

# AMPAkines Have Novel Analgesic Properties in Rat Models of Persistent Neuropathic and Inflammatory Pain

Alexander M. Le, M.S., Michelle Lee, B.S., Chen Su, B.S., Anthony Zou, Jing Wang, M.D., Ph.D.

## ABSTRACT

**Background:** Novel analgesics that do not suppress the respiratory drive are urgently needed. Glutamate signaling through  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors plays important roles in central pain circuits. AMPAkinases 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:** The authors studied AMPAkinases (CX546 and CX516) in rat spared nerve injury (SNI) model of neuropathic pain and Complete Freund's Adjuvant (CFA) model of inflammatory pain. They measured the effect of AMPAkinases on mechanical and cold allodynia. They 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:** The authors found that CX546, compared with dimethyl sulfoxide (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,  $n = 9$ ). They 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,  $n = 10$ ). Finally, they found that CX516, compared with control, also reduced mechanical and cold allodynia in the SNI model (both DMSO and CX516 groups,  $n = 10$ ).

**Conclusions:** AMPAkinases alleviate pain hypersensitivity as well as depression-like behavior associated with long-lasting nerve injury and inflammatory insult. (**ANESTHESIOLOGY 2014; 121:1080-90**)

**D**ESPITE 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.<sup>1-3</sup> In recent years, there has been a sharp rise in opioid-related mortalities in chronic pain patients, mostly due to respiratory depression.<sup>1,4</sup> Even as many of these opioid-related morbidities and mortalities involve inappropriate prescription and over- or mis-use, treatment or prevention of opioid-induced respiratory depression remains lacking. Similarly, the development of new analgesics, particularly agents that do not suppress the respiratory drive, is urgently needed.<sup>2</sup>

Excitatory glutamate signaling in the central nervous system (CNS) plays an important role in the regulation of chronic pain.  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are the primary glutamate receptors in the CNS.<sup>5</sup> Increased transmission through AMPA receptors in the spinal dorsal horn has been shown to contribute to the induction and maintenance of chronic pain.<sup>6-10</sup> AMPA receptor signaling in the anterior cingulate cortex (ACC) and amygdala can also facilitate pain transmission.<sup>11-13</sup> On the other hand, transmission through AMPA receptors is critical for maintaining

### What We Already Know about This Topic

- AMPAkinases are compounds that enhance AMPA receptor transmission in the central nervous system. Importantly, they stimulate respiration in the setting of opiate-induced hypoventilation.
- AMPA receptors also play a significant role in nociceptive transmission. While AMPAkinases might find utility in the treatment of opioid-induced respiratory depression, their effects on nociception are not fully understood.
- In two experimental models of chronic pain, the effect of AMPAkinases on nociception was determined.

### What This Article Tells Us That Is New

- Surprisingly, AMPAkinases reduced mechanical and cold allodynia and attenuated symptoms of depression associated with chronic pain.
- Given the combination of analgesia and reduction of opioid-induced hypoventilation, AMPAkinases may find utility in the treatment of persistent postoperative and chronic pain.

the integrity of the periaqueductal gray-rostral ventromedial medulla (PAG-RVM) descending inhibitory pathway.<sup>14-16</sup> 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.<sup>17,18</sup> Thus, depending on the region in the CNS,

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AMPA receptor signaling may play either a pro-nociceptive or anti-nociceptive role.

AMPAkines are a class of compounds that enhance AMPA receptor transmission.<sup>19,20</sup> AMPAkines bind to an allosteric site on the AMPA receptor to reduce 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,<sup>21</sup> and have been studied in schizophrenia, depression, Huntington disease, and Alzheimer disease.<sup>19,22–25</sup> Interestingly, recent animal and human studies have shown that AMPAkines stimulate the respiratory drive in the context of hypoventilation.<sup>26–28</sup> 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 of acute pain.<sup>26,28</sup> However, the effect of AMPAkines on persistent or chronic pain has not been examined. Given the central role the AMPA receptors play in pain, we hypothesized that the systemic administration of AMPAkines can alter and affect the pain sensitivity.

