Postnatal maturation of the hyperpolarization-activated cation current, \( I_h \), in trigeminal sensory neurons

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Cho HJ, Furness JB, Jennings EA. Postnatal maturation of the hyperpolarization-activated cation current, \( I_h \), in trigeminal sensory neurons. J Neurophysiol 106: 2045–2056, 2011. First published July 13, 2011; doi:10.1152/jn.00798.2010.—Hyperpolarization-activated inward currents (\( I_h \)) contribute to neuronal excitability in sensory neurons. Four subtypes of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels generate \( I_h \), with different activation kinetics and cAMP sensitivities. The aim of the present study was to examine the postnatal development of \( I_h \) and HCN channel subunits in trigeminal ganglion (TG) neurons. \( I_h \) was investigated in acutely dissociated TG neurons from rats aged between postnatal day (P)1 and P35 with whole cell patch-clamp electrophysiology. In voltage-clamp studies, \( I_h \) was activated by a series of hyperpolarizing voltage steps from \(-40 \) mV to \(-120 \) mV in \(-10\)-mV increments. Tail currents from a common voltage step (\(-100 \) mV) were used to determine \( I_h \) voltage dependence. \( I_h \) activation was faster in older rats and occurred at more depolarized potentials; the half-maximal activation voltage \( (V_{1/2}) \) changed from \(-89.4 \) mV (P1) to \(-81.6 \) mV (P35). In current-clamp studies, blocking \( I_h \) with ZD7288 caused membrane hyperpolarization and increases in action potential half-duration at all postnatal ages examined. ZD7288 also reduced the action potential firing frequency in multiple-firing neurons. Western blot analysis of the TG detected immunoreactive bands corresponding to all HCN subtypes. HCN1 and HCN2 band density increased with postnatal age, whereas the low-intensity HCN3 and moderate-intensity HCN4 bands were not changed. This study suggests that functional \( I_h \) are activated in rat trigeminal sensory neurons from P1 during postnatal development, have an increasing role with age, and modify neuronal excitability.

Hyperpolarization-activated cyclic nucleotide-gated channel; trigeminal ganglion; ZD7288

The processing and modulation of somatosensory information change dramatically after birth in both rodent and human. This postnatal maturation of the sensory system is due to changes in the neurochemical phenotype of primary afferent neuronal soma and terminals, myelination of axons, maturation of central synaptic connections with second-order neurons, and the development of interneuron circuits and descending inhibitory inputs (Fitzgerald 2005). The expression of ion channels in primary afferent neurons changes considerably during the first three postnatal weeks in rats, reaching a mature state after this time (Fitzgerald and MacDermott 2005). The excitability of sensory ganglion neurons is determined by ion channel properties and changes with postnatal age (Fitzgerald and Fulton 1992).

One family of ion channels that can strongly influence neuronal excitability, the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, occur in many excitable cells including neurons, cardiac pacemaker cells, and photoreceptors (for reviews, see Pape 1996; Robinson and Siegelbaum 2003). In adult sensory neurons, the current of HCN channels, \( I_h \), contributes to neuronal excitability. The persistent activation of \( I_h \), at resting and more negative membrane potentials counterbalances hyperpolarization and brings the membrane back toward action potential threshold after an afterhyperpolarization (AHP) (Hogan and Poroli 2008; Masuda et al. 2006; Momin et al. 2008; Orio et al. 2009).

Four HCN channel subtypes have been cloned (Ludwig et al. 1998; Santoro et al. 1998), and all subtypes are detected in the trigeminal ganglion (TG) (Cho et al. 2009a, 2009b; Wells et al. 2007) and dorsal root ganglion (DRG) (Antal et al. 2004; Chaplan et al. 2003; Cho et al. 2009b; Jiang et al. 2008; Kouranova et al. 2008; Matsuhashi et al. 2006; Moosmang et al. 2001; Tu et al. 2004), although the different reports are not entirely consistent. Most of these studies show that HCN protein is localized to neuronal membranes. When expressed individually, each subtype shows different activation kinetics, voltage dependence, and sensitivity to cAMP (Moosmang et al. 2001; Stieber et al. 2005). HCN1 has the fastest opening kinetics, followed by HCN2, HCN3, and HCN4. HCN2 and HCN4 are highly sensitive to cAMP, and their activation voltage can be shifted by \( >20 \) mV in a depolarizing direction with elevated cAMP. HCN1 and HCN3 are less sensitive to cAMP but are activated at more depolarized potentials than HCN2 and HCN4. In addition, HCN channels can be assembled as either homomeric or heteromeric tetramers and have mixed characteristics (Chen et al. 2001; Much et al. 2003).

Postnatal changes in HCN channel protein expression and \( I_h \) have been observed in the central nervous system (Bayliss et al. 1994; Bender et al. 2001; McCormick and Prince 1987; Surges et al. 2006), cardiac neurons (Adams et al. 2002; Hogg et al. 2001), and mesencephalic trigeminal neurons (Tanaka et al. 2003). However, it is not clear whether \( I_h \) is developmentally regulated in the major somatosensory ganglion (DRG or TG) neurons or whether there are functional consequences of such regulation.

The aim of this study was to determine whether \( I_h \) or HCN channel subunit expression changes during postnatal development and how such regulation affects the excitability of TG neurons.

METHODS

Ethical approval. Experiments were conducted on Sprague-Dawley rats at postnatal day (P)1–P35 from the Anatomy and Cell Biology colony at the University of Melbourne. All procedures conformed to
the Australian National Health and Medical Research Council code of practice for the use of animals in research and were approved by the University of Melbourne Animal Experimentation Ethics Committee. They also comply with the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP) (Zimmermann 1983).

**Acute dissociation of trigeminal ganglion.** Sprague-Dawley rats were deeply anesthetized with isoflurane (4%) and decapitated. Both TGs were rapidly removed, placed in ice-cold, oxygenated dissociation solution consisting of (in mM) 140 NaCl, 5 KCl, 10 HEPES, 0.5 CaCl₂, 2 MgCl₂, and 10 d-glucose (pH 7.3), and cut into three or four pieces with irisectomy scissors. The tissues were then incubated for 25 min at 37°C in dissociation solution containing 0.6–1.2 mg/ml of collagenase (type 1A, Sigma). After tissues were washed, individual neurons were dissociated by gentle triturating with sterilized Pasteur pipettes with decreasing bore and fire-polished tips in Dulbecco’s modiﬁed Eagle’s medium (Sigma). Cells were plated onto 35-mm plastic tissue culture dishes and allowed to settle down for >2 h in a 5% CO₂-air incubator at 37°C. Experiments were conducted from short-term cultures (2–7 h after plating) to minimize alterations in cellular phenotype (Schlichter et al. 1991).

