Diverse levels of an inwardly rectifying potassium conductance generate heterogeneous neuronal behavior in a population of dorsal cochlear nucleus pyramidal neurons

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Departments of 1Otolaryngology and 2Neurobiology, University of Pittsburgh School of Medicine; 3Center for the Neural Basis of Cognition, University of Pittsburgh and Carnegie Mellon; and 4Department of Mathematics, University of Pittsburgh, Pittsburgh, Pennsylvania; and 5Department of Physiology, School of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

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Leao RM, Li S, Doiron B, Tzounopoulos T. Diverse levels of an inwardly rectifying potassium conductance generate heterogeneous neuronal behavior in a population of dorsal cochlear nucleus pyramidal neurons. J Neurophysiol 107: 3008–3019, 2012. First published February 29, 2012; doi:10.1152/jn.00660.2011.—Homeostatic mechanisms maintain homogeneous neuronal behavior among neurons that exhibit substantial variability in the expression levels of their ionic conductances. In contrast, the mechanisms, which generate heterogeneous neuronal behavior across a neuronal population, remain poorly understood. We addressed this problem in the dorsal cochlear nucleus, where principal neurons exist in two qualitatively distinct states: spontaneously active or not spontaneously active. Our studies reveal that distinct activity states are generated by the differential levels of a Ba2+-sensitive, inwardly rectifying potassium conductance (Kir). Variability in Kir maximal conductance causes variations in the resting membrane potential (RMP). Low Kir conductance depolarizes RMP to voltages above the threshold for activating subthreshold-persistent sodium channels (NaP). Once NaP channels are activated, the RMP becomes unstable, and spontaneous firing is triggered. Our results provide a biophysical mechanism for generating neural heterogeneity, which may play a role in the encoding of sensory information.

THEORETICAL AND EXPERIMENTAL work has revealed that similar circuit and neuronal function can result from substantially different combinations of correlated and compensating intrinsic and synaptic conductances (Gaillard et al. 2009; Marder and Gaillard 2006; Prinz et al. 2004). In accordance with this view, recent studies have shown that variability in one or more conductances is accompanied by coordinated compensatory variations in other conductances, which maintain constant neuronal behavior (Achard and De Schutter 2006; Cao and Oertel 2011; Goldman et al. 2001; Golowasch et al. 2002; Grashow et al. 2010; Schulz et al. 2006, 2007; Sobie 2009; Swensen and Bean 2005; Taylor et al. 2009; Tobin and Cabreze 2006). These homeostatic mechanisms involve coordinated changes in channel densities, which push or pull excitability toward opposite directions, so that intrinsic variability produces an equivalent output response (MacLean et al. 2003; Schulz et al. 2006).

However, less is known about mechanisms that generate a heterogeneous output response within a neuronal population. To answer this question, we studied the cellular mechanisms underlying neural heterogeneity in dorsal cochlear nucleus (DCN) principal neurons. Numerous in vitro and in vivo studies in anesthetized and in awake animals have shown that each DCN principal neuron (fusiform cells) exists in one of two qualitatively distinct intrinsic states: a quiet state, where the membrane potential settles at a resting membrane potential (RMP) value, or a spontaneously active state, where action potentials (APs) are spontaneously discharged (Manis 1990; Parham et al. 2000; Parham and Kim 1992; Portfors and Roberts 2007; Roberts and Trussell 2010; Young and Davis 2001; Zhang and Oertel 1994). This unambiguous, qualitative difference allowed us to study the mechanisms via which the fusiform cell population creates variability in the spontaneous firing mode of its members. Our studies reveal a single conductance-based mechanism for generating neural heterogeneity.

MATERIALS AND METHODS

Brainstem slice preparation and electrophysiology. Coronal brain slices were prepared from ICR mice (postnatal days 17–25). The preparation and use of coronal slices containing DCN have been described in detail (Tzounopoulos et al. 2004). Animals were killed, according to methods approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Single cells were visualized with infrared interference contrast optics and recorded using patch pipettes in either voltage- or current-clamp modes. Fusion cells were identified based on their electrophysiological and morphological characteristics (for more details, see Tzounopoulos et al. 2004). The external solution contained (in mM): 130 NaCl, 3 KCl, 1.2 KH2PO4, 2.4 CaCl2, 1.3 MgSO4, 20 NaHCO3, 3 HEPES, and 10 glucose, saturated with 95% O2/5% CO2. For voltage- and current-clamp experiments, pipettes were filled with a K+-based internal solution containing (in mM): 113 K-glucosate, 4.5 MgCl2, 14 tris-phosphocreatine, 9 HEPES, 0.1 EGTA, 4 Na-ATP, 0.3 Tris-GTP, 10 sucrose, pH 7.3, and ~300 mOsmol. The fluorophore ALEXA 488 (10 µM) was added to the internal solution for visualization for the cell’s morphology after the experiment.

Whole-cell recordings were performed at 33–36°C using an inline heating system (Warner Instruments, Hamden, CT). Access resistance was monitored throughout the experiment from the size and shape of the capacitive transient in response to a 5-mV hyperpolarization. Recordings with access resistance larger than 20 MΩ were not used. All recordings were performed in the presence of the ionotropic GABAergic, glycineric, and glutamatergic antagonists SR95531 (20...
μM), strychnine (0.5 μM), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-
[52x620]j[quinoxaline-2,3-dione (NBQX; 20 μM), and DL-AP5 (50 μM). Data were acquired in Clampex 10.0 (Axon Instruments, Molecular Devices, Sunnyvale, CA) at 10 or 20 kHz and low-pass filtered at 3 kHz (Bessel), using a MultiClamp 700B connected to a Digidata 1440A board (Axon Instruments, Molecular Devices). Drugs were prepared from 1,000× stock solutions and diluted before applications. SR95531, strychnine, NBQX, DL-AP5, ZD7288, and TTX were purchased from Tocris Cookson (Ellisville, MO) and Ascen Scientific (Princeton, NJ). Riluzole and BaCl2 were purchased from Sigma (St. Louis, MO).

