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AMPAkines have novel analgesic properties in rat models of persistent neuropathic and inflammatory pain

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Abstract

Background—Novel analgesics that do not suppress the respiratory drive are urgently needed. Glutamate signaling through α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors plays important roles in central pain circuits. AMPAkines augment AMPA receptor function and have been shown to stimulate the respiratory drive to oppose opioid-induced hypoventilation. However, their role in chronic pain states remains unknown.

Methods—We studied AMPAkines (CX546 and CX516) in rat spared nerve injury (SNI) model of neuropathic pain and Complete Freund's Adjuvant (CFA) model of inflammatory pain. We measured the effect of AMPAkines on mechanical and cold allodynia. We also evaluated their effect on depressive symptoms of pain using the forced swim test, as time of immobility on this test has been used as a measure for behavioral despair, a feature of depression.

Results—We found that CX546, compared with DMSO control, reduced both mechanical and sensory allodynia in SNI(DMSO group, $n = 9$; CX546 group, $n = 11$) and CFA models (Both DMSO and CX546 groups, $n=9$). We found that CX546, compared with control, also reduced depressive symptoms of pain by decreasing immobility on the forced swim test in both SNI (both DMSO and CX546 groups, $n = 8$) and CFA models (both DMSO and CX546 groups, $n = 10$).

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Finally, we found that CX516, compared with control, also reduced mechanical and cold allodynia in the SNI model (both DMSO and CX516 groups, $n = 10$).

Conclusions—AMPAkines alleviate pain hypersensitivity as well as depression-like behaviors associated with long-lasting nerve injury and inflammatory insult.

Introduction

Despite advances in pain research, pharmacologic options remain limited. Opioids remain the most potent postoperative analgesic. A feared complication of opioids is respiratory depression, which can happen in both acute postoperative and chronic pain settings.^{1–3} In recent years, there has been a sharp rise in opioid-related mortalities in chronic pain patients, mostly due to respiratory depression.^{1,4} Even as many of these opioid-related morbidities and mortalities involve inappropriate prescription and over- or mis-use, treatment and/or prevention for opioid-induced respiratory depression remains lacking. Similarly, the development of new analgesics, particularly agents that do not suppress the respiratory drive, is urgently needed.²

Excitatory glutamate signaling in the central nervous system (CNS) plays an important role in the regulation of chronic pain. α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are the primary glutamate receptors in the CNS.⁵ Increased transmission through AMPA receptors in the spinal dorsal horn has been shown to contribute to the induction and maintenance of chronic pain.^{6–10} AMPA receptor signaling in the anterior cingulate cortex (ACC) and amygdala can also facilitate pain transmission.^{11–13} On the other hand, transmission through AMPA receptors is critical for maintaining the integrity of the periaqueductal gray-rostral ventromedial medulla(PAG-RVM) descending inhibitory pathway.^{14–16} In addition, the nucleus accumbens (NAc), a key region for the regulation of mood and motivated behaviors, also utilizes AMPA receptor signaling to generate pain-induced analgesia.^{17,18} Thus, depending on the region in the CNS, AMPA receptor signaling may play either a pro-nociceptive or anti-nociceptive role.

AMPAkines are a class of compounds that enhance AMPA receptor transmission.^{19,20} AMPAkines bind to an allosteric site on the AMPA receptor to slow the kinetics of channel deactivation and desensitization. By preventing AMPA receptors from closing, AMPAkines increase the inward synaptic current. AMPAkines have been found to have cognitive effects in animal and human studies,²¹ and have been studied in schizophrenia, depression, Huntington's disease, and Alzheimer's disease.^{19,22–25} Interestingly, recent animal and human studies have shown that AMPAkines stimulate the respiratory drive in the context of hypoventilation.^{26–28} For reasons not yet mechanistically understood, AMPAkines stimulate respiratory rhythmogenesis only when the respiratory drive has been suppressed, making these compounds ideal drugs for treating opioid-induced respiratory depression. A few limited studies found that AMPAkines do not alter the threshold for acute pain.^{26,28} However, the effect of AMPAkines on persistent or chronic pain has not been examined. Given the role central AMPA receptors play in pain, we hypothesized that systemic administration of AMPAkines can alter pain sensitivity and affect.

Here we studied the effect of AMPAkinases on chronic pain using the spared nerve injury (SNI) model for neuropathic pain and Complete Freund's Adjuvant (CFA) model for inflammatory pain.^{29–31} We found that CX546, an established AMPAkinase which has been studied in hypoventilation, Rett syndrome, anxiety, and autism,^{27,32–35} has significant analgesic properties. We verified these findings using a second AMPAkinase, CX516.^{36,37} Thus, our results suggest a novel analgesic role for AMPAkinases in persistent pain states. This novel analgesic property, combined with the ability to stimulate the respiratory drive, makes AMPAkinases promising drugs for postoperative and chronic pain.

Materials and Methods

Animals

All procedures in this study were approved by the New York University School of Medicine Institutional Animal Care and Use Committee (New York, NY) as consistent with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* (publication number 85–23) to ensure minimal animal use and discomfort. Male Sprague-Dawley rats were purchased from Taconic Farms, Albany, NY and kept in the Mispro Animal Facility in Alexandria Life Science Building (New York, NY), with controlled humidity, room temperature, and 12-h (6:30 AM to 6:30 PM) light-dark cycle. Food and water were available *ad libitum*. Animals arrived to the animal facility at 250 to 300 grams and were given approximately 7 days to adjust to the new environment prior to the onset of any experiments. For each experiment, animals were randomized to either the control or experimental group.

SNI surgery

The SNI surgery has been previously described in detail.²⁹ Briefly, under Isoflurane anesthesia (1.5 to 2%), the skin on the lateral surface of the right thigh of rat was incised and a section made through the biceps femoris muscle to expose three branches of the sciatic nerve: sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were tied with non-absorbent 5.0 silk sutures at the point of trifurcation. The nerves were then cut distal to the knot, and about 3 to 5 mm of the distal ends were removed. In sham surgeries (control), above nerves were dissected but not cut. Muscle and skin layers were then sutured closed in distinct layers.

CFA administration

To produce inflammatory pain, CFA (mycobacterium tuberculosis, Sigma-Aldrich (St. Louis, MO), 0.1cc) was suspended in an oil-saline (1:1) emulsion and injected subcutaneously into the plantar aspect of the right hind paw. Control rats received equal volume of saline injection.

