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Article

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Pain hypersensitivity in a pharmacological mouse model of Attention-Deficit/Hyperactivity Disorder (ADHD).

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Author contribution:

OB, SBM, MB and ML conceive the study and designed the experiments; OB, WS and HM performed the behavioral experiments; OB did the electrophysiology experiments; RBB, LB and ML did the immunohistochemical work; RBB did the western-blot experiments; OB and FA performed the optogenetic work; RBB, PF, SBM, MB and ML supervise the experiments; OB, MB and ML wrote the manuscript; all authors discussed and revised the manuscript.

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Abstract

Recent clinical evidence suggest that pain hypersensitivity develops in subjects with attention deficit hyperactivity disorder (ADHD). However, the mechanisms and neural circuits involved in these interactions remain unknown because of the paucity of studies in animal models. In our previous work, we validated a mouse model obtained by neonatal 6-Hydroxydopamine (6-OHDA) injection, to mimic ADHD syndrome. Here, we demonstrated that 6-OHDA mice exhibited a marked decrease of withdrawal thresholds to thermal and mechanical stimuli, suggesting that ADHD conditions increase nociceptive sensitivity. Moreover, sensitization to pathological inflammatory pain is amplified in 6-OHDA mice as compared to shams. Conversely, ADHD-related hyperactivity and anxiety, but not inattention and impulsivity, are worsened in the inflammatory pain model. Our data indicated that ADHD-induced allodynia and hyperalgesia are accompanied by neuronal hyperexcitability in the dorsal spinal cord. Next, we tested the hypothesis that descending controls are responsible for pain alterations. By combining *in vivo* electrophysiology, optogenetic stimulation and behavioral analyses, we demonstrated that the anterior cingulate cortex (ACC) hyperactivity alters the 'ACC – posterior insula' circuit, and triggers changes in spinal networks that underlie pain sensitization. Altogether, our results point to unprecedented mechanisms underlying the comorbidity between ADHD and pain sensitization. They indicate that ADHD and pain sensitization are mutually worsening comorbid disorders. The identification of shared mechanisms, engaging overlapping neuronal circuits and underlying both disorders, is key to better treatments.

Key words: Attention Deficit/Hyperactivity Disorders, Pain sensitization, Comorbidity, Mouse model, Anterior cingulate cortex, Spinal cord, Posterior insula.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental associated with cognitive, emotional, and behavioral deficits¹. It is one of the most common psychiatric pathologies in children with an estimated prevalence of 8-12% worldwide². Hyperactivity, inattention and impulsivity³ are ADHD core symptoms that persist until adulthood in up to two-third of cases⁴. ADHD patients also often coexisting complains such as social disabilities and emotional deficits^{5,6}.

Individuals that retain greater symptomatology are likely to have a greater symptom burden, functional impairment and psychiatric co-morbidity⁷ including anxiety or mood and antisocial personality disorders^{4,8,9}. Recent studies indicate association between attention deficits and altered sensory processing^{10,11}. Attentional processes have been shown to regulate pain transmission through the modulation of brain networks¹² and descending pathways¹³. In clinics, ADHD patients report increased pain perception¹⁴⁻¹⁷. Clinical studies suggested that adults with ADHD have increased risks of pain disorder^{18,19} and are more likely to have taken pain medication in the past years²⁰. Conversely, chronic pain causes cognitive impairments and worsens ADHD symptoms in humans^{21,22,23,24} and animal models²⁵.

Despite this large body of evidence indicating comorbidity between these pathologies, investigations of ADHD and pain are mostly conducted separately. Few studies have been performed to investigate the mechanisms underlying interactions between ADHD and pain, and the neural systems involved have received little attention so far, especially in animal models^{26,27}. More knowledge of the interactions between cognitive, affective and pain neural circuits is key to developing better treatments. Therefore, the effects of ADHD on pain transmission need to be further clarified and mechanistic insights obtained by elucidating circuits and identity of target regions in brain networks.

Pharmacological, genetic, and imaging studies highlighted the role of dopamine dysregulation in the etiology of ADHD, both in human and in animal models of ADHD²⁸⁻³¹. A loss of dopaminergic signaling is implicated in the symptoms of ADHD hyperactivity³² and anatomofunctional changes have been demonstrated in dopamine-related areas, particularly in the prefrontal cortex, including the anterior cingulate cortex³³⁻³⁸. Among the treatments used to combat ADHD, atomoxetine and methylphenidate block the noradrenalin and dopamine transporters, respectively. Dopamine is also involved in the genesis of chronic pain³⁹ and substances that increase dopaminergic transmission have analgesic properties. More particularly, dopaminergic transmission in the prefrontal cortex is implicated in pain chronification⁴⁰.

We previously validated an ADHD mouse model generated at P5 by neonatal disruption of central dopaminergic pathways with 6-hydroxydopamine (6-OHDA). We demonstrated the validity of the model to mimic major and minor symptoms of ADHD^{41,42}. Here, we address the interplay between ADHD and nociception. Our data provide clear evidence for the increased sensitivity to various pain modalities in 6-OHDA mice, a phenotype also reported in human patients with ADHD. The present results point to the hyperexcitability of the anterior cingulate cortex (ACC) which mediates alterations of descending pain modulatory pathways, therefore increasing the excitability of spinal nociceptive neurons in ADHD-like conditions. Mph treatment proved ineffective in normalizing withdrawal threshold and spinal neuron hyperactivity in the 6-OHDA mouse model. Interestingly, while ADHD conditions enhance pain sensitivity, changes in

the pain status have more complex effects on ADHD-related symptoms. Finally, by using viral approaches, optogenetics and *in vivo* electrophysiology, we demonstrated that the excitatory projections from the ACC to the posterior insula gate dysfunction of spinal cord nociceptive neurons in ADHD conditions.

Altogether, the present results indicate that ADHD and pain sensitization are mutually worsening comorbid disorders. The identification of common mechanisms, engaging overlapping neuronal circuits and underlying both disorders, is key to developing new therapeutic strategies.

Results

ADHD-like conditions influence pain sensitivity. First, we analysed nociceptive behavioral responses to thermal and mechanical stimuli using the hot/cold plate and von Frey tests in the 6-OHDA mouse model (**Fig. 1A-C**). The paw licking latency appeared significantly shorter in 6-OHDA mice in response to hot and cold stimuli in comparison to sham mice (**Fig. 1A-B**). In addition, the von Frey test showed a significant decrease in pain withdrawal threshold to mechanical stimulus in 6-OHDA compared to sham mice (**Fig. 1C**). This indicated that ADHD conditions increased basal pain sensitivity to thermal and mechanical stimuli.

Then, we determined if ADHD-induced pain sensitization is altered under persistent pain conditions, in an inflammatory pain model obtained by CFA injection in the right hind paw of sham and 6-OHDA mice (**Fig. 1D-E**). Intraplantar injection of NaCl in the hind paw has no effect on thermal (heat and cold; **Fig. 1D1-D2**) and mechanical sensitivity (**Fig. 1D3**) in both groups. Sham animals showed a significant decrease in pain thresholds to thermal (heat and cold; **Fig. 1D1-D2**) and mechanical (**Fig. 1D3**) stimuli at 4 days post-CFA in comparison to the pre-CFA baseline conditions. 6-OHDA mice exhibited a further decrease in pain thresholds of thermal (heat and cold; **Fig. 1D1-D2**) and mechanical (**Fig. 1D3**) sensitivity under inflammatory pain conditions compared to the day before (pre-CFA). Importantly, the comparison between sham and 6-OHDA mice indicated that ADHD-like conditions amplifies CFA-induced pain sensitization in response to thermal (heat and cold; **Fig. 1E1-E2**) and mechanical (**Fig. 1E3**) stimuli. This suggested that the effects of ADHD-like conditions depend on the pain status and worsen central sensitization processes.

Next, we explored the capacity of ADHD medication to alter nociception in sham and 6-OHDA mice by injecting a single dose of methylphenidate (i.p. 3.0 or 5.0mg/kg Mph) (**Fig. S1A-C**). Neither 3.0 mg/kg nor 5.0 mg/kg Mph influenced thermal (heat and cold; **Fig. S1A and S1B**) or mechanical sensitivity (**Fig. S1C**) of 6-OHDA or sham mice. We also evaluated the effect of Mph under inflammatory pain conditions (**Fig. S1D-I**). Again, no effect of 3.0 mg/kg or 5.0 mg/kg of Mph was seen on thermal or mechanical sensitivity in control (NaCl) or inflammatory (CFA) conditions in both groups (sham and 6-OHDA). These data suggested that Mph is ineffective on 6-OHDA-induced thermal and mechanical hyperalgesia/allodynia, regardless the pain status.

ADHD conditions changes sensory integration in the spinal cord. We tested whether changes in pain sensitivity of 6-OHDA mice were caused by alterations of the dorsal spinal nociceptive network. Thus, we performed single unit *in vivo* extracellular recording of deep dorsal horn

neurons (DHNs) identified as wide dynamic range (WDR) neurons after peripheral electrical stimulation (**Fig. S2A**). First, 6-OHDA mice DHNs responded to innocuous stimuli (1.4g) in contrast to sham mice (**Fig. 2A and C**). Second, 6-OHDA mice DHNs displayed an increased discharge in response to noxious stimulus (6.0g) (**Fig. 2A and C**). Moreover, we evaluated the effect of 5.0 mg/kg Mph (i.p) on the electrical activity of DHNs (**Fig. 2C**; see also **Fig. S2B**). Mph did not influence DHN firing in sham and 6-OHDA mice (**Fig. 2C**; see also **Fig. S2B**). In inflammatory pain conditions, electrophysiology recordings showed increased spinal neuron activity in response to innocuous (1.4g) and noxious stimuli (6.0g) (**Fig. 2B-C**) in sham mice as compared to controls (NaCl injection). CFA injection caused a further amplification of DHN activity in 6-OHDA mice, in response to both innocuous (1.4g) (**Fig. 2B-C**) and noxious stimuli (6.0g) (**Fig. 2B-C**). Again, no effects of Mph were seen on the electrical activity of DHNs in sham and 6-OHDA mice in inflammatory pain conditions (**Fig. 2C**; see also **Fig. S2C**).

ADHD conditions modify dorsal spinal networks. Given the changes that were observed in spinal neuron activity, we investigated possible anatomical and biochemical modifications in the dorsal spinal cord (**Fig. 3**). At first, we characterized the distribution of synaptic markers of inhibitory and excitatory active zones. We assessed the intensity of the immunostaining for postsynaptic gephyrin (**Fig. 3A**) and homer 1 (**Fig. 3B**), respectively, and their colocalization with presynaptic synaptophysin. Gephyrin (**Fig. 3Aa1-f1, a2-f2**) and homer 1 (**3Ba1-f1, a2-f2**) immunostaining appeared as punctate labeling in the superficial and deeper laminae of the dorsal spinal cord, with the highest density in the lamina II. No changes were observed in the inhibitory synapses (**Fig. 3A**). In contrast, immunostaining for homer 1 was stronger in 6-OHDA mice as compared to sham mice (**Fig. 3Ba1-f1 and a2-f2**). Quantitative assessment confirmed the increased immunolabeling intensity for homer 1 and the enhanced colocalization of the excitatory postsynaptic marker with synaptophysin (**Fig. 3B**). Taken together, these data support the strengthening of excitatory transmission in the dorsal spinal cord.

