SUPPLEMENTARY FIGURE LEGENDS

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. 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. S3: 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. S4: 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. S5. 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. S6. 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.

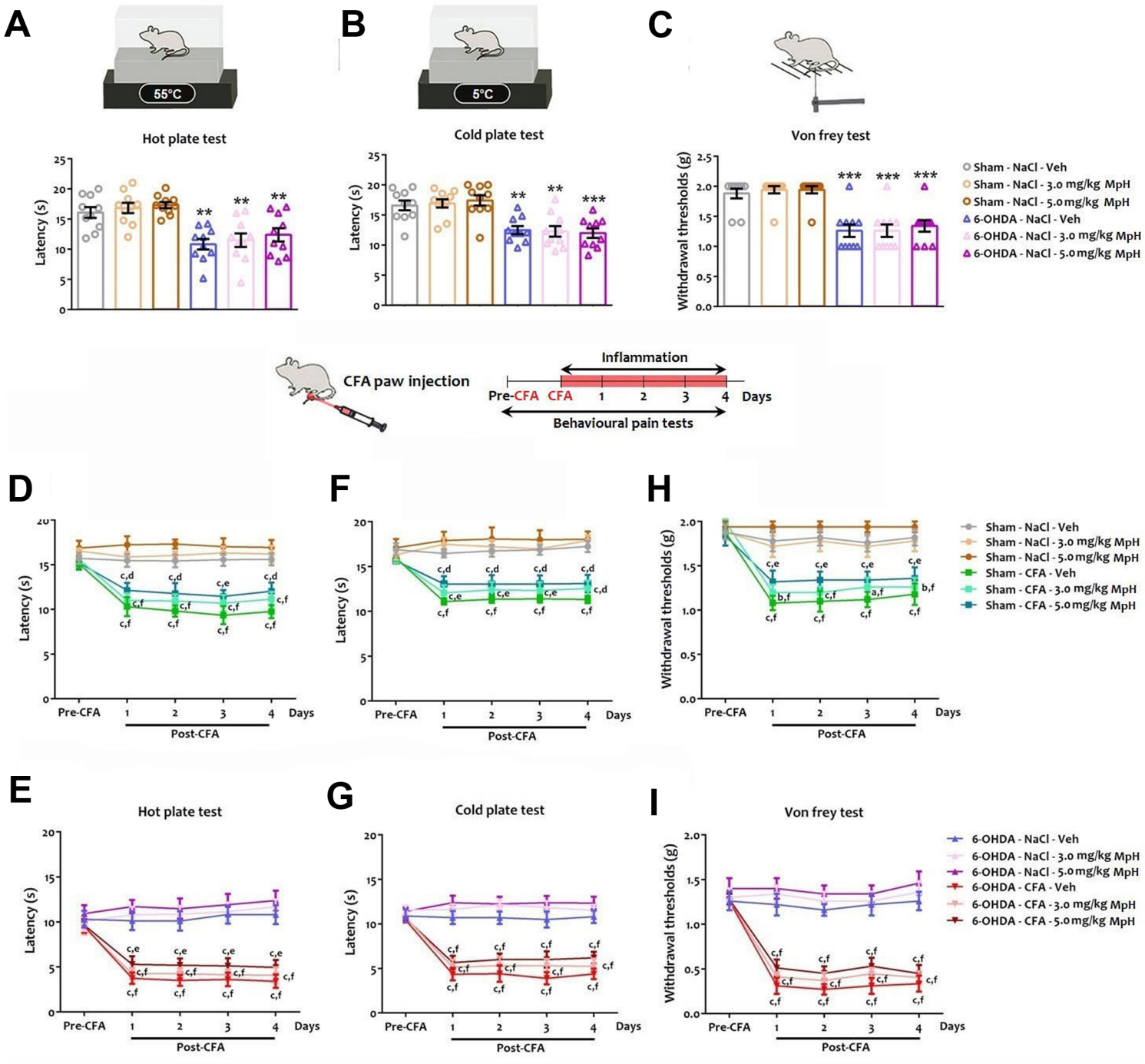
Fig. S7. Schematic representation of the experimental design.