Drugs

CX546 and CX516 were injected intraperitoneally in rats in a volume of 0.5–1cc, while a similar volume of DMSO was injected intraperitoneally to the control group. Injections were given 14 days after SNI surgeries and 7 days after CFA injections. Injections were followed by behavioral tests.

Animal Behavioral Tests

Mechanical allodynia testing—A traditional Dixon up-down method with von Frey filaments was used to measure mechanical allodynia.^{38,39} In brief, rats were individually placed into plexiglass chambers over a mesh table and acclimated for 20 min before the onset of examination. Beginning with 2.55g, von Frey filaments in a set with logarithmically incremental stiffness (0.45, 0.75, 1.20, 2.55, 4.40, 6.10, 10.50, 15.10 g) were applied to the lateral 1/3 of right paws (in the distribution of the sural nerve) of animals prior to and up to 14 days after SNI or sham surgery. Similar tests were done on CFA- or saline-treated rats. 50% withdrawal threshold was calculated as described previously.³⁹ Observers were blinded to the test conditions.

Cold allodynia testing—Animals were individually placed into plexiglass chambers as above and acclimated for 20 min. A drop of acetone (Sigma-Aldrich) was applied to the lateral plantar surface of the paws (in the distribution of the sural nerve). As previously described,^{40,41} the following scoring system was applied. 0: no visible response or startle response lasting <0.5 second; 1: paw withdrawal lasting <5 seconds; 2: withdrawal lasting 5 to 10 seconds, +/- licking of the paws; 3: prolonged repetitive withdrawal lasting > 10s. Acetone was applied 5 times to each paw, and an average score was calculated. Observers were blinded to the test conditions.

Forced swim test (FST)—As described previously,⁴² on the first session of the test, each animal was placed for fifteen minutes into a standard clear Porsolt chamber (Lafayette Instrument Company, Lafayette, IN) with water at 25° C filled to 25 cm. Afterwards, the animal was taken out of the chamber, dried and put back in its home cage. 24 hours later, the animal was placed into the Porsolt chamber again under the same conditions for 5 min. Both sessions were videotaped, but only the second session was analyzed. Immobility was defined as a lack of movement of the hind paws lasting greater than 1 second. An independent observer, blinded to the test conditions, examined and graded the total time of immobility for each rat, and the average grade was presented for each animal. FST was conducted 14 days after SNI/sham surgeries or 7 days after CFA/saline treatments.

Locomotion activities—As described previously,⁴³ rats were acclimated in locomotor measurement chambers (Accuscan, Columbus, OH) prior to testing. On test days, locomotion activities were measured for a total of 30 min. Distance traveled was measured using the VersaMax System (Accuscan) which monitored animal activity via a grid of 16 × 16 infrared light beams that traverse the animal cage (42 × 42 × 30 cm) front to back and left to right. Information about beam status, scanned at a rate of 100 times per second, was stored to disk. Activity was expressed as ambulatory distance measured during 10 different 3-min bins in a 30 min session.

Statistics

The results of behavioral experiments were given as mean ± SEM. For mechanical allodynia, a two-way ANOVA with repeated measures and *post hoc* multiple pair-wise comparison Bonferroni tests was used to compare the 50% withdrawal threshold of the SNI and sham animals and of the CFA and saline treated groups. Cold allodynia was also

analyzed using the two-way ANOVA test with repeated measures and *post hoc* multiple pair-wise comparison Bonferroni tests. Two-way ANOVA with *post hoc* multiple pair-wise Bonferroni comparison tests were also used for the dose-response experiments to compare the effect of AMPAkinines at various doses on mechanical and cold allodynia. In the pharmacokinetic experiment, a two-way ANOVA with repeated measures and *post hoc* multiple pair-wise comparison Bonferroni tests was used to compare mechanical or cold allodynia in AMPAkinine vs. DMSO (control) treatments at various time points. For the FST, unpaired two-tailed Student's *t* tests were used to compare the performances of sham and SNI groups, CFA and saline groups, as well as AMPAkinine and DMSO groups. A two-way ANOVA with repeated measures and *post hoc* multiple pair-wise comparison Bonferroni tests was also used to compare locomotion in SNI vs. sham groups, CFA vs. saline treatment groups, and in AMPAkinine vs. DMSO groups. For all tests, a *p* value <0.05 was considered statistically significant. All data were analyzed using GraphPad Prism Version 5 software (GraphPad, La Jolla, CA).

Results

SNI produced long lasting sensory allodynia and depression-like behavior

The SNI model is commonly used to study chronic neuropathic pain or persistent postoperative pain. Consistent with previous results,^{29,42,44} we found that SNI surgery produced significant mechanical and cold allodynia in the (spared) sural nerve distribution (fig. 1A and B). In contrast, rats that underwent sham operation did not demonstrate any sensory allodynia in the spared nerve distribution.

We have previously shown that chronic neuropathic pain, in addition to producing sensory allodynia as tested by paw withdrawal, also leads to depression-like behaviors in rats.^{42,44} Here, we applied the FST, a widely used test to assess depression-like behaviors in rats.⁴⁵ Numerous studies have demonstrated clinically relevant pharmacological validity of this test. Increased time of immobility (instead of swimming) on the FST is considered a measure of behavioral despair, a salient feature of depression. We performed the FST 14 days after the surgery and found that compared with sham-operated rats, rats after SNI developed a significantly increased time of immobility (fig. 1C). To rule out the possibility that this immobility was caused by locomotor deficits secondary to peripheral neuropathy or by pain associated with movement, we performed locomotion tests on SNI-treated rats. SNI-treated rats, compared with sham-treated rats, did not display a change on the locomotion test over 30 minutes (fig. 1D), and thus these rats were unlikely to have overt locomotor deficits that prevented them from swimming for 5 minutes. Increased immobility on FST, therefore, more likely reflected depression-like behaviors exhibited by these animals in chronic pain.⁴⁵

CX546, an established AMPAkinine, relieves sensory allodynia and depression-like behavior associated with chronic neuropathic pain

We tested the effect of CX546 on pain using the SNI model. CX546 is a well-established AMPAkinine which has been studied in a number of preclinical models of CNS diseases including respiratory depression, Rett syndrome, anxiety, and autism.^{27,32–35} When we

applied this compound systemically (via intraperitoneal injection) to SNI-treated rats, we were surprised to observe a dramatic improvement in mechanical allodynia compared with control (DMSO) injection (fig. 2A). We began to observe anti-allodynic effects at a dose of 5mg/kg, while a higher dose of 10mg/kg produced an even greater effect. We further validated this anti-nociceptive effect in the cold allodynia test, where we similarly observed improvements in cold allodynic scores at 5 and 10mg/kg doses (fig. 2B). Next, we evaluated the duration of this anti-nociceptive effect (fig. 3A and B). We administered a 10mg/kg dose of CX546 (fig. 2A and B), and we found that the effect of CX546 lasted 6 hours on the mechanical allodynia test. Its effect was shorter (2 hours) on the cold allodynia test.