Here we studied the effect of AMPAkines on chronic pain using the spared nerve injury (SNI) model for neuropathic pain and Complete Freund's Adjuvant (CFA) model for inflammatory pain.<sup>29–31</sup> We found that CX546, an established AMPAkin, which has been studied in hypoventilation, Rett syndrome, anxiety, and autism,<sup>27,32–35</sup> has significant analgesic properties. We verified these findings using a second AMPAkin, CX516.<sup>36,37</sup> Thus, our results suggest a novel analgesic role for AMPAkines in persistent pain states. This novel analgesic property, combined with the ability to stimulate the respiratory drive, makes AMPAkines 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–6:30 PM) light-dark cycle. Food and water were available *ad libitum*. Animals arrived to the animal facility at 250–300 g and were given approximately 7 days to adjust to the new environment before the onset of any experiments. For each experiment, the animals were randomized to either the control or experimental group.

### SNI Surgery

The SNI surgery has been previously described in detail.<sup>29</sup> Briefly, under isoflurane anesthesia (1.5–2%), the skin on the lateral surface of the right thigh of rat was incised and a section was 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 3–5 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 closely in distinct layers.

### CFA Administration

To produce inflammatory pain, CFA (*Mycobacterium tuberculosis*, Sigma–Aldrich [St. Louis, MO], 0.1 ml) 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 to a volume of 0.5–1 ml, while a similar volume of dimethyl sulfoxide (DMSO) was injected intraperitoneally to the control group. Injections were given 14 days after SNI surgery and 7 days after CFA injection. 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.<sup>38,39</sup> 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 2.55 g, 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 before and up to 14 days after SNI or sham surgery. Similar tests were done on CFA- or saline-treated rats. About 50% withdrawal threshold was calculated as described previously.<sup>39</sup> 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,<sup>40,41</sup> the following scoring system was applied. 0: no visible response or startle response lasting <0.5 s; 1: paw withdrawal lasting <5 s; 2: withdrawal lasting 5–10 s, ± licking of the paws; 3: prolonged repetitive withdrawal lasting > 10 s. Acetone was applied five times to each paw, and average score was calculated. Observers were blinded to the test conditions.

**Forced Swim Test (FST).** As described previously,<sup>42</sup> in the first session of the test, each animal was placed for 15 min 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. Twenty-four hours later, the animal was placed into the Porsolt chamber again under the same condition 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 more than 1 s. 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,<sup>43</sup> rats were acclimated in locomotor measurement chambers (Accuscan, Columbus, OH) before testing. On test days, locomotion activities were measured for a total of 30 min. The distance traveled was measured using the VersaMax System (Accuscan), which monitored the 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. The 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 was 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 *versus* DMSO (control) treatments at various time points. For the FST, unpaired two-tailed Student *t* test was 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 *versus* sham groups, CFA *versus* saline treatment groups, and in AMPAkinine *versus* 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

