.60 ± 9.24 %, t=2.56, p=0.02) and speed of animal movement (257.68 ± 42.18 % *vs* 101.26 ± 5.20 %, t=3.68, p=0.02) between the sham and 6-OHDA groups with respect to NaCl paw injection. However, no significant difference in mobility mean time (160.63 ± 50.24 % *vs* 66.09 ± 7.78 %, t=1.86, p=0.08). All the data are mean% of sham ± SEM, n = 10 mice per group, *p<0.05; **p<0.01 *vs* NaCl.

B. Anxiety-like behavior. **B1.** Video-tracking data representative of the exploration in EPM. **B2.** Time spent in EPM open arms (OA). **B3.** Number of entries in EPM closed arms (CA). There was a a major effect of lesion (6-OHDA) ($F_{(1,36)}$=70.75, p=0.0001; $F_{(1,36)}$=18.24, p=0.0001) and inflammation (CFA) ($F_{(1,36)}$=15.35, p=0.0004; $F_{(1,36)}$=20.98, p=0.0001) on time spent and number of entries in OA, while interaction 6-OHDA x CFA ($F_{(1,36)}$=0.009, p=0.92; $F_{(1,36)}$=0.006, p=0.94) had no effect. CFA injection decreased time spent ([sham]: 59.31 ± 6.31 s *vs* 77.71 ± 4.41 s; q=4.02, p<0.05; [6-OHDA]: 21.22 ± 3.26 s *vs* 38.72 ± 3.75 s; q=3.82, p<0.05) and number of entries in OA ([sham]: 8.40 ± 0.75 *vs* 11.40 ± 0.54; q=4.66, p<0.05; [6-OHDA]: 5.70 ± 0.60 *vs* 8.60 ± 0.67; q=4.50, p<0.05) in both groups. All the data are means ± SEM, n=10 mice per group, *p<0.05 *vs* NaCl; $^{\#}$p<0.05; $^{\#\#\#}$p<0.001 *vs* Sham.

B4-5. Amplitude of changes in OA duration (**B4**), and number of entries in OA **(B5)**. CFA conditions amplify the difference between sham and 6-OHDA in open arms duration (-64.98 ± 2.34 % *vs* -51.24 ± 2.36 %, t=4.14, p=0.0006) as compared to the NaCl paw injection group. There was no significant effect in the number of entries in OA (-32.32 ± 2.98 % *vs* -25.55 ± 2.72 %, t=1.68, p=0.11). All the data are mean % of sham ± SEM, n=10 mice per group, ***p<0.001 *vs* NaCl.

C. Mice performance (inter-trial interval [ITI] = 5 sec; stimulus duration [SD] = 1 sec) in 5-CSRTT**. C1.** Percentage of accuracy. **C2.** Percentage of omissions. **C3.** Percentage of premature responding. **C4.** Percentage of perseverations. Two-way repeated measures ANOVA showed a significant effect on all 5-CSRTT parameters ([accuracy]: $F_{(3,27)}$=24.68, p=0.0001; [omission]: $F_{(3,27)}$=55.81, p=0.0001; [premature responding]: $F_{(3,27)}$=118.7, p=0.0001; [perseverations]: $F_{(3,27)}$=43.62, p=0.0001). Inflammation had an effect only on perseverations ([accuracy]: $F_{(1,9)}$=0.017; p=0.90; [omission]: $F_{(1,9)}$=0.92, p=0.37; [premature responding]: $F_{(1,9)}$=4.80, p=0.06; [perseverations]: $F_{(1,9)}$=29.04, p=0.0004). In addition, the interaction group x inflammation had an effect on premature responding and perseverations ($F_{(3,27)}$=9.11, p=0.002; $F_{(3,27)}$=11.43,

p=0.0001; respectively), but not on accuracy and omissions ($F_{(3,27)}$=0.66, p=0.58; $F_{(3,27)}$=0.56, p=0.65; respectively).

6-OHDA adult mice displayed less accuracy than sham mice (68.30 ± 1.54 % *vs* 78.70 ± 1.83 %; q=10.38, p=0.0001), more omission errors (27.90 ± 2.14 % *vs* 10.00 ± 1.91 %; q=14.68, p=0.0001), premature responding (10.10 ± 1.16 % *vs* 2.70 ± 0.80 %; q=7.50, p=0.0001) and perseverations (14.00 ± 2.16 *vs* 7.00 ± 1.05; q=4.47, p<0.05) than sham mice. CFA injection did not affect accuracy ([sham]: 81.20 ± 1.27 % *vs* 80.00 ± 1.41 %; q=0.85, p>0.05; [6-OHDA]: 68.00 ± 1.58 % *vs* 69.50 ± 1.14 %; q=1.06, p>0.05) and omissions ([sham]: 8.80 ± 1.25 % *vs* 8.80 ± 1.68 %; q=0.00; p>0.05; [6-OHDA]: 30.10 ± 1.73 % *vs* 27.30 ± 1.54 %; q=1.62, p>0.05) in both groups. Injection had a significant effect *per se* only in 6-OHDA mice, increasing premature responding ([sham]: 1.40 ± 0.83 % *vs* 2.00 ± 0.63 %; q=0.43, p>0.05; [6-OHDA]: 15.80 ± 1.39 % *vs* 8.90 ± 1.35 %; q=4.95, p=0.001) and perseverations ([sham]: 5.00 ± 1.30 *vs* 7.80 ± 1.29; q=1.26, p>0.05; [6-OHDA]: 24.40 ± 1.83 *vs* 14.70 ± 1.37; q=4.38, p=0.001). However, CFA injection had no specific effect on those parameters. All the data are means ± SEM from 10 mice per group, *p<0.05; **p<0.01; ***p<0.001 *vs* Sham; $^{\#\#}$p<0.01; $^{\#\#\#}$p<0.001 *vs* Pre-CFA.

Fig. S7. Direct optogenetic modulation of ACC excitatory neurons potentiates sensitization of the contralateral paw in 6-OHDA mice.

A. Representative diagram of the viral injection site (left) and optic cannula placement (right) in the left ACC. **A1.** Atlas representation of the ACC region targeted in this study (Atlas images from Paxinos and Franklin, 2001). **A2-A3.** Example of viral expression in excitatory neurons (bar: 1 mm and 50 µm, respectively).

B. Activation of neurons in the left ACC of mice injected with AAV5.CaMKII.ChR2.eGFP and behavioral assessment on the contralateral (right) hind paw. **B1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was a significant effect of 473nm light on withdrawal thresholds to mechanical ([sham]: Before: 4.00 ± 0.53 g *vs* Opto: 2.10 ± 0.44 g; t=3.10, p=0.02; [6-OHDA]: Before: 2.43 ± 0.35 g *vs* Opto: 0.93 ± 0.17 g; t=3.62, p=0.009) and thermal stimuli ([sham]: Before: 25.38 ± 4.67 s *vs* Opto: 11.75 ± 4.12 s; t=3.06, p=0.02; [6-OHDA]: Before: 19.50 ± 2.44 s *vs* Opto: 7.25 ± 1.75 s; t=3.81, p=0.007) in both groups. After the light was off (Recovery), the mechanical and thermal withdrawal thresholds of sham mice ([von Frey]: 3.85 ± 0.72 g; t=0.13, p=0.90; [IR40]: 24.88 ± 5.0 s; t=0.10, p=0.93) and 6-OHDA mice ([von Frey]: 2.35 ± 0.37 g; t=0.13, p>0.90; [IR40]: 19.13 ± 2.00 s; t=0.20, p=0.85) returned to their baseline values. All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01 *vs* Before. **B2.** Amplitude of changes in withdrawal threshold and latency between 'Before' and 'Opto' conditions (% of values before illumination). 6-OHDA lesion enhanced behavioral changes induced by optogenetic activation of ACC excitatory neurons in response to mechanical (-61.52 ± 3.81 % *vs* -49.58 ± 4.23 %; t=2.09, p=0.04), but not thermal, stimulus (-64.52 ± 5.08 % *vs* -59.88 ± 7.25 %; t=0.52, p=0.61). All data are means ± SEM (8 mice per group), *p<0.05 *vs* Sham.

C. Activation of neurons in the left ACC of mice injected with AAV5.CaMKII.ChR2.eGFP and contralateral (right) DHN recording. Quantification of action potentials per 5 seconds upon peripheral mechanical stimuli, before, during and at 2 minutes after illumination. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=89.79, p<0.0001 and $F_{(1,7)}$=47.78, p=0.0002) and light stimulation (Opto) ($F_{(2,14)}$=28.44; p<0.0001 and $F_{(2,14)}$=11.58; p=0.001) on DHNs discharges in response to innocuous (1.4g) and noxious (6.0g) peripheral

stimulation, respectively. The interaction 6-OHDA x Opto ($F_{(2,14)}$=1.45, p=0.27 and $F_{(2,14)}$=0.17, p=0.85) had no effect. There was a significant effect of 473 nm light on DHN activity in response to innocuous (1.4g: [Sham]: Before: 1.88 ± 0.44 *vs* Opto: 4.50 ± 0.42; q=3.85, p<0.05; [6-OHDA]: Before: 5.63 ± 0.57 *vs* On: 10.25 ± 0.99; q=6.79, p<0.001) and noxious stimuli (6.0g: [Sham]: Off: 47.88 ± 4.46 *vs* Opto: 65.13 ± 4.39; q=4.99, p<0.01; [6-OHDA]: Before: 68.50 ± 3.81 *vs* Opto: 89.25 ± 3.13; q=6.01, p<0.01) in both groups. When the light was off, the electrical activity of DHNs neurons returned to baseline levels before illumination in sham ([1.4g]: 1.88 ± 0.48; q=0.00, p>0.05; [6.0g]: 46.75 ± 2.98; q=0.30, p>0.05) and 6-OHDA groups ([1.4g]: 5.63 ± 0.60; q=0.00, p>0.05; [6.0g]: 70.75 ± 3.82; q=0.59, p>0.05). All data are means ± SEM (8 neurons per group), **p<0.01; ***p<0.001 *vs* Sham; $^{\#}$p<0.05; $^{\#\#}$p<0.01; $^{\#\#\#}$p<0.001 *vs* Before.