Sensory allodynia tests assay nociception, but amplifying AMPA receptor transmission can affect a number of brain regions that regulate both nociceptive and affective components of pain. Thus, we next used the FST to test whether CX546, in addition to its anti-nociceptive effects, can also treat depression-like behavior associated with pain. Here we found that CX546 also improved immobility on the FST in SNI-treated rats (fig. 4A). In contrast, CX546 did not affect locomotion (fig. 4B). Thus, the effect of CX546 on FST likely reflected an improvement in behavioral despair, rather than improved locomotion. Results from the FST augment and complement results on allodynia tests.

CX546 relieves sensory allodynia and depression-like behavior associated with persistent inflammatory pain

Synaptic and circuit mechanisms for neuropathic pain are distinct in many aspects from other chronic or acute pain conditions.⁴⁶ Thus, we applied the CFA model of inflammatory pain to test whether CX546 also confers analgesic effects in persistent inflammatory pain. As expected, CFA injection, compared with saline injection, resulted in consistent mechanical allodynia that lasted at least 11 days (fig. 5A). In addition, we found that CFA injection also led to a dramatic increase in immobility (fig. 5B). CFA did not alter locomotion (fig. 5C), suggesting that immobility on the FST indicates the development of behavioral despair rather than locomotor deficits. These results provide evidence that inflammatory pain, similar to neuropathic pain, leads to sensory allodynia as well as depression-like behaviors.

Next, we tested whether the analgesic property of CX546 we found in the SNI model of neuropathic pain is preserved in the CFA model of inflammatory pain. After intraperitoneal administration of this AMPAkin, we observed a significant improvement in mechanical allodynia (fig. 6A), in a dose dependent manner that is similar to our findings in the SNI model. Furthermore, CX546, as expected, also reduced immobility on the FST, without altering locomotion (fig. 6B and C). Thus, CX546 treated both sensory allodynia and depression-like behaviors elicited by the CFA model. These results, therefore, demonstrate that the analgesic property of CX546 is preserved in both neuropathic and inflammatory pain conditions.

CX516 relieves sensory allodynia and depression-like behavior associated with chronic pain

To further validate the analgesic effect of AMPAkinines in chronic pain, we applied CX516, another compound with well-known AMPA potentiation properties.^{36,37} Here, we found that CX516 also produced dose-dependent effects on sensory allodynia in SNI-treated rats (fig. 7A, B). The dose required to produce maximal analgesic effects is higher in CX516 (20mg/kg) than required for CX546 (<10mg/kg). The maximal analgesic efficacy, however, is similar in both compounds. Thus, these results suggest that AMPAkinines in general have important analgesic properties in chronic pain states.

Discussion

In this study, we investigated the role of AMPAkinines in chronic pain. We found that CX546 and CX516, two well-studied AMPAkinines, reduce sensory allodynia and depression-like behaviors in rodent neuropathic and inflammatory pain models.

New analgesics that do not suppress the respiratory drive are urgently needed. AMPAkinines slow the kinetics of AMPA receptor deactivation to enhance the inward synaptic current.^{19,20} By increasing AMPA receptor-mediated currents in neurons of the pre-Botzinger complex in the medulla, AMPAkinines directly stimulate the respiratory drive,^{26,27,47–49} and they have been shown to treat or prevent hypoventilation caused by opioids and propofol.^{26,28,50,51} The effect of AMPAkinines in chronic pain, however, has not been previously studied. The analgesic dose for CX546 found in our study is comparable to the dose tested in rats to treat respiratory depression.²⁷ Meanwhile, the time course of analgesia after a single administration (2–6 hours) is slightly longer than the respiratory stimulatory effect of AMPAkinines.^{26,27} Thus, the analgesic property of AMPAkinines overlaps their respiratory stimulatory activity from a pharmacologic and pharmacokinetic standpoint. As a rare analgesic that can stimulate the respiratory drive, AMPAkinines should be useful for postoperative and chronic pain.

The anti-nociceptive effect of AMPAkinines can be explained by their action on AMPA receptors in two descending inhibitory circuits: PAG-RVM and NAc-RVM circuits. The PAG-RVM-spinal descending pathway is a well-known mechanism for pain regulation.^{52–54} In this pathway, neurons from the PAG form glutamatergic projections through AMPA receptors on GABAergic cells in the RVM to inhibit dorsal horn neurons.⁵⁵ The administration of glutamate into the PAG is known to produce analgesia.^{56–58} Furthermore, there is evidence that in the RVM, AMPA receptor upregulation mediates analgesia in inflammatory pain states,^{59,60} whereas their downregulation in neuropathic pain causes hyperalgesia.⁶¹ Thus, transmission through AMPA receptors is required for the intact PAG-RVM descending pathway.^{16,62} A second pain modulating center that depends on AMPA receptor signaling is the NAc. The NAc provides pain-induced analgesia, in part through its projection to the RVM.¹⁸ Intra-NAc administration of AMPA receptor antagonists, however, can disrupt this pain-induced analgesic mechanism.¹⁷

At the same time, however, potentiation of AMPA receptors in neurons of the spinal dorsal horn, ACC and amygdala can have pro-nociceptive effects. In the dorsal horn, chronic

inflammatory pain increases membrane targeting of GluA1 AMPA receptor subunits but decreases GluA2 delivery,^{6,9,63} leading to the formation of GluA2-lacking receptors to augment pain transmission.⁸ Similarly, AMPA receptor signaling in the ACC and amygdala has also been suggested to mediate increased synaptic plasticity and confer hyperalgesia.^{11–13,64,65}

Thus, in chronic pain conditions, AMPA receptor signaling plays both pro-nociceptive and anti-nociceptive roles, depending on the target CNS regions. In our study, we administered AMPAkinines systemically, and we expected them to act peripherally and/or in the brain, with a smaller component of activity in the spinal cord. Thus, the behavioral phenotype we observed likely represents AMPAkinine effects in the brain. Our data indicate that the net result favors analgesia rather than hyperalgesia. A possible reason for this net analgesic effect may lie in the distinct affinity of AMPAkinines for neurons of different brain regions.⁶⁶ AMPAkinines are known to bind to neurons in the NAc and brain stem with high affinity,⁶⁶ thereby facilitating the enhancement of AMPA receptor signaling in these descending inhibitory circuits. Future studies targeting specific regions in the brain and spinal cord are needed to further elucidate the precise roles for these drugs in pain states.