Data analysis. All data are corrected for a measured liquid junction potential of 11 mV. Fusiform cells were classified as active when their spontaneous AP firing rate was >0.5 Hz. Fusiform cells, which did not display any spontaneous firing or displayed spontaneous firing rates below 0.2 Hz, were classified as quiet. This assessment was performed at (1) = 0 mode. For the frequency (f)-I relationships (see Fig. 2B) and for calculation of activity and AP thresholds (see Fig. 2, C and D), we hyperpolarized active neurons by injecting negative current (-20 to -200 pA, depending on the cell) to stop spontaneous firing and set the membrane potential to similar values. Subsequently, AP firing was elicited by 500 ms current injections in 20 pA increments up to 140 pA. We normalized the curves by defining the point of no spiking as zero current injection point: zero current injection is a negative current injection in active cells (see Fig. 2B). AP thresholds were calculated using phase plots consisting of the first derivative (dV/dt) of the membrane potential vs. the membrane potential (see Fig. 2C). The membrane potential, at which the phase plot slope reached 10 V/s, was considered the AP threshold (see Fig. 2, C and D). Activity threshold was estimated in the f-I relationships as the mean membrane potential at the current step immediately before the appearance of APs (see Fig. 2Di). Voltage-clamp experiments were performed at a holding potential of -65 mV. To measure subthreshold sodium-persistent conductances, 4-s ramps from -80 to -50 mV were applied. The TTX (or riluzole)-sensitive component of the ramp-evoked currents represents the persistent sodium current ($I_{\text{NaP}}$). Subthreshold potassium inwardly rectifying currents ($I_{\text{KIR}}$) and hyperpolarization-activated cationic current ($I_{\text{KAP}}$) were elicited by 4-s hyperpolarizations from -65 to -120 mV and quantified by subtracting the currents before and after the application of BaCl2 (200 μM) and ZD7288 (20 μM). $I_{\text{KAP}}$ was measured at ∼200 ms after the voltage-step onset to account for the decay of the transient capacitive currents, and $I_{\text{KIR}}$ was measured at the steady-state current at the end of a 4-s hyperpolarization pulse. The rectification index of $I_{\text{KAP}}$ is the ratio between the Ba2+-sensitive current at -65 mV and the current value at the same potential calculated from a straight line, extrapolated from the linear (ohmic) part of the current (from -120 to -100 mV). For Fig. 7, the BaCl2-sensitive conductance ($G_{\text{KAP}}$) and the ZD7288-sensitive conductance ($G_{\text{KIR}}$) were determined as the slope of the current-voltage (I-V) relationship between -100 and -120 mV (see Fig. 7). To measure the minimal amount of $I_{\text{NaP}}$ necessary to produce spontaneous firing (see Fig. 8E), we monitored $I_{\text{NaP}}$ and the activity state of the cell during application of increasing concentrations of riluzole (2.5–10 μM). When the neuron stopped firing, we switched to voltage clamp to measure the remaining $I_{\text{NaP}}$ (transition $I_{\text{NaP}}$). $I_{\text{NaP}}$ was measured at -50 mV and then converted to a conductance (see Fig. 8E). We chose active neurons, in which the RMP (estimated by the 0-pA intercept of the I-V relationship) was at least 4 mV above the activity threshold. Data are presented as mean ± SE. The means were compared using appropriate parametric statistic tests using GraphPad Prism (GraphPad Software, La Jolla, CA).

Computational modeling. A point soma model of the fusiform cell was built using a standard Hodgkin-Huxley formalism. Specifically, the membrane potential ($V_m$) obeyed the following differential equation

$$
C_m \frac{dV_m}{dt} + I_{\text{Na}}(V_m, t) + I_{\text{Kd}}(V_m, t) + I_{\text{K}}(V_m, t) + I_{\text{NaP}}(V_m, t)
$$

$$
+ I_{\text{KAP}}(V_m, t) + I_{\text{L}}(V_m) = 0
$$

Here, $I_{\text{Na}}$ is a fast sodium current, $I_{\text{Kd}}$ is a delayed rectifier potassium current, $I_{\text{K}}$ is a hyperpolarization-activated cation current, $I_{\text{NaP}}$ is a persistent sodium current, $I_{\text{KAP}}$ is an inward-rectifier potassium current, $I_{\text{L}}$ is a leak current, and $C_m$ is the membrane capacitance. Kinetics of a time- and voltage-dependent current ($I_{\text{L}}$) were determined by activation ($A_l$-) and inactivation ($B_l$-)gating variables and were described by the first-order differential equation

$$
\frac{dY}{dt} = \frac{Y_e(V_m) - Y}{\tau_y(V_m)} \left( Y = A_l, B_l \right)
$$

where $Y_e(V_m)$ is the steady-state value, and $\tau_y(V_m)$ is the time constant. $Y_e(V_m)$ was described by the Boltzmann equation

$$
Y_e(V_m) = \frac{1 + e^{(V_m-V_e^{1/2})/\sigma}}{1 + e^{(V_m-V_e^{1/2})/\sigma}}^{-1}
$$

All gating variables, except for $A_{\text{KAP}}$, were assumed to have voltage-independent time constants. $V_{1/2}$ and $k$ are the half activation and slope, respectively, of the steady-state conductance curve. $I_{\text{NaP}}$ and $I_{\text{KAP}}$ AP generation was generated with the combination of $I_{\text{NaP}}$ and $I_{\text{KAP}}$ dynamics, where channel parameters were taken from a previous DCN fusiform cell model (Kanold and Manis 2001). The Kanold and Manis model incorporated fast- and slow-inactivated potassium channels; however, these channels had little influence on the spontaneously active state and were replaced by a voltage-independent leak conductance for model simplicity. To accurately model the RMP and spontaneous firing property, we included $I_{\text{KIR}}$ and $I_{\text{NaP}}$. Parameters describing these conductances were obtained by experimental measurements.