In a second step, we evaluated in adult mice the depletion in catecholamines induced by 6-OHDA injection at P5 (**Fig. 3C**). For this purpose, we performed TH immunodetection in various brain areas. As expected, TH immunolabeling decreased in various areas, e.g. striatum (**Fig. 3Ca-b**). In the ACC, 6-OHDA injection only modifies the area labeled for TH (**Fig. 3Ce-f**) but not the labeling intensity. In contrast, no variations of TH immunostaining were detected in hypothalamic nuclei including the hypothalamic A11 area projecting to the spinal cord⁴³ (**Fig. 3Cc-d**). Accordingly, no changes were observed in TH innervation of the dorsal spinal cord (**Fig. 3Cg-h**).

Finally, we analysed the ERK1/2 MAP kinase and CaMKII intracellular signalling pathways involved in CREB activation⁴⁴ and spinal cord sensitization to pain^{45,46} by using western blot (**Fig. 3D**) and immunohistochemistry (**Fig. 3E**). blotting of ipsilateral dorsal spinal cord revealed an increased expression of the excitatory marker CaMKII in 6-OHDA mice (**Fig. 3D**). Similarly, the labeling for the phosphorylated forms of ERK1/2 MAP kinase and CREB transcription factor (**Fig. 3D**) was significantly stronger in 6-OHDA mice. Immunolabeling for pERK (**Fig. 3E**) confirmed the increased expression of the phosphorylated kinase in the ipsilateral dorsal horn of 6-OHDA mice.

Pain status influences ADHD symptoms. We determined if pain conditions influenced ADHD behavioral symptoms by analysing locomotor activity, anxiety, attention and impulsivity in mice with increased (CFA injection) (**Fig. 4**) pain sensitivity. First, we analysed spontaneous locomotor

activity of 6-OHDA and sham mice with the open field test (**Fig. 4A**). CFA injection decreased the distance traveled (**Fig. 4A1** and **A2**), mobility mean time (**Fig. 4A3**) and speed of animal movement (**Fig. 4A4**) in sham and 6-OHDA mice. Notably, CFA amplified hyperactivity symptoms of 6-OHDA mice. Indeed, the difference in distance traveled (**Fig. 4A5**) and velocity (**Fig. 4A7**) between the sham and 6-OHDA groups is higher in CFA conditions than after NaCl paw injection. Next, we evaluated anxiety-like behavior, one of the ADHD comorbid complains (**Fig. 4B**). In the elevated plus maze test (EPM), CFA injection decreased the time spent in open arms (**Fig. 4B1-B2**) in sham and 6-OHDA groups. In addition, CFA injection also increased the number of entries in open arms (**Fig. 4B1** and **B3**). Therefore, anxiety-like behavior caused by ADHD-like conditions⁴² was further worsened by CFA treatment. The ADHD-induced decrease in the time spent in the open arms is amplified in CFA conditions as compared to the NaCl paw injection group (**Fig. 4B4**). Finally, we performed the 5-choice serial reaction time task (5-CSRTT) test to analyse inattention and impulsivity (**Fig. 4C**). During the standard sessions of the 5-CSRTT (SD = 1 s, ITI = 5 s), 6-OHDA adult mice were less accurate than sham mice (**Fig. 4C1**), making more omission errors (**Fig. 4C2**), premature responding (**Fig. 4C3**) and perseverations (**Fig. 4C4**) than sham mice. Importantly, inflammatory pain does not affect accuracy and omissions in both groups (**Fig. 4C1-2**). Taken together, these data suggested that inflammatory pain conditions worsened some of the ADHD-related symptoms including hyperactivity and anxiety, but not impulsive-like behaviors or inattention.

The Anterior Cingulate Cortex (ACC) is at the origin of descending control dysfunctions in ADHD conditions. We tested the hypothesis that descending controls are responsible for pain alterations through the modulation of spinal circuits in 6-OHDA mice. The ACC is at the cross-road of pain and ADHD disorders, being involved in inattentiveness, impulsiveness and anxiety^{33,34}. Meanwhile, the ACC sends projections to the dorsal horn of spinal cord⁴⁷. Therefore, we explored whether ACC is critically involved in the dysfunction of descending controls that were revealed by *in vivo* electrophysiology in 6-OHDA mice (**Fig. 5**). For this purpose, we recorded ACC spontaneous (**Fig. 5A**) and evoked (**Fig. 5B**) activity in urethane-anesthetized animals. *In vivo* single unit extracellular recordings indicated that ACC neurons are hyperactive in 6-OHDA mice (**Fig. 5A1**), with higher action potential frequencies (**Fig. 5A2**). Evoked activity was generated by applying mechanical stimuli on the posterior hind paw contralateral to the recorded ACC. In these conditions, our data showed that mechanical stimuli caused a significantly stronger increase of ACC neuronal activity in 6-OHDA animals as compared to sham (**Fig. 5B1**). The frequency of action in ACC neurons was greater in 6-OHDA mice than in sham mice subjected to innocuous stimuli (1.4g) (**Fig. 5B2**). Moreover, the increase in ACC neuron discharge is stronger in 6-OHDA mice than in sham mice, when animals receive noxious peripheral stimuli (6.0g) (**Fig. 5B2**). These observations indicated that ACC hyperactivation seen in 6-OHDA mice may contribute to alter sensory processing in the dorsal spinal cord.

Then, we explored the effects of ACC modulation on the activity of DHNs. First, we analyzed the effect of ACC activation on contralateral spinal cord responses evoked by peripheral mechanical stimuli (**Fig. 5C**). *In vivo* electrophysiology recording showed that the electrical stimulation (100 Hz) of ACC at different intensity (10, 20, 30 V) increased contralateral DHNs activity in the sham group, in response to peripheral innocuous (1.4g) and noxious (6.0g) mechanical stimuli (**Fig. 5C**). This effect was significantly higher in 6-OHDA mice (**Fig. 5C**). Interestingly, spinal neuron activity returned to baseline activity within 2 min after the last electrical stimulation in sham mice,

whether the stimulus was painful or not, while the potentiation of spinal neuron activity in 6-OHDA mice was maintained up to 2 minutes (**Fig. 5C**). Next, we evaluated the effect of pharmacological inhibition of ACC by local muscimol injection in order to potentiate GABA-A inhibition (**Fig. 5D**). Our data indicated that muscimol partially blocked electrical activity of contralateral DHNs in the sham and 6-OHDA groups, in response to innocuous (1.4g) and noxious (6.0g) peripheral mechanical stimuli, at 30 minutes after the drug injection (**Fig. 5D**). The proper location of the stimulation/injection site in ACC was confirmed histologically (**Fig. 5E**). Collectively, these data demonstrated the regulatory role of ACC in the dysfunction of spinal sensory integration in ADHD conditions.

ACC excitatory neurons amplify pain sensitization in ADHD conditions. To further explore the role of ACC in 6-OHDA mice pain mechanisms, we injected AAV₅.CamKII.ChR₂ or AAV₅.CamKII.ArchT_{3.0}, and AAV₅.CamKII.eGFP unilaterally in the left ACC (area 24b) (**Fig. 6A1**). We then used optogenetic tools to activate or inhibit excitatory ACC neurons (**Fig. 6A**). The transduction efficiency was checked *a posteriori* by eGFP visualization (**Fig. 6A2 and A3**).

1. Optogenetic activation. At 4 weeks after injection, we tested whether specific activation of ACC excitatory neurons was sufficient to modulate nociceptive responses in awake, freely moving, animals (**Fig. 6B**; see also **Fig. S3A**). ChR₂ activation induced a significant decrease in withdrawal thresholds to mechanical and thermal stimuli of ipsilateral (left) (**Fig. S3A1**) and contralateral (right) hind paw of sham mice during the light phase (**Fig. 6B1**). When the light was turned off, the mechanical and thermal withdrawal thresholds of ipsilateral (**Fig. S3A1**) and contralateral hind paw (**Fig. 6B1**) returned to their baseline values. The overall behavior was similar in 6-OHDA mice although the thresholds were lowered as compared to sham mice. The mechanical and thermal pain thresholds of ipsilateral (**Fig. S3A1**) and contralateral hind paw (**Fig. 6B1**) decreased when the light was on and returned to their baseline values when the light was off (**Fig. 6B1**; see also **Fig. S3A1**). Importantly, the comparison of sham and 6-OHDA mice suggested that ADHD-like conditions enhanced behavioral changes induced by optogenetic activation of ACC excitatory neurons, in response to mechanical, but not thermal, stimulus to contralateral hind paw (**Fig. 6B2**). By contrast, no significant changes were seen in ipsilateral hind paw whether the application of mechanical or thermal stimulus (**Fig. S3A2**).

We also evaluated DHN activity using *in vivo* single unit recording in the dorsal horn of the spinal cord, contralateral to the AAV injection site in the ACC (**Fig. 6C**). The results were in line with the behavioral tests. In sham animals, optogenetic activation of the ACC increased the activity of DHNs to innocuous (1.4g) and noxious (6.0g) mechanical stimuli (**Fig. 6C**). When the light was off, the electrical activity of nociceptive neurons decreased and returned to baseline levels before illumination (**Fig. 6C**). In 6-OHDA animals, ACC optogenetic activation increased the activity of spinal neurons to innocuous (1.4g) and noxious (6.0g) mechanical stimuli (**Fig. 6C2**). By contrast, when the light was off, the electrical activity of spinal neurons returned to the baseline levels recorded before illumination (**Fig. 6C**).

2. Optogenetic inhibition. Conversely, we silenced ACC excitatory neurons expressing AAV₅.CaMKII.ArchT_{3.0} and determined the withdrawal threshold in sham and 6-OHDA mice (**Fig. 6D**; see also **Fig. S3B**). Optogenetic inhibition of ACC neurons had no significant

effect on pain thresholds to mechanical or thermal stimuli of ipsilateral (**Fig. S3B1**) and contralateral hind paw (**Fig. 6D1**) of sham animals. By contrast, we found that optogenetic inhibition of ACC neuron activity increased mechanical threshold and withdrawal latency of ipsilateral (**Fig. S3B1**) and contralateral hind paw (**Fig. 6D1**) of 6-OHDA mice. After the light was turned off, both mechanical and thermal baseline sensitivity of ipsilateral (**Fig. S3B1**) and contralateral hind paw (**Fig. 6D1**) were fully restored. The comparison of sham and 6-OHDA mice indicated that ADHD-like conditions enhanced the analgesic effects induced by optogenetic inhibition of ACC excitatory neurons in response to mechanical stimulus of the contralateral hind paw (**Fig. 6D2**). In contrast, no significant changes were seen in ipsilateral hind paw whether the application of thermal or mechanical stimulus (**Fig. S3B2**).

In line with the behavioral results, *in vivo* recordings indicated no changes in the electrical activity of sham DHNs in response to innocuous (1.4g) or noxious stimuli (6.0g) when light was on (**Fig. 6E**). By contrast, ACC optogenetic inhibition significantly decreased DHN electrical activity of in response to innocuous (1.4g) and noxious stimuli (6.0g) in 6-OHDA mice (**Fig. 6E**). When the light was off, the electrical activity of spinal neurons returned to the baseline levels recorded before illumination (**Fig. 6E**).