Symptoms of depression occur in many chronic pain patients,^{67,68} and our finding that AMPAkinines can treat pain-induced depression is consistent with the role of central AMPA receptors in depression⁶⁹. For example, GluA1 subunits of AMPA receptors are reduced in the amygdala, prefrontal cortex, and hippocampus in rodent models of depression.^{70–72} Ketamine has been shown to elevate GluA1 levels in the PFC to provide fast-acting relief from depression,⁷³ and it is effective in treating pain-induced depression.⁴² Thus, increased AMPA receptor signaling (especially in the hippocampus and prefrontal cortex) has antidepressant effects. By directly amplifying postsynaptic currents through AMPA receptors, AMPAkinines have been shown to treat depression in animal models.⁷⁴ Our results show that this antidepressant effect of AMPAkinines is preserved in pain models.

Distinct molecular mechanisms are found in the maintenance of neuropathic and inflammatory pain. The dose requirement for CX546 is higher in the CFA model than the SNI model, and the maximal anti-allodynia effect, is also quantitatively lower. Qualitatively, however, CX546 clearly has anti-nociceptive and antidepressant effects in both models. Our results, therefore, suggest a shared analgesic mechanism mediated by AMPA receptors in both neuropathic and inflammatory pain states.

In comparison to our finding here, previous studies on AMPAkinine failed to demonstrate pro- or anti-nociceptive effects.^{26,28} However, these studies were performed in wild-type rodents and healthy human subjects with acute stimuli. In one study, nociceptive threshold was tested in rats by withdrawal to acute thermal stimuli.²⁶ In another study, pain was tested by electric shock and thermal stimuli in healthy volunteers.²⁸ The difference between these findings and ours may be due to the mechanistic differences between acute and chronic pain. Synaptic plasticity in the CNS is thought to mediate the transition from acute to chronic pain,^{46,75} and a general feature of synaptic plasticity is amplification of excitatory postsynaptic currents conducted through AMPA receptors.⁷⁶ Thus, it is not surprising that

while potentiating these receptors in acute pain has an insignificant effect,^{26,28} it has a more profound effect in chronic pain states.

Both withdrawal tests and the FST can be confounded by locomotor deficits. Worse performance on the FST may reflect a decrease in locomotion due to movement-induced pain or neuropathy. Our locomotion tests, however, do not show any deficits, compatible with earlier findings.⁴⁴ If rats do not demonstrate locomotor deficits over 30 minutes, it is unlikely that they will have deficits while swimming for 5 minutes during the FST. Thus, results on the FST likely reflect the phenotype of depression, rather than deficiencies in locomotion. In addition, compatible with our findings, other studies on AMPAkinases also demonstrate no effects on locomotion.²¹

The timing of development of affective pain symptoms in rodents is not completely known. In our study, immobility on the FST manifested two weeks after SNI, compatible with two previous reports.^{42,44} In another study using this model, however, deficits on the FST were detected at 7 weeks, but data from earlier time points were not shown.⁷⁷ In the spinal nerve ligation model, Kontinen *et al.* reported no changes in immobility 14 days after surgery,⁷⁸ whereas Suzuki *et al.* found increased immobility 15 days after surgery.⁷⁹ In another study, rats developed immobility 7 days after chronic constriction injury.⁸⁰ Sham surgery can induce transient reversible depressive pain symptoms, likely caused by acute pain from skin and/or muscle incision⁴². Thus, depending on the rate of recovery from sham surgeries, the detectable differences on the FST between neuropathy and control groups at earlier time points may be masked by acute pain-induced changes in the control group. In terms of inflammatory pain, we found the rats developed deficits on the FST one week after subcutaneous CFA injections. Similar to what we observe here, another study using CFA to induce peripheral inflammatory pain also demonstrated deficits on the FST within a week after pain induction.⁸¹ A study on monoarthritic pain, meanwhile, found deficits at 4 weeks after CFA injection into the tibiotarsal joint.⁸² In that study, however, injection was deep into the joint. Thus, the exact onset of affective pain symptoms may vary depending on the nature of pain models.

In our study, we used DMSO to deliver AMPAkinases according to the manufacturer recommendation, as has been done in previous studies.²⁷ In our study, there were small differences in the average allodynia measures for the SNI group without any treatment (fig. 1A, B) and the group that received DMSO (fig. 2A, B). These differences were small, however, compared with the changes in allodynia as the result of AMPAkinase treatments (fig. 2A, B). We did not observe any anti-allodynic effect for DMSO in the CFA model (fig. 5A, 6A). To make sure that the use of DMSO as a solvent would not confound our findings, we used DMSO as control throughout our study. Thus, the minor anti-allodynic effect of DMSO should not have affected our results that the AMPAkinases have significant pain-relieving properties.

A potential side effect of AMPAkinases is CNS hyperexcitability due to increased AMPA receptor activities.^{19,20} AMPAkinases are classified into two classes: low- and high-impact AMPAkinases. Low-impact AMPAkinases (e.g. CX717 and CX1739) are less likely to cause hyper-excitability and have a broader therapeutic window. High-impact AMPAkinases are

more potent, but they have a narrower therapeutic window. We used low doses of high-impact AMPAkinases and did not observe any overt behavioral deficits. Nevertheless, in the future, it will be important to investigate the pain-inhibiting properties of low-impact AMPAkinases which have safer pharmacologic profiles.

