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Expression of background potassium channels in rat DRG is cell-specific and down-regulated in a neuropathic pain model

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Abstract

Neuropathic pain is associated with hyperexcitability of DRG neurons. Despite the importance of leakage potassium channels for neuronal excitability, little is known about their cell-specific expression in DRGs and possible modulation in neuropathic pain. Multiple leakage channels are expressed in DRG neurons, including TASK1, TASK3, TRESK, TRAAK, TWIK1, TREK1 and TREK2 but little is known about their distribution among different cell types. Our immunohistochemical studies show robust TWIK1 expression in large and medium size neurons, without overlap with TRPV1 or IB4 staining. TASK1 and TASK3, on the contrary, are selectively expressed in small cells; TASK1 expression closely overlaps TRPV1-positive cells, while TASK3 is expressed in TRPV1- and IB4-negative cells. We also studied mRNA expression of these channels in L4-L5 DRGs in control conditions and up to 4 weeks after spared nerve injury lesion. We found that TWIK1 expression is much higher than TASK1 and TASK3 and is strongly decreased 1, 2 and 4 weeks after neuropathic injury. TASK3 expression, on the other hand, decreases 1 week after surgery but reverts to baseline by 2 weeks; TASK1 shows no significant change at any time point. These data suggest an involvement of TWIK1 in the maintenance of the pain condition.

Keywords

KCNK; PCR; immunohistochemistry; nerve injury, TWIK1

Introduction

Neuronal signal transmission is mediated by action potentials generated by voltage-gated ion channels (Hodgkin and Huxley, 1952). The diverse functional properties of voltage-gated channels lead to cell-to cell variability in action potential phenotype, including differences in threshold, width, frequency and spiking pattern (McCormick, 2004). However, leakage channels are also critically involved in the modulation of neuronal excitability (Bayliss and Barrett, 2008). Particularly, work over the last 15 years has led to the characterization of KCNK, a family of leakage potassium channels abundantly expressed in neuronal and nonneuronal tissues (reviewed by Lesage, 2003; Lesage and Lazdunski, 2000). KCNK

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channels influence cell excitability by setting the resting potential and by setting neuronal membrane potential and input resistance (Berg and Bayliss, 2007; Taverna et al. 2005) and are modulated by a vast array of transmitters and modulators (Lesage and Lazdunski, 2000; Talley et al. 2000). These channels also regulate several other electrophysiological properties; for instance, in thalamocortical neurons, KCNK blockade shifts the firing mode from bursting to tonic (Meuth et al. 2003), while, in cortical basket cells, TASK3 channels regulate the maximum firing frequency (Goldberg et al, 2011).

Dorsal root ganglia neurons (DRGs) are the primary sensory neurons that convey information from the skin, muscles and joints to the spinal cord. Nociceptive fibers can be myelinated A fibers, originating from large DRGs, or unmyelinated C fibers originating from small and medium DRGs. Unmyelinated fibers are classified (Julius and Bausbaum, 2001) into the substance P expressing peptidergic population and another population, which does not express substance P, but binds the lectin IB4 (Stucky and Lewin, 1999). Both these populations are further divided into TRPV1-positive and negative (Aoki et al. 2005; Michael and Priestley, 1999).

