Distinctive Neurophysiological Properties of Embryonic Trigeminal and Geniculate Neurons in Culture

ARTURAS GRIGALIUNAS, ROBERT M. BRADLEY, DONALD K. MACCALLUM, AND CHARLOTTE M. MISTRETTA
1Department of Biologic and Materials Sciences, School of Dentistry and 2Department of Cell and Developmental Biology, Medical School, University of Michigan, Ann Arbor, Michigan 48109

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Grigaliunas, Arturas, Robert M. Bradley, Donald K. Mac Callum, and Charlotte M. Mistretta. Distinctive neurophysiological properties of embryonic trigeminal and geniculate neurons in culture. J Neurophysiol 88: 2058–2074, 2002; 10.1152/jn.00889.2001. Neurons in trigeminal and geniculate ganglia extend neurites that share contiguous target tissue fields in the fungiform papillae and taste buds of the mammalian tongue and thereby have principal roles in lingual somatosensation and gustation. Although functional differentiation of these neurons is central to formation of lingual sensory circuits, there is little known about electrophysiological properties of developing trigeminal and geniculate ganglia or the extrinsic factors that might regulate neural development. We used whole cell recordings from embryonic day 16 rat ganglia, maintained in culture as explants for 3–10 days with neurotrophin support to characterize basic properties of trigeminal and geniculate neurons over time in vitro and in comparison to each other. Each ganglion was cultured with the neurotrophin that supports maximal neuronal survival and that would be encountered by growing neurites at highest concentration in target fields. Resting membrane potential and time constant did not alter over days in culture, whereas membrane resistance decreased and capacitance increased in association with small increases in trigeminal and geniculate soma size. Small gradual differences in action potential properties were observed for both ganglion types, including an increase in threshold current to elicit an action potential and a decrease in duration and in rise and fall slopes so that action potentials became shorter and sharper with time in culture. Using a period of 5–8 days in culture when neural properties are generally stable, we compared trigeminal and geniculate ganglia and revealed major differences between these embryonic ganglia in passive membrane and action potential characteristics. Geniculate neurons had lower resting membrane potential and higher input resistance and smaller, sharper action potentials with lower thresholds than trigeminal neurons. Whereas all trigeminal neurons produced a single action potential at threshold depolarization, 35% of geniculate neurons fired repetitively. Furthermore, all trigeminal neurons produced TTX-resistant action potentials, but geniculate action potentials were abolished in the presence of low concentrations of TTX. Both trigeminal and geniculate neurons had inflections on the falling phase of the action potential that were reduced in the presence of various pharmacological blockers of calcium channel activation. Use of nifedipine, ω-conotoxin-MVIIA and GVIA, and ω-agatoxin-TK indicated that currents through L-, N-, and P/Q-type calcium channels participate in the action potential inflection in embryonic trigeminal and geniculate neurons. The data on passive membrane, action potential, and ion channel characteristics demonstrate clear differences between trigeminal and geniculate ganglion neurons at an embryonic stage when target tissues are innervated but receptor organs have not developed or are still immature. Therefore these electrophysiological distinctions between embryonic ganglia are present before neural activity from differentiated receptive fields can influence functional phenotype.

I N T R O D U C T I O N

One of the principal taste organs on the mammalian tongue is the set of fungiform papillae and resident taste buds innervated by nerve fibers from two main sensory ganglia (Mistretta 1991; Mistretta and Hill 1995). The trigeminal ganglion, via the lingual branch of the mandibular nerve root innervates both the lingual epithelium that lies between fungiform papillae and the papilla epithelium that surrounds taste bud cells but not taste buds per se. In contrast, the geniculate ganglion, via the chorda tympani nerve branch, provides innervation only to the taste bud. Thus in this taste organ, nerve fibers from the geniculate ganglion travel to and innervate cells in a highly circumscribed location, whereas fibers from the trigeminal ganglion essentially encompass a geniculate-derived nerve basket to innervate surrounding papilla and lingual epithelium (Miller 1974; Whitehead et al. 1985). Although the lingual receptors are not the only peripheral target organs for trigeminal and geniculate ganglia (Brodal 1981), neurites from these ganglia have contiguous but distinct receptive fields within the fungiform papillae that require precise developmental controls to establish appropriate innervation patterns.

The trigeminal and geniculate ganglia not only have distinctive target organs but also have different embryonic derivations (Graham and Begbie 2000). The trigeminal placode is neurogenic and forms from CNS tissue near the midbrain-hindbrain junction. The geniculate placode is epibranchial, forming between the branchial arches in close proximity to neural crest and pharyngeal endoderm (Begbie et al. 1999). Although trigeminal and geniculate ganglion function is integral to formation of taste circuits and to gustatory and related somatosensory sensation, there is little known about electrophysiological differentiation of these ganglion cells. To begin to understand early ganglion cell function and the factors that regulate emerging function, we have studied basic neural properties of embryonic trigeminal and geniculate ganglia in rat.