**Electrophysiology.** After incubation, cells were perfused with oxygenated (100% O₂) HEPES-buffered saline consisting of (in mM) 140 NaCl, 5 KCl, 10 HEPES, 2 CaCl₂, 2 MgCl₂, and 10 d-glucose (pH = 7.3, 300 mosM) at ~1.0 ml/min. Experiments were carried out at room temperature (RT) to enable comparison to previous studies and at 33–35°C because Iₚ is highly temperature sensitive (Q₁₀ = 3–5; Hogg et al. 2001; Magee 1998; Orio et al. 2009; Pape 1996). Standard whole cell patch-clamp electrophysiology was done with an Axopatch 200B amplifier, a Digidata 1200 analog-to-digital converter interface, and pCLAMP 8.0 software (Axon Instruments, Foster City, CA). Signals were digitized at 25 kHz and low-pass filtered at 3 kHz for action potential measurement, and 10-kHz sampling with 1-kHz low-pass filtering was done for slow inward current measurement. Electrodes were pulled from borosilicate glass capillaries (P-97, Sutter instruments, Novato, CA). Initial resting membrane potential (V̇res) was measured immediately after whole cell configuration was obtained, and the recording was only continued when the V̇rest was more negative than ~45 mV. Liquid junction potentials were calculated with pClamp in pCLAMP software, and all membrane potential measurements were corrected during analyses. Cell capacitance, membrane resistance, and series resistance were measured in voltage-clamp configuration with the membrane test of pCLAMP software 5 min after whole cell configuration was achieved. Only neurons having series resistance lower than 10 MΩ were included in analysis.

**Voltage clamp.** In voltage-clamp experiments, the pipette resistances were 2–4 MΩ when ﬁlled with internal solution consisting of (in mM) 140 KCl, 20 HEPES, 10 EGTA, 1 MgCl₂, and 2 Mg-ATP (pH = 7.3, 280 mosM). Iₚ was activated by stepping the voltage from a holding potential of ~60 mV to between ~40 mV and ~120 mV for 2 s in ~10-mV increments, followed by ~100 mV for 1 s. Each current trace was digitally subtracted with Clampfit with parallel trace subtraction in the presence of 4-[(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinum chloride (ZD7288, 100 μM; Tocris), a specific Iₚ inhibitor (BoSmith et al. 1993; Harris and Constanti 1995), to acquire the Iₚ component only. Only neurons conducting with a slowly activating inward current after ZD7288 subtraction were used. The time course of the Iₚ activation at steady state was examined by measuring the tail current at a ~100 mV final common step after a series of different potentials was applied to activate various degrees of Iₚ (Banks et al. 1993; Doan and Kunze 1999). The tail current amplitudes were normalized to the maximal tail current amplitude and plotted against the corresponding preceding voltage step. The normalized current-voltage (I-V) relationship was ﬁtted in GraphPad Prism (GraphPad Software, San Diego, CA) with a Boltzmann equation in the following form:

\[ I(t) = A_{\text{fast}} \exp(-t/T_{\text{fast}}) + A_{\text{slow}} \exp(-t/T_{\text{slow}}) \]

where \( I(t) \) is the amplitude of the current at time \( t \), \( A_{\text{fast}} \) and \( A_{\text{slow}} \) are the amplitudes of the fast and slow components, and \( T_{\text{fast}} \) and \( T_{\text{slow}} \) are the activation time constants, respectively.

**Current clamp.** In current-clamp experiments, the pipette resistances were 8–10 MΩ when ﬁlled with internal solution consisting of (in mM) 120 K-methanesulfonate, 20 KCl, 5 NaCl, 10 HEPES, 10 EGTA, 1 MgCl₂, and 2 Mg-ATP (pH 7.3, 280 mosM). The stability of the V̇rest was checked for at least 1 min prior to evoking action potentials and was monitored throughout the recording. Then a series of brief depolarizing current steps (100-pA step, 1 ms) were injected to evaluate action potentials in a fast current-clamp mode (Magistretti et al. 1996). Action potential parameters such as the duration at 50% of amplitude (action potential half-duration), maximal rate of rise (dV/dtmax), maximal rate of fall (−dV/dtmax), and the AHP 10–90% recovery time (AHP repolarization time) were measured.

The pattern and the number of action potentials discharged were determined by injecting a series of depolarizing current steps (500-ms duration). The minimum current required to evoke the ﬁrst action potential (rheobase) was determined by current injection steps (50–100-pA increments), and then a current step three times rheobase was applied. A series of hyperpolarizing current steps (25–50-pA increment, 500-ms duration) were also applied to check the rectiﬁcation of the cell. A rectiﬁcation index (%) of the voltage responses to hyperpolarizing current was calculated as \([1 \times (V_{\text{peak}} - V_{\text{steady-state}})] / V_{\text{peak}} \times 100 \) in both control and ZD7288-applied conditions (Viana et al. 2002). The rectiﬁcation index increased as V̇peak was more hyperpolarized, and the maximum index value with stable recording (V̇peak = −100 mV to −120 mV) was compared. The rectiﬁcation index in the presence of ZD7288 was between 1% and 4%; therefore the neurons with a rectiﬁcation index of >5% were considered to have Iₚ (Cabanes et al. 2002).

**Drug application.** ZD7288 was dissolved in distilled water (100 mM, stock concentration), and capsaicin (Sigma) stock solution was made in 100% ethanol (10 mM) and stored at 4°C. Both stock solutions were diluted to working concentrations (dilution factor = 1:1,000) in HEPES-buffered saline before use. Drugs were perfused by a gravity-driven valve controller system (VC-6M, Warner Instruments). The ﬂow rate was ~0.25–0.3 ml/min, and the application tube was placed at ~0.5–1 mm to minimize the ﬂow pressure effect on the cell and to reduce drug diffusión.