Under normal conditions sensory signals originate in DRG's peripheral endings, where the transducers are located, whereas somatic initiation of firing is sporadic and has minor sensory consequences (Devor, 1999). In the presence of nerve injury or pain, however, ectopic (somatic) DRG firing is strongly enhanced and is believed to represent a major cause of neuropathic pain (Nordin et al. 1984; Sukhotinsky et al. 2004) additionally, nociceptors become intrinsically firing and their intrinsic firing frequency correlates with spontaneous pain (Djouhri et al. 2006). Much work has been performed to investigate changes in the expression of voltage-gated sodium channels correlated with nerve injury; these studies show a complex scenario of up- and down-regulation of different channel subunits (reviewed by (Rogers et al. 2006; Waxman, 1999). In addition, nerve injury has been shown to induce downregulation in the expression of voltage gated potassium channels; in particular, downregulation of several members of the shaker-homologous class (Kv 1.1,1.2, Kv1.4) has been reported after nerve injury (Kim et al. 2002; Rasband et al. 2001; Yang et al. 2004). However, far less is known about neuropathic injury-associated changes in leakage channel expression in DRG neurons. TASK channels were shown to regulate excitability in sensory neurons from humans with neuropathic pain (Baumann et al. 2004). Accordingly, DRG expression of several members of the KCNK family has been found to be down regulated in the first 4 days following an inflammatory response (Marsh et al. 2012) while expression of TRESK was diminished 3 weeks after injury in a rat neuropathic injury model (Tulleuda et al. 2011). Yet, a characterization of KCNK subunit expression among the various DRG neuron subtypes is still lacking, as is any knowledge of their long-term regulation in neuropathic pain. Because several studies have demonstrated changes in extracellular pH due to inflammation following nerve injury (Reeh and Steen 1996, Steen et al 1999) we focused on expression of TASK1 and TASK3 subunits, which are sensitive to even small changes of pH in the physiological range (Berg et al. 2004) and on TWIK1, which represents an intriguing molecule whose expression has not been previously investigated in DRGs. We investigated the expression of these subunits in different DRG neuron subclasses and its regulation up to 4 weeks after neuropathic injury.

Materials and Methods

Spared nerve injury (SNI) model

All studies were performed in conformity with national guidelines and were approved by the Animal Care and Use Committee of Northwestern University. 20- to 21-day-old male, Sprague Dawley rats (from Harlan or Charles Rivers) were anesthetized using gas anesthesia (isoflurane 1-2%, and 30% N₂O, 70% O₂). For induction of SNI-neuropathy, the left sciatic

nerves were exposed at the level of the trifurcation into the sural, tibial and common peroneal nerves. The tibial and common peroneal nerves were tightly ligated and severed, leaving the sural nerve intact (Decosterd and Woolf, 2000). A second group of animals was used for control sham surgery. In this case, the left sciatic nerve was exposed just as in the SNI procedure but was not further manipulated.

Behavioral test for tactile sensitivity

Tactile sensitivity of the spared region of the operated paws was measured from the withdrawal responses to mechanical stimulation with von Frey filaments. Animals were placed in a cage with wire grid floor and allowed to habituate to the environment for 15 minutes. Filaments (Stoelting) of varying forces were applied to the plantar surface of the hind paw in ascending order. Each filament was applied for a maximum of 10 seconds. Paw withdrawal during the application was considered a positive response. 50% response thresholds were calculated according to Chaplan et al. (1994). All animals that underwent surgery developed tactile allodynia that persisted until they were sacrificed. The left foot von Frey thresholds for sham-operated and SNI rats, respectively, were 4.01 ± 1.62 g and 0.74 ± 0.51 g at 1 week 5.27 ± 1.67 g and 0.84 ± 0.60 g at 2 weeks and 10.82 ± 3.62 g and 0.99 ± 1.06 g at 4 weeks after surgery. The experimenter assessing mechanical allodynia was blinded to the condition (SNI vs. sham) of each rat.

RT-PCR analysis

L4 and L5 DRGs were dissected from sham operated and SNI rats; DRGs both ipsilateral and contralateral to the injury were collected 1 week, 2 weeks, and 4 weeks after onset of SNI injury and frozen in liquid nitrogen. RNA was extracted using a Qiagen RNeasy RNA extraction kit that includes a column that binds DNA while allowing RNA to flow through in order to prevent DNA contamination, and stored in the -80°C . RNA was then reverse transcribed into cDNA using Roche's First Strand cDNA Synthesis kit and oligo dT primers. The primers used for quantitative RT-PCR are reported in Table 1 with their slopes and efficiencies. Quantitative RT-PCR was performed using a Roche Lightcycler 480 (LC480) with Roche SYBR Green or Probes master mix, primers ($0.4 \mu\text{M}$), and DRG cDNA. All PCR reactions were run using a 5 minute hot start at 95°C , followed by 45 cycles of 10 sec. at 95°C , 15 sec. at 60°C , and 10 sec. at 72°C . All samples were run in duplicate. The specificity of these amplifications was tested by melt curve analysis, which demonstrated a single peak for each gene product and by electrophoresis on 1.8% agarose gels (Figure 1A). Although all primers were intron spanning, cDNA negative and reverse transcriptase negative controls were done for genes of interest and were always negative.