In general, immature ganglion neurons have electrophysiological properties that include action potentials with a rela-
tively long duration, a smaller amplitude, and a resistance to the sodium channel blocking agent, tetrodotoxin (Spitzer and Ribera 1998; Vincent et al. 2000). There follows a gradual maturation to briefer, sodium-dependent action potentials. However, in adult mammals, ganglia typically have heterogeneous subpopulations of neurons with varying action potential, ion channel and neuropeptide characteristics (De Armentia et al. 2000; Hall et al. 1997). In dorsal root ganglia (DRG), functional subpopulations have been related to specific peripheral receptor type (Fedulova et al. 1998; Koeber et al. 1988), but initial neuropeptide expression in sensory subtypes apparently emerges in advance of connections with receptor targets (Hall et al. 1997). On the other hand, neurotrophins in target tissues have known roles in regulating differentiation of neuron morphology, ion channel expression, and neurotransmitters (Hall et al. 1997). Electrical stimulation is also important in developmental expression of neuropeptides and signaling pathways in embryonic rat DRG cells (Ai et al. 1998; Fields 1998). Furthermore, patterned electrical stimulation versus chronic depolarization differentially alters ion channel activity and gene expression in embryonic petrosal ganglion (Brosenitsch and Katz 2001), suggesting potential for receptor input to alter ganglion neuron properties.

Although there are some basic neural properties that characterize neurons as immature and relatively homogeneous (Spitzer and Ribera 1998), the extent to which these generalizations apply to all sensory ganglion neurons and the age at which ganglia demonstrate divergent neural properties are not clear. Both the trigeminal (Liu and Simon 1996; Liu et al. 2001) and geniculate (King and Bradley 2000; Koga and Bradley 2000) ganglia have been studied in postnatal or adult ages to discern whether neurons projecting to defined receptive fields have distinctive biophysical characteristics that correlate with neuron subtypes. Action potential properties in postnatal geniculate ganglion neurons that are primarily gustatory differ in some aspects from those that are primarily somatosensory (King and Bradley 2000). In adult rat, trigeminal neurons are subtyped based on responses to nociceptive chemicals, and action potential and ion channel properties (Liu and Simon 1996; Liu et al. 2001).

Discovering how and when the gradual process of neuro-physiological differentiation is regulated by intrinsic and extrinsic factors, including interactions with target organs, requires basic knowledge of neurophysiological development in pre- and postnatal ganglia. There are no experimental data comparing embryonic trigeminal or geniculate ganglion neural properties. Therefore, it is not clear how intrinsic ganglion neuron development, emerging neural activity from developing sense organs, and molecular factors in the target environment might interact to effect the basic neurobiology of these two sensory ganglia and their participation in formation of taste and taste-related sensory circuits.

To study the trigeminal and geniculate ganglia, we used an explant system of ganglia maintained in culture for several days and made whole cell recordings from single ganglion neurons. Explant cultures were used to retain a biological environment more similar to that in vivo than with dissociated neurons. Ganglia were dissected at gestational day 16, which is an embryonic stage when the cell soma extend neurites to provide robust innervation of the early fungiform papilla (Farbman and Mbiene 1991; Mbiene and Mistretta 1997) but is a period in advance of taste bud formation. To sustain ganglion neurons in the explant cultures, we added the appropriate neurotrophin to maximally support neuron survival (Al Hadlaq et al. 2001a; Davies 1997). Thus trigeminal explants were maintained with nerve growth factor (NGF) and geniculate explants with brain-derived neurotrophic factor (BDNF). Furthermore NGF is a principal neurotrophin in lingual receptive fields of the trigeminal ganglion, whereas BDNF is in circumscribed target fields of geniculate ganglion neurons (Nosrat and Olson 1995; Nosrat et al. 2001).

Our experiments had two main objectives. First, to understand neurophysiological function over time in culture, we tested the hypothesis that basic cell membrane and action potential properties for each ganglion type remain stable over days in vitro. Second, with basic information about the explant system in hand, we tested the hypothesis that trigeminal and geniculate ganglion cells cultured at E16 and maintained with optimal neurotrophin support, have similar neural properties. Early reports of these studies have appeared in abstracts (Gri- galiunas et al. 1999, 2000).

**METHODS**

**Ganglion cultures**

Timed-pregnant Sprague Dawley rats at gestational day 16 (day 0, vaginal plug detected) were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), which effectively anesthetizes the embryos also. Embryos were removed and placed in cold (4°C), Earle’s balanced salt solution with gentamicin sulfate (20 μg/ml) and 20 mM HEPES buffer (pH 7.4). Embryo heads were hemisected in a sagittal plane and ganglia were exposed from the medial side of each half. Trigeminal and geniculate ganglia were dissected and explanted onto glass coverslips covered by a matrix deposited by cultured bovine corneal endothelial cells (MacCallum et al. 1982). The relatively large trigeminal ganglia were cut in half before explanting, and the two halves were placed on one coverslip. Two individual geniculate ganglia were placed on one coverslip. Coverslips with ganglia were placed in a culture dish in medium containing a 1:1 mixture of Dulbecco’s modified Eagle’s minimum essential medium and Ham’s nutrient mixture F12. 1% fetal bovine serum, 20 μg/ml gentamicin sulfate, and 2% B27 culture supplement (Gibco, Life Technologies). The medium was supplemented with 10 ng/ml NGF and 10 ng/ml BDNF (Alomone Labs, Jerusalem, Israel) for trigeminal and geniculate ganglia, respectively, to optimally sustain cells and promote neurite outgrowth. Explanted ganglia were maintained in an incubator in humidified 5% CO2 in air, at 37°C, for up to 10 days. The culture medium was changed every second day.