In summary, we show that AMPAkinases have novel analgesic properties in rat models of neuropathic and inflammatory pain. A combination of analgesic and respiratory stimulatory properties can make AMPAkinases ideal drugs for the treatment of persistent postoperative and chronic pain.

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References

1. Dahan A, Overdyk F, Smith T, Aarts L, Niesters M. Pharmacovigilance: A review of opioid-induced respiratory depression in chronic pain patients. *Pain Physician*. 2013; 16:E85–94. [PubMed: 23511694]
2. Dahan A, Aarts L, Smith TW. Incidence, Reversal, and Prevention of Opioid-induced Respiratory Depression. *Anesthesiology*. 2010; 112:226–38. [PubMed: 20010421]
3. Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R. Opioid complications and side effects. *Pain Physician*. 2008; 11:S105–20. [PubMed: 18443635]
4. Paulozzi LJ, Budnitz DS, Xi Y. Increasing deaths from opioid analgesics in the United States. *Pharmacoepidemiol Drug Saf*. 2006; 15:618–27. [PubMed: 16862602]
5. Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci*. 2002; 25:578–88. [PubMed: 12392933]
6. Park JS, Yaster M, Guan X, Xu JT, Shih MH, Guan Y, Raja SN, Tao YX. Role of spinal cord alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in complete Freund's adjuvant-induced inflammatory pain. *Mol Pain*. 2008; 4:67. [PubMed: 19116032]
7. Stanfa LC, Dickenson AH. The role of non-N-methyl-D-aspartate ionotropic glutamate receptors in the spinal transmission of nociception in normal animals and animals with carrageen an inflammation. *Neuroscience*. 1999; 93:1391–8. [PubMed: 10501464]
8. Hartmann B, Ahmadi S, Heppenstall PA, Lewin GR, Schott C, Borchardt T, Seeburg PH, Zeilhofer HU, Sprengel R, Kuner R. The AMPA receptor subunits GluR-A and GluR-B reciprocally modulate spinal synaptic plasticity and inflammatory pain. *Neuron*. 2004; 44:637–50. [PubMed: 15541312]
9. Katano T, Furue H, Okuda-Ashitaka E, Tagaya M, Watanabe M, Yoshimura M, Ito S. N-ethylmaleimide-sensitive fusion protein (NSF) is involved in central sensitization in the spinal cord through GluR2 subunit composition switch after inflammation. *Eur J Neurosci*. 2008; 27:3161–70. [PubMed: 18598260]
10. Lu Y, Sun YN, Wu X, Sun Q, Liu FY, Xing GG, Wan Y. Role of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunit GluR1 in spinal dorsal horn in inflammatory nociception and neuropathic nociception in rat. *Brain Res*. 2008; 1200:19–26. [PubMed: 18289517]
11. Chen J, Song Y, Yang J, Zhang Y, Zhao P, Zhu XJ, Su HC. The contribution of TNF-alpha in the amygdala to anxiety in mice with persistent inflammatory pain. *Neurosci Lett*. 2013; 541:275–80. [PubMed: 23415758]
12. Li W, Neugebauer V. Block of NMDA and non-NMDA receptor activation results in reduced background and evoked activity of central amygdala neurons in a model of arthritic pain. *Pain*. 2004; 110:112–22. [PubMed: 15275758]

13. Xu H, Wu LJ, Wang H, Zhang X, Vadakkan KI, Kim SS, Steenland HW, Zhuo M. Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. *J Neurosci*. 2008; 28:7445–53. [PubMed: 18632948]
14. van Praag H, Frenk H. The role of glutamate in opiate descending inhibition of nociceptive spinal reflexes. *Brain Res*. 1990; 524:101–5. [PubMed: 1976028]
15. Spinella M, Cooper ML, Bodnar RJ. Excitatory amino acid antagonists in the rostral ventromedial medulla inhibit mesencephalic morphine analgesia in rats. *Pain*. 1996; 64:545–52. [PubMed: 8783320]
16. Urban MO, Coutinho SV, Gebhart GF. Involvement of excitatory amino acid receptors and nitric oxide in the rostral ventromedial medulla in modulating secondary hyperalgesia produced by mustard oil. *Pain*. 1999; 81:45–55. [PubMed: 10353492]
17. Ghalandari-Shamami M, Hassanpour-Ezatti M, Haghparsat A. Intra-accumbal NMDA but not AMPA/kainate receptor antagonist attenuates WIN55,212–2 cannabinoid receptor agonist-induced antinociception in the basolateral amygdala in a rat model of acute pain. *Pharmacol Biochem Behav*. 2011; 100:213–9. [PubMed: 21924283]
18. Gear RW, Aley KO, Levine JD. Pain-induced analgesia mediated by mesolimbic reward circuits. *J Neurosci*. 1999; 19:7175–81. [PubMed: 10436070]
19. Arai AC, Kessler M. Pharmacology of ampakine modulators: From AMPA receptors to synapses and behavior. *Curr Drug Targets*. 2007; 8:583–602. [PubMed: 17504103]
20. Lynch G. Glutamate-based therapeutic approaches: Ampakines. *Curr Opin Pharmacol*. 2006; 6:82–8. [PubMed: 16361116]
21. Lynch G, Gall CM. Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci*. 2006; 29:554–62. [PubMed: 16890999]
22. Tuominen HJ, Tiihonen J, Wahlbeck K. Glutamatergic drugs for schizophrenia: A systematic review and meta-analysis. *Schizophr Res*. 2005; 72:225–34. [PubMed: 15560967]
23. Skolnick P, Popik P, Trullas R. Glutamate-based antidepressants: 20 years on. *Trends Pharmacol Sci*. 2009; 30:563–9. [PubMed: 19837463]
24. Zheng YW, Balabhadrapatruni S, Masumura C, Darlington CL, Smith PF. Effects of the Putative Cognitive-Enhancing Ampakine, CX717, on Attention and Object Recognition Memory. *Current Alzheimer Research*. 2011; 8:876–82. [PubMed: 22171951]
25. Simmons DA, Rex CS, Palmer L, Pandeyarajan V, Fedulov V, Gall CM, Lynch G. Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knock in mice. *Proc Natl Acad Sci U S A*. 2009; 106:4906–11. [PubMed: 19264961]
26. Ren J, Ding X, Funk GD, Greer JJ. Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology*. 2009; 110:1364–70. [PubMed: 19461299]
27. Ren J, Poon BY, Tang Y, Funk GD, Greer JJ. Ampakines alleviate respiratory depression in rats. *Am J Respir Crit Care Med*. 2006; 174:1384–91. [PubMed: 16973981]
28. Oertel BG, Felden L, Tran PV, Bradshaw MH, Angst MS, Schmidt H, Johnson S, Greer JJ, Geisslinger G, Varney MA, Lotsch J. Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. *Clin Pharmacol Ther*. 2010; 87:204–11. [PubMed: 19907420]
29. Decosterd I, Woolf CJ. Spared nerve injury: An animal model of persistent peripheral neuropathic pain. *Pain*. 2000; 87:149–58. [PubMed: 10924808]
30. Ruda MA, Iadarola MJ, Cohen LV, Young WS 3rd. In situ hybridization histochemistry and immunocytochemistry reveal an increase in spinal dynorphin biosynthesis in a rat model of peripheral inflammation and hyperalgesia. *Proc Natl Acad Sci U S A*. 1988; 85:622–6. [PubMed: 2893375]
31. Iadarola MJ, Brady LS, Draisci G, Dubner R. Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: Stimulus specificity, behavioral parameters and opioid receptor binding. *Pain*. 1988; 35:313–26. [PubMed: 2906426]
32. Nagarajan N, Quast C, Boxall AR, Shahid M, Rosenmund C. Mechanism and impact of allosteric AMPA receptor modulation by the ampakine CX546. *Neuropharmacology*. 2001; 41:650–63. [PubMed: 11640919]