A. Animal experimentation design related to figures 1, 2, 3, S1 and S2.

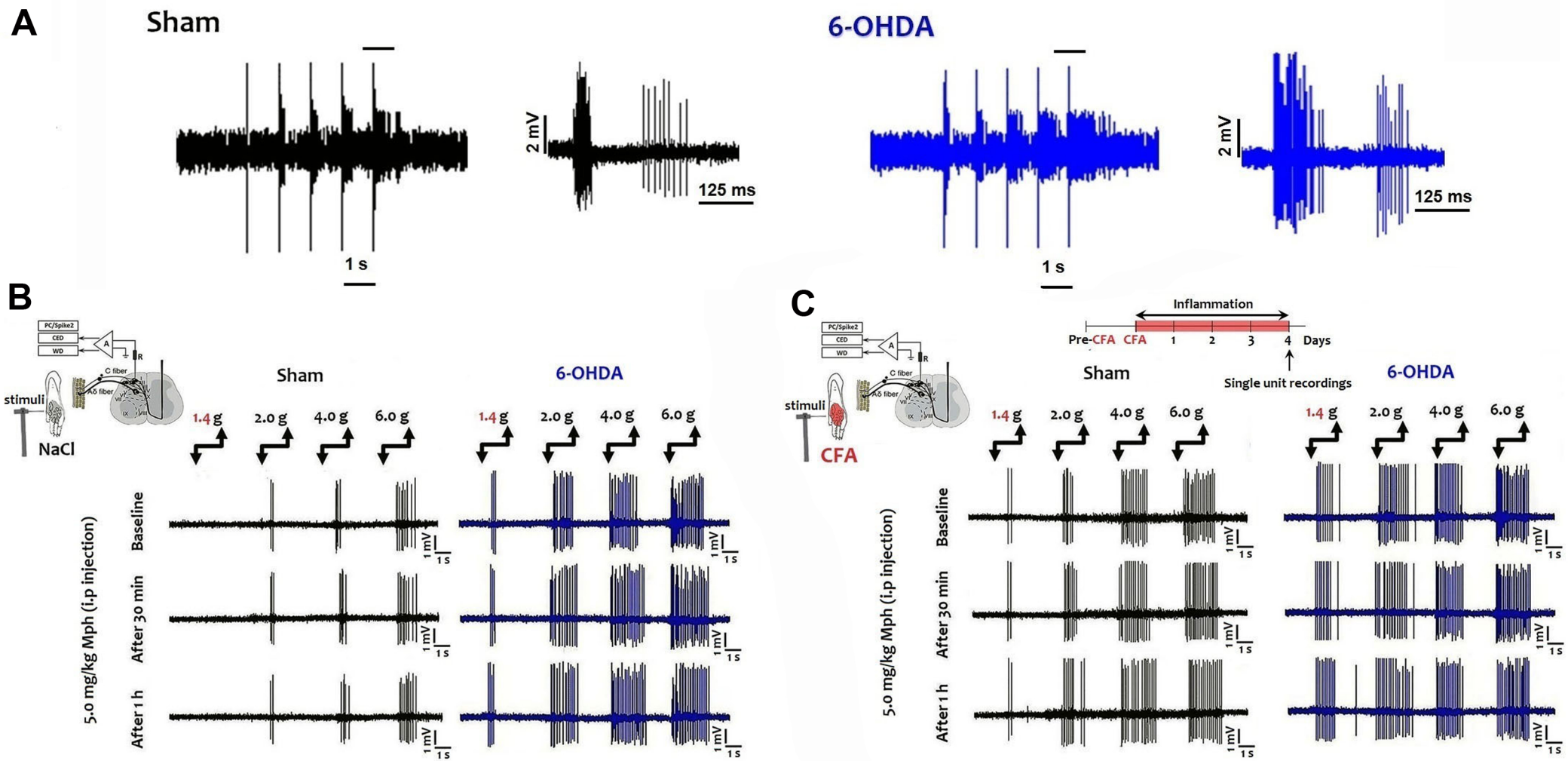
B. Animal experimentation design related to figures 4.