$I_{\text{L}}$ $I_{\text{L}}$ was confirmed by the characteristic “sag”, appearing during long, hyperpolarizing pulses. Modeling of $I_{\text{L}}$ was built on a previous description of the current (Destexhe and Babloyantz 1993; Destexhe et al. 1993). We used two activation variables ($A_{\text{L1}}, A_{\text{L2}}$) that were described with same stable state dynamics ($A_{\text{L1}}$) but different voltage dependence of time constant ($\tau_{\text{L1}}, \tau_{\text{L2}}$). The kinetic equations were as follows

$$
I_{\text{L}}(V_m, t) = g_{\text{L}} A_{\text{L1}} A_{\text{L2}}(V_m, t)(V_m - E_{\text{L}})
$$

$$
A_{\text{L1}} = \left[ 1 + e^{(V_m-V_e^{1/2})/\sigma} \right]^{-1}
$$

$$
\tau_{\text{L1}}(V_m) = \left[ 1 + e^{(V_m-V_e^{1/2})/\sigma} \right]^{-1}
$$

$$
\tau_{\text{L2}}(V_m) = \left[ 1 + e^{(V_m-V_e^{1/2})/\sigma} \right]^{-1}
$$

We obtained the maximum conductance ($0.54 \text{ mS/cm}^2$) and reversal potential ($-43 \text{ mV}$) of $I_{\text{L}}$ by the I-V relationship of the $G_{\text{L}}$. $V_{1/2}$ and $k$ were also determined by our experimental measurements.

$I_{\text{NaP}}$. To enable the spontaneous firing of a cell at a subthreshold membrane potential, $I_{\text{NaP}}$ was incorporated into the model. Kinetic equations for $I_{\text{NaP}}$ were given by

$$
I_{\text{NaP}}(V_m, t) = g_{\text{NaP}} A_{\text{NaP}}^3(V_m, t)(V_m - E_{\text{Na}}),
$$

$$
A_{\text{NaP}}(V_m) = \left[ 1 + e^{-(V_m-V_e^{1/2})/\sigma} \right]^{-1}
$$

The maximum conductance $g_{\text{NaP}}$ was set to 0.1 mS/cm², which gives a spontaneous firing frequency within physiological range. The time constant of the persistent sodium channel ($\tau_{\text{NaP}}$) was voltage-independent and fixed to a value of 5.3 ms. $I_{\text{KAP}}$. As the key component influencing the RMP, the voltage dependence of $I_{\text{KAP}}$ was given by

$$
I_{\text{KAP}}(V_m, t) = g_{\text{KAP}} A_{\text{KAP}}(V_m, t)(V_m - E_{\text{K}}),
$$

$$
A_{\text{KAP}}(V_m) = \left[ 1 + e^{(V_m-V_e^{1/2})/\sigma} \right]^{-1}
$$

Maximum conductance of $I_{\text{KAP}}$, $g_{\text{KAP}}$, was obtained by the I-V curve of $g_{\text{KAP}}$. This value was 0.5 mS/cm² for spontaneously firing neurons.
and 1.0 mS/cm² for not spontaneously firing fusiform cells. Both quiet and spontaneously firing fusiform cells have the same (13.3 ms) \( \tau_{K_{ir}} \).

\( I_{k} \). The \( I_{k} \) had a reversal potential of \(-51.32 \) mV (due to the mixed potassium and sodium leak conductance, which were experimentally observed) and a maximum conductance of 0.15 mS/cm². Based on the experimental data, the model cell for the quiet vs. spontaneously active states differed only in the maximum conductance of \( I_{k} \). Modeling was performed with programs implemented in XPP, version 2. Results were also confirmed with the same equations in NEURON, version 7.1. All model simulations assumed a cell of diameter of 22 \( \mu \)m. The cell capacitance was set at 1 \( \mu \)F/cm².

**RESULTS**

**Diverse firing states depend on intrinsic properties.** In accordance with previous reports, our whole-cell recordings revealed that 55% of the fusiform cells were spontaneously active (Fig. 1A). The generation of quiet and active fusiform cells is due to the intrinsic properties of fusiform cells, because neurotransmitter receptor blockers did not affect the firing properties of fusiform cells (Fig. 1B). These results suggest that DCN principal neurons express different ionic conductances, which produce quiet and active neurons. Spontaneous firing of principal neurons was not affected by our whole-cell recording techniques, because cell-attached recordings revealed a similar distribution of quiet and active neurons (Fig. 1C). When a whole-cell recording mode was successfully attained after cell-attached recordings in the same fusiform cell, firing rate and firing state (quiet/active) were not affected by the recording mode (Fig. 1D). We conclude that artifacts introduced by a whole-cell configuration do not affect the variability of the firing mode of fusiform cells. Therefore, we used whole-cell techniques to determine the biophysical mechanisms underlying the heterogeneity in the activity state of the fusiform cell population.

