[Original Research]

Effects of Anticonvulsants on Neuropathic Pain-like State and Pain-induced Anxiety in Mice

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Abstract: We investigated whether anticonvulsants could suppress mechanical allodynia and anxiety-like behavior during sciatic nerve cuffing in mice. A neuropathic pain model was induced by the unilateral insertion of a polyethylene cuff around the main branch of a sciatic nerve, which induced an ipsilateral mechanical allodynia for at least 4 weeks. Anxiety-like behaviors then became evident, and the anxiolytic effects of several anticonvulsants were examined in naive mice. When we administered carbamazepine, gabapentin, valproate, or ethosuximide, each agent suppressed both cuff-induced allodynia and anxiety-like behaviors. Although diazepam had no effect on mechanical sensitivity, it improved cuff-induced anxiety-like behaviors. In naive mice, carbamazepine, gabapentin, valproate, and diazepam each exhibited anxiolytic effects, and although ethosuximide improved cuff-induced anxiety-like behaviors, it did not produce anxiolytic effects. We conclude that anticonvulsants with an inhibitory effect on excitatory transmitters are effective for treating not only neuropathic pain but also the anxiety associated with chronic pain.

Key words: cuff model, anticonvulsants, anxiety, neuropathic pain

Neuropathic pain is defined as chronic pain resulting from damage or abnormal function of the central or peripheral nervous system.[1] Patients with neuropathic pain frequently report sensory abnormalities, including burning, hyperalgesia, allodynia, and dysesthesia.[2] Clinicians typically treat these conditions using agents that were initially developed for other disorders of the nervous system, including anticonvulsants or antidepressants.[3] Indeed, these are now recommended as the first-line treatment options for neuropathic pain.[4] Anticonvulsants, in particular, have pharmacological effects on various putative pathophysiologic mechanisms underlying the development of neuropathic pain. The mechanisms of action include the modulation of sodium and calcium channels, the activation of gamma amino butyric acid (GABA) receptors, and the suppression of neuronal hyper-excitability.[5] Gabapentin and pregabalin, calcium channel alpha-2-delta subunit ligands, are recommended as first-line treatment for neuropathic pain, while the sodium channel blocker carbamazepine has been used for the treatment of both trigeminal neuralgia and neuropathic pain.[4]

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Neuropathic pain can alter a patient's quality of life by interfering with emotional well-being. Sleep disturbance, anxiety, and depression are frequent and severe in patients with neuropathic pain, and quality of life is impaired to a greater degree when patients have chronic neuropathic pain than when they have chronic non-neuropathic pain.[6] Several animal studies have demonstrated that neuropathic pain models in mice (e.g., cuff-implantation model) develop an anxiety-like phenotype, as shown by reduced time being spent in the open arms of the elevated plus maze (EPM) test.[7–9] Anxiety disorder comprises a diverse category of pathologies in which excessive and debilitating anxiety presents as the primary symptom; however, anxiety is also a prominent symptom of other psychiatric disorders and often occurs in medical and neurological conditions. There have been preliminary reports suggesting the anxiolytic efficacy of anticonvulsant drugs, including carbamazepine, valproate, lamotrigine, and gabapentin, with further research being advocated.[10] Furthermore, it is unknown whether the optimal analgesic dose of anticonvulsants could show an anxiolytic effect on anxiety-related behavior induced by chronic pain.

The aim of the present study was to investigate whether certain anticonvulsants (carbamazepine, diazepam, gabapentin, valproate, and ethosuximide) could suppress both mechanical allodynia and anxiety-like

behavior during sciatic nerve cuffing in mice. We also examined the anxiolytic effect of these anticonvulsants in naive mice.

METHODS

1. Animals

Eight-week-old male C57BL/6 J mice were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in cages in groups of five or six and given free access to food and water. The room temperature was controlled (23℃ ± 1℃), and a 12-h light-dark cycle (light on 8 : 00–20 : 00) was maintained. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and studies were conducted according to the guidelines of the National Institute of Health and the Japan Neuroscience Society. All behavioral experiments were carried out in an isolated behavioral room, between 10 am and 6 pm.