**Western blot.** Sprague-Dawley rats were deeply anesthetized with isoflurane (4%) and decapitated. Both TGs were quickly removed and sonicated immediately in ice-cold lysis buffer (CellLytic MT, Sigma) with phenylmethylsulfonyl ﬂuoride (50 μM, Sigma) and protease inhibitor cocktail (1:100, Sigma P8340). Lysates were centrifuged, and the protein concentration of the supernatant was measured with the Bio-Rad Protein Assay (Regent’s Park, Australia). Equal amounts of protein (30 μg) were separated on an 8% sodium dodecyl sulfate-polyacrylamide gel. The protein was then transferred overnight at 30 mV and 4°C onto a polyvinylidene difluoride membrane (Hybond-P, Amersham, Melbourne, Australia). Membranes were blocked overnight at 4°C in a blocking solution consisting of 5% skim milk powder in PBS plus 0.1% Tween 20 (PBS-T). Blots were then incubated with rabbit anti-HCN antibody (dilution 1:250 for anti-HCN1, 1:2,000 for anti-HCN2, 1:500 for anti-HCN3, and 1:200 for anti-HCN4) and rabbit anti-α-actin antibody (dilution 1:2,000, Sigma) overnight at 4°C.
RESULTS

In the present study, whole cell voltage-clamp or current-clamp recordings from acutely dissociated TG neurons from rats at P1, P7, P14, P21, and P35 were used for analysis. For simplicity, data from cells at P1 and P35 are presented in this section and all data, including P7, P14, and P21, are summarized in the tables.

Passive properties of \( I_h \) positive neurons. Small- to medium-sized capsaicin-sensitive neurons were investigated, as these sensory neurons have previously been shown to express \( I_h \) (Momin et al. 2008; Scroggs et al. 1994; Tu et al. 2004). \( I_h \) could be recorded from all neurons examined. In addition, ZD7288 (100 \( \mu \)M) was applied to all neurons after the initial characterization. The \( V_{rest} \) was more depolarized in older animals compared with younger animals; it was \(-62.1 \pm 0.8 \text{ mV} \) (\( n = 21 \)) to \(-59.4 \pm 0.9 \text{ mV} \) (\( n = 21 \)) at P35 (1-way ANOVA of P1, P7, P14, P21, and P35, \( P < 0.01 \)). The cell capacitance, an indication of cell size, increased from 24.0 ± 2.0 \( \text{pF} \) (\( n = 21 \), P1) to 52.6 ± 5.0 \( \text{pF} \) (\( n = 21 \), P35; ANOVA, \( P < 0.0001 \)), and the membrane resistance significantly decreased with postnatal maturity, from a median of 266 \( \text{M}\Omega \) [25th percentile (212 \( \text{M}\Omega \))–75th percentile (395 \( \text{M}\Omega \))] at P35 (\( P < 0.05 \); Table 1).

Consistent with the decrease in membrane resistance, the minimum current to evoke the first action potential (rheobase) increased from 382 ± 239 \( \text{pA} \) (P35, \( n = 10 \)) to 722 ± 239 \( \text{pA} \) (P35, \( n = 10 \)) but was not significant (ANOVA, \( P > 0.05 \); Table 1).

Table 1. Summary of passive membrane properties and rectification of sampled TG neurons

<table>
<thead>
<tr>
<th>( V_{rest} ), mV</th>
<th>( n )</th>
<th>( C_c ), ( \text{pF} )</th>
<th>( R_m ), ( \text{M}\Omega )</th>
<th>( I_h ), pA</th>
<th>( R_{index} ), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>-62.1 ± 0.8</td>
<td>21</td>
<td>24.0 ± 2.0</td>
<td>21</td>
<td>266 (212–395)</td>
</tr>
<tr>
<td>P7</td>
<td>-60.0 ± 1.2</td>
<td>19</td>
<td>36.0 ± 2.4</td>
<td>19</td>
<td>181 (117–331)</td>
</tr>
<tr>
<td>P14</td>
<td>-58.8 ± 1.0</td>
<td>26</td>
<td>42.8 ± 3.4</td>
<td>26</td>
<td>170 (81–310)</td>
</tr>
<tr>
<td>P21</td>
<td>-55.7 ± 1.0</td>
<td>14</td>
<td>47.5 ± 2.9</td>
<td>14</td>
<td>152 (91–203)</td>
</tr>
<tr>
<td>P35</td>
<td>-59.4 ± 0.9</td>
<td>21</td>
<td>52.6 ± 5.0</td>
<td>21</td>
<td>148 (92–205)</td>
</tr>
<tr>
<td>Overall</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
<td>NE</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means ± SE or medians (25th–75th percentile) are given for each parameter. TG, trigeminal ganglion; \( P \), postnatal day; \( V_{rest} \), resting membrane potential; \( C_c \), membrane capacitance; \( R_m \), membrane resistance; \( I_h \), rheobase; \( R_{index} \), rectification index; NE, no experimental data; NS, not significant.

4°C. Washes were done with PBS-T (3 times, 15, 5, and 5 min each). Blots were then incubated with an horseradish peroxidase (HRP)-conjugated donkey anti-rabbit antiserum (dilution 1:2,000, Amersham) for 1 h at RT. After a final wash, protein immunoreactivity was visualized by enhanced chemiluminescence (ECL, Amersham) and exposed to Hyperfilm ECL (Amersham). Molecular weight was estimated with Precision Plus Protein Standards (Bio-Rad). The band intensity signal was quantified with Image J software (v1.32). National Institutes of Health.

\( HCN \) antibodies. The following antibodies were used in this study: 1) rabbit anti-HCN1 raised against 549–862 (QAGSRTVPQVRQTL) of the rat HCN1 protein; 2) rabbit anti-HCN2 raised against 849–863 (CLDPPLSDARSLSNL) of the rat HCN2 protein; 3) rabbit anti-HCN3 raised against 586–599 (RGLAPGTLARGLSKLG) of the rat HCN3 protein; 4) rabbit anti-HCN4 raised against 119–155 (HGHLHDSAEERRLIAEGDASPGEDRTPPGLAAEPERP) of the human HCN4 protein and with rat HCN4 protein sharing 35/37 residues. Anti-HCN1, anti-HCN2, and anti-HCN3 were gifts from GlaxoSmithKline (Harlow, UK), and peptide preincubation tests for HCN1 and HCN2 were done previously in our lab (Cho et al. 2009a; Xiao et al. 2004). The antigen peptide that the antibody to HCN3 was raised against was synthesized (Auspep, Parkville, VIC, Australia) for a 2004. The antigen peptide that the antibody to HCN3 was raised against was synthesized (Auspep, Parkville, VIC, Australia) for a 2004. The antigen peptide that the antibody to HCN3 was raised against was synthesized (Auspep, Parkville, VIC, Australia) for a 2004. The antigen peptide that the antibody to HCN3 was raised against was synthesized (Auspep, Parkville, VIC, Australia) for a 2004.
voltage sag following hyperpolarizing current steps (Fig. 1A, top). The I-V relationship of peak and steady-state potentials of P1 and P35 is shown in Fig. 1B. In neonatal neurons, the peak hyperpolarized voltage was greater than that of adults, indicating a higher membrane resistance (Table 1). The changes in voltage response from peak hyperpolarization (i.e., depolarizing sag, between −100 mV and −120 mV) to steady state were calculated and presented as a rectification index (see Cabanes et al. 2002; Viana et al. 2002). The rectification index was not different between adults and neonates: 24.5 ± 2.1% (P1, n = 11) and 19.7 ± 2.8% (P35, n = 10; ANOVA, P > 0.05; Table 1). A few minutes after ZD7288 application, the depolarizing sag was abolished and less current was required to cause hyperpolarization, indicating that input resistance was increased (Fig. 1A, middle).