**Quiet and active neurons display similar excitability.** To test for differences in intrinsic excitability between quiet and active neurons, we measured their responsiveness to depolarizing current (Fig. 2A). To stop spontaneous firing of active neurons and to reach a common membrane potential for all fusiform cells, we injected negative current (Fig. 2, B–D). Depolarizing current injections revealed that the firing rate increased similarly in quiet and active neurons (Fig. 2B). Spontaneously active (but silenced here by hyperpolarizing current) and quiet fusiform cells responded to current injection with a slow but steady depolarization in their membrane potential, which eventually reached the AP threshold, characterized by an abrupt rise in the depolarization rate (Fig. 2, C and Di). By applying small, depolarizing steps, we measured the membrane potential, above which fusiform cells started to produce spontaneous firing (termed activity threshold; Fig. 2Dii). We observed that the activity threshold was significantly more hyperpolarized than the AP threshold (Fig. 2, D and E), and the spike occurred significantly later than the membrane-potential crossing of the activity threshold (Fig. 2D). Activity and AP thresholds were similar in quiet and active cells (Fig. 2E), thus suggesting that heterogeneous firing modes (quiet vs. active) arise from variability of different biophysical parameters.
Steady-state subthreshold $I_{\text{Nap}}$ drives active neurons to spiking, yet $I_{\text{Nap}}$ variability is not the source of heterogeneous activity states. Next, we determined the biophysical mechanisms determining the transition from activity threshold to AP threshold. AP threshold was significantly more depolarized than the activity threshold (Fig. 2, C–E), suggesting that some depolarizing conductance activates around $-60$ to $-65$ mV and drives the final phase of depolarization to the spike threshold. One obvious candidate is the subthreshold TTX-sensitive “persistent” sodium current ($I_{\text{Nap}}$), which is often present in this voltage range in central neurons. Pharmacological blockade of voltage-dependent sodium channels with TTX (1 μM) not only abolished spontaneous AP firing but also abolished the subthreshold depolarization and oscillations preceding AP (Fig. 3A1) in response to a 3-s ramp of depolarizing current (Fig. 3A2), thus suggesting the existence of TTX-sensitive subthreshold Na$^+$ conductances in fusiform cells (Manis et al. 2003). We characterized the voltage dependence and the magnitude of subthreshold TTX-sensitive sodium current using voltage ramps (Fig. 3B). The ramp-evoked current was linear when the membrane potential was between $-80$ and $-65$ mV, but at potentials that were more positive to $-65$ mV, an inward current appeared (Fig. 3B). Application of TTX (Fig. 3B1) or 10 μM riluzole (a blocker of persistent and transient sodium currents; Fig. 3B2) (Bellingham 2011; Ptak et al. 2005) blocked the inward component of a ramp-evoked current, suggesting that this component is carried by subthreshold activation of $I_{\text{Nap}}$ channels.

The activation threshold of fusiform cells (Fig. 2E) coincides with the voltage, where $I_{\text{Nap}}$ activates ($-65$ mV), suggesting that activation of $I_{\text{Nap}}$ sets the activation threshold. Consistent with this hypothesis, blockade of $I_{\text{Nap}}$ by riluzole shifted the activity threshold toward more depolarized potential values, which are equal to AP threshold (Fig. 4A). Blockade of $I_{\text{Nap}}$ channels abolished spontaneous firing in active cells (Fig. 4B), indicating that $I_{\text{Nap}}$ channels are necessary for the establishment of distinct activity states. However, application of riluzole did not block the ability of fusiform cells to fire current-evoked spikes (Fig. 4B). Moreover, application of riluzole did not change the spike threshold of current-evoked spiking (control: $-47.7 \pm 1.3$ mV; riluzole: $-44.0 \pm 1.8$ mV; $n = 6$), suggesting that riluzole (2.5 – 10 μM) is a preferential
inhibitor of the persistent sodium current in DCN fusiform cells (Bellingham 2011). Together, these results suggest that Na$_p$ channels are necessary for the establishment of distinct activity states through their effects on activity threshold. However, variability in maximal Na$_p$ conductance (gNa$_p$) values between quiet and active cells is not the source for the generation of heterogeneous firing states, because quiet and active fusiform cells displayed: 1) the same activation threshold value (Fig. 2E), 2) the same average amplitude of I$_{Nap}$ (between −50 and −80 mV; Fig. 4C), and 3) the same voltage dependence (Fig. 4C). Moreover, whereas variability in I$_{Nap}$ amplitude is observed among different neurons, this variability is not correlated with distinct activity states (Fig. 4D). We conclude that whereas Na$_p$ expression in fusiform cells is required for heterogeneous neuronal behavior, the source of cellular variability, which determines spontaneously active vs. quiet dynamics, cannot be attributed to variability of gNa$_p$ levels. Our results suggest that Na$_p$ sets the activity threshold, which can be crossed or not, depending on the variable expression of another conductance.

Active neurons have a more depolarized RMP than quiet neurons. We hypothesized that the RMP and the conductances that control RMP may be the cause of heterogeneity among different neurons displaying different activity states. According to this hypothesis, active neurons are expected to show more depolarized RMP than quiet neurons. When RMP is more depolarized than the activity threshold, then subsequent activation of Na$_p$ channels is expected to lead to the generation of spontaneous firing. A spontaneously active neuron does not have a true RMP, but an approximate measurement can be made if spontaneous firing is silenced by application of TTX to block the voltage-dependent sodium channels underlying spiking. Therefore, we measured the RMP of quiet and active neurons in the presence of TTX. Such measurements revealed that the RMP of active neurons was significantly more depolarized than quiet neurons (Fig. 5A), thus suggesting that the activity mode of fusiform cells (quiet or active) is determined by the RMP of the principal neurons. In agreement with this hypothesis, the average RMP of active neurons fluctuates around the mean activity threshold (−63 mV; Fig. 5A), whereas the mean RMP of quiet neurons is below this value. Comparison of the RMP and activity threshold values measured from the same neuron indicates that the RMP lies at a more hyperpolarized value than the activity threshold in quiet neurons, yet RMP lies at a more depolarized value than the activity threshold in active neurons (Fig. 5B). Given that activity threshold is determined by the existence and the voltage dependence of Na$_p$ channels, these results indicate that neurons whose RMP is equal or depolarized to the range for activation of INap are spontaneous active, whereas neurons whose RMP is hyperpolarized to the range of activation of INap are quiet. These results also suggest that heterogeneity observed in the RMP of principal neurons acts with respect to the threshold set by Na$_p$ channels to generate quiet and active fusiform cells.