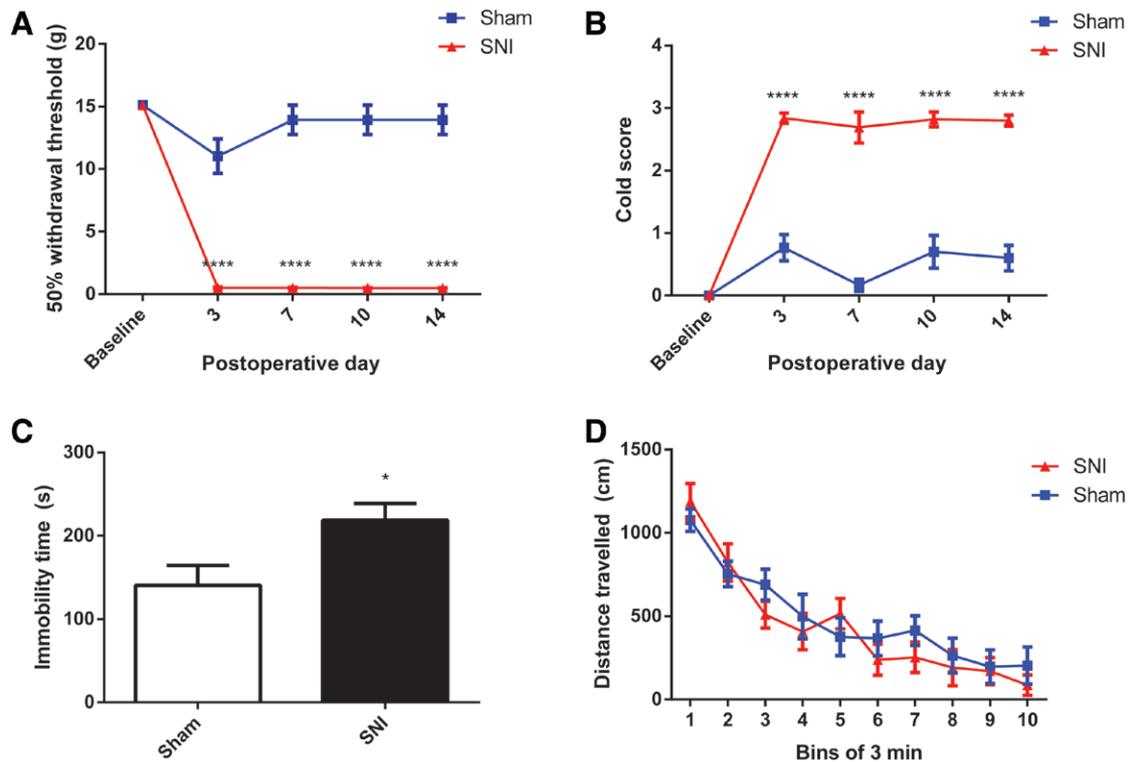
The SNI model is commonly used to study chronic neuropathic pain or persistent postoperative pain. Consistent with previous results,<sup>29,42,44</sup> we found that SNI surgery produced significant mechanical and cold allodynia in the (spared) sural nerve distribution (fig. 1, A and B). In contrast, 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 behavior in rats.<sup>42,44</sup> Here, we applied the FST, a widely used test to assess depression-like behavior in rats.<sup>45</sup> Numerous studies have demonstrated clinically relevant pharmacologic 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 for 30 min (fig. 1D), and thus these rats were unlikely to have overt locomotor deficits, which prevented them from swimming for 5 min. Increased immobility on FST, therefore, more likely reflected depression-like behavior exhibited by these animals in chronic pain.<sup>45</sup>

### 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.<sup>27,32–35</sup> When we applied this compound systemically (*via* intraperitoneal injection) to SNI-treated rats, we were surprised to observe a drastic improvement in mechanical allodynia compared with control (DMSO) injection (fig. 2A). We began to observe anti-allodynic effects at a dose of 5 mg/kg, while a higher dose of 10 mg/kg produced an even greater effect. We further validated this antinociceptive effect in the cold allodynia test, where we similarly observed improvements in cold allodynic scores at 5 and 10 mg/kg doses (fig. 2B). Next, we evaluated the duration of this anti-nociceptive effect (fig. 3, A and B). We administered a 10 mg/kg dose of CX546 (fig. 2, A and B), and we found that the effect of CX546 lasted 6 h in the mechanical allodynia test. Its effect was shorter (2 h) in the cold allodynia test.

Sensory allodynia tests assay nociception, but amplifying AMPA receptor transmission can affect a number of brain



**Fig. 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 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).

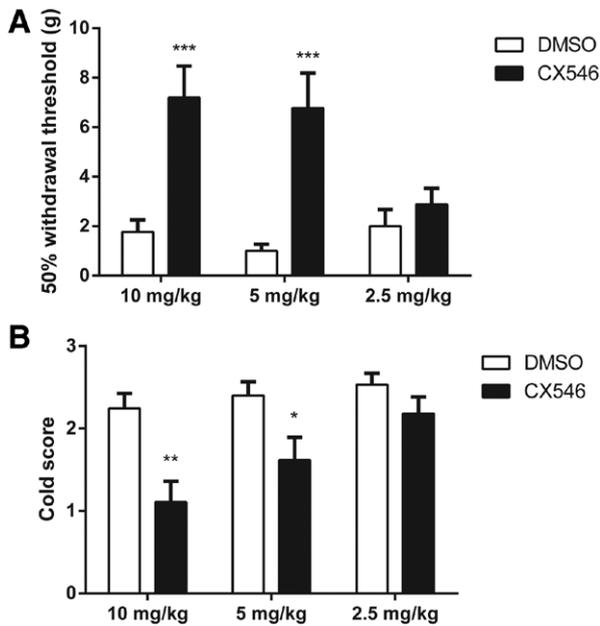
regions that regulate both nociceptive and affective components of pain. Thus, we next used the FST to test whether CX546, in addition to its anti-nociceptive effects, can also treat depression-like behavior associated with pain. Here we found that CX546 also improved immobility on the FST in SNI-treated 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 the 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.<sup>46</sup> 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 for at least 11 days