3. Control experiments. Pain thresholds of ipsilateral (**Fig. S4A2**) and contralateral hind paws (**Fig. S4A1**) injected with the AAV₅.CamKII.eGFP control virus are not affected by the light stimulation in sham mice. Similarly, no effects of light stimulation were seen on pain sensitivity in 6-OHDA mice, whether for ipsilateral or contralateral paw (**Fig. S4A1-2**). Accordingly, optogenetic modulation did not induce changes in the activity of contralateral spinal neurons after transduction of AAV₅.CamKII.eGFP in sham or 6-OHDA mice (**Fig. S4B1-2**).

Taken together, these data confirmed that ACC excitatory neurons regulate pain behavior and spinal neuron activity, both in sham and 6-OHDA mice. Notably, these effects are amplified in ADHD-like conditions.

The ACC – Posterior Insula (PI) excitatory pathway potentiates pain sensitization in ADHD conditions. We then sought to understand the circuit from ACC to spinal cord that is involved in ADHD-induced pain sensitization. First, we verified that unilateral viral injection in the left 24b area (**Fig. 7A2**) triggered virus expression in the ipsilateral ACC projection areas of the PI (**Fig. 7A3**). To manipulate the ACC–PI pathway, we used the same optogenetic strategy as previously described but the optical fiber was placed in the ipsilateral PI (left side) (**Fig. 7A**).

1. Optogenetic activation. In the sham group, the activation of ChR₂ resulted in a significant decrease in withdrawal thresholds for both ipsilateral (**Fig. S5A1**) and contralateral hind paw (**Fig. 7B1**) in response to mechanical and thermal stimuli. The changes in mechanical and thermal pain thresholds of the ipsilateral (**Fig. S5A1**) and contralateral hind paw (**Fig. 7B1**) were maintained until 2 minutes after the optogenetic stimulation was stopped, but returned to the pre-illumination baseline levels after 5 minutes (**Fig. S5A1** and **Fig. 7B1**). In 6-OHDA mice, withdrawal threshold or latency of both ipsilateral (**Fig. S5A1**) and contralateral hind paws (**Fig. 7B1**) decreased significantly in response to thermal and mechanical stimuli, when the light was on. Interestingly, 2 minutes after optogenetic stimulation, the modification of mechanical and thermal pain thresholds of the ipsilateral

(**Fig. S5A1**) and controlateral hind paw (**Fig. 7B1**) was further amplified, in comparison to the thresholds measured during optogenetic manipulations. After 5 minutes, thermal pain thresholds for the ipsilateral (**Fig. S5A1**) and contralateral hind paws (**Fig. 7B1**) returned to baseline levels, while the mechanical thresholds were not fully restored (**Fig. S5A1** and **Fig. 7B1**). As compared to sham mice, changes in controlateral and ipsilateral paw withdrawal threshold to thermal stimuli were stronger in ADHD-like conditions than in sham at 2 minutes recovery (**Fig. 7B2** and **Fig. S5A2**).

In line with the behavioral data, single unit *in vivo* recording in sham mice after ACC – PI pathway optogenetic activation revealed increased DHN activity after innocuous (1.4g) and noxious (6.0g) mechanical stimuli (**Fig. 7C**). At 2 minutes after the light is turned off, DHN activity remained unchanged and returned to the pre-illumination baseline levels after 5 minutes (**Fig. 7C**). In 6-OHDA mice, *in vivo* recording also indicated that optogenetic activation of the ACC – PI excitatory pathway increased the activity of spinal neurons to innocuous (1.4g) and noxious (6.0g) mechanical stimuli (**Fig. 7C**). Contrary to the situation in sham mice, the activity of spinal neurons was further amplified at 2 minutes after the light is turned off (**Fig. 7C**) and returned to the baseline levels at 5 minutes after the illumination ended.

2. Optogenetic inhibition. Optogenetic inhibition of the ACC – PI excitatory pathway was performed by yellow light illumination of the ArchT_{3.0} (**Fig. 7D**; see also **Fig. S5B**). In the sham group, it resulted in a significant increase in pain threshold for both the ipsilateral (**Fig. S5B1**) and contralateral hind paws (**Fig. 7D1**) in response to mechanical and thermal stimuli. At 2 minutes after the light was turned off, the increase of mechanical and thermal pain thresholds of both hind paws was maintained (**Fig. 7D1** and **Fig. S5B1**). It returned to baseline levels after 5 minutes (**Fig. 7D1** and **Fig. S5B1**). In 6-OHDA mice, mechanical threshold and withdrawal latency of both the ipsilateral (**Fig. S5B1**) and contralateral (**Fig. 7D1**) hind paws increased significantly when the light was on. Again, 2 minutes after the optogenetic inhibition ended, we found that both mechanical and thermal pain thresholds of the ipsilateral (**Fig. S5B1**) and contralateral (**Fig. 7D1**) hind paws were further increased. After 5 minutes, this increase was fully abolished (**Fig. 7D1** and **Fig. S5B1**). The behavioral effects of optogenetic inhibition were amplified in 6-OHDA mice as compared to sham mice at 2 minutes recovery in response to both mechanical and thermal stimuli (**Fig. 7D2**) of contralateral hind paw, while only to mechanical stimulus of ipsilateral hind paw (**Fig. S5B2**).

Single unit *in vivo* recordings (**Fig. 7E**) revealed that optogenetic inhibition of the ACC – PI excitatory pathway in sham mice partially blocked the electrical activity of spinal neurons in response to noxious stimuli (**Fig. 7E**). No effects were detected upon innocuous stimulus (1.4g) (**Fig. 7E**). Reduced DHN activity upon noxious stimuli was maintained at 2 minutes after the light was turned off (**Fig. 7E**) and returned to baseline levels after 5 minutes (**Fig. 7E**). In 6-OHDA mice, the optogenetic inhibition of ACC – PI excitatory pathway also blocked the activity of spinal neurons in response to noxious (6.0g) but also to innocuous (1.4g) mechanical stimuli (**Fig. 7E**). In contrast to sham mice, spinal neuron activity was further decreased at 2 minutes after optogenetic inhibition ended (**Fig. 7E**) but returned to the baseline levels after 5 minutes (**Fig. 7E**).

3. *Control experiments.* To check for the specificity of the optogenetic modulation on pain behavior and spinal neuron activity, we used a control AAV₅.CamKII.eGFP. In sham mice injected with this control virus, pain thresholds were not affected by light stimulation whether for ipsilateral (**Fig. S6A2**) or contralateral (**Fig. S6A1**) hind paw. Similarly, light stimulation had no effect in both hind paws of 6-OHDA mice (**Fig. S6A1-2**). In addition, *in vivo* recordings confirmed the absence of light effect on spinal neurons in AAV₅.CamkII.eGFP-expressing sham or 6-OHDA mice (**Fig. S6B1-2**).

Overall, these data indicated that ADHD-like conditions impaired the regulation exerted by the ACC-PI excitatory pathway on pain behavior and spinal neuron activity in 6-OHDA mice.

Discussion

The present study reports hypersensitivity to mechanical, heat and cold stimuli in a mouse model of ADHD obtained by postnatal disruption of dopaminergic systems. Behavioral testing also provides evidence that inflammatory pain-induced sensitization of withdrawal reflexes is further amplified in 6-OHDA mice. Behavioral changes are accompanied by increased excitability of spinal neurons and changes in spinal networks. The effects of inflammatory pain on ADHD-like symptoms are complex, pointing to changes in hyperactivity and anxiety, but not impulsivity or attention. Physiological and behavioral alterations are correlated to ACC hyperactivity in 6-OHDA mice. Manipulating the ACC or the ACC-PI excitatory pathway has bidirectional effects on spinal neuron activity and withdrawal reflexes. These effects are significantly amplified in 6-OHDA mice revealing an impaired tuning of pain modulation processes in ADHD-like conditions.

In clinics, ADHD patients reported impaired perceptual function, and particularly pain¹⁴. Studies in children and adults with ADHD described both hypo- and hyper-responsiveness to sensory stimuli⁴⁸⁻⁵⁰ and point to an increased prevalence of generalized pain in adults with ADHD as compared to a control population¹⁸. In agreement, experimental studies demonstrate changes in pain perception in patients with ADHD¹⁵. Reciprocally, chronic pain increases impulsivity³² and induces attentional and cognitive deficits in human patients^{22,23,51} and preclinical animal models^{25,52}. Increased pain sensation in ADHD patients is thought to be caused by brain mechanisms leading to exacerbated perception. However, data also point to alterations in sensory transmission, and recent studies indicate problems modulating sensory inputs¹¹ and reciprocal association between altered sensory processing and attention deficits¹⁰.

Strong arguments underline the role of a deregulation of the dopaminergic systems in the etiology of ADHD^{28,29}. In addition, a loss of dopaminergic signaling is implicated in the symptoms of ADHD hyperactivity³² and the D2 dopamine receptor is frequently associated with neuropsychiatric disorders and in particular with ADHD⁵³. Dopamine is also involved in the genesis of chronic pain^{39,54} and many substances that increase dopaminergic transmission have analgesic properties⁵⁵. Furthermore, the painful hypersensitivity observed in the context of various pathologies such as Parkinson's disease, fibromyalgia, restless legs syndrome, is likely related to dopaminergic system dysfunctions³⁹. The 6-OHDA mouse model replicates the alterations of dopaminergic systems and has been validated by us^{2,41,42} and others⁵⁶⁻⁶¹ as a model of ADHD with good face, construct and predictive validity.

A key finding of the present study is that pain sensitization observed in 6-OHDA mice is supported by impairment of sensory integration in the spinal cord. Electrophysiology data in the

spinal cord suggest that increased pain sensitivity is caused by DHN hyperexcitability in the 6-OHDA model. The augmented activity of DHNs is a hallmark of central sensitization leading to chronic pain in rat and mouse models of chronic inflammatory^{62,63} and neuropathic^{46,64} pain. The present study indicates increased immunoreactivity for homer 1 at synaptic sites that has been also observed in inflammatory conditions⁶⁵ and after nerve injury⁶⁶ in rodents. The long forms of homer are located in the post-synaptic density and their up-regulation may result from an increase in the number and/or size of glutamatergic synapses since homer immunostaining was shown to reliably reveal most of excitatory synapses in the mouse dorsal spinal cord⁶⁷. In addition, the accumulation of homer 1 is in agreement with increased expression of other excitatory post-synaptic scaffolding proteins, e.g. shank or PSD95, in chronic pain conditions^{68,69}. In conjunction with increased excitability and excitatory synaptic connections, immunistochemistry and biochemistry data in 6-OHDA mice reveal the activation of intracellular signalling pathways causing spinal sensitization to pain^{44,70-72}. Taken together, these data suggest increased excitation that may underlie the development spinal central sensitization processes⁷³⁻⁷⁵ in the dorsal spinal cord of 6-OHDA mice.

The primary source of catecholaminergic innervation of the spinal cord is the A11 hypothalamic area^{43,76}. However, spinal circuits are not directly affected by the depletion in dopamine induced by postnatal 6-OHDA injection since TH immunoreactivity is not modified in the A11 area nor in the dorsal spinal cord in 6-OHDA mice. Therefore, the modifications of spinal cord physiology in ADHD-like conditions, and their consequences on withdrawal reflexes, are likely caused by alterations of brain circuits that engage descending pathways to the spinal cord. Indeed, descending facilitation has been recently indicated to play key roles in central sensitization for nociceptive inputs and maintenance of chronic pain in different animal models⁷⁷.

