Differential Expression of Three Distinct Potassium Currents in the Ventral Cochlear Nucleus

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Rothman, Jason S. and Paul B. Manis. Differential expression of three distinct potassium currents in the ventral cochlear nucleus. J Neurophysiol 89: 3070-3082, 2003; 10.1152/jn.00125.2002. In the ventral cochlear nucleus (VCN), neurons transform information from auditory nerve fibers into a set of parallel ascending pathways, each emphasizing different aspects of the acoustic environment. Previous studies have shown that VCN neurons differ in their intrinsic electrical properties, including the K$^+$ currents they express. In this study, we examine these K$^+$ currents in more detail using whole cell voltage-clamp techniques on isolated VCN cells from adult guinea pigs at 22°C. Our results show a differential expression of three distinct K$^+$ currents. Whereas some VCN cells express only a high-threshold delayed-rectifier-like current (I$_{HT}$), others express I$_{HT}$ in combination with a fast inactivating current (I$_{A}$) and/or a slow-inactivating low-threshold current (I$_{LT}$). I$_{HT}$, I$_{LT}$, and I$_{A}$ were partially blocked by 1 mM 4-aminopyridine. In contrast, only I$_{LT}$ was blocked by 10–100 nM dendrotoxin-I. A surprising finding was the wide range of levels of I$_{LT}$ suggesting I$_{LT}$ is expressed as a continuum across cell types rather than modally in a particular cell type. I$_{A}$, on the other hand, appears to be expressed only in cells that show little or no I$_{HT}$, the Type I cells. Boltzmann analysis shows I$_{LT}$ activates with 164 ± 12 (SE) nS peak conductance, −14.3 ± 0.7 mV half-activation, and 7.0 ± 0.5 mV slope factor. Similar analysis shows I$_{LT}$ activates with 171 ± 22 nS peak conductance, −47.4 ± 1.0 mV half-activation, and 5.8 ± 0.3 mV slope factor.

INTRODUCTION

The central auditory system has a remarkable ability to extract information from the relatively simple sensory representation provided by the auditory nerve (AN). At the first central processing center, the cochlear nucleus, neurons transform the relatively uniform discharge of AN fibers into a set of parallel ascending pathways that each emphasize different features of the sensory environment. In the ventral cochlear nucleus (VCN), neurons differentially represent stimulus phase, fine temporal periodicity, envelope modulation, and stimulus intensity by their discharge patterns. These transformations are achieved by a variety of mechanisms, including exceptionally rapid synaptic receptor kinetics, dendritic filtering, coincidence detection, and specialized membrane currents in the postsynaptic neurons.

One of the major constituent cell types in the VCN is the bushy cell. Bushy cells typically have one or two apical dendrites that branch profusely within several hundred microns of their cell body. Based on their location in the cochlear nucleus, their AN innervation pattern, and their projections to the superior olivary complex, bushy cells are subclassified as being either spherical or globular. Spherical bushy cells are located in the rostral anterior VCN, receive their AN input via a few (1–3) large calycoidal synapses located on their soma (Brawer and Morest 1975; Brawer et al. 1974; Lorente de Nó 1981; Sento and Ryugo 1989), and project to the lateral superior olive. Globular bushy cells, on the other hand, are located near the AN root region, receive anywhere between 5 and 25 convergent somatic AN calycoidal synapses (Liberman 1991, 1993; Spirou et al. 1990), and project both to the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (Cant and Casseday 1986).

The second major constituent cell type in the VCN is the multipolar, or stellate cell (Brawer and Morest 1975; Brawer et al. 1974; Cant 1992; Lorente de Nó 1981). In contrast to bushy cells, stellate cells have long, sparsely branched dendrites that receive significant synaptic innervation via small bouton endings; physiological measurements suggest a minimum of five convergent inputs from AN fibers (Ferragamo et al. 1998). Rather than projecting to the superior olivary complex, as bushy cells do, stellate cells project to the inferior colliculus and/or the dorsal cochlear nucleus (Adams 1979; Cant 1982; Doucet and Ryugo 1997; Ostapoff et al. 1999).

Given the heterogeneous morphology and innervation pattern of VCN neurons, it has long been postulated that different classes of VCN neurons perform distinct information processing functions (Browell 1975; Pfeiffer 1966; Rhode and Smith 1986). VCN neurons of different morphology are in fact associated with different responses to acoustic stimuli (Feng et al. 1994; Ostapoff et al. 1994; Rhode et al. 1983; Rouiller and Ryugo 1984; Smith and Rhode 1987, 1989). Spherical and globular bushy cells, for example, respond to tone bursts with a “primary-like” and “primary-like-with-notch” response. Both cell types exhibit excellent phaselocking; in fact, globular bushy cells with convergent inputs can phaselock to low-frequency tones better than AN fibers (Joris et al. 1994). It has been suggested that these two cell types provide distinct information about the fine timing structure of complex stimuli (Shofner 1999). Such information is useful in determining formant structure and pitch. Similarly, octopus cells of the posterior VCN are thought to provide timing information to higher auditory centers, and although their innervation patterns are quite distinct from bushy cells (they receive convergent
input from a large array of AN fibers tuned to different frequencies), they appear to use membrane mechanisms similar to those of bushy cells (Golding et al. 1995, 1999). Stellate cells, in contrast, respond to tone bursts with a “chopping” response; these cells presumably report information about the stimulus envelope, or low-frequency amplitude modulation of a sound, an analysis useful in identifying vowels (Frisina et al. 1985, 1990; Kim et al. 1990; Rhode 1998; Shofner 1999; Wang and Sachs 1992, 1994).

The specific complement of K⁺ currents in neurons has been shown to be tremendously important in controlling not only spike shape, but also spike rate, spike adaptation, and regularity of discharge. This is undoubtedly true for VCN neurons, which are already known to possess different complements of K⁺ channels (Manis and Marx 1991). The response of bushy cells to injected current steps, for example, is much different to that of stellate cells; whereas stellate cells respond with a regular train of action potentials, bushy cells respond with a phasic discharge of one to three action potentials (Francis and Manis 2000; Oertel 1983; Schwarz and Puil 1997; White et al. 1994; Wu and Oertel 1984). After this initial discharge of one to three action potentials, bushy cells enter a high conductance state until the applied current is terminated. The high conductance state is generated by a low-threshold, relatively non-inactivating K⁺ current (I₅₋₇) (Manis and Marx 1991). I₅₋₇ differs from the conventional delayed rectifier found in the mammalian brain in that it has a particularly low activation voltage (~70 vs. ~30 mV) and differs from other K⁺ currents active at subthreshold potentials (e.g., the transient A-type K⁺ current, I₆ₓ) in that it shows little inactivation for short test pulses. A detailed model including I₅₋₇ replicates many characteristics of VCN bushy cells, including their ability to phase-lock at frequencies up to 5 kHz (Rothman et al. 1993). Experimental evidence suggests that blocking I₅₋₇ with 4-aminopyridine (4-AP) degrades the ability of these cells to phaselock (Reyes et al. 1994). Other studies suggest I₅₋₇ allows cells to act as precise coincidence detectors (Joris et al. 1994; Rathouz and Trussell 1998; Reyes et al. 1994; Rothman and Young 1996; Rothman et al. 1993).

In addition to I₅₋₇, a high-threshold delayed-rectifier-like current (I₅₋₇) has been characterized in both bushy and stellate cells (Manis and Marx 1991). The likely source of I₅₋₇ is the KCNC1 channel, which is highly expressed in mammalian VCN cells (Grigg et al. 2000; Perney and Kaczmarek 1997; Perney et al. 1992). This channel is also highly expressed in cells homologous in structure to spherical bushy cells, the MNTB cells (Grigg et al. 2000; Perney et al. 1992; Wang et al. 1998). It has been proposed that I₅₋₇ allows neurons to fire at high rates by providing a rapid repolarization of their action potential (Wang et al. 1998).

