Characteristics of Calcium Currents in Rat Geniculate Ganglion Neurons

Shiro Nakamura1 and Robert M. Bradley1,2
1Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor; and 2Department of Molecular and Integrative Physiology, Medical School, University of Michigan, Ann Arbor, Michigan

Submitted 19 July 2010; accepted in final form 8 November 2010

Nakamura S, Bradley RM. Characteristics of calcium currents in rat geniculate ganglion neurons. J Neurophysiol 105: 224–234, 2011. First published November 10, 2010; doi:10.1152/jn.00636.2010. Geniculate ganglion (GG) cell bodies of chorda tympani (CT), greater superficial petrosal (GSP), and posterior auricular (PA) nerves transmit orofacial sensory information to the rostral nucleus of the solitary tract (rNST). We used whole cell recording to study the characteristics of the Ca2+ channels in isolated Fluorogold-labeled GG neurons that innervate different peripheral receptive fields. PA neurons were significantly larger than CT and GSP neurons, and CT neurons could be further subdivided based on soma diameter. Although all GG neurons possess both low voltage-activated (LVA) “T-type” and high voltage-activated (HVA) Ca2+ currents, CT, GSP, and PA neurons have distinctly different Ca2+ current expression patterns. Of GG neurons that express T-type currents, the CT and GSP neurons had moderate and PA neurons had larger amplitude T-type currents. HVA Ca2+ currents in the GG neurons were separated into several groups using specific Ca2+ channel blockers. Sequential applications of L, N, and P/Q-type channel antagonists inhibited portions of Ca2+ current in all CT, GSP, and PA neurons to a different extent in each neuron group. No difference was observed in the percentage of L- and N-type Ca2+ currents reduced by the antagonists in CT, GSP, and PA neurons. Action potentials in GG neurons are followed by a Ca2+ current initiated afterdepolarization (ADP) that may influence intrinsic firing patterns. These results show that based on Ca2+ channel expression the GG contains a heterogeneous population of sensory neurons possibly related to the type of sensory information they relay to the rNST.

Introduction

Cell bodies of afferent fibers of the chorda tympani (CT), greater superficial petrosal (GSP), and posterior auricular nerves (PA) are contained in the geniculate ganglion (GG). These nerves relay chemosensory, thermal, and mechanosensitive information from the anterior two thirds of the tongue (CT), soft palate and nasoincisor duct (GSP), and the skin surrounding the pinna of the ear (PA) (Brodal 1981). The central process of the ganglion—the nervus intermedius—enters the lateral brain stem and contributes to the solitary tract (May and Hill 2006). Collateral branches of the solitary tract make monosynaptic contact with second-order neurons of the rostral nucleus of the solitary tract via glutamatergic synapses (Li and Smith 1997; Wang and Bradly 1995, 2010; Whitehead and Frank 1983).

The few anatomical studies of GG neurons reported that the CT and GSP cell soma have a typical pseudounipolar morphology (Grigaliunas et al. 2002; Kitamura et al. 1982) and are 15–25 μm in diameter. The PA neuron somata are larger than the CT and GSP neurons (Gomez 1978; Semba et al. 1984). A few larger neurons ranging from 30 to 40 μm in diameter are also reported in the GG (Gomez 1978). In ultrastructural studies of guinea pig, monkey, and human GG, the neurons are described as being similar to those in other sensory ganglia (Lieberman 1976) and are classified as either light or dark cells based on their appearance in electron micrographs (Kitamura et al. 1982; Nawar et al. 1980).

Most research on GG neuron function has involved extracellular recordings of responses to tongue stimulation with chemicals (Boudreau et al. 1983, 1985; Breza et al. 2006; Lundy and Contreras 1999; Sollars and Hill 2005). Intracellular in vitro recordings of isolated GG ganglion cells have been used to characterize their biophysical properties. CT, GSP, and PA neurons were found to have similar passive membrane properties (King and Bradley 2000; Koga and Bradley 2000). However, there were significant differences in cell excitability between the three groups based on action potential threshold current magnitudes, and PA neurons often responded with a burst of action potentials when depolarized. All action potentials of GG neurons were “sharp” with rapid rise and fall times (King and Bradley 2000; Koga and Bradley 2000). The “broad spikes” with a deflection on the repolarizing phase of the action potential described in dorsal root and trigeminal ganglion neurons were not observed in the GG (Grigaliunas et al. 2002; Koerber and Mendell 1992). Isolated GG neurons responded to glutamate receptor agonists (King and Bradley 2000) and substance P and GABA (Koga and Bradley 2000). In immunocytochemical study, GG neurons expressed several glutamate receptor subunits (Caicedo et al. 2004) and subpopulations of GG neurons are positive for ATP receptors (P2X2 and P2X3). Most GG neurons stain positive for TrkB neurotrophin receptors and BDNF is required for GG neuron survival (Matsumoto et al. 2001; Ohmoto et al. 2008; Patel and Krinn 2010).

It is apparent therefore that, despite the importance of the GG in a number of orofacial sensory functions, its basic neurobiological properties have received relatively little attention compared with other sensory ganglia. For example, heterogeneity of GG sensory neurons, a characteristic of other sensory ganglia (Lawson 2002; McMahon and Priestley 2005) has never been fully explored. Unlike other sensory ganglia, no information is available on the relationship between GG neuron size and peripheral fiber size and expression of different neuroproteins. Neurons in other ganglia also differ in their ion channels distribution possibly related to the different sensory modalities they transmit (Kim and Chung 1999). More specifically, multiple types of Ca2+ channel have been described in all sensory ganglion cells studied thus far (Bean 1989; Mendelowitz and Kunze 1992; Miller 1987; Sher et al. 1991;
Swandulla et al. 1991). Importantly, these channels are involved in intracellular calcium-dependent processes, can influence the properties of the action potential, play a role in the pattern of action potential discharge, and are necessary for the release of neurotransmitters from the central synapses (Miller 1988).