Variability in inwardly rectifying potassium channels determines heterogeneous RMP and generates heterogeneous activity states. Next, we investigated the conductances that generate heterogeneous RMP among fusiform cells. Among obvious candidates are potassium conductances, which are open at rest and hyperpolarize RMP in fusiform cells. Inwardly rectifying potassium currents (Kir) are expressed in fusiform cells (Irie and Ohmori 2008) and are important in setting the RMP in many neuronal types (Hibino et al. 2010). We isolated IKir currents by using BaCl$_2$ (Ba$^{2+}$ 200 μM; Fig. 6A). We com-

Fig. 3. Subthreshold-persistent sodium channels are expressed in fusiform cells and drive active fusiform cells to spiking threshold. A1: response of a fusiform cell to a 3-s ramp of depolarizing current at a rate of 20 pA/100 ms (A2), before (black trace) and after (gray trace) application of TTX (1 μM). Note that subthreshold oscillations are blocked by TTX. B: current in response to a 4-s ramp from −80 to −50 mV, before (black) and after (gray) application of TTX (B1) or riluzole (10 μM; B2).
pared Ba$^{2+}$-sensitive currents in active and quiet neurons to
determine whether differential-expression K$_{ir}$ underlies the
differences in the RMP (Fig. 6, A and B). Quiet neurons display
significantly more robust K$_{ir}$ currents (Fig. 6B). Based on these
findings, we suggest that variability in the density of K$_{ir}$
channels generates heterogeneous RMP among fusiform cells;
lower K$_{ir}$ density depolarizes the RMP and promotes repetitive,
spontaneous firing, whereas higher K$_{ir}$ density hyperpolarizes
the RMP and generates quiet neurons. If the observed differ-
ences in IKir were mediating the observed differences in the
RMP and firing mode, then we expect that blockade of Kir
channels would eliminate the difference in RMP between quiet
and active fusiform cells. To test this hypothesis, we deter-
mined the effect of Kir channels on the RMP. Blockade of Kir
equalized the RMP between quiet and active neurons (Fig. 6C),
suggesting that the variability in channel density of a Ba$^{2+}$-
sensitive inwardly rectifying potassium conductance (gK$_{ir}$)
causes the diversity in the RMP and the firing mode of fusiform
cells.

Although Ba$^{2+}$ is commonly used as a specific blocker of
the K$_{ir}$ conductances (Hibino et al. 2010), 200 μM Ba$^{2+}$ blocks
other conductances with a lower Ba$^{2+}$ affinity, such as the leak
potassium conductance (Day et al. 2005; Goldstein et al. 2001).
To test this possibility, we used a lower concentration of BaCl$_2$
(50 μM). Our experiments indicate that Ba$^{2+}$-sensitive cur-
rents for 50 and 200 μM Ba$^{2+}$ are similar in both quiet and
active neurons (Fig. 6D). The difference of Ba$^{2+}$-sensitive
currents between quiet and active neurons was similar for both

Fig. 4. Subthreshold-persistent sodium channels are
required for heterogeneous neuronal behavior, yet
their variability is not correlated with distinct activ-
ity states. A: comparison of activity thresholds be-
fore and after application of riluzole (2.5 × 10 μM;
activity threshold: control = −63.0 ± 1.5 mV;
riluzole = −50.2 ± 0.4 mV; *P < 0.05, paired
t-test). B: application of riluzole blocks spontaneous
firing of active neurons without blocking current-
evoked APs (n = 12). C: average TTX-sensitive current (I_{NaP}) in response to a 4-s ramp from −80
to −50 mV for quiet and active neurons (n = 5). D: I_{NaP} amplitude at −50 mV (I_{NaP} @ −50 mV) for
quiet and active neurons. I_{NaP} mean amplitude is not
different between quiet and active neurons (quiet: 446 ± 85 pA, n = 6; active: 552 ± 108 pA, n = 6).
In addition, the I_{NaP} variances in quiet and active
neurons are equal (Levene’s test, P = 0.7530).

