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Attenuated pain responses in mice lacking $Ca_V3.2$ T-type channels

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Although T-type Ca^{2+} channels are implicated in nociception, the function of specific subtypes has not been well defined. Here, we compared pain susceptibility in mice lacking $Ca_V3.2$ subtype of T-type Ca^{2+} channels ($Ca_V3.2^{-/-}$) with wild-type littermates in various behavioral models of pain to explore the roles of $Ca_V3.2$ in the processing of noxious stimuli *in vivo*. In acute mechanical, thermal and chemical pain tests, $Ca_V3.2^{-/-}$ mice showed decreased pain responses compared to wild-type mice. $Ca_V3.2^{-/-}$ mice also displayed attenuated pain responses to tonic noxious stimuli such as intraperitoneal injections of irritant agents and intradermal injections of formalin. In spinal nerve ligation-induced neuropathic pain, however, behavioral responses of $Ca_V3.2^{-/-}$ mice were not different from those of wild-type mice. The present study reveals that the $Ca_V3.2$ subtype of T-type Ca^{2+} channels are important in the peripheral processing of noxious signals, regardless of modality, duration or affected tissue type.

Keywords: Chemical pain, mechanical pain, neuropathic pain, spinal nerve ligation (SNL), thermal pain, tonic inflammatory pain

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Pain, such as acute somatic, tonic inflammatory and visceral pain, is often found to be insensitive to conventional remedies. Therefore, a better understanding of the molecular mechanisms of pain is needed to assist in the discovery of novel therapeutics. Changes in the expression and the excitability of Ca^{2+} channels has been suggested to play a pivotal role in the generation of pain (Bourinet & Zamponi 2005; Heinke *et al.* 2004; Newton *et al.* 2001). In particular, low voltage-activated T-type Ca^{2+} channels, originally identified in peripheral sensory neurons of the dorsal root ganglion (DRG), have been thought to modulate pain processing because they play a crucial role in controlling neuronal excitability of the DRG (Carbone & Lux 1984; White *et al.* 1989). Based on studies of the effects of mibefradil, a preferential T-type Ca^{2+} channel blocker, and redox modulation of T-channels, Todorovic *et al.* suggested a peripheral pronociceptive role for T-type Ca^{2+} channels in pain transmission (Todorovic *et al.* 2001, 2002, 2004). In addition, blocking of spinal T-type Ca^{2+} channels with ethosuximide depressed neuronal responses in the spinal dorsal horn (Matthews & Dickenson 2001). T-type Ca^{2+} channels have also been shown to be involved in the development of long-term potentiation (LTP) at synapses between nociceptive afferents and dorsal horn neurons of the spinal cord (Heinke *et al.* 2004; Ikeda *et al.* 2003). Although T-type Ca^{2+} channels are known to be important in pain perception, the understanding of their function is still at an early stage.

Three subtypes of T-type channel have been cloned, namely, $Ca_V3.1$ (α_{1G}), $Ca_V3.2$ (α_{1H}) and $Ca_V3.3$ (α_{1I}) (Cribbs *et al.* 1998, 2000; Gomora *et al.* 2002; Lee *et al.* 1999; Zhuang *et al.* 2000). Gene expression analysis demonstrated that the $Ca_V3.2$ subtype is predominantly found at sites essential for pain transmission, such as medium- and small-sized sensory neurons of the DRG and the superficial laminae of the dorsal horn (Talley *et al.* 1999). This expression pattern for $Ca_V3.2$ indicates that this subtype may be important for peripheral pain perception. Although Bourinet *et al.* recently showed reduced mechanical nociception in healthy or mononeuropathic rats following antisense-mediated knockdown of $Ca_V3.2$ (Bourinet *et al.* 2005), further exploration of the role of this channel in various pain modalities is still needed to fully validating it as a target for analgesic drug development. In the present study, we therefore investigated the roles of the $Ca_V3.2$ subtype in the perception of various noxious signals as well as mechanical noxious stimuli. Our results revealed that the $Ca_V3.2$ subtype of T-type Ca^{2+} channels

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has a pronociceptive role not only in mechanical nociception but also in thermal, chemical and tonic inflammatory pain but that it may not be critical for the development of neuropathic pain.

