A HYPERTIRXITABILITY PHENOTYPE IN MOUSE TRIGEMINAL SENSORY NEURONS EXPRESSING THE R192Q CACNA1A MISSENSE MUTATION OF FAMILIAL HEMIPLEGIC MIGRAINE TYPE-1

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Abstract—Missense mutation R192Q in the CACNA1A gene causes familial hemiplegic migraine type-1 (FHM1), a monogenic subtype of migraine with aura. Using knock-in (KI) gene targeting we introduced this mutation into the mouse gene and generated a transgenic mouse model to investigate basic mechanisms of migraine pathophysiology. While FHM1 R192Q KI trigeminal ganglia were previously shown to exhibit constitutive up-regulation of ATP-gated P2X3 receptors, little is known about the firing properties of trigeminal sensory neurons, which convey nociceptive inputs to higher brain centers. We patch-clamped trigeminal sensory neurons to search for differences in firing properties between wildtype (WT) and KI cells in culture. Although various subclasses of trigeminal neurons were observed with respect to their firing patterns evoked by intracellular current injection, their distribution among WT and KI cells was similar with only small differences in rheobase or input resistance values. However, when neurons were excited by either α,β-methyl-ATP to stimulate P2X3 receptors or capsaicin, two functional classes of WT or KI neurons were distinguished according to the first spike latency, which suggests that a subgroup of neurons may be indirectly activated, probably via crosstalk between neurons and satellite glial cells. Thus, our results are consistent with reported facilitated trigeminal pain behavior of FHM1 R192Q KI mice.

Key words: P2X3 receptors, capsaicin, TRPV1 receptors, ATP, purinergic.

INTRODUCTION

Familial hemiplegic migraine type-1 (FHM1) is a rare monogenic form of common migraine with aura that is characterized by some degree of hemiparesis during the aura phase (International Headache Society, 2004) and caused by missense mutations in the CACNA1A gene (Ophoff et al., 1996). A transgenic knock-in (KI) mouse model of FHM1, in which we introduced the human pathogenic R192Q missense mutation in the orthologous mouse Cacna1a gene by a gene targeting approach, expresses mutant α1A subunits of neuronal voltage-gated calcium channel type 2.1 (CaV2.1) (van den Maagdenberg et al., 2004). Main neurobiological features in FHM1 R192Q KI mice include a gain-of-function of CaV2.1 channels both at peripheral (van den Maagdenberg et al., 2004; Kaja et al., 2005) and central synapses (van den Maagdenberg et al., 2004; Tottene et al., 2009; Gao et al., 2012; Uchitel et al., 2012), and an increased susceptibility to cortical spreading depression (CSD) (van den Maagdenberg et al., 2004; Eikermann-Haerter et al., 2009; Tottene et al., 2009), the mechanism underlying the migraine aura (Lauritzen, 1994). Increased excitatory, but not inhibitory, neurotransmission of cortical neurons appeared to be the underlying cause of the CSD susceptibility in these KI mice (Tottene et al., 2009). Our previous experiments have indicated that, in KI trigeminal sensory neurons, the R192Q mutation, via enhanced CaV2.1 activity, leads to sensitization of ATP-gated purinergic ionotropic receptor 3 (P2X3) receptors that mediate nociceptive responses (Nair et al., 2010). Recently, and in line with this, migraine-like pain behavior that seemed stress-related was reported in FHM1 R192Q KI mice (Chanda et al., 2013).

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Abbreviations: α,β-methATP, α,β-methylene adenosine 5-triphosphate; AHP, after-hyperpolarization; BDNF, brain derived neurotrophic factor; CaV2.1, voltage-gated calcium channel type 2.1; CGRP, calcitonin gene-related peptide; CSD, cortical spreading depression; EGTA, ethylene glycol tetra-acetic acid; FA, fast adaptation; FHM1, familial hemiplegic migraine type-1; HEPES, hydroxyethyl piperazine-ethanesulfonic acid; KI, knock-in; MF, multiple firing; P2X3, purinergic ionotropic receptor 3; RF, rapid firing; SEM, standard error of the mean; SS, single spike; TRPV1, transient receptor potential vaniloid receptor; WT, wildtype.