Amplification curves were done to calculate reaction efficiency for each gene product using progressive dilutions of cDNA (Table 1). As recommended in published guidelines for qPCR methods, all data were efficiency corrected (Bustin et al. 2010). Roche LC480 software and the comparative Ct method were employed to normalize potassium channel expression to the reference housekeeping gene GAPDH (Schmittgen et al. 2008). GAPDH is highly expressed and is commonly used as a PCR reference gene. Importantly, GAPDH transcripts have been shown to be stably expressed in DRGs of rat models of both inflammatory and neuropathic pain (Abbadie et al. 2003; da Costa et al. 2010; Marsh et al. 2012; Molliver et al. 2005).

For the gene expression analysis one week post surgery (4 weeks old), 8 SNI and 8 sham rats were used; at the two week post surgery (5 weeks old) time point, 4 SNI and 4 sham rats; and at the 4 week post surgery time point (7 weeks old animals) 6 SNI and 6 sham rats were used.

The relative expression analysis in Figure 1B represents data from 5 naïve animals aged one month. L4 and L5 DRGs were collected and RNA purified as described above. RNA was reverse transcribed into cDNA using Roche's First Strand cDNA Synthesis kit with both random hexamer and oligo dT primers. qPCR for Kv1.2, TWIK1, TASK1, and TASK3 was run as described above, using SYBR green for all reactions.

Statistical significance in expression data was determined at the 0.05 level using Analysis of Variance with post-hoc correction (Student-Newman-Keuls). Because no expression difference was ever detected between ipsi- and contra-lateral DRGs of control animals, these data were pooled and used for comparison with contra- and ipsi-lateral SNI. The "Sham" expression level in the figures represents these pooled data.

Immunohistochemistry

Four week-old naïve male Sprague Dawley rats were anesthetized using ketamine/xylazine (80 and 10 mg/0.1 kg, respectively) and perfused transcardially with saline and then with 4% paraformaldehyde. L4 and L5 DRGs were dissected out and post fixed in paraformaldehyde for one hour after which they were cryoprotected with 10% sucrose overnight followed by 20% sucrose. DRGs were then embedded in OCT, frozen on dry ice, and cut into 30 μ m slices using a Microtome Cryostat HM 505E. Sections were frozen for later use. Sections were incubated at 40°C for 15 minutes prior to staining and then incubated for 36 hours in primary antibody in TBS with 0.2% triton-X and 3% NGS. Sections were then washed with TBS and incubated for 2 hours at 22 to 24°C in secondary antibodies in TBS. Sections were again washed (6-8 times) in TBS with one final wash in PBS. Sections were mounted and imaged on a Zeiss UV LSM 510 META confocal microscope. Z-stacks (8 to 12 in 2 micron steps) were taken for all image analyses. Primary antibodies used were: TASK1 (1:300) and TASK3 (1:300) generously provided by Dr. Doug Bayliss (University of Virginia); TWIK1 (1:3000) generously provided by Dr. Florian Lesage (CNRS, France); TRPV1 (1:1000) Neuromics (cat. Number GP14100), and biotinylated IB4 (1:500) Vector Labs (cat. number B-1205). Secondary antibodies (Molecular Probes) were Goat anti-Rabbit 488, Goat anti-Guinea pig 568, and anti-Streptavidin 568. Controls with primary antibody alone and secondary antibody alone were negative throughout. For TASK cell counting experiments n=3 naïve rats were used.

