Identification and modelling of fast and slow $I_h$ current components in vestibular ganglion neurons

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Abstract

Previous experimental data indicates the hyperpolarization-activated cation ($I_h$) current, in the inner ear, consists of two components [different hyperpolarization-activated cyclic nucleotide-gated (HCN) subunits] which are impossible to pharmacologically isolate. To confirm the presence of these two components in vestibular ganglion neurons we have applied a parameter identification algorithm which is able to discriminate the parameters of the two components from experimental data. Using simulated data we have shown that this algorithm is able to identify the parameters of two populations of non-inactivated ionic channels more accurately than a classical method. Moreover, the algorithm was demonstrated to be insensitive to the key parameter variations. We then applied this algorithm to $I_h$ current recordings from mouse vestibular ganglion neurons. The algorithm revealed the presence of a high-voltage-activated slow component and a low-voltage-activated fast component. Finally, the electrophysiological significance of these two $I_h$ components was tested individually in computational vestibular ganglion neuron models (sustained and transient), in the control case and in the presence of cAMP, an intracellular cyclic nucleotide that modulates HCN channel activity. The results suggest that, first, the fast and slow components modulate differently the action potential excitability and the excitatory postsynaptic potentials in both sustained and transient vestibular neurons and, second, the fast and slow components, in the control case, provide different information about characteristics of the stimulation and this information is significantly modified after modulation by cAMP.

Introduction

The identification and characterisation of neuronal ionic conductances remains a major component of electrophysiological research. Classically, individual ionic conductances are pharmacologically isolated, and their current responses are recorded by the voltage-clamp technique. For each voltage step, the corresponding current response is fitted in order to identify its magnitude and time constant parameters (Hodgkin & Huxley, 1952). This method extracts these characteristics from each voltage-clamp trace independently. Consequently we will call this parameter identification approach the single-trace method. An improved method that simultaneously extracts the conductance characteristics using all voltage-clamp traces at once has been shown to produce more accurate solutions in the identification of sodium currents (Willms et al., 1999). This algorithm is called the full-trace method.

In some cases, pharmacological tools to isolate individual ionic currents are not available. This is the case for the hyperpolarisation-activated cation currents in the cochlea (Chen, 1997; Yi et al., 2010). In the vestibular ganglion, the fitting of these currents was significantly better with a two-component exponential model (Meredith et al., 2012), and biochemical analysis showed the presence of different HCN subunits in the vestibular periphery (Horwitz et al., 2010, 2011). These studies could not draw conclusions about the differences in activation of the two apparent components, due to the limited performance of the single-fitting approach. Given its better constraints, we assumed that the full-trace method should be a good tool for analysing the vestibular ganglion $I_h$ currents, first, in order to corroborate the presence of two components and, second, to isolate analytically rather than pharmacologically these two components and so permit their individual modelling to test their electrophysiological significance.

We first validated on simulated data the ability of the full-trace method to discriminate two populations of non-inactivated voltage-dependent ionic currents. Then, we applied the method to $I_h$ currents recorded from mouse vestibular ganglion neurons and identified the activation and kinetics of both components. Finally, these two $I_h$ components were tested individually in a computational auditory neuron model (Rothman & Manis, 2003a) modified to...
reproduce the regular and irregular (sustained and transient) vestibular ganglion neuron firing patterns (Kalluri et al., 2010; Yoshimoto et al., 2015), in the control case and in presence of cAMP, an intracellular cyclic nucleotide that modulates hyperpolarization-activated cyclic nucleotide-gated (HCN) channel activity (Chen, 1997; Yi et al., 2010). The results suggest that the fast and the slow components modulate differently action potential (AP) excitability and excitation post synaptic potentials (EPSPs) in the sustained and transient vestibular ganglion neurons. In addition, the fast component allows the neuron to fire a single AP at the end of a hyperpolarizing stimulation whatever its duration or amplitude, whereas the slow component, in the control case, allows the neuron to fire a number of APs correlated with both the stimulation duration and amplitude and, after modulation by cAMP, it results in a number of APs exclusively correlated to the stimulation amplitude.

Materials and methods

Patch-clamp recordings

Whole-cell recordings of hyperpolarisation-activated currents were performed in postnatal day (P)5–P8, male and female, wild-type Swiss mice (CERI, Le Genest, France), specifically in the superior branch of the vestibular nerve, innervating the utricular macula and the horizontal and lateral cristae, using an isolation procedure previously described (Chabbert et al., 2001a). Ganglia were asceptically dissected from mice rapidly killed by decapitation (the day of birth was considered P0). About twenty ganglia for each experiment were collected in phosphate-buffered saline (PBS; Life Technologies). We tested different incubation times and trypsin concentrations and collected in phosphate-buffered saline (PBS; Life Technologies). We were able to dissociate mouse vestibular neurons within 12 min at 37 °C in PBS containing 0.25% EDTA-trypsin (Life Technologies). Ganglia were triturated with fire-polished Pasteur pipettes of three decreasing diameters in a cell culture medium containing Neurobasal medium (Life Technologies), 10% B27 (Life Technologies), 25 μM glutamate and 0.25 mM glutamine. Neurons were plated onto 35-mm culture dishes (Nunc) coated with 10 μg/ml poly-D-ornithine (Sigma) in cell culture medium. Cells were used between 1 and 4 h after dissociation. Under phase-contrast microscopy, dissociated neurons had a spherical shape and birefringent cytoplasm as previously reported (Desmadryl et al., 1997). Cell diameters ranged between 10 and 25 μm in a sample of 20 animals. Only isolated neurons exhibiting no processes were chosen for the electrophysiological studies.