Following the pioneer work of Reynolds⁷⁸, both inhibitory and facilitatory descending influences on spinal nociceptive processing has been widely studied in rodent models and humans^{77,79,80}. However, most of previous studies focused on descending modulation from the brainstem and it is only recently that authors considered the role of cortical and subcortical structures in the control of spinal neuron activity^{81,82} and pain behavior in pathological conditions^{83,84}. ACC is an important cortical area for pain perception, and the development of pathological pain⁸⁵⁻⁸⁷, and has clinical relevance in chronic pain sufferers⁸⁸. In addition, neuroimaging studies in ADHD patients identified structural and functional abnormalities in networks comprising the ACC and parietal cortices^{89,90}. Persistent pain in rodents is characterized by the potentiation of synaptic responses⁹¹, disinhibition⁹² and increased excitability⁹³ of the ACC. ACC hyperactivity is also crucial in bridging pain and its comorbid emotional disorders^{94,95}. The effects on pain transmission depend on the ACC areas that are investigated⁹⁶. Contrary to other studies that described a role of rostral ACC in the negative affect and depression-like behavior associated to chronic pain but not in mechanical hypersensitivity^{95,97}, our data were obtained in the dorsal part of the ACC and indicate that these areas (24a and 24b^{98,99}) actually control sensory transmission in the spinal cord. At a mechanistic level, future experiments will be required to determine the cellular and molecular mechanisms underpinning ACC dysfunction in ADHD-like conditions.

Taken together, these data provide a framework that strongly support our findings of a causal relationship between ADHD-like conditions and pain. Postnatal injection of 6-OHDA in mice results in ACC hyperactivity in adulthood. The up- or down-regulation of ACC activity, and more particularly of glutamatergic neurons, can increase or decrease, respectively, spinal neuron excitability and withdrawal reflex in agreement with previous studies showing that the

persistently activated ACC neurons cause tonic potentiation effect on the spinal sensory transmission and contribute to the maintenance of behavioral hyperalgesia¹⁰⁰. In addition, we established that the ACC-PI excitatory pathway controlling spinal networks and pain behavior⁸⁴ is impaired in ADHD-like conditions and underpin changes in pain sensitization. The anterior and mid-subdivisions of the cingulate cortex gate sensory hypersensitivity in mouse models of inflammatory pain via facilitatory descending projections to the spinal cord⁸⁴. This effect engages an ACC–posterior insula pathway and the downstream recruitment of serotonergic projections, probably from the rostroventral medulla (RVM) to the spinal cord. Interestingly, elevated serotonin is also involved in hyperactivity induced by 6-OHDA neonatal injection in mouse⁶⁰. The agranular insula participates in nociceptive processing in rodent and humans¹⁰¹⁻¹⁰³. Noteworthy are the multiple lines of evidence linking the PI to intensity coding, including pain intensity¹⁰⁴. Furthermore, it has been proposed that the insula subserves a general function as a general magnitude detector¹⁰⁵. ADHD patients also displayed structural alterations of the insula supporting a role for the insula in modulating attention and inhibitory capacity in ADHD¹⁰⁶.

Our study demonstrates that the ACC-posterior insula excitatory circuit bidirectionally modulates spinal neuron activity and withdrawal reflex. However, this does not exclude the existence of other, top-down pathways that may also contribute to this modulation. Previous studies reported that ACC-mediated descending facilitation of pain behavior requires rostroventral medulla (RVM) relay and provokes facilitation of a spinal nociceptive reflex⁴⁷. Moreover, a recent work from the same group demonstrated a direct, RVM-independent, ACC-spinal cord pathway, that facilitates activity of laminae I–II spinal neurons and potentiates withdrawal reflex⁸¹. Future studies will be needed to assess the respective contribution of these different parallel top-down pathways, arising from the ACC, in the control of spinal networks in ADHD-like conditions.

Mph is effective to relieve ADHD-related symptoms also induced by neonatal dopamine depletion in human¹⁰⁷ and mouse⁴². However, administration of Mph does not alleviate allodynia and hyperalgesia in 6-OHDA mice. Therefore, pain and ADHD-like symptoms are likely underpinned by partially different brain circuits. This is further confirmed by the observation that enhancing pain states with CFA has different consequences on the various ADHD-like symptoms. CFA-induced inflammatory pain modifies hyperactivity and anxiety behavior of 6-OHDA. Surprisingly, modulating pain has no effect on attention. This is in contrast with several studies that have demonstrated tight links between pain and attentional states that also involve ACC and insular cortex^{13,108,109}. However, in line with our results, some findings point to a lack of pain-induced effects on a visual search task²¹, and to the differential effects of pain on various attentional tasks²². Importantly, most of the studies to date considered the effect of acute painful stimuli on attention and only rare articles reported the consequences of chronic pain states²³. In patients with fibromyalgia, only selective attentional tasks were impaired thus suggesting here again that pain differentially regulates various attentional processes²³. Whether modulation of the ACC-PI circuit also alters ADHD-like symptoms also remains to be established. In a context of high prevalence of chronic pain¹¹⁰ and ADHD², characterized by the ineffectiveness of currently available treatments, the present study provides novel insights in the understanding of cross-effects between these pathologies. ADHD and pain may be critically involved in regulating specific sub-circuits originating from the ACC and underpinning spinal sensitization to nociceptive transmission and ADHD-like symptoms. An increased understanding of mechanisms

that underlie the overlap of psychiatric disorders with pain are key factors in the development of new therapies.

Methods

The various work flows are depicted in **Fig. S7**.

Animals.

We used male Swiss mice, 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^{41,42,111}. 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 200 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^{112,113}.

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.

Behavioral tests.

Von Frey Test. The von Frey test measures paw withdrawal responses to a range of mechanical stimuli^{112,114}. 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, with increasing bending forces ranging from 0.02 to 8.0 g, were used to deliver mechanical pressure to the right hind paw according to the ascending method¹¹⁴. 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 sensitivity¹¹⁵. 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 is 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 removal¹¹⁶. 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 protocols⁴². 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., Cornellà, Barcelona, Spain) and data were collected via a computer. Pre-training and training procedures were identical to previous studies^{41,117}. 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 $37.5^{\circ}\text{C} \pm 0.5$ 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 M Ω 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 field⁴⁶ (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 stimulation¹¹⁸. 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 M Ω 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 μ s; 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 μ l of muscimol (1 μ g/ μ l) 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 μ l) 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: 1×10^{13} ng/ml; AAV5.CamKII.ArchT3.0.eGFP: 3×10^{12} ng/ml; AAV5.CamKII.eGFP: 1×10^{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 μ m (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/mm² 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/mm² according to published protocols^{96,119,120}. 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 18 μ m 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 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). 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.

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 protocols¹²¹⁻¹²³. Background was subtracted by thresholding. The mean gray level corresponding to fluorescence intensity, and the immunolabeled area, were measured in sham and 6-OHDA¹²³. 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, mouse anti-CamK2 antibody (Biotechne; 1/1000) 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 $\pm$ 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.

References

- 1 Biederman, J. Attention-deficit/hyperactivity disorder: a selective overview. *Biol Psychiatry* **57**, 1215-1220, doi:10.1016/j.biopsych.2004.10.020 (2005).
- 2 Faraone, S. V., Sergeant, J., Gillberg, C. & Biederman, J. The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry* **2**, 104-113 (2003).
- 3 Press, A. P. (American Psychiatric Association, Washington DC, 2013).
- 4 Kooij, J. J. *et al.* Distinguishing comorbidity and successful management of adult ADHD. *J Atten Disord* **16**, 3S-19S, doi:10.1177/1087054711435361 (2012).
- 5 Barkley, R. A. Major life activity and health outcomes associated with attention-deficit/hyperactivity disorder. *J Clin Psychiatry* **63 Suppl 12**, 10-15 (2002).
- 6 Schatz, D. B. & Rostain, A. L. ADHD with comorbid anxiety: a review of the current literature. *J Atten Disord* **10**, 141-149, doi:10.1177/1087054706286698 (2006).
- 7 Alexander, L. & Farrelly, N. Attending to adult ADHD: a review of the neurobiology behind adult ADHD. *Ir J Psychol Med* **35**, 237-244, doi:10.1017/ipm.2017.78 (2018).
- 8 Konofal, E., Lecendreux, M. & Cortese, S. Sleep and ADHD. *Sleep Med* **11**, 652-658, doi:10.1016/j.sleep.2010.02.012 (2010).
- 9 Mao, A. R. & Findling, R. L. Comorbidities in adult attention-deficit/hyperactivity disorder: a practical guide to diagnosis in primary care. *Postgrad Med* **126**, 42-51, doi:10.3810/pgm.2014.09.2799 (2014).
- 10 Dellapiazza, F. *et al.* Sensory processing related to attention in children with ASD, ADHD, or typical development: results from the ELENA cohort. *Eur Child Adolesc Psychiatry*, doi:10.1007/s00787-020-01516-5 (2020).
- 11 Panagiotidi, M., Overton, P. G. & Stafford, T. The relationship between ADHD traits and sensory sensitivity in the general population. *Compr Psychiatry* **80**, 179-185, doi:10.1016/j.comppsy.2017.10.008 (2018).
- 12 Kucyi, A., Salomons, T. V. & Davis, K. D. Mind wandering away from pain dynamically engages antinociceptive and default mode brain networks. *Proc Natl Acad Sci U S A* **110**, 18692-18697, doi:10.1073/pnas.1312902110 (2013).
- 13 Villemure, C. & Bushnell, M. C. Cognitive modulation of pain: how do attention and emotion influence pain processing? *Pain* **95**, 195-199, doi:10.1016/s0304-3959(02)00007-6 (2002).
- 14 Fuermaier, A. B. M. *et al.* Perception in attention deficit hyperactivity disorder. *Atten Defic Hyperact Disord* **10**, 21-47, doi:10.1007/s12402-017-0230-0 (2018).
- 15 Treister, R., Eisenberg, E., Demeter, N. & Pud, D. Alterations in Pain Response are Partially Reversed by Methylphenidate (Ritalin) in Adults with Attention Deficit Hyperactivity Disorder (ADHD). *Pain Pract*, doi:10.1111/papr.12129 (2013).
- 16 Northover, C., Thapar, A., Langley, K. & van Goozen, S. H. Pain Sensitivity in Adolescent Males with Attention-Deficit/Hyperactivity Disorder: Testing for Associations with Conduct Disorder and Callous and Unemotional Traits. *PLoS One* **10**, e0134417, doi:10.1371/journal.pone.0134417 (2015).
- 17 Stickley, A., Koyanagi, A., Takahashi, H. & Kamio, Y. ADHD symptoms and pain among adults in England. *Psychiatry Res* **246**, 326-331, doi:10.1016/j.psychres.2016.10.004 (2016).
- 18 Stray, L. L. *et al.* Motor regulation problems and pain in adults diagnosed with ADHD. *Behav Brain Funct* **9**, 18, doi:10.1186/1744-9081-9-18 (2013).
- 19 Lensing, M. B., Zeiner, P., Sandvik, L. & Opjordsmoen, S. Quality of life in adults aged 50+ with ADHD. *J Atten Disord* **19**, 405-413, doi:10.1177/1087054713480035 (2015).
- 20 Vingilis, E. *et al.* Attention Deficit Hyperactivity Disorder Symptoms, Comorbidities, Substance Use, and Social Outcomes among Men and Women in a Canadian Sample. *Biomed Res Int* **2015**, 982072, doi:10.1155/2015/982072 (2015).
- 21 Veldhuijzen, D. S., Kenemans, J. L., de Bruin, C. M., Olivier, B. & Volkerts, E. R. Pain and attention: attentional disruption or distraction? *J Pain* **7**, 11-20, doi:10.1016/j.jpain.2005.06.003 (2006).
- 22 Moore, D. J., Keogh, E. & Eccleston, C. The interruptive effect of pain on attention. *Q J Exp Psychol (Hove)* **65**, 565-586, doi:10.1080/17470218.2011.626865 (2012).
- 23 Moore, D. J. *et al.* The Effect of Induced and Chronic Pain on Attention. *J Pain* **20**, 1353-1361, doi:10.1016/j.jpain.2019.05.004 (2019).