The slowly developing, noninactivating inward current, $I_h$, recorded in voltage-clamp mode, was activated by stepping the membrane voltage from a holding potential (−60 mV) in a series of voltage steps (−40 mV to −120 mV). Membrane voltages more negative than −120 mV usually resulted in

![Diagram](image_url)

Fig. 2. Age-related characteristics of $I_h$ revealed in voltage-clamp experiments. A: time course of the onset of the inward current activated by hyperpolarizing voltage steps in a voltage-clamp experiment from a P35 rat trigeminal ganglion (TG) neuron. B: no slowly activating inward current was apparent when the same voltage step protocol was run in the presence of ZD7288 (100 μM). C: the residual current (A − B). Insets on left show the early phases of current activation on an expanded time scale. D: example of $I_h$ activated by a −90-mV hyperpolarizing voltage step, that did not reach a steady state even at 4 s when recorded at room temperature (RT) but was almost fully activated by 2 s at 35°C. Current traces were normalized to the maximum current amplitude of each trace for better comparison of the time course. E: current density (steady-state current amplitude (squares) − instantaneous current amplitude (circles))/cell capacitance) of $I_h$ activated by hyperpolarizing voltage steps. Error bars have been omitted for clarity, and these values are shown in Table 2. F: maximum $I_h$ density at −120 mV (35°C) and at −130 mV (RT) at different postnatal ages. F and H: *significance of data compared to P1; #significance compared within the same age group to recordings at 35°C. ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). G: normalized $I_h$ voltage activation curves, derived from tail currents. H: $I_h$ half-maximal activation voltage ($V_{1/2}$) at different ages. $V_{1/2}$ was significantly more depolarized at older postnatal ages.
unstable current traces at 35°C, but at RT hyperpolarizing the membrane to −130 mV produced a stable current trace. However, at RT, Ih often did not reach a steady state, even after 4 s, while it reached steady state within 2 s in most cases at 35°C. These observations are consistent with the previous findings that Ih is highly temperature dependent (Q10 = 3–5; Hogg et al. 2001; Magee 1998; Orio et al. 2009; Pape 1996; Fig. 2, D–F).

This slow inward current completely disappeared after ZD7288 application in a time-dependent manner (the full effect required at least 1-min exposure to ZD7288; Fig. 2B). Control experiments were carried out in which rundown was recorded. The ZD7288 effect was within 2–3 min of application and completely abolished Ih, while rundown is not obvious during this short period of time and was usually only a small reduction of Ih. The remaining current is thought to comprise an inward-rectifying potassium current and a leak current (Scroggs et al. 1994). Ih density was calculated from the amplitude of ZD7288-specific current (Fig. 2C) divided by cell capacitance (an indirect measure of membrane surface area). Although Ih density was highly variable, the maximum current density measured at −120 mV was not significantly different between age groups, such that the median was −15.8 pA/pF (−3.7 to −26.8 pA/pF) at P1 (n = 9) and −8.7 pA/pF (−6.5 to −18.8 pA/pF) at P35 (n = 11, Kruskal-Wallis test, P > 0.05; Fig. 2, E and F, Table 2). While the median Ih density decreased during maturation, there was no significant correlation between age and density (Spearman test, P > 0.05). In the studies done at RT, the maximum current amplitude was measured 4 s after a −130-mV hyperpolarizing voltage step. The Ih density, however, was smaller than that measured at −120 mV at 35°C [significant at P7, −8.6 ± 1.3 pA/pF, n = 14 compared with −15.4 (−10.5 to −35.9 pA/pF), n = 13, Mann-Whitney, P < 0.01]. At RT, there was also no difference in Ih density (Kruskal-Wallis test, P > 0.05) during postnatal maturation (Fig. 2F, Table 2).

The voltage dependence of Ih activation was presented as an activation curve derived from tail current normalization (Fig. 2G). Ih activation was observed from around −50 mV and reached a maximum at −120 mV (−130 mV at RT), the most hyperpolarized potentials used in this study. The activation curve shifted to more depolarized potentials with postnatal age such that the V1/2 moved from −89.4 ± 2.3 mV (n = 9, P1) to −81.6 ± 1.4 mV (n = 11, P35; ANOVA, P < 0.001) without changing the slope factor (7.8 ± 1.0 at P1 and 8.3 ± 0.7 at P35; ANOVA, P > 0.05). At RT, V1/2 was more hyperpolarized compared with that recorded at 35°C (e.g., −97.8 ± 2.4 mV, n = 15, P1; Student’s t-test, P > 0.05). At RT, V1/2 also became significantly more depolarized with postnatal age (−85.8 ± 2.9 mV at P35, n = 8; ANOVA, P < 0.001; Fig. 2, G and H, and Table 2).

To determine the reversal potential of Ih, the linear instantaneous current-voltage (Iinst–V) relationship at voltage steps (−60 mV to −100 mV, −10-mV increment) from a holding potential, −60 mV or −100 mV, was obtained to get the chord conductance at each holding potential. Extrapolation of the two lines yields a reversal potential of Ih where the conductance difference becomes zero. The reversal potential of Ih estimated with this method was −37.1 ± 3.0 mV in TG neurons (n = 14 from animals 14–35 days old; there was no significant age-dependent difference).