The present study was undertaken to more thoroughly characterize the K⁺ currents in VCN neurons and especially to provide detailed kinetic data that can be used for modeling. We present this work as a series of three papers. In this, the first paper, we describe the separation of the three different classes of K⁺ currents in VCN neurons. We show that the magnitude of I₅₋₇ appears to vary between neurons in a way that suggests a continuum of cell types rather than a collection of discrete classes. We also find a gradation of expression of I₆ₓ. In the second paper, we present kinetic analysis of the separated currents and derive equations useful for representing them mathematically. In the final paper, we present a somatic model that incorporates these currents into a single electrical compartment, thereby allowing us to elucidate the role each current plays in controlling the discharge pattern of VCN neurons. Portions of this work have been presented previously in abstract form (Rothman and Manis 1996, 1997).

**METHODS**

**Cell isolation and whole cell recordings**

Isolated VCN cells were obtained by previously published methods (Harty and Manis 1996). Briefly, young adult guinea pigs (4–12 wks old) weighing 110–340 g were anesthetized with pentobarbital and decapitated. The brain stem was quickly removed from the cranial cavity and immersed in an oxygenated dissection solution ([in mM] 112 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 30 glucose, 20 PIPES, 1 kynurenic acid, and 0.5 mg/ml bovine serum albumin). Each cochlear nucleus was blocked and cut into 300-μm-thick slices. From these slices the VCN was cut away and placed in a spinner flask containing oxygenated dissection solution (30°C) supplemented with bovine pancreatic trypsin (0.6 mg/ml). After 30 min of enzymatic treatment, VCN slices were thoroughly rinsed in enzyme-free dissection solution and allowed to incubate at room temperature for ≥1 h. Dissociated VCN cells were obtained by gently triturating one or two slices through a series of fire-polished Pasteur pipettes with decreasing tip diameter. The resulting cell suspension was then plated onto a petri dish coated with poly-co-lysine. Cells were allowed to settle and attach to the bottom of the dish for ~15 min before starting a continuous flow of a HEPES-buffered extracellular solution in (in mM) 130 NaCl, 5 KCl, 2.5 CaCl₂, 1.3 MgCl₂, 30 glucose, and 10 HEPES.

Whole cell voltage-clamp recordings were made at room temperature (22°C) with an Axopatch 200 amplifier (Axon Instruments). Electrodes were pulled from glass capillaries (KG-33, Garner Glass), fire polished, coated with silicone elastomer (Sylgard 184; Dow Corning), and filled with a K-glutamate electrode solution ([in mM] 130 K-glutamate, 4 NaCl, 1 EGTA, 5 sucrose, 10 HEPES, and 4 Mg₂ATP). Electrode resistance was typically 3–10 MΩ. Access resistance (Rₓ) was in the range 5–20 MΩ. 75–95% of which was compensated on-line. Cell capacitance (Cₛ) was also compensated on-line. Recordings were filtered at 1–5 kHz and digitized at 2–10 kHz with a 12-bit A/D converter (Digidata 1200, Axon Instruments). Current records are averages of three records except where noted.

To block specific ionic currents, pharmacological agents were often added to the HEPES-buffered extracellular solution. For example, 1 μM tetrodotoxin (TTX) and 50 μM cadmium (Cd²⁺) were routinely added to block sodium and calcium currents respectively. To this HEPES-CTX/Cd²⁺ solution, various concentrations of 4-AP or dendrotoxin-I (DTX) were sometimes added to block specific K⁺ currents.

**Voltage and current corrections**

Under whole cell voltage clamp, the voltage command (Vc) and the transmembrane voltage (Vm) are not identical. For the most part, this discrepancy arises from two sources: the voltage drop arising from the flow of membrane current (Iₚ) across the series resistance (Rₛ) and the charging of the membrane capacitance (Cₛ) during a step change in Vc. The first source of error was partially compensated online (75–95%) using the “correction” compensation circuitry of the Axopatch amplifier. The remaining error due to uncompensated portion of Rₛ was then corrected offline, assuming the following current-voltage relation: 

\[ V_m = V_c - \left( I_p + \frac{C_s}{R_s} \right) \]

where Rₛ is the residual uncompensated fraction of Rₛ. In this paper, all figures display the estimated Vₚ rather than Vₚ, in which case the voltage traces often show small deviations at the beginning of the command step. The second source of error was also partially compensated on-line (40–95%) using the “prediction”
compensation circuitry of the amplifier. There were, however, a number of cells where prediction compensation was not employed (n = 55). With no prediction compensation, Vmax will respond to a step change in Vi in the following exponential manner: 

\[ V_{\text{max}} = V_i \left[ 1 - \exp(-t/\tau) \right] \]

where \( \tau = R_i C_{\text{m}} \). For the average cell in this study, \( \tau = (13 \text{ MF})(12 \text{ pF}) = 160 \mu\text{s} \), meaning \( V_{\text{max}} \) will have a 10–90% rise time of 340 \( \mu\text{s} \) and will settle to within 5% of its final value 0.5 ms after the start of the command step. For the analysis of steady-state current-voltage (I-V) relations presented in this paper, the difference in rise times is inconsequential.

Another voltage correction performed offline was the subtraction of the liquid junction potential between the pipette and the extracellular bath solutions, measured to be \(-12\text{ mV}\).

Finally, current traces were routinely corrected for leakage current by computing I-V relations at hyperpolarized potentials, fitting a linear function to the resulting data, then subtracting estimates of the leakage current from the raw current traces at each voltage step (linear interpolation and extrapolation). Because some cells possessed \( I_{\text{leak}} \) and/or \( I_{\text{p}} \), whereas others did not, the specified range of hyperpolarizing voltage steps was adjusted on a cell-by-cell basis. Typically, the range fell between \(-55\) and \(-100\text{ mV}\) for Type I cells, and \(-75\) and \(-100\text{ mV}\) for Type II cells.

**Boltzmann functions**

Steady-state activation of a two-state voltage-dependent conductance \( g \) can be described by the Boltzmann equation

\[ g = g_{\text{max}} \left[ 1 + \exp(-V - V_{\text{h}})/k \right]^{-1} \]

where \( g_{\text{max}} \) is the maximum conductance, \( V \) the membrane potential, \( V_{\text{h}} \) the half-activation voltage point, and \( k \) the slope parameter. Because the \( K^+ \) currents investigated in this study showed linear instantaneous I-V relations in the form \( I = g(V - V_i) \) (from -110 to 0 mV; data not shown), Eq. 1 can be redefined as

\[ Y_i(V) = g_{\text{max}}(V - V_i) \left[ 1 + \exp(-V - V_{\text{h}})/k \right]^{-1} \]

where \( Y_i \) is the reversal potential of the \( K^+ \) current under investigation. Eq. 2, referred to as the modified Boltzmann function, proved useful in this case since I-V relations could be directly fit without converting current to conductance: an undesirable conversion since it leads to large deviations in the data near \( V_i \). Because \( K^+ \) channels are usually closed near their \( V_i \) in which case \( V_i \) is not evident in steady-state I-V relations, \( V_i \) had to be predetermined from instantaneous I-V relations (i.e., tail currents), or, if that was not possible, set equal to \(-70\text{ mV}\), the average value computed from our tail current analysis (Rothman and Manis 2003a).