Results

Immunochemical analysis was performed to study cell-specific expression of TASK1, TASK3 and TWIK1, three KCNK family members in adult rat L4/5 DRGs. Additionally, the temporal profile of gene expression of these channel subunits was examined in DRGs in the spared nerve injury model of neuropathic pain at 1, 2 and 4 weeks after the peripheral injury.

For these experiments, DRGs ipsilateral and contralateral to the nerve lesion were collected as well as DRGs from sham controls and mRNA expression of the gene of interest was quantified relative to GAPDH using real-time PCR.

Relative abundance of background channels

1 month old naïve rats were used to determine the relative abundance of transcripts of the background channels of interest in L4-L5 DRGs (Figure 1B). Kv1.2 was included in the analysis because its expression has previously been characterized in DRGs and it was also shown to be reduced after nerve injury (Kim et al. 2002; Rasband et al. 2001; Yang et al. 2004). Interestingly, TWIK1 transcripts showed the highest expression, approximately 80% higher than Kv1.2, which had the second highest expression level. TASK1 was

approximately 3 fold higher than TASK3, which had the lowest transcript expression. These measurements confirmed that TASK1, TASK3 and TWIK1 genes are expressed in rat DRGs but did not provide any information concerning the cells in which the genes are expressed. Immunohistochemical staining was thus performed to answer this question.

KCNK channel expression is cell-type specific

Immunohistochemical staining was performed on 30 μm -thick slices obtained from L4/5 DRG of 1 month old naïve rats. Distribution of TASK1, TASK3 and TWIK1 protein was assessed in parallel with IB4 and TRPV1, two widely used cellular markers of C-fiber nociceptive neurons. In agreement with previous reports, IB4 and TRPV1 stained partially overlapping populations of small DRG neurons (Aoki et al. 2005; Stucky and Lewin, 1999).

TWIK-1 expression is abundant in large neurons and does not overlap with IB4 and TRPV1

PCR data showed an abundant expression of TWIK1 in DRG tissue. Immunostaining confirmed that expression is robust and showed that it predominates in large and medium size neurons (Figure 2A, E) in agreement with the outcome of a previous in-situ characterization (Talley et al. 2001). TWIK1 expression was seldom found in small size neurons (2 of 28 cells Figure 2I). Interestingly, co-staining studies showed no overlap of TWIK1 with IB4 and TRPV1, even in the few cases when expression was detected in small size neurons (D,H,L). In 12 z-stacks encompassing 28 TWIK1 positive cells, no overlap with IB4 or TRPV1 was detected.

TASK1 expression overlaps with IB4 and TRPV1 positive cells

Our data showed that TASK1 expression was largely limited to small size ($<25 \mu\text{m}$) neurons (Figure 3). Because preliminary studies revealed some overlap of TASK1 with IB4 and TRPV1, a more detailed co-staining and cell counting study was undertaken, as detailed in Table 2. 93% of TASK1 expressing cells also expressed TRPV1, while 43% of TRPV1 positive cells expressed TASK1 (Figure 3C and Table 2). In a separate staining 58% of TASK1 positive cells co-localized with cells positive for IB4, while 45% of the IB4 population expressed TASK1 (Figure 3G and Table 2). Thus TASK1 showed very high selective expression in TRPV1-positive cells and substantial overlap with the IB4 population within the DRG.

TASK3 expression is sparse and limited to a subpopulation of small cells

In keeping with the PCR data that showed much lower expression of TASK3 compared to TASK1 in DRG tissue, TASK3 staining was sparse. Similar to TASK1, TASK3 expression was mostly limited to small (presumably C-fiber) DRGs (Figure 4). Contrary to TASK1, however, TASK3 expression was almost exclusively limited to the TRPV1- and IB4-negative population. Indeed, only 6% (5/85) of the TASK3-positive cells expressed TRPV1 (Figure 4C and Table 2) and 4% (3/86) expressed IB4 (Figure 4G and Table 2). Thus, expression of TASK1 and TASK3 appeared to delineate different sub-populations within small DRG neurons.