C. Animal experimentation design related to figure 5.

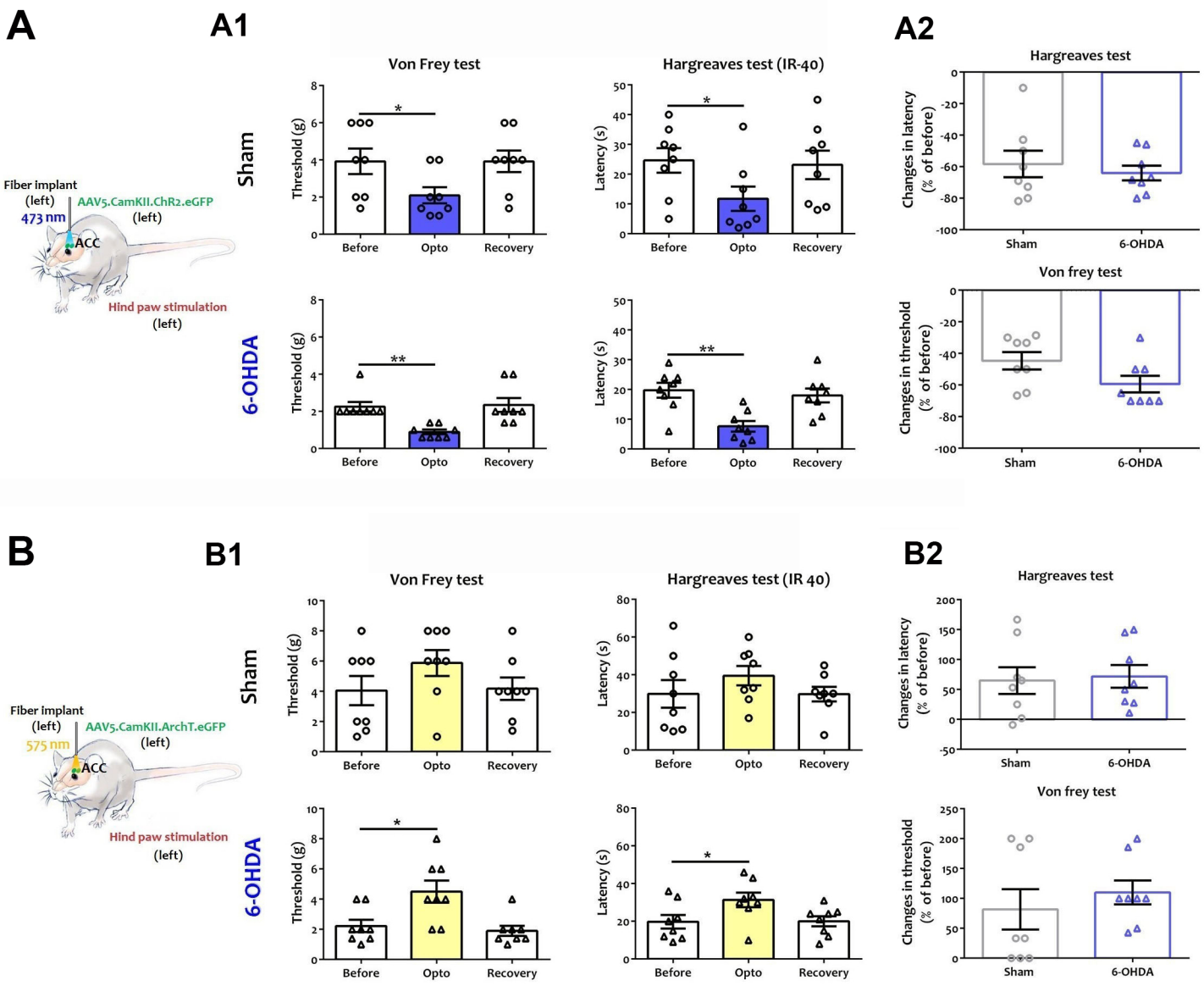
D. Animal experimentation design related to figure 6, 7, S4, S4, S5 and S6.