- 24 Kasahara, S. *et al.* Attention-Deficit/Hyperactivity Disorder and Chronic Pain. *Psychosom Med* **82**, 346-347, doi:10.1097/PSY.0000000000000789 (2020).
- 25 Higgins, G. A. *et al.* Enduring attentional deficits in rats treated with a peripheral nerve injury. *Behav Brain Res* **286**, 347-355, doi:10.1016/j.bbr.2015.02.050 (2015).
- 26 Ogata, M., Noda, K., Akita, H. & Ishibashi, H. Characterization of nociceptive response to chemical, mechanical, and thermal stimuli in adolescent rats with neonatal dopamine depletion. *Neuroscience* **289**, 43-55, doi:10.1016/j.neuroscience.2015.01.002 (2015).
- 27 Liu, M. G. & Chen, J. Preclinical research on pain comorbidity with affective disorders and cognitive deficits: Challenges and perspectives. *Prog Neurobiol* **116**, 13-32, doi:10.1016/j.pneurobio.2014.01.003 S0301-0082(14)00013-6 [pii] (2014).
- 28 Sagvolden, T., Johansen, E. B., Aase, H. & Russell, V. A. A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behav Brain Sci* **28**, 397-419; discussion 419-368, doi:10.1017/S0140525X05000075 (2005).
- 29 Oades, R. D. *et al.* The control of responsiveness in ADHD by catecholamines: evidence for dopaminergic, noradrenergic and interactive roles. *Dev Sci* **8**, 122-131, doi:10.1111/j.1467-7687.2005.00399.x (2005).
- 30 Swanson, J. M. *et al.* Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev* **17**, 39-59, doi:10.1007/s11065-007-9019-9 (2007).
- 31 Tripp, G. & Wickens, J. R. Neurobiology of ADHD. *Neuropharmacology* **57**, 579-589, doi:10.1016/j.neuropharm.2009.07.026 (2009).
- 32 Ko, I. G. *et al.* Swimming exercise alleviates the symptoms of attention-deficit hyperactivity disorder in spontaneous hypertensive rats. *Mol Med Rep* **8**, 393-400, doi:10.3892/mmr.2013.1531 (2013).
- 33 Bledsoe, J. C., Semrud-Clikeman, M. & Pliszka, S. R. Anterior cingulate cortex and symptom severity in attention-deficit/hyperactivity disorder. *J Abnorm Psychol* **122**, 558-565, doi:10.1037/a0032390 (2013).
- 34 Vogt, B. A. Cingulate impairments in ADHD: Comorbidities, connections, and treatment. *Handb Clin Neurol* **166**, 297-314, doi:10.1016/B978-0-444-64196-0.00016-9 (2019).
- 35 Ernst, M. *et al.* High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder. *Am J Psychiatry* **156**, 1209-1215, doi:10.1176/ajp.156.8.1209 (1999).
- 36 Lou, H. C., Henriksen, L., Bruhn, P., Borner, H. & Nielsen, J. B. Striatal dysfunction in attention deficit and hyperkinetic disorder. *Arch Neurol* **46**, 48-52, doi:10.1001/archneur.1989.00520370050018 (1989).
- 37 Schweitzer, J. B. *et al.* Alterations in the functional anatomy of working memory in adult attention deficit hyperactivity disorder. *Am J Psychiatry* **157**, 278-280, doi:10.1176/appi.ajp.157.2.278 (2000).
- 38 Vaidya, C. J. *et al.* Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. *Proc Natl Acad Sci U S A* **95**, 14494-14499, doi:10.1073/pnas.95.24.14494 (1998).
- 39 Jarcho, J. M., Mayer, E. A., Jiang, Z. K., Feier, N. A. & London, E. D. Pain, affective symptoms, and cognitive deficits in patients with cerebral dopamine dysfunction. *Pain* **153**, 744-754, doi:10.1016/j.pain.2012.01.002 (2012).
- 40 Ong, W. Y., Stohler, C. S. & Herr, D. R. Role of the Prefrontal Cortex in Pain Processing. *Mol Neurobiol* **56**, 1137-1166, doi:10.1007/s12035-018-1130-9 (2019).
- 41 Bouchatta, O., Manouze, H., Ba-M'Hamed, S., Landry, M. & Bennis, M. 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, doi:10.3389/fnbeh.2020.00027 (2020).
- 42 Bouchatta, O. *et al.* Neonatal 6-OHDA lesion model in mouse induces Attention-Deficit/ Hyperactivity Disorder (ADHD)-like behaviour. *Scientific Reports* **8**, 15349, doi:10.1038/s41598-018-33778-0 (2018).
- 43 Koblinger, K. *et al.* Characterization of A11 neurons projecting to the spinal cord of mice. *PLoS One* **9**, e109636, doi:10.1371/journal.pone.0109636 (2014).

- 44 West, A. E. *et al.* Calcium regulation of neuronal gene expression. *Proc Natl Acad Sci U S A* **98**, 11024-11031, doi:10.1073/pnas.191352298 (2001).
- 45 Woolf, C. J. & Salter, M. W. Neuronal plasticity: increasing the gain in pain. *Science* **288**, 1765-1769, doi:10.1126/science.288.5472.1765 (2000).
- 46 Fossat, P. *et al.* Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. *J Neurosci* **30**, 1073-1085, doi:10.1523/JNEUROSCI.3145-09.2010 (2010).
- 47 Calejesan, A. A., Kim, S. J. & Zhuo, M. Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex. *Eur J Pain* **4**, 83-96, doi:10.1053/eujp.1999.0158 (2000).
- 48 Egger, H. L., Costello, E. J., Erkanli, A. & Angold, A. Somatic complaints and psychopathology in children and adolescents: stomach aches, musculoskeletal pains, and headaches. *J Am Acad Child Adolesc Psychiatry* **38**, 852-860, doi:10.1097/00004583-199907000-00015 (1999).
- 49 Scherder, E. J., Rommelse, N. N., Broring, T., Faraone, S. V. & Sergeant, J. A. Somatosensory functioning and experienced pain in ADHD-families: a pilot study. *Eur J Paediatr Neurol* **12**, 461-469, doi:10.1016/j.ejpn.2007.11.004 (2008).
- 50 Wolff, N. *et al.* Reduced pain perception in children and adolescents with ADHD is normalized by methylphenidate. *Child Adolesc Psychiatry Ment Health* **10**, 24, doi:10.1186/s13034-016-0112-9 (2016).
- 51 Moriarty, O., McGuire, B. E. & Finn, D. P. The effect of pain on cognitive function: a review of clinical and preclinical research. *Progress in neurobiology* **93**, 385-404, doi:10.1016/j.pneurobio.2011.01.002 (2011).
- 52 Pais-Vieira, M., Lima, D. & Galhardo, V. Sustained attention deficits in rats with chronic inflammatory pain. *Neurosci Lett* **463**, 98-102, doi:10.1016/j.neulet.2009.07.050 (2009).
- 53 Bowton, E. *et al.* Dysregulation of dopamine transporters via dopamine D2 autoreceptors triggers anomalous dopamine efflux associated with attention-deficit hyperactivity disorder. *J Neurosci* **30**, 6048-6057, doi:10.1523/JNEUROSCI.5094-09.2010 (2010).
- 54 Pelissier, T., Laurido, C., Hernandez, A., Constandil, L. & Eschalier, A. Biphasic effect of apomorphine on rat nociception and effect of dopamine D2 receptor antagonists. *Eur J Pharmacol* **546**, 40-47, doi:10.1016/j.ejphar.2006.06.081 (2006).
- 55 Wood, P. B. Role of central dopamine in pain and analgesia. *Expert Rev Neurother* **8**, 781-797, doi:10.1586/14737175.8.5.781 (2008).
- 56 Davids, E., Zhang, K., Kula, N. S., Tarazi, F. I. & Baldessarini, R. J. Effects of norepinephrine and serotonin transporter inhibitors on hyperactivity induced by neonatal 6-hydroxydopamine lesioning in rats. *J Pharmacol Exp Ther* **301**, 1097-1102, doi:10.1124/jpet.301.3.1097 (2002).
- 57 Davids, E., Zhang, K., Tarazi, F. I. & Baldessarini, R. J. Animal models of attention-deficit hyperactivity disorder. *Brain Res Brain Res Rev* **42**, 1-21, doi:10.1016/s0165-0173(02)00274-6 (2003).
- 58 Zhang, K., Tarazi, F. I., Davids, E. & Baldessarini, R. J. Plasticity of dopamine D4 receptors in rat forebrain: temporal association with motor hyperactivity following neonatal 6-hydroxydopamine lesioning. *Neuropsychopharmacology* **26**, 625-633, doi:10.1016/S0893-133X(01)00404-3 (2002).
- 59 Avale, M. E. *et al.* The dopamine D4 receptor is essential for hyperactivity and impaired behavioral inhibition in a mouse model of attention deficit/hyperactivity disorder. *Mol Psychiatry* **9**, 718-726, doi:10.1038/sj.mp.4001474 (2004).
- 60 Avale, M. E., Nemirovsky, S. I., Raisman-Vozari, R. & Rubinstein, M. Elevated serotonin is involved in hyperactivity but not in the paradoxical effect of amphetamine in mice neonatally lesioned with 6-hydroxydopamine. *J Neurosci Res* **78**, 289-296, doi:10.1002/jnr.20245 (2004).
- 61 Bari, A. & Robbins, T. W. Animal models of ADHD. *Curr Top Behav Neurosci* **7**, 149-185, doi:10.1007/7854_2010_102 (2011).
- 62 Aby, F., Whitestone, S., Landry, M., Ulmann, L. & Fossat, P. Inflammatory-induced spinal dorsal horn neurons hyperexcitability is mediated by P2X4 receptors. *Pain Rep* **3**, e660, doi:10.1097/PR9.0000000000000660 (2018).
- 63 Eliava, M. *et al.* A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron* **89**, 1291-1304, doi:10.1016/j.neuron.2016.01.041 (2016).