2. Surgical procedures

All surgeries were done under aseptic conditions and anesthesia was with medetomidine (0.03 mg/mL) midazolam (0.4 mg/mL) and butorphanol 0.5 mg/mL given intraperitoneally at a dose of 10 mL/kg. The common branch of the right sciatic nerve was exposed and a split section of polyethylene tubing (length = 2 mm, internal diameter = 0.38 mm, external diameter = 1.09 mm; PE-20, Imamura Co., Ltd., Tokyo, Japan) was placed around the nerve (cuff group), as described previously.[7] Sham-operated mice underwent the same procedure, but without cuff implantation (sham group).

3. Measurement of tactile threshold

The mechanical threshold for ipsilateral hind paw withdrawal was determined using von Frey filaments (Aesthesio®; DanMic Global, California, USA). A series of von Frey filaments (0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0 g) was applied. The 50% withdrawal threshold was calculated using the up–down method[11] starting with the 0.6 g filament. If a positive response was observed, the next lower force filament was applied and so on until a change in response was observed. Four subsequent filaments were then assessed, according to the up–down sequence, and the 50% paw withdrawal value was calculated using a previously described method.[12]

The mechanical thresholds in sham and cuff groups were carefully measured in a non-blind manner.

4. Nociceptive test

A von Frey filament was applied to the middle of the plantar surface of the ipsilateral hind paw with a weight of 0.16 g, and a nociceptive test was performed as previously described.[13,14] Briefly, mice were placed individually in a plastic cage with a wire mesh bottom. When mice had adapted to the testing environment for 60 min, the von Frey filaments were pressed perpendicularly against the mid-plantar surface of the hind paw from below the mesh floor and held for 3–5 s such that the filament buckled slightly. Lifting of the paw was recorded as a positive response. Stimulation of the same intensity was applied ten times to the plantar surface of the ipsilateral hind paw per mouse at 5 s intervals.

The experimenters were blind to drug treatment condition during the nociceptive testing.

5. Elevated plus maze (EPM) test

The EPM test was performed as previously described.[15] The plus maze consisted of four arms: two open arms (6 × 29.5 cm^2) and two closed arms (6 × 29.5 cm^2) enclosed by walls that were 55 cm in height. Each arm had a delimited central area of 6 × 6 cm^2 The entire maze was elevated to a height of 55 cm above the floor and illuminated by a dim light (12 or 35 lux) at the end of each open arm. The mice were brought to the corner of the experimental room at least 60 min before the experiment started and were placed in the center of the maze facing one of the open arms at the start of the testing phase. Entry into an arm was defined as the animal placing two front paws over the line marking that area. The numbers of open-arm entries, closed-arm entries, and the time spent in the open arms were recorded during the 5 min testing period. The percentage of time spent in the open arm of the maze [(open arm time/total test time) ×100] and the total number of entries (open arm entries + closed-arm entries) were calculated for each animal.

The anxiolytic effects of drugs were examined based on the timing of their analgesic potency. The pretreatment times and doses of drugs used were 30 min for ethosuximide (100, 300 mg/kg, s.c.) and valproate (100, 300 mg/kg, i.p.); 60 min for carbamazepine (10, 30 mg/kg, s.c.), diazepam (1, 2 mg/kg, i.p.), and gabapentin (3, 10 mg/kg, i.p.).

6. Drugs

All drugs used in the present study were purchased from the following manufacturers: carbamazepine, diazepam, gabapentin, and sodium valproate from Wako Pure Chemical Industries (Osaka, Japan), and ethosuximide from Tokyo Chemical Industry (Tokyo, Japan). Carbamazepine and diazepam were dissolved in water containing 5% dimethyl sulfoxide (Wako Pure Chemical Industries), 5% Tween 80 (Tokyo Chemical Industry), and 90% saline. Other drugs were diluted in saline. All drugs were administered at the volume of 10 mL/kg.

7. Data analysis

Data are expressed as means ± standard errors of the mean and were evaluated by one- or two-way analysis of variance followed by the Bonferroni multiple comparisons test. Statistical analysis for two-group comparisons was performed using unpaired *t*-tests with Welch's correction. All statistical analyses were performed with Prism version 5.0 (GraphPad Software, Inc., CA, USA).

RESULTS

1. Sciatic nerve cuffing and mechanical response

The mechanical sensitivity of the hind paws was evaluated by using von Frey filaments. Unilateral cuff implantation induced an ipsilateral mechanical allodynia that was not seen in the sham group (Fig. 1a). This mechanical allodynia persisted for at least 4 weeks.