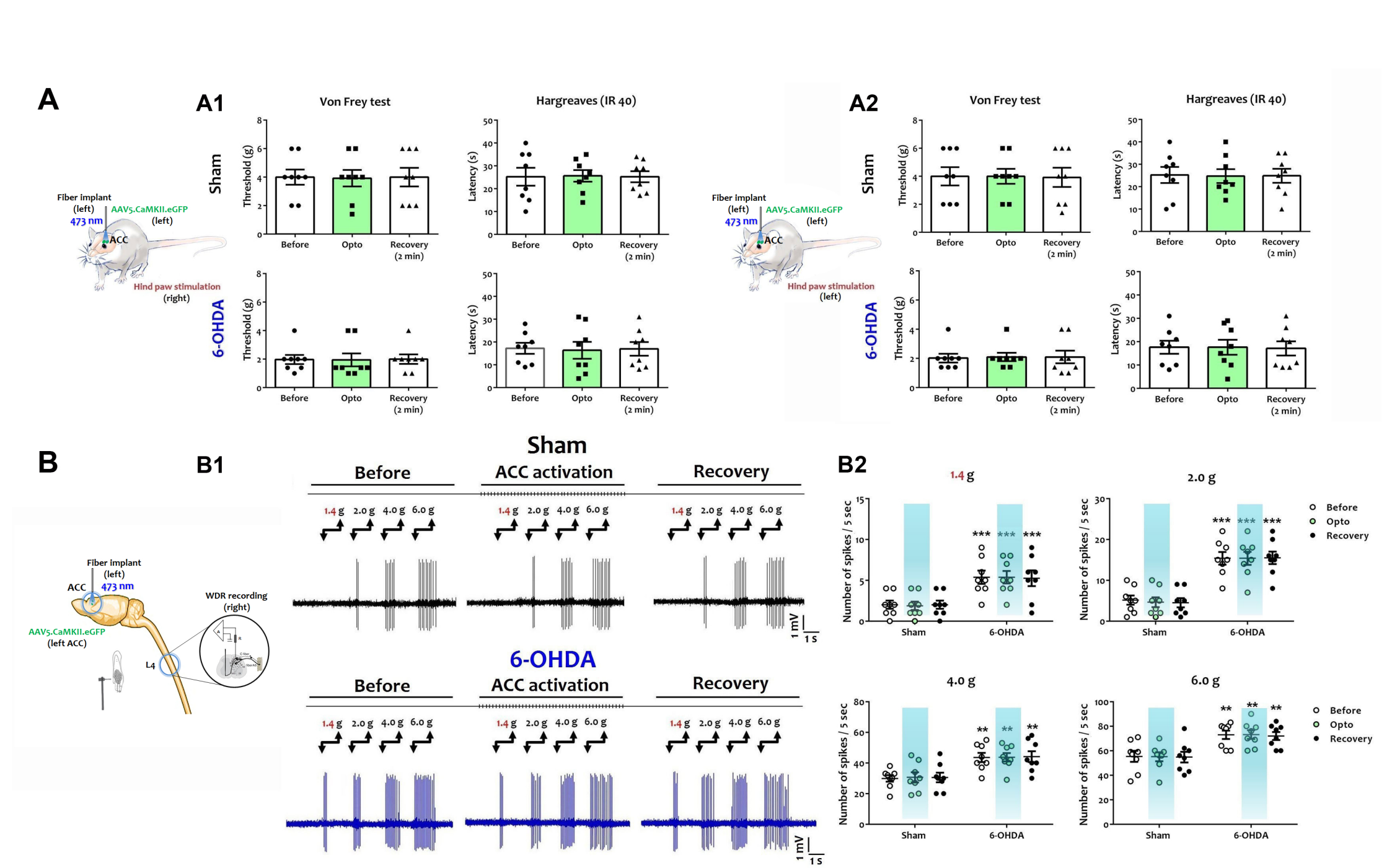
Bouchatta et al., Figure S1



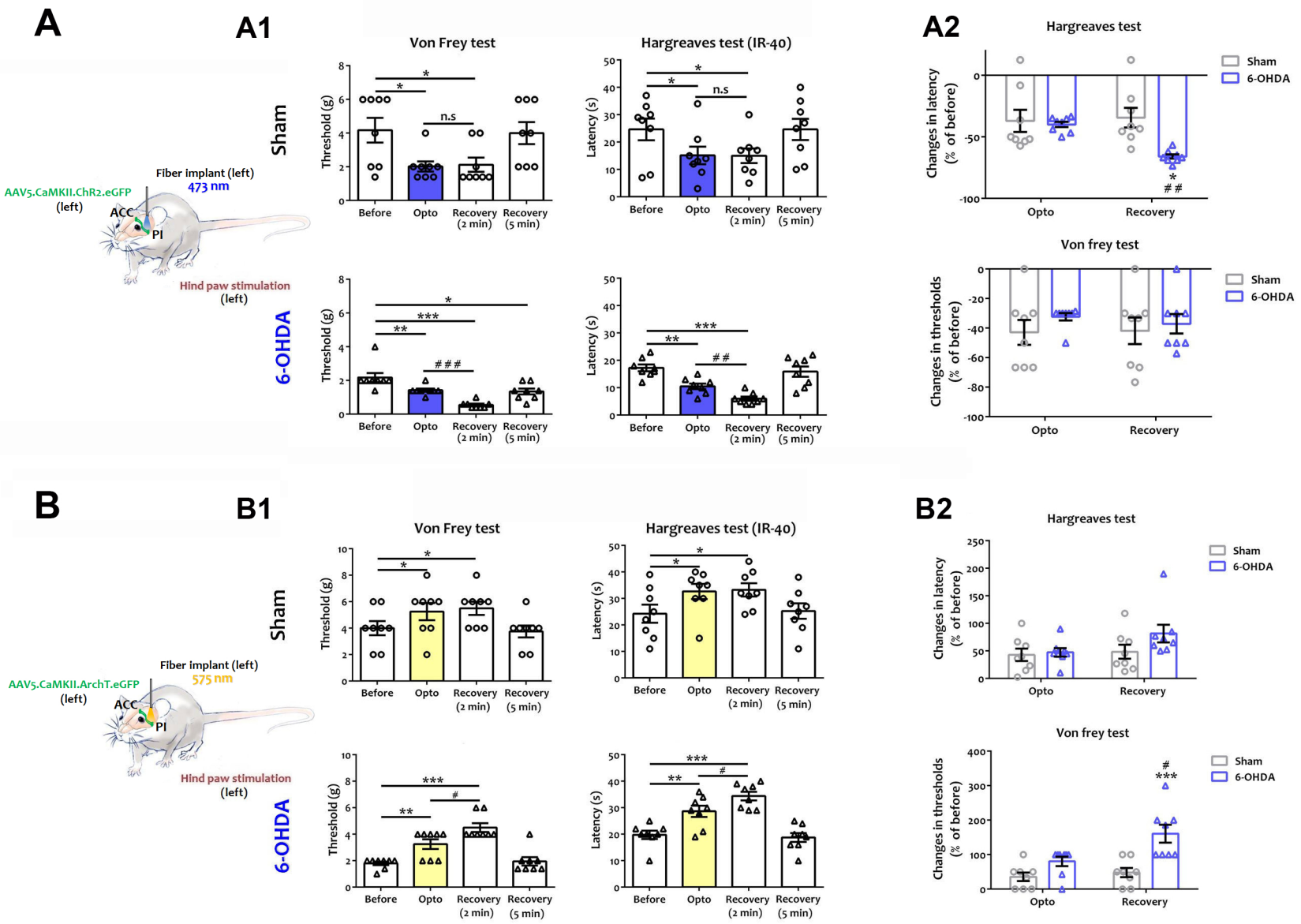