D. Silencing of neurons in the left ACC of mice injected with AAV5.CaMKII.ArchT.eGFP and behavioral assessment on the contralateral (right) hind paw. **D1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was no significant effect of 575 nm light on withdrawal thresholds to mechanical (Before: 4.43 ± 0.77 g *vs* Opto: 5.88 ± 0.85 g; t=1.34, p=0.22) and thermal stimuli (Before: 29.75 ± 5.26 s *vs* Opto: 39.13 ± 5.89 s; t=0.96, p=0.37) in sham mice. In contrast, there was a significant effect of 575 nm light in withdrawal threshold to mechanical (Before: 1.95 ± 0.31 g *vs* Opto: 4.00 ± 0.65 g; t=2.51, p=0.04) and thermal stimuli (Before: 18.13 ± 2.65 s *vs* Opto: 30.63 ± 2.88 s; t=2.47, p=0.04) in the 6-OHDA group. After the light was off, the mechanical and thermal withdrawal threshold of 6-OHDA mice returned to their baseline values ([von Frey]: 2.03 ± 0.30 g; t=0.18, p=0.86; [IR40]: 18.13 ± 2.17 s; t=0.00, p=0.99). All data are means ± SEM (8 mice per group), *p<0.05 *vs* Before. **D2.** Amplitude of changes in withdrawal threshold and latency between 'Before' and 'Opto' conditions (% of values before illumination). ADHD-like conditions enhanced the analgesic effects induced by optogenetic inhibition of ACC excitatory neurons in response to mechanical stimulus (108.04 ± 26.43 % *vs* 36.01 ± 13.47 %; t=2.43, p=0.03), but not thermal stimulus (76.63 ± 9.61 % *vs* 41.17 ± 16.59 %; t=1.85, p=0.09). All data are means ± SEM (8 mice per group), *p<0.05 *vs* Sham.

E. Silencing of neurons in the left ACC of mice injected with AAV5.CaMKII.ArchT.eGFP and contralateral (right) DHN recording. Quantification of action potentials per 5 seconds of peripheral stimulus (Von Frey filament: 1.4g, 2.0g, 4.0g, 6.0g), before, during and at 2 minutes after illumination. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=128.00, p<0.0001) and light stimulation (Opto) ($F_{(2,14)}$=4.83; p=0.03) on DHNs discharges in response to innocuous (1.4g) peripheral stimulation. Interaction 6-OHDA x Opto ($F_{(2,14)}$=2.87, p=0.09) had no effect. In addition, there were a significant effect of 6-OHDA ($F_{(1,7)}$=64.67, p<0.0001) and the interaction 6-OHDA x Opto ($F_{(2,14)}$=5.04, p=0.02) on DHNs discharges in response to noxious (6.0g) peripheral stimulation, while light stimulation (Opto) ($F_{(2,14)}$=3.22; p=0.07) had no effect. There was no significant effect of laser light in DHNs activity to innocuous ([1.4g]: Before: 2.88 ± 0.30 *vs* Opto: 2.75 ± 0.31; q=0.32, p>0.05) and noxious stimuli ([6.0g]: Before: 50.75 ± 4.07 *vs* Opto: 51.25 ± 3.06; q=0.18, p>0.05) in sham mice. By contrast, there was a significant effect of laser light the activity of DHNs to innocuous ([1.4g]: Before: 6.00 ± 0.46 *vs* Opto: 4.38 ± 0.32; q=4.11, p<0.05) and noxious stimuli ([6.0g]: Before: 82.88 ± 2.84 *vs* Opto: 67.63 ± 3.81; q=5.53, p<0.01) in the 6-OHDA group. When the light was off, the electrical activity of DHNs neurons returned to baseline levels before illumination in 6-OHDA mice ([1.4g]: 6.13 ± 0.40; q=0.40, p>0.05; [6.0g]: 83.00 ± 3.54; q=0.04, p>0.05). All data are means ± SEM (8 neurons per group), *p<0.05, ***p<0.001 *vs* Sham; $^{\#}$p<0.05 *vs* Before.

Fig. S8: Direct optogenetic modulation of ACC excitatory neurons potentiates sensitization of the ipsilateral paw in 6-OHDA mice.

A. Activation of neurons in the left ACC of mice injected with the AAV5.CaMKII.ChR2.eGFP and behavioral assessment on the ipsilateral (left) hind paw. **A1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was a significant effect of 473 nm light on withdrawal thresholds to mechanical ([sham]: Before: 3.93 ± 0.69 g *vs* Opto: 2.10 ± 0.44 g; t=2.56, p=0.04; [6-OHDA]: Before: 2.25 ± 0.25 g *vs* Opto: 0.90 ± 0.13 g; t=4.28, p=0.004) and thermal stimuli ([sham]: Before: 24.63 ± 4.16 s *vs* Opto: 11.75 ± 4.12 s; t=2.65, p=0.03; [6-OHDA]: Before: 19.75 ± 2.47 s *vs* Opto: 7.63 ± 1.76 s; t=3.79, p=0.007) in both groups. After the light was turned off (Recovery), the mechanical and thermal withdrawal thresholds of sham mice ([von Frey]: 3.93 ± 0.58 g; t=0.00, p>0.99; [IR40]: 23.13 ± 4.82 s; t=0.32, p=0.76) and 6-OHDA mice ([von Frey]: 2.35 ± 0.37 g; t=0.35, p=0.74; [IR40]: 18.00 ± 2.31 s; t=0.65, p=0.54) returned to their baseline values before illumination. All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01 *vs* Before. **A2.** Amplitude of changes in pain thresholds (% of values before illumination). There was no significant effect caused by ADHD-like conditions in behavioral changes elicited by optogenetic activation of ACC excitatory neurons in response to mechanical (-59.38 ± 5.31 % *vs* -44.61 ± 5.49 %; t=1.95, p=0.07) and thermal (-63.95 ± 4.70 % *vs* -58.30 ± 8.44 %; t=0.59, p=0.57) stimuli. All data are means ± SEM (8 mice per group).

B. Silencing of neurons in the left ACC of mice injected with the AAV5.CaMKII.ArchT.eGFP and behavioral assessment on the ipsilateral (left) hind paw. **B1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was a tendency but no significant effect of 575 nm light on withdrawal thresholds to mechanical (Before: 4.05 ± 0.96 g *vs* Opto: 5.88 ± 0.85 g; t=1.65, p=0.14) and thermal stimuli (Before: 29.88 ± 7.30 s *vs* Opto: 39.50 ± 5.12 s; t=1.53, p=0.17) in sham mice. In contrast, there was a significant effect of 575 nm light on withdrawal thresholds to mechanical (Before: 2.23 ± 0.41 g *vs* Opto: 4.50 ± 0.73 g; t=2.89, p=0.02) and thermal (Before: 19.75 ± 3.58 s *vs* Opto: 31.38 ± 3.85 s; t=3.07, p=0.02) stimuli in the 6-OHDA group. After the light was off, the mechanical and thermal withdrawal thresholds of 6-OHDA mice returned to their baseline values ([von Frey]: 1.90 ± 0.33 g; t=1.24, p=0.25; [IR40]: 20.00 ± 2.71 s; t=0.04, p=0.97). All data are means ± SEM (8 mice per group), *p<0.05 *vs* Before. **B2.** Amplitude of changes in pain thresholds (% of values before illumination). There was no significant effect caused by ADHD-like conditions in behavioral changes elicited by optogenetic inhibition of ACC excitatory neurons in response to mechanical (109.82 ± 19.97 % *vs* 81.55 ± 33.68 %; t=0.72, p=0.48) and thermal (71.83 ± 19.04 % ± 64.80 ± 22.37 %; t=0.24, p=0.81) stimuli. All data are means ± SEM (8 mice per group).

Fig. S9: Control of the effects of ACC neurons optogenetic modulation on nociceptive sensitization.

A. Illumination of neurons in the left ACC of mice injected with the AAV5.CaMKII.eGFP and behavioral assessment. **A1.** Von Frey and Hargreaves tests on contralateral hind paw. **A2.** Von Frey and Hargreaves tests on ipsilateral hind paw. There was no significant effect of 473 nm light before (Before), during (Opto) and at 2 minutes after (Recovery) illumination on mechanical or thermal thresholds of hind paw of sham mice (upper panels; ipsilateral: [von Frey]: t=0.00, p>0.99; [IR40]: t=0.09, p=0.93; contralateral: [von Frey]: t=0.11, p=0.92; [IR40]: t=0.06, p=0.95) and 6-OHDA mice (lower panels; ipsilateral: [von Frey]: t=0.17, p=0.87; [IR40]: t=0.00, p>0.99;

contralateral: [von Frey]: t=0.04, p=0.97; [IR40]: t=0.24, p=0.82). All data are means ± SEM (8 mice per group).

B. Illumination of neurons in the left ACC of mice injected with the AAV5.CaMKII. eGFP and contralateral (right) DHN recording. **B1.** Single unit *in vivo* extracellular recording of DHN activity in response to peripheral mechanical stimuli. **B2.** Quantification of action potentials per 5 seconds upon peripheral mechanical stimulus, before, during and after 2 minutes of 473 nm light. Two-way repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=91.31, p<0.0001 and $F_{(1,7)}$=34.94, p=0.0006) on DHN discharge in response to innocuous (1.4g) and noxious (6.0g) peripheral stimulation, respectively. Light stimulation (Opto) ($F_{(2,14)}$=0.09; p=0.92 and $F_{(2,14)}$=0.02; p=0.98) and the interaction 6-OHDA x Opto ($F_{(2,14)}$=0.27, p=0.77 and $F_{(2,14)}$=0.007, p=0.99) had no effect. There was no significant effect of 473 nm light on DHN activity in response to innocuous ([1.4g]: sham: q=0.74, p>0.05; 6-OHDA: q=0.00, p>0.05) and noxious stimuli ([6.0g]: sham: q=0.08, p>0.05; 6-OHDA: q=0.04, p>0.05) in both groups. All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01; ***p<0.001 *vs* Sham.