**Defining threshold of activation**

The voltage threshold \( V_{\text{th}} \) for activation of a current is typically determined as the voltage at which a predefined fraction of a maximum conductance \( (g_{\text{max}}) \) has been reached. This definition, however, requires knowing \( g_{\text{max}} \), which is not always possible. For example, whole cell currents in this study sometimes exceeded the upper limit of the recording amplifier (20 nA) at the highest command steps. This current could be achieved before saturation of the conductance was evident, preventing an accurate estimate of \( g_{\text{max}} \) via Eq. 2. \( V_{\text{th}} \) was therefore defined as the voltage at which the current reached 0.1 nA. This definition is desirable in that it does not require knowing \( g_{\text{max}} \) is small enough to lay within the knee of conductance activation but large enough to lay above the baseline noise, and is small enough that any voltage error due to \( R_s \) is negligible. Yet, as Fig. 1 shows, this definition can be problematic. This figure shows three I-V relations of three hypothetical cells sharing the same delayed-rectifier-like conductance \( \text{(inset)} \) but with varying \( g_{\text{max}} \). Because the cells share the same conductance, they should have the same activation threshold.

![Graph showing voltage threshold and conductance](http://jn.physiology.org/)

**Statistics**

Statistical significance was assessed using a two-sided \( t \)-test for unpaired samples at the significant level \( (P) \) indicated. Significant differences are denoted with asterisks as follows: \(*P < 0.05\), \(**P < 0.01\), \(***P < 0.001\). All results are reported as means \( \pm SE \).

**RESULTS**

Voltage-clamp recordings were obtained from 173 isolated VCN cells. Due to the mechanically disruptive nature of the dissociation technique, these cells typically had few dendritic or axonal processes. Of those processes that remained, most were shorter than 50 \( \mu\text{m} \) in length. Cell bodies were either round, eccentric, or teardrop-shaped, with the shortest diameter ranging 16–30 \( \mu\text{m} \) (average 21 \( \pm \) 3 \( \mu\text{m} \)). There was no correlation between cell type, as defined in the following text,
and cell body shape or size, except Type II cells were never teardrop shaped. Because cell diameters exceeded 16 μm in this study, it is unlikely that any recordings were made from VCN granule cells.

Given an average cell diameter of 21 μm, and a specific membrane capacitance of 0.9 μF/cm² (Gentet et al. 2000), whole cell capacitance \( C_m \) should be on average 12.5 pF, assuming a spherical soma. While this was true for our population of Type I cells, the average \( C_m \) of our Type II cells was slightly less (Table 1; 9.8 pF), most likely due to the difficulty of estimating \( C_m \) in the presence of active conductances near the resting membrane potential.

VCN neurons exhibit four characteristic voltage-clamp responses

Previously, isolated VCN cells have been categorized as either Type I or Type II, where Type II cells display \( I_{HT} \) at a resting membrane potential near \(-60 \text{ mV} \) and Type I cells do not (Harty and Manis 1996; Manis and Marx 1991). Although the isolated cells in this study could also be categorized as either Type I or Type II, the Type I cells appeared more heterogeneous than originally reported. A number of Type I cells, for example, showed clear signs of a rapidly inactivating A-type current \( (I_A; 65\%, \text{ see also Manis et al. 1996}) \), whereas others appeared intermediate in type in that they shared characteristics of both Type I and Type II cells (12%). Hence, the following subclassification of Type I cells was adopted: Type I cells that displayed \( I_A \) were subclassified as Type I-t (transient), and those intermediate in character, between Type I and Type II, were subclassified as Type I-i (intermediate). The remaining Type I cells that displayed neither \( I_A \) nor \( I_{HT} \) were subclassified as Type I-c (classic). The methods of classification are described in more detail in the next section. Here we give a general description of each cell type.

Figure 2A1 shows a characteristic voltage-clamp response of a Type I-c cell. In HEPES-TTX/Cd²⁺ solution, this cell displayed a single macroscopic K⁺ current: a non-inactivating high-threshold current \((I_{HT})\) that activated at \( V > -40 \text{ mV} \) (Fig. 2A2). Here, the name “high threshold” is used to distinguish it from \( I_{HT} \), whose threshold of activation is somewhere between \(-70 \text{ and } -58 \text{ mV} \) (see following text). Although \( I_{HT} \) may undergo slow inactivation on the order of seconds, we refer to it as non-inactivating because inactivation was not apparent during the 100- to 150-ms voltage command steps used in this study.

**Figure 2.** Four voltage-clamp response types of ventral cochlear nucleus (VCN) neurons. Outward currents elicited by the voltage-clamp protocol shown in A1–D1, bottom, recorded in HEPES-TTX/Cd²⁺ solution. Electrode capacity transients at the beginning of each step have been deleted. A1: outward currents from a Type I classic (Type I-c) cell. Only \( I_{HT} \) is apparent. The returning command step was set to \(-52 \text{ mV} \) instead of \(-62 \text{ mV} \) to increase the size of the tail currents for later kinetic analysis. A2: \( I-V \) relation of the steady-state current in A1. Dashed line denotes \( V_m \) at \(-40 \text{ mV} \). B1: outward currents recorded from a Type I transient (Type I-t) cell, consisting of \( I_{HT} \) and \( I_A \) (arrow). Arrowhead denotes \( I_A \) that activated at \( V < -80 \text{ mV} \). B2: \( I-V \) relation of the non-inactivating current in B1 (\( V_m = -48 \text{ mV} \)). C1: outward currents recorded from a Type I-i cell. Here, 5 clear signs of \( I_A \) are apparent (numbered arrows; see text for description). Inset: comparison of the activation time course of the outward current from this Type I cell (solid line) to the Type I-c cell in A1 (dotted line; step from \(-106 \text{ to } -30 \text{ mV} \)). The activation time course is clearly faster in the Type I cell. C2: \( I-V \) relation of the current in C1 (\( V_m = -61 \text{ mV} \)). D1: outward currents recorded from a Type I intermediate (Type I-i) cell, in which case only subtle signs of \( I_A \) are apparent (arrows 2, 3, and 5). Current traces are not averaged. D2: \( I-V \) relation of the current in D1 (\( V_m = -51 \text{ mV} \)).

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>I-c</th>
<th>I-t</th>
<th>I-i</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>23 (13.3)</td>
<td>80 (46.2)</td>
<td>19 (11.0)</td>
<td>49 (28.3)</td>
</tr>
<tr>
<td>( S_{max} ) / mV</td>
<td>1.9 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>8.0 ± 1.1***</td>
<td>71.7 ± 8.4***</td>
</tr>
<tr>
<td>( V_{th} ) / mV</td>
<td>-45.1 ± 1.1</td>
<td>-46.0 ± 0.7</td>
<td>-53.2 ± 0.7***</td>
<td>-63.1 ± 0.5***</td>
</tr>
<tr>
<td>( C_m ) / pF</td>
<td>11.6 ± 0.9</td>
<td>13.6 ± 0.5</td>
<td>9.8 ± 0.5*</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>( D ) / μm</td>
<td>20.2 ± 0.6</td>
<td>21.6 ± 0.3</td>
<td>20.3 ± 0.4</td>
<td>21.2 ± 0.4</td>
</tr>
<tr>
<td>( I_{HT} ) / %</td>
<td>78</td>
<td>90</td>
<td>100</td>
<td>86</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are percentages. VCN, ventral cochlear nucleus; \( S_{max} \)/mV, slope conductance computed from steady-state \( I-V \) relations from \(-70 \text{ to } -50 \text{ mV} \); \( V_{th} \), 0.1 nA activation threshold of the steady outward current; \( C_m \), whole cell capacitance; \( D \), shortest-width diameter; \( I_{HT} \), percentage of cells with visual signs of a hyperpolarization-activated inward current. *, ***, significant differences in comparison to Type I-c cells.
and Manis 2003a). This analysis shows that \( I_A \) activates \( \sim 15 \) mV below \( V_{IT} \) (see Table 2, \( V_{0.5} \) values) and inactivates significantly faster than all other currents. Also apparent in the current traces in Fig. 2B1 is a hyperpolarization-activated inward current (\( I_n \), arrowhead) that showed slow time-dependent activation at \( V < -80 \) mV. Although \( I_n \) was often found in Type I-t cells, it was found in the other cell types as well. Unfortunately, because \( I_n \) was only weakly expressed in our dissociated cells (possibly due to the enzymatic treatment), it was not possible to analyze it systematically.