(fig. 5A). In addition, we found that CFA injection also led to a drastic 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 behavior.

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



**Fig. 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 dimethyl sulfoxide (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.

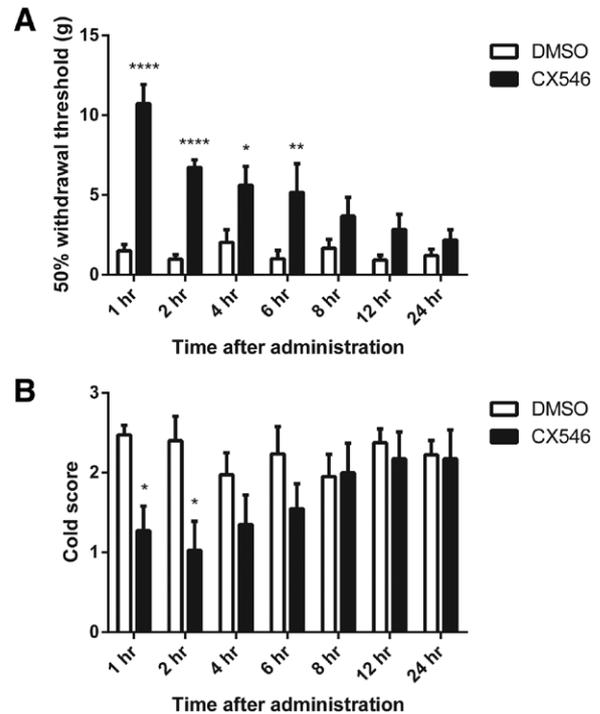
### CX516 Relieves Sensory Allodynia and Depression-like Behavior Associated with Chronic Pain

To further validate the analgesic effect of AMPAkinines in chronic pain, we applied CX516, another compound with well-known AMPA-potential properties.<sup>36,37</sup> Here, we found that CX516 also produced dose-dependent effects on sensory allodynia in SNI-treated rats (fig. 7, A and B). The dose required to produce maximal analgesic effects is higher in CX516 (20 mg/kg) than required for CX546 (<10 mg/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 behavior in rodent neuropathic and inflammatory pain models.

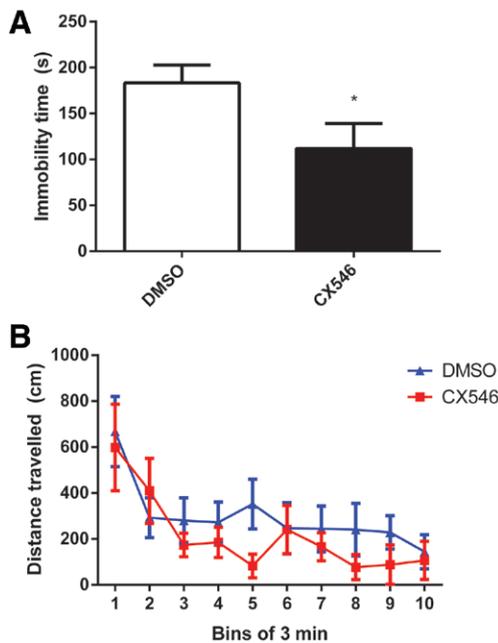
New analgesics that do not suppress the respiratory drive are urgently needed. AMPAkinines slow the kinetics of



**Fig. 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 h 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. Dimethyl sulfoxide (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 h. \* $P < 0.05$ , 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.

