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Peripheral indoleamine 2,3-dioxygenase¹ is required for comorbid depression-like behavior but does not contribute to neuropathic pain in mice

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Abstract

Chronic pain frequently co-occurs with major depressive disorder but the mechanisms are poorly understood. We investigated the contribution of indoleamine-2,3-dioxygenase-1 (IDO1), a rate-limiting enzyme in the conversion of tryptophan to neurotoxic metabolites to this comorbidity using the spared nerve injury (SNI) model of neuropathic pain in mice.

SNI resulted in unilateral mechanical allodynia, reduced social interaction, and increased immobility in the forced swim test without changes in locomotor activity. These findings indicate SNI-induced pain and comorbid depression-like behavior. These behavioral responses were accompanied by increases in plasma kynurenine/tryptophan ratios and increased expression of *Ido1* and *Il1b* mRNA in the liver. Interestingly, SNI did not induce detectable changes in spinal cord or brain *Ido1* mRNA levels after SNI. SNI was associated with spinal cord inflammatory activity as evidenced by increased *Il1b* mRNA expression. The SNI-induced increase of liver *Ido1* and *Il1b* mRNA was abrogated by intrathecal administration of the IL-1 inhibitor IL-1RA. Intrathecal IL-1RA also inhibited both mechanical allodynia and depression-like behavior. We also show that *Ido1* is required for the development of depression-like behavior because *Ido1*^{-/-} mice do not develop increased immobility in the forced swim test or decreased social exploration in response to SNI. Mechanical allodynia was similar in WT and *Ido1*^{-/-} mice.

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In conclusion, our findings show for the first time that neuropathic pain is associated with an increase of *Ido1* in liver, but not brain, downstream of spinal cord IL-1 β signaling and that *Ido1* mediates co-morbid depression. Moreover, comorbidity of neuropathic pain and depression are only partially mediated by a common mechanism because mechanical hyperalgesia develops independently of *Ido1*.

Keywords

neuropathic pain; depression; interleukin-1; indoleamine 2, 3 dioxygenase 1; liver; brain

Introduction

Pain and depression frequently occur together. Clinically, more than 50% of patients suffering from chronic pain also experience symptoms of depression (Arnow et al., 2006; Bair et al., 2003). Specifically, patients with multiple pain symptoms (e.g., back pain, headache, abdominal pain, chest pain and facial pain) are 3-5 times more likely to be depressed than individuals without pain (Bair et al., 2003; Magni et al., 1993; Von Korff 1988). Conversely, depressed patients are more likely to develop low back pain, neck-shoulder pain, and musculoskeletal symptoms than non-depressed individuals (Arnow et al., 2006; Trivedi et al., 2004). Comorbid chronic pain and depression are difficult to treat, partially because of a lack of knowledge on the commonalities and specificities of the underlying mechanisms.

In models of persistent peripheral inflammation or in models of neuropathic pain, inflammatory cytokines produced by spinal cord microglia and astrocytes contribute to the pain response (Clark et al., 2010; Ikeda et al., 2012; Eijkelkamp et al., 2010). Inflammatory cytokines produced in the brain mediate depression-like behavior (e.g. increased immobility time in the forced swim test) induced by systemic administration of lipopolysaccharide (O'Connor et al., 2009a; Corona et al., 2010). A role for cerebral inflammation in depression-like behavior associated with neuropathic pain in the spared nerve injury (SNI) model has already been demonstrated. Norman et al. showed that intracerebroventricular (i.c.v.) injection of the IL-1 receptor antagonist (IL-1RA) (Norman et al., 2010a, 2010b) blocks depression-like behavior in this model. Collectively, these findings indicate neuroinflammatory activity in the brain as a common underlying mechanism in comorbid pain and depression.