**Solutions and drugs**

For whole cell recording, coverslips with cultured ganglia were removed from the culture dish, placed in a petri dish, and superfused (3 ml/min) with oxygenated solution containing (in mM) 124 NaCl, 5 KCl, 5 MgCl2, 10 Na-succinate, 15 dextrose, 15 HEPES, and 2 CaCl2 (Du and Bradley 1996). The pH was adjusted to 7.4 with NaOH. In experiments with Na⁺-free solution, Na⁺ was substituted by choline to maintain osmolality, and pH was adjusted with KOH. The sodium channel blocker, tetrodotoxin (TTX, Sigma, St. Louis, MO), was kept in citrate buffer (pH 4.3) at –20°C and diluted in the external bath solution to the required concentration before each experiment. The L-type calcium channel blocker, nifedipine (Sigma), was dissolved in dimethylsulfoxide (20 mM stock solution) before adding to the bath solution. Verapamil hydrochloride (Tokris Cookson, Ballwin, MO) was added directly to the external solution. Specific calcium channel
Electrophysiological recordings

Pipettes were pulled in two stages from 1.5 mm OD borosilicate filament glass (WPI, MTW150F-4) using a Narishige PP-83 electrode puller and were filled with a solution containing (in mM) 130 K-glucanate, 10 HEPES, 10 EGTA, 1 MgCl₂, 1 CaCl₂, and 2 ATP; pH was adjusted to 7.2 with KOH. Electrodes had tip resistances of 6–8 MΩ. All experiments were performed at room temperature (20–22°C).

Coverslips with cultured ganglion explants were placed in a petri dish mounted on the stage of an inverted microscope, equipped with Nomarski optics and epifluorescent illumination. Neurons for whole cell recording were selected among cells migrating from the central explant core and electrodes were positioned with a three-coordinate, hydraulic manipulator under visual control. Whole cell recordings in current- and voltage-clamp modes were made using an Axoclamp-2A amplifier (Axon Instruments). The measured liquid junction potential (10 mV) between pipette filling and bath solutions was subtracted from the recorded membrane voltages (Neher 1992). Criteria for successful recording were a minimum 10-min recording time, with a stable resting membrane potential more negative than −40 mV, an action potential amplitude of 70 mV or greater, and 100 MΩ or higher input resistance.

To examine morphology of neuronal processes, more than 30 trigeminal and 30 geniculate neurons were filled with Lambda yellow CH (dipotassium salt, Sigma), added to the pipette solution. Recording were made to confirm neuron function, and cells were photographed to analyze the neurite branch pattern.

Data analysis

Data were acquired and analyzed using pCLAMP software (Axon Instruments). Input resistance was estimated from current-clamp recordings of the voltage response to 400-ms-long hyperpolarizing, 25-pA current steps. Membrane time constant was measured by fitting a single exponential function to the charging transient of the same recording. Membrane capacitance was calculated by dividing the time constant by input resistance. To evaluate action potential properties, a short (3 ms) 300-pA current step protocol was used for excitation. Action potential variables analyzed were: amplitude, from resting membrane to peak potential; half-duration, spike duration at half-amplitude; decay time, duration of the falling phase between 90 and 10% of spike amplitude; maximal right and left slopes, slope at the steepest point of spike rising or falling phases. Threshold of excitation was determined from recordings after application of an increasing series of depolarizing, 400-ms current steps at 25 pA. Afterhyperpolarization (AHP) amplitude was measured from the level of resting membrane potential to the lowest point, and AHP duration was defined as recovery time from the lowest point to the level of resting membrane potential. Average soma diameter was calculated from the mean of the longest and shortest axes of the cell measured with an eyepiece micrometer. No changes in the cell size were observed during recording.

Data are presented as means ± SD for passive membrane and action potential properties. Differences across data for days in culture for either trigeminal or geniculate ganglia were tested using ANOVA with Scheffe’s post hoc tests. Differences in electrophysiological properties between trigeminal and geniculate ganglia were evaluated using the Student’s t-test. Reported differences were significant at P ≤ 0.05, but actual P values are included in the text.

In addition to the parametric statistical tests reported here, we tested for differences in neuron properties across days in culture for the trigeminal or geniculate ganglion with the Kruskal-Wallis nonparametric test and for differences between ganglia with the Mann-Whitney U test. Results for statistical significance did not differ with use of parametric or nonparametric measures.