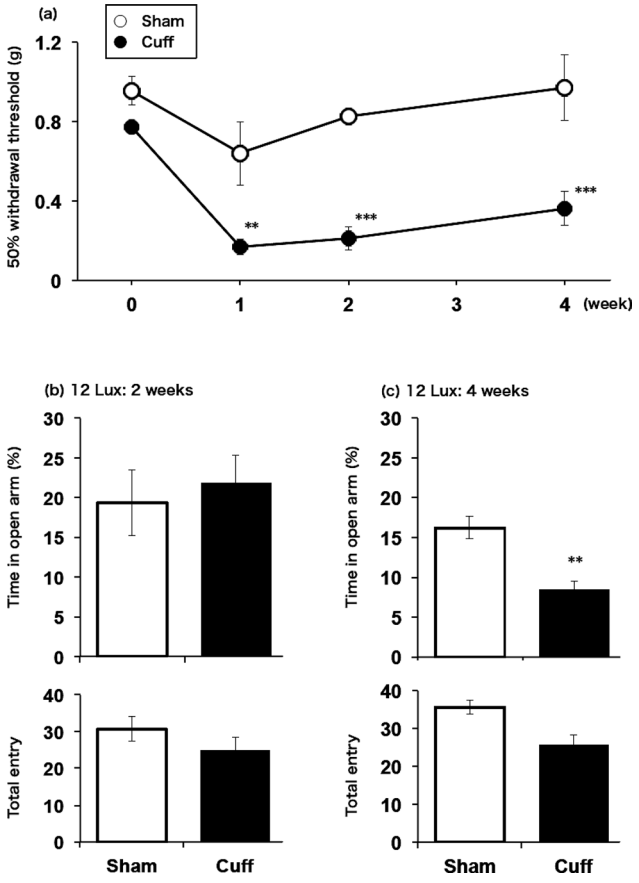

Fig. 1 Changes in the withdrawal threshold after mechanical stimulation to the ipsilateral hind paw induced by sciatic nerve cuffing in mice (a); Each point represents the mean ± *SEM* of the 50% withdrawal threshold (n = 6); $^{**}p < 0.01$, $^{***}p < 0.005$ versus Sham group; Mice evaluated 2 weeks (b) or 4 weeks (c) after surgery in the EPM test; Each column represents the mean ± *SEM* of the percentage of time spent in the open arms (upper panel) or number of total entries (bottom panel) for 5 min (n = 6); $^{**}p < 0.01$ versus Sham group. Abbreviations: EPM, Elevated plus maze; *SEM*, Standard error of the mean.

2. Anxiety-like behavior during sciatic nerve cuffing

Two weeks after surgery for sciatic nerve cuffing, the mice did not spend different amounts of time in the open arms of the EPM test compared with the sham-operated mice (Fig. 1b). By contrast, at 4 weeks after surgery, mice in the cuff group spent less time in the open arms of the EPM test compared with mice in the sham group (Fig. 1c).

3. Antinociceptive effects of anticonvulsants in the neuropathic pain-like state

The acute injection of a vehicle did not affect mechanical sensitivity in the cuff group at 4 weeks after surgery (Fig. 2a–e). However, acute carbamazepine treatment at either 10 mg/kg or 30 mg/kg suppressed the cuff-induced allodynia compared with the vehicle (Fig. 2a). A similar result was obtained after acute gabapentin at both doses (Fig. 2b). Interestingly, although valproate at a dose of 100 mg/kg had no effect on mechanical sensitivity, treatment at 300 mg/kg did suppress the cuff-induced allodynia compared with vehicle (Fig. 2c). Similarly, ethosuximide treatment was only effective at suppressing the cuff-induced allodynia at 300 mg/kg (Fig. 2d). Acute treatment with diazepam had no effect on mechanical sensitivity at either of the tested doses (Fig. 2e).

4. Anxiolytic effect of anticonvulsants in the neuropathic pain-like state

Carbamazepine (30 mg/kg; Fig. 3a), gabapentin (10 mg/kg; Fig. 3b), valproate (300 mg/kg; Fig. 3c), and ethosuximide (300 mg/kg; Fig. 3d) reversed the anxiety-like behavior induced by cuffing, as shown by significant increases in the percentage of time spent in the open arms of the EPM test. Diazepam (2 mg/kg) did not affect mechanical sensitivity, but it did increase the time spent in the open arms of the EPM test (Fig. 3e).