Fig. S10. Optogenetic modulation of the ACC – Posterior Insula (PI) excitatory pathway potentiates sensitization of the ipsilateral paw in 6-OHDA mice.

A. Activation of the left ACC-PI excitatory pathway in mice injected with the AAV5.CaMKII.ChR2.eGFP and behavioral assessment on the ipsilateral (left) hind paw. **A1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was a significant effect of 473 nm light on withdrawal thresholds to mechanical ([Sham]: Before: 4.18 ± 0.74 g *vs* Opto: 2.03 ± 0.30 g; t=2.97, p=0.02; [6-OHDA]: Before: 2.18 ± 0.27 g *vs* Opto: 1.43 ± 0.10 g; t=3.70, p=0.008) and thermal ([Sham]: Before: 24.63 ± 3.94 s *vs* Opto: 15.13 ± 3.25 s; t=3.43, p=0.02; [6-OHDA]: Before: 17.25 ± 1.22 s *vs* Opto: 10.50 ± 1.02 s; t=3.56, p=0.009) stimuli in both groups. After the light was off (Recovery), changes in mechanical and thermal withdrawal thresholds of sham mice ([von Frey]: 2.13 ± 0.42 g; t=2.43, p=0.04; [IR40]: 15.00 ± 2.69 s; t=2.46, p=0.04) were maintained until 2 minutes after the optogenetic stimulation was stopped, and returned to the baseline values before illumination after 5 minutes ([von Frey]: 4.00 ± 0.65 g; t=0.14, p=0.89; [IR40]: 24.63 ± 3.86 s; t=0.00, p>0.99). In contrast, changes in mechanical and thermal withdrawal thresholds of the 6-OHDA group were further amplified at 2 minutes after the illumination was off ([von Frey]: 0.55 ± 0.07 g; t=5.40, p=0.001; [IR40]: 6.00 ± 0.73 s; t=7.83, p=0.0001). After 5 minutes, thermal withdrawal latency returned to baseline levels ([IR40]: 15.88 ± 1.90 s; t=0.52, p=0.62), while the mechanical threshold was not fully restored ([von Frey]: 1.35 ± 0.17 g; t=3.43, p=0.02). All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01; ***p<0.001 *vs* Before; $^{\#\#}$p<0.01; $^{\#\#\#}$p<0.001 *vs* Opto. **A2.** Amplitude of changes in withdrawal threshold and latency between 'Before' and 'Opto' or 'Recovery (2 min)' conditions (% of values before illumination). Two-way ANOVA showed no significant effect on the mechanical withdrawal threshold of lesion (6-OHDA) ($F_{(1,28)}$=1.16, p=0.29), light stimulation (Opto) ($F_{(1,28)}$=0.07; p=0.79) and interaction 6-OHDA x Opto ($F_{(1,28)}$=0.17, p=0.68). In contrast, there was a significant effect of lesion (6-OHDA) $F_{(1,28)}$=7.67, p=0.001), but not light stimulation (Opto) $F_{(1,28)}$=3.53; p=0.07), on thermal withdrawal latency. The interaction 6-OHDA x Opto had a main effect on thermal withdrawal latency $F_{(1,28)}$=5.32, p=0.03). Changes were greater in 6-OHDA conditions than in sham at 2 minutes recovery in response to thermal (-65.91 ± 1.78 % *vs* -34.39 ± 8.04 %; q=5.08, p<0.01), but not to mechanical (-37.14 ± 6.59 % *vs* -41.88

± 9.01 %; q=0.67, p>0.05) stimulus. All data are means ± SEM (8 mice per group), *p<0.05 *vs* Sham; $^{\#\#}$p<0.01; *vs* Opto.

B. Silencing of the left ACC-PI excitatory pathway in mice injected with AAV5.CaMKII.ArchT.eGFP and behavioral assessment on the ipsilateral (left) hind paw. **B1.** Von Frey and Hargreaves tests before (Before), during (Opto) and at 2 minutes after (Recovery) illumination. There was a significant effect of 575 nm light on withdrawal thresholds to mechanical ([sham]: Before: 4.00 ± 0.53 g *vs*, Opto: 5.25 ± 0.65 g; t=3.42, p=0.02; [6-OHDA]: Before: 1.80 ± 0.14 g *vs* Opto: 3.25 ± 0.37 g; t=3.71, p=0.008) and thermal ([sham]: Before: 24.25 ± 3.41 s *vs* Opto: 32.63 ± 2.83 s; t=2.51, p=0.04; [6-OHDA]: Before: 19.75 ± 1.54 s *vs* Opto: 28.63 ± 2.10 s; t=3.56, p=0.009) stimuli in both groups. After the light was off, changes in mechanical and thermal withdrawal in sham mice were maintained during 2 minutes ([von Frey]: 5.50 ± 0.50 g; t=2.39, p=0.04; [IR40]: 33.25 ± 2.48 s; t=2.46, p=0.04), and returned to the baseline values after 5 minutes ([von Frey]: 3.75 ± 0.45 g; t=0.31, p=0.76; [IR40]: 25.25 ± 2.90 s; t=0.20, p=0.85). In contrast, changes in mechanical and thermal pain threshold of the 6-OHDA group were further amplified at 2 minutes after the illumination was off ([von Frey]: 4.50 ± 0.33 g; t=5.87, p=0.0006; [IR40]: 34.38 ± 1.65 s; t=8.21, p<0.0001). After 5 minutes, this increase was fully abolished in the 6-OHDA group ([von Frey]: 1.95 ± 0.31 g; t=0.52, p=0.62; [IR40]: 18.75 ± 1.70 s; t=0.44, p=0.67). All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01; ***p<0.001 *vs* Before; $^{\#}$p<0.05 *vs* Opto. **B2.** Amplitude of changes in withdrawal threshold and latency between 'Before' and 'Opto' or 'Recovery (2 min)' conditions (% of values before illumination). Two-way ANOVA showed no significant effect on the mechanical withdrawal threshold of lesion (6-OHDA) ($F_{(1,28)}$=20.84, p<0.0001), light stimulation (Opto) ($F_{(1,28)}$=7.22; p=0.01), and interaction 6-OHDA x Opto ($F_{(1,28)}$=3.86, p=0.06). Similarly, there was no significant effect on thermal thresholds of lesion (6-OHDA) $F_{(1,28)}$=2.37, p=0.13), light stimulation period (Opto) $F_{(1,28)}$=2.64; p=0.12) and interaction 6-OHDA x Opto $F_{(1,28)}$=1.36, p=0.25). Changes were greater in 6-OHDA conditions than in sham at 2 minutes recovery in response to mechanical (160.71 ± 26.00 % *vs* 47.92 ± 13.52 %; q=6.53, p<0.001), but not thermal (81.58 ± 16.06 % *vs* 48.46 ± 12.59 %; q=2.71, p>0.05) stimulus. All data are means ± SEM (8 mice per group), ***p<0.001 *vs* Sham; $^{\#}$p<0.05 *vs* Opto.

Fig. S11. Control of the effects of optogenetic modulation of the ACC-PI pathway on nociceptive sensitization.

A. Illumination of the left ACC-PI pathway of mice injected with the AAV5.CaMKII.eGFP and behavioral assessment. **A1.** Von Frey and Hargreaves tests on contralateral hind paw. **A2.** Von Frey and Hargreaves tests on ipsilateral hind paw. There was no significant effect of 473 nm light before (Before), during (Opto) and at 2 minutes after (Recovery) illumination on mechanical or thermal thresholds of hind paw of sham mice (upper panels; ipsilateral: [von Frey]: t=0.24, p=0.82; [IR40]: t=0.09, p=0.93; contralateral: [von Frey]: t=0.00, p>0.99; [IR40]: t=0.16, p=0.88) and 6-OHDA mice (lower panels; ipsilateral: [von Frey]: t=0.15, p=0.88; [IR40]: t=0.00, p>0.99; contralateral: [von Frey]: t=0.00, p=0.99; [IR40]: t=0.14, p=0.89) hind paw. All data are means ± SEM (8 mice per group).

B. Illumination of the left ACC-PI pathway of mice injected with the AAV5.CaMKII.eGFP and contralateral (right) DHN recording. **B1.** Single unit *in vivo* extracellular recording of DHN activity in response to peripheral mechanical stimuli. **B2.** Quantification of action potentials per 5 seconds upon peripheral stimulus, before, during and after 2 minutes of 473 nm light. Two-way

repeated measures ANOVA showed a significant effect of lesion (6-OHDA) ($F_{(1,7)}$=111.30, p<0.0001 and $F_{(1,7)}$=92.60, p<0.0001) on DHN discharges in response to innocuous (1.4g) and noxious (6.0g) peripheral stimulation, respectively. Light stimulation (Opto) ($F_{(3,21)}$=0.08; p=0.97 and $F_{(3,21)}$=0.09; p=0.96) and the interaction 6-OHDA x Opto ($F_{(3,21)}$=0.02, p=0.99 and $F_{(3,21)}$=0.04, p=0.99) had no effect. There was no significant effect of 473 nm light on DHN activity in response to innocuous ([1.4g]: sham: q=0.00, p>0.05; 6-OHDA: q=0.38, p>0.05) and noxious stimuli ([6.0g]: sham: q=0.58, p>0.05; 6-OHDA: q=0.13, p>0.05) in both groups. All data are means ± SEM (8 mice per group), *p<0.05; **p<0.01; ***p<0.001 *vs* Sham.