To date, there is no information on the distribution of different Ca\(^{2+}\) channels in different populations of GG neurons and how these channels influence action potential discharge properties. Based on anatomy and central tracing studies, GG neurons are heterogeneous, relaying different modalities and qualities of sensory information to the sensory relay nuclei in the brain stem and may therefore express a diversity of Ca\(^{2+}\) channels. We used whole cell recordings of GG neurons innervating diverse orofacial receptive fields to characterize the Ca\(^{2+}\) currents based on voltage-dependent activation kinetics and sensitivity to Ca\(^{2+}\) channel antagonists.

METHODS

Fluorescent labeling

Male Sprague-Dawley rats (Charles River Laboratories) aged 28–37 days were used in this study. All surgical procedures were carried out under National Institutes of Health and University of Michigan Animal Care and Use Committee–approved protocols. The ganglion cell labeling technique is based on methods developed in earlier studies (King and Bradley 2000; Koga and Bradley 2000). Rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg) and xylazine (16 mg/kg). The lower jaw was retracted and the tongue depressed. Under a dissecting microscope, 10 l of a 3–5% aqueous solution of Fluorogold (Fluorochrome) was injected bilaterally into the anterior tongue or soft palate from a Hamilton microsyringe. To label a strictly mechanoreceptive subpopulation of neurons, a 3–5 mm-diameter incision was made in the soft palate of a rat anesthetized with halothane and decapitated, and the head was secured in a stereotaxic device and kept at room temperature. Recordings were performed between 30 min and 7 h after perfusing. Fluorogold-labeled GG neurons were identified by brief exposure to ultraviolet light. Patch electrodes were pulled using a two-stage puller (Narishige PP-83 puller) from borosilicate filament glass (1.5 OD, World Precision Instruments). Electrode tip resistance was between 1.5 and 2.5 M\(\Omega\) when filled with internal solution.

Electrodes were positioned under visual control onto the membrane of neurons using a hydraulic three-axis micromanipulator (Narishige). A gigahm seal between the electrode and neuron was established in a HEPES buffer. Neurons were recorded in the standard whole cell patch-clamp configuration with an Axopatch 1D amplifier (Axon Instruments) for voltage-clamp experiments or an Axoclamp 2A amplifier (Axon Instruments) in bridge mode for current-clamp recordings. Current- and voltage-clamp protocols, data acquisition, and analysis were performed using pCLAMP 8 software (Axon Instruments). Signals were low-pass filtered at 2–50 kHz (DigitData 1200, Axon Instruments), and stored on a computer.

For voltage-clamp recordings, series resistance and cell membrane capacitance were electronically compensated. Leak subtraction was performed with an on-line P/4 or P/5 protocol using pCLAMP software. Measurements of cell capacitance were calculated by fitting a single exponential curve to the uncompensated current trace from a voltage step from −60 to −70 mV. For current-clamp experiments, bridge balance was carefully monitored throughout the experiments and adjusted when necessary. Recordings were made at room temperature.

For voltage-clamp experiments, the external solution used to isolate Ca\(^{2+}\) currents contained (in mM) 140 tetraethylammonium (TEA)-Cl, 2 MgCl\(_2\), 3 CaCl\(_2\), 10 dextrose, and 10 HEPES adjusted to pH 7.4 with TEA-OH. HEPES buffer served as the external solution for current-clamp recordings. In some experiments, extracellular Ca\(^{2+}\) concentration was raised from 2 to 6 mM. The internal solution used for voltage-clamp experiments contained (in mM) 120 CsCl, 1 MgCl\(_2\), 10 HEPES, 10 EGTA, 4 Mg-ATP, and 0.3 Na-GTP (pH 7.3 adjusted with CsOH). The internal solution for current-clamp recordings contained (in mM) 130 K-gluconate, 10 HEPES, 10 EGTA, 1 MgCl\(_2\), 1 CaCl\(_2\), and 2 Mg-ATP buffered to pH 7.3 with KOH. The liquid junction potential of the internal solution for current-clamp experiments (10 mV) was subtracted from the membrane potential values. The liquid junction potential for the internal solution used for voltage-clamp experiments was not compensated.

Dissection and neuronal dissociation

Acutely dissociated GG neurons were prepared as described previously (King and Bradley 2000; Koga and Bradley 2000). Three to 12 days after fluorescent labeling, the rats were reanesthetized with halothane and decapitated, and the head was secured in a stereotaxic bite bar. After removal of the superior skull, the forebrain was removed just rostral to the brain stem. The facial nerves were visualized at their exit from the brain stem, and the petrous portion of the temporal bones was removed to expose the GG. Both left and right GG were collected and placed in oxygenated HEPES buffer containing (in mM) 124 NaCl, 5 KCl, 5 MgCl\(_2\), 10 sodium succinate, 15 dextrose, 15 HEPES, and 2 CaCl\(_2\) (pH adjusted to 7.4 with NaOH). The ganglia were transferred to HEPES buffer containing 1.5 mg/ml trypsin (Sigma) and 2.5 mg/ml collagenase (type IA, Sigma) and incubated for 60 min at 37°C. After incubation, ganglia were washed three times with HEPES buffer at room temperature. The ganglia were triturated with a series of progressively decreasing diameter, fire-polished Pasteur pipettes to produce a suspension of dissociated neurons. The resulting suspension was placed in a 35-mm-diameter petri dish containing poly-d-lysine/laminin–precoated coverslips and kept for ≥30 min before making electrophysiological recordings.