33. Ogier M, Wang H, Hong E, Wang Q, Greenberg ME, Katz DM. Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. *J Neurosci*. 2007; 27:10912–7. [PubMed: 17913925]
34. Silverman JL, Oliver CF, Karras MN, Gastrell PT, Crawley JN. AMPAKINE enhancement of social interaction in the BTBR mouse model of autism. *Neuropharmacology*. 2013; 64:268–82. [PubMed: 22801296]
35. Procaccini C, Aitta-aho T, Jaako-Movits K, Zharkovsky A, Panhelainen A, Sprengel R, Linden AM, Korpi ER. Excessive novelty-induced c-Fos expression and altered neurogenesis in the hippocampus of GluA1 knockout mice. *Eur J Neurosci*. 2011; 33:161–74. [PubMed: 21073553]
36. Arai A, Kessler M, Rogers G, Lynch G. Effects of a memory-enhancing drug on DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor currents and synaptic transmission in hippocampus. *J Pharmacol Exp Ther*. 1996; 278:627–38. [PubMed: 8768713]
37. Lynch G, Kessler M, Rogers G, Ambros-Ingerson J, Granger R, Schehr RS. Psychological effects of a drug that facilitates brain AMPA receptors. *Int Clin Psychopharmacol*. 1996; 11:13–9. [PubMed: 8732309]
38. Bourquin AF, Suveges M, Pertin M, Gilliard N, Sardy S, Davison AC, Spahn DR, Decosterd I. Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. *Pain*. 2006; 122:14, e1–14. [PubMed: 16542774]
39. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods*. 1994; 53:55–63. [PubMed: 7990513]
40. Hao JX, Shi TJ, Xu IS, Kaupilla T, Xu XJ, Hokfelt T, Bartfai T, Wiesenfeld-Hallin Z. Intrathecal galanin alleviates allodynia-like behaviour in rats after partial peripheral nerve injury. *Eur J Neurosci*. 1999; 11:427–32. [PubMed: 10051743]
41. Jorum E, Warncke T, Stubhaug A. Cold allodynia and hyperalgesia in neuropathic pain: The effect of N-methyl-D-aspartate (NMDA) receptor antagonist ketamine--a double-blind, cross-over comparison with alfentanil and placebo. *Pain*. 2003; 101:229–35. [PubMed: 12583865]
42. Wang J, Goffer Y, Xu D, Tukey DS, Shamir DB, Eberle SE, Zou AH, Blanck TJ, Ziff EB. A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology*. 2011; 115:812–21. [PubMed: 21934410]
43. Cabeza de Vaca S, Carr KD. Food restriction enhances the central rewarding effect of abused drugs. *J Neurosci*. 1998; 18:7502–10. [PubMed: 9736668]
44. Goffer Y, Xu D, Eberle SE, D'Amour J, Lee M, Tukey D, Froemke RC, Ziff EB, Wang J. Calcium-permeable AMPA receptors in the nucleus accumbens regulate depression-like behaviors in the chronic neuropathic pain state. *J Neurosci*. 2013; 33:19034–44. [PubMed: 24285907]
45. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci*. 2010; 13:1161–9. [PubMed: 20877280]
46. von Hehn CA, Baron R, Woolf CJ. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. *Neuron*. 2012; 73:638–52. [PubMed: 22365541]
47. Greer JJ, Smith JC, Feldman JL. Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rat. *J Physiol*. 1991; 437:727–49. [PubMed: 1653855]
48. Funk GD, Smith JC, Feldman JL. Generation and transmission of respiratory oscillations in medullary slices: Role of excitatory amino acids. *J Neurophysiol*. 1993; 70:1497–515. [PubMed: 8283211]
49. Pace RW, Mackay DD, Feldman JL, Del Negro CA. Inspiratory bursts in the preBotzinger complex depend on a calcium-activated non-specific cation current linked to glutamate receptors in neonatal mice. *J Physiol*. 2007; 582:113–25. [PubMed: 17446214]
50. Greer JJ, Ren J. Ampakine therapy to counter fentanyl-induced respiratory depression. *Respir Physiol Neurobiol*. 2009; 168:153–7. [PubMed: 19712906]
51. Ren J, Lenal F, Yang M, Ding X, Greer JJ. Coadministration of the AMPAKINE CX717 with propofol reduces respiratory depression and fatal apneas. *Anesthesiology*. 2013; 118:1437–45. [PubMed: 23542802]
52. Fields HL, Anderson SD, Clanton CH, Basbaum AI. Nucleus raphe magnus: A common mediator of opiate- and stimulus-produced analgesia. *Trans Am Neurol Assoc*. 1976; 101:208–10. [PubMed: 195380]