Fig. 5. Active fusiform cells display more depolarized resting membrane potential (RMP) than quiet fusiform cells. A: RMP (in the presence of TTX) of quiet and active neurons. Bars represent the average RMP value of quiet and active fusiform cells (quiet: −70.9 ± 1.3 mV, n = 13; active: −62.8 ± 1.3 mV, n = 10; *P = 0.0003, unpaired t-test). Horizontal dashed line represents the mean activity threshold. B: comparison of the RMP and activity threshold (act. thres.) of quiet (left; black circles) and active (right; gray circles) neurons (quiet: activity threshold = −63.2 ± 2 mV, RMP = −67.8 ± 2 mV, n = 6, *P = 0.025;
active: activity threshold = −63.7 ± 1.4 mV, RMP = −61.2 ± 1 mV, n = 6, *P = 0.04).
Fig. 6. Differential levels of inwardly rectifying potassium (\(K_{ir}\)) currents among fusiform cells generates differential RMPs and thus produces quiet and active fusiform cells. A: representative traces of BaCl_2-sensitive currents (\(I_{K_{ir}}\)), obtained by subtraction of the current before and after application of BaCl_2 (200 \(\mu\)M). i: currents produced by hyperpolarization of fusiform cells; ii: BaCl_2 (Ba\(^{2+}\) (Ba\(^{2+}\)))-sensitive currents; iii: voltage protocol. B: current-voltage plot of \(I_{K_{ir}}\) from quiet (black circles) and active (gray circles) neurons (\(K_{ir}\) amplitude at -65 mV: quiet = 156 ± 18 pA, \(n = 18\); active = 82 ± 13 pA, \(n = 20\); \(P < 0.05\), unpaired \(t\)-test; \(K_{ir}\) amplitude at -120 mV: quiet = -698.1 ± 79 pA, \(n = 18\); active = -397.4 ± 67 pA, \(n = 20\); \(P < 0.05\), unpaired \(t\)-test). C: effect of 200 \(\mu\)M BaCl_2 on RMP, measured in the presence of TTX in quiet (black circles) and active (gray circles) neurons (quiet: RMP in BaCl_2 = -56.1 ± 1.5 mV, \(n = 11\); active: RMP in BaCl_2 = -54.0 ± 1.1 mV, \(n = 15\); \(P = 0.27\)). D: comparison of the Ba\(^{2+}\)-sensitive current (50 \(\mu\)M, open circles; 200 \(\mu\)M, solid circles) in quiet (black circles) and active (gray circles) neurons. Ba\(^{2+}\)-sensitive currents were equal for either 50 or 200 \(\mu\)M BaCl_2 (Ba\(^{2+}\) 50 \(\mu\)M active: -342 ± 133 pA; Ba\(^{2+}\) 50 \(\mu\)M quiet: -810 ± 127 pA Ba\(^{2+}\), 136 ± 37% increase; Ba\(^{2+}\) 200 \(\mu\)M active: -433 ± 142 pA; Ba\(^{2+}\) 200 \(\mu\)M quiet: -937 ± 144 pA, 116 ± 33% increase; \(n = 5\)). The rectification index (RI) of Ba\(^{2+}\)-sensitive currents was similar for both Ba\(^{2+}\) concentrations (RI active 50 \(\mu\)M: 0.27 ± 0.11; active 200 \(\mu\)M: 0.16 ± 0.02; quiet 50 \(\mu\)M: 0.14 ± 0.006; quiet 200 \(\mu\)M: 0.16 ± 0.03; \(P = 0.4\), one-way ANOVA). E: effect of Ba\(^{2+}\) 50 \(\mu\)M (open circles) and 200 \(\mu\)M (solid circles) in the RMP of quiet and active neurons (quiet: RMP in 50 \(\mu\)M Ba\(^{2+}\): -53.9 ± 2.3 mV; RMP in 200 \(\mu\)M Ba\(^{2+}\): -52.5 ± 2 mV, \(n = 6\); active: RMP in 50 \(\mu\)M Ba\(^{2+}\): -54.4 ± 1.6 mV; RMP in 200 \(\mu\)M Ba\(^{2+}\): -54.1 ± 1.6 mV, \(n = 7\); \(P = 0.9\), one-way ANOVA). F: comparison of the tertiapin-Q-sensitive current (60 nM, open symbols) and Ba\(^{2+}\) 200 \(\mu\)M (solid symbols) in quiet (\(n = 3\)) and active (\(n = 3\)) neurons. Tertiapin-Q did not affect the hyperpolarization-activated current (\(P = 0.86\); \(n = 6\)). G: effect of tertiapin-Q (60 nM) on RMP, measured in the presence of TTX in quiet and active neurons (quiet: control = -62.5 ± 1 mV, tertiapin = -61 ± 2 mV, active: control = -57.3 ± 2 mV, tertiapin = -57.3 ± 2 mV; \(n = 3\) each).

Ba\(^{2+}\) concentrations (Fig. 6D). Moreover, RMPs of quiet and active neurons were equal in both Ba\(^{2+}\) 50 and 200 \(\mu\)M (Fig. 6E), further suggesting that the differences in the size of the Ba\(^{2+}\)-sensitive current in quiet and active neurons are not due to differential expression of Ba\(^{2+}\)-sensitive leak conductances. Given that the amount of the inward rectification of the Ba\(^{2+}\)-sensitive current was equal for the two different Ba\(^{2+}\) concentrations (Fig. 6D), these results suggest that the ob-
served rectification is due to the expression of a $K_{ir}$ inwardly rectifying conductance and not to a differential $Ba^{2+}$ block of inward- and outward-going currents of various conductances (Day et al. 2005).

To further determine the type of the inward rectifier that generates differential RMPs in fusiform neurons, we used a specific blocker of G-protein-coupled inwardly rectifying potassium channels (GIRK). Application of 60 nM tertiapin-Q, a specific antagonist of GIRK channels (Jin and Lu 1998), did not affect the hyperpolarization-activated currents in either quiet or active neurons (Fig. 6F), suggesting that GIRK channels are not involved in setting differential RMPs in fusiform cells. Additionally, tertiapin-Q did not have any significant effect in RMP of either quiet or active neurons (Fig. 6G). We conclude that differential expression of a non-GIRK $K_{ir}$ conductance generates heterogeneous RMPs in fusiform cells.

If the observed variability in a non-GIRK $K_{ir}$ conductance determines the observed variability in RMP of fusiform cells, then we expect to see a correlation between RMP and $g_{K_{ir}}$ conductance for different fusiform cells. In addition, if variability in $g_{K_{ir}}$ were the only conductance that generates RMP and firing mode variability, then variability in other subthreshold conductances is not expected to correlate with the firing state of fusiform cells. To test these hypotheses, we measured $g_{K_{ir}}$ and $g_{Nh}$ (Ih, another subthreshold conductance) simultaneously from the same fusiform cells. Our results indicate that variability in $g_{K_{ir}}$ was well correlated with the RMP and the activity state of fusiform cells (Fig. 7, A and C), whereas variability in $g_{Nh}$ was not correlated with the activity state or the RMP of fusiform cells (Fig. 7, B and C). Taken together, these results suggest that $K_{ir}$ currents are larger in quiet neurons, and observed between $Gh$ and activity state ($Gh$ quiet: 4.95 nS; $Gh$ active: 6.99 nS; $n = 6$ for both conditions; $P < 0.01$, unpaired $t$-test). In addition, the variances of $Gh$ in quiet and active cells are equal (Levene’s test, $P = 0.92$). No correlation is observed between $Gh$ and activity state ($Gh$ quiet: 4.95 ± 1.27 nS; $Gh$ active: 6.99 ± 1.15 nS; $n = 6$ for both conditions). The variances of $Gh$ in quiet and active cells are equal (Levene’s test; $P = 0.91$).