5. Anxiolytic effects of the included anticonvulsants on naive mice

Carbamazepine (10, 30 mg/kg; Fig. 4a), gabapentin (3, 10 mg/kg; Fig. 4b), valproate (100, 300 mg/kg; Fig. 3c), and diazepam (1, 2 mg/kg; Fig. 3e) each dose-dependently and significantly increased the time spent in the open arms of the EPM test compared with vehicle. Although ethosuximide (300 mg/kg) treatment increased the time spent in the open arms in the cuff group, treatment did not result in mice spending different times in the open arms compared with control mice receiving the vehicle (Fig. 3d).

DISCUSSION

Anticonvulsants were introduced for the treatment of neuropathic pain based on their ability to reduce the synchronous firing of neurons that is characteristic of neuropathic conditions.[16] However, both antidepressants and anticonvulsants are broadly recommended for first-line treatment of neuropathic pain.[4] A previous report has indicated that sciatic nerve cuffing in mice offers a simple neuropathic pain model that is sensitive to chronic, but not acute, treatment with the antidepressants.[17] In the present study, we added to this research base by showing that giving certain anticonvulsants could suppress cuff-induced allodynia acutely. These results suggest that anticonvulsants could offer rapid analgesic effects.

In the present study, neuropathic pain was shown to induce anxiety-related behaviors in a time-dependent manner, with cuff-implanted mice only developing anxiety-related behaviors by 4 weeks after surgery. This is consistent with a report showing that neuropathic model mice developed anxiety-like behaviors after 4 weeks and depression-like behaviors after 6–8 weeks.[8] In this study, we did not evaluate either the development of depression-like behaviors or the impact of therapy on those behaviors, which must be considered a limitation. Some functional changes in the emotionality-related brain regions occur in association with chronic pain. Under the control of the anterior cingulate cortex, the