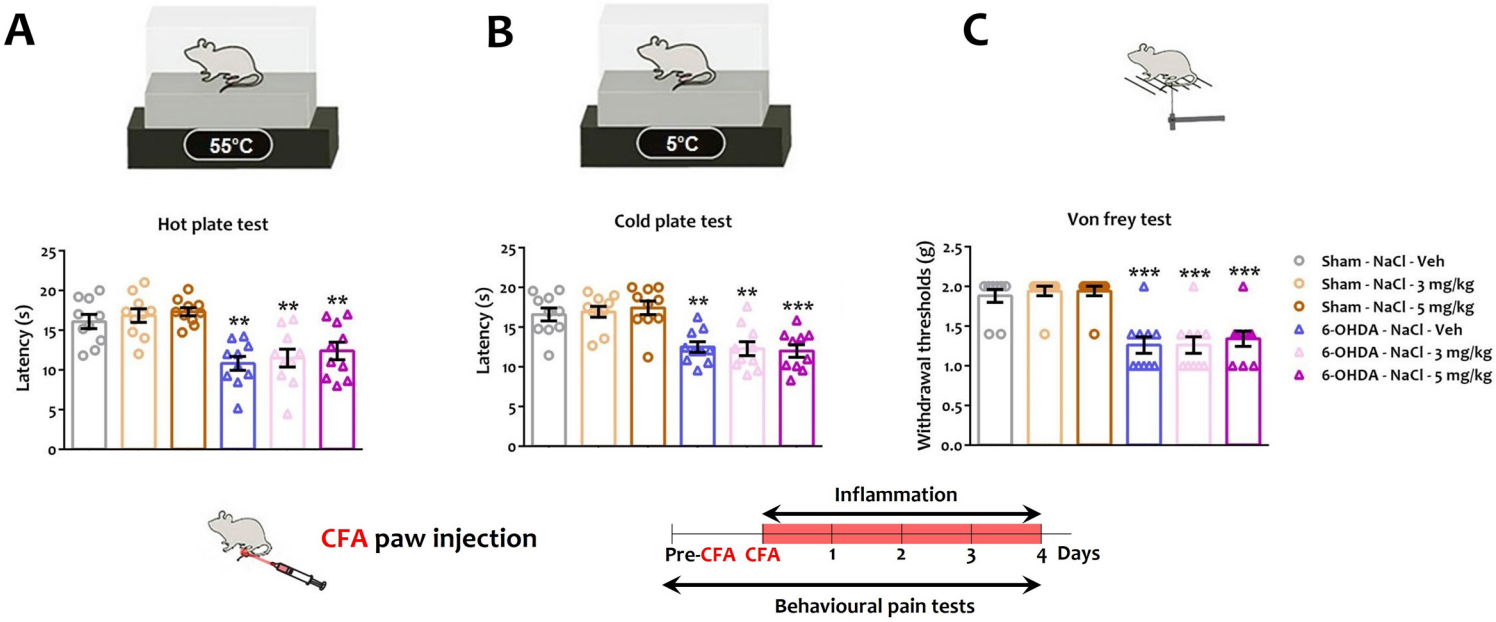
Fig. S12. Schematic representation of the experimental design.

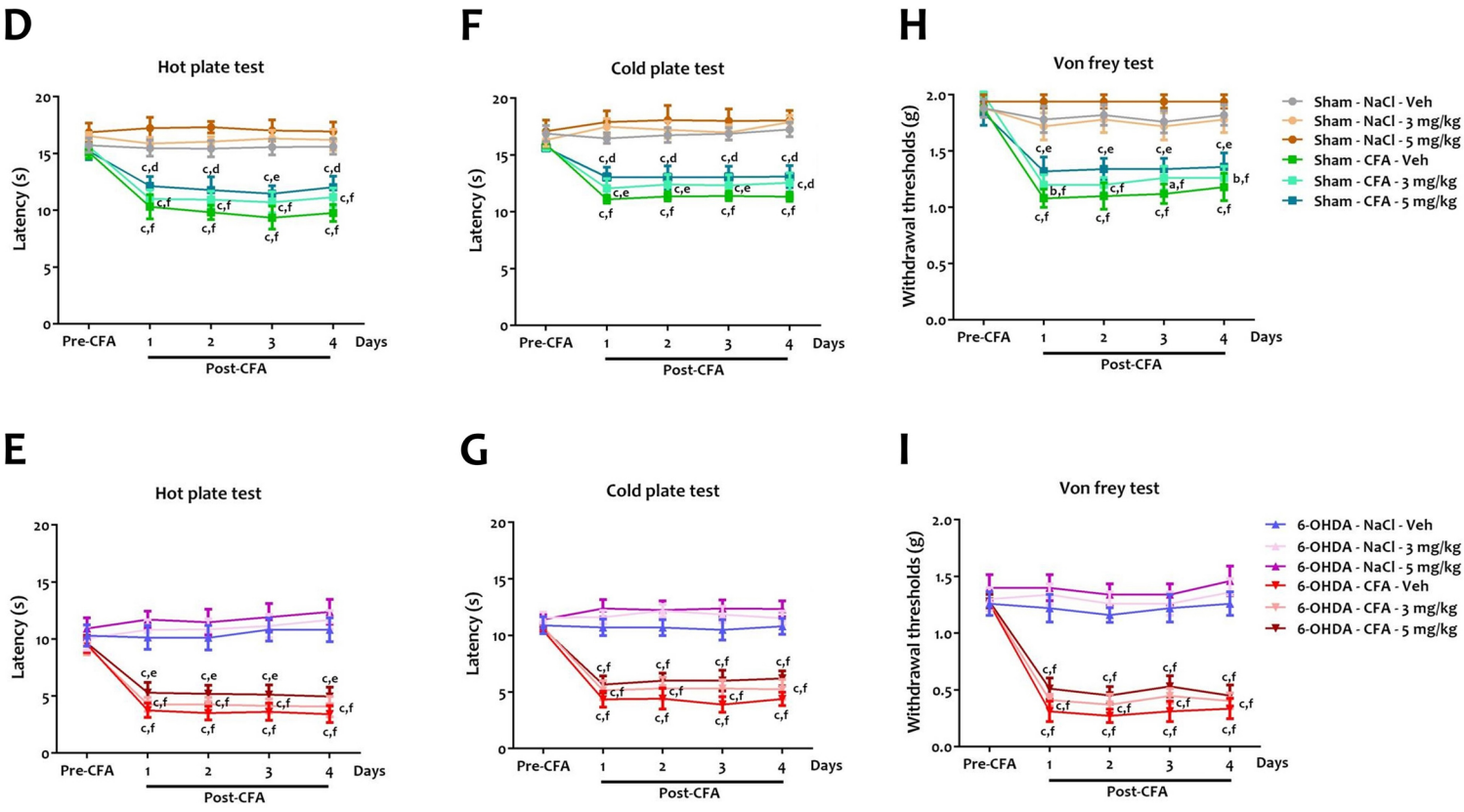
A. Animal experimentation design related to figures 1, 2, 3, S2 and S3.

B. Animal experimentation design related to figures 4.

C. Animal experimentation design related to figure 5.

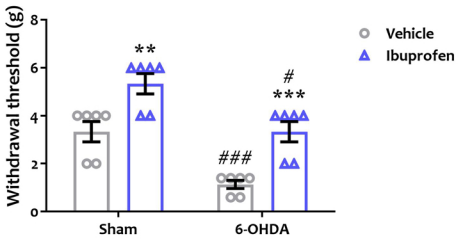
D. Animal experimentation design related to figure 6, 7, S4, S5, S6 and S7.





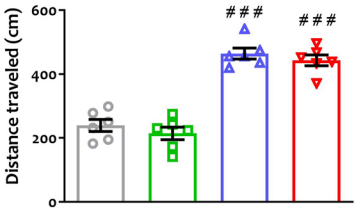
Bouchatta et al., Figure S1

A



B

B1



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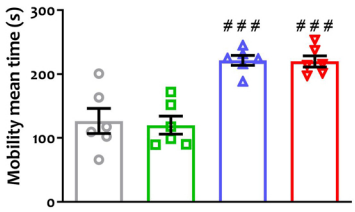