AMPA receptor deactivation to enhance the inward synaptic current.<sup>19,20</sup> By increasing AMPA receptor-mediated currents in neurons of the pre-Botzinger complex in the medulla, AMPAkinines directly stimulate the respiratory drive,<sup>26,27,47–49</sup> and they have been shown to treat or prevent hypoventilation caused by opioids and propofol.<sup>26,28,50,51</sup> The effect of AMPAkinines in chronic pain, however, has not been previously studied. The analgesic dose of CX546 found in our study is comparable with the dose tested in rats to treat respiratory depression.<sup>27</sup> Meanwhile, the time course of analgesia after a single administration (2–6 h) is slightly longer than the respiratory stimulatory effect of AMPAkinines.<sup>26,27</sup> 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.

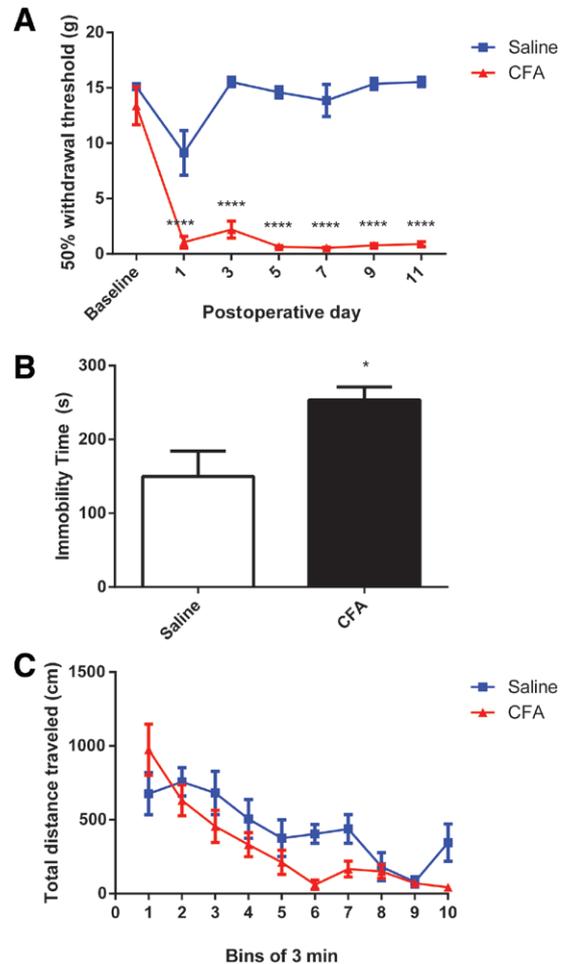
The anti-nociceptive effect of AMPAkinines can be explained by their action on AMPA receptors in two descending inhibitory circuits: PAG-RVM and NAc-RVM circuits.



**Fig. 4.** Intrapерitoneal 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 dimethyl sulfoxide (DMSO) control. \* $P < 0.05$ , unpaired two-tailed Student 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.  $n = 6$  for both DMSO and CX546 groups. Error bars represent SEM.

The PAG-RVM-spinal descending pathway is a well-known mechanism for pain regulation.<sup>52–54</sup> In this pathway, neurons from the PAG form glutamatergic projections through AMPA receptors on GABAergic cells in the RVM to inhibit dorsal horn neurons.<sup>55</sup> The administration of glutamate into the PAG is known to produce analgesia.<sup>56–58</sup> Furthermore, there is evidence that in the RVM, AMPA receptor up-regulation mediates analgesia in inflammatory pain states,<sup>59,60</sup> whereas their down-regulation in neuropathic pain causes hyperalgesia.<sup>61</sup> Thus, transmission through AMPA receptors is required for the intact PAG-RVM-descending pathway.<sup>16,62</sup> 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.<sup>18</sup> Intra-NAc administration of AMPA receptor antagonists, however, can disrupt this pain-induced analgesic mechanism.<sup>17</sup>