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Both common migraine and rare FHM are episodic disorders defined by attacks which are likely triggered by so-called “migraine mediators”, namely soluble factors that can facilitate the activation of trigeminal sensory neurons and headache mechanisms (Goadsby et al., 2002; Giniatullin et al., 2008; Ho et al., 2010). It remains to be established whether trigeminal sensory neurons harbor intrinsic alterations in their electrochemical responsiveness to such mediators that could account for migraine-relevant neuronal hyperexcitability. In this report we investigated voltage- and ligand-activated responses by studying firing patterns of trigeminal sensory neurons of wildtype (WT) and FHM1 R192Q KI (KI) mice activated by intracellular current pulses, by brief application of α,β-methyl-ATP to stimulate P2X3 receptors, or by application of capsaicin to activate transient receptor potential vanilloid receptors (TRPV1) as the latter have also been proposed to mediate trigeminal pain (Bradbury et al., 1998; Barclay et al., 2002; Meents et al., 2010). Different classes of trigeminal neurons have been described in WT mouse trigeminal ganglia based on their firing patterns (Catacuzzeno et al., 2008) or on voltage-dependent Ca2+ currents (Fioretti et al., 2011). Here we investigated whether these firing characteristics may differ between WT and KI trigeminal sensory neurons.

**EXPERIMENTAL PROCEDURES**

**Mouse trigeminal ganglion cultures**

FHM1 R192Q KI and WT mouse littermates were used for the experiments. Our colony of mice is bred locally, after an initial transfer of mice from Leiden University Medical Centre (van den Maagdenberg et al., 2004). Mice were maintained in accordance with the Italian Animal Welfare Act. The experimental protocols were approved by the SISSA ethical committee. Genotyping was performed by polymerase chain reaction (PCR), as previously reported (Nair et al., 2010). Trigeminal ganglion cultures were obtained from 2-week-old animals. Ganglia were isolated from mice killed by cervical dislocation under general anesthesia produced by slow gas breathing with isoflurane anesthesia (Simonetti et al., 2006; Nair et al., 2010; Hullugundi et al., 2013). For all experiments, KI and WT cultures were used in parallel at the same time to allow for direct comparison of experimental data.

**Electrophysiology**

As previously described in detail (Nair et al., 2010; Hullugundi et al., 2013), after 1 day in culture, trigeminal neurons were superfused continuously (2 mL/min) with physiological solution containing (in mM): 125 NaCl, 5 KCl, 1 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES (pH adjusted to 7.4 with NaOH). Cells were patch-clamped in the whole-cell configuration. Recordings were performed on neurons with capacitance below 22 pF. In all recording sessions, the culture medium was washed out with physiological solution and patch-clamp recording was commenced within 10 min. Ten kilohertz filtering was used for current-clamp, using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) and acquired by means of a DigiData 1200 Interface and pClamp 8.2 software (Molecular Devices).