HCN protein subtype changes in developing TG. We also tested for the presence of all subtypes of HCN protein at different postnatal ages (P1, P7, P14, P21, and P35) in rat TG with Western blot (Fig. 3). Cortical brain tissue from a P21 rat was used in every experiment as a positive tissue control (Notomi and Shigemoto 2004). Bands immunoreactive for each of the HCN subtypes were detected in both rat TGs and brain around the corresponding molecular mass for rat HCN1 (102 kDa), rat HCN2 (95 kDa), rat HCN3 (87 kDa), and rat HCN4 (129 kDa) (Fig. 3A). The optical density (OD) of each HCN band was normalized against the α-actin band density, which was used as a loading control (Fig. 3B). The HCN1 band density increased most quickly to P14 and then reached a plateau such that the HCN1 band at P1 was visible (0.47 ± 0.10 OD, n = 6), although lighter than the mature levels at P14 (0.93 ± 0.15 OD, n = 6; Student’s t-test, P < 0.05 compared with P1). The HCN2 band density was hardly detected at P1 (0.19 ± 0.03 OD, n = 6) but continuously increased until P35 (1.27 ± 0.26 OD, n = 5; Student’s t-test, P < 0.05 compared with P1). HCN3 was very faint and did not significantly change with postnatal maturation (0.14 ± 0.08 OD at P1 and 0.28 ± 0.05 OD at P35, n = 5 each; Student’s t-test, P > 0.05 compared with P1). The HCN4 protein band was visible at P1 (0.59 ± 0.13 OD, n = 4) and did not change significantly over

Table 2. Summary of Ih properties of sampled TG neurons

<table>
<thead>
<tr>
<th></th>
<th>Ih Density, pA/pF</th>
<th>n</th>
<th>V1/2, mV</th>
<th>Slope Factor</th>
<th>n</th>
<th>τinst, ms</th>
<th>τslow, ms</th>
<th>Aτinst/(Aτinst+Aτslow)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>35°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>−15.8 (−3.7 to −26.8)</td>
<td>9</td>
<td>−89.4 ± 2.3</td>
<td>7.8 ± 1.0</td>
<td>9</td>
<td>198.8 ± 42.9</td>
<td>98.6 ± 95.7</td>
<td>0.45 ± 0.06</td>
<td>10</td>
</tr>
<tr>
<td>P7</td>
<td>−15.4 (−10.5 to −25.9)</td>
<td>13</td>
<td>−85.8 ± 0.6</td>
<td>9.0 ± 0.5</td>
<td>13</td>
<td>124.3 ± 24.3</td>
<td>92.8 ± 155.6</td>
<td>0.55 ± 0.06</td>
<td>13</td>
</tr>
<tr>
<td>P14</td>
<td>−12.0 (−5.9 to −27.4)</td>
<td>15</td>
<td>−85.6 ± 1.0</td>
<td>8.2 ± 0.8</td>
<td>14</td>
<td>104.2 ± 14.5</td>
<td>86.3 ± 99.3</td>
<td>0.51 ± 0.06</td>
<td>15</td>
</tr>
<tr>
<td>P21</td>
<td>−8.9 (−4.2 to −19.4)</td>
<td>14</td>
<td>−82.6 ± 0.9</td>
<td>8.9 ± 1.0</td>
<td>14</td>
<td>94.7 ± 12.3</td>
<td>616.7 ± 38.3</td>
<td>0.53 ± 0.05</td>
<td>15</td>
</tr>
<tr>
<td>P35</td>
<td>−8.7 (−6.5 to −18.8)</td>
<td>11</td>
<td>−81.6 ± 1.4</td>
<td>8.3 ± 0.7</td>
<td>11</td>
<td>65.1 ± 7.6</td>
<td>490.2 ± 68.3</td>
<td>0.58 ± 0.04</td>
<td>11</td>
</tr>
<tr>
<td>Overall P</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<td>RT</td>
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<tr>
<td>P1</td>
<td>−11.3 ± 1.8</td>
<td>12</td>
<td>−98.7 ± 2.4</td>
<td>7.3 ± 0.5</td>
<td>13</td>
<td>425.0 ± 39.9</td>
<td>2,603.7 ± 418.2</td>
<td>0.44 ± 0.06</td>
<td>12</td>
</tr>
<tr>
<td>P7</td>
<td>−8.6 ± 1.3</td>
<td>14</td>
<td>−99.1 ± 1.6</td>
<td>9.0 ± 0.5</td>
<td>14</td>
<td>401.0 ± 44.9</td>
<td>2,469.4 ± 305.0</td>
<td>0.25 ± 0.05</td>
<td>13</td>
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<tr>
<td>P14</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
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<tr>
<td>P21</td>
<td>−9.5 ± 2.5</td>
<td>11</td>
<td>−91.3 ± 1.1</td>
<td>7.9 ± 0.3</td>
<td>14</td>
<td>286.0 ± 47.3</td>
<td>1,717.4 ± 211.5</td>
<td>0.45 ± 0.05</td>
<td>13</td>
</tr>
<tr>
<td>P35</td>
<td>−6.7 ± 1.3</td>
<td>7</td>
<td>−85.8 ± 2.9</td>
<td>8.1 ± 0.6</td>
<td>8</td>
<td>228.2 ± 33.5</td>
<td>1,657.0 ± 281.9</td>
<td>0.41 ± 0.08</td>
<td>8</td>
</tr>
<tr>
<td>Overall P</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Means ± SE or medians (25th–75th percentile) are given for each parameter. NE, not examined; NS, not significant. Ih density was measured at −120 mV (35°C) and at −130 mV (room temperature (RT)).
functions, and two time constants, voltage steps was well fitted with a sum of two exponential
(Ih in repetitive firing of developing TG neurons by applying a depolarizing current step (500 ms, 3
times rheobase). Most neurons studied at P1 (10/11) showed only a single spike even at three times rheobase current
injection, while multiple-firing neurons were frequently observed in older animals (8/10 at P35; Fig. 6). After ZD7288

animals (65.1 ± 7.6 ms and 490.2 ± 68.3 ms, n = 11, P35) than in younger animals (198.8 ± 42.9 ms and 980.6 ± 95.7 ms, n = 10, P1; ANOVA, P < 0.01 for both τ_fast and τ_slow). There was no significant change in the amplitude ratio of fast and slow components [A_fast/(A_fast + A_slow)] between ages (ANOVA, P > 0.05; Fig. 4, B and C, and Table 2).

In experiments at RT, current activation time constants were significantly slower than those at 35°C. For example, at P1, τ_fast and τ_slow were 425.0 ± 39.9 ms and 2,603.7 ± 418.2 ms, respectively (Student’s t-test, P < 0.001 and P < 0.01, respectively; Fig. 4, B and C, and Table 2). As in the experiments at 35°C, both time constants were also faster with postnatal maturation. Although the time constants decreased and cell capacitance increased with age (see negative correlation in Fig. 4, D and E), the correlation between the time constant and cell capacitance at an individual age group was not obvious and was only significant for τ_fast at P1 (RT) and τ_slow at P7 (35°C; Fig. 4, D and E).