The standard extracellular solution contained (in mM): NaCl, 135; KCl, 5; HEPES, 10; glucose, 10; and MgCl₂, 1. Patch pipettes (2 and 3 MΩ) were filled with the following intracellular solution (in mM): KCl, 135; EGTA, 10; HEPES, 25; MgATP, 3; NaGTP, 1; and glucose, 10. The pH of the solution was adjusted to 7.35 and the osmolarity to 300 mOsm/L. Data were recorded with an Axopatch 200B and analysed with Pclamp software (Axon Instrument, Foster City, CA, USA; now Molecular Devices, part of Danaher). Series resistances in the range 5–9 MΩ were 80% compensated after cancellation of the capacitive transients. Data presented are corrected online for junction potential (~7 mV). Delayed-rectifier and low-voltage-activated potassium currents are not activated under ~60 mV (Chabbert et al., 2001a), and KCNQ currents are not active at these ages at potentials under ~70 mV (Hurley et al., 2006). Ih currents were isolated by subtracting traces evoked in the presence of 5 mM BaCl₂, known to abolish the instantaneous component, from those elicited in control external solution (Chabbert et al., 2001b). The care and use of animals followed the animal welfare guidelines of the Institut National de la Sante et de la Recherche Medecale (Inserm), and was approved by the Ministere Francais de l’Agriculture et de la Peche (authorization number A 3417231).

Current, activation and kinetic models

The form of current equation initially proposed by Hodgkin & Huxley (1952) in the context of sodium and potassium channels is adapted here for the hyperpolarisation-activated cation current Ih to give:

\[ I_h(t) = G_h(r_{\infty}(V_i) + (r_{\infty}(V_{ps}) - r_{\infty}(V_i))\exp(-t/\tau_r(V_i)))/(V_i - E_h) \]  

where \( G_h \) is the maximal conductance, \( r_{\infty} \) is the activation function, \( \tau_r \) is the activation kinetic function, \( V_{ps} \) is the voltage pre-step value, \( V_i \) is the voltage-step value and \( E_h \) is the reversal potential. Param \( \tau_r \) ete r identification involves determining values for the parameters \( G_h, r_{\infty} \) and \( \tau_r \) from recordings of \( I_h \). The reversal potential is predetermined and here is set to \( E_h = -36 \) mV to correspond to physiological values (Chen, 1997; Meredith et al., 2012).

Functional forms for \( r_{\infty} \) and \( \tau_r \) as functions of the membrane voltage \( V \) are assumed. The conductance activation \( r_{\infty} \) as a function of voltage is commonly described by a Boltzmann function of the form:

\[ r_{\infty}(V) = (1 + \exp(-(V - V_0)/k))^{-1} \]

where \( V_0 \) is the half-activation voltage and \( k \) is the slope factor that determines the steepness of the Boltzmann function.

The conductance kinetic \( \tau_r \) as a function of voltage can be described by a Gaussian function (Izhikevich, 2010) of the form:

\[ \tau_r(V) = B + A\exp\left(-\frac{(M - V)^2}{2S^2}\right) \]

where \( M, S \) and \( A \) are respectively the location of the maximal value, the width (\( \sigma \) for a Gaussian) and the amplitude of the Gaussian, and \( B \) is an offset from the abscissa axis (base time constant). These two curves (activation and kinetic) are called the characteristic curves of a voltage-dependent channel population and, along with the maximal conductance \( G \), they are sufficient to fully describe the resulting current.

Algorithms

This study aims to show that the full-trace method (Willms et al., 1999) is more reliable than the more widely used single-trace approach for identifying the parameters of currents composed of two components with differing activations and kinetics. In other words, the recorded membrane currents are the sum of two conductances described by the above equations, so the parameter identification algorithms have to identify the parameters of currents described by the equation:

\[ I_{total} = I_{hs}(G_1, r_{\infty}(V), \tau_r(V)) + I_{hf}(G_1, r_{\infty}(V), \tau_r(V)) \]

Here \( I_{hs} \) and \( I_{hf} \) are the slow and fast components of the total current and \( r_{\infty}(V), \tau_r(V) \) are their respective activation and kinetic functions.
The single-trace method

A voltage-clamp protocol consists of a pre-step potential $V_{ps}$ imposed on the cell followed by several values of a step potential $V_s$, completed with a return to the pre-step potential (deactivation). When $V_{ps}$ is different from $V_s$, a current flows through the membrane (activation) and is measured by the patch-clamp setup. The traces of a voltage-clamp experiment are the individual currents recorded in response to different voltage steps in the voltage-clamp protocol. The single-trace method used here fits each current trace (Fig. 1A) of a voltage-clamp experiment with the current Eqn (1) and identifies, for each value of $V_s$, the variables $r_h(V_s)$, $r_f(V_s)$, and $G(V_s)$ (Rothman & Manis, 2003b; Gerstner, 2014). To enable identification of these parameters, we set the initial value of activation to the steady-state value at the pre-step potential $V_{ps}$ (Willms et al., 1999), which is 0 for the $I_h$ current, and a value for the ionic reversal potential is assumed. From the resulting sets of parameter values, first the maximal conductances are averaged to give a single estimate of the maximal conductance $G$. Second, the set of values for $r_h(V_s)$ are fitted with the activation Eqn (2), yielding values for the parameters $V_h$ and $k$. Finally, the set of identified values for $r_f(V_s)$ are fitted with the kinetic Eqn (3) in order to evaluate the parameters $M$, $S$, $A$, and $B$.