Figure 2Cl shows a characteristic voltage-clamp response of a Type II cell. Unlike the previous Type I cells, this cell showed prominent signs of \( I_{LT} \). As the \( I-V \) relation in Fig. 2C2 shows, \( I_{LT} \) in this Type II cell began to activate near \(-60 \) mV, \(-20 \) mV negative to the activation of \( I_{HT} \) in Fig. 2A1. Because \( I_{LT} \) is partially activated at \(-60 \) mV, two unusual features appear in the current traces of this Type II cell (numbered arrows): a small steady outward current at a holding potential near \(-60 \) mV (1) and small deactivating inward currents in response to voltage steps below the \( K^+ \) reversal potential (2; \( V < -70 \) mV). Another characteristic sign of \( I_{LT} \) is its inactivation. In Fig. 2Cl, development of inactivation of \( I_{LT} \) is evident in the largest current traces during the depolarizing command steps (3), and removal of inactivation of \( I_{LT} \) is evident in the unusual “crossing tail currents” at the end of the hyperpolarizing command steps (4). Such unusual crossing tail currents arise from the removal of inactivation of \( I_{LT} \) during the different hyperpolarizing command steps, in which case the final step to \(-50 \) mV produces different activation levels of \( I_{LT} \). Due to the slow inactivation kinetics of \( I_{LT} \) and the use of \(-55 \) mV holding potentials, both development and removal of inactivation of \( I_{LT} \) were not evident in previous experiments (Manis and Marx 1991). A final distinguishing characteristic of \( I_{LT} \) is that it activates more rapidly than \( I_{HT} \). This is evident in the inset to Fig. 2Cl (5) where similar magnitude current traces of \( I_{LT} \) and \( I_{HT} \) in response to a voltage step near 0 mV are compared directly. Kinetic analysis shows that \( I_{LT} \) possesses a slowly activating component, whereas \( I_{LT} \) is not (Rothman and Manis 2003a). Hence, as pointed out with arrows in Fig. 2Cl, Type II cells exhibit as many as three distinct signs of \( I_{LT} \).

Figure 2DI shows a characteristic voltage-clamp response of a Type I-i cell. This response is similar to the Type II cell in Fig. 2Cl in that the whole cell current shows fast activation kinetics (5) and slow inactivation (3). There are, however, several differences from the Type II response, including almost no steady outward current at a holding potential near \(-60 \) mV (1); barely discernable deactivating inward currents in response to voltage steps below the \( K^+ \) reversal potential (2); tail currents that are small, showing no signs of removal of inactivation (4; no “crossing tail currents”); and a high activation threshold (Fig. 2D2, solid line; \( V_{th} = -51 \) mV). These similarities and differences are best explained by the presence of \( I_{LT} \), but one whose magnitude is significantly less than that shown in Fig. 2Cl and, perhaps, whose activation is shifted more positive. Evidence in support of this conclusion is given in the following text, where we show dendrotoxin specifically blocks a small \( I_{LT} \) in this Type I-i cell, as well as in 4 other Type I-i cells.

### Classification of isolated VCN neurons

As previously mentioned, isolated VCN cells have been classified as Type II if they displayed \( I_{LT} \) near rest, or Type I if they did not (Harty and Manis 1996; Manis and Marx 1991). The basis of these classifications was that Type II cells (putative bushy cells), which fired only one or two action potentials in response to depolarizing current pulses, possessed both \( I_{LT} \) and \( I_{HT} \), whereas Type I cells (putative stellate cells), which fired regular trains of action potentials in response to depolarizing current pulses, possessed only \( I_{HT} \). Hence, when studying \( K^+ \) currents under voltage clamp, \( I_{LT} \) could be used as the delineating factor between Type I and Type II cells. In the present study, the same “low-threshold” criterion was used initially to classify isolated VCN cells: cells with visible signs of \( I_{LT} \) at a holding potential near \(-60 \) mV were classified as Type II, and all other cells were classified as Type I. As shown in Fig. 2Cl, visible signs of \( I_{LT} \) at \(-60 \) mV include: a small steady outward current at the holding potential (1) and small deactivating inward currents (tail currents) in response to voltage steps below the \( K^+ \) reversal potential (2). After visually classifying cells as Type I or Type II, two subsequent criteria were used to further subclassify Type I cells. The first criterion was the presence of \( I_A \). In 84% of the Type I cells, \( I_A \) was readily apparent when the membrane potential was stepped positive from a holding potential near \(-60 \) mV (e.g., Fig. 2B1). In the remaining instances (16%), \( I_A \) was not apparent until the positive command steps were preceded by a hyperpolarizing prepulse to potentials below \(-100 \) mV, in which case inactivation of \( I_A \) was removed [data not shown, but see Fig. 1 of Rothman and Manis (2003a) for an example of the prepulse protocol used to isolate \( I_A \)]. Hence, in this study, prepulses were routinely used to verify the presence of \( I_A \). If a Type I cell showed visible signs of \( I_A \) with or without a prepulse, it was classified as Type I-t. The second criterion for subclassifying Type I cells was the presence of fast activation and slow inactivation in the outward current traces (putative signs of \( I_{LT} \); see Fig. 2DI); such cells were classified as Type I-i. All remaining Type I cells, which presumably possess only \( I_{HT} \), were classified as Type I-c.

Of the 173 cells investigated in this study, 49 were classified as Type II, 23 as Type I-c, 80 as Type I-t, and 19 as Type I-i. Only two cells failed to fit into any category, and were there-

### Table 2. Summary of activation analysis

<table>
<thead>
<tr>
<th>Cell type</th>
<th>( I_{DTX} )</th>
<th>( I_V )</th>
<th>( I_{HT} )</th>
<th>( I_A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>( g_{max} ), nS</td>
<td>171 ± 22</td>
<td>148 ± 12</td>
<td>171 ± 21</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>( k ), mV</td>
<td>5.8 ± 0.3</td>
<td>7.6 ± 0.8</td>
<td>6.7 ± 0.9</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>( V_{0.5} ), mV</td>
<td>-47.4 ± 1.0</td>
<td>-10.0 ± 2.3</td>
<td>-11.2 ± 1.5</td>
<td>-30.8 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( I_{DTX} \) and \( I_V \) data from modified Boltzmann fits in Fig. 6, A and C. \( I_A \) data from Rothman and Manis (2003a).
fore classified as unusual. These two cells were similar to each other in that, besides \( I_{HT} \), they possessed a slowly activating, slowly inactivating \( K^+ \) current that was quite distinct from the rapidly activating, rapidly inactivating \( I_A \), or the rapidly activating, slowly inactivating \( I_{LT} \).

4-AP does not specifically block \( I_{LT} \)

To quantify the expression of \( I_{LT} \) in VCN neurons, it was first necessary to isolate the current pharmacologically. We first attempted to use 4-AP because this drug has previously been shown to block \( I_{LT} \) (Manis and Marx 1991; Rathouz and Trussell 1998; Reyes et al. 1994; Zhang and Trussell 1994). However, as the results in this section indicate, 4-AP not only blocks \( I_{LT} \), but \( I_{HT} \) and \( I_A \) as well. Thus 4-AP is not an effective tool to isolate \( I_{LT} \) in VCN neurons (see also Rathouz and Trussell 1998).