Whole cell patch-clamp recording

The petri dish containing dissociated neurons was mounted on the stage of an inverted microscope (ECLIPSE TE300, Nikon) equipped with epifluorescence and phase contrast optics. The cell suspension was continuously perfused at about 2 ml/min with HEPES buffer at room temperature. Recordings were performed between 30 min and 7 h after perfusing. Fluorogold-labeled GG neurons were identified by brief exposure to ultraviolet light. Patch electrodes were pulled using a two-stage puller (Narishige PP-83 puller) from borosilicate filament glass (1.5 OD, World Precision Instruments). Electrode tip resistance was between 1.5 and 2.5 M\(\Omega\) when filled with internal solution.

Electrodes were positioned under visual control onto the membrane of neurons using a hydraulic three-axis micromanipulator (Narishige). A gigahm seal between the electrode and neuron was established in a HEPES buffer. Neurons were recorded in the standard whole cell patch-clamp configuration with an Axopatch 1D amplifier (Axon Instruments) for voltage-clamp experiments or an Axoclamp 2A amplifier (Axon Instruments) in bridge mode for current-clamp recordings. Current- and voltage-clamp protocols, data acquisition, and analysis were performed using pCLAMP 8 software (Axon Instruments). Signals were low-pass filtered at 2–50 kHz (DigitData 1200, Axon Instruments), and stored on a computer.

For voltage-clamp recordings, series resistance and cell membrane capacitance were electronically compensated. Leak subtraction was performed with an on-line P/4 or P/5 protocol using pCLAMP software. Measurements of cell capacitance were calculated by fitting a single exponential curve to the uncompensated current trace from a voltage step from −60 to −70 mV. For current-clamp experiments, bridge balance was carefully monitored throughout the experiments and adjusted when necessary. Recordings were made at room temperature.

For voltage-clamp experiments, the external solution used to isolate Ca\(^{2+}\) currents contained (in mM) 140 tetraethylammonium (TEA)-Cl, 2 MgCl\(_2\), 3 CaCl\(_2\), 10 dextrose, and 10 HEPES adjusted to pH 7.4 with TEA-OH. HEPES buffer served as the external solution for current-clamp recordings. In some experiments, extracellular Ca\(^{2+}\) concentration was raised from 2 to 6 mM. The internal solution used for voltage-clamp experiments contained (in mM) 120 CsCl, 1 MgCl\(_2\), 10 HEPES, 10 EGTA, 4 Mg-ATP, and 0.3 Na-GTP (pH 7.3 adjusted with CsOH). The internal solution for current-clamp recordings contained (in mM) 130 K-gluconate, 10 HEPES, 10 EGTA, 1 MgCl\(_2\), 1 CaCl\(_2\), and 2 Mg-ATP buffered to pH 7.3 with KOH. The liquid junction potential of the internal solution used for current-clamp experiments (10 mV) was subtracted from the membrane potential values. The liquid junction potential for the internal solution used for voltage-clamp experiments was not compensated.

Drug application

Stock solutions of the Ca\(^{2+}\) channel antagonists ω-conotoxin GVIA (ω-CgTX: 100 μM; Alomone Labs), ω-agatoxin IVA (ω-Aga IVA: 20 μM; Tocris Bioscience), CdCl\(_2\) (Cd\(^{2+}\): 10 mM; Sigma), and NiCl\(_2\) (Ni\(^{2+}\): 20 mM; Sigma) were dissolved in distilled H\(_2\)O, and ω-CgTX and ω-Aga IVA were kept in frozen aliquots. Stock solution of nimodipine (Sigma) was prepared by dissolving in 100% ethanol at concentrations of 0.5 mM. All experiments using nimodipine were carried out in the dark to avoid photosensitive degradation. Antagonist stock solutions (2.5 μl) were pipetted near the cell body after the HEPES buffer flow was stopped, achieving a final concentration indicated in the text. All antagonists were tested on the same neuron. We applied subsequent calcium channel antagonist to the GG neurons once relatively stable current amplitude was observed in the presence of last antagonist (Baccei and Kocsis 2000; Scroggs and Fox 1991). The blocking effects of high voltage–activated (HVA) Ca\(^{2+}\) currents by antagonists took into consideration rundown of current amplitudes (Scroggs and Fox 1992). After the current amplitude was relatively stable, the rate of rundown before drug application was calculated, and this rate was used for correction of the drug effects to indicate the approximate extent of current rundown during drug application.