- 64 McGaraughty, S., Chu, K. L. & Xu, J. Characterization and pharmacological modulation of nociceptive deep dorsal horn neurons across diverse rat models of pathological pain. *J Neurophysiol* **120**, 1893-1905, doi:10.1152/jn.00325.2018 (2018).
- 65 Yao, Y. X., Jiang, Z. & Zhao, Z. Q. Knockdown of synaptic scaffolding protein Homer 1b/c attenuates secondary hyperalgesia induced by complete Freund's adjuvant in rats. *Anesth Analg* **113**, 1501-1508, doi:10.1213/ANE.0b013e31822c0b98 (2011).
- 66 Obara, I. *et al.* Nerve injury-induced changes in Homer/glutamate receptor signaling contribute to the development and maintenance of neuropathic pain. *Pain* **154**, 1932-1945, doi:10.1016/j.pain.2013.03.035 (2013).
- 67 Gutierrez-Mecinas, M. *et al.* Immunostaining for Homer reveals the majority of excitatory synapses in laminae I-III of the mouse spinal dorsal horn. *Neuroscience* **329**, 171-181, doi:10.1016/j.neuroscience.2016.05.009 (2016).
- 68 Kuner, R. Central mechanisms of pathological pain. *Nat Med* **16**, 1258-1266, doi:10.1038/nm.2231 (2010).
- 69 Miletic, G., Dumitrascu, C. I., Honstad, C. E., Micic, D. & Miletic, V. Loose ligation of the rat sciatic nerve elicits early accumulation of Shank1 protein in the post-synaptic density of spinal dorsal horn neurons. *Pain* **149**, 152-159, doi:10.1016/j.pain.2010.02.001 (2010).
- 70 Fang, L., Wu, J., Lin, Q. & Willis, W. D. Calcium-calmodulin-dependent protein kinase II contributes to spinal cord central sensitization. *J Neurosci* **22**, 4196-4204, doi:20026343 (2002).
- 71 Ji, R. R., Gereau, R. W. t., Malcangio, M. & Strichartz, G. R. MAP kinase and pain. *Brain Res Rev* **60**, 135-148, doi:10.1016/j.brainresrev.2008.12.011 (2009).
- 72 Wu, J. *et al.* Protein kinases mediate increment of the phosphorylation of cyclic AMP-responsive element binding protein in spinal cord of rats following capsaicin injection. *Mol Pain* **1**, 26, doi:10.1186/1744-8069-1-26 (2005).
- 73 Ji, R. R., Kohno, T., Moore, K. A. & Woolf, C. J. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* **26**, 696-705, doi:10.1016/j.tins.2003.09.017 (2003).
- 74 Sandkuhler, J. Understanding LTP in pain pathways. *Mol Pain* **3**, 9, doi:10.1186/1744-8069-3-9 (2007).
- 75 Latremoliere, A. & Woolf, C. J. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain* **10**, 895-926, doi:10.1016/j.jpain.2009.06.012 (2009).
- 76 Reinig, S., Driever, W. & Arrenberg, A. B. The Descending Diencephalic Dopamine System Is Tuned to Sensory Stimuli. *Curr Biol* **27**, 318-333, doi:10.1016/j.cub.2016.11.059 (2017).
- 77 Ossipov, M. H., Morimura, K. & Porreca, F. Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* **8**, 143-151, doi:10.1097/SPC.0000000000000055 (2014).
- 78 Reynolds, D. V. Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* **164**, 444-445, doi:10.1126/science.164.3878.444 (1969).
- 79 Porreca, F., Ossipov, M. H. & Gebhart, G. F. Chronic pain and medullary descending facilitation. *Trends Neurosci* **25**, 319-325, doi:10.1016/s0166-2236(02)02157-4 (2002).
- 80 Gebhart, G. F. Descending modulation of pain. *Neurosci Biobehav Rev* **27**, 729-737, doi:10.1016/j.neubiorev.2003.11.008 (2004).
- 81 Chen, T. *et al.* Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. *Nat Commun* **9**, 1886, doi:10.1038/s41467-018-04309-2 (2018).
- 82 Roca-Lapirot, O. *et al.* Acquisition of analgesic properties by the cholecystokinin/CCK2 receptor system within the amygdala in a persistent inflammatory pain condition. *Pain*, doi:10.1097/j.pain.0000000000001408 (2018).
- 83 Huang, J. *et al.* A neuronal circuit for activating descending modulation of neuropathic pain. *Nat Neurosci* **22**, 1659-1668, doi:10.1038/s41593-019-0481-5 (2019).
- 84 Tan, L. L. *et al.* A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat Neurosci* **20**, 1591-1601, doi:10.1038/nn.4645 (2017).
- 85 Bliss, T. V., Collingridge, G. L., Kaang, B. K. & Zhuo, M. Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nature reviews. Neuroscience* **17**, 485-496, doi:10.1038/nrn.2016.68 (2016).
- 86 Vogt, B. A. Pain and emotion interactions in subregions of the cingulate gyrus. *Nature reviews. Neuroscience* **6**, 533-544, doi:10.1038/nrn1704 (2005).

- 87 Koga, K. *et al.* Coexistence of two forms of LTP in ACC provides a synaptic mechanism for the interactions between anxiety and chronic pain. *Neuron* **85**, 377-389, doi:10.1016/j.neuron.2014.12.021 (2015).
- 88 Zhuo, M. Cortical excitation and chronic pain. *Trends Neurosci* **31**, 199-207, doi:10.1016/j.tins.2008.01.003 (2008).
- 89 Faraone, S. V. Attention deficit hyperactivity disorder and premature death. *Lancet* **385**, 2132-2133, doi:10.1016/S0140-6736(14)61822-5 (2015).
- 90 Vetter, N. C. *et al.* Anterior insula hyperactivation in ADHD when faced with distracting negative stimuli. *Hum Brain Mapp* **39**, 2972-2986, doi:10.1002/hbm.24053 (2018).
- 91 Chen, T. *et al.* Adenylyl cyclase subtype 1 is essential for late-phase long term potentiation and spatial propagation of synaptic responses in the anterior cingulate cortex of adult mice. *Mol Pain* **10**, 65, doi:10.1186/1744-8069-10-65 1744-8069-10-65 [pii] (2014).
- 92 Blom, S. M., Pfister, J. P., Santello, M., Senn, W. & Nevian, T. Nerve injury-induced neuropathic pain causes disinhibition of the anterior cingulate cortex. *J Neurosci* **34**, 5754-5764, doi:10.1523/JNEUROSCI.3667-13.2014 (2014).
- 93 Cordeiro Matos, S., Zhang, Z. & Seguela, P. Peripheral Neuropathy Induces HCN Channel Dysfunction in Pyramidal Neurons of the Medial Prefrontal Cortex. *J Neurosci* **35**, 13244-13256, doi:10.1523/JNEUROSCI.0799-15.2015 (2015).
- 94 Humo, M. *et al.* Ketamine induces rapid and sustained antidepressant-like effects in chronic pain induced depression: Role of MAPK signaling pathway. *Prog Neuropsychopharmacol Biol Psychiatry* **100**, 109898, doi:10.1016/j.pnpbp.2020.109898 (2020).
- 95 Sellmeijer, J. *et al.* Hyperactivity of anterior cingulate cortex areas 24a/24b drives chronic pain-induced anxiodepressive-like consequences. *J Neurosci* **38**, 3102-3115, doi:10.1523/JNEUROSCI.3195-17.2018 (2018).
- 96 Kang, S. J. *et al.* Bidirectional modulation of hyperalgesia via the specific control of excitatory and inhibitory neuronal activity in the ACC. *Mol Brain* **8**, 81, doi:10.1186/s13041-015-0170-6 (2015).
- 97 Barthas, F. *et al.* The anterior cingulate cortex is a critical hub for pain-induced depression. *Biol Psychiatry* **77**, 236-245, doi:10.1016/j.biopsych.2014.08.004 (2015).
- 98 Fillinger, C., Yalcin, I., Barrot, M. & Veinante, P. Efferents of anterior cingulate areas 24a and 24b and midcingulate areas 24a' and 24b' in the mouse. *Brain Struct Funct* **223**, 1747-1778, doi:10.1007/s00429-017-1585-x (2017).
- 99 Vogt, B. A. & Paxinos, G. Cytoarchitecture of mouse and rat cingulate cortex with human homologies. *Brain Struct Funct* **219**, 185-192, doi:10.1007/s00429-012-0493-3 (2014).
- 100 Chen, T. *et al.* Postsynaptic potentiation of corticospinal projecting neurons in the anterior cingulate cortex after nerve injury. *Mol Pain* **10**, 33, doi:10.1186/1744-8069-10-33 (2014).
- 101 Burkey, A. R., Carstens, E. & Jasmin, L. Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. *J Neurosci* **19**, 4169-4179 (1999).
- 102 Jasmin, L., Rabkin, S. D., Granato, A., Boudah, A. & Ohara, P. T. Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. *Nature* **424**, 316-320, doi:10.1038/nature01808 (2003).
- 103 Peyron, R., Laurent, B. & Garcia-Larrea, L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol Clin* **30**, 263-288, doi:10.1016/s0987-7053(00)00227-6 (2000).
- 104 Segerdahl, A. R., Mezue, M., Okell, T. W., Farrar, J. T. & Tracey, I. The dorsal posterior insula subserves a fundamental role in human pain. *Nat Neurosci* **18**, 499-500, doi:10.1038/nn.3969 (2015).
- 105 Baliki, M. N., Geha, P. Y. & Apkarian, A. V. Parsing pain perception between nociceptive representation and magnitude estimation. *J Neurophysiol* **101**, 875-887, doi:10.1152/jn.91100.2008 (2009).
- 106 Lopez-Larson, M. P., King, J. B., Terry, J., McGlade, E. C. & Yurgelun-Todd, D. Reduced insular volume in attention deficit hyperactivity disorder. *Psychiatry Res* **204**, 32-39, doi:10.1016/j.psychres.2012.09.009 (2012).
- 107 Jonkman, L. M. *et al.* Perceptual and response interference in children with attention-deficit hyperactivity disorder, and the effects of methylphenidate. *Psychophysiology* **36**, 419-429 (1999).