53. Basbaum AI, Fields HL. Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci.* 1984; 7:309–38. [PubMed: 6143527]
54. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev.* 2009; 60:214–25. [PubMed: 19146877]
55. Morgan MM, Whittier KL, Hegarty DM, Aicher SA. Periaqueductal gray neurons project to spinally projecting GABAergic neurons in the rostral ventromedial medulla. *Pain.* 2008; 140:376–86. [PubMed: 18926635]
56. Behbehani MM, Fields HL. Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia. *Brain Res.* 1979; 170:85–93. [PubMed: 223721]
57. Jensen TS, Yaksh TL. Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. *Brain Res.* 1984; 321:287–97. [PubMed: 6149792]
58. Urca G, Nahin RL, Liebeskind JC. Glutamate-induced analgesia: Blockade and potentiation by naloxone. *Brain Res.* 1980; 192:523–30. [PubMed: 7378801]
59. Guan Y, Guo W, Zou SP, Dubner R, Ren K. Inflammation-induced upregulation of AMPA receptor subunit expression in brain stem pain modulatory circuitry. *Pain.* 2003; 104:401–13. [PubMed: 12855351]
60. Guan Y, Guo W, Robbins MT, Dubner R, Ren K. Changes in AMPA receptor phosphorylation in the rostral ventromedial medulla after inflammatory hyperalgesia in rats. *Neurosci Lett.* 2004; 366:201–5. [PubMed: 15276247]
61. Ho YC, Cheng JK, Chiou LC. Hypofunction of glutamatergic neurotransmission in the periaqueductal gray contributes to nerve-injury-induced neuropathic pain. *J Neurosci.* 2013; 33:7825–36. [PubMed: 23637174]
62. Guan Y, Terayama R, Dubner R, Ren K. Plasticity in excitatory amino acid receptor-mediated descending pain modulation after inflammation. *J Pharmacol Exp Ther.* 2002; 300:513–20. [PubMed: 11805211]
63. Park JS, Voitenko N, Petralia RS, Guan X, Xu JT, Steinberg JP, Takamiya K, Sotnik A, Kopach O, Haganir RL, Tao YX. Persistent inflammation induces GluR2 internalization via NMDA receptor-triggered PKC activation in dorsal horn neurons. *J Neurosci.* 2009; 29:3206–19. [PubMed: 19279258]
64. Li XY, Ko HG, Chen T, Descalzi G, Koga K, Wang H, Kim SS, Shang Y, Kwak C, Park SW, Shim J, Lee K, Collingridge GL, Kaang BK, Zhuo M. Alleviating neuropathic pain hypersensitivity by inhibiting PKMzeta in the anterior cingulate cortex. *Science.* 2010; 330:1400–4. [PubMed: 21127255]
65. Ji G, Sun H, Fu Y, Li Z, Pais-Vieira M, Galhardo V, Neugebauer V. Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. *J Neurosci.* 2010; 30:5451–64. [PubMed: 20392966]
66. Montgomery KE, Kessler M, Arai AC. Modulation of agonist binding to AMPA receptors by 1-(1,4-benzodioxan-6-ylcarbonyl)piperidine (CX546): Differential effects across brain regions and GluA1–4/transmembrane AMPA receptor regulatory protein combinations. *J Pharmacol Exp Ther.* 2009; 331:965–74. [PubMed: 19717789]
67. Dworkin RH, Gitlin MJ. Clinical aspects of depression in chronic pain patients. *Clin J Pain.* 1991; 7:79–94. [PubMed: 1809423]
68. Miller LR, Cano A. Comorbid chronic pain and depression: Who is at risk? *J Pain.* 2009; 10:619–27. [PubMed: 19398383]
69. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology.* 2012; 62:63–77. [PubMed: 21827775]
70. Chandran A, Iyo AH, Jernigan CS, Legutko B, Austin MC, Karolewicz B. Reduced phosphorylation of the mTOR signaling pathway components in the amygdala of rats exposed to chronic stress. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013; 40:240–5. [PubMed: 22889863]

71. Duric V, Banasr M, Stockmeier CA, Simen AA, Newton SS, Overholser JC, Jurjus GJ, Dieter L, Duman RS. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. *Int J Neuropsychopharmacol*. 2012;1–14.
72. Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z. Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*. 2012; 73:962–77. [PubMed: 22405206]
73. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 2010; 329:959–64. [PubMed: 20724638]
74. Skolnick P. AMPA receptors: A target for novel antidepressants? *Biol Psychiatry*. 2008; 63:347–8. [PubMed: 18237615]
75. Latremoliere A, Woolf CJ. Central sensitization: A generator of pain hypersensitivity by central neural plasticity. *J Pain*. 2009; 10:895–926. [PubMed: 19712899]
76. Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron*. 2009; 61:340–50. [PubMed: 19217372]
77. Goncalves L, Silva R, Pinto-Ribeiro F, Pego JM, Bessa JM, Pertovaara A, Sousa N, Almeida A. Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat. *Exp Neurol*. 2008; 213:48–56. [PubMed: 18599044]
78. Kontinen VK, Kauppila T, Paananen S, Pertovaara A, Kalso E. Behavioural measures of depression and anxiety in rats with spinal nerve ligation-induced neuropathy. *Pain*. 1999; 80:341–6. [PubMed: 10204747]
79. Suzuki T, Amata M, Sakaue G, Nishimura S, Inoue T, Shibata M, Mashimo T. Experimental neuropathy in mice is associated with delayed behavioral changes related to anxiety and depression. *Anesth Analg*. 2007; 104:1570–7. [PubMed: 17513660]
80. Zeng Q, Wang S, Lim G, Yang L, Mao J, Sung B, Chang Y, Lim JA, Guo G. Exacerbated mechanical allodynia in rats with depression-like behavior. *Brain Res*. 2008; 1200:27–38. [PubMed: 18289511]
81. Kim H, Chen L, Lim G, Sung B, Wang S, McCabe MF, Rusanescu G, Yang L, Tian Y, Mao J. Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. *J Clin Invest*. 2012; 122:2940–54. [PubMed: 22751107]
82. Borges G, Neto F, Mico JA, Berrocoso E. Reversal of Monoarthritis-induced Affective Disorders by Diclofenac in Rats. *Anesthesiology*. 2014; 120:1476–90. [PubMed: 24534905]

Figure 1.