J Neurophysiol. • VOL 105 • JANUARY 2011 • www.jn.org
Data analysis

Electrophysiological data were analyzed using Clampfit 8 (Axon Instruments) and Origin 6.1 software (Originlab). The amplitude of the Ca$^{2+}$ current at each test potential for current-voltage ($I$-$V$) plots was measured as the difference between the peak and the baseline values. The amplitude of the low voltage–activated (LVA) T-type Ca$^{2+}$ current was calculated from the peak subtracted from the current at the end of the test potential to avoid small contamination with residual HVA currents. The HVA Ca$^{2+}$ current amplitude was obtained from the peak value to baseline of the current. The peak conductance ($G$) at each potential was calculated from the corresponding peak current using the equation $G = I/(E - E_{\text{rev}})$, where $E_{\text{rev}}$ is the reversal potential measured for each cell, $I$ is the peak current amplitude, and $E$ is the membrane potential. Normalized peak conductance ($G_{\text{max}}$) during the activation and the peak current amplitude during the steady-state inactivation ($I_{\text{max}}$) were fitted using a Boltzmann function of the following form: $G/G_{\text{max}} = 1 + \exp(V_{\text{SO}} - V)/k$, where $G_{\text{max}}$ is the maximal conductance and $I_{\text{max}}$ is the maximal amplitude of the Ca$^{2+}$ current, $V_{\text{SO}}$ is the membrane potential for 50% of Ca$^{2+}$ current activation and inactivation, and $k$ is the voltage-dependent slope factor. Average soma diameter was calculated from the mean of the longest and shortest axes of the neuron measured with an eyepiece micrometer. No changes in cell size were observed during recording.

Statistical analysis was conducted using the PASW statistics program (SPSS). Data are expressed as mean ± SE, and statistical significances ($P < 0.05$) between groups were assessed using Student’s $t$-test or one-way ANOVA, which was followed by Scheffe’s post hoc tests for comparison of values and $\chi^2$ test or Fisher’s exact test for comparison of proportion.

RESULTS

Identification of neurons labeled with Fluorogold

Recordings were made from neurons that project to the anterior tongue via the chorda tympani (CT neurons, $n = 76$); the soft palate via the greater superficial petrosal nerve (GSP neurons, $n = 61$); and the inner surface of the ear via the posterior auricular branch of the facial nerve (PA neurons, $n = 55$). The Fluorogold-labeled neurons were easily identified under ultraviolet illumination and were spherical or ovoid in shape, with a diameter between 22 and 32.5 µm. Some of neurons had short processes and became loosely attached to the bottom of the dish. The average diameters of the CT, GSP, and PA neurons were 27.6 ± 0.3, 27.2 ± 0.3, and 29.3 ± 0.2 µm, respectively. PA neurons were significantly larger than CT and GSP neurons ($P < 0.01$). Once a labeled neuron was identified, whole cell recordings were performed under brightfield illumination.

Whole cell Ca$^{2+}$ currents of GG neurons

Ca$^{2+}$ currents were recorded under conditions that suppressed K$^+$ and Na$^+$ by the replacements of internal KCl with CsCl and external NaCl with TEA-Cl, respectively (see Methods). Figure 1 shows whole cell Ca$^{2+}$ current from a GSP neuron activated by depolarizing step pulses (150 ms) from a holding potential of –90 mV to test potentials ($V_t$) from –80 to 60 mV as shown at the top right in each trace. A fast decaying component of current was observed first at a $V_t$ of –50 mV (arrowhead). B: current traces elicited from a holding potential of –40 mV as shown at the top right in each trace. Note the disappearance of transient inactivating components shown in A. Current traces in A and B were obtained from the same neuron. C: $I$–$V$ relationship obtained from the current traces shown in A and B.

FIG. 1. Whole cell Ca$^{2+}$ currents in a geniculate ganglion (GG) neuron. A: example of current traces in a greater superficial petrosal (GSP) neuron activated by depolarizing step pulses (150 ms) from a holding potential of –90 mV to test potentials ($V_t$) from –80 to 60 mV as shown at the top right in each trace. A fast decaying component of current was observed first at a $V_t$ of –50 mV (arrowhead). B: current traces elicited from a holding potential of –40 mV as shown at the top right in each trace. Note the disappearance of transient inactivating components shown in A. Current traces in A and B were obtained from the same neuron. C: $I$–$V$ relationship obtained from the current traces shown in A and B.
neuron activated by 150-ms depolarizing step pulses from a holding potential of either −90 or −40 mV to test potentials from −80 to 60 mV in 10-mV increments. A fast transient inactivating current component was first observed at a test potential of −50 mV from a holding potential of −90 mV (filled arrowhead in Fig. 1A) and followed by more slowly inactivating component at more positive test potentials. In this study, a noninactivating low-threshold current, which was sensitive to nimodipine, L-type channel antagonist (Durante et al. 2004; Kline et al. 2009), was not observed from a holding potential of around −90 mV to test potentials of −50 or −40 mV, further supported by the data that application of 5 μM nimodipine did not affect the currents activated at test potentials of either −50 or −40 mV in the CT (n = 7), GSP (n = 6), and PA neurons (n = 6). From a holding potential of −40 mV, transient inactivating components could no longer be evoked over the whole range of test potentials, but a sustained, slowly inactivating component was apparent (Fig. 1B). A plot of the I-V relationship of the peak currents at two different holding potentials (Fig. 1C) indicates two GG neuron current components with different activation ranges: LVA T-type and HVA Ca2+ currents.

To determine the characteristics of Ca2+ currents in different subpopulations of GG neurons, we evoked Ca2+ current in CT (n = 45), GSP (n = 32), and PA neurons (n = 28). The inward Ca2+ currents were typically activated at a test potential of around −50 mV and reached the maximum at 0 mV in CT, GSP, and PA neurons (Figs. 2A–C). CT and GSP neurons had moderate transient T-type Ca2+ currents at hyperpolarized test potentials of about −40 mV and large sustained HVA Ca2+ currents at more depolarized test potentials (Fig. 2A and B). In contrast, PA neurons expressed relatively large T-type Ca2+ currents at hyperpolarized potentials of around −40 mV compared with HVA Ca2+ currents evoked by larger depolarizing test potentials (Fig. 2C). This resulted in a prominent shoulder at negative test potentials of the I-V relationship in PA neurons (Fig. 2D).