Figure 3A shows five \( I-V \) relations of a Type II cell when exposed to various concentrations of extracellular 4-AP (0–4 mM). Inspection of these \( I-V \) relations above and below -48 mV (the dividing line between \( I_{LT} \) and \( I_{HT} \), as shown in the following text) shows that at no concentration of 4-AP was there a specific block of \( I_{LT} \) in this Type II cell; that is, either \( I_{LT} \) appeared only partially blocked (0.1 mM) or both \( I_{LT} \) and \( I_{HT} \) appeared to be significantly blocked by 4-AP (1 and 4 mM).

Because \( I_{HT} \) activates at \( V > -50 \) mV, we were able to quantify the effects of 4-AP on \( I_{HT} \) alone in five Type II cells by measuring the slope of their \( I-V \) relations, such as those in Fig. 3A, from -70 to -50 mV (\( S_{-50/-70} \)) at various concentrations of 4-AP (0.01, 0.1, 1, and 4 mM). These \( S_{-50/-70} \) values were then normalized to control conditions and fit to Eq. 3 (Fig. 3B), yielding an \( IC_{50} = 79 \mu M \).

Unfortunately, it was not possible to quantify the effects of 4-AP on \( I_{HT} \) in Type II cells because \( I_{HT} \) coactivates with \( I_{HT} \) at \( V > -50 \) mV. However, it was possible to quantify the effects of 4-AP on \( I_{HT} \) in Type I-c and Type I-t cells because the primary \( K^+ \) current in Type I-c cells was \( I_{HT} \), and the presence of \( I_A \) in Type I-t cells was effectively eliminated by computing \( I-V \) relations after most of \( I_A \) was inactivated (\( t > 100 \) ms after the command step onset). To further ensure our analysis pertained to \( I_{HT} \), and not some small \( I_{LT} \), only cells with high activation threshold (\( V_{th} > -48 \) mV) were included in the analysis. Similar to \( I_{HT} \), we computed \( I-V \) relations and slope conductance values from -40 to -20 mV as a function of 4-AP concentration and then fit these data to Eq. 3 (Fig. 3B). Results of this analysis yielded an \( IC_{50} = 54 \mu M \) (\( n = 14 \)). These results then suggest that it is not possible to pharmacologically separate \( I_{LT} \) and \( I_{HT} \) in VCN neurons with 4-AP, due to their similar \( IC_{50} \) values.

Using prepulse protocols (see Rothman and Manis 2003a), we were also able to compute the sensitivity of \( I_A \) to 4-AP. Results of this analysis revealed an \( IC_{50} \) near 1 mM (\( n = 5 \) Type I-t cells), which is considerably higher than the \( IC_{50} \) for \( I_{HT} \) and \( I_{LT} \).

Dendrotoxin specifically blocks \( I_{LT} \)

As a second attempt to isolate \( I_{LT} \) pharmacologically, we used Toxin-I, a dendrotoxin (DTX) from black mamba snake venom. This drug was chosen because several studies have already demonstrated its ability to specifically block \( I_{LT} \) (Robertson et al. 1996; Stansfeld et al. 1986), including \( I_{LT} \) in MNTB neurons (Brew and Forsythe 1995) and chick \( n. \) magnocellularis neurons (Rathouz and Trussell 1998). As Fig. 4 demonstrates, this is also true for \( I_{LT} \) in VCN neurons. In Fig. 4A, whole cell currents recorded from a Type II cell show the five characteristic signs of \( I_{LT} \) (numbered arrows). Computing peak current as a function of voltage resulted in an \( I-V \) relation with low activation threshold (Fig. 4D, open circles; \( V_{th} = -69 \) mV), consistent with the presence of \( I_{LT} \). In Fig. 4B, whole cell currents recorded from the same Type II cell after bath application of 100 nM DTX no longer shows signs of \( I_{LT} \). Moreover, the remaining current, referred to as \( I_{DTX} \), resembles \( I_{HT} \) in both kinetics of activation (compare to Fig. 2A1) and its \( I-V \) relation (Fig. 4D, closed circles; \( V_{th} = -50 \) mV). In Fig. 4C, the current obtained by subtracting \( B \) from \( A \) (see legend for details), referred to as \( I_{DTX}^{s} \), shows all characteristic signs of \( I_{LT} \). Thus in this Type II cell, \( I_{LT} \) has been selectively blocked by 100 nM DTX.

The sensitivity of \( I_{LT} \) to DTX was investigated in 12 other Type II cells. Whereas a concentration of 1 nM DTX produced a partial block of \( I_{LT} \) (\( n = 5 \); not shown), a concentration of 10 nM DTX (\( n = 2 \)) or 100 nM DTX (\( n = 8 \)) produced a near complete block of \( I_{LT} \). Computing \( S_{-50/-70} \) values from the \( I-V \) relations of these 12 cells resulted in the following estimates for the percent conductance block of \( I_{LT} \) at concentrations of 1,
Comparison of DTX-sensitive and insensitive currents

First, we address the question of whether \(I_{\text{DTX}}\) is the same in all cell types. This question is addressed in Fig. 6A where I-V relations of \(I_{\text{DTX}}\) from 9 Type I cells (○) and 13 Type II cells (●) are plotted together. As this figure shows, I-V relations of both Type I and Type II cells overlap within the region highlighted in gray. To directly compare the I-V relations, the data were interpolated onto a common voltage scale and averaged as a function of voltage. The relations for Type I cells again overlap the Type II cells (inset). Except at −15 mV, the interpolated data points are not statistically different. Similarly, \(V_{\text{th}}\) values of the Type I cells overlap those of Type II cells (Fig. 6B) and are not statistically different \((P = 0.54)\). Hence these results suggest Type I and Type II cells possess similar, if not identical DTX-insensitive high-threshold currents. To further support this claim, we fit modified Boltzmann functions (Eq. 2) to the I-V relations in Fig. 6A and compared \(g_{\text{max}}, V_{0.5}, \) and \(k\) values. Results of this analysis show very similar values (Fig. 6D and Table 2, \(I_{\text{DTX}}\)), suggesting the two currents are the same.

Next, we address the question of whether \(I_{\text{DTX}}\) is the same in all cell types. This question is addressed in Fig. 6C where I-V relations of \(I^+_\text{DTX}\) from 9 Type I cells (△) and 13 Type II cells (▲) are plotted together (see legend for methods); cell classi-

![Fig. 4. Pharmacological separation of \(I_{\alpha}\) and \(I_{\text{HT}}\) with DTX. A: outward currents from a Type II cell bathed in HEPES-TTX/Cd\(^{2+}\) solution. Voltage-clamp protocol is shown above C. Numbered arrows denote 5 distinct signs of \(I_{\text{LT}}\), as explained in text. B: outward currents from the same Type II cell now in 100 nM DTX. Only a small \(I_{\text{LT}}\) remained. The bulk of the remaining DTX-insensitive current \((I_{\text{DTX}})\) activated at \(V > -50 \text{ mV}\). C: DTX-sensitive current \((I_{\text{DTX}})\) obtained by subtracting traces in \(B\) from \(A\). Prior to subtraction, traces in \(B\) were linearly interpolated to the command voltages in \(A\) at each point in time. D: I-V relations of the currents in \(A\) (open circles), \(B\) (closed circles), and \(C\) (closed triangles). Y axis denotes maximum current during the command step. Solid line denotes \(V_{\text{sh}}\) of the control current. Dotted line denotes \(V_{\text{sh}}\) of \(I_{\text{DTX}}\).](http://www.jn.physiology.org/doi/10.1152/jn.00454.2002)
and those for Type II cells as parameters). Average Boltzmann functions for Type I cells are denoted as ...<−25 mV, and therefore did not fit well to a Boltzmann function. We believe the non-monotonic I-V relations are probably due to a change in magnitude of \( I_{\text{RT}} \) measured between control and DTX conditions, a time that could take ≥15 min. Nevertheless, the I-V relations in Fig. 6C indicate at a minimum that \( g_{\text{max}} \) is smaller in Type I cells.