Materials and methods

Animals

Mice lacking the $Ca_V3.2$ subtype of T-type Ca^{2+} channels were generated by gene targeting (Chen *et al.* 2003), and $Ca_V3.2$ null mutations were maintained in 129/sv or C57BL/6J mice, which have different genetic backgrounds. Heterozygotic mice obtained from chimeras were backcrossed into each genetic background more than six times ($\geq$N6). To evaluate the gene knockout effect on general behaviors and nociceptive responses, hybrid F_1 $Ca_V3.2^{-/-}$ and wild-type littermate control mice were generated by mating heterozygotes from the two genetic backgrounds (129/sv and C57BL/6J). Mice were given free access to food and water and maintained under a 12-h light/12-h dark cycle, with the light cycle beginning at 0600 h. The genotype was confirmed by polymerase chain reaction analysis of tail biopsies. Animal care and all behavior tests including pain tests were conducted under the ethical guidelines of the Institutional Animal Care and Use Committee of the Korea Institute of Science and Technology and the International Association for the Study of Pain. In all pain tests, different groups of $Ca_V3.2^{-/-}$ mice and their wild-type littermates were used except in the spinal nerve ligation (SNL)-induced neuropathic pain test.

Behavioral assays of various pains

Tail clip test

A tail clip test was used to assess sensitivity to noxious mechanical stimuli. An alligator clip (exerting a force of 250*g*; Fine Science Tools Inc., North Vancouver, Canada) was applied to the tail 1 cm from the tip. The latency to licking/biting of the clip or trembling of the clipped tail within 1 cm from the nose was measured.

Tail flick test

To assess tail heat sensitivity, a tail flick test was performed using a plantar test device (7370 plantar test; Ugo Basile, Italy) at high-intensity infrared radiation (infrared intensity of 50) with a maximum cutoff time of 15 seconds. Before the test, mice were habituated for 1 h in transparent restrainers with a glass floor. To obtain the mean latency of tail flicks, five trials of heat stimulation were applied with a 10-min interval using a radiant heat source.

Hot plate test

A hot plate test was conducted to assess hind paw heat sensitivity. Mice were habituated for 1 h in a transparent testing box with a metal floor (15 $\times$ 15 $\times$ 25 cm). A mouse was then placed in a box that was preheated to 52.5°C in a thermoregulated water bath, and the latency to hind paw licking or jumping was assessed.

Capsaicin test

To assess acute pain responses to a chemical, 1-μg capsaicin (10 μl) was injected subcutaneously into the dorsal region of the hind paw. The time spent licking or biting the injected paw was measured over 5 min.

Formalin test

To assess chemical-induced acute and tonic pain, 2% paraformaldehyde (10 μl) was injected subcutaneously into the dorsal region of the hind paw. The cumulative time that an animal spent licking or biting the injected paw was measured every 5 min for 1 h.

Visceral pain test

To assess visceral pain, mice were injected intraperitoneally with 0.6% acetic acid (5 ml/kg body weight), which evokes an inflammatory reaction-induced persistent pain, or 12 mg/ml $MgSO_4$ (10 ml/kg body weight), which induces an immediate visceral pain response independent of inflammation. The number of abdominal writhes such as stretches and contractions was counted within 20 min or 5 min of injection of acetic acid or $MgSO_4$, respectively.

Behavioral assay of neuropathic pain by SNL

Surgical procedure for SNL

Neuropathic surgery was performed under gaseous anesthesia with a mixture of enflurane (2% for induction and 0.5% for maintenance) and oxygen. The L5 spinal nerve was ligated according to the procedure of Kim and Chung (1992). Briefly, following surgical preparation and exposure of the dorsal vertebral column from L4 to S2, the L6 vertebral transverse process was removed and the L5 spinal nerve was tightly ligated with a 6-0 silk thread under a dissecting microscope. Complete hemostasis was confirmed, and the wound was sutured closed. These animals were used in a series of SNL-induced neuropathic pain tests in the following order: (1) spontaneous pain test, (2) mechanical allodynia and (3) thermal hyperalgesia at low-intensity (IR 30) and high-intensity (IR 60) infrared radiation.

Spontaneous pain test

To measure the spontaneous pain response in the hind paw following L5 SNL, we used a modification of the formalin test rating system (Dubuisson & Dennis 1977). Following surgery, a mouse was placed in an open-topped transparent plastic cylinder (6 cm diameter $\times$ 16 cm height) and allowed to move freely for 20 min before being observed. During the subsequent 3-min observation period, the cumulative time during which the mouse held its foot off the floor was measured.