**Patch pipettes with a resistance of 4-5 MΩ were filled with solution containing (in mM): 125 K-gluconate, 5 KCl, 2 MgCl2, 2 Mg2ATP3, 10 HEPES, and 10 EGTA (pH-adjusted to 7.2 with KOH). The calculated K+ equilibrium potential with the Nernst equation was –105 mV. The calculated liquid junction potential was 14.6 mV and data were corrected accordingly. To minimize voltage-dependent changes in ionic currents, all neurons were held at –90 mV. Input resistance was measured by applying hyperpolarizing pulses of –5 or –3 pA, while cell capacitance was estimated from the whole-cell capacitance facility. Current pulses lasting for 300 ms were applied with 5-pA increments starting from 0 up to 45 pA. Firing thresholds can be precisely characterized by the properties of the membrane potential as a dynamical system (Platkiewicz and Brette, 2010). We developed an algorithm that allowed automated threshold detection based on first and second discrete time derivatives of the voltage time-series. The parameters used for detecting the threshold events were empirically determined and are consistent with the values reported in literature (Sekerli et al., 2004). Rheobase was calculated as the average of minimum current injected to generate a single action potential for each class of neurons. The P2X3 receptor selective agonist α,β-methylene adenosine 5-triphosphate (α,β-meATP; Sigma, Milan, Italy) (Sokolova et al., 2006) was applied at the concentration of 10 μM (for 2 s) to produce near-maximal P2X3 receptor activation (Sokolova et al., 2006; Hullugundi et al., 2013) using a fast superfusion system (Rapid Solution Changer RSC-200; BioLogic Science Instruments, Claix, France). In accordance with our previous studies (Simonetti et al., 2006; Nair et al., 2010; Hullugundi et al., 2013), capsaicin (Sigma) was applied at a concentration of 1 μM (for 3–5 s) to elicit a large TRPV1 receptor-mediated response. The experimental protocol usually started with current injection tests followed by application of α,β-meATP and capsaicin. The selective P2X3 antagonist A-317491 (Jarvis et al., 2002) was used at 1 μM concentration (Sigma; 6 min pre-application; Simonetti et al., 2006).

**Statistics**

Data are expressed as mean ± SEM (standard error of the mean), where n indicates the number of independent experiments or the number of investigated cells. Statistical analysis was performed using Student’s t-test or the Mann–Whitney rank sum test after the software-directed choice of parametric or nonparametric data, respectively (Matlab; Sigma Plot & Sigma Stat,
Chicago, IL, USA). A \( p \)-value of 0.05 was accepted as indicative of a statistically significant difference.

RESULTS

Distinct firing patterns of trigeminal sensory neuron evoked by intracellular current pulses

We observed four distinct types of firing pattern in our cultured WT trigeminal neurons that had a similar somatic size (10–20 \( \mu \)m) and into which we injected the same rectangular current pulse (45 pA), starting from the same holding potential of –90 mV (Fig. 1A). Larger current pulses led to voltage-dependent spike inactivation and no conversion of one firing pattern into another one. One group of neurons generated a single spike (SS) followed by a large after-hyperpolarization (AHP). Another group consisted of neurons that exhibited a cluster of early spikes followed by fast adaptation (FA) and AHP. Neurons that showed spiking throughout the current pulse application (although with decreasing frequency) followed by the AHP were, therefore, termed multiple firing (MF) cells. Finally, a few neurons showed sustained high-frequency firing (rapid firing; RF) despite early partial inactivation, and had a short-lasting AHP. Fig. 1B summarizes the percent distribution of these firing patterns among the sample population under control culturing conditions whereby the largest number belonged to the MF type and the smallest to the RF class. A very similar distribution was observed when investigating KI neurons.

Next we compared the basic characteristics of WT and KI neurons in terms of rheobase, input resistance, spike threshold and cell capacitance, while holding all cells at –90 mV to minimize voltage-dependent inactivation as summarized in Fig. 2. MF neurons of both WT and KI ganglia had the lowest rheobase values (Fig. 2A), suggesting that they require the smallest current amplitude to fire. Conversely, within the SS and FA groups, KI cells had significantly higher rheobase values compared with WT cells. FA cells from KI mice showed a significantly lower input resistance than WT cells (Fig. 2B). Fig. 2C demonstrates that there was no difference in threshold for firing between WT and KI neurons across the four classes. In accordance with the relatively narrow range of somatic size of recorded neurons, we observed average capacity values for these cells comprised between 11 and 15 pF (Fig. 2D). These data suggest that, when cells were cultured under standard conditions, only very subtle differences between WT and KI genotypes exist in the distribution of neurons with specific firing patterns.