Spared nerve injury (SNI) induces long-lasting mechanical and cold allodynia and increases immobility during forced swim tests in rats. (A) Animals that underwent SNI surgery developed mechanical allodynia that lasted at least 14 days. Mechanical allodynia was tested using von Frey filaments. In comparison, sham-treated rats (control) did not demonstrate mechanical allodynia. A 50% withdrawal threshold was calculated (see Methods for details). $***P < 0.0001$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. Sham group, $n = 6$. SNI group, $n = 10$ (B) SNI-treated animals, in contrast to control rats, developed cold allodynia that lasted at least 14 days. $***P < 0.0001$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. Sham group, $n = 6$. SNI group, $n = 10$ (C) SNI-treated rats demonstrated increased immobility during the forced swim test compared to controls. $*P < 0.05$, unpaired two-tailed Student's *t* test. Sham group, $n = 12$. SNI group, $n = 10$. (D) Locomotion was unaffected by SNI surgery. $P > 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni tests. $n = 6$ for both Sham and SNI groups. Error bars represent standard error mean (SEM).

Figure 2.

CX546 relieves mechanical and cold allodynia in spared nerve injury (SNI)-treated animals in a dose-dependent manner. (A) SNI-treated animals after intraperitoneal administration of CX546 demonstrated reduced mechanical allodynia compared to DMSO control at doses of 10 mg/kg and 5 mg/kg, but not at 2.5 mg/kg. *** $P < 0.001$, two-way ANOVA with *post hoc* Bonferroni multiple comparison tests. DMSO group, $n = 9$. CX546 group, $n = 11$. (B) SNI-treated animals after intraperitoneal administration of CX546 demonstrated reduced cold allodynia compared to DMSO control at doses of 10 mg/kg and 5 mg/kg, but not at 2.5 mg/kg. ** $P < 0.01$, * $P < 0.05$, two-way ANOVA with *post hoc* Bonferroni multiple comparison tests. DMSO group, $n = 9$. CX546 group, $n = 11$. Error bars represent SEM.

Figure 3.

A single administration of CX546 (10 mg/kg) improves mechanical and cold allodynia in spared nerve injury (SNI)-treated animals over several hours. (A) SNI-treated animals had reduced mechanical allodynia at 1, 2, 4, and 6 hrs after administration of CX546 (10 mg/kg). **** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. DMSO group, $n = 10$. CX546 group, $n = 9$. (B) SNI-treated animals administered with CX546 (10 mg/kg) had reduced cold allodynia compared to DMSO control at 1 and 2 hrs. * $P < 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. $n = 8$ for both DMSO and CX546 groups. Error bars represent SEM.

Figure 4.

Intraperitoneal injection of CX546 (10 mg/kg) in spared nerve injury (SNI)-treated animals reduces immobility time during forced swim tests without altering locomotor activities. (A) Animals with intraperitoneal injection of CX546 (10 mg/kg) exhibited reduced immobility compared to DMSO control. $*P < 0.05$, unpaired two-tailed Student's *t* test. $n = 8$ for both DMSO and CX546 groups. (B) Locomotion in SNI-treated rats was not affected by CX546 injection (10 mg/kg) compared to DMSO injection. $P > 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. $n = 8$ for both DMSO and CX546 groups. Error bars represent SEM.

Figure 5.

Subcutaneous injection of Complete Freund's Adjuvant (CFA) in the right hind paw of animals induces long-lasting mechanical allodynia and increased immobility during forced swim tests. (A) CFA-treated rats exhibited increased and long-lasting mechanical allodynia compared to saline-treated rats up to post-operative day 11. $****P < 0.0001$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. $n = 6$ for both saline and CFA groups. (B) CFA-treated rats exhibited increased immobility during the forced swim test compared to saline-treated rats. $*P < 0.05$, unpaired two-tailed Student's *t* test. $n = 6$ for both saline and CFA groups. (C) CFA treatment did not alter the locomotor activity of animals. $P > 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. Saline group, $n = 6$. CFA group, $n = 8$. Error bars represent SEM.

Figure 6.

Intraperitoneal administration of CX546 in Complete Freund's Adjuvant (CFA)-treated animals relieves mechanical allodynia and reduces immobility during forced swim tests without altering locomotor activities. (A) Intraperitoneal administration of CX546 in CFA-treated animals decreases mechanical allodynia at doses of 25 mg/kg and 12.5 mg/kg, but not at 6.25 mg/kg or 3.125 mg/kg dose. *** $P < 0.001$, * $P < 0.05$, two-way ANOVA with *post hoc* Bonferroni multiple comparison tests. For the 25, 12.5, and 6.25 mg/kg dose, $n = 9$. For the 3.125 mg/kg dose, $n = 6$ (B) CFA-treated animals administered with CX546 (25 mg/kg) showed decreased immobility compared with control during the forced swim test. * $P < 0.05$, unpaired two-tailed Student's *t* test. $n = 10$ for both DMSO and CX546 groups. (C) Administration of CX546 (25 mg/kg) did not affect locomotor activities of CFA-treated animals. $P > 0.05$, two-way ANOVA with repeated measures and *post hoc* Bonferroni multiple comparison tests. $n = 7$ for both DMSO and CX546 groups. Error bars represent SEM.

Figure 7.

Intraperitoneal administration of CX516 in spared nerve injury (SNI)-treated animals relieves mechanical and cold allodynia. (A) Intraperitoneal administration of CX516 in SNI-treated animals decreases their mechanical allodynia in a dose-dependent manner. Mechanical allodynia was decreased at CX516 doses of 40 mg/kg and 20 mg/kg, but not at 10 mg/kg. *** $P < 0.001$, * $P < 0.05$, two-way ANOVA with *post hoc* Bonferroni multiple comparison tests. For 10 mg/kg, $n = 7$. For 20 mg/kg, $n = 10$. For 40 mg/kg, $n = 7$. (B) Intraperitoneal administration of CX516 in SNI-treated animals decreases their cold allodynia in a dose-dependent manner. Cold allodynia was decreased at CX516 doses of 40 mg/kg, but not at 20 mg/kg or 10 mg/kg. * $P < 0.05$, two-way ANOVA with *post hoc* Bonferroni multiple comparison tests. For 10 mg/kg, $n = 7$. For 20 mg/kg, $n = 10$. For 40 mg/kg, $n = 7$. Error bars represent SEM.