Foot lifts during locomotion or body repositioning were not included in the measurement. The average score for two trials was calculated.

Mechanical allodynia (von Frey test)
To assess hind paw mechanical sensitivity, the withdrawal threshold of the hind paw was measured using the up–down method (Chaplan *et al.* 1994). For each test, the animal was placed under a transparent plastic chamber (9.5 × 5.5 × 5 cm) on a metal mesh floor. The 50% withdrawal threshold was determined using a set of von Frey filaments (0.02, 0.07, 0.16, 0.4, 1, 2, 4 and 6 g; Stoelting, Wood Dale, IL, USA). A brisk foot lift upon filament application was regarded as a withdrawal response. Mice first received a stimulus using the 0.4-g filament, and, if there was a withdrawal response, the next smallest filament was used. If there was no response, the next largest filament was used.

Thermal hyperalgesia (paw withdrawal test)
To assess hind paw heat sensitivity, Hargreaves' test was conducted using a plantar test device (7371 plantar test; Ugo Basile, Italy) (Hargreaves *et al.* 1988). Animals were allowed to freely move within an open-topped transparent plastic cylinder (6 cm diameter × 16 cm height) on a glass floor 20 min before the test. A mobile radiant heat source was then placed under the glass floor and focused onto the hind paw. Paw withdrawal latencies were measured with a cutoff time of 15 seconds. Two different intensities were employed: low (IR 30) and high (IR 60). The heat stimulation was repeated three times with a 10-min interval to obtain the mean latency of paw withdrawal.

General behavioral assays

Open-field test
A cubic box was made of polyacrylic plastic plates without a ceiling (40 × 40 × 40 cm), the arena of which was divided into 16 equal squares. After being placed in a holding cage for 10 min, each mouse was transferred to the center of the arena and the activity of animals was recorded with a video camera. Horizontal activities (number of squares crossed) and vertical activities (number of rearing) were assessed for 1 h.

Rotarod test
A rotarod apparatus (Rota-rod LE 8200; Letica Scientific Instrument, Spain) was used to explore coordinated locomotor function in mice. Mice were placed on a 3-cm diameter rod that slowly accelerated from 0 rpm to constant speeds of rotation. The speed of the rotating rod in each test session was set at 5–35 rpm, with a 5-rpm increment. After a 1-min training session on the rod, each mouse was tested in a single session at a specific speed for 1 min. The latency to falling from the rotating rod at each speed was recorded by the rotarod timer.

Light/dark activity test
The test box (45 cm × 25 cm × 25 cm) for the light/dark activity test was composed of two compartments: one compartment (2/3 of the space) was illuminated by bright light (illumination >500 lux), the other compartment was painted black, not illuminated and covered by a black lid. The compartments were connected by an opening (7.5 cm × 7.5 cm) located in the center of a partition at the floor level. Each mouse was placed in the center of the dark compartment facing the opening, and the activity of the animals was recorded by a video camera fixed 30 cm above the floor. The latency to movement into the light compartment and the time spent in the light compartment were measured during 10 min.

Data analysis

SAS version 8.02 (SAS, Inc., Cary, NC, USA) was used for statistical analysis, and Sigma plot (SPSS, Inc., Chicago, IL, USA) was used for creating graphs. The paw-licking times following formalin injections were analyzed by two-way analysis of variance (ANOVA). Tukey's honestly significant difference method was used for *post hoc* comparisons. A Mann–Whitney rank sum test, which is a nonparametric analog to the *t*-test, was used to investigate all behavioral responses induced by neuropathic pain, spontaneous pain, mechanical allodynia and thermal hyperalgesia. All other comparisons between two genotypes were performed by Student's *t*-test.

Results

Pronociceptive role of $Ca_V3.2$ in mechanical, thermal and chemical cutaneous pain

We examined the behavioral responses in $Ca_V3.2^{-/-}$ mice to mechanical, thermal or chemical stimuli to determine the role of $Ca_V3.2$ in acute pain. In a tail clip test to assess the susceptibility to mechanical pain, $Ca_V3.2^{-/-}$ mice displayed a dramatic increase in the latency to tail licking/biting or flicking reactions (Fig. 1a; $P < 0.001$ by Student's *t*-test). In tail flick (Fig. 1b) and hot plate tests (Fig. 1c), $Ca_V3.2^{-/-}$ mice showed a significantly prolonged latency to heat-induced behavior ($P < 0.01$ and $P < 0.001$, respectively, by Student's *t*-test). We also examined the response of mice to chemical stimulation using capsaicin. We found that $Ca_v3.2^{-/-}$ mice showed a dramatic reduction in paw-licking response to capsaicin compared to wild-type littermates (Fig. 1d; $P < 0.001$ by Student's *t*-test). Collectively, these results indicate that, compared to wild-type mice, $Ca_V3.2^{-/-}$ mice have a reduced sensitivity to acute pain induced by mechanical, thermal and chemical means.