When the current recorded during a voltage-clamp experiment is the sum of two components, as it is here, the fitting has to identify twice the variables (one set for each component): $r_{hs}(V_s)$, $r_{hf}(V_s)$, $G_h(V_s)$, and $r_{hs}(V_s)$, $r_{hf}(V_s)$, $G_f(V_s)$, and then twice the characteristic parameters: $V_{hs}$, $k_h$, $M_h$, $S_h$, $A_h$, $B_h$, $V_{hf}$, $k_f$, $M_f$, $S_f$, $A_f$, and $B_f$.

The full-trace method

The full-trace method is an iterative algorithm that estimates directly and simultaneously the activation and kinetic functions, and the maximal conductance from all the currents recorded during a voltage-clamp experiment. Initially, values for the elements of the characteristic curve parameter vector $[V_h, k, M, S, A, B]$ are drawn randomly from a prescribed range for each parameter. The algorithm then calculates the values of $r_{hs}(V_s)$, $r_{hf}(V_s)$, for each value of $V_s$, with equations (2) and (3). Next, the set of step currents are reconstructed from Eqn (1). Iteratively, the error between all the modelled current traces and the experimental data traces is minimized with a standard nonlinear least-squares optimization algorithm by adjusting the characteristic curve parameters vector until the error between the modelled and experimental current traces is minimal. The procedure is exactly the same in the presence of two components, now with the extended characteristic curve parameter vector $[V_{hs}, k_h, M_h, S_h, A_h, B_h, V_{hf}, k_f, M_f, S_f, A_f, B_f]$.

Application to simulated data

In order to evaluate the performance of the single-trace and the full-trace methods, we simulated an $I_h$ current that was the sum of two

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components with the model described in section 3.2 and the parameters shown in Table 1. These parameters were chosen to correspond, as far as possible, to known physiological values in the inner ear (Chen, 1997; Yi et al., 2010; Meredith et al., 2012).

The currents were simulated in response to a voltage protocol with a holding potential of −60 mV and stepped in 10-mV decrements from −60 to −150 mV, then the post-step potential was −60 mV. Physiological white noise of 10-pA amplitude was added.

To determine the best solutions provided by the identification method, we analysed the distribution of the residual error reached from 500 trials. The best solutions that reached successfully the global minima were associated with the first mode of the distribution (28% of the total number of trials), whereas local minima were associated with additional modes clearly spaced from the first mode. To avoid solutions from local minima, we only retained this first mode (smallest 28%) of the total number of trials.

Both identification methods were applied to these simulated data currents for 50 repetitions with random initial parameter values chosen with a uniform distribution inside the search region, which also constrains the minimization function, of size tol = 80% around the simulated current parameter values (Table 1). The search ranges for the single-trace method are the same for the maximal conductances, between 0 and 1 for the activation gates, and limited to the sum $(A + B) \pm$ tol, for the time constants.

The individual parameter identification error was estimated using $\varepsilon = (p - p_i)/p \times 100$, where $p_i$ is the identified parameter value and $p$ is the simulated current parameter value.

The goodness of fit (GoF) was calculated from the equation:

$$GoF = 1 - \frac{\sum (y(t) - y_i(t))^2}{\sum (y(t) - \text{mean}(y(t)))^2},$$

where $y$ is the original current trace and $y_i$ is the identified current trace.

In all experiments, values are presented as mean ± SEM and statistical significance was assessed using Student’s $t$-test at a significance level of 0.05.

**Neural simulation model**

A single-compartment excitable neuron model was used to demonstrate the effects of the two $I_h$ currents identified in this study. The description of the membrane current flow follows models of cochlear nucleus neurons (Rothman & Manis, 2003a) in the auditory pathway, where maximal conductances are modified to reproduce the firing patterns of sustained and transient vestibular ganglion neurons (Kalluri et al., 2010; Yoshimoto et al., 2015). We added the $I_h$ components identified in this study to models.

$$C \frac{dV}{dt} = -(G_{Na} m^3 h (V - E_{Na}) + G_{K} h (0.85 n^2 + 0.15 p) (V - E_K) + G_{K} h (V - E_K) + G_{I_h} I_{h} + I_{h}) + I(t)$$

where $E_{Na} = 50$ mV, $E_K = -77$ mV, $E_{h} = -36$ mV (Meredith et al., 2012) and $E_{i} = -67$ mV are the reversal potentials. $G_{K} h = 140$ nS, $G_{I_h} = 0$ nS for the sustained vestibular ganglion neuron model type, and $G_{K} h = 50$ nS for the transient model type, are the modified maximal conductances; for details see Rothman & Manis (2003a). The values of the $I_h$ component maximal conductances, $G_{h}$ and $G_{I_h}$, are identified in this study.

**Temperature**

All the conductances were modelled at physiological temperature, using classical $Q_{10}$ values of 3 (Hodgkin & Huxley, 1952).

**Effect of cAMP**

The effect of cAMP, an intracellular cyclic nucleotide that modulates HCN channel activity (Yi et al., 2010), on $I_h$ currents was modelled using data obtained from patch-clamp recordings of the inner hair cell afferent synapse (Yi et al., 2010). Typically, with $200 \mu$m cAMP added to the pipette solution and, additionally, with $200 \mu$m 8-Br-cAMP (cAMP analog) added to the external solution, the $I_h$ current amplitude increases by a factor 1.91, which we utilized to multiply the $I_h$ model maximal conductances in the case of cAMP presence; the activation curves are shifted by 12 mV toward depolarisation (no effect on the slope), which we modelled by shifting the half-activations; the fast-component time constant (probably due to HCN1/HCN2 subunits in the vestibule; Horwitz et al., 2010, 2011), like in the cochlea, is reduced by a factor 2.87, and the slow-component kinetic is reduced by a factor 5.31, which values we used to divide the magnitude of the respective component kinetic functions).