Inflammation-induced depression is thought to be mediated by increased expression of the tryptophan-metabolizing enzyme indoleamine 1,3 deoxygenase (IDO1) downstream of pro-inflammatory cytokines (O'Connor et al., 2009a, 2009b; Raison et al., 2010) and the formation of kynurenine and its metabolites in the brain (Walker et al., 2013). To our knowledge, only one study has examined the role of IDO1 in inflammatory pain. In that study, chronic pain induced by administration of complete Freund's adjuvant (CFA) in the joints of rats and mice was associated with increased expression of IDO1 in the brain (Kim et al., 2012). Blockade of brain IDO1 activity or genetic deletion of IDO1 abrogated both pain and depression-like behavior in this model. However, these results cannot easily be

generalized, as CFA induces a state of severe persistent inflammation (Raghavendra et al., 2004), while chronic pain and depression in humans are associated with persistent low grade inflammation rather than severe systemic inflammation (Dowlati et al., 2010). Therefore, we elected to use the SNI model of chronic neuropathic pain in mice to further study the involvement of inflammation and IDO-1 in pain and comorbid depression.

Methods

Animals

Male C57/BL6J and *Ido1*^{-/-} mice (10-16 weeks old, The Jackson Laboratory, Bar Harbor, ME) were individually housed on a 12h reversed light/dark cycle (lights on 10:00 pm). Water and food were available *ad libitum*. The study was conducted in accordance with NIH guidelines for the care and use of animals and under protocols approved by the University of Illinois Urbana Champaign and Texas A&M Institutional Animal Care and Use Committees.

Surgery

SNI surgery was performed as described (Willemsen et al., 2012). Briefly, the sural common peroneal and tibial branches of the left sciatic nerve were exposed under isoflurane anesthesia. The tibial and common peroneal nerves were transected. The sural nerve was kept intact. For sham surgery, nerves were exposed but not transected.

Behavior

Mechanical hyperalgesia was monitored using von Frey hairs. The 50% paw withdrawal threshold was calculated using the up-and-down method as previously described (Eijkelkamp et al., 2010; Wang et al., 2013).

Social exploration was tested on days 3 and 5 after SNI surgery in a two-compartment arena with a removable divider as previously described (Zhou et al., 2011). For habituation, mice explored the two compartments freely for 25 min under red light (40 lux). On the test day, mice were placed in one compartment for 5 min. Subsequently, an unfamiliar weight-matched male mouse was placed in the other compartment in a small wire cage and the divider was removed. Behaviors in the test period (10 min) were recorded (Sony DCR-SR100). The total time the test mouse spent interacting with the other mouse (grooming, sniffing and observing), was counted by a trained person blinded to treatment.

Forced swim test (FST) was performed on day 7 after SNI surgery. Behavior was recorded for 6 min after mice were placed in a bucket (diameter: 23cm) of water at 25±0.2°C and the immobility time was scored as described (Walker et al., 2013).

Locomotor Activity was tested two hours prior to the social exploration test or forced swim test during the dark cycle. Mice were individually placed in a clean, cage identical to its home cage but without beddings and were permitted to freely explore the whole cage for 10 min. For each mouse, a new cage was used. The cage was virtually divided into four quadrants. Locomotor activity was quantified by counting the number of quadrants entries during the last 5 min. The number of full rears was also counted during the same time interval. Scoring was conducted by a well-trained observer who was blind to treatments.

On days 3 and 5 after SNI surgery, mice were given a 2 hours interval between mechanical hyperalgesia and the depression-like behavior tests. On day 7, mice were given a 4 hours interval between i.t. injection of drugs and behavioral tests. On day 8, mice were sacrificed for tissue collection.

Drug Administration

IL-1RA (R&D Systems, Minneapolis, MN) was given daily intrathecally (50 ng/5 µl/day in saline (Willemen et al., 2012; Chamberlain et al., 2014) for 7 days from day 1 after SNI surgery).

Plasma IL-6

IL-6 levels in heparin-plasma diluted 1:2 in assay buffer were determined by ELISA (BD Biosciences, San Diego, CA) according to manufacturer's instructions. The lower limit of detection was 15.6pg/ml.