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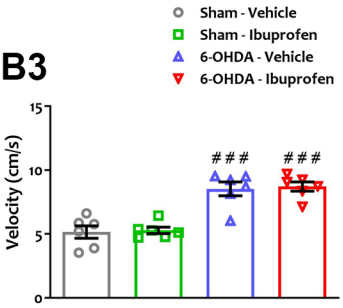
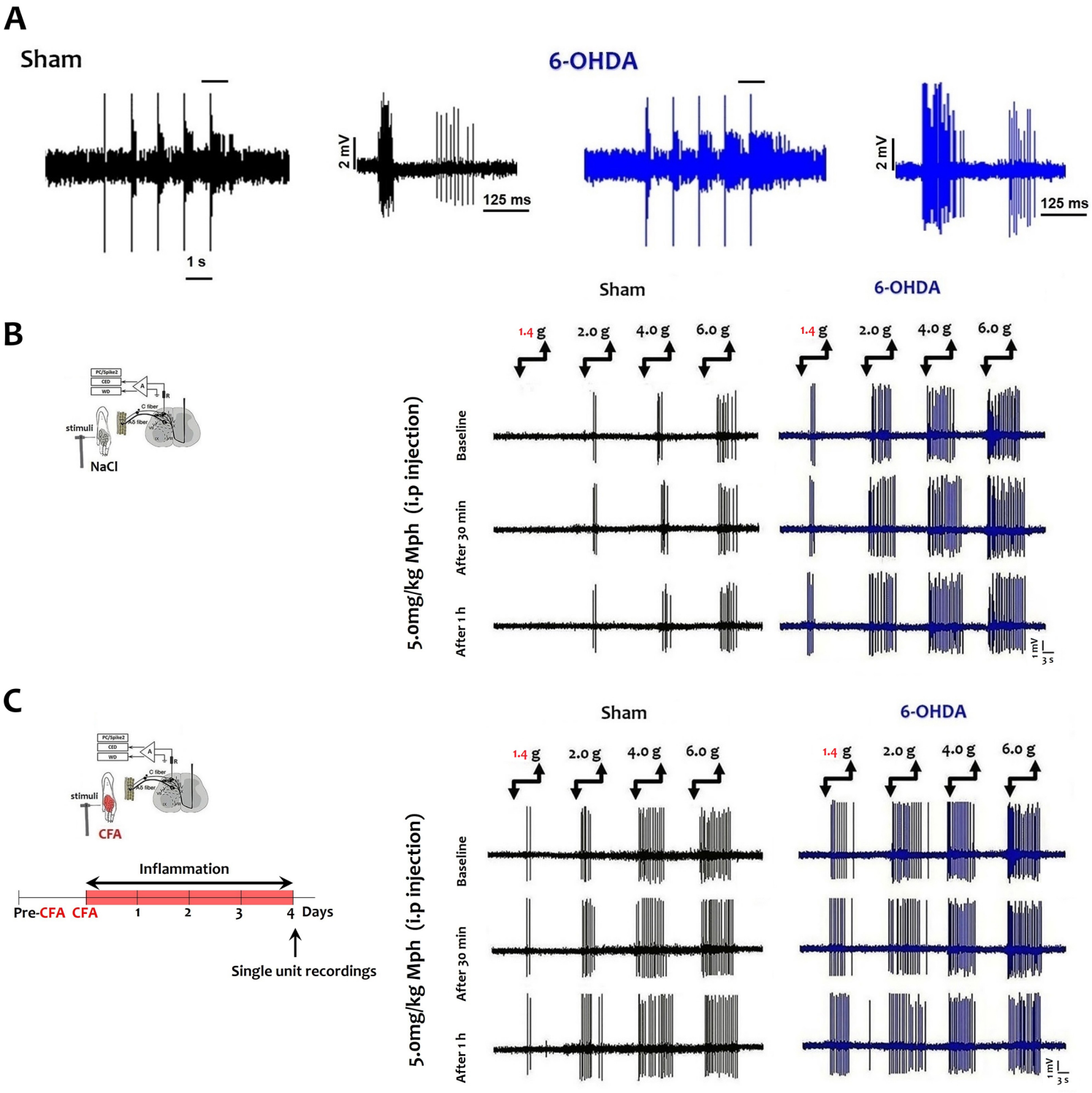
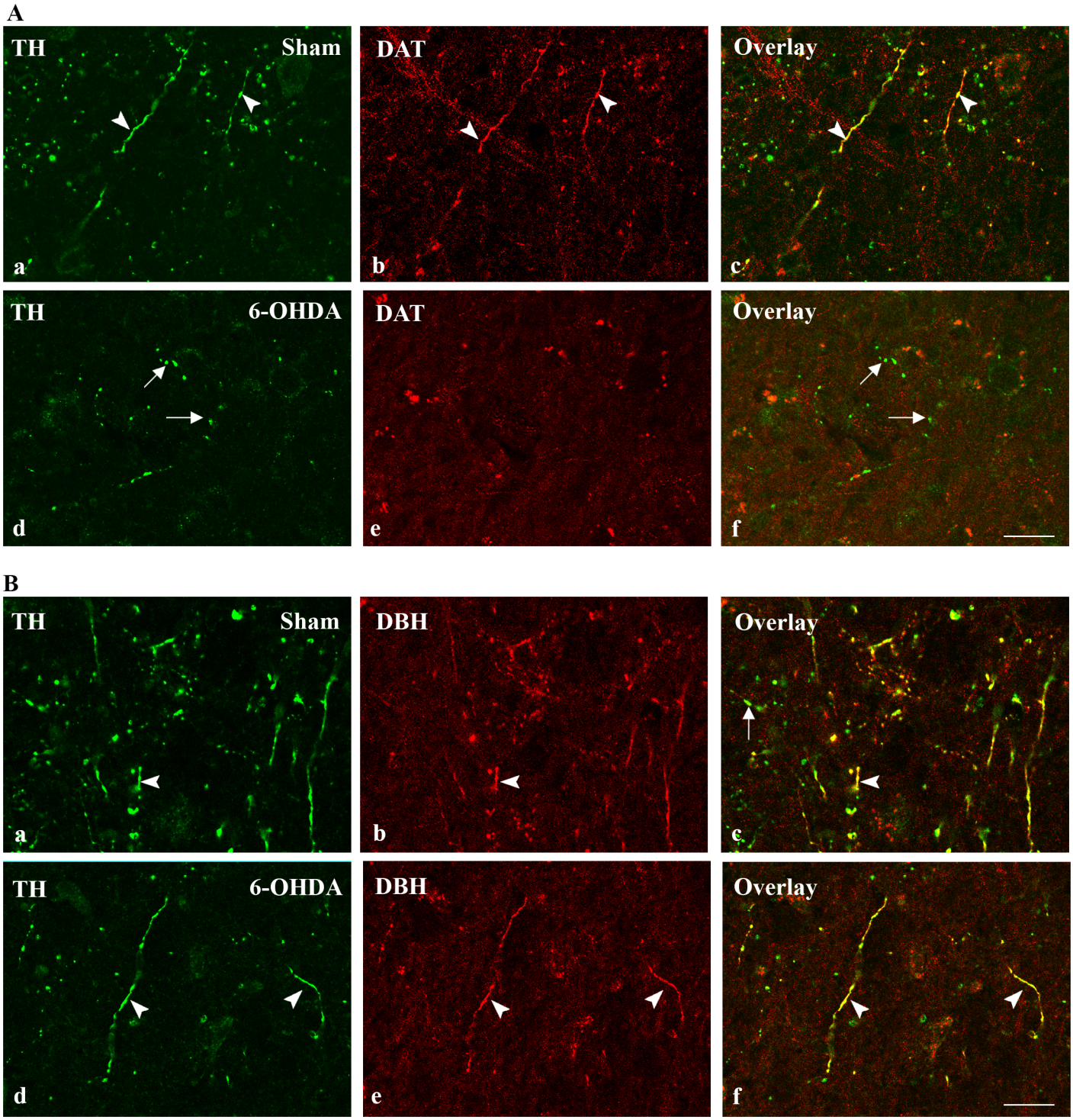
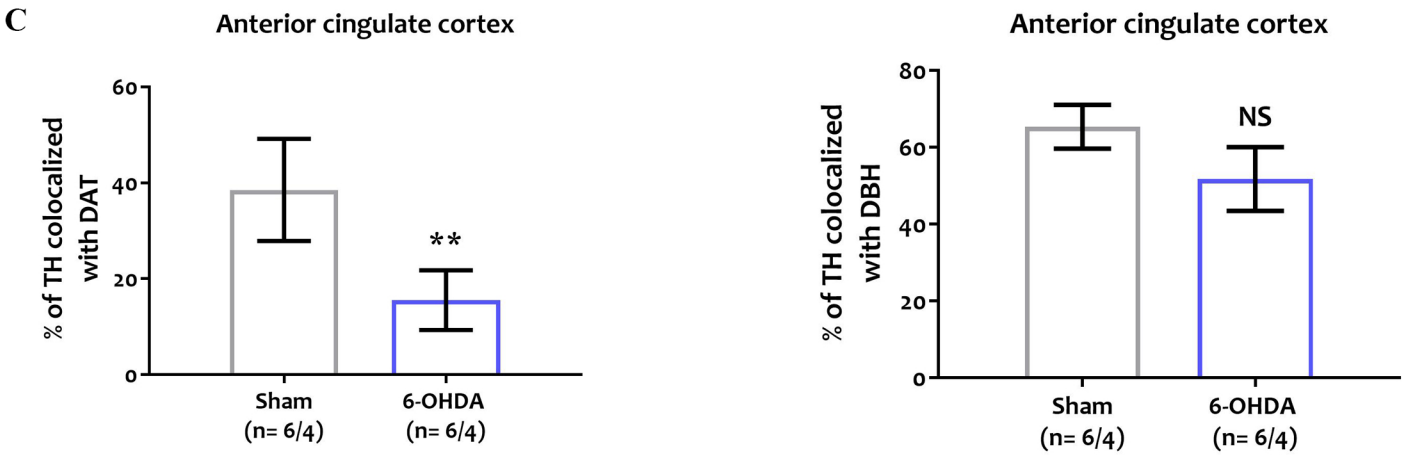