We calculated the current ratio of the Ca2+ current evoked at a test potential of −40 mV to the current evoked at a test potential of 0 mV in CT, GSP, and PA neurons (Fig. 2E). The mean current ratio of PA neurons (0.55 ± 0.03) was significantly higher than that of CT (0.32 ± 0.03) and GSP neurons (0.31 ± 0.03), reflecting a larger contribution of T-type Ca2+ current to total Ca2+ current in PA neurons than in CT and GSP neurons. The I-V curves indicated a significant enhancement of Ca2+ current at test potentials from −50 to 30 mV in PA neurons compared with CT and GSP neurons (Fig. 2D).

**T-type Ca2+ currents of GG neurons**

There were differences in the functional expression of T-type Ca2+ currents in the GG neuron subpopulations. T-type Ca2+ currents were present in 62.2% (28 of 45 neurons) of CT, 81.3% (26 of 32 neurons) of GSP, and 96.4% (27 of 28 neurons) of PA neurons (Fig. 3A). Neurons with T-type Ca2+ currents were more frequently encountered in PA neurons than in CT neurons (χ² test: P < 0.01).

---

**FIG. 2.** Whole-cell Ca2+ currents in different subpopulations of GG neurons. A–C: families of Ca2+ currents evoked in representative chorda tympani (CT) (A), GSP (B), and posterior auricular (PA) (C) neurons by depolarizing steps from a holding potential of −90 mV to test potentials from −80 to 60 mV in 10-mV increments (top) and the representative current traces evoked from a holding potential of −90 mV to test potentials of −40 and 0 mV (bottom). Current traces in the bottom were obtained from the same neurons as in the top. Voltage protocols used to activate Ca2+ currents in A–C are shown below the current traces in column A. Note that CT and GSP neurons had small T-type Ca2+ currents and large high voltage-activated (HVA) Ca2+ currents, whereas PA neurons had large T-type Ca2+ currents and equally prominent HVA Ca2+ currents. D: average I-V curve obtained from CT, GSP, and PA neurons. The I-V curve for PA neurons displays a prominent shoulder at hyperpolarized test potentials. All points are the mean values from 45 CT, 32 GSP, and 28 PA neurons, respectively. E: histogram of the average ratio of currents at a test potential of −40 mV (I−40mV) to currents at a test potential of 0 mV (I0mV) for CT, GSP, and PA neurons (n = 45, 32, and 28 neurons, respectively). The ratio of T-type Ca2+ current to total Ca2+ current was significantly higher in PA neurons than in CT and GSP neurons (P < 0.01). In this and subsequent figures, error bars indicate mean ± SE and significant differences are marked by *P < 0.05 or **P < 0.01.
Distribution of average soma diameter differed in CT neurons with and without T-type Ca\(^{2+}\) currents (Fig. 3B). The average diameter was 28.8 ± 0.3 \(\mu\)m for CT neurons with T-type Ca\(^{2+}\) currents (\(n = 28\)) and 25.7 ± 0.7 \(\mu\)m for CT neurons without T-type Ca\(^{2+}\) currents (\(n = 17\)), respectively, a significant difference (\(P < 0.01\)). Diameters of GSP and PA neurons with T-type Ca\(^{2+}\) currents (GSP: 27.2 ± 0.3 \(\mu\)m, \(n = 26\); PA: 29.3 ± 0.3 \(\mu\)m, \(n = 27\)) did not differ from diameter of GSP and PA neurons that did not express T-type Ca\(^{2+}\) currents (GSP: 27.2 ± 1.6 \(\mu\)m, \(n = 6\); PA: 28.0 ± 1.5 \(\mu\)m, \(n = 1\)). However, this result may be influenced by the low numbers of GSP and PA neurons without T-type Ca\(^{2+}\) currents that we encountered.

We compared T-type Ca\(^{2+}\) current amplitudes evoked by a step pulse from a holding potential of −90 mV to a test potential of −40 mV in the neurons expressing T-type Ca\(^{2+}\) currents in three subpopulations of GG neurons (Fig. 3C). The T-type Ca\(^{2+}\) current amplitude was significantly larger in PA neurons (1.042 ± 70.1 pA, \(n = 27\)) than in CT (729.6 ± 50.4 pA, \(n = 28\); \(P < 0.01\)) and GSP neurons (595.8 ± 63.2 pA, \(n = 26\); \(P < 0.01\)). Because differences in cell size of GG neurons tested could affect the amplitude of T-type Ca\(^{2+}\) currents, we compared the current density of T-type Ca\(^{2+}\) current by dividing the current amplitude (pA) by the cell capacitance (pF). The T-type Ca\(^{2+}\) current density was significantly larger in PA neurons (31.3 ± 1.5 pA/pF, \(n = 27\)) than in CT (24.9 ± 1.6 pA/pF, \(n = 28\); \(P < 0.05\)) and GSP neurons (19.4 ± 1.6 pA/pF, \(n = 26\); \(P < 0.01\); Fig. 3D). Furthermore, GSP neurons had a significantly smaller T-type Ca\(^{2+}\) current density than CT neurons (\(P < 0.01\)). There was a weak correlation between cell size and T-type Ca\(^{2+}\) current density in neurons with T-type Ca\(^{2+}\) currents (\(R = 0.29\)).