$g_{Na_{p}}$ values do not reach low enough values, which could allow for the transition from an active to quiet state. The variability of $K_{ir}$ channels and their effect on the RMP are well correlated with the spontaneous states of fusiform cell excitability (Fig. 7, A and C). Although $Na_{p}$ channels are a critical determinant of the activation threshold and are required for spontaneous activity (Fig. 4B), the variability in their expression is not correlated with the distinct activity states (Fig. 4D). This is a surprising result, suggesting a complex interaction between $Na_{p}$ and $K_{ir}$ conductance levels, which determines the firing mode of fusiform cells. To further understand the interaction of $Na_{p}$ and $K_{ir}$ channels in regulating RMP and firing mode of fusiform cells, we constructed a single-compartment computational model of a fusiform cell. We incorporated channel descriptions, measured from the somatic voltage-clamp experiments (see MATERIALS AND METHODS). Our model allowed us to test the combined effect of $Na_{p}$ and $K_{ir}$ on the RMP and membrane potential excitability over a broad range of maximal conductance values. Varying $K_{ir}$ maximal conductance revealed that for large $g_{K_{ir}}$ values ($>0.5 \text{ mS/cm}^{2}$), the membrane potential shows two equilibrium values. One equilibrium value is stable (termed as a node) and represents the RMP ($V_{RMP}$; Fig. 8A). The second value ($V_{AT}$) is unstable (termed as a saddle point) and represents the activity threshold (Fig. 8A1). If the membrane potential exceeds $V_{AT}$, then an AP will occur before the membrane potential approaches $V_{RMP}$ whereas membrane potential values below $V_{AT}$ converge to $V_{RMP}$ without producing an AP. Our model indicates that fusiform cells have a stable RMP for large values of $g_{K_{ir}}$ (Fig. 8A2). As $g_{K_{ir}}$ is reduced, $V_{RMP}$ depolarizes to coalesce with $V_{AT}$ in a saddle-node bifurcation (Izhikevich 2007; Rinzel 1989) at a specific value of $g_{K_{ir}}$ ($\sim 0.55 \text{ mS/cm}^{2}$). For values of $g_{K_{ir}}$ which are below the saddle-node point, there are no stable RMP values; rather, we observed repetitive neural firing (Fig. 8A2). The experimentally determined mean values of $g_{K_{ir}}$ for quiet and active neurons do straddle the saddle-node bifurcation.
Both means and variances were significantly different in active cells during application of increasing concentrations of riluzole (2.5–10 μM). When the active neuron stopped firing during riluzole application, the remaining INaP at this exact time point represents the INaP value, where the transition between active and quiet state occurs (Fig. 8D; transition INaP). The variability of INaP in control (prior to riluzole application) and INaP trans was measured after riluzole application and at the time point where active neurons transition from active to quiet state. INaP control and INaP trans were measured at −50 mV, and these values were converted to conductances (gNaP, control: 4.86 ± 1 nS; gNaP trans: 1.03 ± 0.3 nS; n = 7). Both means and variances were significantly different (P < 0.05).

Apart from capturing our experimental findings, the model makes an important prediction that explains our paradoxical finding on the lack of correlation of gNaP variability with the distinct activity states. According to this prediction, variability of gNaP values does not co-vary with activity states, because fusiform cells fall at the saturated part of the curve shown in Fig. 8C, thus exhibiting saturating gNaP values, which are far from the minimal gNaP conductance needed for generating spontaneous firing (termed transition point). Based on the curvature of the active-quiet boundary in gKir and gNaP parameter space (Fig. 8C), the difference in the amount of gNaP between active cells in control conditions is expected, on average, to be significantly larger than the difference between their gNaP values at their respective transition point (Fig. 8D).

This compression in variability is illustrated for a pair of cells, which is spontaneously active (Fig. 8A). The variability of INaP in control (prior to riluzole application) and INaP trans was measured after riluzole application and at the time point where active neurons transition from active to quiet state. INaP control and INaP trans were measured at −50 mV, and these values were converted to conductances (gNaP, control: 4.86 ± 1 nS; gNaP trans: 1.03 ± 0.3 nS; n = 7). Both means and variances were significantly different (P < 0.05).

**Fig. 8. Variability in maximal Na+ conductance (gNaP) expression levels does not reach low-enough values, which allow for the transition from active to quiet state. Quiet and spontaneously active dynamics in a Hodgkin-Huxley style model of fusiform cell membrane potential activity (A–D). A: model bifurcation diagram as the maximal Kir conductance, gK_{ir}, is varied (A1). Activity threshold (dashed trace) and the stable RMP (solid trace) coalesce in a saddle-node bifurcation. The experimentally measured gK_{ir} in quiet and spontaneously active cells straddles the bifurcation (marked with * and **), with the model producing either resting or rhythmic firing dynamics (A2). B: model bifurcation diagram as the gNaP is varied. C: 2-parameter (gNaP, gKir) bifurcation set, with a curve of saddle-node bifurcations separating quiet and active behavior. D: the concave, downward shape of the boundary between quiet and active dynamics predicts that gNaP variability (ΔgNaP) across control, spontaneously active cells will contract when gNaP is blocked with sufficient applied riluzole to transition the cell from active to quiet dynamics. E: comparison of experimentally measured gNaP values in control active state with gNaP values measured at the transition point from active to quiet state. INaP control was measured at the beginning of the experiment, and INaP trans was measured after riluzole application and at the time point where active neurons transition from active to quiet state. INaP control and INaP trans were measured at −50 mV, and these values were converted to conductances (gNaP, control: 4.86 ± 1 nS; gNaP trans: 1.03 ± 0.3 nS; n = 7). Both means and variances were significantly different (P < 0.05).
was significantly larger than the variability of $I_{\text{NaP}}$ at the transition point (Fig. 8E), consistent with the compression of variability predicted by our model (Fig. 8D). These results indicate that whereas $\text{Na}_p$ expression is critical for setting activity threshold, its expression levels in fusiform cells do not reach low enough levels, which would allow for the transition from an active to a quiet cell. This is an important finding, suggesting that variability in $g_{\text{Na}_p}$ does not lead to changes in activity states (quiet or active) due to homeostatic adjustment of some other conductance, but instead, variability is set above a critical minimal value.