**Implementation**

All the simulations and algorithms of this study were implemented in Python. The current-trace errors were calculated as the sum-of-squares difference between the model and experimental traces at all recorded time points. Parameter identification to minimise this error was done with the _fmin_powell_ function of the _scipy.optimize_ Python package.

**Results**

**Discrimination of the different components in simulated data**

Both parameter identification methods were applied to simulated $I_h$ currents, as described in the methods. Figure 1A, B, E and F shows the individual best trial results (grey lines) and the averages (black bold lines), which are the final results for both methods. The single-trace method individual trial activation function identifications (Fig. 1A) are confounded and do not permit, when averaged, to distinguish the activations of the two current components. The kinetic components are well separated for the two methods (Fig. 1B and F) but for the single-trace method they are not a close fit to the kinetics used to generate the simulated data (Fig. 1B), unlike with the full-trace method (Fig. 1F). Figure 1C and D shows the simulated (grey traces) and reconstructed (black traces) currents (without deactivation currents for clarity) with parameters identified with the single-trace method (Fig. 1A and B). Figure 1C shows the quality of reconstruction when the two components are together and Fig. 1D when the two components are separated. The single-trace method permits a very good reconstruction of the combined currents (GoF = 0.99) but shows a poor reconstruction for the separated currents (fast-component GoF = 0.82, slow-component GoF = 0.91) due to the misidentification of the activation curves (Fig. 1A).

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Table 1. Parameters of the two component currents for the evaluation of the single-trace and full-trace methods

| Component | $V_h$(mV) | $k$ | $M$(mV) | $S$ | $A$(ms) | $B$(ms) | $G$(nS) |
|-----------|-----------|-----|---------|-----|---------|---------|---------|
| $I_{h}$   | −100      | −6  | −80     | 80  | 1000    | 60      | 3       |
| $I_{h}$   | −130      | −9  | −80     | 40  | 250     | 40      | 4       |

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The full-trace method individual trial activation identifications (Fig. 1E) are well separated and permit, when averaged, to distinguish the two components of activation. The full-trace method shows a very good reconstruction of the combined currents (GoF = 0.99; Fig. 1G) as well as the separated currents (fast component GoF = 0.99, slow component GoF = 0.99; Fig. 1H).

Table 2 relates the relative error between each simulated and identified parameter by the single-trace and full-trace methods. The averaged error per parameter value for the single-trace method is 23.4%. In addition, it is impossible, between the slow and fast components, to discriminate their half-activations, $V_{\text{hs}}$ and $V_{\text{hf}}$ ($P = 0.13$), their widths, $S_f$ and $S_s$, or their maximal conductances, $G_f$ and $G_s$. The averaged error for the full-trace method is 4.14%. This method is particularly better than the single-trace method on activation (compare Fig. 1A with Fig. 1E) but also on kinetic identification (the single-trace kinetic amplitude errors are largely > 20% against < 10% for the full-trace method; compare Fig. 1B and F). Most of the parameter identification errors for the full-trace method are < 5%, and a few are < 1%. Furthermore, statistically significant differences between the two $I_h$ components are found in all relevant cases.

To test the sensitivity of the two identification methods, tests were made with either (i) different sizes of the initial search zone (tolerance), (ii) different distances between the half-activations, $V_{\text{hs}}$, of the two current components (by varying $V_{\text{hs}}$ from −130 to −90 mV), or (iii) different distances between the kinetic amplitudes (by varying $A_h$ from 250 to 1250 ms). Figure 2 shows this analysis for the two methods. Identification errors of the principle parameters ($V_{\text{hs}}$, $V_{\text{hf}}$, $A_h$, and $A_f$) are shown as a function of the size of the search zone (tol), the difference between half-activations (Delta act: $|V_{\text{hs}}-V_{\text{hf}}|$), or the difference between the kinetic amplitudes (Delta kin: $|A_f-A_h|$). Unsurprisingly, for all the shown parameters the identification becomes more and more difficult when the search zone increases, but more substantially for the single-trace method (Fig. 2A). Variation of the difference between half-activations does not affect the half-activation parameter identification by the full-trace method but increases the error of the single-trace method (Fig. 2B). The fast and slow kinetics amplitudes are mostly more easily identified by both methods when the difference between the half-activations is large. Increasing the difference between kinetics amplitudes results in slightly better identification of the half-activations by both methods and, for the full-trace method, identification of the slow kinetic amplitude, contrary to the fast kinetic amplitude (Fig. 2C). The averaged error values ($\sigma$) are always smaller for the full-trace method compared to the single-trace method.