Noxious chemical stimulation, for example, by intradermal injection of formalin into the hind paw, induces a biphasic pain behavioral response: an early-phase paw-licking

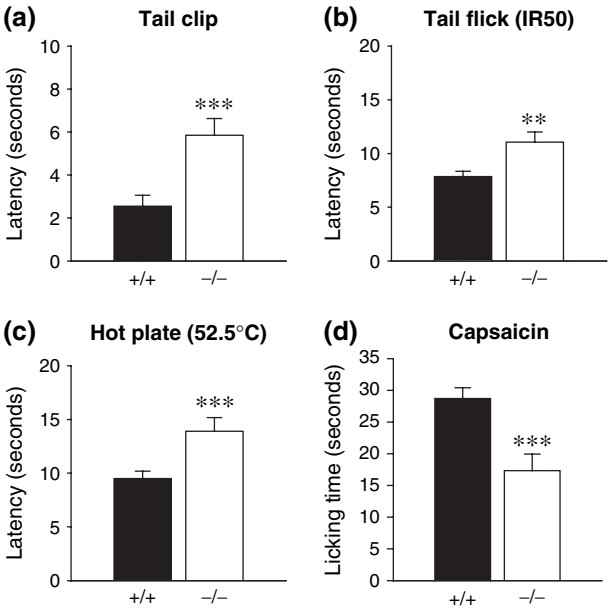

Figure 1: $Ca_v3.2^{-/-}$ mice show hypoalgesic responses to various acute stimuli. (a) Tail clip test ($n = 21$ for wild-type mice; $n = 29$ for mutant). Time (seconds) represents the latency to tail licking or trembling after tail clipping using an alligator clip. (b) Tail flick latency in noxious thermal stimuli testing (at infrared intensity IR 50; $n = 18$ for wild-type mice; $n = 14$ for mutants). (c) Paw withdrawal latency in hot plate testing (at 52.5°C; $n = 21$ for wild-type mice; $n = 24$ for mutants). (d) Capsaicin-induced pain behavior in $Ca_v3.2^{-/-}$ mice (3 μg/10 μl; $n = 14$ for wild-type mice; $n = 14$ for mutants). Time (seconds) spent paw licking or biting within the first 5 min following capsaicin injection into the hind paw. All data represent means ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ by Student's *t*-test.

response due to direct stimulation of peripheral nociceptors, followed by late-phase responses due to inflammation-induced persistent input of pain signals at the supraspinal level (Abbadie *et al.* 1997; Taylor *et al.* 1995). We administered an intradermal injection of formalin into the hind paws of $Ca_V3.2^{-/-}$ and wild-type mice and recorded their biphasic licking responses. $Ca_V3.2^{-/-}$ mice exhibited a markedly shorter duration of paw licking (*i.e.*, reduced pain response) in both phases compared to wild-type littermates (Fig. 2 line graph, $F_{1,31} = 15.25$ and $P = 0.0005$ by two-way ANOVA with Tukey's *post hoc* test; Fig. 2 bar graph, $P < 0.05$ by Student's *t*-test).

Reduced abdominal writhing in $Ca_V3.2$-deficient mice in response to chemical visceral pain

To confirm the role of $Ca_V3.2$ channels as enhancers of pain signals, we performed abdominal writhing (stretching or contracting) tests in $Ca_V3.2^{-/-}$ mice. Writhing was induced by injecting mice with acetic acid, which evokes persistent inflammatory reaction-induced pain, or by intraperitoneal