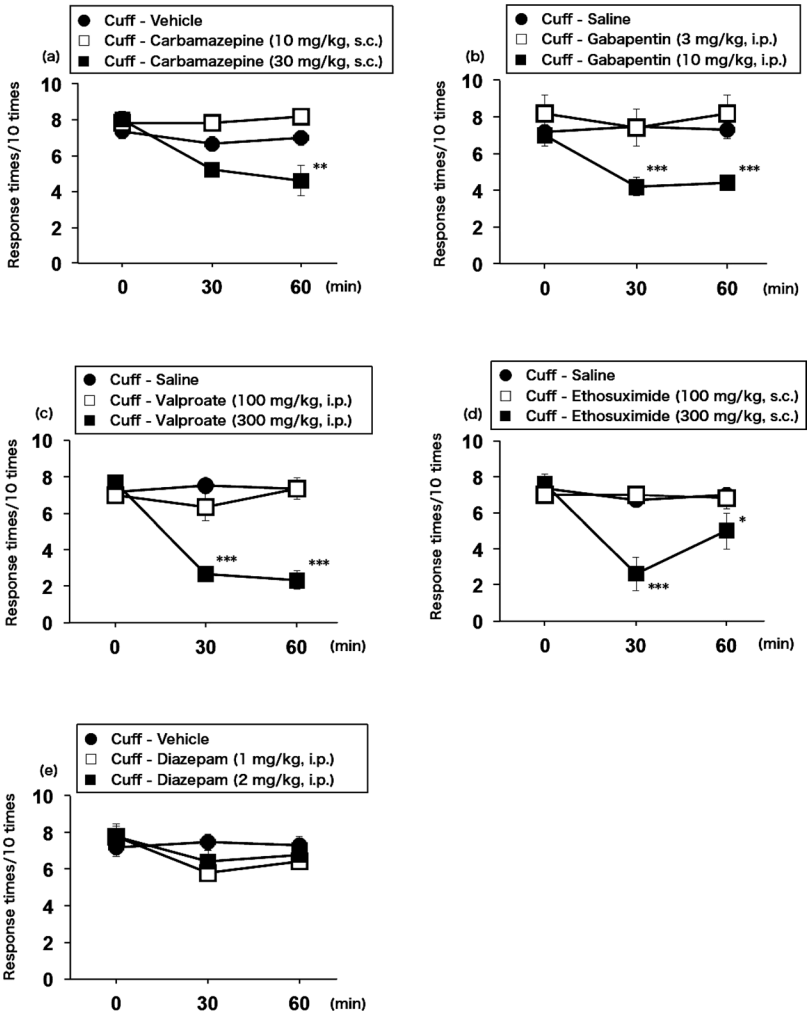

Fig. 2 Effects of carbamazepine (a), gabapentin (b), valproate (c), ethosuximide (d), and diazepam (e) on mechanical allodynia of the ipsilateral hind paw 4 weeks after surgery; Tests were performed 30 and 60 min after drug administration. A tactile stimulus was applied using filaments with a bending force of 0.16 g. Each point represents the mean ± *SEM* of the paw withdrawal scores; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus saline or vehicle treatment; (a) cuff–vehicle: $n = 6$, cuff–carbamazepine: $n = 5$; (b) cuff–saline: $n = 6$, cuff–gabapentin: $n = 5$; (c) cuff–saline: $n = 6$, cuff–valproate: $n = 5$; (d) cuff–saline: $n = 6$, cuff–ethosuximide: $n = 5$; (e) cuff–vehicle: $n = 6$, cuff–diazepam: $n = 5$. Abbreviations: *SEM*, Standard error of the mean.

medial prefrontal cortex participates in signaling the unpleasantness of pain as its affective component. *In vivo* microdialysis in awake rats has been used to indicate that extracellular levels of glutamate were increased in the contralateral medial prefrontal cortexes of neuropathic pain model rats, with no measurable change in GABA levels.[18] These data suggest a neuropathic pain-induced imbalance between excitatory and inhibitory neurotransmissions, causing increased excitability of the medial prefrontal cortex. Interestingly, the extracellular glutamate levels of the prelimbic medial prefrontal cortex in mice were found to be significantly increased after local perfusion of the sodium channel activator veratrine. Perfusing veratrine in this region induced anxiety-like behaviors simultaneously.[19] Therefore, it is likely that the anxiety-like behavior induced by cuffing was due to activation of the glutamate neuron in the medial prefrontal cortex.

The selective benzodiazepine receptor agonist, etizolam (1 mg/kg, s.c.), has shown a significant anxiolytic effect in naive mice, being able to cause a complete suppression of anxiety in neuropathic pain-like states. However, it has been reported that it failed to improve the increased pain-sensitivity observed after nerve injury.[20] We also found that treatment with diazepam (2 mg/kg) had no effect on mechanical sensitivity, but that treatment did increase the time mice spent in the open arms of the EPM test. These results indicate that chronic pain is not effectively suppressed by treatment with anxiolytics.

Sodium or calcium channel blockade may not only provide anticonvulsant actions and chronic pain treatment but also mood-stabilizing actions. Carbamazepine and valproate, the sodium channel blockers used to treat