Scanning electron microscopy

A sample of ganglion explants (n = 4 trigeminal, 3 geniculate) was prepared for examining high-resolution culture topography with scanning electron microscopy. Cultures were rinsed briefly in phosphate buffered saline and fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.025 M cacodylate buffer (pH 7.3) for 6 h at 4°C. Cultures were then rinsed in buffer and postfixed for 1 h each in a sequence of 1% OsO₄, 1% tannic acid, and 1% OsO₄. Dehydration was through an ascending series of alcohols, and alcohol displacement was accomplished with three changes of hexamethyldisilazane (HMDS). Residual HMDS was evaporated in a fume hood overnight before mounting cultures on specimen stubs for light sputter coating with gold/palladium.

RESULTS

Cellular characteristics of ganglion explants

Cultured trigeminal and geniculate ganglion explants were characterized by a core of cell soma from which a dense mat of neurites extended at 30 h in culture (Fig. 1A). From the central mass of cells, individual neurons began to migrate within 30 h of culture (Fig. 1B), and migration continued over days in culture although a central mass of neurons was retained. Ganglion neurons were clearly identified by a round to oval shape and neurite extensions (Fig. 1C). The explant perimeter had a fine network of processes (Fig. 1D), extending more than 400 μm from ganglion soma. Cultured ganglion neurons retained characteristics of actively growing cells, with neurite processes that formed varied growth-cone morphologies (Fig. 1E).

Soma diameter, measured for all recorded neurons, was 29 ± 4 (SD) μm (range = 18–40 μm) for trigeminal (n = 254) and 27 ± 5 μm (range = 16–44 μm) for geniculate (n = 150) ganglion neurons. A small, gradual but statistically significant (P < 0.001) increase in average soma diameter across days in culture was observed for neurons in both types of ganglia (Fig. 1, bottom right). Average trigeminal soma diameter increased from 25 ± 3.0 μm at day 3 to 32 ± 2.8 μm at day 10; and, geniculate soma from 22 ± 4.3 μm at day 4 to 29 ± 4.6 μm at day 10. Our measures of ganglion cell size overlap in range with data on adult neurons. Trigeminal soma diameters in adult rat range from 20 to 51 μm (Janigro et al. 1997; Kim and Chung 1999; Liu and Simon 1996). Acutely isolated, adult rat geniculate ganglion neurons have a reported average soma diameter of 26–30 μm (King and Bradley 2000; Koga and Bradley 2000). Cell survival in trigeminal and geniculate explants was not obviously altered with time in culture, and indeed counts of geniculate neurons in explants demonstrate that there is no decrease in total number of neurons from 1 to 12 days in culture with BDNF (Altadill et al. 2001a).

Trigeminal and geniculate neurons filled with Lucifer yellow at time of recording exhibited a densely filled soma and fine processes (Fig. 2). Branch characteristics of neurites ranged from simple, bipolar and pseudounipolar extensions to more
complex, multipolar configurations. Neurite patterns for geniculate ganglion neurons were generally pseudounipolar (Fig. 2, gg) and more homogeneous than in trigeminal neurons which exhibited multipolar and bipolar branches (Fig. 2, tg).

**Neurophysiological properties of ganglion cells in explants cultured for 3–10 days**

Whole cell recordings were made from 254 trigeminal and 150 geniculate ganglion neurons to characterize their passive membrane and action potential properties. To determine whether neurophysiological properties changed during several days in vitro, electrophysiological characteristics were measured from ganglion neurons in culture for 3–10 days for trigeminal (neuron numbers at each day = 16, 19, 24, 43, 55, 51, 26 and 20) and 4–10 days for geniculate (neuron numbers at each day = 18, 20, 19, 28, 22, 20 and 23). Results are organized to present data for trigeminal neurons across days in culture first, followed by data for geniculate neurons.
TRIGEMINAL GANGLION. Passive membrane properties. Resting membrane potential (from $-60 \pm 5$ to $-60 \pm 3$ mV over 3–10 days) and membrane time constant (from $31 \pm 14$ to $29 \pm 15$ ms) did not alter during 3–10 days in culture for trigeminal neurons (Fig. 3). However, a decrease in input resistance, from $552 \pm 227$ to $313 \pm 154$ MΩ ($P = 0.002$) and increase in capacitance, from $57 \pm 13$ to $96 \pm 30$ pF ($P < 0.001$) were observed (Fig. 3), perhaps reflecting the gradual increase in average soma diameter (Fig. 1, graph). Indeed, there was an inverse correlation between soma size and input resistance ($Pearson’s r = -0.93, P = 0.001$) and a positive correlation between soma size and capacitance ($Pearson’s r = 0.88, P = 0.004$).

Action potential properties. When depolarized, trigeminal neurons produced a single action potential at threshold that had a stable amplitude (106 ± 6 to 108 ± 6 mV) over days in culture (Fig. 4). Whereas the threshold of excitation (mV) did not alter over days (from $-36 \pm 5$ to $-36 \pm 3$ mV), the current required to reach threshold increased (from 94 ± 29 to 143 ± 62 pA; $P = 0.009$; Fig. 4).