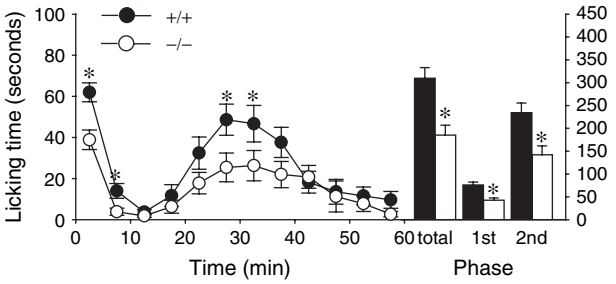

Figure 2: Formalin-induced pain behavior in $Ca_v3.2^{-/-}$ mice ($n = 18$ for wild-type mice; $n = 16$ for mutants). Values represent time (seconds) spent paw licking or biting in each 5-min interval following formalin injection (2%; 10 μl). In the bar graph, values represent licking or biting during the first phase (0–10 min), second phase (10–60 min) and total time (0–60 min) after formalin injection. Data represent means ± SEM. Significance of differences was analyzed by two-way ANOVA with Tukey's *post hoc* test for line graph data, and Student's *t*-test for bar graph data. $^{*}P < 0.05$.

injection with $MgSO_4$, which induces an immediate visceral pain response independent of inflammation. Compared to wild-type controls, $Ca_V3.2^{-/-}$ mice showed fewer incidents of writhing in response to both types of visceral pain (Fig. 3a,b; $P < 0.01$ and $P < 0.05$, respectively, by Student's *t*-test).

Normal neuropathic pain responses in $Ca_V3.2^{-/-}$ mice

To define the roles of $Ca_V3.2$ in neuropathic pain, $Ca_V3.2^{-/-}$ mice and their wild-type littermates underwent L5 SNL, and a variety of behavioral responses relevant to neuropathic pain were evaluated. We found that both genotypes of mice developed similar levels of spontaneous pain, mechanical allodynia and thermal hyperalgesia following L5 SNL.

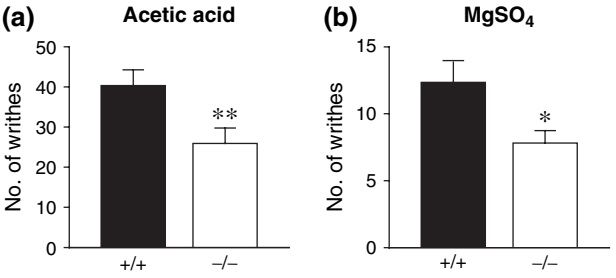

Figure 3: $Ca_v3.2^{-/-}$ mice show attenuated abdominal writhing following visceral toxic stimulation. Visceral pain in response to 0.6% acetic acid (a; 5 ml/kg; $n = 12$ for wild-type mice; $n = 14$ for mutants) and $MgSO_4$ (b; 120 mg/kg; $n = 21$ for wild-type mice; $n = 21$ for mutants). Values represent the number of abdominal writhes (body stretches and contractions). All data represent means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ by Student's *t*-test.

$Ca_V3.2^{-/-}$ mice did not differ from wild-type mice in the overall development of neuropathic pain (Fig. 4), except for a modest decrease in thermal hyperalgesia at high-intensity (IR 60) irradiation on day 21 after L5 SNL (Fig. 4d; $P < 0.05$ by Mann–Whitney rank sum test).

Normal general behaviors in $Ca_V3.2$-deficient mice

We next examined the general behavior of the mutant mice. In an open-field box test, the horizontal and vertical activities of $Ca_V3.2^{-/-}$ mice were indistinguishable from those of wild-type mice (Fig. 5a,b). In a rotarod test, we did not observe any difference between mutant and wild-type mice in their duration on the rotating rotarod at various speeds (Fig. 5c). In the light/dark transition test to measure the level of anxiety, the latency to move into the light compartment and the time spent in the light compartment did not differ between $Ca_V3.2^{-/-}$ mice and wild-type littermates (Fig. 5d). These results indicate that the deletion of $Ca_V3.2$ does not cause significant abnormalities in motor-associated and emotion-related functions that could have biased interpretation of the results from the various pain tests.