Effect of the P2X3 antagonist A-317491 on firing by WT and KI cells

Because WT or KI trigeminal cultures release different concentrations of endogenous ATP (Franceschini et al., 2002), we investigated the effect of the P2X3 antagonist A-317491 on WT and KI cells. We exposed cells to 10 \( \mu \)M of A-317491 and monitored their firing pattern after 15 min. The results showed that A-317491 reduced the number of SS and FA neurons and increased the number of MF and RF neurons. Fig. 3A shows the percent distribution of firing patterns before and after A-317491 treatment. Fig. 3B demonstrates that A-317491 significantly reduced the number of neurons with SS and FA firing patterns and increased the number of neurons with MF and RF firing patterns. These findings suggest that the P2X3 receptor plays a role in the regulation of firing patterns in trigeminal neurons.

Fig. 1. Distinct firing patterns of trigeminal sensory neuron evoked by intracellular current pulses. (A) Representative traces (from WT cells; similar pattern of firing also found with KI cells) of the four firing patterns evoked by 300 ms 45 pA pulses, namely single spiking (SS), fast adaptive (FA), multiple firing (MF) and rapid firing (RF) observed in trigeminal neurons. Cells were always held at –90 mV. (B) Pie charts showing the percent distribution of neurons with the above mentioned firing patterns in WT and KI trigeminal neurons. Note similar distribution in WT (left) and KI (right) samples. Number of cells for each group are in Fig. 2 legend.
we investigated if basal P2X3 receptor activity might influence firing induced by electrical stimuli. For this test, a selective P2X3 antagonist was used. Fig. 3 A shows example traces (on the same cells) of the spike discharge before and during continuous application of 1 μM A-317491: there was no significant difference in firing for WT or KI neurons as also confirmed by the cumulative probability plots for number of spikes (Fig. 3 B). On average, with 45-pA current pulse, WT neurons fired 5.7 ± 2.2 spikes in control solution and 5.2 ± 2.0 spikes in the presence of A-317491 (n = 4), while under comparable experimental conditions KI neurons fired 4.2 ± 1.3 and 3.7 ± 1.3 spikes, respectively (n = 4). These data show that P2X3 receptor activity did not contribute to firing evoked by current pulses. The effect of the classical TRPV1 antagonist capsazepine (O’Neill et al., 2012) was not investigated because it has been reported to block K⁺ and Ca²⁺ channels (Kuenzi and Dale, 1996; Docherty et al., 1997).

Trigeminal sensory neuron firing evoked by α, β-meATP or capsaicin

Under physiological conditions, sensory neurons are expected to be depolarized by activation of their ionotropic receptors, such as P2X3 receptors (activated by the selective agonist α,β-meATP; Jarvis and Khakh, 2009; Fabbretti and Nistri, 2012) or TRPV1 receptors (activated by their agonist capsaicin; Meents et al., 2010; O’Neill et al., 2012). We investigated what firing patterns could be generated by pulse application of these agonists to the culture medium.
Two-second pulse application of α,β-meATP (10 μM) to elicit near-maximal P2X3 receptor activity (Sokolova et al., 2006; Nair et al., 2010) produced either an early and transient cluster of spikes emerging during the rising phase of the depolarization (Fig. 4A), or a longer train of spikes appearing with a few hundred ms delay (Fig. 4C). The ‘early pattern of firing’ was most commonly observed (35 (82%) cells), as the other seven cells showed a delayed onset of spikes. A similar distribution was observed when recording from KI neurons (29 (78%) cells were of the early discharge type, the other eight cells showed a delayed onset of spikes). Despite the difference in impulse discharge, α,β-meATP-mediated responses were always accompanied by membrane depolarization that, on a random sample of cells, reached an average peak of 45 ± 1 mV for WT neurons (n = 24) and 46 ± 2 mV for KI neurons (n = 22).

In most cells (n = 22; see example trace in Fig. 4B), neuronal firing evoked by 1 μM capsaicin developed slowly and was terminated only after a few seconds, despite persistent depolarization. In a minority of cells (n = 9; see example trace in Fig. 4D), firing appeared with a strong delay of 1.5 s and persisted for at least
3 s. The same pattern of response was observed with KI neurons (18 (64%) cells showed early onset of spikes, 10 cells (36%) a delayed onset of spikes). Membrane depolarization elicited by capsaicin was, on average, 63 ± 2 mV for WT neurons (n = 21) and 60 ± 3 mV for KI neurons (n = 17). In summary, firing properties of trigeminal sensory neurons following chemical stimuli were different from those found with intracellular current pulses.