Bouchatta et al., Figure S2



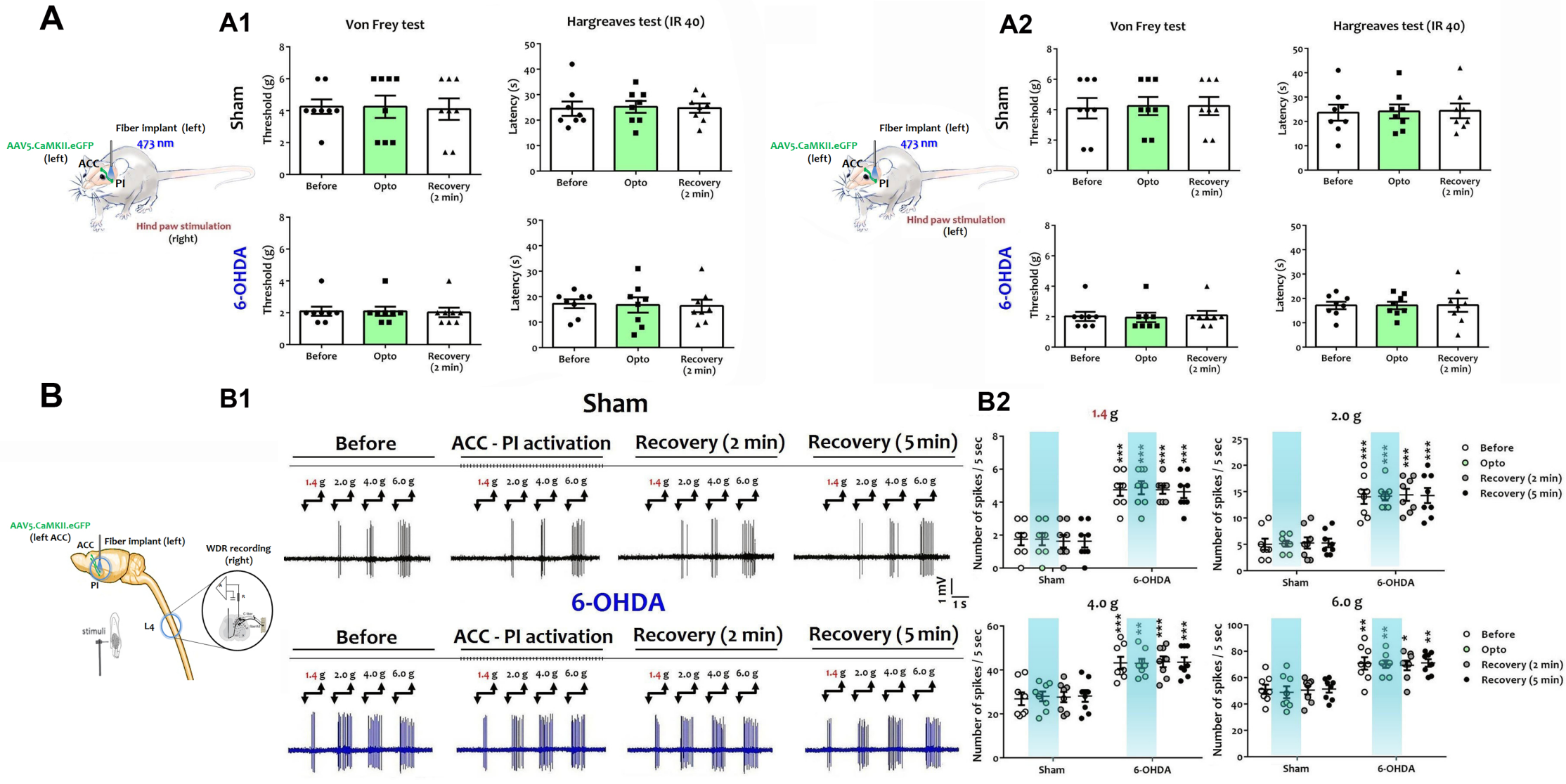