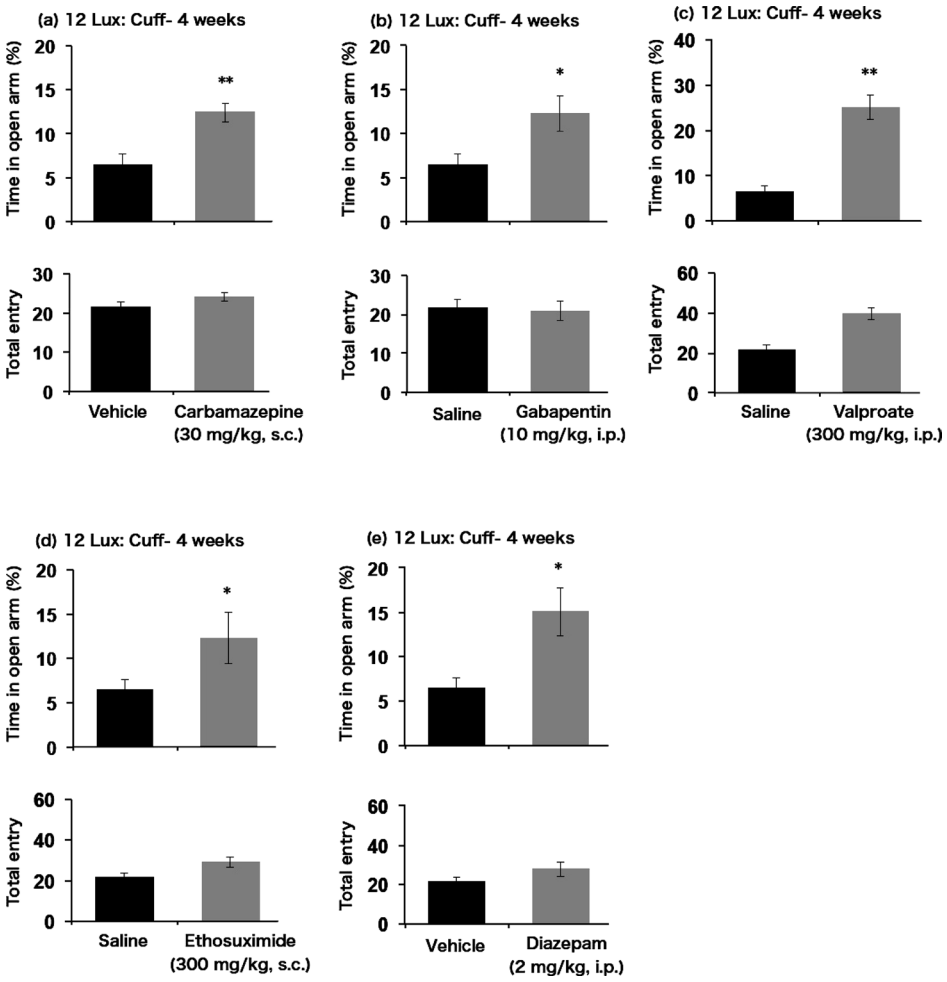

Fig. 3 Effects of carbamazepine (a), gabapentin (b), valproate (c), ethosuximide (d), and diazepam (e) on the EPM test in the cuff group. Each column represents the mean ± *SEM* of the percentage of time spent in the open arms (upper panel) or the total number of entries (bottom panel) for 5 min. $^{*}p < 0.05$, $^{**}p < 0.01$, versus saline or vehicle treatment; (a) cuff–vehicle: $n = 6$, cuff–carbamazepine: $n = 5$; (b) cuff–saline: $n = 6$, cuff–gabapentin: $n = 5$; (c) cuff–saline: $n = 6$, cuff–valproate: $n = 5$; (d) cuff–saline: $n = 6$, cuff–ethosuximide: $n = 5$; (e) cuff–vehicle: $n = 6$, cuff–diazepam: $n = 5$. Abbreviations: EPM, Elevated plus maze; *SEM*, Standard error of the mean.

neuropathic pain in humans,[4] also have efficacy in bipolar disorder.[21] Similarly, gabapentin has analgesic[22] and anxiolytic activities.[23] The most important action of gabapentin appears to be its binding to the alpha-2-delta subunit of voltage dependent calcium channels, where it inhibits the release of excitatory neurotransmitters and reduces glutamate availability at *N*-methyl-D-aspartate and other receptors.[22] In the present study, carbamazepine, valproate, and gabapentin each suppressed both cuff-induced allodynia and anxiety-like behaviors. These findings suggest that these anticonvulsants, which inhibit excitatory transmitters, are effective for treating not only neuropathic pain but also the anxiety associated with chronic pain.

Interestingly, although ethosuximide treatment increased the time spent by mice from the cuff group in the open arms, there was no difference compared with the control mice that receive treatment with the vehicle only. Although the mechanisms underlying this phenomenon are unclear, changes in neurotransmitters in the central nervous system were probably required. T-type calcium channels are broadly involved in many physiological processes, including neuronal firing, epilepsy, and pain.[24] The present findings suggest that T-type calcium channels, at least in part, contribute to pain-related anxiety-like behavior.