The vast majority of WT and KI neurons was depolarized and excited by α,β-meATP (Fig. 5A) in accordance with their strong and widespread expression of neuronal P2X3 receptors (Simonetti et al., 2006; Nair et al., 2010). Conversely, capsaicin-mediated effects were detected only in approximately half of the tested neurons (Fig. 5A) in keeping with the limited expression of TRPV1 receptors in trigeminal ganglia and the rather low degree of co-expression of P2X3 and TRPV1 channels (Simonetti et al., 2006).

We next examined whether the firing pattern observed with intracellularly injected current pulses could help predicting responses to either α,β-meATP or capsaicin, and whether there was a significant difference between WT and KI neurons. Fig. 5B shows the distribution of α,β-meATP-mediated firing among the four classes of neurons. FA, SS and MF neurons almost invariably responded to α,β-meATP, while only few RF neurons from WT cultures were activated by α,β-meATP, and none in KI cultures. The scant occurrence of this firing pattern might explain its absence in KI cells. Fig. 5C

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**Fig. 4.** Trigeminal sensory neuron firing evoked by α,β-meATP or capsaicin. (A, B) Representative traces (from WT cells, similar pattern of firing also found with KI cells) of the firing patterns induced by 10 μM α,β-meATP or capsaicin. Note early and transient cluster of spikes during depolarizing phase in panel A and longer train of spikes (with a few hundred ms delay) in panel B. (C, D) Examples of slow onset firing elicited by α,β-meATP or capsaicin on WT cells.
compares analogous data for capsaicin application. FA neurons were the largest capsaicin-sensitive class in WT cultures, while, in KI cultures, the majority of FA and RF neurons was activated by this drug. Although we did not observe a difference in spiking threshold between WT and KI neurons (Fig. 3C) when activated by intracellular current pulses, a significantly more negative spike threshold was detected when α,β-meATP or capsaicin was tested on either WT or KI neurons (Fig. 6A). Hence, while neurons clearly started firing at more negative membrane potentials when stimulated by either agonist, KI neurons had a significantly more negative threshold value, both for α,β-meATP and capsaicin tests (Fig. 6B). To validate this observation, cumulative spike probability plots were constructed for α,β-meATP (Fig. 6C) and capsaicin (Fig. 6D). These plots show that the probability of observing a large number of spikes was always higher for KI cells, regardless of the total number of spikes that substantially differed depending on whether α,β-meATP or capsaicin was applied to the culture medium.

**DISCUSSION**

The main finding of this study is that trigeminal sensory neurons from a transgenic migraine KI mouse model expressing R192Q-mutated CaV2.1 channels, a mutation that causes FHM1 in patients, constitutively show a significantly lower firing threshold and generate a larger number of action potentials in response to α,β-meATP and capsaicin. Taken together, our data suggest that trigeminal sensory neurons of KI mice exhibit enhanced basal excitability that is not immediately apparent when testing responses with intracellular current pulses. It seems likely that such increases in neuronal excitability can be transduced as
facilitated activation of second-order neurons in the trigeminal complex, which would be consistent with the enhanced trigeminal pain observed in these mice in vivo (Chanda et al., 2013) and which would further validate the KI mice as a valuable migraine model.