### Application to recorded $I_h$ currents

We applied the full-trace method on $I_h$ current recordings from mouse vestibular neurons ($n = 9$) assumed to be composed of at least two activated components given the two exponentials needed for single-trace fitting (Meredith et al., 2012), and the presence of several HCN subunits, including in the vestibular periphery (Horwitz et al., 2010, 2011), not isolated pharmacologically in the cochlea (Chen, 1997; Yi et al., 2010). Figure 3 shows the application of the full-trace method to the recorded $I_h$ currents, the parameters identified, and the simulations of the sustained and transient vestibular neuron models provided by the newly identified $I_h$ component models. Figure 3A shows an example of the $I_h$ current (grey trace) in response to a voltage-clamp protocol with a holding potential of −60 mV and a step potential from −60 to −160 mV stepped in decrements of 10 mV. The black trace shows the reconstruction of these currents after identification. For each recorded cell, the fitting of the experimental traces involves the averaging of the 14 best repetitions from 50 trials, resulting in a GoF of 0.98 in the example shown in Fig. 3A and an average GoF of 0.97 over the nine recordings. In Fig. 3, panels B and C show, respectively, the activation and kinetic curves obtained for all the $I_h$ recordings ($n = 9$). The thin lines represent the averaging of the 14 best trials on one particular cell (the final result of the algorithm applied to one particular cell, or the equivalent of the bold line in the Fig. 1E). The bold lines represent the averaging of the results obtained for the nine cells. Figure 3D shows the identified parameter values, which indicate a fast component that is a low-voltage-activated current ($V_{\text{hf}} = −130.6 \text{ mV}$) and slow component that is a high voltage activated current ($V_{\text{hs}} = −108.6 \text{ mV}$), with significantly different activation slopes $k_f$ and $k_s$, of, respectively, −5.1 and −9.6. The principle kinetic characteristics are also significantly different, with fast and slow maximum time constants of, respectively, 0.244 and 0.995 s, and widths $S_f$ and $S_s$, of, respectively, 59.8 and 31.2. The pairwise parameters of the two components are significantly different in all cases apart from the kinetic bases ($B_f$ and $B_s$) and the maximal conductances ($G_f$ and $G_s$).

Figure 3E shows the responses of the vestibular ganglion neuron models (sustained, left panel and transient, right panel; see Materials and Methods) provided by both identified $I_h$ components, evoked by positive and negative current-clamp steps. The firing patterns differentiating the two basic categories of vestibular ganglion neurons (Kalluri et al., 2010; Yoshimoto et al., 2015) are well reproduced by the models (left and right upper panels). The sag which is known to reflect the presence of the $I_h$ currents in vestibular ganglion neurons (Chabbert et al., 2001b), in response to hyperpolarising current steps, is reproduced by the model in both neuron model types (left and right lower panels).

### Table 2. Relative parameter identification evaluation for single and full-trace methods

| Method       | $V_{\text{hs}}$ | $K$     | $M$     | $S$     | $A$     | $B$     | $G$     |
|--------------|----------------|---------|---------|---------|---------|---------|---------|
| Single trace |                |         |         |         |         |         |         |
| Slow         | 16 ± 7.1       | 30 ± 24.0 | 21 ± 10.1 | 36 ± 7.9 | 18 ± 14.5 | 28 ± 28.6 | 23 ± 5.8 |
| Fast         | 7 ± 4.0        | 34 ± 17.7 | 22 ± 9.0  | 20 ± 23.7 | 26 ± 15.5 | 35 ± 17.4 | 12 ± 7.0 |
| $P$          | 0.13           | < 0.01   | 0.7      | 0.31     | < 0.01   | < 0.05   | < 0.01   |
| Full trace   |                |         |         |         |         |         |         |
| Slow         | 0 ± 0.1        | 4 ± 6.3  | 2 ± 21.2 | 7 ± 20.6 | 10 ± 15.7 | 16 ± 29.2 | 3 ± 5.3  |
| Fast         | 0 ± 0.5        | 0 ± 5.2  | 0 ± 14.2 | 2 ± 20.5 | 9 ± 27.6  | 5 ± 20.6  | 0 ± 8.3  |
| $P$          | < 0.01         | < 0.01   | 0.57     | < 0.01   | < 0.01   | < 0.01   | < 0.01   |

The first two lines for both methods show the errors in percentage, compared to the original parameters. The mean single-trace method error per parameter value is 23.4%. The mean full-trace method error is 4.14%. The third line indicates the significance of difference ($P$-value). The single-trace method does not find significant differences between half-activations and the Gaussian width of kinetics. When relevant, the differences are always significant for the full-trace method.
Thus, if the actual different from the GoF obtained with the two-component model; the three-component current model is slightly better but not significantly different with two components, $P_{\text{one current component}}$ and the GoF was 0.93 (significantly different than with two components).

The half-activations, $M$, and the kinetics amplitudes, $A$, errors (% of the genuine parameters) are shown as a function of (A) tolerance (tol), (B) difference between half-activations (delta act) and (C) difference between kinetics amplitude (delta kin). The solid and open points correspond respectively to the single- and full-trace identification methods. The lines show the tendencies. The single-trace method results in averaged errors (m) that are always larger than the full-trace method, whatever the tested parameter.

To confirm that the two-component model is indeed the most parsimonious fit to the data, we applied the full-trace method with only one current component and the GoF was 0.93 (significantly different than with two components, $P < 0.02$). The GoF obtained with a three-component current model is slightly better but not significantly different from the GoF obtained with the two-component model; thus if the actual $I_h$ currents are composed of more than two components, their effect is negligible.

$I_h$ components shape action potential excitability and EPSPs

The $I_h$ currents are known, in the inner ear, to shape EPSPs and modulate excitability in neurons (Yi et al., 2010). To see whether the two components contribute differently to shaping neuronal excitability and EPSPs, we have simulated the sustained and transient neuron models with both $I_h$ components and with each isolated component, to evaluate AP latencies due to the $I_h$ currents and, in the case of $G_{\text{Na}} = 0$ nS (modelling the presence of TTX), to see the influence they have on EPSP shape.