There was a significant decrease in action potential duration at half-amplitude ($2.7 \pm 0.3$ to $2.4 \pm 0.6$ ms) and an increase in maximal left slope ($140 \pm 35$ to $168 \pm 46$ V/s) and decrease in right slope ($-42 \pm 7$ to $-50 \pm 11$ V/s; $P < 0.001$) with days in culture (Fig. 5). Post hoc analyses between days indicated that these differences relate mainly to the contribution of data at 10 days (Table 1). Action potential decay time decreased across days in culture ($3.2 \pm 0.5$ to $2.7 \pm 0.7$ ms; Fig. 5; $P = 0.029$); however, the difference was small and post hoc analysis did not reveal differences between specific days (Table 1).

Action potentials of trigeminal neurons were followed by a long AHP, apparent in Fig. 4. Whereas the AHP amplitude did not change during days in culture (from $-10 \pm 2$ to $-11 \pm 2$ mV, days 3–10), there was a large increase ($P < 0.001$) in AHP duration ($82 \pm 49$ to $245 \pm 113$ ms; Fig. 5).

GENICULATE GANGLION. Passive membrane properties. The resting membrane potential of geniculate neurons became slightly more negative (from $-55 \pm 3$ to $-58 \pm 4$ mV, days 4–10; $P = 0.007$) during days in culture (Fig. 3). However, with post hoc analysis, no differences between specific days were found. As in trigeminal cells, the average membrane time constant was stable (32 ± 14 to 31 ± 14 ms; Fig. 3). A decrease in input resistance (691 ± 297 to 487 ± 186 MΩ; $P = 0.001$) and increase in membrane capacitance (48 ± 20 to 64 ± 14 pF; $P < 0.001$) were similar to data trends in trigeminal neurons (Fig. 3) and probably reflect the increase in cell soma size. Soma size correlated inversely with input resistance ($Pearson’s r = -0.96, P = 0.001$) and positively with capacitance ($Pearson’s r = 0.96, P = 0.001$).

Action potential properties. In contrast to trigeminal neurons that generated single action potentials, 35% of geniculate neurons had multiple action potentials when depolarized at threshold level (Fig. 4). This percentage did not alter with days in culture nor did action potential amplitude alter (from 102 ± 7 to 102 ± 12 mV, days 4–10; Fig. 4). An observed decrease ($P = 0.014$) in threshold of excitation ($-37 \pm 5$ to $-40 \pm 4$ mV) was small (Fig. 4), and post hoc analyses did not demonstrate differences between specific times in culture (Table 1). There was also a difference ($P = 0.011$) in current required to reach the threshold of excitation across days in culture (36 ± 18 to 42 ± 28 pA; Fig. 4).

There was a decrease in the action potential duration (2.2 ± 0.3 to 2.0 ± 0.3 ms at day 9; $P = 0.013$; Fig. 5), similar to that in trigeminal neurons. Maximal left slope increased (150 ± 29 to 167 ± 55 V/s; $P < 0.001$) and right slope decreased ($-49 \pm 9$ to $-55 \pm 10$ V/s at day 9; $P = 0.024$) during days in culture (Fig. 5). Action potential decay time did not change (3.2 ± 0.6 to 3.1 ± 0.7 ms; Fig. 5).

Similarly to trigeminal neurons, action potentials of geniculate neurons were followed by a long AHP, seen in Fig. 4. AHP amplitude decreased during time in culture ($-11 \pm 2$ to $-9 \pm 3$ mV; $P = 0.004$); however, duration did not alter (74 ± 33 to 98 ± 67 ms, days 4–10) in contrast to trigeminal neurons (Fig. 5).

To summarize, gradual changes in input resistance and membrane capacitance, apparently associated with an increased cell size, were observed during time in culture for trigeminal and geniculate neurons. Although several action potential properties also altered significantly across 10 days in culture, the magnitude of these differences was small and largely attributed to extending cultures to 9 and 10 days (Table
Overall, the differences across days in culture suggested a tendency for both trigeminal and geniculate ganglion neurons to become somewhat less excitable and to generate action potentials that were shorter in duration, with steeper rising and falling slopes.

**Comparisons between trigeminal and geniculate ganglion cells**

To study differences in neurophysiological properties between embryonic trigeminal and geniculate neurons, data for each ganglion were pooled across culture days 5–8, when neurophysiological characteristics were relatively stable. Comparisons of passive membrane and action potential properties between ganglia are presented in Table 2.