Role of Ih in neuronal excitability. The V_rest measured 5 min after achieving whole cell configuration was depolarized in older rats (−60.5 ± 8.0 mV, n = 10, P35) compared with neonates (−65.1 ± 1.3 mV, n = 11, P1; ANOVA, P < 0.001). As Ih activation can be observed from around −50 mV in postnatal sensory neurons (Fig. 2G), we hypothesized that blocking Ih (a depolarizing current) would hyperpolarize the V_rest. Within 3 min of ZD7288 application, the membrane potential of cells of all ages examined was significantly hyperpolarized in a time-dependent manner, for example, at P1, from −65.1 ± 1.3 mV to −67.5 ± 1.4 mV (n = 11, Student’s t-test, P < 0.05; Fig. 5 and Table 3).

To investigate how Ih activation affected the active membrane properties of developing TG nociceptive neurons, the shapes of single action potentials evoked by a short depolarizing pulse (1 ms) before and during application of ZD7288 were compared. Figure 5A shows an example of an action potential in a TG neuron with a spike followed by an AHP. ZD7288 increased the action potential half-duration (from 0.5 ± 0.1 ms to 0.8 ± 0.1 ms at P1 and from 0.7 ± 0.1 to 1.1 ± 0.1 ms at P35; Student’s t-test, P < 0.001 in both groups) and slowed the maximal rate of rise and fall of action potentials significantly at P1, P14, and P35. The rise rate decreased from 240.6 ± 29.8 to 187.1 ± 23.3 mV/ms at P1 (Student’s t-test, P < 0.01) and from 201.3 ± 18.3 to 138.4 ± 8.1 mV/ms at P35 (Student’s t-test, P < 0.01; Fig. 5B). The falling rate also decreased from −107.4 ± 15.1 to −88.6 ± 9.3 mV/ms at P1 (Student’s t-test, P < 0.05) and from −93.5 ± 5.2 to −70.4 ± 4.2 mV/ms at P35 (Student’s t-test, P < 0.01; Fig. 5B). The 10–90% decay time of the AHP (from its peak back to V_rest) also increased from 0.19 ± 0.03 to 0.29 ± 0.05 ms at P1 and from 0.24 ± 0.02 to 0.43 ± 0.04 ms at P35 (Student’s t-test, P < 0.05 at P1, P < 0.01 at P35; Fig. 5A). Parameters at all postnatal time points are summarized in Table 3.

As previous studies in adult sensory neurons have revealed an important role of Ih in generating repetitive discharge (Hogan and Poroli 2008; Momtaz et al. 2008), we investigated the involvement of Ih in repetitive firing of developing TG neurons by applying a depolarizing current step (500 ms, 3 times rheobase). Most neurons studied at P1 (10/11) showed only a single spike even at three times rheobase current injection, while multiple-firing neurons were frequently observed in older animals (8/10 at P35; Fig. 6). After ZD7288

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**Fig. 3.** Western blot of hyperpolarization-activated cyclic nucleotide-gated channel (HCN) subtypes in TG at different postnatal ages. A: immunoreactive bands of all 4 isoforms were detected with different densities dependent on age. In right lane, P21 cortical brain tissue (Br) was used in every experiment as a positive tissue control. Each HCN band density was normalized to the α-actin band density examined from the same membrane. B: normalized band density [optical density (OD)] of HCN subtypes at different postnatal ages (labeled as gray to black as indicated at top). *Significance compared with P1. C: ratio of individual HCN subtypes (as % of total HCN) expression levels at different postnatal ages.

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The 5-wk time period examined (0.84 ± 0.22 OD, n = 4; Student’s t-test, P > 0.05 compared with P1). Comparison of the relative ratio of each subtype revealed that the ratio of HCN4 showed a clearly decreasing pattern until P14, after which it remained stable (Fig. 3C).

As all HCN subtypes were detected in TGs in the present study (Fig. 3) and each subtype has been shown to have different activation kinetics when expressed heterologously (Moosmang et al. 2001; Stieber et al. 2005), the onset kinetics of Ih were analyzed and compared with the protein level changes during development. Ih evoked by hyperpolarizing voltage steps was well fitted with a sum of two exponential functions, and two time constants, τ_fast and τ_slow, were derived (Fig. 4A). Both of the time constants were voltage dependent and were quicker at more hyperpolarized holding potentials. They were also age dependent such that at the −90 mV voltage step both τ_fast and τ_slow were significantly faster in older
The firing frequency of the multiple-firing neurons at P35 was significantly reduced from a median of 26 Hz (15–95 Hz) to 10 Hz (4–49 Hz, Wilcoxon matched pairs test, \( P < 0.05 \); Fig. 6B).

At the end of the above current-clamp recordings, the amplifier mode was switched to voltage clamp to examine responses to capsaicin (30 \( \mu \)M), an agonist of transient receptor potential vanilloid 1 (TRPV1).

**DISCUSSION**

The present study shows that HCN channel protein and \( I_h \) conducted through the channel are present from birth and are able to influence the excitability of sensory neurons at all postnatal ages. The present observation that \( I_h \) both becomes active at more depolarized potentials and has faster activation kinetics suggests that \( I_h \) contribution to somatosensory and nociceptive processing in sensory neurons is also likely to increase during development.

**Discussion**

The present study shows that HCN channel protein and \( I_h \) conducted through the channel are present from birth and are able to influence the excitability of sensory neurons at all postnatal ages. The present observation that \( I_h \) both becomes active at more depolarized potentials and has faster activation kinetics suggests that \( I_h \) contribution to somatosensory and nociceptive processing in sensory neurons is also likely to increase during development.

\( I_h \) is activated in developing TG neurons. \( I_h \) was found in TG neurons of rats from newborn to 5 wk old (the oldest investigated). Other studies have reported \( I_h \) in adult rat DRG and TG neurons, where it occurs in 100%, 92%, and 45% of large-, medium-, and small-sized neurons, respectively (Cabanes et al. 2002; Momin et al. 2008; Scroggs et al. 1994; Tu et al. 2004).
In the present study, small- to medium-sized capsaicin-sensitive neurons were preferentially recorded at every age and the properties of $I_h$ were compared. Our data, cell diameter (17.8 ± 0.5 μm at P1 and 25.3 ± 0.9 μm at P35) and membrane capacitance (52.6 ± 5.0 pF at P35), fall within the parameter described by previous studies for small- to medium-sized cells (Cabanes et al. 2002; Scroggs et al. 1994).