Real-Time PCR

Total RNA was extracted in TRIzol reagent (Life Tech., Carlsbad, CA (9)) and reverse transcribed to cDNA using a high capacity cDNA reverse transcription kit (Life Tech., Foster City, CA). Real-time qPCR was performed on an Applied Biosystems ViiA using PrimeTime qPCR assays for *Ido1* (exon3-4, NM_008324 3-4), *Il1b* (exon3-4, NM_008361 3-4), *Il6* (exon2-3, NM_031168 2-3), *Ifng* (exon1-2, NM_008337(1)) and *Gapdh* (exon2-3, NM_008084 2-3; all from Integrated DNA Technologies, Coraville, IA). Amplifications without template were included as negative controls. Relative quantitative measurement of target gene levels corrected for GAPDH was performed using the C_t method.

High-Performance Liquid Chromatography and Mass Spectrum (HPLC-MS): High-Performance Liquid chromatography and mass-spectrometry (HPLC-MS)

Assessment of brain and plasma metabolites was conducted in the following manner by collaborators at Lundbeck Research USA (Paramus, New Jersey). Brain samples were homogenized (2 min) using an Omni-Prep Multi-Sample Homogenizer after addition of a 4× mass of an aqueous solution containing 0.2% acetic acid and internal standards (see below). Resultant samples were then filtered using a 3kDa 0.5 mL Millipore Amicon Ultra filter which was spun down at 13500 g for 60 min at 4°C, followed by triplicate analysis of the filtrate. Plasma samples (10-50µL) were diluted 5× with 0.2% acetic acid prior to filtration with the 3kDa filter. Injection of the resulting solution was performed in triplicate for analysis of each sample.

Standard curves were prepared using pure components (Tryptophan (TRP), Kynurenine (KYN), 5-HT, kynurenic acid (KYNA), 3-hydroxykynurenine (3HK), xanthurenic acid (XT), quinolinic acid (QA), 5-hydroxytryptophan (5HTP), nicotinamide (NTA), picolinic acid (PA) anthranilic acid (AA), and 3-hydroxyanthranilic acid (3HAA) purchased from Sigma dissolved in 0.2% acetic acid. Internal standards (2H5-TRP, 2H5-KYNA, 2H4-NTA, 2H4-PA, 2H4-5HT, 13C6-KYN) were added to each standard and sample (final concentration of 100ng/mL except 2H4-5HT at 50ng/mL) to examine and correct for sample matrix and instrument variation.

Samples were analyzed with a Waters Acquity HPLC system equipped with an YMC ODS AQ 2×100mm, 3μm particle column which provided separation of the kynurenine analytes prior to detection by a Waters Quattro Premier XE triple quadrupole mass spectrometer operating in the MS/MS configuration. Full loop injections with a 3 time overfill were performed with a 5μL loop requiring a total sample volume of 15μL. Column and pre-column tubing were maintained at 40°C while eluting (0.2mL/min flow rate) kynurenine metabolites with a mobile phase consisting of an aqueous component (A: 0.5% formic acid in milliQ water) and an organic component (B: 1% formic acid in UV grade acetonitrile from B and J). Gradient elution included a 2 min hold at 100% A followed by a shallow gradient of 0-30% B over 4.4min. Later, eluting materials were then brought off the column using a stronger gradient of 30-70% B over 0.5 min with a total run time of 9 min. The final 2.1 min were utilized for rinsing and re-equilibration of the column.