Finally, we address the question of whether \( I_{\text{DTX}} \) and \( I_{\text{DTX}} \) can be statistically separated using \( V_{\text{th}} \) values. This question is addressed in Fig. 6B, where \( V_{\text{th}} \) values of \( I_{\text{DTX}} \) in Fig. 6A (●, ○) are plotted with \( V_{\text{th}} \) values of \( I_{\text{DTX}} \) in Fig. 6C (∆, ▲). As this figure shows, \( V_{\text{th}} < −48 \) mV for \( I_{\text{DTX}} \) and \( V_{\text{th}} > −48 \) mV for \( I_{\text{DTX}} \). Because \( I_{\text{DTX}} \) in Type I cells is small, the line of separation is less dramatic for Type I cells (○ and ∆) than it is for Type II cells (● and ▲). These results then indicate that a cell with \( V_{\text{th}} < −48 \) mV is likely to possess \( I_{\text{DTX}} \) (i.e., \( I_{\text{LT}} \)).

Quantitative analysis reveals a differential expression of \( I_{\text{LT}} \)

Using the preceding DTX results, we were able to compare the expression of \( I_{\text{LT}} \) in our population of Type I and Type II cells. In this analysis, two measures were computed from each cell’s I-V relation: \( V_{\text{th}} \) and \( S_{−50−70} \) (Fig. 7, inset). \( V_{\text{th}} \) was used because, as the above results indicate, a cell with \( V_{\text{th}} < −48 \) mV is likely to possess \( I_{\text{LT}} \). \( S_{−50−70} \) was used because it provided an estimate of the magnitude of \( I_{\text{LT}} \) from −70 to −50 mV. Also, in a previous study, \( S_{−50−70} \) was shown to be larger in Type II cells than in Type I cells (23.8 ± 35.8 nS as compared to 2.0 ± 1.6 nS) (Harty and Manis 1996), suggesting a possible means of separating the two cell types quantitatively.

Results of the analysis are shown in Fig. 7, where cell types, as denoted in the figure legend, are plotted with respect to \( V_{\text{th}} \) and \( S_{−50−70} \). Three important points are to be noted about Fig. 7. First, there is a significant number of Type I cells with \( V_{\text{th}} < −48 \) mV (44%), suggesting the presence of \( I_{\text{LT}} \). Ten of these Type I cells were exposed to DTX (4 shown in Fig. 5: cells 1–4), and all 10 showed the presence of a small DTX-sensitive current that activated at \( V < −48 \) mV. The second point is that there appears to be a gradual change in both \( V_{\text{th}} \) and \( S_{−50−70} \) values between Type I cells (circles) and Type II cells (triangles). Only at \( V_{\text{th}} = −58 \) mV does there appear to be a small separation between cell types. These results suggest a gradation in magnitude of \( I_{\text{LT}} \) from zero (\( V_{\text{th}} > −48 \) mV; Type I-c and Type I-t cells) to modest (−58 < \( V_{\text{th}} < −48 \) mV; Type I-t and Type I-i cells) to significant (−58 mV < \( V_{\text{th}} \); Type II cells). Third, Fig. 7 confirms our visual cell classification; that is, Type II cells show significant \( I_{\text{LT}} \) (\( V_{\text{th}} < −58 \) mV and \( S_{−50−70} > 8 \) nS), whereas Type I-c cells do not (\( V_{\text{th}} > −52 \) mV and \( S_{−50−70} < 8 \) nS). Note that 7 of the 23 Type I-c cells have \( V_{\text{th}} < −48 \) mV; hence, these cells may possess a small \( I_{\text{HT}} \) that was difficult to detect. It is also possible that these cells possess only \( I_{\text{HT}} \), but one whose \( V_{\text{th}} \) is more negative than usual. In either case, most if not all of the K⁺ current in these seven Type I-c cells represents \( I_{\text{HT}} \). Finally, the Type I-i cells...
appear truly intermediate in type, in that they fall between Type II and Type I-c cells with respect to both \( V_{\text{th}} \) (−58 to −48 mV) and \( S_{-50-70} \) values (2–17 nS). This finding again suggests the presence of a small \( I_{HT} \) in the Type I-i cells.

Results from this section are further summarized in Table 1, where average values of \( S_{-50-70} \) and \( V_{\text{th}} \) are tabulated with respect to cell type. Statistical differences in comparison to the Type I-c cells are denoted with asterisks. In summary, Type I-t cells were not significantly different from Type I-c cells for either measure, whereas Type I-i and Type II cells were significantly different from Type I-c cells.

Quantitative analysis reveals a differential expression of \( I_{HT} \)

In this section, we compare the steady-state activation of \( I_{HT} \) across cell types. This analysis shows two things. First, the voltage dependence of \( I_{HT} \) is similar across cell types, suggesting the same “high-threshold” \( K^+ \) channels in VCN neurons. Second, the magnitude of \( I_{HT} \) is similar in Type II, Type I-i, and Type I-c cells but somewhat smaller in Type I-t cells.

First, we compare \( I_{HT} \) in Type I-c cells to \( I_{DTX} \) in Type I and Type II cells. Here, \( I_{DTX} \) is used since \( I_{HT} \) coactivates with \( I_{HT} \) at \( V > -50 \) mV and therefore cannot be independently examined under control conditions. Figure 8A shows the first comparison, where \( I-V \) relations of 14 Type I-c cells are plotted (○). These \( I-V \) relations reflect the steady-state activation of \( I_{HT} \) because \( V_{\text{th}} > -48 \) mV for this sample of Type I-c cells, suggesting the absence of \( I_{LT} \) (Fig. 6B). In the background of Fig. 8A is the shaded region from Fig. 6A, which defines the boundaries of \( I_{DTX} \) in Type I and Type II cells. Hence, steady-state activation of \( I_{HT} \) is similar to that of \( I_{DTX} \). Of the 14 \( I-V \) relations of Type I-c cells in Fig. 8A, 10 could be satisfactorily fit to a modified Boltzmann function (Fig. 8B), resulting in the following parameter estimates for steady-state activation of \( I_{HT} \) in Type I-c cells: \( g_{\text{max}} = 164.2 \pm 11.5 \) nS, \( V_{0.5} = -14.3 \pm 0.7 \) mV, \( k = 7.0 \pm 0.5 \) mV. Although these results resemble those of \( I_{DTX} \) in Type I and Type II cells (see Table 2), they are significantly different (Fig. 8B, inset), largely due to a difference in \( V_{0.5} \). \( V_{0.5} \) of \( I_{HT} \) sits ~3 mV negative to that of \( I_{DTX} \). The difference in \( V_{0.5} \) values could be due to small approximation errors, small effects of DTX on \( I_{HT} \), or the presence of small amounts of \( I_{LT} \) in the Type I-c cells. Regardless, the difference in \( V_{0.5} \) values is small. As reported in our next paper (Rothman and Manis 2003a), there are enough kinetic similarities between \( I_{HT} \) and \( I_{DTX} \) to suggest they constitute the same current.