Geniculate neurons were smaller in diameter (27 μm geniculate; 29 μm trigeminal) and had a slightly more positive resting membrane potential (−57 mV geniculate; −59 mV trigeminal) than trigeminal ganglion cells. Whereas the time constant did not differ between the two ganglia (30 ms geniculate; 27 ms trigeminal), the higher input resistance (525 MΩ geniculate; 392 MΩ trigeminal) and lower capacitance (59 pF geniculate; 69 pF trigeminal) of geniculate cells correspond with their smaller soma size. Geniculate neurons also demonstrated an action potential of smaller amplitude (99 mV geniculate; 105 mV trigeminal) and shorter duration (2.2 ms geniculate; 3.1 ms trigeminal) than trigeminal. Average maximal left (145 V/s geniculate; 127 V/s trigeminal) and right slopes (−47 V/s geniculate; −40 V/s trigeminal) of the action potential were steeper in geniculate neurons. Furthermore, geniculate neurons had a lower threshold of excitation (−39 mV geniculate; −36 mV trigeminal) and needed less current (49 pA geniculate; 128 pA trigeminal) to reach threshold of excitation compared with trigeminal. Whereas 35% of geniculate cells were multiple spiking, all trigeminal cells generated just a single action potential at threshold level. In general, comparisons of action potential properties suggest that geniculate neurons are more excitable than trigeminal at this embryonic stage.

AHP characteristics were not investigated in detail, but both
ganglion types had long AHPs. However, geniculate neurons had an AHP that was smaller in amplitude (−9 mV geniculate; −11 mV trigeminal) and shorter in duration (88 ms geniculate; 135 ms trigeminal) than trigeminal cells. This would suggest a more rapid recovery for subsequent depolarization in keeping with the multiple spiking characteristics of many geniculate cells.

In summary, virtually all membrane and action potential characteristics differed substantially between embryonic trigeminal and geniculate ganglion neurons. To learn whether subgroups of neurons were present within either ganglion type based on electrophysiology, frequency histograms were generated for each neurophysiological property from either trigeminal or geniculate recordings. No distributions were seen to suggest obvious subpopulations of neurons in E16 ganglia (data not shown).

**Ionic currents in trigeminal and geniculate neurons**

Observed differences in passive membrane and action potential properties between embryonic trigeminal and geniculate ganglion neurons suggest that different ionic currents are expressed in these two ganglia. We used several ion channel blockers to determine and compare sodium and calcium currents in trigeminal and geniculate ganglion cells. Potassium currents were not studied in detail. However, all trigeminal and geniculate cells had a time-dependent “depolarizing sag” during hyperpolarization, reflecting the activation of an inward rectifying current (Figs. 4 and 6). This current, presumably caused by mixed cation conductance, was reversibly abolished after the application of 1–3 mM Cs⁺ in eight experiments each with trigeminal and geniculate neurons (Fig. 6).

SODIUM CURRENTS. To compare currents involved in generation of action potentials in cultured embryonic trigeminal and
geniculate neurons, we examined effects of the specific sodium channel blocker, TTX, in recordings from 14 geniculate and 18 trigeminal neurons. Concentrations of 0.3–3 μM TTX for geniculate and 3–10 μM for trigeminal cells were used. In all geniculate neurons application of TTX abolished the generation of action potentials (Fig. 7). This effect was completely reversible. In contrast, all trigeminal neurons demonstrated TTX-resistant action potentials, even after application of 10 μM TTX; however, the threshold of excitation was increased in the presence of TTX (Fig. 7). This effect was completely reversible as well. The abolished spiking ability in geniculate cells and increased threshold of excitation in trigeminal neurons after application of TTX suggest that TTX-sensitive sodium currents participate to different extents in the generation of action potentials for these two types of ganglion cells.

Although we cannot exclude the possible participation of other currents, a TTX-sensitive sodium current is clearly essential for spiking in geniculate cells. However, it is obvious that a TTX-sensitive current is just one of the participating components in eliciting action potentials for trigeminal neurons. To understand whether TTX-resistant sodium currents participate in generation of the action potential in trigeminal neurons, sodium in the bath solution was replaced with choline. In recordings from eight neurons, the ability to spike was reversibly abolished in a sodium-free bathing solution (Fig. 7). Therefore we assume that for trigeminal neurons, TTX-resistant as well as TTX-sensitive sodium currents participate in generation of the action potential.

TTX had no effect during hyperpolarization in either trigeminal or geniculate neurons; that is, the input resistance, time constant, and sag from the inward rectifying current remained unchanged. However, there was a considerable increase in input resistance in the sodium-free solution.

CALCIUM CURRENTS. All E16 trigeminal and most geniculate (95%) neurons have an inflection (or “hump”) in the falling phase of the action potential. However, these inflections have a different shape in the two types of neurons. Maximal slopes of “upper” and “lower” levels of the action potential falling
phase usually are comparable in trigeminal neurons (Fig. 8). In contrast, for geniculate neurons the slope closer to the peak of the action potential usually is considerably steeper than at the lower level (Fig. 8). The first-order derivative of these slopes clearly illustrates this difference (Fig. 8, bottom). Because currents through voltage-gated calcium channels contribute to formation of this hump (Gallego 1983; Schild et al. 1994), we investigated the effect of voltage-gated calcium channel blockers on action potential properties of trigeminal and geniculate neurons.