Bouchatta et al., Figure S2



Bouchatta et al., Figure S3





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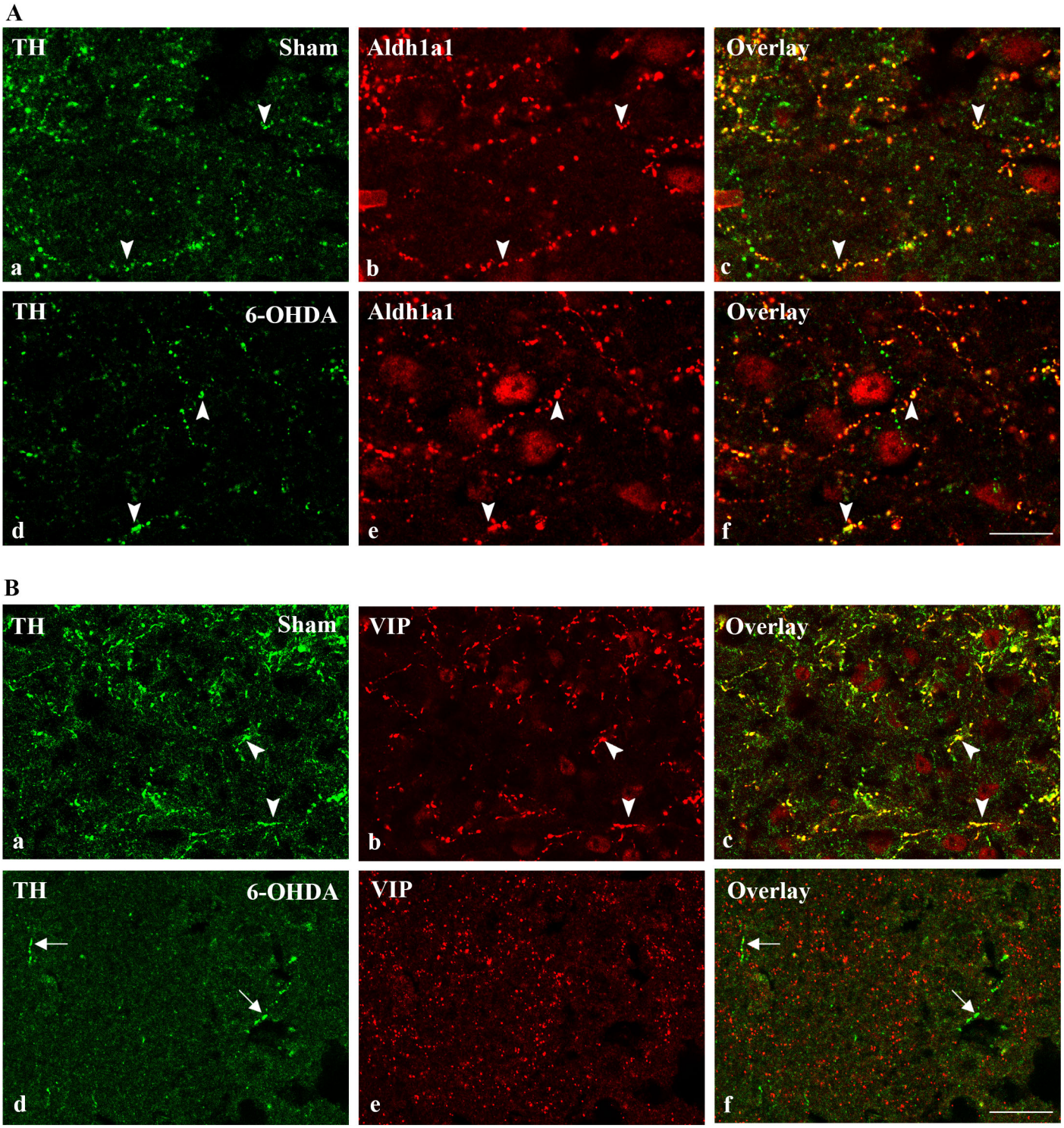

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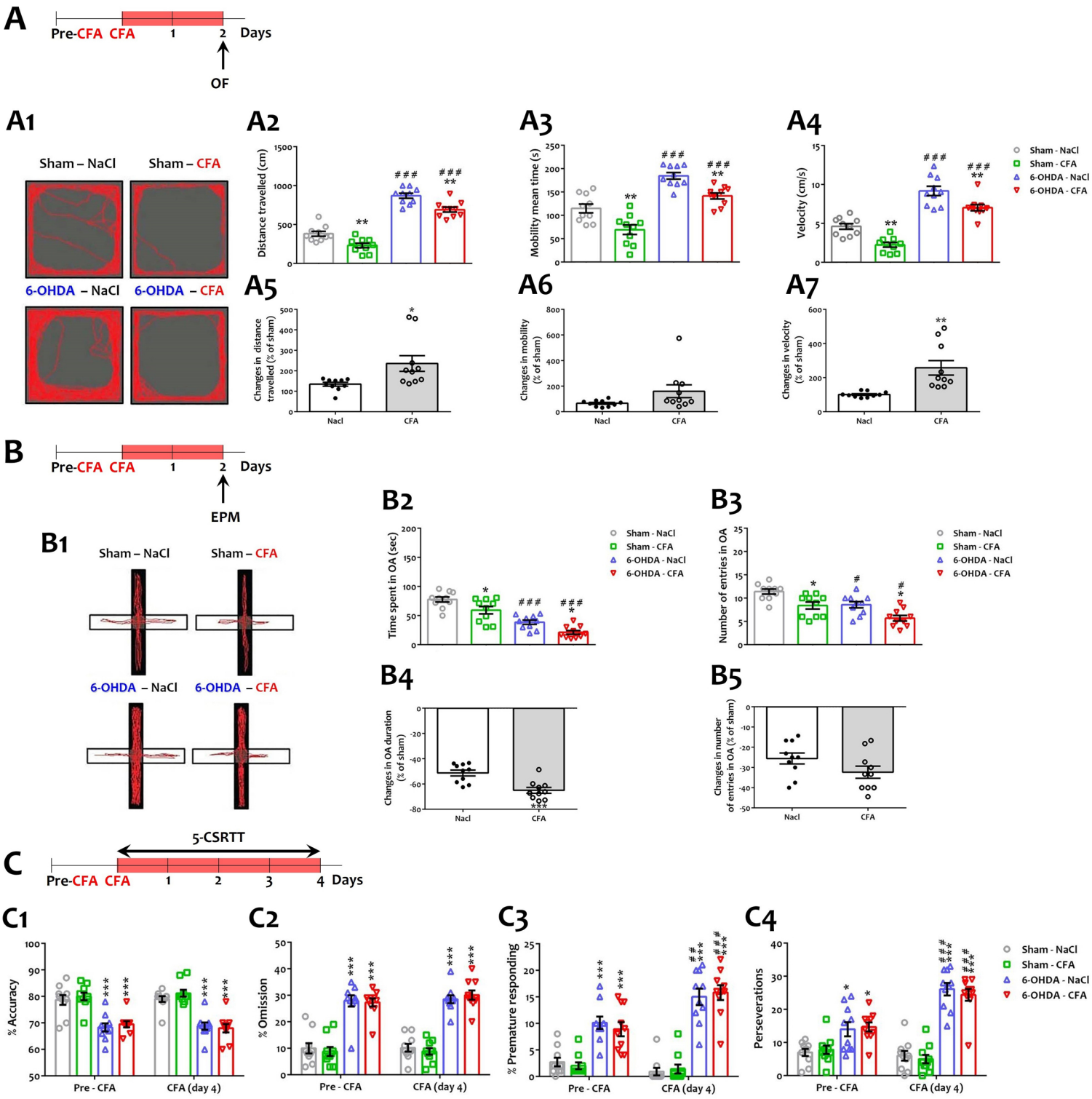

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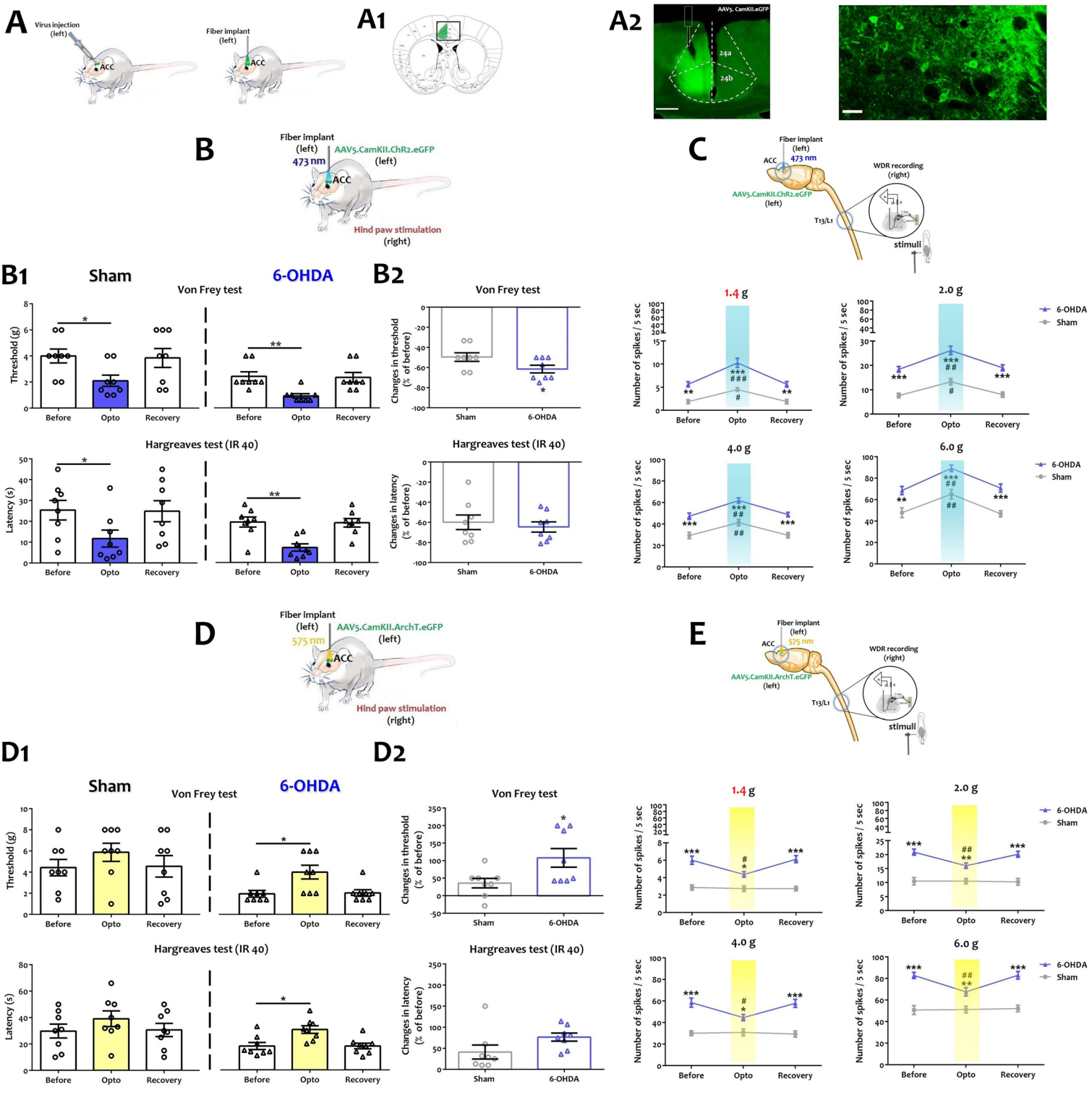