Also in Fig. 8A are the \( I-V \) relations of 40 Type I-t cells (○). Again, the \( I-V \) relations reflect the steady-state activation of \( I_{HT} \) because \( V_{\text{th}} > -48 \) mV for all cells; furthermore, the \( I-V \) relations were computed for \( t > 100 \) ms after the command step onset, at which time \( I_{LT} \) in these cells had been inactivated. As Fig. 8A shows, data points from both Type I-c and Type I-t cells predominantly fall within the region defined by \( I_{DTX} \). However, data from the Type I-c cells tend to fall toward the upper boundary, whereas data from the Type I-t cells tend to fall toward the lower boundary. When these \( I-V \) relations were interpolated onto a common voltage scale and averaged as a function of voltage, averages of the Type I-t cells were smaller than those of the Type I-c cells at \( V > -30 \) mV (Fig. 8A, inset); these differences were significant (\( P < 0.05 \)). The difference in current magnitudes suggest Type I-c and Type I-t cells either possess two distinct \( I_{HT} \) or possess the same \( I_{HT} \) but with different \( g_{\text{max}} \) values. Although the former scenario cannot be ruled out as a possibility, there are several reasons to suspect the latter scenario is true. First, average \( I-V \) relations of Type I-c and Type I-t cells are well described by the same modified Boltzmann function but with different \( g_{\text{max}} \) values (Fig. 8A, inset, —). Second, modified Boltzmann fits to individual \( I-V \) relations result in similar Boltzmann functions for Type I-c and Type I-t cells (Fig. 8C). Although there are differences between these Boltzmann functions, the differences do not appear large enough to suggest two distinct currents. Third, tail current analysis shows that \( I_{HT} \) in Type I-c cells is kinetically similar to \( I_{HT} \) in Type I-t cells (Rothman and Manis 2003a). Hence, \( I_{HT} \) in Type I-t cells is probably the same as that in Type I-c cells, but expressed in smaller magnitude.

Differential expression of \( I_A \)

Because the isolation of \( I_A \) required the use of prepulse protocols, which worked better on cells that expressed relatively large \( I_A \) (e.g., the Type I-t cell in Fig. 2B), we were not able to quantify the magnitude of \( I_A \) in our entire population of VCN cells. However, we do note here two observable instances of a differential expression of \( I_A \). First, within our population of Type I-t cells, the magnitude of \( I_A \) was clearly different from

![Image](http://jn.physiology.org/doi/fig/10.1152/jn.00493.2002)
cell to cell. In some instances (16%), $I_A$ was so small, it was not apparent until a prepulse was used to remove its inactivation. Second, $I_A$ was not apparent in any of our Type II cells. This was true even after $I_{LT}$ was blocked with DTX ($n = 13$ Type II cells; DTX-insensitive currents observed with and without prepulses).

**Discussion**

Our results suggest that VCN neurons can be subdivided according to their $K^+$ currents further than previously indicated. In total, we identified four separate cell groups: the Type I-c cells, which express a high-threshold delayed-rectifier-like current ($I_{HT}$) with neither a DTX-sensitive low-threshold current ($I_{LT}$) nor a rapidly inactivating transient A-type current ($I_A$); the Type I-t cells, which express both $I_{HT}$ and $I_A$, and sometimes a weak $I_{LT}$; the Type I-i cells, which express $I_{HT}$ and a strong $I_{LT}$, but no $I_A$; and the Type II cells, which express $I_{HT}$ and a strong $I_{LT}$, but no $I_A$. The latter two groups appear to belong to a continuum of cells with varying levels of $I_{LT}$. We also found that only $I_{LT}$ was sensitive to the peptide blocker DTX, whereas all three $K^+$ currents were sensitive to 4-AP.

**Limitations of the cell classification**

Two obvious limitations of our cell classification are the absence of correlation to morphology and the absence of correlation to current-clamp responses. Previous studies correlated current-clamp responses with cell morphology (Wu and Oertel 1984) or voltage-clamp responses with current-clamp responses (Manis and Marx 1991). However, because we wished to obtain detailed kinetic descriptions of $I_A$, $I_{LT}$, and $I_{HT}$, these limitations were unavoidable for the following reasons. First, cells are far better voltage clamped in an isolated-cell preparation than in a slice preparation because most of their dendritic and axonal processes are removed. Second, obtaining voltage-clamp recordings from mature VCN neurons is more feasible in an isolated-cell preparation than in a slice preparation. Third, most amplifiers with good voltage-clamp performance have only fair current-clamp performance due to the headstage design (Magistretti et al. 1998). Nevertheless, based on results from previous in vitro studies, it can be inferred that the Type I cells reported in this study would have a Type I current-clamp response (i.e., repetitive firing in response to depolarization) (Manis and Marx 1991) and a stellate morphology (Wu and Oertel 1984), whereas the Type II cells would have a Type II current-clamp response (i.e., a phasic discharge in response to depolarization) and a bushy morphology. In the future it will be of significant interest to determine how our four physiologically defined categories parse against cell morphology.

**DTX reveals $I_{LT}$ in Type I cells**

One interpretation of our DTX results is that as many as 47% of the Type I cells possess small amounts of $I_{LT}$ (Fig. 7; $-58 < V_th < -48$ mV). Of these Type I cells, 54% were classified as Type I-t and 33% were classified as Type I-i. The magnitude of $I_{LT}$ in Type I cells was on average six times smaller than that in Type II cells. Consequently, the difference between Type I and Type II cells is not simply the absence or presence of $I_{LT}$, as was previously supposed, but rather a difference in magnitude of $I_{LT}$. However, there is no clear boundary between cells

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**FIG. 8. Steady-state activation of $I_{HT}$**

A: $I-V$ relations of the steady-state current ($t > 100$ ms after command step onset) for 14 Type I-c cells (C) and 40 Type I-t cells (○). For all cells, $V_{th} > -48$ mV, the boundaries of $I_{DTX}$ in Fig. 6A. Inset: mean and SE as a function of voltage after $I-V$ relations in A were interpolated onto a common voltage scale. Significant differences between Type I-c and Type I-t cells are denoted (*). ——, the results of a simultaneous fit to a modified Boltzmann function when $g_{max}$ and $k$ were shared between data sets (Type I-c data: $g_{max} = 157.2$ nS, $V_{0.5} = -15.9$ mV, $k = 7.6$ mV; Type I-t data: $g_{max} = 77.9$ nS, $V_{0.5} = -15.9$ mV, $k = 7.6$ mV). B: modified Boltzmann fits (—→) to the Type I-c data in A (○). Both data and fits were normalized to $I_{max}$ values, where $I_{max} = g_{max}(V - V_{th})$. Inset: mean and SE as a function of voltage after normalized $I-V$ relations in B were interpolated onto a common voltage scale. ——, average Boltzmann function of the data ($V_{0.5} = -14.3$ mV, $k = 7.0$ mV). For comparison, means and SE are shown for $I_{DTX}$ (●) computed from the data in Fig. 6D (Type I and Type II cells averaged together). ——, average Boltzmann function of $I_{DTX}$ in Type I and Type II cells ($V_{0.5} = -11.4$ mV, $k = 7.0$ mV). * significant differences between $I_{HT}$ and $I_{DTX}$. C: modified Boltzmann fits (—→) to the data in A (○, ●). As in B, data and fits were normalized to $I_{max}$ values. Inset: mean and SE as a function of voltage after normalized $I-V$ relations in C were interpolated onto a common voltage scale. ——, average Boltzmann function for Type I-c cells ($V_{0.5} = -11.3$ mV, $k = 9.7$ mV). * significant differences between Type I-c and Type I-t cells.
with large and small $I_{LT}$, as Fig. 7 illustrates. The line drawn at \(-58\) mV in this figure represents an arbitrary definition: Type II cells were defined as those possessing visual signs of $I_{LT}$ near \(-60\) mV, the average resting potential of VCN neurons. If the line at \(-58\) mV is ignored, then the data reflect a near continuum of responses between \(-32\) to \(-68\) mV. Models of the cells using our measured kinetics (Rothman and Manis 2003b) show that this continuum of responses with increasing slope conductance values ($S_{50-\gamma_0}$) can be accounted for by a continuum of $I_{LT}$ expression. Furthermore, this continuum of $I_{LT}$ can in turn generate a range of discrete current-clamp responses, including current-clamp responses intermediate in character between Type I and Type II. Cells with intermediate discharge patterns and action potential shapes have in fact been observed experimentally in the rat and gerbil VCN (Francis and Manis 2000; Schwarz and Puil 1997).