**Functional characteristics of trigeminal sensory neurons of KI mice**

Using intracellular current pulses, we could distinguish four types of small- to medium-size neurons that are typically considered trigeminal nociceptors (Sessle, 1999) with distinctive discharge patterns. The vast majority of neurons possessed spike adaptation which ranged from SS generation to a gradual deceleration of spike discharge during continuous current injection. These results extend former observations of three subgroups of mouse trigeminal neurons obtained from acutely dissociated ganglia (Catacuzzeno et al., 2008). To the classification by these authors, we added one further subgroup, namely that of rapid-firing cells, that, admittedly, is the smallest and amounts to less than 20% of the total neuron population we analyzed. Notably, WT and KI neurons were virtually indistinguishable for their spike responses to current injection as well as for the subgroup classification. There were, however, subtle differences in threshold values as SS and FA of KI neurons had somewhat higher rheobase values than corresponding WT neurons. FA KI neurons showed, however, comparably lower input resistance. The neuronal sub-threshold properties that control excitability and resting potential are largely regulated by a wide family of leak K^+^ channels with cell-restricted expression and differential electrophysiological properties in terms of activation and deactivation (Goldstein et al., 2005). Furthermore, a

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**Fig. 6.** Firing threshold of trigeminal sensory neurons to chemical stimuli. (A) Plot of threshold values (mean ± SEM) for firing action potential with current (n = 66, WT; n = 49, KI) or chemical stimuli (α,β-meATP n = 52, WT, *p* < 0.001; n = 46, KI, *p* < 0.001 and capsaicin; n = 41, WT, *p* = 0.014; n = 37, KI, *p* < 0.001). (B) Comparison of threshold values (mean ± SEM) between WT and KI neurons activated by α,β-meATP (*p* = 0.04) and capsaicin (*p* = 0.02). (C, D) Kolmogorov–Smirnov plots of cumulative firing probability of WT and KI neurons sensitive to α,β-meATP (left, n = 38, WT; n = 33, KI; *p* = 0.047) and capsaicin (right, n = 29, WT; n = 29, KI; *p* = 0.042) respectively. KI showed significantly higher probability of firing with both chemical stimuli.
subclass of trigeminal sensory neurons expresses \( h \), a mixed cationic conductance activated by membrane hyperpolarization to control their spike generating ability (Oro et al., 2009). The interplay among these conductances that show distinct voltage- and time-dependent activation characteristics is likely to determine the dissociation between rheobase and input resistance values observed for SS neurons and, in particular, for KI FA neurons. While future studies are necessary to dissect out the relative role of these conductances, it is interesting that a mutation in KCNK18 gene that encodes a protein member of the TRESK subclass of leak K\(^+\) channels was reported to be linked to familial migraine (Lafreniere et al., 2010), although it was later suggested that KCNK18 might act as a modifier instead of being alone sufficient to cause typical migraine (Andres-Enguix et al., 2012). Regardless, there is sufficient evidence that such K\(^+\) channels impact mouse trigeminal neuronal firing (Liu et al., 2013) and, therefore, are of importance to migraine. The consequences of the Ca\(_{\text{a,2.1}}\) R192Q mutation on the subthreshold conductances of KI sensory neurons require further detailed studies.

In the present investigation it was possible to detect major differences between WT and KI trigeminal sensory neurons when they were activated by pulse application of the agonists \( \alpha,\beta\text{-meATP} \) or capsaicin. First, the firing threshold for activation with either of these agonists was lower than for current pulses regardless of the genotype investigated or the agonist applied, as the evoked membrane depolarization was always sufficient to exceed the spike threshold.