- 108 Petrovic, P., Petersson, K. M., Ghatan, P. H., Stone-Elander, S. & Ingvar, M. Pain-related cerebral activation is altered by a distracting cognitive task. *Pain* **85**, 19-30, doi:10.1016/s0304-3959(99)00232-8 (2000).
- 109 Peyron, R. *et al.* Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. *Brain* **122** (Pt 9), 1765-1780, doi:10.1093/brain/122.9.1765 (1999).
- 110 Bouhassira, D., Lanteri-Minet, M., Attal, N., Laurent, B. & Touboul, C. Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* **136**, 380-387, doi:S0304-3959(07)00449-6 [pii] 10.1016/j.pain.2007.08.013 (2008).
- 111 Sontag, T. A., Tucha, O., Walitza, S. & Lange, K. W. Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. *Atten Defic Hyperact Disord* **2**, 1-20, doi:10.1007/s12402-010-0019-x (2010).
- 112 Abboud, C. *et al.* Animal models of pain: diversity and benefits. *J Neurosci Methods*, 108997, doi:10.1016/j.jneumeth.2020.108997 (2020).
- 113 Bortalanza, L. B. *et al.* Anti-allodynic action of the tormentic acid, a triterpene isolated from plant, against neuropathic and inflammatory persistent pain in mice. *Eur J Pharmacol* **453**, 203-208, doi:10.1016/s0014-2999(02)02428-7 (2002).
- 114 Deuis, J. R., Dvorakova, L. S. & Vetter, I. Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* **10**, 284, doi:10.3389/fnmol.2017.00284 (2017).
- 115 Shi, M., Qi, W. J., Gao, G., Wang, J. Y. & Luo, F. Increased thermal and mechanical nociceptive thresholds in rats with depressive-like behaviors. *Brain Res* **1353**, 225-233, doi:10.1016/j.brainres.2010.07.023 (2010).
- 116 O'Brien, D. E., Brenner, D. S., Gutmann, D. H. & Gereau, R. W. t. Assessment of pain and itch behavior in a mouse model of neurofibromatosis type 1. *The journal of pain* **14**, 628-637, doi:10.1016/j.jpain.2013.01.770 (2013).
- 117 Humby, T., Wilkinson, L. & Dawson, G. 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, doi:10.1002/0471142301.ns0805hs31 (2005).
- 118 Senapati, A. K., Huntington, P. J. & Peng, Y. B. Spinal dorsal horn neuron response to mechanical stimuli is decreased by electrical stimulation of the primary motor cortex. *Brain Res* **1036**, 173-179, doi:10.1016/j.brainres.2004.12.043 (2005).
- 119 Madisen, L. *et al.* A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nat Neurosci* **15**, 793-802, doi:10.1038/nn.3078 (2012).
- 120 Zhang, F. *et al.* Multimodal fast optical interrogation of neural circuitry. *Nature* **446**, 633-639, doi:10.1038/nature05744 (2007).
- 121 Landry, M., Bouali-Benazzouz, R., El Mestikawy, S., Ravassard, P. & Nagy, F. Expression of vesicular glutamate transporters in rat lumbar spinal cord, with a note on dorsal root ganglia. *J Comp Neurol* **468**, 380-394, doi:10.1002/cne.10988 (2004).
- 122 Papon, M. A. *et al.* Spinal Inhibition of GABAB Receptors by the Extracellular Matrix Protein Fibulin-2 in Neuropathic Rats. *Front Cell Neurosci* **14**, 214, doi:10.3389/fncel.2020.00214 (2020).
- 123 Zaky, A., Bouali-Benazzouz, R., Favereaux, A., Tell, G. & Landry, M. APE1/Ref-1 redox function contributes to inflammatory pain sensitization. *Exp Neurol* **307**, 1-11, doi:10.1016/j.expneurol.2018.05.014 (2018).
- 124 Paxinos, G. & Franklin, K. B. J. (Academic Press, San Diego, 2001).

Figure legends

Fig. 1. Hypersensitivity to pain in ADHD-like conditions.

A & D1-2. Paw licking latency in hot plate (55°C). **B & E1-2.** Paw licking latency in cold plate (5°C). **C & F1-2.** Paw withdrawal thresholds using von Frey filaments.

A-C. A significant decrease was found in the paw licking latency of 6-OHDA mice to hot (A, 10.81 ± 0.89 s vs 16.07 ± 0.90 s; $t=4.16$, $p=0.0006$), cold (B, 12.51 ± 0.66 s vs 16.60 ± 0.83 s; $t=3.87$, $p=0.002$) and mechanical stimuli (C, 1.26 ± 0.10 g vs 1.88 ± 0.08 g; $t=4.74$, $p=0.0002$) in comparison to sham mice. All data are means $\pm$ SEM (10 mice per group), ** $p<0.01$; *** $p<0.001$ vs Sham.

D1-3. We evaluated ADHD-induced pain sensitization under inflammatory pain conditions. After the pre-test (one day before CFA injection; Pre-CFA), CFA or saline was injected in the sole of the right hind paw. The post-test (Post-CFA) was done four days after CFA injection. Two-way repeated measures ANOVA showed a significant effect of the lesion (6-OHDA) ([heat]: $F_{(3,27)}=98.48$, $p=0.0001$; [cold]: $F_{(3,27)}=257.0$, $p=0.0001$; [von Frey]: $F_{(3,27)}=243.4$, $p=0.0001$), inflammation (CFA) ([heat]: $F_{(4,36)}=9.69$; $p=0.0001$; [cold]: $F_{(4,36)}=14.64$, $p=0.0001$; [von Frey]: $F_{(4,36)}=20.40$, $p=0.0001$) and interaction 6-OHDA $\times$ CFA ([heat]: $F_{(12,108)}=3.62$, $p=0.0001$; [cold]: $F_{(12,108)}=5.47$, $p=0.0001$; [von Frey]: $F_{(12,108)}=3.60$, $p=0.0002$) on thermal and mechanical sensitivity. There was a significant decrease in pain licking latency to thermal stimuli in sham ([heat]: 9.77 ± 0.75 s vs 15.11 ± 0.54 s; $q=6.87$, $p=0.0001$; [cold]: 11.33 ± 0.41 s vs 15.95 ± 0.29 s; $q=7.72$, $p=0.0001$) and 6-OHDA mice ([heat]: 3.42 ± 0.75 s vs 9.53 ± 0.72 s; $q=7.86$, $p=0.0001$; [cold]: 4.38 ± 0.59 s vs 10.43 ± 0.13 s; $q=10.11$, $p=0.0001$) 4 days post-CFA in comparison to the pre-CFA. CFA injection also resulted in a lowering of the mechanical threshold in sham (1.18 ± 0.12 g vs 1.82 ± 0.09 g; $q=6.12$, $p=0.0001$) and 6-OHDA mice (0.34 ± 0.09 g vs 1.26 ± 0.10 g; $q=8.87$, $p=0.0001$) 4 days post-CFA in comparison to the pre-CFA. In contrast, intraplantar injection of NaCl in the hind paw had no effect on thermal (sham: [heat]: 15.62 ± 0.70 s vs 15.73 ± 0.63 s; $q=0.15$, $p>0.05$; [cold]: 17.24 ± 0.64 s vs 16.89 ± 0.71 s; $q=0.59$, $p>0.05$; 6-OHDA: [heat]: 10.80 ± 1.05 s vs 10.31 ± 0.93 s; $q=0.63$, $p>0.05$; [cold]: 10.81 ± 0.71 s vs 10.90 ± 0.74 s; $q=0.16$, $p>0.05$) and mechanical sensitivity (sham: 1.82 ± 0.09 g vs 1.88 ± 0.08 g; $q=0.57$, $p>0.05$; 6-OHDA: 1.26 ± 0.10 g vs 1.30 ± 0.10 g; $q=0.38$, $p>0.05$) in both groups. All data are means $\pm$ SEM (10 mice per group), * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs Pre-CFA; ## $p<0.01$; ### $p<0.001$ vs NaCl; §§ $p<0.01$; §§§ $p<0.001$ vs Sham-CFA.

E1-3. Amplitude of changes in latency (D2, hot plate; E2, cold plate) or threshold (F2, von Frey). ADHD-like conditions increases CFA-induced pain sensitization in response to thermal ([heat]: -67.01 ± 4.60 % vs -36.20 ± 3.21 %; $t=10.44$, $p=0.0001$; [cold]: -58.48 ± 5.22 % vs -29.12 ± 1.49 %; $t=6.92$, $p=0.0001$) and mechanical (-75.86 ± 4.15 % vs -35.43 ± 5.32 %; $t=12.19$, $p=0.0001$) stimuli. All data are mean % of Pre-CFA $\pm$ SEM (10 mice per group), ** $p<0.01$; *** $p<0.001$ vs Sham.

Fig. 2. Hyperexcitability of deep dorsal horn neurons (DHNs) in ADHD-like conditions.

A & B. Single unit *in vivo* extracellular recordings of deep dorsal horn neurons (DHNs) in response to peripheral mechanical stimulation (1.4 to 6.0 g von Frey filament) under control (NaCl) and inflammatory pain conditions (CFA). **C.** Quantification of the number of action potentials (number of spikes/5 sec.) after peripheral mechanical stimulation. Two-way repeated measures ANOVA showed a significant effect of the group ([1.4g]: $F_{(7,63)}=207.7$, $p=0.0001$; [2.0g]: $F_{(7,63)}=184.3$, $p=0.0001$; [4.0g]: $F_{(7,63)}=159.0$, $p=0.0001$; [6.0g]: $F_{(7,63)}=108.5$, $p=0.0001$) on DHNs electrical discharges. In contrast, Mph treatment (5.0mg/kg Mph) ([1.4g]: $F_{(3,27)}=0.13$; $p=0.094$; [2.0g]: $F_{(3,27)}=0.20$, $p=0.90$; [4.0g]: $F_{(3,27)}=0.21$; $p=0.89$; [6.0g]: $F_{(3,27)}=0.63$, $p=0.60$) and interaction

group x Mph ([1.4g]: $F_{(21,189)}=0.85$, $p=0.66$; [2.0g]: $F_{(21,189)}=0.34$, $p=0.99$; [4.0g]: $F_{(21,189)}=0.20$, $p=0.99$ and [6.0g]: $F_{(21,189)}=0.29$, $p=0.099$) had no effect. There was a significant increase in discharge of 6-OHDA mice DHNs in response to innocuous ([1.4g]: 10.25 ± 0.83 vs 1.0 ± 0.73 ; $q=12.73$, $p=0.0001$) and noxious stimulus ([6.0g]: 37.63 ± 3.42 vs 16.88 ± 1.57 ; $q=7.77$, $p=0.0001$) in comparison to sham mice. In inflammatory pain conditions, there was a significant increase of DHNs activity in response to innocuous ([1.4g]: 5.67 ± 0.79 vs 1.00 ± 0.73 ; $q=6.43$, $p=0.001$) and noxious stimuli ([6.0g]: 29.02 ± 1.56 vs 16.88 ± 1.57 ; $q=4.54$, $p<0.05$) in sham mice as compared to their controls (NaCl-injected sham mice). In contrast, CFA injection caused a further increase of DHN activity in 6-OHDA mice in response to both innocuous ([1.4g]: 15.60 ± 1.03 vs 10.25 ± 0.83 ; $q=7.36$, $p=0.0001$) and noxious stimuli ([6.0g]: 56.49 ± 3.30 vs 37.63 ± 3.42 ; $q=7.06$, $p=0.0001$) as compared to their controls (NaCl-injected 6-OHDA mice).