We studied the voltage-dependent activation of the T-type Ca\(^{2+}\) currents by applying 150-ms step pulses to test potentials from −80 to −40 mV in 10-mV increments from a holding potential of −90 mV (Fig. 4A). Resulting data for 8 CT, 7 GSP, and 12 PA neurons were fitted with a Boltzmann equation. The T-type Ca\(^{2+}\) currents showed similar (\(P > 0.05\)) half-activation values (CT −46.1 ± 1.3 mV; GSP −45.5 ± 0.4 mV; PA −46.9 ± 0.7 mV) and slopes of the activation curves (Fig. 4C).

We also measured the steady-state inactivation by varying the holding potentials at voltages from −100 to −40 mV for 2 s in 10-mV increments before changing the voltage to the test potential of −40 mV for 250 ms (Fig. 4B). The half-inactivation voltages (CT −67.1 ± 1.1 mV; GSP −66.9 ± 0.5 mV; PA −65.8 ± 0.7 mV) and slopes of the inactivation curves were also similar (\(P > 0.05\)) between the three subpopulations of GG neurons (Fig. 4D).

**HVA Ca\(^{2+}\) currents of GG neurons**

We used pharmacological antagonists to characterize subtypes of HVA calcium channels in subpopulations of GG neurons (10 CT, 10 GSP, and 10 PA). HVA Ca\(^{2+}\) currents were evoked by depolarization from −60 to −10 mV for 80 ms and the peak amplitude of the current plotted as a function of time (Fig. 5, A–C). Sequential application of 5 \(\mu\)M nifedipine (L-type channel antagonist), 1 \(\mu\)M \(\omega\)-CgTX (N-type channel antagonist), and 200 nM \(\omega\)-AgA IVA (P/Q-type channel antagonist) inhibited part of the current in all CT (Fig. 5A), GSP (Fig. 5B), and PA neurons (Fig. 5C). A residual current remained, resistant to the combined antagonist application. Application of the nonselective Ca\(^{2+}\) current antagonist Cd\(^{2+}\) (100 \(\mu\)M) completely blocked the remaining Ca\(^{2+}\) currents, indicating the presence of R-type Ca\(^{2+}\) currents. Inhibition by the antagonists was partially reversible. Figure 6 summarizes the effects of the antagonists as a mean percentage of total current in the different subpopulations of GG neurons. Nimodipine application blocked 21.0 ± 2.5 (CT), 22.8 ± 3.7 (GSP), and 13.9 ± 1.8% (PA) of the total currents in GG neuron subpopulations. Subsequent application of \(\omega\)-CgTX reduced 31.7 ± 2.8 (CT), 26.0 ± 3.0 (GSP), and 24.4 ± 1.5% (PA) of the current and \(\omega\)-AgA IVA produced an additional 17.6 ± 1.9 (CT), 37.8 ± 4.4 (GSP), and 24.7 ± 1.5% (PA) reduction of...
FIG. 4. Voltage dependence of activation and steady-state inactivation for T-type Ca\(^{2+}\) currents in different subpopulations of GG neurons. A: representative current traces evoked by 150 ms step pulses to test potentials from \(-80\) to \(-40\) mV in 10 mV increments from a holding potential of \(-90\) mV to investigate the voltage dependence of activation. B: representative current traces by varying the holding potentials from \(-100\) to \(-40\) mV for 2 s in 10 mV increments before changing the voltage to the test potential of \(-40\) mV for 250 ms for the voltage dependence of steady-state inactivation. The bottom panels indicate voltage protocols. C: activation curve obtained from CT (□), GSP (○), and PA neurons (△). Relative conductance (G/G\(_{\text{max}}\)) of T-type Ca\(^{2+}\) current was plotted against membrane potential. D: steady-state inactivation curve from CT (□), GSP (○), and PA neurons (△). Normalized current (I/I\(_{\text{max}}\)) was plotted against membrane potential. Data were fitted with a Boltzmann curve. There were no significant differences in the voltage dependence of activation and inactivation of the T-type Ca\(^{2+}\) currents between 3 subpopulations of GG neurons (P > 0.05).

Action potential and afterdepolarization of GG neurons

We used current-clamp recordings to study the contribution of Ca\(^{2+}\) currents to the spike firing of GG neuron subpopulations. Current-clamp recordings were performed on 14 CT, 13 GSP, and 11 PA neurons. Resting membrane potentials of all neurons ranged from \(-41\) to \(-64\) mV, with a mean of \(-56 ± 1\) (CT neurons), \(-56 ± 1\) (GSP neurons), and \(-54 ± 2\) mV (PA neurons), respectively. There were no significant differences in the resting membrane potential between three subpopulations of GG neurons (P > 0.05). Figure 7A shows examples of action potential elicited from various membrane potentials in GG neurons. A single action potential was evoked by injection of brief depolarizing current pulses (3 ms), which was followed by an afterhyperpolarization (AHP) at resting membrane potential (Fig. 7A, left). When the cell membrane potential was hyperpolarized at \(-77\) mV, a humplike afterdepolarization (ADP) was elicited following the rapid repolarizing phase of the spike (filled arrowhead in Fig. 7A, middle). Holding the cell membrane at a more negative potential of \(-80\) mV produced a strong enhancement of the ADP that elicited another spike (Fig. 7A, right). Figure 7B exhibits the effect of change in extracellular Ca\(^{2+}\) concentration on ADP. The ADP was strongly enhanced by increasing the extracellular Ca\(^{2+}\) concentration from 2 (Fig. 7B, left) to 6 mM (Fig. 7B, middle).