Figure 4A shows APs fired in response to a 10%-above-threshold double-exponential current stimulation (rise time constant 0.05 ms, decay time constant 5 ms; Sadeghi et al., 2014). The black trace is the control case, without $I_h$ currents in the model, and the grey traces are the response of the sustained neuron, after a conditioning protocol activating the $I_h$ current at $-105$ mV (a 500-ms hyperpolarisation, which is long enough to reach a steady-state value for the $I_h$ currents but not large enough to induce spontaneous AP firing, followed by a delay of 5 ms at rest before inducing the AP). Left central, and right panels show the response of the model provided with both $I_h$ components, fast component only, and slow component only, respectively. The results show that the latencies of neuronal firing are reduced, mainly driven by the slow component. Figure 4B shows the simulations done with the transient neuron model. Now the $I_h$ currents introduce a very small increase in the latency.

Figure 4C and D shows the same simulations but without the sodium conductance, to evaluate the influence of both $I_h$ components on the shape of the EPSP, with the sustained and transient neuron models, respectively. Changes in the time course or amplitude of the EPSP could influence the temporal requirements for transmitter release to generate an AP. Once again, the slow component contributes more than the fast component in affecting the shape of the EPSPs, resulting in a smaller amplitude and faster time course. In addition, the simulations show that the EPSP shape and the resting membrane potential are more influenced by the $I_h$ currents in the sustained neuron than in the transient neuron model.

Quantification of these effects for several conditioning potentials (Fig. 4E) shows that in sustained neurons (black traces), as a function of the conditioning potential, the AP latencies (measured from the stimulation to the peak) vary from 0.65 to 1.6 ms, the resting potentials decrease from $-59$ to $-66$ mV, the EPSP exponential decays vary from 3.9 to 5.0 ms, compatible with data (Meredith et al., 2012; Sadeghi et al., 2014), and the EPSP amplitudes increase slightly from 5.5 to 7.5 mV. In transient neurons (grey traces), as a function of the conditioning potential, the AP latencies vary from 0.65 to 0.50 ms, the resting potentials decrease from $-64.5$ to $-68$ mV, the EPSP exponential decays vary from 5.4 to 6.8 ms, and the EPSP amplitudes increase significantly from 10.5 to 15.5 mV. All these variations are principally due to the slow component in both sustained and transient neuron models.

Neural simulations with identified $I_h$ currents

To further highlight the effect of each component (Fig. 5), we added only one of the two $I_h$ components at a time to the sustained-neuron model. The model was stimulated with either a moderate negative...
Vestibular fast and slow \( I_h \) components

Fig. 3. The two-component \( I_h \) current parameters identification. (A) Recorded \( I_h \) currents (grey trace) in response to a voltage-clamp protocol with a holding potential of \(-60\) mV and a step potential from \(-60\) to \(-150\) mV decreased in steps of \(10\) mV. Reconstruction by the model (black trace). (B) Half-activation of the slow (grey) and fast (black) activated components are respectively at \(-108.6\) and \(-130.6\) mV, the slopes are respectively of \(-5.0\) and \(-9.5\). Thin lines are best fits for each of the nine cells (average of 14 best tries from 50); thick lines are average fit over the nine cells. (C) The kinetic amplitudes of the slow- and fast-activated components are respectively 0.99 and 0.24 s and the widths (parameters \( S \)) are, respectively, 59.8 and 31.2. (D) Parameter values and lines are best fits for each of the nine cells (average of 14 best tries from 50); thick lines are average fit over the nine cells. (E) Firing patterns and hyperpolarisation sags of sustained and transient vestibular neurons models, provided with both \( I_h \) components, in response to \(-120\), \(-60\), 20 and 40 pA current-clamp stimulation. The models reproduce actual data (Kalluri et al., 2010; Yoshimoto et al., 2015).

hyperpolarising current, \( A = -250\) pA (Fig. 5A, left panels) or a slightly stronger current, \( A = -300\) pA (Fig. 5A, right panels), with varying durations. Whatever the stimulation amplitudes or durations, the fast component is able to fire only one AP (Fig. 5A, upper panels). The slow component induces bursts of one to seven APs as a function of the stimulation duration and this effect increases with stimulation strength (Fig. 5A, lower panels). The results with both components present (not shown) are very similar to those with the slow component only.

In the same conditions of stimulation, we simulated the firing of the cells with the modelled effect of cAMP (Fig. 5B), which increases current amplitude, shifts the activation curves and reduces the two kinetic components differently (see Materials and Methods). Whatever the stimulation amplitudes and durations (Fig. 5B, upper panels) the fast component still is able to fire only one single AP. Interestingly, the slow component induces either bursts of two or three APs as a function of the stimulation strength, now independently of the stimulation duration (Fig. 5B, lower panels).

To better understand the role of the slow component, which is clearly involved in shaping the stimulation coding, we have fully tested the neural response (number of APs fired after inhibitory stimulation) of this model as a function of the stimulation duration and amplitude, in control conditions (Fig. 5C) and in the presence of cAMP (Fig. 5D). In control conditions the neural response is not clearly correlated with one or the other variable. The neuron seems to code for the duration of stimuli > 350 pA, and for the amplitude of stimuli > 200 ms. However, in the presence of cAMP the neural response becomes totally independent of the duration for stimuli > 50 ms and the neuron codes then exclusively for the stimulus amplitude.

In addition, we tested the transient vestibular neuron model with the same protocols, but the large amplitude of a low-voltage-activated potassium current, probably composed of Kv1 and KCNQ channels, in this cell, precluded rebound firing of more than one AP, as already shown (Kalluri et al., 2010; Yoshimoto et al., 2015) whatever the activated \( I_h \) component.