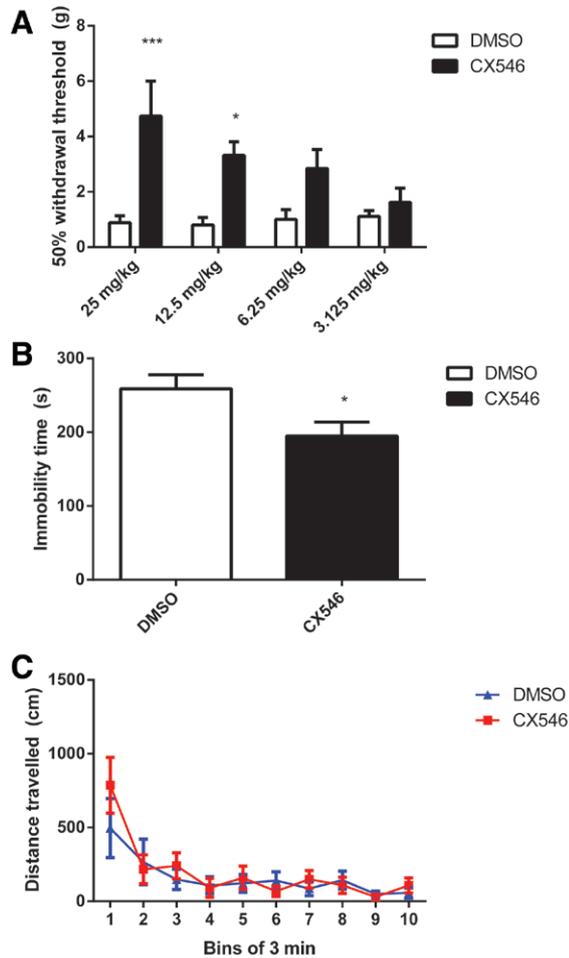
At the same time, however, potentiation of AMPA receptors in neurons of the spinal dorsal horn, ACC and amygdala can have pro-nociceptive effects. In the dorsal horn, chronic inflammatory pain increases membrane targeting of GluA1 AMPA receptor subunits, but decreases GluA2 delivery,<sup>6,9,63</sup> leading to the formation of GluA2-lacking receptors to augment pain transmission.<sup>8</sup> Similarly, AMPA



**Fig. 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 postoperative 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 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.

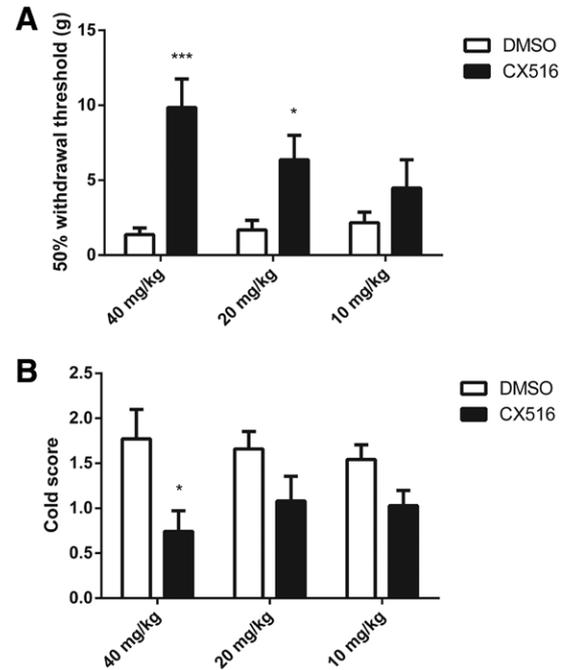
receptor signaling in the ACC and amygdala has also been suggested to mediate increased synaptic plasticity and confer hyperalgesia.<sup>11–13,64,65</sup>

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 AMPA agonists systemically, and we expected them to act peripherally or in the brain, with a smaller component of activity in the spinal cord. Thus, the behavioral phenotype we observed likely represents AMPA effects



**Fig. 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 test.  $n = 10$  for both dimethyl sulfoxide (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.

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.<sup>66</sup> AMPAkinines are known to bind to neurons in the NAc and brain stem with high affinity,<sup>66</sup> thereby facilitating the enhancement of AMPA receptor signaling in these descending inhibitory



**Fig. 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. DMSO = dimethyl sulfoxide.

circuits. Future studies targeting specific regions in the brain and spinal cord are needed to further elucidate the precise roles of these drugs in pain states.