After recording the baseline electrical activity of DHNs, Mph or vehicle (0.9% NaCl) was injected intraperitoneally and the single unit extracellular recording was done every 20 minutes post-injection during one hour. There was no significant effect of ADHD medication on electrical DHNs activity in both sham and 6-OHDA groups in response to peripheral innocuous and noxious mechanical stimulation under control (sham: [1.4g]: 1.41 ± 0.65 vs 1.28 ± 0.57 ; $q=0.18$, $p>0.05$; [6.0g]: 15.42 ± 1.77 vs 15.37 ± 1.49 ; $q=0.02$, $p>0.05$; 6-OHDA: [1.4g]: 8.96 ± 0.87 vs 10.38 ± 1.07 ; $q=1.95$, $p>0.05$; [6.0g]: 38.84 ± 2.07 vs 41.33 ± 2.55 ; $q=0.93$, $p>0.05$) and inflammatory pain conditions (sham: [1.4g]: 4.63 ± 0.76 vs 4.55 ± 0.64 ; $q=0.11$, $p>0.05$; [6.0g]: 28.86 ± 2.80 vs 28.97 ± 2.07 ; $q=0.04$, $p>0.05$; 6-OHDA: [1.4g]: 28.86 ± 2.81 vs 28.97 ± 2.08 ; $q=0.26$, $p>0.05$; [6.0g]: 54.90 ± 4.08 vs 57.87 ± 4.07 ; $q=1.11$, $p>0.05$). In addition, vehicle injection had no effect on DHNs discharge in response to peripheral innocuous and noxious mechanical stimulation under control and inflammatory pain conditions in both sham and 6-OHDA groups. All data are means $\pm$ SEM (10 neurons per group), ^a $p<0.05$; ^b $p<0.01$; ^c $p<0.001$ vs NaCl; ^d $p<0.001$ vs Sham.

Fig. 3. ADHD-like conditions modify dorsal spinal networks.

A-B. Expression of synaptic markers in the dorsal spinal cord. Co-expression of post-synaptic gephyrin (A, red; a1, d1, c1, f1) or homer 1 (B, red; a1, d1, c1, f1) and pre-synaptic synaptophysin (A and B, green; b1, c1, e1, f1) in the dorsal horn of sham and 6-OHDA mice.

Frames in Aa1-f1 and Ba1-f1 are displayed at higher magnification in Aa2-f2 and Ba2-f2. Arrowheads point to close apposition between markers in Aa2-f2 and Ba2-f2. Quantification of post-synaptic immunolabeling intensity (left), and colocalisation with the pre-synaptic marker synaptophysin (right), are shown in A and B. Homer 1, but not gephyrin, is overexpressed at synaptic sites in 6-OHDA mice. ($n=7$ sections in 4 mice; t-test, A: $p>0.05$; B: intensity: $p=0.0056$, colocalization: $p=0.0019$). Bar: 50 μ m (a1-f1), 20 μ m (a2-f2). Geph: gephyrin, Syn: synaptophysin.

C. TH immunodetection of fibres (arrowheads) or cell bodies (arrows) in adult sham (a, c, e, g) and 6-OHDA (b, d, f, h) mice in the striatum (a, b), the A11 hypothalamic area (c, d), the ACC (e, f) and the spinal cord (g, h).

The quantification shows that TH intensity is lowered in the striatum but not in the other areas, while TH immunolabeled area is decreased in striatum and ACC but not affected in the A11 or the spinal cord. ($n=9$ (striatum), 7 (ACC and spinal cord), or 5 (A11) sections in 4 mice; t-test, intensity in the striatum: $p=0.000028$; area in the striatum: $p<0.00001$; area in the ACC: $p=0.0048$). Bar: 50 μ m. Str: striatum, ACC: anterior cingulate cortex, SC: spinal cord. ** $p<0.01$, *** $p<0.001$, NS = non-significant.

D. Western-blot detection of total and phosphorylated forms of activation-dependent intracellular kinases (CaMKII, ERK) and transcription factor (CREB).

Phosphorylated CaMKII, ERK and CREB are overexpressed in 6-OHDA mice. (n=4 mice; t-test, CaMKII: p=0.00086; pERK/ERK: p=0.0063; pCREB/CREB: p=0.00072).

E. Immunodetection of pERK (arrows) in the dorsal horn of sham (a) and 6-OHDA (b) mice. The quantification of pERK labeling intensity and area indicates ERK activation in 6-OHDA mice. (n=7 sections in 4 mice; t-test, intensity: p=0.0057, area: p=0.0069). Bar: 25 μ m.

Fig. 4. 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 ($68.30 \pm 1.54\%$ vs $78.70 \pm 1.83\%$; $q=10.38$, $p=0.0001$), more omission errors ($27.90 \pm 2.14\%$ vs $10.00 \pm 1.91\%$; $q=14.68$, $p=0.0001$), premature responding ($10.10 \pm 1.16\%$ vs $2.70 \pm 0.80\%$; $q=7.50$, $p=0.0001$) 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]: $81.20 \pm 1.27\%$ vs $80.00 \pm 1.41\%$; $q=0.85$, $p>0.05$; [6-OHDA]: $68.00 \pm 1.58\%$ vs $69.50 \pm 1.14\%$; $q=1.06$, $p>0.05$) and omissions ([sham]: $8.80 \pm 1.25\%$ vs $8.80 \pm 1.68\%$; $q=0.00$; $p>0.05$; [6-OHDA]: $30.10 \pm 1.73\%$ vs $27.30 \pm 1.54\%$; $q=1.62$, $p>0.05$) in both groups. Injection had a significant effect *per se* only in 6-OHDA mice, increasing premature responding ([sham]: $1.40 \pm 0.83\%$ vs $2.00 \pm 0.63\%$; $q=0.43$, $p>0.05$; [6-OHDA]: $15.80 \pm 1.39\%$ vs $8.90 \pm 1.35\%$; $q=4.95$, $p=0.001$) 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 $\pm$ 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. 5: Anterior cingulate cortex is the key brain area of descending control dysfunctions in ADHD-like conditions.

A. Representative diagram of the *in vivo* recording procedure of ACC neuron spontaneous activity (left side). **A1.** Single unit *in vivo* extracellular recording of spontaneous activity of ACC neuron in urethane-anesthetized animals. **A2.** Quantification of the number of action potential measured per second. 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 ; $t=3.70$, $p=0.002$). All data are means $\pm$ SEM (10 neurons per group), ** $p<0.01$ vs Sham.

B. Representative diagram of the *in vivo* recording procedure of ACC neuron evoked activity. **B1.** Single unit extracellular recording of evoked activity recording of ACC neuron in urethane-anesthetized animals. **B2.** Quantification of the number of action potential measured per 5 seconds upon peripheral mechanical stimulus of the hind paw, contralateral to the recorded ACC (von Frey filament: 1.4g, 2.0g, 4.0g, 6.0g and pressure). 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. All

data are means $\pm$ SEM (10 neurons per group), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs Sham; ## $p < 0.01$; ### $p < 0.001$ vs 1.4g.

C. Representative diagram of contralateral DHNs neuron recording upon ACC electrical stimulation in response to different peripheral mechanical stimuli. Quantification of the number of action potentials per 5 seconds upon peripheral stimulus (von Frey filament: 1.4g, 2.0g, 4.0g, 6.0g) at different ACC electrical stimulation intensities (100Hz: 10V, 20V, 30V), and after 2 minutes of recovery. 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 $\times$ 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 $\times$ 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 ; $q=12.40$; $p=0.0001$). All data are means $\pm$ SEM (10 neurons per group), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs Baseline; ### $p < 0.001$ vs 30V; NS = non-significant.

D. Representative diagram of contralateral DHNs neuron recording upon ACC inhibition in responses to different peripheral mechanical stimuli. Quantification of the number of action potentials per 5 seconds upon peripheral stimulus (Von Frey filament: 1.4g, 2.0g, 4.0g, 6.0g), every 15 minutes before and one hour after muscimol infusion in the ACC. 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 $\times$ 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 $\times$ 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). All data are means $\pm$ SEM (10 neurons per group), * $p<0.05$ vs Pre-infusion; ### $p<0.001$ vs Sham.

E. Histological verification of the infusion site in the ACC.

Fig. 6. 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 from¹²⁴). **A2-A3.** Example of viral expression in excitatory neurons (bar: 1 mm and 50 μ m, 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ SEM (8 neurons per group), * $p < 0.05$, *** $p < 0.001$ vs Sham; # $p < 0.05$ vs Before.

Fig. 7. Optogenetic modulation of the ACC – Posterior Insula (PI) excitatory pathway potentiates sensitization of the contralateral paw in 6-OHDA mice.

A. Representative diagram of viral injection site in the left ACC (left) and optic cannula placement (right) in the left PI. **A1.** Atlas representation of the ACC – PI pathway targeted in this study (Atlas images from¹²⁴). **A2 & A3.** Examples of viral expression of ChR2-transduced ACC excitatory neurons (**A2**) projecting to the PI (**A3**). Bar: ACC (1 mm), PI (200 μ m).

B. Activation of the left ACC–PI pathway in 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 and 5 minutes after (Recovery) illumination. 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; $t=2.48$, $p=0.04$). All data are means $\pm$ SEM (8 mice per group), * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs Before; ## $p<0.01$; ### $p<0.001$ 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) ($F_{(1,28)}=1.23$, $p=0.28$), 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. All data are means $\pm$ SEM (8 mice per group), *** $p<0.001$ vs Sham; ## $p<0.01$; vs Opto.

C. Activation of the left ACC–PI pathway of mice injected with AAV5.CaMKII.ChR2.eGFP and contralateral (right) DHN recording. Quantification of action potentials per 5 seconds upon peripheral stimulus before, during and at 2 or 5 minutes after illumination. 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. All data are means $\pm$ SEM (8 neurons per group), * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs Sham; # $p<0.05$; ## $p<0.01$; ### $p<0.001$ vs Before; § $p<0.05$ vs Opto.

D. Silencing of the left ACC–PI pathway in 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 or 5 minutes after (Recovery) illumination. 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 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; $t=8.06$, $p=0.0001$; [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; $t=0.62$, $p=0.55$). All data are means $\pm$ SEM (8 mice per group), * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs Before; # $p<0.05$ vs Opto. **D2.** 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 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. All data are means $\pm$ SEM (8 mice per group), ** $p<0.01$; *** $p<0.001$ vs Sham; # $p<0.05$; vs Opto.

E. Silencing of the left ACC–PI pathway in mice injected with AAV5.CaMKII.ArchT.eGFP and contralateral (right) DHN recording. Quantification of action potentials per 5 seconds upon peripheral stimulus before, during and at 2 or 5 minutes after illumination. 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 ; $q=1.10$, $p>0.05$). All data are means $\pm$ SEM (8 neurons per group), * $p<0.05$; *** $p<0.001$ vs Sham; # $p<0.05$; ## $p<0.01$; ### $p<0.001$ vs Before; § $p<0.05$; §§ $p<0.01$ vs Opto.

A **B** **C** **D** **E**

Figures

Figure 1

Hypersensitivity to pain in ADHD-like conditions. (see Manuscript file for full figure caption)

Figure 2

Hyperexcitability of deep dorsal horn neurons (DHNs) in ADHD-like conditions. (see Manuscript file for full figure caption)

Figure 3

ADHD-like conditions modify dorsal spinal networks. (see Manuscript file for full figure caption)

Figure 4

Pain conditions influences ADHD-like symptoms. (see Manuscript file for full figure caption)

Figure 5

Anterior cingulate cortex is the key brain area of descending control dysfunctions in ADHD-like conditions. (see Manuscript file for full figure caption)

Figure 6

Direct optogenetic modulation of ACC excitatory neurons potentiates sensitization of the contralateral paw in 6-OHDA mice. (see Manuscript file for full figure caption)

Figure 7

Optogenetic modulation of the ACC – Posterior Insula (PI) excitatory pathway potentiates sensitization of the contralateral paw in 6-OHDA mice. (see Manuscript file for full figure caption)

Supplementary Files

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