**DISCUSSION**

Our study unmasks a biophysical mechanism that enables the generation of heterogeneous activity states of DCN principal neurons. Specifically, variability in $K_r$ conductance leads to heterogeneous RMP values at approximately $-60$ and $-70$ mV. Whereas this variability is not expected to produce qualitatively distinct firing states, the expression of $\text{Na}_p$ channels, which activate approximately $-65$ mV, allows for this variability to generate quiet and spontaneously active fusiform cells with diverse firing rates. Fusiform cells, with resting potential at above or approximately $-65$ mV, activate $\text{Na}_p$ channels and initiate spontaneous firing, whereas neurons, which are slightly more hyperpolarized, remain quiet. Therefore, $\text{Na}_p$ channels create the conditions upon which variable expression of $K_r$ channels can now create heterogeneous activity states.

**Homeostasis vs. heterogeneity.** Numerous studies have established that one of the consequences of the fact that neurons express a large number of different conductances is that similar neuronal behavior can arise from diverse combinations of conductance densities of different channels (Cao and Oertel 2011; Gaoillard et al. 2009; Goldman et al. 2001; Golowasch et al. 2002; Marder and Gaoillard 2006; Parham et al. 2000; Swensen and Bean 2005). In these cases, compensatory mechanisms allow for different expression levels of multiple conductances to produce similar cellular or circuit behavior. In contrast, our study demonstrates that changes in the expression of a single conductance mediate the establishment of heterogeneous neuronal behavior in a population of primary sensory neurons. Moreover, our study establishes that the observed heterogeneity is not the result of nonspecific ups and downs of random conductances. On the contrary, variability of $K_r$ conductance, assisted by $\text{Na}_p$ conductance, establishes heterogeneous activity states. Therefore, here, we unmask a mechanism of single-channel variability, which is not homeostatically compensated and is designed to produce heterogeneous neuronal behavior.

Our results are consistent with previous studies illustrating that observed heterogeneity within a single identified neuronal type can generate diverse, spontaneous neuronal behavior in cortical and subcortical areas (Ma et al. 2006; Parham et al. 2000; Yassin et al. 2010) and/or can diversify an output response to similar input (Marsat and Maler 2010; Padmanabhan and Urban 2010; Wang et al. 2006). These findings have suggested that this diversity is not the random product of biological imprecision and that populations of heterogeneous neurons display enhanced stimulus representation compared with homogeneous populations (Chelaru and Dragoi 2008; Liu et al. 2009; Marsat and Maler 2010; Osborne et al. 2008; Padmanabhan and Urban 2010; Shamir and Sompolinsky 2006). However, in previous studies, the diverse neuronal behavior within populations of pyramidal neurons has been attributed to the variability of synaptic excitatory or inhibitory drive (Pouille et al. 2009; Wang et al. 2006; Yassin et al. 2010) but not to individual conductances. Our studies in DCN reveal a single conductance-based mechanism for generating neuronal heterogeneity.

**Mechanisms that establish differences in the amounts of $K_r$ conductance in different fusiform cells.** Molecular mechanisms, used to establish differences between neuronal types, such as gene-regulatory pathways, may also be used to produce heterogeneous ion channel expression among fusiform cells. Alternatively, heterogeneity of $K_r$ conductance may be the result of an activity-dependent, homeostatic process. However, in this case, the activity sensor, which reports the difference between the set point of the system and the actual output of the system, must be responsive to average population neuronal activity. In this case, a homeostatic signaling system would provide information regarding deviations from the set point, and the neuron would modify its $K_r$ conductance so that the target state is achieved. However, these two possibilities are not mutually exclusive. Moreover, given that fusiform cells exhibit multiple forms of activity-dependent synaptic plasticity (Doiron et al. 2011; Fujino and Oertel 2003; Zhao et al. 2009, 2011; Zhao and Tzounopoulos 2011), we propose that $K_r$ conductances are potential targets for activity-dependent, intrinsic plasticity. Future experiments examining the developmental profile and the activity dependence of $K_r$ heterogeneity are expected to provide answers to these questions.

**Implications for auditory processing.** One function of the DCN is discrimination of head-related transfer functions (HRTFs), spectral cues used for vertical sound localization. Spectral notches are important sources of acoustic information about the location of sounds. In cats, HRTFs are characterized by a midfrequency notch, which shifts systematically in frequency with sound source azimuth and elevation (Musicant et al. 1990; Rice et al. 1992). This notch produces a sharp minimum in the sound spectrum at the eardrum, which is an important cue for vertical sound localization in cats (Huang and May 1996). Fusiform cells generally respond to broadband noise, but they show a dip in response rate when the notch is centered at the frequency to which the neuron is most sensitive (Yu and Young 2000). Notch-evoked inhibition will be coded by a reduction of firing rate of spontaneously active cells, whereas notch-evoked inhibition will not be coded by quiet cells. Nonetheless, both quiet and spontaneously active cells respond with a peak in discharge rate when a rising spectral edge is aligned near their best frequency (Reiss and Young 2005). Therefore, the diversity of firing states of the fusiform cell population may permit the fusiform population response to code for inputs that both excite and inhibit, thereby broadening the overall coding capacity of the population.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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MECHANISMS GENERATING NEURAL HETEROGENEITY