Tuning of the triple quadrupole in the +ve ESI mode was performed by direct injection of the analyte standards with preference given to the lower abundant/important analytes such as 3HK and QA. This resulted in the following conditions: capillary voltage 1.5V, cone voltage 15V, source temperature 150°C and desolvation temperature 500°C. Included are the mass spectral transitions utilized to measure the levels of each analyte along with the collision energy utilized to achieve the transition and the cone voltage when it differed from the conditions indicated for the tune: NTA (122.85 > 79.90, 18, 28V), PA (123.75 > 77.70, 17), QA (167.85 > 77.70, 20), 3HK (225.10 > 109.80, 18), 5HT (176.9 > 114.8, 22 and 176.9 > 131.8, 22), 5HTP (220.91 > 161.8, 18, 15V), KYN (209.05 > 93.70, 15), 3HAA (153.85 > 79.75, 25), TRP (205.10 > 145.80, 18), XT (206.05 > 131.80, 27, 28V), KYNA (189.90 > 143.75, 18, 28V), AA (137.82 > 64.75, 25 and 137.82 > 91.75, 20). Stable labeled internal standards were monitored with the following mass spectral parameters: 2H4-NTA (126.80 > 83.70, 20, 28V), 2H4-PA (127.8 > 81.75, 15), 2H4-5HT (181 > 118, 24 and 181 > 136, 22), 2H5-TRP (210.20 > 150.10, 20), 2H5-KYNA (195.10 > 149.00, 20, 28V), 13C6-KYN (215.12 > 99.95, 15).

Statistical Analysis

The data are expressed as means ± SEM. Testing of statistical significance was performed using independent Student *t*-test, one- or two-way ANOVA, or two-way repeated-measures ANOVA according to the experimental design. *Post-hoc* analysis was conducted using Tukey or LSD test. All experiments were repeated 3-4 times, with the exception of the analysis of the effect of genetic deletion of *Ido1* on behavior at 21 days after SNI; this experiment was performed twice.

Results

Comorbid pain and depression-like behaviors in response to SNI

SNI induced mechanical allodynia in the ipsilateral hind paw (Figure 1A, Surgery×Time interaction: $F(7,63)=8.39$, $p<0.01$) but not in the contralateral hind paw (Figure 1B). SNI decreased the time spent in social interactions with an adult conspecific placed (Figure 1C, $t(17)=3.03$, $p<0.01$) and increased duration of immobility in the forced swim test on day 7 post-surgery (Figure 1D, $t(31)=3.89$, $p<0.001$). These findings are in line with earlier studies

examining duration of immobility in the forced swim test at 7 -14 days after SNI in mice or rats (Norman et al., 2010a, 2010b; Wang et al., 2013). Spontaneous locomotor activity was not affected by SNI, indicating that the increased immobility in the forced swim test and the reduced social exploration did not result from motor deficits (Figure 1E).

Effect of SNI on tryptophan metabolism and IDO1 expression

In contrast to the data on severe inflammation induced by CFA, SNI did not induce *Ido1* mRNA expression in the ipsi- or contralateral frontal cortex (Figure 2A), hippocampus (not detectable), lumbar dorsal horn (Figure 2B) and total brain (data not shown), but led to a significant increase in *Ido1* mRNA expression in the liver (Figure 2C, $t(18)=2.17$, $p<0.05$). The increase in liver *Ido1* mRNA in SNI mice was associated with an increase in liver *Il1b* mRNA (Figure 2D, $t(24)=2.28$, $p<0.05$).

In accordance with the *Ido1* mRNA expression data, higher KYN (Figure 2E, $t(14)=2.17$, $p<0.05$) and KYN-to-TRP ratios (Figure 2F, $t(14)=2.51$, $p<0.05$) were only observed in the plasma but not in contralateral and ipsilateral hippocampus and frontal cortex (Table 1).

Contribution of spinal cord IL-1 β signaling to mechanical allodynia, depression-like behavior, and IDO1 expression

SNI increased mRNA expression of *Il1b* in the ipsilateral but not contralateral spinal cord (Figure 3A, Surgery: $F(1,38)=6.85$, $p<0.05$). Intrathecal administration of the IL-1 receptor antagonist IL-1-RA inhibited SNI-induced mechanical hyperalgesia (Figure 3B, Surgery $\times$ Drug $\times$ Time interaction: $F(5,187)=3.72$, $p<0.01$). Intrathecal injection of IL-1-RA did not influence the pain threshold in the contralateral paw (Figure 3C). In addition, intrathecal IL-1-RA prevented the increase in immobility in the forced swim test in SNI mice (Figure 3D, Surgery $\times$ Drug interaction: $F(1,32)=11.8$, $p<0.01$). The inhibition of depression-like behavior by IL-1-RA was associated with abrogation of the increase in expression of *Ido1* (Figure 3E, Surgery: $F(1,29)=5.39$, $p<0.05$) and *Il1b* mRNA (Figure 3F, Drug $\times$ Surgery interaction: $F(1,29)=4.41$, $p<0.05$) in the liver of SNI mice.