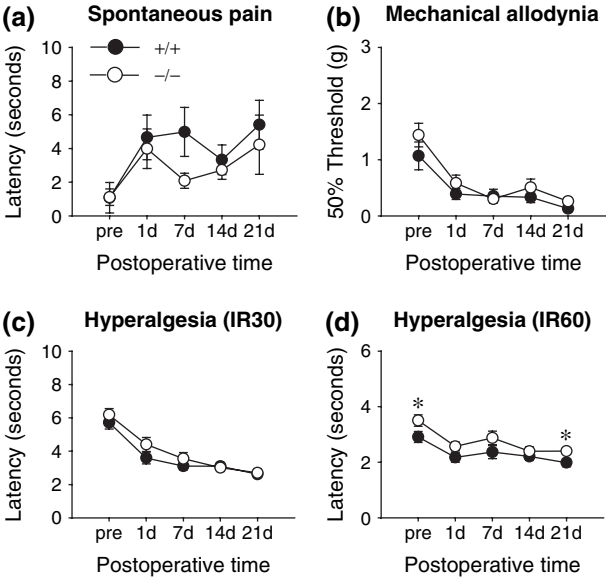

Figure 4: L5 spinal nerve ligation (SNL)-induced neuropathic pain behavior in $Ca_V3.2^{-/-}$ mice ($n = 18$ for wild-type mice; $n = 18$ for mutants). Spontaneous behavioral pain response induced by SNL in $Ca_V3.2^{-/-}$ mice (a). 50% hind paw withdrawal thresholds to von Frey filament stimuli are plotted against the days after injury (b). Paw withdrawal latency in response to low-intensity (IR 30) and high-intensity (IR 60) infrared radiation after SNL was compared in $Ca_V3.2^{-/-}$ mice and wild-type mice (c) and (d), respectively. All data represent means ± SEM. Mann–Whitney rank sum tests were used to compare values between mutant and wild-type mice. $^*P < 0.05$.

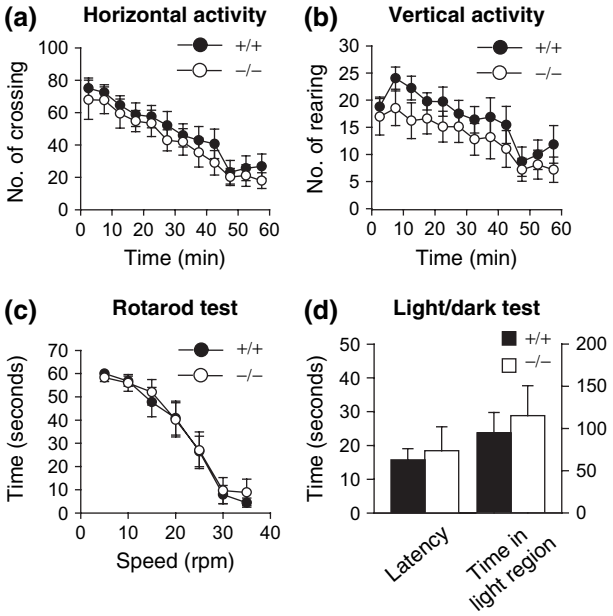

Figure 5: General behavioral assessments in $Ca_V3.2^{-/-}$ mice (a) and (b). Open-field test for a total of 1 h ($n = 18$ for both genotypes). (c) Rotarod test. Duration (seconds) that mice remain on the rotating rod at various speeds ($n = 12$ for wild-type mice; $n = 10$ for mutants). (d) Light/dark transition box test. Latency (seconds) is the time to first entry into the light region from the dark region. Time (seconds) represents the total time spent in the light region during 10 min. Values indicate the total number of entries into the light region from the dark region over 10 min ($n = 10$ for both genotypes). All data are presented as means ± SEM. Locomotor activity, motor coordination and balance, and anxiety levels were not significantly different between wild-type mice and mutants according to two-way ANOVA (a)–(c) or Student's *t*-test (d).

Discussion

Using mice lacking $Ca_V3.2$ T-type Ca^{2+} channels, we have shown that the $Ca_V3.2$ subtype plays a pronociceptive role in acute and tonic cutaneous pain caused by a variety of noxious stimuli as well as in chemical visceral pain. Previous studies have shown that the viability, fertility and ratios of specific tissues to total body weight are normal in $Ca_V3.2^{-/-}$ mice, although they have abnormal coronary function and are slightly smaller than littermate controls (Chen *et al.* 2003). There were no significant differences in heart rate between wild-type mice and mutant mice in the current study. In addition, deletion of $Ca_V3.2$ did not result in abnormalities of locomotion, motor coordination and skill, or anxiety, which indicate normal behavioral performance of the mutant mice. Thus, the observed reductions in behavioral pain responses in $Ca_V3.2^{-/-}$ mice evoked by various noxious stimuli or stimulation of somatic or visceral tissues appears to be due to an alteration in nociceptive processing rather than other defects.