The inflection was reduced or eliminated for both types of neurons by adding 5 mM Co²⁺ and/or reducing to 0.2 mM the Ca²⁺ concentration in the bathing solution (data not shown) or by applying 10 μM of the L-type calcium channel blocker, verapamil (Fig. 9). After application of verapamil, the action potential amplitude was reduced in both trigeminal (n = 10) and geniculate (n = 4) cells. However, another L-type calcium channel blocker, nifedipine, had a different effect on the two types of neurons. Application of 20 μM nifedipine had a minimal effect on shape of the action potential in all four trigeminal cells that were investigated (Fig. 9). On the other hand, in four geniculate cells, after application of 15 μM nifedipine, the action potential inflection and amplitude were reduced as after application of verapamil (Fig. 9). Verapamil may be acting as a nonspecific blocker of ionic currents in these neurons, whereas the different effect of nifedipine indicates different types of calcium currents contributing to the hump formation in trigeminal and geniculate neurons. In summary, results suggest that currents through L-type channels contribute to the action potential inflection in trigeminal and geniculate neurons, but possible differences between ganglia in magnitudes of the contribution of these channels remain to be investigated.

We also examined the effect of specific N-type calcium channel blockers, ω-conotoxin-MVIIC and ω-conotoxin-GVIA, on the action potential inflection in trigeminal and geniculate neurons. For all neurons (13 trigeminal; 7 geniculate) application of 0.5–2 μM ω-conotoxin-MVIIC substantially reduced the inflection in the falling phase of the action potential (Fig. 10). The effect of ω-conotoxin-MVIIC was partially reversible. Application of 1 μM N-type blocker ω-conotoxin-GVIA also had the same (but nonreversible) effect on 13 trigeminal and 5 geniculate neurons (Fig. 10). These results suggest that currents through conotoxin-sensitive N-type calcium channels contribute to the action potential inflection in both trigeminal and geniculate neurons.

To evaluate the contribution of P/Q-type calcium channels, we studied nine trigeminal and three geniculate neurons by blocking N-type calcium channels using 1 μM ω-conotoxin-GVIA and subsequently applying 200 nM of the P/Q-type calcium channel blocker ω-agatoxin-TK. The agatoxin altered the falling phase of the action potential in both types of neurons (Fig. 10). However, in trigeminal neurons, the agatoxin had a smaller effect than conotoxin, whereas in geniculate neurons agatoxin had a comparable effect to conotoxin. These results indicate the presence of P/Q-type calcium channels in embryonic day 16 trigeminal and geniculate ganglion neurons.

### Table 1. Analysis of variance and post hoc Sheffe tests for trigeminal and geniculate ganglion cell properties across days in culture

<table>
<thead>
<tr>
<th>Ganglion cell property</th>
<th>Trigeminal ANOVA</th>
<th>Sheffe</th>
<th>Geniculate ANOVA</th>
<th>Sheffe</th>
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<tr>
<td>Average soma diameter, μm</td>
<td>F = 11.76 P &lt; 0.001</td>
<td>3 from 6, 7, 8, 9, 10</td>
<td>F = 8.48 P &lt; 0.001</td>
<td>4 from 6, 7, 8, 9, 10</td>
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<tr>
<td>Resting membrane potential</td>
<td>F = 0.49 P = 0.84</td>
<td>—</td>
<td>F = 3.1 P = 0.007</td>
<td>No differences</td>
</tr>
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<tr>
<td>Input resistance</td>
<td>F = 3.44 P = 0.002</td>
<td>3 from 8, 9</td>
<td>F = 3.84 P = 0.001</td>
<td>4 from 9</td>
</tr>
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<tr>
<td>Time constant</td>
<td>F = 0.47 P = 0.86</td>
<td>—</td>
<td>F = 0.53 P = 0.78</td>
<td>—</td>
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<tr>
<td>Membrane capacitance</td>
<td>F = 13.41 P &lt; 0.001</td>
<td>3, 4, 5, 6 from 8, 9, 10</td>
<td>F = 4.41 P &lt; 0.001</td>
<td>4 from 8</td>
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<tr>
<td>AP amplitude</td>
<td>F = 1.68 P = 0.114</td>
<td>—</td>
<td>F = 1.58 P = 0.157</td>
<td>—</td>
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<tr>
<td>AP maximal right slope</td>
<td>F = 6.34 P &lt; 0.001</td>
<td>6, 7 from 10</td>
<td>F = 2.79 P = 0.013</td>
<td>6 from 9</td>
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<tr>
<td>AP maximal left slope</td>
<td>F = 5.13 P &lt; 0.001</td>
<td>6, 7, 8 from 10</td>
<td>F = 2.51 P = 0.024</td>
<td>6 from 9</td>
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<tr>
<td>AP 10–90% decay time</td>
<td>F = 5.03 P &lt; 0.001</td>
<td>6, 7, 8 from 10</td>
<td>F = 5.47 P &lt; 0.001</td>
<td>6 from 9, 10</td>
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<tr>
<td>Threshold of excitation, mV</td>
<td>F = 2.29 P = 0.029</td>
<td>No differences</td>
<td>F = 0.5 P = 0.81</td>
<td>—</td>
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<tr>
<td>Threshold of excitation, pA</td>
<td>F = 0.58 P = 0.77</td>
<td>—</td>
<td>F = 2.77 P = 0.014</td>
<td>No differences</td>
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<tr>
<td>AHP amplitude</td>
<td>F = 2.75 P = 0.009</td>
<td>3 from 8, 10</td>
<td>F = 2.88 P = 0.011</td>
<td>No differences</td>
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<tr>
<td>AHP duration</td>
<td>F = 1.06 P = 0.39</td>
<td>—</td>
<td>F = 3.4 P = 0.004</td>
<td>No differences</td>
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<tr>
<td></td>
<td>F = 9.66 P &lt; 0.001</td>
<td>3, 4, 5, 6 from 9, 10</td>
<td>F = 0.98 P = 0.44</td>
<td>—</td>
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</tbody>
</table>