Changes in DRG KCNK channel expression in a model of neuropathic pain

Having established that these KCNK subunits are expressed, although at widely different levels and location, in DRG neurons, we wondered whether their expression levels are affected in a neuropathic injury condition. To this end, real time RT-PCR was performed on DRG tissue of control (sham operated) and SNI rats. In particular, we investigated gene expression of these channels in L4-L5 DRGs ipsi- and contra-lateral to the neuropathic lesion at 1, 2 and 4 weeks after the SNI surgery; additionally, Kv1.2 expression, which has

been previously shown to be importantly decreased in neuropathic pain (Kim et al. 2002; Rasband et al. 2001; Yang et al. 2004) was used as a positive control.

At one week post surgery, consistent with other reports (Kim et al. 2002; Rasband et al. 2001), Kv1.2 expression showed a significant, 28% reduction in ipsilateral DRGs of SNI rats relative to sham controls (data from 3 animals; $p < 0.05$). DRGs ipsilateral to the nerve lesion also showed a significant reduction in TWIK1 (36%) and TASK3 (68%) transcripts (data from 8 animals, $p < 0.05$, Figure 5). TASK1, on the other hand, did not show any significant reduction.

In order to better understand the functional changes occurring in DRG neurons during chronic neuropathic conditions, and the evolution of these changes, transcript levels were also measured at 2 weeks and 4 weeks post surgery. Interestingly, at two weeks post surgery the ipsilateral expression level of both Kv1.2 and TWIK1 transcripts remained significantly reduced (47% for Kv1.2 and 35% for TWIK1) compared to either the contralateral SNI or the sham DRGs, while TASK1 and TASK3 levels did not show significant expression changes (Figure 6).

At 4 weeks post surgery the relative expression levels of Kv1.2 and TWIK1 transcripts in ipsilateral SNI DRGs were reduced (45% for Kv1.2 and 37% for TWIK1) very similarly to the 2 week time point, while TASK1 and TASK3 did not differ significantly from controls (Figure 7). Thus, these data suggest that, at this time point, neuropathic injury-associated changes in channel expression in ipsilateral DRGs have reached a steady state.

Discussion

We show that TWIK1 expression is high in rat lumbar DRGs and, in agreement with previous studies (Talley et al. 2001) is robust in large and medium size neurons and does not overlap with IB4 or TRPV1; TASK1 expression, on the contrary, is lower, predominantly limited to small cells and enriched in TRPV1-positive neurons (93% of TASK1-positive cells are also TRPV1-positive); finally, TASK3 expression is sparse and largely limited to small TRPV1- and IB4-negative cells. Additionally, in ipsilateral DRGs of spared nerve injury (SNI) model of neuropathic pain TWIK1 expression decreases early and remains depressed for at least 1 month after the injury, while expression of TASK3 is only transiently reduced and there is no detectable change in TASK1 expression at any time point.

Cell-specific expression of TASK1, TASK3 and TWIK1 subunits

In situ hybridization studies had shown widespread expression of TASK-1, TASK-2, and TRAAK in DRG neurons while TWIK1 expression appeared mostly in large neurons and TASK3 in a few small cells (Talley et al. 2001). Yet, no information was available concerning the distribution of different KCNK channels among the nociceptor populations (e.g. TRPV1 vs. IB4 neurons). Here we show that TASK1 and TASK3 channels are predominantly expressed in small (C-fiber) neurons. TASK1 was detected in all populations (TRPV1 positive and negative, IB4 positive and negative). TASK3, on the other hand was found mostly in TRPV1 and IB4 negative neurons, suggesting that these channels may have mediated the TASK-like currents described in capsaicin-insensitive nociceptors (Cooper et al. 2004). Because both TASK1 and TASK3 antibodies were raised in rabbits, potential co-localization of these two subunits could not be tested directly although the staining data strongly suggest that TASK1 expression defines a subpopulation of TRPV1-positive neurons. TWIK-1, on the other hand, appears highly expressed in large and medium size neurons, with only sparse labeling of small neurons, and is not co-expressed with IB4 or TRPV1.