Previous studies indicate that T- and R-type Ca\(^{2+}\) currents are involved in the generation of the ADP and additional spikes that trigger burst firing in some acutely isolated rat dorsal root ganglion neurons (Metz et al. 2005; White et al. 1989). Therefore we applied the antagonist to the GG neurons. As shown in Fig. 7, application of 200 μM Ni\(^{2+}\), which blocks both T- and
current-clamp experiments, a brief depolarization generated an ADP in neurons of the CT, GSP, and PA nerves. In addition, in CT neurons, ADPs were significantly more frequent during hyperpolarizing current pulses injection (500 ms) compared to PA neurons (Fisher’s exact test: \( P < 0.05 \)).

Neither R-type calcium currents, abolished the ADP with burst firing (Fig. 7C, middle). The effect of Ni\(^{2+}\) was partially reversible (Fig. 7C, right). Furthermore, the number of rebound spikes elicited after hyperpolarizing current pulses injection (500 ms) from a resting membrane potential was reduced by application of Ni\(^{2+}\) (Fig. 7D, middle). ADPs were observed in 72% (11 of 14 neurons) of CT, 58% (7 of 13 neurons) of GSP, and 100% (11 of 11 neurons) of PA at a membrane potential of \(-70\) mV, whereas only 14% (2 of 14 neurons) of CT, 0% (0 of 13 neurons) of GSP, and 27% (3 of 11 neurons) of PA neurons displayed ADPs at a hyperpolarized membrane potential (Fig. 8). All PA neurons expressed ADPs at a hyperpolarized membrane potential, whereas GSP neurons with ADPs were less frequent encountered compared with PA neurons (Fisher’s exact test: \( P < 0.05 \)).

**DISCUSSION**

Our data provide a compelling demonstration of heterogeneity among GG neurons with different receptive fields on the anterior tongue, soft palate, and ear innervated by the CT, GSP, and PA branches of the facial nerve. Results establish that isolated CT, GSP, and PA neurons differ in size, expression of calcium channels, and action potential characteristics. PA neurons were significantly larger than CT and GSP neurons, and CT neurons could be further subdivided based on soma diameter. Both LVA and HVA Ca\(^{2+}\) currents were expressed in all GG neuron groups, but there were significant differences in biophysical properties and percentage of different Ca\(^{2+}\) currents in neurons of the CT, GSP, and PA nerves. In addition, in current-clamp experiments, a brief depolarization generated an ADP that was Ca\(^{2+}\) current initiated. This heterogeneity is a fundamental characteristic of the basic neurobiology of the GG related to the diverse function of the sensory information transmitted by the CT, GSP, and PA branches of the facial nerve.

**GG neuron differ in several properties related to function**

Heterogeneity in other sensory ganglia neurons has been correlated with different sensory function. Most available information on ganglion cell properties is derived from studies of the dorsal root ganglion. Neurons in this ganglion differ in soma size and peripheral fiber diameter and conduction velocity (Harper and Lawson 1985) and can also be subdivided based on their response to adequate stimuli (Lawson 2002). The biophysical properties of the ganglion soma differ, and these differences correlate with the type of peripheral receptors they innervate (Koerber et al. 1988). In addition, dorsal root ganglion nociceptive fibers have been found to be separable into two major classes based on molecular and anatomical criteria. These two classes also express different ion channels and have different central and peripheral terminations (Braz et al. 2005; Woolf and Ma 2007; Zylka et al. 2005). Based on this and considerable other information on the dorsal root ganglion, it is reasonable to assume that the different properties we reported for GG neurons relate to similar correlations between ganglion cell properties and function.

Peripheral fibers of GG neurons differ in size. Investigators have reported a bimodal distribution of CT axon diameters (Jang and Davis 1987). Axon diameters of the CT in rat and hamster have been measured after the parasympathetic efferent fiber component was eliminated by section of the central process (nervus intermedius). Seventy-nine percent of the different processes of the CT were myelinated fibers and only 30% were unmyelinated (Farbman and Hellekant 1978). The myelinated axons range from 1 to 5 \(\mu m\) in diameter and the unmyelinated range from 0.2 to 1.5 \(\mu m\). Data from the hamster CT show a bimodal distribution of axon diameters with equal proportions of myelinated and unmyelinated fibers (Jang and Davis 1987). Thus there is considerable variability in the distribution of CT axon diameters. No information is available for GSP and PA axon diameters.

Using extracellular recording from rat GG soma, investigators provided information on chemical and thermal responses of neurons with afferent fibers in the CT and GSP (Breza et al. 2006; Lundy and Contreras 1999; Sollars and Hill 2005). Stimuli representing the taste qualities (sweet, sour, salty, bitter, and umami) and thermal stimuli were applied to the anterior tongue and soft palate. Neurons of the GG innervating the tongue were divided into those that responded to a single stimulus (either NaCl or sucrose and called “specialists”) and those that responded to several of the applied stimuli and were classified as “generalists” (Lundy and Contreras 1999; Sollars and Hill 2005). The temperature sensitivity of the CT GG neurons was also examined (Breza et al. 2006). The majority of the neurons that responded to either warming or cooling were either NaCl specialists or acid-sensitive generalists. When responses of GG somata innervating the tongue and soft palate were compared, differences in response characteristics were found and assumed to reflect the response characteristics of the taste buds in the two receptive fields (Hendricks et al. 2002; Nejad 1986; Sollars and Hill 1998, 2000, 2005). However, it is also possible that these differences in response characteristics may relate to the biophysical properties of the GG neurons reported in these experiments.