Symptoms of depression occur in many chronic pain patients,<sup>67,68</sup> and our finding that AMPAkinines can treat pain-induced depression is consistent with the role of central AMPA receptors in depression.<sup>69</sup> For example, GluA1 subunits of AMPA receptors are reduced in the amygdala, prefrontal cortex, and hippocampus in rodent models of depression.<sup>70–72</sup> Ketamine has been shown to increase GluA1 concentration in the prefrontal cortex to provide fast-acting relief from depression,<sup>73</sup> and it is effective in treating pain-induced depression.<sup>42</sup> 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.<sup>74</sup> 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 AMPAkinases failed to demonstrate pro- or anti-nociceptive effects.<sup>26,28</sup> 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.<sup>26</sup> In another study, pain was tested by electric shock and thermal stimuli in healthy volunteers.<sup>28</sup> 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,<sup>46,75</sup> and a general feature of synaptic plasticity is the amplification of excitatory postsynaptic currents conducted through AMPA receptors.<sup>76</sup> Thus, it is not surprising that while potentiating these receptors in acute pain has an insignificant effect,<sup>26,28</sup> 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 previous findings.<sup>44</sup> If rats do not demonstrate locomotor deficits above 30 min, it is unlikely that they will have deficits while swimming for 5 min 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.<sup>21</sup>

The timing of development of affective pain symptoms in rodents is not completely known. In our study, immobility on the FST manifested 2 weeks after SNI, compatible with two previous reports.<sup>42,44</sup> In another study using this model, however, deficits on the FST were detected at 7 weeks, but data from previous time points were not shown.<sup>77</sup> In the spinal nerve ligation model, Kontinen *et al.*<sup>78</sup> reported no changes in immobility 14 days after surgery, whereas Suzuki *et al.*<sup>79</sup> found increased immobility 15 days after surgery. In another study, rats developed immobility 7 days after chronic constriction injury.<sup>80</sup> Sham surgery can induce transient reversible depressive pain symptoms, likely caused by acute pain from skin or muscle incision.<sup>42</sup> Thus, depending on the rate of recovery from sham surgeries, the detectable differences on the FST between neuropathy and control groups at previous 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 1 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.<sup>81</sup> A study on monoarthritic pain, meanwhile, found deficits at 4 weeks after CFA injection into the tibiotarsal joint.<sup>82</sup> 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's recommendation, as has been done in previous studies.<sup>27</sup> In our study, there were small differences in the average allodynia measures for the SNI group without any treatment (fig. 1, A and B) and the group that received DMSO (fig. 2, A and B). These differences were small, however, compared with the changes in allodynia as the result of AMPAkinase treatment (fig. 2, A and B). We did not observe any anti-allodynic effect for DMSO in the CFA model (fig. 5A and, 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 owing to increased AMPA receptor activities.<sup>19,20</sup> AMPAkinases are classified into two classes: low- and high-impact AMPAkinases. Low-impact AMPAkinases (*e.g.* CX717 and CX1739) are less likely to cause hyperexcitability 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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## Competing Interests

Dr. Wang has filed a patent for the use of AMPA receptor potentiation in the treatment of pain and pain-induced depression. The other authors declare no competing financial interests.

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exandria Life Science Building, 450 East 29th Street, Room 823, New York, New York 10016. jing.wang2@nyumc.org. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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## ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

### From \$1 a Pound to \$1 a Grain—Coca Leaf to Cocaine in 1885



In June of 1885, New York's *American Agriculturist* magazine published that "The discovery that Cocaine will produce local anaesthesia, or insensibility to pain, is next in importance to the discovery of the properties of ether." The article cites the genus of the Coca shrub (left) as *Erythroxylon* [sic] which means "red-wood." In 1885, a pound (454 g) of dried coca leaves sold for \$1. However, at 1/7000 of that weight, a grain (65 mg) of cocaine isolated from the coca leaf (right) also sold in 1885 for that same \$1, which is more than \$25 in today's U.S. dollars. The *American Agriculturist* notes that in "view of the probable increased demand for Coca, ... our Department of Agriculture [should] consider the possibility of successfully cultivating the shrub within our territory." (Copyright © the American Society of Anesthesiologists, Inc.)

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