Pain and depression-like behavior in mice with genetic deletion of *Ido1*

To determine the importance of *Ido1* for development of depression-like behavior and mechanical allodynia in response to SNI, we compared WT and *Ido1*-deficient (*Ido1*^{-/-}) mice. The data in figure 4A show that SNI significantly decreased the social exploration time (Figure 4A, Surgery $\times$ Genotype, $F(1,29)=4.92$, $p<0.05$) and prolonged the immobility time (Figure 4B, Surgery $\times$ Genotype, $F(1,49)=8.25$, $p<0.01$) in the FST. However, SNI did not affect social exploration in *Ido1*^{-/-} mice. Moreover, *Ido1*^{-/-} mice also no longer displayed any increase in the duration of immobility in the forced swim test at either 7 days or 3 weeks (Figure 4C, Surgery $\times$ Genotype, $F(1,10)=6.06$, $p<0.05$) days after SNI in contrast to WT mice. However, mechanical allodynia as measured at 7 days (Figure 4D, Surgery $\times$ Time, $F(4,139)=50.0$, $p<0.01$) or 3 weeks after SNI was unaffected by genetic deletion of *Ido1* (Figure 4E, Surgery, $F(1,21)=1106.8$, $p<0.01$).

Discussion

The frequent co-occurrence of chronic pain and depression is highly challenging for both clinicians and researchers as little is known about the underlying mechanisms, and treatment options are limited. Here we used the SNI model of comorbid pain and depression to investigate the contribution of the tryptophan metabolizing enzyme IDO-1 to this comorbidity. SNI activated inflammatory signaling in the spinal cord as attested by the increased expression of IL-1 β . IL-1 β -signaling in the spinal cord was required for the development of both mechanical allodynia and depression-like behavior in response to SNI. Depression-like behavior was dependent on the induction of IDO-1 at the periphery and not in the brain. Specifically, SNI increased liver *Ido1* mRNA and the plasma KYN/TRP ratio without changes in brain *Ido1* and these changes occurred downstream of spinal cord IL-1 β -signaling as evidenced by the blockade of these effects by intrathecal IL-1RA. Moreover, *Ido1*^{-/-} mice did not develop depression-like behavior in response to SNI while mechanical allodynia developed normally in *Ido1*^{-/-} mice.

There is already ample evidence that IDO1 is required for the development of depression-like behavior in response to systemic inflammation induced by e.g. systemic administration of LPS (O'Connor et al., 2009a, 2009b; Raison et al., 2010). However, the role of IDO-1 in neuropathic pain and comorbid depression-like behavior has not been investigated. Depression-like behavior in models of systemic inflammation is associated with increased *Ido1* expression in the brain. Moreover, blockade of IDO-1 activity by peripheral administration of 1-methyl tryptophan, a competitive inhibitor of the enzyme, or genetic deletion of *Ido1* abrogates depression-like behavior induced by systemic inflammation. As increased brain *Ido1* mRNA expression was observed in all these studies, the assumption was that IDO1 is needed to be active in the brain in order for depression-like behavior to develop in the context of peripheral inflammation. However, a study in gerbils indicated that the majority of the kynurenine that is found in the brain during LPS-induced acute systemic inflammation is coming from the periphery (Kita et al., 2002). Our finding that *Ido1* expression is increased in the periphery and not in the brain and contributes to depression-like behavior in response to SNI surgery is in accordance with a model in which kynurenine is formed in the periphery. In contrast, in the context of strong cerebral inflammatory activity, the prevailing idea is that most of the brain kynurenine is derived from local synthesis (Kita et al., 2002). For example, depression-like behavior induced by i.c.v. administration of LPS was blocked by i.c.v. administration of 1-methyl tryptophan (Lawson et al., 2013). Brain IDO1 activity also contributes to depression-like behavior in a model of chronic inflammatory pain induced by intra articular administration of complete Freund's adjuvant (Kim et al., 2012). In this model, the development of mechanical allodynia and depression-like behavior occurred in a context of an intense and prolonged inflammatory response at the periphery and in the brain, as evidenced by increased plasma IL-6 and hippocampal IL-6 and *Ido1*. Immunoneutralization of IL-6 blocked the increase in hippocampal *Ido1* and abrogated the development of both mechanical allodynia and depression-like behavior (Kim et al., 2012).