Discussion

Hyperpolarisation-activated currents

This study shows that the \( I_h \) current in vestibular ganglion neurons is composed of a slow and a fast component which are activated at different voltages, quite similar to the \( I_h \) current in the guinea pig spiral ganglion (Chen, 1997), the rat primary auditory afferent dendrites (Yi et al., 2010) and the vestibular calyx afferent of Mongolian gerbils (Meredith et al., 2012). This is the first identification and modelling of separate \( I_h \) components.

Suprathreshold EPSPs (slightly larger than spontaneous EPSPs from data) that generated an AP were modelled to measure the effect of the \( I_h \) current components on ganglion cell firing properties. We have also modelled EPSPs, in the absence of cell firing, to provide information about the effect of the two \( I_h \) current components on the response to synaptic input from hair cells to afferent terminals (independently of their effect on firing). These \( I_h \) currents influence both AP firing and EPSP shape. In sustained neurons, the
AP latency could be strongly decreased by the activation of the $I_h$ current, due to depolarisation of the resting potential, and the EPSP time course also is decreased, sharpening the temporal response to transmitter release. Both of these effects could allow the regulation of firing rate in vestibular neurons, as well as in the cochlea (Yi et al., 2010). In transient neurons, surprisingly, the AP latency very slightly increases, but the resting potential, decay time and amplitude vary similarly to the sustained neurons. Interestingly, the presence of low-voltage-activated potassium conductance in these neurons results in EPSPs with a double exponential decay (Fig. 4D), like the calyx EPSCs (Sadeghi et al., 2014). For all these conclusions, the main effect is due to the slow $I_h$ component.

Further computer simulations indicated that these two $I_h$ components influence the firing behaviour of these cells in response to inhibitory (hyperpolarising) stimulation of sufficient magnitude and duration to generate rebound APs. The components allow the coding of different characteristics of the stimulation. The fast component permits the firing of a single AP at the end of the stimulation, regardless of stimulation duration or amplitude, whereas the slow component rather permits coding of the stimulation duration or amplitude by the number of rebound APs fired (and this is further modified in the presence of cAMP; see below). These results are valid only for the sustained vestibular ganglion neurons. The high sensitivity of the rebound AP to the low-voltage-activated potassium current (Kv1 and KCNQ; Hurley et al., 2006), means the transient neurons do not fire more than one AP, even for high stimulation duration and amplitude.

The stimulation potentials used in the computer simulations underpinning the conclusions of this study, in particular concerning the firing of rebound APs, may seem surprisingly long and hyperpolarised, but the spontaneous APs recorded in the vestibular calyx terminal (transient neurons) are followed by a strong hyperpolarisation reaching $-160$ mV with duration of $\sim 100$ ms (Meredith et al., 2012). This form of hyperpolarisation drives the electrophysiological behaviour presented in this study. However, it would be worth knowing whether this strong hyperpolarisation is also present in the sustained neurons, which are more likely to fire rebound APs. Such hyperpolarisations have not yet been seen in these neurons and this could confirm the eventual functional role of the $I_h$ currents in these neurons. That said, the sustained neurons do not need to give information about the stimulus duration because of their regularity, but this functionality could be interesting to code the strength of the preceding hyperpolarisation.

$I_h$ currents are thought to contribute to setting the resting membrane potential (He et al., 2014). This is the case for the slow component, because of its 'high' voltage activation (closer to the resting membrane potential).
membrane potential) but still only after conditioning induced by preceding strong hyperpolarisation; however, it is more difficult for the fast component because of the very low voltage activation (Fig. 3B and C). So, if the slow component could be expected to depolarise the membrane resting potential, the fast component could be only expected to regulate the firing of the neurons, for example by inducing rebound APs.

The most popular hypothesis concerning the presence of two components in the hyperpolarisation-activated cationic current is that the different electrophysiological behaviours arise from channel populations consisting of different mixtures of HCN subunits (Liu et al., 2014). Concerning the mouse vestibular ganglion, here we show the presence, at room temperature, of a fast activated component (200–400 ms) and a slow activated component (800–1200 ms) that could be principally induced respectively by HCN1/HCN2 and HCN4 subunits, knowing their kinetics (Santoro et al., 2000), and this would be compatible with previous results in the vestibular periphery (Horwitz et al., 2010, 2011).

A study in the superior paraolivary nucleus showed that an \( I_h \) current has to be associated with a transient calcium current for a neuron to be able to burst in response to inhibitory current stimulation (Kopp-Scheinpflug et al., 2011). Our study shows, without neuro-modulation, that the fast component effectively provides only one AP even for strong or long inhibitory stimulation, but in these conditions the slow component can stimulate bursting responses (without any other association) where the number of APs is dependent on the stimulation amplitude or duration. A next step in the study of ionic channel associations could be this calcium effect with each of the \( I_h \) components, and the specific HCN subunits with which it is required to allow an inhibitory current to cause stimulation bursting.

**cAMP modulation**

In the inner ear, cAMP has been shown to strongly reduce the time constants and to shift the activation curves towards depolarisation differently according to the related \( I_h \) current component (Yi et al., 2010). With our cell model, we have shown that the presence of cAMP does little to change the effect of the fast component. However, the slow component loses its ability to signal stimulus duration during cAMP modulation.
and amplitude and becomes only a clear stimulus amplitude indicator. Again, these results are valid only for the sustained-type neur- 
on, and transient-type neurones do not fire more than one AP, even for high stimulation duration or amplitude. It has also been show in 
this study that the cAMP modulation does not affect the Ih influence on AP excitability nor the shape of EPSPs.