In conclusion, we demonstrated that anticonvulsants with inhibitory effects on excitatory transmitters are effective for treating not only neuropathic pain but also the anxiety associated with chronic pain. However, although most agents suppressed anxiety, diazepam did not suppress the cuff-induced allodynia, indicating that chronic pain is not always suppressed by treatment with anxiolytics. Ethosuximide was also shown to exhibit anxiolytic activity under the pain-induced anxiety. Although further investigation is necessary, our results suggest that T-type calcium channels might be important drug targets for the treatment of neuropathic pain and

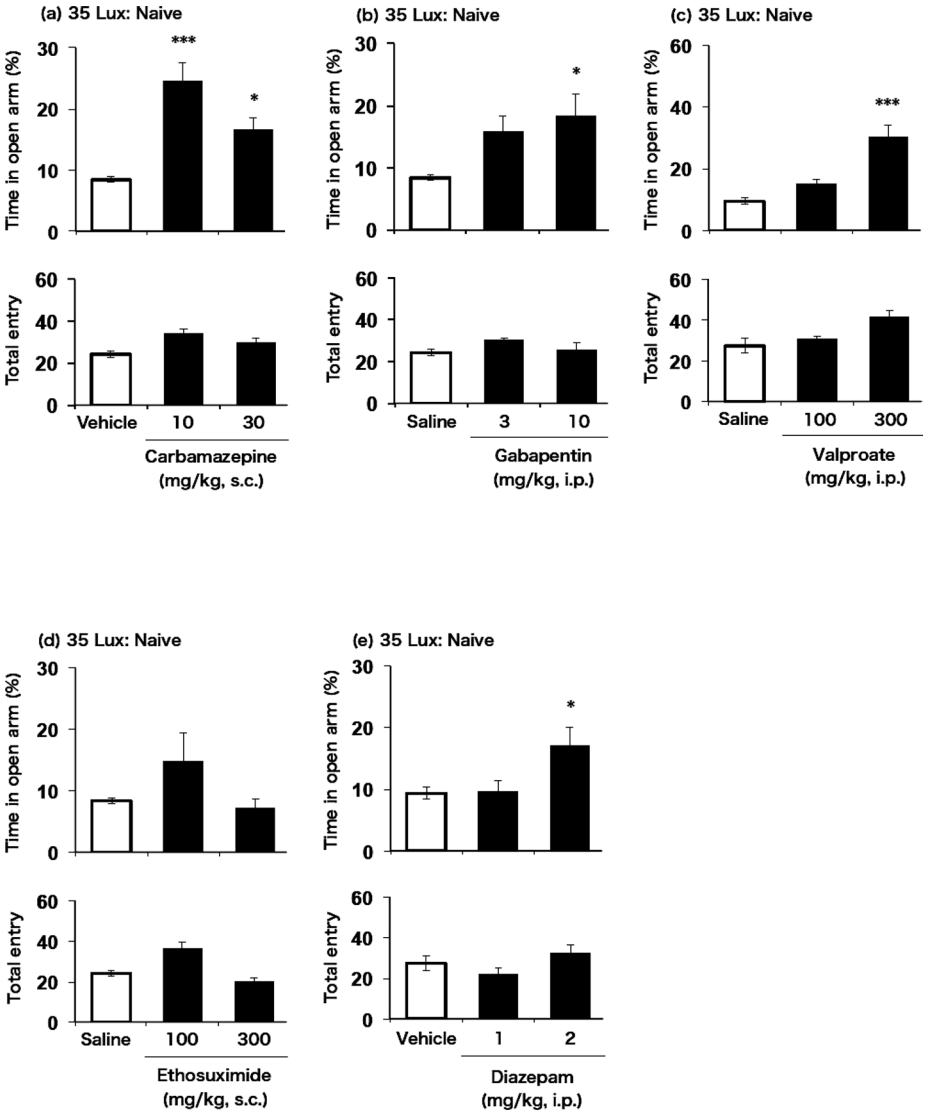

Fig. 4 Effects of carbamazepine (a), gabapentin (b), valproate (c), ethosuximide (d), and diazepam (e) on the EPM test in naive mice; Each column represents the mean ± *SEM* of the percentage of time spent in the open arms (upper panel) or the total number of entries (bottom panel) for 5 min ($n = 5$); $^{*}p < 0.05$, $^{***}p < 0.005$ versus saline or vehicle treatment. Abbreviations: EPM, Elevated plus maze; *SEM*, Standard error of the mean.

chronic pain-induced anxiety.

Conflict of interest: The authors declare that they have no conflicts of interest.

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