Inflammation is much milder in the model of spared nerve injury. In line with other studies, we did not detect any increase in plasma IL-6 protein or brain IL-6 mRNA (data not shown).

and Norman et al., 2010a; Guillemin et al., 2003) in SNI mice. This mild inflammatory response probably accounts for the lack of induction of brain IDO1 in the SNI model. The mild inflammatory response that develops in the SNI model, possibly in conjunction with the chronic stress induced by SNI, is still sufficient to propagate to the liver and increase hepatic *Ido1* mRNA and circulating levels of kynurenine. This peripheral kynurenine is probably transported into the brain (Kita et al., 2002) where it can be further metabolized via two major pathways; the kynurenine amino transferase pathway mediates formation of the neuroprotective metabolite kynurenic acid (Guillemin et al., 2001); the kynurenine monooxygenase (KMO) pathway produces the neurotoxic metabolite 3-hydroxykynurenine that is further converted into quinolinic acid, an NMDA receptor agonist that is thought to promote depression-like behavior (Guillemin et al., 2003; Alberati-Giani et al., 1996). We have preliminary evidence indicating that SNI induces an increase in KMO in the brain. Increased KMO-mediated metabolism of kynurenine in the brain may explain why we did not observe increased kynurenine levels in the brain.

The role of IDO1 in pain is much less well documented than its role in depression. Clinical evidence for a relation between tryptophan/kynurenine metabolism and pain is emerging. For example, in patients with complex regional pain syndrome, increased plasma KYN/TRP ratios are related to higher pain scores, but information on depression was not provided (Alexander et al., 2013). Similarly, Kim et al. reported that in patients with chronic back pain and depression KYN/TRP ratios in the plasma were increased as compared to healthy individuals (Kim et al., 2012), but patients with chronic back pain without depression were not included in that study. Thus, although there is evidence for increased peripheral kynurenine or kynurenine/tryptophan ratios in patients with chronic pain and in patients with depressive disorders, these clinical studies do not allow conclusions on the role of the tryptophan/kynurenine pathway in the comorbidity of pain and depression. There is also some limited evidence for a role of IDO1 in pain at the preclinical level. As mentioned above, in the model of intra-articular CFA-induced inflammation, genetic deletion of *Ido1* attenuated both mechanical allodynia and the increase in immobility in the forced swim test (Kim et al., 2012). However, we did not find any evidence for a role of *Ido1* in mechanical allodynia in response to spared nerve injury, when comparing WT and *Ido1*^{-/-} mice. These findings indicate that under conditions of low grade inflammation as induced by SNI, *Ido1* does not contribute to pain. This does not allow dismissing a possible role of kynurenine metabolites downstream of IDO1 in mechanical allodynia. For example, there is evidence that elevating endogenous kynurenic acid has been shown to alleviate neuropathic pain in rats after spinal nerve ligation (Vécsei et al., 2013), and this effect is likely due to antagonism of the NMDA receptor by kynurenic acid (Pineda-Farias et al., 2013).