Intrinsic DRG excitability and neuropathic injury

Hyperexcitability of primary nociceptors is well documented in neuropathic injury and chronic pain (Djouhri et al. 2006; Kajander et al. 1992; Liu et al. 2000; Zhang et al. 1999). As is often the case, the change in electrophysiological phenotype is the consequence of multiple factors that, in the case of DRG excitability, include a complex regulation of sodium channel expression, increased expression of calcium channels and decreased expression of voltage-gated potassium channels. In particular, while the total density of sodium channels was suggested to increase (Gurtu and Smith, 1988), TTX-resistant sodium currents mediated by Nav1.8 and Nav1.9 appear to be down regulated (Dib-Hajj et al. 1998; Dib-Hajj et al. 1999), whereas the TTX-sensitive current is potentiated, most likely through the up-regulation of Nav1.3 sodium channels (Waxman et al. 1994). This scenario is even more complex considering that different pain-models may have opposite effects on the regulation of certain sodium channel genes (reviewed by Waxman, 1999). Accordingly, the use of sodium channel blockers in pain treatment has been only partly successful (Hargus and Patel, 2007).

DRG expression of voltage-gated potassium channels is also modified in pain conditions. Immunohistochemical data show a strong reduction in expression of potassium channel subunits mediating delayed rectifier and A-type currents in models of neuropathic pain (Ishikawa et al. 1999; Rasband et al. 2001) and a reduction of A-type current has been found to be associated with increased DRG excitability following chronic compression of dorsal root ganglia (Tan et al. 2006). In agreement with prior studies, our PCR data show that Kv1.2 expression is decreased already 1 week after neuropathic injury and remained decreased for the duration of our study (up to 1 month after surgery). While these and many other studies focused on the regulation of voltage-gated currents more recently the expression of several background potassium channels, which are critical modulators of neuronal activity, was also found to be modified in DRG neurons in pain conditions (Marsh et al. 2012; Tulleuda et al. 2011). In line with these results, we found that gene expression of two of the three genes we studied was significantly reduced in ipsilateral DRGs of SNI animals 1 week after surgery. However, while TWIK1 expression remained low at 2 and 4 weeks after the injury, TASK3 expression reverted to baseline by two weeks post injury. This may suggest that in later stages DRGs undergo some form of homeostatic plasticity or that the TASK3 subunit, while important in establishing the pain behavior, is not critically involved in maintaining the neuropathic phenotype.

It is intriguing that our data show a clear lateralization as all changes were found exclusively in the DRGs ipsilateral to the lesion. In contrast, a recent paper in which KCNK transcript expression was investigated after cutaneous inflammation (Marsh et al. 2012) showed that 4 days after the onset of inflammation TASK3 transcript was decreased bilaterally. Although these data are not directly comparable as our shortest time point was seven days after the lesion, the ipsilateral only change in neuropathic injury may suggest a role of this channel in initiating the neuropathic response (which is behaviorally ipsilateral only).

Neuropathic lesion is associated with decreased TWIK1 expression

Contrary to TASK3 gene expression, which returned to baseline 2 weeks after SNI surgery, TWIK1 expression remained significantly lower 1, 2 and 4 weeks after the neuropathic injury, suggesting a more important role in the maintenance of the neuropathic phenotype. Our data cannot distinguish whether the decreased TWIK1 expression in SNI DRGs is due to decreased number of TWIK1-positive cells, or to decreased expression within the same pool of cells. In order to address this point, we counted in histological sections the number of TWIK1-positive cells relative to the total neuronal population (the NeuN-positive cells) for L4 and L5 DRGs at 2 and 5 weeks post surgery. This type of analysis failed to detect any

change in the number of TWIK1-expressing cells in SNI versus sham animals (data not shown). This suggests that the reduction in TWIK1 transcript results in reduced expression level in TWIK1 positive cells rather than in a loss of TWIK1-positive cells.