Few other studies have examined $I_h$ development in sensory neurons. In one study, $I_h$ was first detected around embryonic day 10 in both TG and DRG of quail and was present in 75% of neurons during early postnatal development (Schlichter et al. 1991). In another study (published in abstract form only), $I_h$ was present in 20% of rat DRG neurons at P1, increasing to 100% at P14 (Fulton 1987). All of these studies were done at RT. In the present study, a few small-sized neurons with no $I_h$ were found at each postnatal age in recordings at RT; however, all cells sampled at 35°C exhibited an $I_h$.

The density of $I_h$ (the amplitude of $I_h$ per unit of cell capacitance) showed a great degree of variability at 35°C but was not significantly different in neurons of any postnatal age. The variability of current density has been previously reported to change significantly over the postnatal period, it is likely that $I_h$ has a greater role in shaping neuronal responses as rats mature (see below).

**HCN subtype proteins and $I_h$ properties change in developing postnatal TG neurons.** The relative levels of the HCN channel proteins were developmentally regulated. All HCN channel protein subtypes were detected by Western blot at every postnatal age, with levels of HCN1 and HCN2 proteins increasing and the proportions of HCN4 protein decreasing with age (Fig. 3). The relative abundance of HCN protein is associated with differences in the biophysical properties of $I_h$, e.g., kinetics, voltage dependence, and cAMP sensitivity (Kanyshkova et al. 2009; Santoro et al. 2000; Surges et al. 2006).

The increasing levels of HCN1 and HCN2 proteins and/or the decreasing proportions of HCN4 protein decrease with age (Fig. 3). The relative abundance of HCN protein is associated with differences in the biophysical properties of $I_h$, e.g., kinetics, voltage dependence, and cAMP sensitivity (Kanyshkova et al. 2009; Santoro et al. 2000; Surges et al. 2006).

Despite the greater amount of protein that was revealed by Western blot analysis, $I_h$ current density remained the same during development. This implies that the proportion of protein in the ganglion that is functional, in that it contributes to membrane current, decreases during development. This could mean that there is more protein in reserve that could be inserted into the membrane and contribute to plastic changes in the neurons. A proportion of protein that is nonfunctional in the nerve cells could be destined for export to the nerve terminals.

Experiments in heterologous expression systems have shown that both homomeric and heteromeric HCN channels can form (Much et al. 2003). In individual DRG neurons, there are no depolarized potentials (Fig. 2) since HCN1 has the most accelerated channel activation kinetics seen in older neurons (Fig. 4). In addition, significant increases of HCN1 and HCN2 proteins are likely to contribute to the $V_{1/2}$ shift to more depolarized potentials (Fig. 2E) since HCN1 has the most depolarized $V_{1/2}$ under basal conditions (Stieber et al. 2005).

![Fig. 5.](http://jn.physiology.org/figures/2052a.png) Shape of action potential before and after ZD7288. A: single action potential (AP) evoked by brief (1 ms) depolarizing current injection shown in black (control) and gray (after ZD7288). B: 1st derivative ($dV/dt$) of the voltage trace shown in A. Bottom: current injected. Parameters measured: resting membrane potential (a), AP duration at 50% of amplitude (b), AP maximum rate of rise (c); AP maximum rate of fall (d), afterhyperpolarization (AHP) decay time between 10% and 90% of amplitude (e).

**Table 3. Summary of parameters measured as shown in Fig. 5.**

<table>
<thead>
<tr>
<th></th>
<th>$V_{rest}$, mV (a)</th>
<th>AP half-width, ms (b)</th>
<th>$dV/dt_{max}$, mV/ms (c)</th>
<th>$-dV/dt_{max}$, mV/ms (d)</th>
<th>AHP depol time, ms (e)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
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<tr>
<td>P1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-65.1 ± 1.3$</td>
<td>0.5 ± 0.1</td>
<td>$240.6 ± 29.8$</td>
<td>$-107.4 ± 15.1$</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>ZD7288</td>
<td>$-67.5 ± 1.4^*$</td>
<td>0.8 ± 0.1~</td>
<td>$187.1 ± 23.3^+$</td>
<td>$-88.6 ± 9.3^*$</td>
<td>0.29 ± 0.05*</td>
</tr>
<tr>
<td>P7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-63.7 ± 1.7$</td>
<td>0.6 ± 0.1</td>
<td>$260.1 ± 25.6$</td>
<td>$-106.9 ± 12.5$</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>ZD7288</td>
<td>$-65.4 ± 2.1$</td>
<td>0.8 ± 0.1~</td>
<td>$220.6 ± 22.7$</td>
<td>$-109.2 ± 9.4$</td>
<td>0.26 ± 0.04*</td>
</tr>
<tr>
<td>P14</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>$-62.5 ± 1.0$</td>
<td>0.7 ± 0.1</td>
<td>$223.1 ± 12.5$</td>
<td>$-108.6 ± 12.7$</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>ZD7288</td>
<td>$-64.1 ± 1.0$</td>
<td>1.1 ± 0.1~</td>
<td>$170.7 ± 14.3^+$</td>
<td>$-85.1 ± 9.2^*$</td>
<td>0.36 ± 0.03~</td>
</tr>
<tr>
<td>P35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-60.5 ± 0.8$</td>
<td>0.7 ± 0.1</td>
<td>$201.3 ± 18.3$</td>
<td>$-93.5 ± 5.2$</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>ZD7288</td>
<td>$-62.6 ± 1.5^*$</td>
<td>1.1 ± 0.1~</td>
<td>$138.4 ± 8.1$</td>
<td>$-70.4 ± 4.2^+$</td>
<td>0.43 ± 0.04~</td>
</tr>
</tbody>
</table>

Means ± SE are given for each parameter; n = 6–11 in each group. $V_{rest}$, resting membrane potential; AP, action potential; $dV/dt$, maximal rate of rise; $-dV/dt$, maximal rate of fall; AHP, afterhyperpolarization; depol, depolarization. Significance between control and ZD7288 treated at each age group: *P < 0.05, †P < 0.01, and ‡P < 0.001. Lower case letters refer to presentation in Fig. 5.

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is immunohistochemical evidence for more than one HCN channel subtype, suggesting the presence of heteromeric channels (Chaplan et al. 2003; Tu et al. 2004). Colocalization was not investigated in individual TG neurons in the three studies published to date (Cho et al. 2009a, 2009b; Wells et al. 2007). However, we have shown that HCN1 and HCN2 are found in similar-sized neurons (Cho et al. 2009a). A previous study reported that \( I_h \) activation kinetics were faster in large-sized than in small-sized DRG neurons recorded at RT (Momin et al. 2008). In the present study this relationship between cell size and activation kinetics was only apparent in studies at RT at P1 and in studies at 35°C at P7. However, our sample did not cover the entire range of sensory neuron sizes, and there were developmental changes in the relative distribution of HCN channel subtypes and significant increases in activation kinetics with age.