It is not clear at present why *Ido1* contributes to CFA-induced mechanical allodynia without affecting mechanical allodynia in the SNI model of neuropathic pain. One possibility would be that there is a difference in the effect of kynurenine and its metabolites on the specific subclasses of peripheral afferents that are involved in mechanical allodynia in response to inflammation as compared to nerve injury (Abrahamsen et al., 2008). Alternatively, binding of kynurenine to the arylhydrocarbon receptor on immune cells is known to affect the peripheral inflammatory response (Nguyen et al., 2014; Stockinger et al., 2014). It is thus

possible that immunomodulatory effects of peripheral kynurenine contribute to joint inflammation in CFA model and thereby indirectly affects mechanical allodynia. It is also possible that the increase in Idol and its products in the brain as observed in the CFA model, but not in the SNI model, influences descending pathways that may contribute to mechanical allodynia.

Collectively, our findings point to a pathophysiological mechanism of comorbid pain and depression that begins with the spinal inflammatory response to SNI. This response propagates to the liver where it recruits the IDO-1 pathway leading to increased production of KYN. The activity of IDO-1 is required for the development of depression-like behavior, but does not contribute to mechanical allodynia. In view of the clinical importance of comorbid depression in patients suffering from chronic pain, targeting peripheral tryptophan metabolism may represent a novel way to treat this comorbidity.

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Highlights

- The spared injury (SNI) model of neuropathic pain increases liver but not brain IDO1
- Spared nerve injury increases plasma kynurenine and the kynurenine/tryptophan ratio
- Spinal cord IL-1 β signaling induces liver IL-1 β , IDO1 and depression-like behavior
- IDO1^{-/-} mice develop pain, but not depression-like behavior after SNI

Figure 1. Comorbid pain and depression-like behaviors in response to SNI

Mice were exposed to either SNI or sham surgery, and mechanical hyperalgesia and depressionlike behavior were analyzed. Mechanical hyperalgesia in the paw ipsilateral (Surgery $\times$ Time, $F(7,63)=8.39$, $p<0.01$) (A) and contralateral (B) to the surgical site was monitored using von Frey hairs and the up/down method. Data represent mean 50% withdrawal threshold and were analyzed by one-way repeated measures ANOVA followed by LSD. (C) Social exploration behavior was examined on day 5 after SNI. Data were analyzed by independent t -test ($t(17)=3.03$, $p<0.01$). (D) On day 7 after SNI or sham surgery, the duration of immobility time in the forced swim test was examined. The data represent time of immobility during the last 4 minutes of the test and were analyzed by independent t -test ($t(31)=3.89$, $p<0.001$). SNI and sham operated mice were tested for spontaneous locomotor activity on day 7 after surgery. Data were analyzed by Student t -test and expressed as mean $\pm$ SEM. SNI surgery did not influence the locomotor activity in mice. In all panels, data represent mean $\pm$ SEM. *, $p<0.05$; **, $p<0.01$. $n=6-20$ per group.

Figure 2. Effects of SNI on tryptophan metabolism and IDO1 expression

Effect of SNI surgery on mRNA expression for *Idol* in frontal cortex (A) and lumbar dorsal horn (B) from WT mice. Two-way ANOVA analysis shows there is no significant Surgery × Side interaction. Livers were homogenized and analyzed for expression level of *Idol* (C) and *IL-1β* (D) mRNA. Data were analyzed by Student *t*-test. (*Idol*: $t(18)=2.17$, $p<0.05$; *IL-1β*: $t(24)=2.28$, $p<0.05$). (E,F) Effect of SNI on the plasma level of KYN (E) and on plasma Kynurenine/Tryptophan (KYN/TRP) ratio (F) measured by HPLC-MS. Data were analyzed by Student *t*-test. (KYN: $t(14)=2.17$, $p<0.05$; KYN/TRP: $t(14)=2.51$, $p<0.05$). In all panels, data represent mean ± SEM. *, $p<0.05$; **, $p<0.01$. $n=6-15$ per group.