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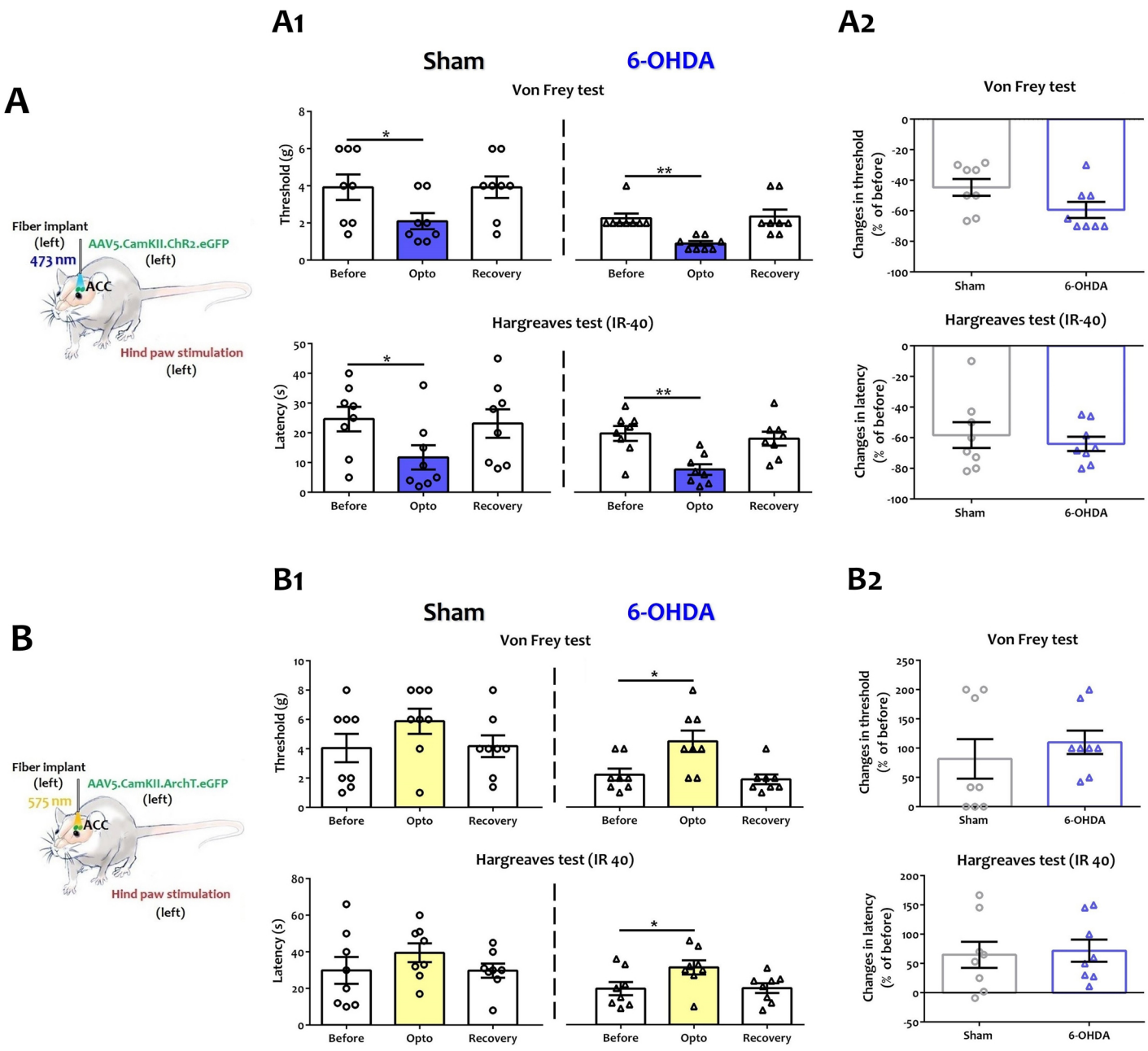