Bouchatta et al., Figure S3



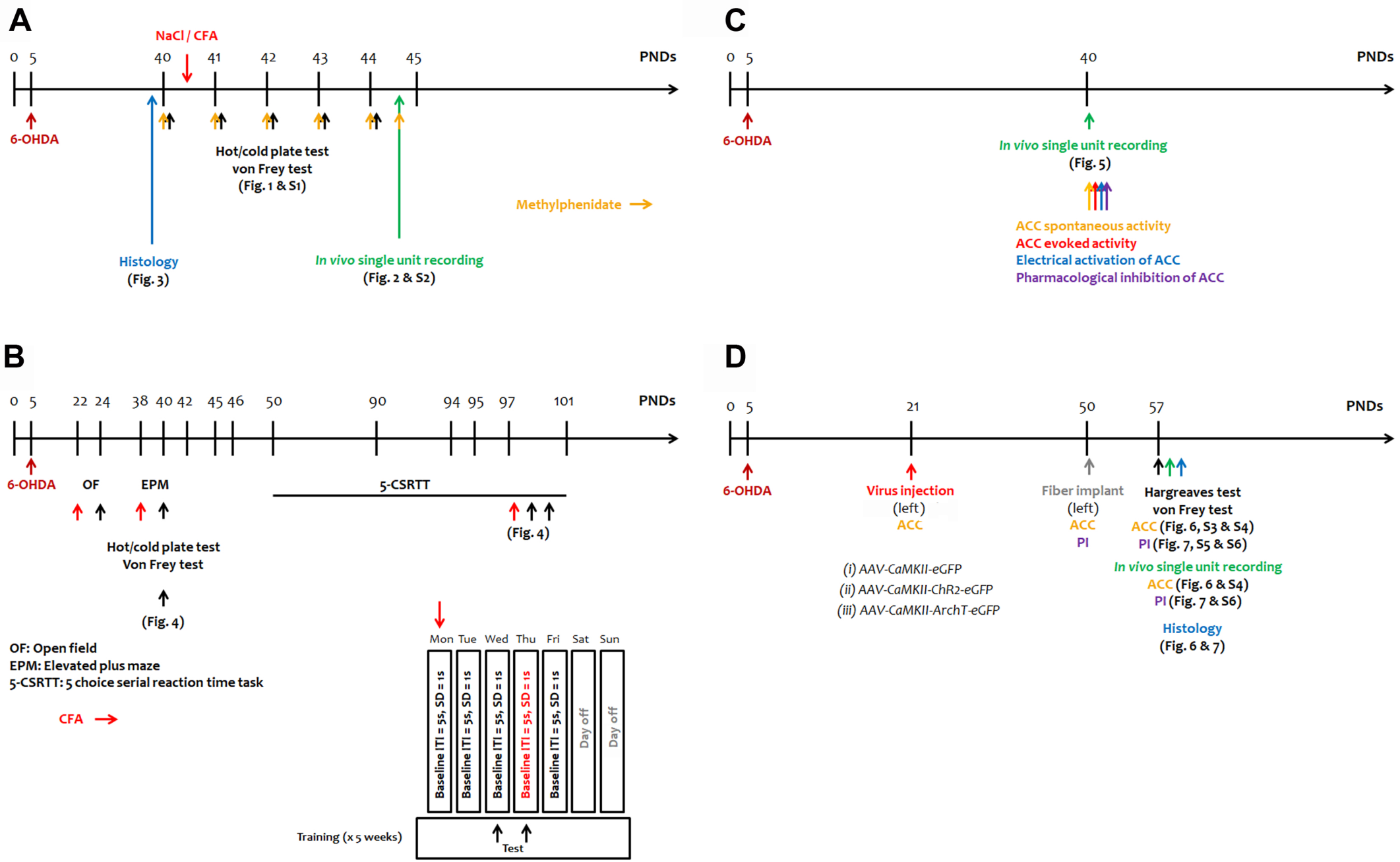
Bouchatta et al., Figure S4



Bouchatta et al., Figure S5



Bouchatta et al., Figure S6



Bouchatta et al., Figure S7