Figure 3. Contribution of spinal cord IL-1 β signaling to mechanical allodynia, depressionlike behavior, and IDO1 expression

(A) *IL-1 β* mRNA in the ipsi- and contralateral dorsal horn of the lumbar spinal cord were quantified by real-time qPCR analysis. Data were analyzed by two-way ANOVA followed by LSD. (Surgery, $F(1,38)=6.84$, $p<0.02$). (B,C) Effect of daily intrathecal injection of IL-1RA (50ng/5 μ l/day) from day 1 after SNI or sham surgery on mechanical hyperalgesia in the ipsilateral (B) and contralateral (C) hind paw. Data were analyzed by two-way repeated measures ANOVA (ipsilateral paw: Surgery $\times$ Drug $\times$ Time, $F(5,187)=3.72$, $p<0.01$) followed by LSD. (D) Effect of intrathecal IL-1RA on immobility time in the forced swim test. Mice were treated with IL-1RA as in panel 3B and tested in the forced swim test on day 7 after surgery. Data were analyzed by two-way ANOVA (Surgery $\times$ Drug, $F(1,32)=11.767$, $p<0.01$) followed by LSD. (E,F) Effect of intrathecal injection of IL-1RA on mRNA expression of *Ido1* (E) and *IL-1 β* (F) in liver. Data were analyzed by two-way ANOVA followed by LSD (IDO1: Surgery, $F(1,29)=6.56$, $p<0.05$; Drug, $F(1,29)=5.39$, $p<0.05$; IL-1 β : Surgery $\times$ Drug, $F(1,29)=4.41$, $p<0.05$). In all panels, data represent mean $\pm$ SEM. *, $p<0.05$; **, $p<0.01$. $n=6-15$ per group.

Figure 4. Effects of genetic deletion of *Idol1* on pain and depression-like behavior in mice

(A) On days 5 after SNI or sham surgery we compared social interaction time of WT and *Idol1*^{-/-} mice. Data were analyzed by two-way ANOVA (Surgery×Genotype, $F(1,29)=4.92$, $p<0.05$) followed by LSD. (B,C) On day 7 (B) or three weeks (C) after SNI or sham surgery in WT or *Idol1*^{-/-} mice, the immobility time in the forced swim test was quantified. Data were analyzed by two-way ANOVA (Days 7: Surgery×Genotype, $F(1,49)=8.25$, $p<0.01$; Days 21: $F(1,10)=6.06$, $p<0.05$) followed by LSD. (D) Mechanical allodynia induced by SNI in the ipsilateral hind paw of WT and *Idol1*^{-/-} mice. Data were analyzed by two-way repeated ANOVA (Surgery×Time, $F(4,139)=50$, $p<0.01$) followed by LSD. (E) Mechanical allodynia in the ipsilateral hind paw of WT and *Idol1*^{-/-} mice at days 21 after SNI. Data were analyzed by two-way ANOVA (Surgery, $F(1,21)=1106.8$, $p<0.01$). In all panels, data represent mean $\pm$ SEM. *, $p<0.05$; **, $p<0.01$. $n=8-15$ per group.

Table 1

Tryptophan and Kynurenine in the brain after SNI

	Frontal Cortex				Hippocampus			
	<i>Ipsi</i>		<i>Contra</i>		<i>Ipsi</i>		<i>Contra</i>	
	Sham	SNI	Sham	SNI	Sham	SNI	Sham	SNI
KYN(pg/mg)	17.8±0.9	15.9±0.8	16.4±0.9	14.6±0.5	20.3±0.8	18.5±0.5	17.3±0.8	16.1±0.7
TRP (ng/mg)	10.0±0.9	8.7±0.5	9.9±0.9	8.6±0.3	7.7±0.9	6.0±0.3	7.1±0.8	5.8±0.2
KYN/TRP	1.8±0.1	1.8±0.1	1.7±0.1	1.7±0.1	2.9±0.3	3.1±0.2	2.4±0.2	2.8±0.2

Mice were sacrificed on days 8 after SNI surgery and tryptophan and kynurenine levels in frontal cortex and hippocampus were analyzed by HPLC-Mass spectrometry.