Yet, while the decreased expression of TWIK1 in the pain condition is an intriguing observation, the functional implications may seem less straightforward because although TWIK1 mediates a background potassium current (Lesage et al. 1996), this current is usually small in heterologous expression systems and a native current with the properties of TWIK1 has not been unequivocally identified yet (Feliciangeli et al. 2007). It has been proposed that modulation and posttranslational modification may regulate the membrane insertion of these subunits although the precise mechanisms remain debated (Feliciangeli et al. 2007). The search for the main functional role of these subunits has been further complicated from data suggesting that TWIK1 subunits are expressed in intracellular compartments rather than in the plasma membrane (Decressac et al. 2004) and even the ionic selectivity of the pores formed by TWIK1 subunits has been questioned, as it was shown that it may vary in different extracellular pH conditions (Chatelain et al. 2012). On the basis of these observations it may be suggested that TWIK1 function differs from other KCNKs in DRG neurons. However, recent work on TWIK1 channels expressed in recombinant systems (Feliciangeli et al. 2010) may offer an alternative hypothesis, as these authors reported that TWIK1 membrane insertion and current are strongly enhanced by a G_i-coupled dephosphorylation, which can be initiated by activation of the 5HT₁ receptor. Interestingly, 5HT₁ receptors are expressed at relatively high density in lumbar DRGs, including in large neurons (Classey et al. 2010). Thus, TWIK1 may indeed mediate an inhibitory current in DRG neurons, at least in response to serotonergic modulation. If this is the case, the long-term downregulation of these channels in neuropathic pain animals may lead to increased excitation due to diminished background current, although other explanations, possibly involving metabolic roles of TWIK1 are also possible. Interestingly, DRG TWIK1 expression was correlated with tactile threshold, but the correlation was only present in the uninjured DRGs (sham and contralateral SNI) of older animals (4 weeks after surgery). In the ipsilateral DRGs of SNI rats, in which TWIK1 expression is dramatically reduced, this correlation was lost (Figure 8). This suggests that the role of TWIK1 may be different in physiological condition and after neuropathic injury. In this context it is worth reporting that we found similar correlations (that is, positive correlation between tactile threshold and subunit expression in the uninjured, but not in the injured DRGs at 4 weeks post surgery) also for TASK1 and TASK3 subunits (not shown). This seems to support the hypothesis that background channels have different roles in physiological and pathological conditions. Future work may be able to shed more light onto the functional role of DRG TWIK1 subunits in these conditions.

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Figure 1. Relative abundance of TWIK1, TASK1, TASK3 and Kv1.2 in rat DRG

A, PCR products run on a 1.8% agarose gel demonstrated a single amplification product for each gene of interest. B, Relative abundance of genes of interest detected in naïve animals at 1 month of age. Because no difference was observed between ipsi- and contra-lateral DRGs, the data were pooled for this bar chart. Genes of interest were normalized to GAPDH and plotted on a logarithmic scale. Note the very high expression level for and TWIK1 and Kv1.2, while TASK1 and TASK3 have lower expression levels. Bar chart represents average and SEM; circles represent measurements from individual animals. Data are from 5 naïve rats.

Figure 2. TWIK1 expression in rat DRG

DRG slices from naïve rats were co-stained with antibodies selective for TWIK1 (A,E,I; green), IB4 (B,F,J; red), and TRPV1 (C,G,K, blue). D,H,L; merged images. TWIK1 expression is predominant in large and medium size neurons (A,E), with only very sparse labeling of small size neurons (I). From 12 z-stacks in which 28 TWIK1 positive cells were counted, no TWIK1 overlap was found with IB4 or TRPV1 (D,H,L), even in the few instances where staining was detected in small neurons (L). Scale bar: 50 μ M.

Figure 3. TASK1 is co-expressed with IB4 and TRPV1 in rat DRGs

DRG slices from naïve rats were co-stained with antibodies selective for TASK1 (A,E; green), TRPV1 (B, red), IB4 (F, red). (C) Merged image of TASK1 and TRPV1. (G) Merged image of TASK1 and IB4. TASK1 expression is largely limited to small cells with robust (93%) overlap of TASK1 with TRPV1 positive cells, as well as a substantial overlap (58%) of TASK1 with IB4 positive cells (see Table 2). (D,H) DIC images of the slices. Scale bar: 20 μ M.