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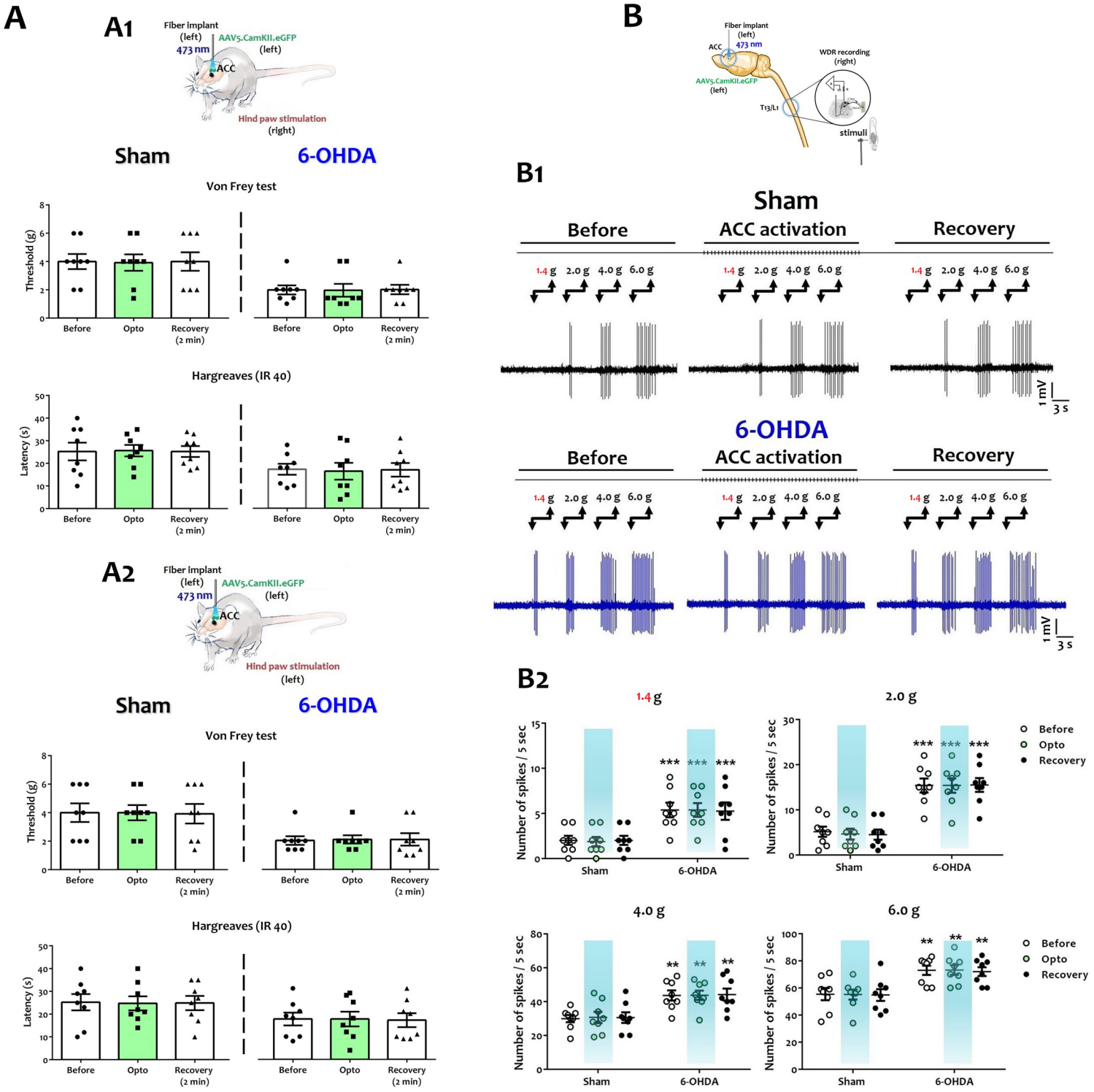
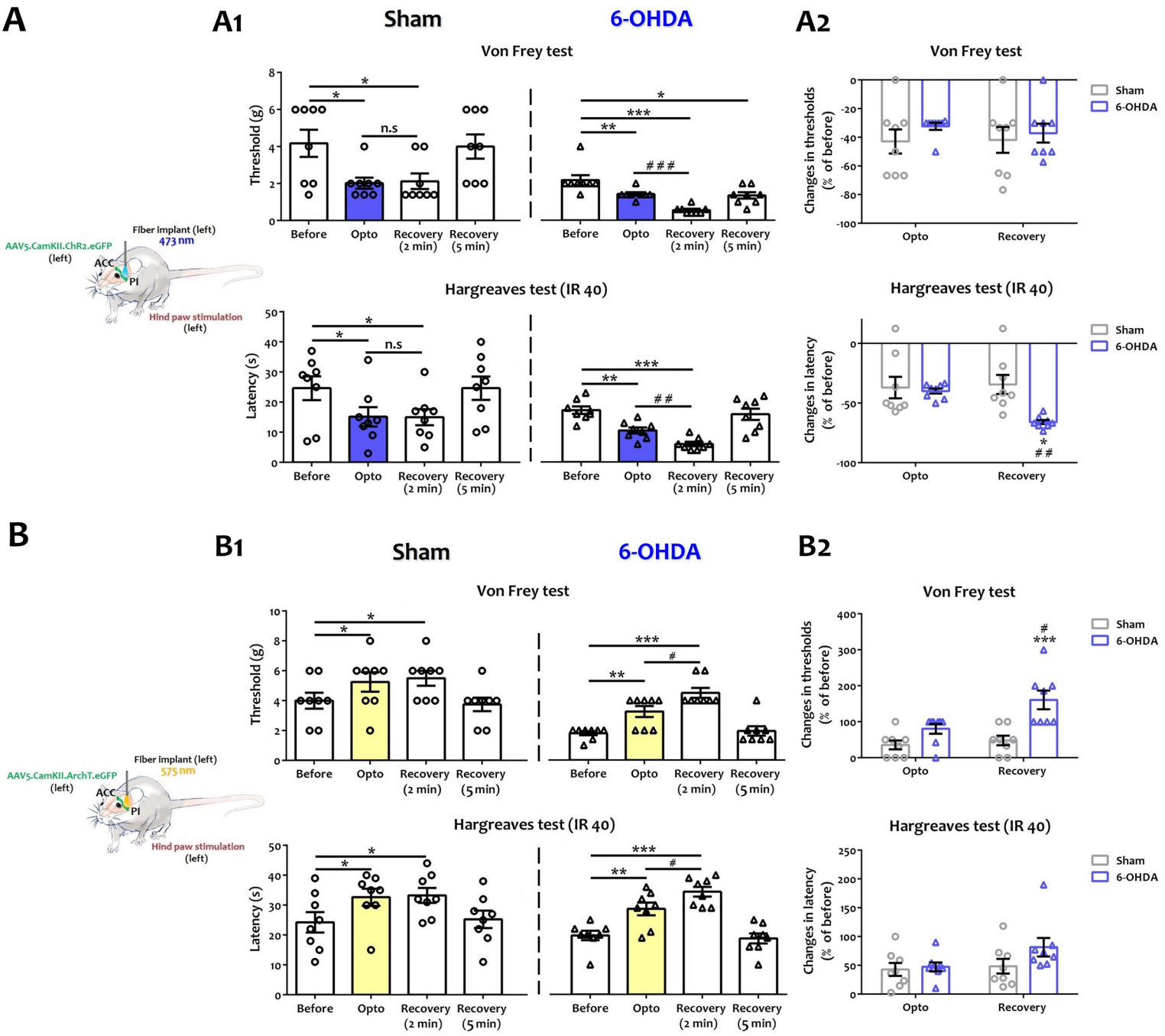
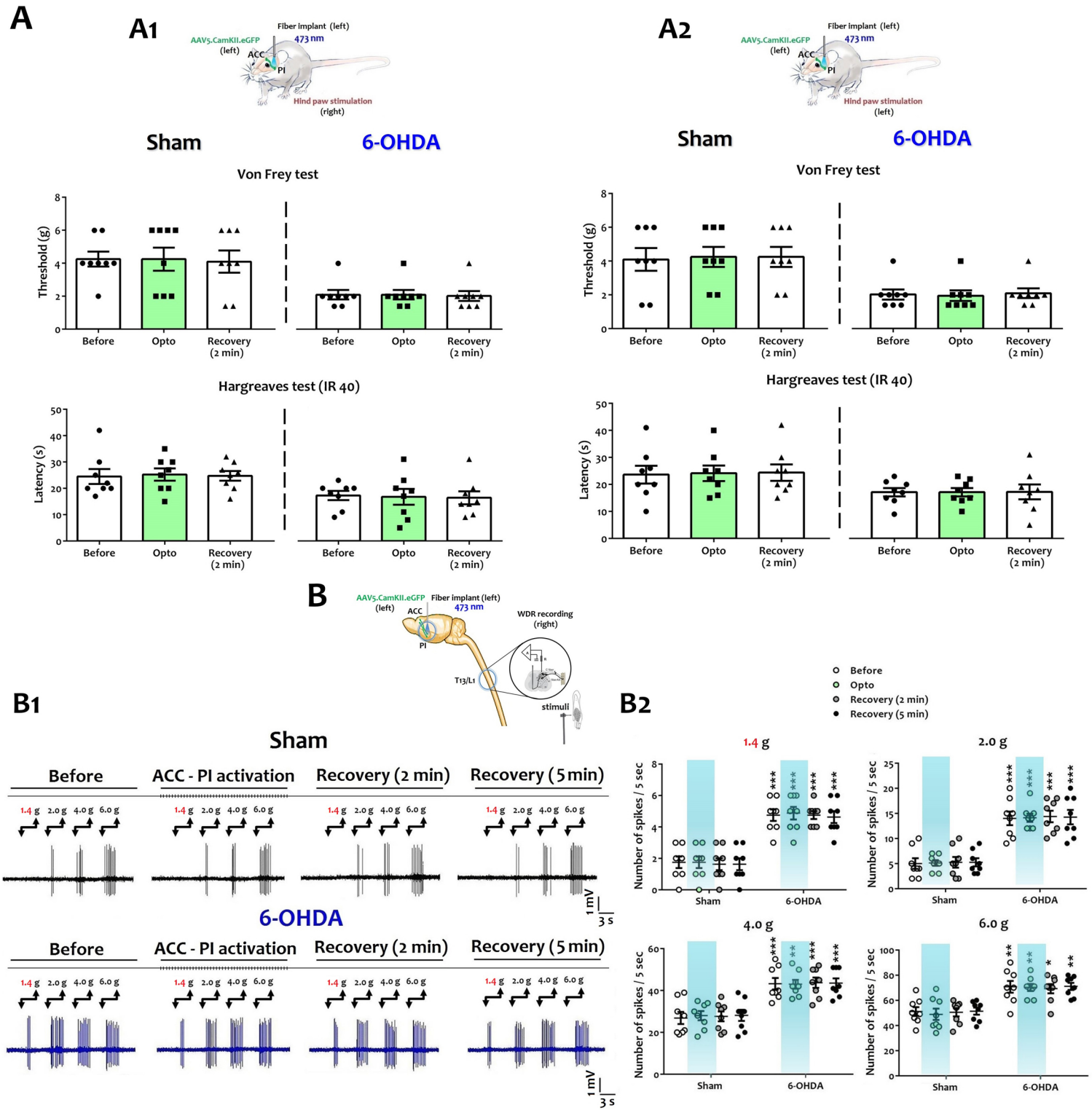


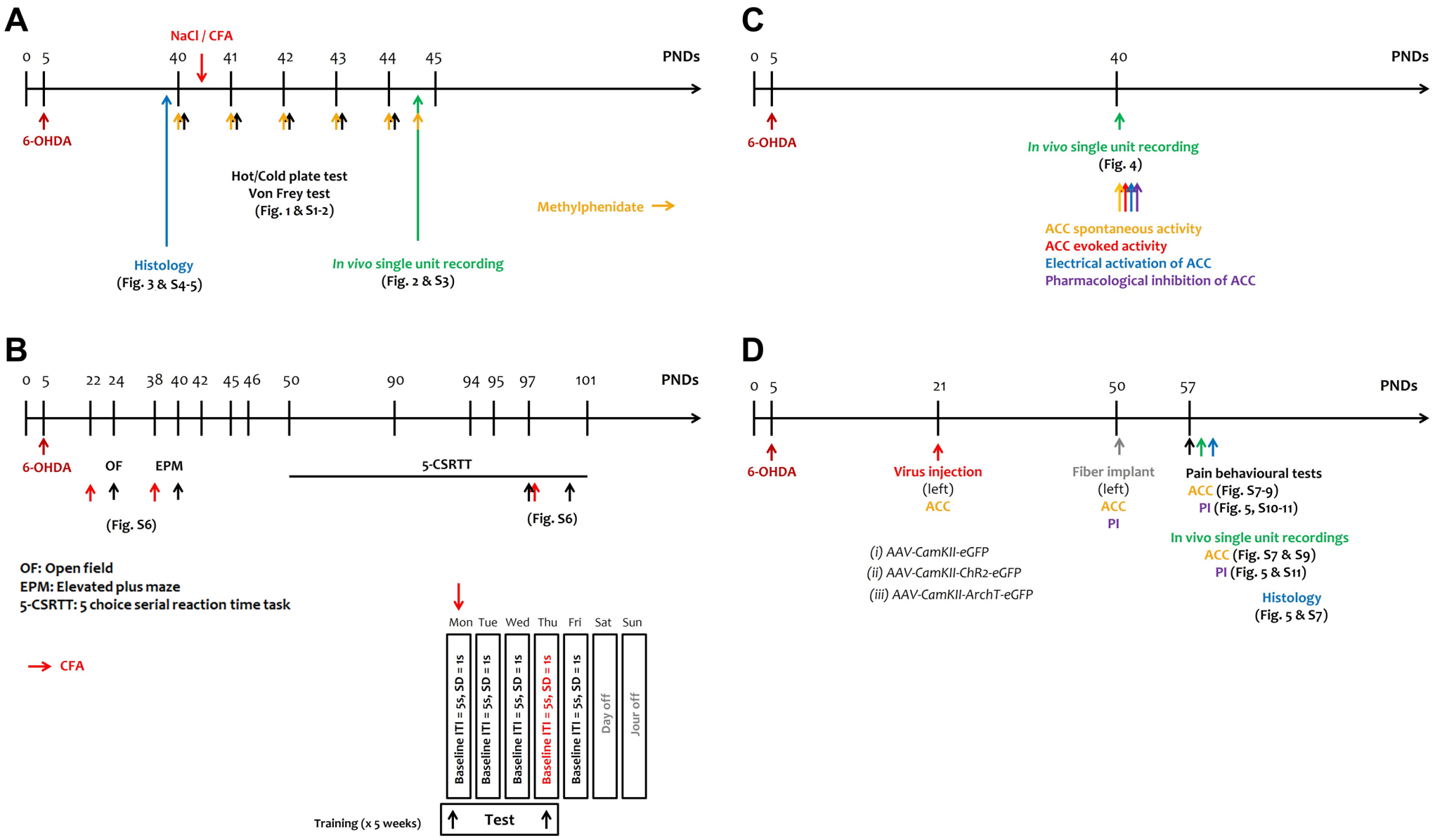
Bouchatta et al., Figure S9



Bouchatta et al., Figure S10



Bouchatta et al., Figure S11



Bouchatta et al., Figure S12