\( I_h \) is involved in neuronal excitability of developing TG neurons. The best-characterized role of \( I_h \) is in rhythm generation in cardiac cells (DiFrancesco 1993) and in other spontaneous firing neurons (Maccabberi and McBain 1996; McCormick and Pape 1990). In some excitable neurons, \( I_h \) contributes more to determining passive membrane properties such as \( V_{\text{rest}} \) and input resistance (Williams et al. 2002). In these cells \( I_h \) tends to be a membrane-stabilizing current, increasing at hyperpolarized potentials and decreasing with depolarization.

In the present study, \( I_h \) was active at rest, and it enhanced repetitive action potential firing, as shown by the action of ZD7288, which hyperpolarized the neurons by 2 mV and reduced the frequency of action potentials elicited by a current at three times rheobase from 26 to 10 Hz. Some studies have reported that ZD7288 increased the frequency of action potentials as a result of increased input resistance (Doan et al. 2004; Li et al. 2008). In our study, input resistance was slightly increased after ZD7288, but that was not enough to increase the excitability. This discrepancy could be due to the different cell types that were studied. Both papers that showed increased excitability used nodose ganglion neurons, while all studies in sensory neurons, DRG and TG neurons, have found decreased excitability.

In response to prolonged intracellular injection of depolarizing current, mature TG neurons either discharge repetitively or fire only single action potentials (Catacuzzeno et al. 2008). We found that most P1–P7 TG neurons fired a single action potential and observed multiple-firing neurons more frequently at later developmental stages. This increase in the firing frequency of DRG neurons at older postnatal ages has been observed in vivo (Fitzgerald 1987) and is likely to reflect the developmental regulation of voltage-gated ion channels, including \( \text{Na}^+ \), \( \text{K}^+ \), and \( \text{Ca}^{2+} \) channels (Fedulova et al. 1991; Rush et al. 2007; Seifert et al. 1999). The results of the present work suggest that HCN channels also contribute to these postnatal changes in sensory neuron firing frequency.

Roles of \( I_h \) in shaping the action potential and in determining firing properties. ZD7288 also changed the action potential shape, which had a longer half-duration, slower rates of rise and fall, and a prolonged recovery of the AHP (Fig. 5, Table 3). These findings are in agreement with previous studies (Hogan and Poroli 2008; Kouranov et al. 2008; Masuda et al. 2006; Orio et al. 2009; Tanaka et al. 2003). Slower action potential rising and falling rates and longer half-durations were observed after ZD7288 by Tanaka et al. (2003) and in the present work. Because \( I_h \) kinetics are considerably slower than the action potential, the sculpting of the action potential is probably due to persistent \( I_h \) activity. During rapid depolarization (negative to the reversal potential of the HCN channel, −37.1 mV; see results), an inward conductance through the open HCN channels may contribute to depolarization. On the other hand, when the membrane potential is positive to the HCN channel reversal potential during the action potential peak, the outward conductance could speed action potential repolarization. A longer half-width was also shown when \( I_h \) conductance was set at zero in a DRG neuron computational model (Kouranov et al. 2008).

During the AHP, the HCN channel is reactivated and the inward \( I_h \) depolarizes the membrane until counterbalanced by outward potassium current (Pape 1996). Blocking \( I_h \) slowed the AHP decay time in TG neurons (present study) and DRG neurons (Hogan and Poroli 2008; Masuda et al. 2006). The slower AHP decay time in the presence of ZD7288 indicated that other currents contribute to the AHP. These may include a conductance through \( \text{Ca}^{2+} \)-activated potassium channels (Sarantopoulos et al. 2007). This, however, needs to be clarified, as ZD7288 has been found to partially block T-type voltage-gated calcium channels in some studies (Felix et al. 2003; Sanchez-Alonso et al. 2008).
Physiological relevance. In the present study, we showed that $I_h$ is important in determining the excitability of sensory neurons in newborn as well as adult rats. Since all neurons responded to capsaicin, an agonist at TRPV1 receptor (predominantly expressed in nociceptive afferents) (Caterina et al. 2000), it is likely that $I_h$ might modify nociception during postnatal maturation. In adult rats, $I_h$ has been shown to contribute to peripheral nerve hyperexcitability (Chaplan et al. 2003; Lee et al. 2005). In adult DRG and TG neurons, nerve injury (Chaplan et al. 2003; Kitagawa et al. 2006; Yao et al. 2003) as well as inflammation (Ingram and Williams 1996; Momin et al. 2008) result in increased $I_h$ conductance, accelerated current activation, and positively shifted $I_h$ activation voltages, thus contributing to the hyperexcitability of neurons in these pain states. The present observation that $I_h$ both becomes active at more depolarized potentials and has faster activation kinetics suggests that the $I_h$ contribution to somatosensory and nociceptive processing in sensory neurons is also likely to increase during postnatal development. This may contribute to the poor following characteristics of neonatal rat sensory neurons in the first postnatal week (Fitzgerald 1987).

It should be noted that the majority of in vitro studies that have examined $I_h$ in somatosensory neurons have been done at RT (Cabanes et al. 2002; Chaplan et al. 2003; Kouranova et al. 2008; Momin et al. 2008; Petruska et al. 2000; Schlichter et al. 1991; Scroggs et al. 1994; Yao et al. 2003). Results from the present study show that at RT $I_h$ has slower onset kinetics and more hyperpolarized half-activation voltages compared with recordings at 35°C, and probably contributes less to neuron excitability as a result. In addition, a greater percentage of the population showed $I_h$ at 35°C than at RT. This temperature relationship of $I_h$ has previously been reported in TG neurons (Cabanes et al. 2003) and intracardiac neurons (Hogg et al. 2001).

Conclusions. In this study, we have identified that the neonatal TG neuron expresses $I_h$ with density similar to adult neurons but with different voltage dependence and kinetics. The activation curve shifted to the right with age (Fig. 2G) and activation was more rapid (Fig. 5), yielding a greater prominence of $I_h$ with postnatal maturation. Thus $I_h$ has a role in trigeminal primary afferent neurons at birth, and its role increases as rats mature.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

H-j. Cho and E. A. Jennings designed these experiments. Data were collected and analyzed by H-j. Cho. E. A. Jennings and J. B. Furness also contributed to analysis; all authors contributed to interpretation. H-j. Cho prepared the first draft of the article. H-j. Cho, J. B. Furness, and E. A. Jennings revised the article critically for intellectual content.