The principal difference between WT and KI trigeminal sensory neurons was the lower spike threshold for the latter when tested with \( \alpha,\beta\text{-meATP} \) or capsaicin, and which was associated with a significantly larger number of spikes in KI than in WT cells, even when the membrane depolarization induced by each agonist was similar for WT and KI cells. The reason for the clear difference between agonist-mediated and electrical excitation is not fully understood. Nevertheless, it seems possible that ligand-gated channels such as P2X3 (Gnanasekaran et al., 2011) or TRPV1 (Szoke et al., 2010) receptors are compartmentalized at the plasma membrane to discrete micro-domains, like lipid rafts where they are clustered together with voltage-gated Na\(^+\) and Ca\(^{2+}\) channels (Davies et al., 2006; Robinson et al., 2010; Pristera et al., 2012; Head et al., 2014). This association would make excitation with receptor agonists more efficient to activate these channels and initiate spikes. In terms of ‘chemosensitivity’, the vast majority of neurons were excited by \( \alpha,\beta\text{-meATP} \), whereas about half of them were activated by capsaicin, which accords with our previously generated voltage-clamp and immunocytochemical data (Simonetti et al., 2006; Nair et al., 2010). Although the overall number of TRPV1-expressing neurons was similar in WT and KI cultures, the R192Q mutation increased the number of RF cells firing in response to capsaicin at the expense of MF and SS cells. This phenomenon might be related to a discrete alteration in the function of voltage-dependent channels in a subpopulation of trigeminal sensory neuron with intense and extended spike discharges and appears to be consistent with the TRPV1-mediated enhanced firing of trigeminal neurons after a strong inflammatory stimulus (Lambert et al., 2009) reminiscent of the neuroinflammatory profile of R192Q ganglia (Franceschini et al., 2012). A previous study has reported that, when P2X3 and TRPV1 receptors are co-expressed by rat dorsal root ganglion neurons, they interact with each other in an inhibitory manner so that the membrane current generated by either receptor type is depressed (Stanchev et al., 2009). This process may be a compensatory system to diminish excessive pain caused by the strong, simultaneous activation of the two receptor classes (Stanchev et al., 2009).

The functional classification observed with current pulses was replaced by a simpler subdivision of neurons that, in most cases, possessed transient firing of rapid onset elicited by \( \alpha,\beta\text{-meATP} \). Few (<20%) cells showed a delayed firing discharge induced by \( \alpha,\beta\text{-meATP} \). Following capsaicin application, again two categories emerged: one (and most frequent) with transient firing and a less common one with delayed, more persisting spiking. A significant firing delay in a minority of neurons might be the expression of crosstalk between neurons and tightly associated glial cells as observed in culture (Ceruti et al., 2011). The latter can sense depolarization and release ATP to stimulate neurons, a phenomenon that seems more pronounced in KI ganglia (Ceruti et al., 2011; Franceschini et al., 2012) and endogenous modulators, such as like calcitonin gene-related peptide (CGRP), brain-derived neurotrophic factor (BDNF) and TNF\(\alpha\) (Hullugundi et al., 2013) that can further potentiate these ATP-mediated effects.

It is noteworthy that the present study, for the first time, describes significant differences in firing threshold and spike discharges between WT and KI trigeminal ganglion neurons. It emphasizes that future studies, carried out with molecular biology and voltage-clamp electrophysiology methods, will be necessary to dissect out the multiple membrane mechanisms that are responsible for the distinctive changes in spike threshold and firing length.

**CONCLUSION**

FHM1 R192Q KI trigeminal sensory neurons showed facilitated firing following activation of either P2X3 or TRPV1 receptors by their respective agonists \( \alpha,\beta\text{-meATP} \) and capsaicin. The low firing threshold of KI neurons would ensure rapid signaling of nociceptive inputs to brainstem second-order neurons whenever P2X3 or TRPV1 receptors become activated by their specific agonists. On the one hand, this observation further contributes to the complex role exerted by P2X3 receptor activity in the stronger purinergic signaling of KI ganglia that includes selective up-regulation of P2X3 currents in the vast majority of KI neurons and the specific gain of P2X3 receptor function and trafficking...
triggered by migraine mediators, such as CGRP, BDNF and NGF (Giniaultin et al., 2008; Hullugundi et al., 2013). In this transgenic mouse model of migraine, P2X3 receptors, therefore, appear as a particularly attractive common transducer of migraine-relevant nociceptive inputs mediated by extracellular ATP. On the other hand, the stronger firing evoked by capsaicin highlights the additional component by TRPV1 receptors to the process of rapid signaling of nociceptive inputs to brainstem second-order neurons. Hence, to block the onset of trigeminal pain it might be important to broadly inhibit these ligand-gated receptors especially when various algogenic substances not only amplify purinergic function (Fabbretti and Nistri, 2012), but also act on TRPV1 receptors (Benemei et al., 2013; Iwashita et al., 2013).

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