Figure 4. TASK3 expressing DRG neurons are mostly TRPV1 and IB4 negative

Immunohistochemical analysis of TASK3 in naïve rat lumbar DRGs. Slices were stained with antibodies selective for TASK3 (A,E; green), TRPV1 (B, red), IB4 (F, red). (C) Merged image of TASK3 and TRPV1. (G) Merged image of TASK3 and IB4. TASK3 immunostaining displayed sparse overall staining mostly in small cells with very little overlap (5.9%) of TASK3 with TRPV1 positive cells, as well as TASK3 and IB4 positive cells (3.5%). Scale bar: 20 μ M.

Figure 5. Reduced expression of KV1.2, TWIK1 and TASK3 one week post injury

Relative abundance for genes of interest in SNI rat DRG are shown normalized to sham, showing a significant (* $p < 0.05$) reduction of Kv1.2 (positive control), TWIK1 and TASK3 in the DRG ipsilateral to nerve injury. Note that TASK1 expression was not significantly reduced ($p = 0.08$). There was no significant change in the DRG contra-lateral to nerve injury. All data is from one week post injury.

Figure 6. Expression of TASK3 recovers two weeks post injury

Relative abundance for genes of interest are shown normalized to sham, and show a significant (* $p < 0.05$) reduction for Kv1.2 (positive control) and TWIK1 in DRGs ipsilateral to nerve lesion. Note the recovery of TASK3 in the ipsilateral DRG. All data is from two weeks post injury. Contra-lateral SNI and sham did not show any significant difference for any genes tested.

Figure 7. Kv1.2 and TWIK1 expression is still reduced at 4 weeks post injury

Relative abundance for genes of interest in SNI rat DRG are shown normalized to sham, and demonstrate a significant (* $p < 0.05$) reduction in Kv1.2 and TWIK1 transcript in ipsilateral DRGs. Note that no difference is detectable between channel transcript levels of contralateral SNI and sham DRGs.

Figure 8. TWIK1 expression in uninjured DRGs is correlated with tactile threshold

TWIK1 expression was plotted against von Frey threshold; panel A shows TWIK1 expression in DRGs from sham animals; panel B, contralateral DRGs from SNI animals, and panel C ipsilateral DRGs of SNI rats. A significant correlation between TWIK1 expression and tactile threshold was only present in the older animals (4 weeks after surgery) and in uninjured DRGs. The correlation was lost in ipsilateral DRGs of SNI rats (C). Asterisks indicate statistically significant correlation ($p < 0.05$).

Table 1

Primer sequences, efficiencies, and slopes for genes of interest.

Gene	Primers		Slope	Efficiency
Kv1.2 (Kcna2)	L cgggtacccatctgcaag	R agatgcacagctcagcaaaa	-3.27	2.02
TWIK1 (Kcnk1)	L gttccgggagctgtacaaga	R acaaccagcatggctatgagt	-3.25	2.03
TASK1 (Kcnk3)	L cgccggctccttctactt	R ggagccgcatgaccatag	-3.29	2.01
TASK3 (Kcnk9)	L agctggagctggtaatcctg	R caggtgcagcatgtccata	-3.23	2.04
GAPDH	L ctgcaccaccaactgcttag	R tgatggcatggactgtgg	-3.32	2.00

Table 2

Cell-type specific expression of TASK1 and TASK3.

TASK1 positive cells that are IB4 positive	114/196 (58.2%)
IB4 positive cells that are TASK1 positive	117/263 (44.5%)
TASK1 positive cells that are TRPV1 positive	116/125 (92.8%)
TRPV1 positive cells that are TASK1 positive	116/274 (42.3%)
TASK3 positive cells that are IB4 positive	3/86 (3.5%)
TASK3 positive cells that are TRPV1 positive	5/85 (5.9%)