Previous studies showed that $Ca_V3.2^{-/-}$ mice lost almost all T-type Ca^{2+} currents in DRG neurons, which are involved

in the transmission of pain signals from the periphery, and did not show any significant change in other Ca^{2+} currents (Chen *et al.* 2003). In the present study, we found that $Ca_V3.2^{-/-}$ mice exhibit hypoalgesic responses to acute somatic, visceral and tonic inflammatory pain. Thus, the hypoalgesic responses of $Ca_V3.2^{-/-}$ mice suggest that the T-type Ca^{2+} currents supported by $Ca_V3.2$ play a critical role in pain perception. The present findings are consistent with recent results obtained using intrathecal delivery of antisense oligonucleotides directed against $Ca_V3.2$, which showed that this channel participates in the response to noxious mechanical stimuli (Bourinet *et al.* 2005). The abundant expression of $Ca_V3.2$ in small- and medium-sized DRG neurons (Talley *et al.* 1999) appears to be a possible cause of the hypoalgesic response in the mutant mice. Our previous study revealed a thalamic antinociceptive function of the $Ca_V3.1$ subtype; specifically, $Ca_V3.1^{-/-}$ mice showed increased visceral pain but no significant difference in peripheral pain (Kim *et al.* 2003). In addition, in agreement with the visceral pain results, following intradermal injection of formalin into the hind paw of $Ca_V3.1^{-/-}$ mice, there was no difference in the licking duration of the early phase, which is elicited by direct stimulation of peripheral nociceptors (Le Bars *et al.* 2001), whereas the licking duration of the late phase was increased (data not shown).

In spinal cord, T-type Ca^{2+} channels mediate sensitivity to pain by controlling synaptic efficiency between primary afferent nerve fibers and intrinsic spinal cord neurons, which was suggested to be the mechanism for neuropathic (Heinke *et al.* 2004; Ikeda *et al.* 2003). Recently, Bourinet *et al.* demonstrated that selective silencing of $Ca_V3.2$ elevates the paw withdrawal threshold for tactile stimuli after chronic constriction injury (CCI) suggesting the involvement of $Ca_V3.2$ in this model of neuropathic pain (Bourinet *et al.* 2005). These findings appear to be in conflict with our present data showing no significant role for $Ca_V3.2$ channels in neuropathic pain induced by L5 SNL. This discrepancy could be due to the differences in the types of pain models used, namely CCI and SNL. Although these two animal models show behavioral signs of neuropathic pain, there is a considerable difference between them both in the magnitude of each pain component and the effect of sympathectomy (Kim *et al.* 1997). It is also conceivable that developmental compensatory mechanisms might have eliminated the need for $Ca_V3.2$ in $Ca_V3.2^{-/-}$ mice. On the other hand, several lines of evidence support the idea that $Ca_V3.2$ T-type channels are not essential for induction of central sensitization following peripheral nerve injury. Although persistent tonic afferent drive of small- and medium-sized DRG neurons resulting from peripheral nerve injury are known to be important for the generation of neuropathic pain (Zimmermann 2001), T-type calcium currents in medium-sized DRG neurons, probably supported by Cav3.2, are unaffected or downregulated following peripheral nerve injury (Andre *et al.* 2003; Baccei & Kocsis 2000; Hogan *et al.* 2000; McCallum *et al.* 2003). Thus, T-currents mediated by Cav3.2 may be unrelated to the neuropathic pain caused by peripheral nerve injury.

In the present study, exploration of various pain behaviors in $Ca_V3.2^{-/-}$ mice was conducted to validate $Ca_V3.2$ as a molecular target for analgesic drugs. Although antisense-mediated knockdown studies by Bourinet *et al.* have already shown that this subtype plays an important role in mechanical nociception (Bourinet *et al.* 2005), our results revealed that $Ca_V3.2$ also participates in the perception of various noxious signals as well as mechanical noxious stimuli. Our results with respect to neuropathic pain, however, seem to contradict those of Bourinet *et al.* (Bourinet *et al.* 2005). Further studies are required to determine the extent to which $Ca_V3.2$ T-type channels contribute to the development of neuropathic pain and the role each subtype plays in the modulation of LTP between primary sensory neurons and neurons of the spinal dorsal horn. In conclusion, our results suggest that the $Ca_V3.2$ subtype of T-type Ca^{2+} channels plays a pronociceptive role in not only mechanical nociception but also thermal, chemical and tonic inflammatory pain.

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