It has been proposed that the Ih conductance regulates the firing activity or resting membrane potential in the inner ear (Chen, 1997; Liu et al., 2014). However, we found that the half-activation 

potentials of both components are too hyperpolarised to be 

substantially associated with these. This discrepancy could be due 
to the young age of the tested mice. To go further, it was shown 
hyperpolarization-activated cation currents are shifted at adult ages 
by ∼30 mV toward depolarisation (Khurana et al., 2012). It could be interesting to apply the algorithm at different stages of develop- 

ment to see at which moment the Ih currents are able to regula- 

tate firing rate and, in this regard, be effectively modulated by 
cAMP.

Seven different neurotransmitters are known to be released by the lateral efference in the cochlea and vestibule (Ryugo et al., 2011). Their effect on cAMP and further on Ih currents could be the way by which lateral efference in the cochlea or brainstem vestibular 
nuclei modulates the firing pattern or the resting potential of the 
affenter pathways. In fact, efferent transmitters could potentially 
influence Ih gating by modulating intracellular levels of cyclic 

nucleotides. HCN channels can be potentiated by direct binding of 
cAMP through the CNBD, and intracellular cAMP levels could 

change via G-protein-coupled inhibition or activation of adenyl cyclase (Meredith et al., 2012). It was speculated that elevating 

levels of intracellular cyclic nucleotides could enhance firing in 
calyx afferents through Ih (Meredith et al., 2012). In our study, 
however, given the very low potentials at which they are activated, 
it is not likely the Ih current would be able to influence directly the 
vestibular afferent’s firing, and it would more probably affect the 
rebound APs, AP excitability or EPSP shaping after hyperpolarisa- 
tion, and this would be principally driven by the slow component. 
Once again, this is valuable at P5–P8, and the activation shifting at 
adult ages could strongly modulate this assumption.

A future study could model the different neurotransmitters 
involved in the regulation of the neural afferent activities in the 
inner ear and evaluate their impact associated with the presence of 
different HCN channels on the afferent pathway. Also, it would be interesting to know whether and what kind of neuromodulation 
would be responsible for the switching of the slow component to 
be a specific stimulus duration indicator, as an antagonist effect of 
the switching to a specific stimulus amplitude indicator, as with 
cAMP.

Algorithm

Several studies have revisited the full-trace method: using data from 
patch-clamp experiments to identify the parameters of Markov mod- 
els on rat cortex pyramidal neurons (Gurkiewicz & Komgreen, 
2007); to highlight the neural dynamics in lobster lateral pyloric 
neurons (Nowotny et al., 2008); and to estimate a conductance gra- 
dient along a dendrite (Keren et al., 2009). Only one study of this 
type concerns the identification of two ionic populations, one inacti- 

vated and one non-inactivated potassium conductance (Vavoulis et al., 2010). This context is more favourable than the one of our 
study, indeed the presence of one inactivated conductance implies 
easier identification of the non-inactivated conductance once the first is inactivated. Furthermore, this study does not give any details of 
the protocols or comparison with a single-trace method. Our study 
shows that the full-trace method also identifies the conductance 
parameters in the new context of two non-inactivated voltage-depen- 
dent ionic currents, whatever the characteristics of the two compo- 
nents, much more accurately than the single-trace method based on 
the fitting procedure of Hodgkin & Huxley (1952), from voltage-

clamp data.

This study is focused on Ih currents but would be appropriate for 
other neurons or sensory cells where different ionic currents are 
involved that may not be able to be perfectly pharmacologically iso- 
lated. In particular, there are two potassium conductances (low-volt-
age-activated and high-voltage-activated) all along the auditory 
pathway (Santos-Sacchi, 1993; Mo et al., 2002; Szabó et al., 2002; 
Rothman & Manis, 2003a; Rusznák & Szucs, 2009). The full-trace 
method could be used to verify the good identification of the activa- 
tions and kinetics of these potassium conductances, and to evaluate 
the selectivity of the pharmacological blockers.

Moreover, neuronal activity depends sometimes on more than 
seven voltage-dependent ionic currents, for example, in the devel- 

oping inner hair cell AP firing (Marcotti, 2012). Pharmacological 
tools do not necessarily exist for all these channels. Further, even 
if the pharmacological tools do exist, because of the sigmoidal 
shaped dose–response curve, the inhibition of a current is never 
total, even with very strong blocker concentration. Thus, the ability 
of the full-trace method to identify conductance parameters was 
shown here in the presence of two non-inactivating ionic compo- 
nents but could be explored in tougher conditions. A study has 
shown the full-trace method could be applied with one inactivating 
and one non-inactivating ion channel population (Vavoulis et al., 
2010). A novel study could examine the full-trace method’s ability 
to identify two non-inactivating and one inactivating population, 
enzymatically separated, such as in the spiral ganglion neurons 
(Szabó et al., 2002).

We have tested the algorithm on patch-clamp data that does not 
include a separate de-activation protocol, apart from a return to the 
pre-step potential following the activation step. To overcome this 
lack, we used a symmetric kinetic model. In order to improve 
knowledge of the Ih current (for depolarised potentials) it would be 
interesting to use this algorithm on data including a de-activation 
protocol with an asymmetrical kinetic model.

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flits of interest.

Abbreviations

AP, action potential; EPSP, excitatory postsynaptic potential; GoF, goodness of fit; HCN, hyperpolarization-activated cyclic nucleotide-gated; P, postnatal day.

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