Although the data in Fig. 7 suggest VCN neurons might be viewed as a continuum of cells according to their expression of $I_{LT}$, this conclusion should be tempered by the experimental conditions under which the data was obtained. The cells are isolated, so they possess variable (but limited) amounts of dendritic membrane, which might distort the apparent current density if channels are not uniformly distributed on the cell surface and if some fraction of the dendritic membrane collapses into the somatic membrane as a result of the cell isolation. The cells were also treated with a proteolytic enzyme during the dissociation procedure, whereby channel function might have been variably compromised. Finally, adult cells in a dissociated situation are metabolically fragile, so it is possible that the variability of current density results from differences in cell metabolic status. We suspect this last possibility does not strongly contribute to our measurements since there were no concomitant variations in input resistance or holding currents that would be consistent with such an interpretation.

Another argument against this interpretation is that some of the cells in the intermediate range ($V_{in} \sim 50$ mV) exhibited weak $I_{LT}$, while expressing normal levels of $I_{HT}$, suggesting any such metabolic compromise would have to selectively suppress $I_{LT}$ and not $I_{HT}$. With these caveats in mind, however, we suggest that the distribution of $I_{LT}$ in VCN cells varies across the cell populations, yielding both obvious differences (e.g., the segregation between Type I and Type II responses to current steps) and more subtle variations in response properties.

$I_{LT}$ in Type II cells is larger than previously reported

While our estimate of half-activation of $I_{LT}$ ($V_{0.5} = -47$ mV) is similar to that previously reported ($V_{0.5} = -53$ mV) (Manis and Marx 1991), our estimate of the mean maximal conductance $g_{LT}$ is significantly larger (171 vs. 20 nS; same source). The most likely explanation for this discrepancy is methodological: series access resistance was corrected online in the present study (75–95%) but not in the earlier study. In the earlier study, it was explicitly stated that $I$-$V$ relations and conductance plots failed to show saturation, in which case the estimate of $g_{LT}$ was to be considered a lower bound. Even in the present study, some $I$-$V$ relations and conductance plots failed to show saturation; hence, estimates of $g_{LT}$ in this study should also be considered lower bounds.

$I_{LT}$ is similar to other low-threshold currents

Low-threshold $K^+$ currents, like $I_{LT}$ described in this study, have been observed in a variety of other neurons outside the VCN, including medial nucleus of the trapezoid body neurons (Brew and Forsythe 1995), avian nucleus magnocellularis neurons (chick bushy cell equivalents) (Rathouz and Trussell 1998; Reyes et al. 1994), lateral and medial olivocochlear nucleus neurons (Fujino et al. 1997), hippocampus neurons (Storm 1988; Wu and Barish 1992), mesencephalic trigeminal sensory neurons (Del Negro and Chandler 1997), vestibular hair cells (Correa and Lang 1990; Ruch and Eatoek 1996), dorsal root ganglion cells (Gold et al. 1996; Hall et al. 1994; Stansfield and Feltz 1988), and peripheral myelinated nerve fibers (Benoit and Dubois 1986; Koh and Vogel 1996; Saffarov et al. 1993). In general, these low-threshold currents tend to share the following six characteristics: activation at subthreshold potentials ($V < -50$ mV), rapid activation, slow, incomplete inactivation, TEA insensitivity (mM concentrations), 4-AP sensitivity (µM concentrations), and DTX sensitivity (nM concentrations). Many, if not all, of these neurons have been implicated in the conveyance of rapid temporal information.

The description of $I_{LT}$ in this study is strikingly similar to that of $I_{LT}$ in VCN octopus cells (Bal and Oertel 2001) sharing nearly the same voltage dependence of activation and inactivation. The one difference between the currents is the estimate of the peak conductance $g_{LT}$, which is approximately three times larger in octopus cells (171 vs. 514 nS). Hence, octopus cells are even more specialized than bushy cells when it comes to expressing $I_{LT}$. This would explain why octopus cells have a lower input resistance and faster membrane time constant in comparison to bushy cells (Golding et al. 1999), allowing them to act as precise coincidence detectors (Golding et al. 1995).

$I_{HT}$ is common to all VCN neurons

$I_{HT}$ appears to be expressed in all VCN neurons. As will be discussed in the next paper (Rothman and Manis 2003a), the characteristics of this current are similar in some respects to KCNCl, which is highly expressed in the mammalian VCN (Grigg et al. 2000; Perney and Kaczmarek 1997; Perney et al. 1992). It has been proposed that the KCNCl channel allows VCN cells to fire at high rates by providing a rapid repolarization of their action potential (Grigg et al. 2000; Wang et al. 1998).

The magnitude of $I_{HT}$ is similar in all VCN neurons (Table 2), except for those cells that also express $I_A$, the Type I-t cells, in which case it is somewhat smaller. The explanation for a smaller $I_{HT}$ in Type I-t cells is unclear. Our modeling results indicate no significant difference in action potential shape or firing properties between smaller $I_{HT}$ in Type I-t cells and the larger $I_{HT}$ in Type I-c cells (Rothman and Manis 2003b).

$I_A$ is expressed in a subset of Type I cells, but not in Type II cells

Previously, based on a small sample, it was reported that $I_A$ was present in some VCN Type I cells (Manis et al. 1996). The present experiments demonstrate that $I_A$ is present in more than half of the Type I cells. The presence or absence of $I_A$ in a subset of Type I cells suggests these cells may be further
subdivided according to their membrane currents. The specific role $I_A$ might play in Type I cells is addressed in our modeling paper (Rothman and Manis 2003b).

The absence of $I_A$ in Type II cells is consistent with previous experimental observations in guinea pigs (Manis and Marx 1991). However, it contrasts to the independent observations of a rapidly-inactivating current in young chicken n. magnocellularis neurons (Rathouz and Trussell 1998; Reyes et al. 1994), which are generally considered close homologs of VCN bushy cells. It is unclear whether this differential expression of $I_A$ is due to species or developmental differences. Our data also contrast with a previous conclusion that spherical bushy cells express $I_A$ (Schwarz and Puil 1997). However, this conclusion was based on the experimental finding that 4-AP blocks a transient component of the spherical bushy cell response to depolarizing current injection. In this study, we find that 4-AP not only blocks $I_A$, but also $I_{HT}$ and $I_{LT}$, and the latter with much more potency. Hence, the effects of 4-AP in previous current-clamp experiments were probably due to the block of $I_{LT}$. The activation of $I_{LT}$ at the onset of a depolarizing current pulse can in fact generate a transient response similar to that reported by Schwarz and Puil (Manis and Marx 1991).

**Summary**

Overall, these data indicate that VCN neurons have a variety of expression patterns of voltage-dependent $K^+$ currents. It is likely that the expression patterns of the currents are at least partially associated with particular subsets of VCN cell types. However, the overall distribution of activation thresholds suggests that the expression of at least $I_{LT}$ may be quantitative rather than strictly qualitative or categorical. On the other hand, there do appear to be discrete populations of cells that express or do not express $I_A$.

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