Recordings from peripheral fibers also show the heterogeneous characteristics of the GG neurons. CT fibers are rela-
tively nonspecific in their response to chemicals representing the taste qualities (Erickson et al. 1965; Fishman 1957; Frank et al. 1988; Ogawa et al. 1968; Pfaffmann 1941, 1955). However, even though fibers were not found to respond exclusively to one of the taste qualities, CT fibers could be grouped based their “best” response to lingual application of taste stimuli (Frank 1974; Frank et al. 1988). Furthermore, despite the fact that the CT is often referred to as a “pure taste” nerve (Arai et al. 2010), numerous electrophysiological studies using chemical, thermal, and tactile stimuli report that, in rat, hamster, and several other species, many CT fibers are multimodal (Breza et al. 2006, 2010; Kosar and Schwartz 1990; Ogawa et al. 1968; Sato et al. 1975; Shimatani et al. 2002). Afferents fibers of GG neurons also transmit different sensory information. However, investigators exploring response properties of GG neurons have restricted their analysis to CT neurons of rats and hamsters with the goal of determining how chemosensory information is encoded.

There are a few reports of whole nerve responses of the GSP nerve to chemical stimulation of the soft palate (Harada and Smith 1992; Nejad 1986; Sollars and Hill 2000), but no reports using single fiber recordings, and to date, no studies of the response properties of the PA nerve have appeared.

**Correlation of GG cell soma size with afferent fiber characteristics**

Early investigators correlated CT fiber diameter with response characteristics to chemical stimuli. CT fibers in dogs responding to sweet tasting stimuli were reported to be larger than fibers responsive to tongue application of bitter tasting compounds (Andersson et al. 1951). In a later study, all the CT taste fibers were reported to be in the Aδ conducting range, although they varied between 17.8 and 1.6 m/s (Iriuchijima and Zotterman 1961). Taste fibers that responded to bitter tasting...
stimuli were smaller than the fibers responding to the other taste qualities, and their conduction velocities were in the upper range of C fibers. The data from the CT were restricted to chemical stimuli, and no data are available on conduction velocity measures for GSP and PA afferent fibers. These early studies have to be evaluated based on many later studies. For example, rat GG tongue neurons that respond to bitter tasting chemicals were classified as generalists, and thus it is not possible to conclude that all small diameter fibers relay information only on bitter taste. In a recent study in which response latency was accurately measured, part of which is contributed by fiber conduction velocity, GG neurons with the shortest latency were those that responded best to a single stimulus (Breza et al. 2010). This suggests that the short latency GG neurons have the larger diameter peripheral fibers.

Potential role of GG neurons in sensory processing

We showed that the GG contains a diverse population of sensory neurons that encode a variety of stimuli with distinctly different electrophysiological properties. The calcium channels characterized in this study are responsible in part for these distinct properties. Besides a number of important roles, calcium influx through voltage-gated calcium channels is required for the release of neurotransmitter from synaptic endings (Hille 2001). However, the calcium currents in GG neurons were characterized in the ganglion cell and not the central synaptic terminals. Calcium currents have also been characterized in aortic baroreceptor neurons in the nodose ganglion (Mendelowitz and Kunze 1992). These currents were later determined to correspond to calcium channels in the central synapses (Mendelowitz et al. 1995), suggesting that calcium channels characterized in the soma reflect those at the central synapses. It is likely therefore that the calcium channels we described in GG soma play a role in synaptic transmission between the central processes and the second-order neurons in the nNST. GG calcium channels may also be required for the release of neurotransmitters and neuropeptidomodulators involved in nonsynaptic communication between ganglion cells (Amir and Devor 1996; Utzschnieder et al. 1992). Ganglion cells do not have synapses, yet respond to a number of neurotransmitters and neuropeptidomodulators (Lieberman 1976). Calcium channels have been shown to be required for release of ATP and Substance P from trigeminal ganglion cells (Matsuka et al. 2001). GG ganglion cells respond to Substance P (Koga and Bradley 1996) and stain positive for ATP receptors (Ohmoto et al. 2000) and TRPV1 immunoreactive fibers have been shown in taste papillae and taste buds innervated by the CT (Ishida et al. 2002; Kido et al. 2003). It is possible therefore that the calcium channels of the GG neurons play a similar role in TRPV1 channel desensitization.

Investigators recording from the GG cells do not mention the possibility that the discharge pattern of GG neurons differ from discharge pattern of action potentials traveling to the GG. However, our finding that a subgroup of GG neurons responds to depolarization by generating an ADP suggests possible modification of the discharge pattern. Neurons in several brain areas including the dorsal root ganglion respond with an ADP that elicits burst firing (White et al. 1989). As in this study, the ADP is eliminated after application of calcium channel antagonists. Both T- and R-type calcium channels have been shown to play a role in the generation of the ADP (Metz et al. 2005; White et al. 1989). Thus GG neurons may not play a “platonic” role (Llinás 1988) in sensory processing and by depolarization-initiated burst discharges add to the frequency of action potentials transmitted from the periphery.

GRANTS
This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-000288 to R. M. Bradley.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES
Boudreau JC, Hoang NK, Oravec J, Do LT. Rat neurophysiological taste responses to salt solutions. Chem Senses 8: 131–150, 1983.
CALCULATIONS OF CURRENTS OF GENICULATE GANGLION SENSORY NEURONS


May OL, Hill DL. Gustatory terminal field organization and developmental plasticity in the nucleus of the solitary tract. Chem Senses 7: 35–64, 2005.


J Neurophysiol • VOL 105 • JANUARY 2011 • www.jn.org

Downloaded from http://jn.physiology.org/ by 10.220.33.1 on September 24, 2016