For Sheffe tests, culture days with significant differences in ganglion properties are noted. AP, action potential; AHP, afterhyperpolarization.
In summary, experiments with sodium and calcium channel blockers demonstrate the participation of currents through at least two types of sodium channels (TTX-sensitive and -resistant), as well as N- and P/Q-type calcium channels, in action potential formation of trigeminal neurons. They demonstrate the participation of a TTX-sensitive sodium current and currents through L-, N-, and P/Q-type calcium channels in formation of the action potential in geniculate neurons. Although there are not sufficient numbers of neurons to dissect differences in types of currents between trigeminal and geniculate ganglia, these studies indicate that diverse currents participate in the neurophysiological characteristics of E16 trigeminal and geniculate neurons.

DISCUSSION

In explant cultures of embryonic trigeminal and geniculate ganglia, neurons extend neurites with complex terminal extensions over several days, and a number of electrophysiological characteristics gradually alter. Over a period of 5–8 days, neural properties are relatively stable. Therefore this system allows neurophysiological comparisons between different embryonic ganglia in an in vitro environment that supports biological function. The data demonstrate that explants of trigeminal and geniculate ganglia have very different passive membrane and action potential properties at a stage well in advance of differentiation of their respective target sensory organs. This indicates that differences in neural characteristics emerge without electrophysiological input from differentiated receptors.

By E16, in vivo neurons from both ganglia have extended neurites to densely innervate the fungiform papillae on anterior tongue (Mbiene and Mistretta 1997). Although geniculate neurons do not yet encounter specialized target organs, because taste buds have not developed, trigeminal and geniculate neurites already occupy essentially separate tissue locations in papillae (Farbman and Mbiene 1991). Therefore these neurons have differentiated in numerous ways from ganglion emergence at E10 in rat, and time of peak neuron production in the trigeminal at E14 and geniculate at E15 (Altman and Bayer 1982), to E16 in rat. Although further functional and morphological differentiation is expected during the lengthy remaining period of embryonic and postnatal development of tongue and taste target organs (Mistretta 1991; Mistretta and Hill 1995), by E16, these two sensory ganglia already have very different neurophysiological characteristics when studied in an explant system.

To compare functional properties between explanted embryonic trigeminal and geniculate ganglia, it was essential to maintain maximal numbers of neurons. Therefore we added specific neurotrophins to the culture medium for each ganglion.
type: NGF maximally supports rodent trigeminal ganglia at later embryonic stages, after E13 in mouse (Buchman and Davies 1993; Davies 1997), and the NGF receptor TrkA mRNA or the TrkA protein is the predominant trk receptor in the mouse trigeminal from E13.5 (Wyatt and Davies 1993; Huang et al. 1999). BDNF is optimal for survival of the geniculate ganglion at E16 in rat (Al Hadlaq et al. 2001a), and most or all cells express the BDNF receptor, TrkB mRNA, in rat geniculate at E16–18 (Ernfors et al. 1992). If NGF was used to support both neuron types at E16, only about 11% of the “original” population of geniculate ganglion neurons would survive for recording. If BDNF was used for both ganglia, only about 10–25% of trigeminal neurons would survive over days in explant (based on extrapolation from mouse data at E14, Paul and Davies 1995; rat data at E14, Ibanez et al. 1993). These maximally supportive neurotrophins also are those localized at high concentration in the innervating fields of the respective ganglia (Nosrat and Olson 1995; Nosrat et al. 2001). On the other hand, mRNA for NGF is not within mouse trigeminal ganglion at E15.5 (Schecterson and Bothwell 1992), whereas data conflict for BDNF mRNA in the geniculate ganglion on reported presence (E15.5 mouse) (Schecterson and Bothwell 1992) or absence of expression (E16–18 rat) (Ernfors et al. 1992). By extrapolation from mouse data, in the rat trigeminal ganglion, E16 would very probably be a period of programmed cell death, presumably due to competition for limited amounts of NGF (Figueiredo et al. 2001). It is likely, therefore, that naturally occurring cell death is prevented in our explant systems.

FIG. 7. Whole cell recordings from geniculate and trigeminal neurons in control bathing solution and after application of TTX and in control and sodium-free bathing solutions (trigeminal only, at bottom of figure). TTX (0.3–3 μM) eliminated the ability to generate an action potential in all 14 recorded geniculate neurons. However, in all 18 recorded trigeminal neurons even higher concentrations of TTX (3–10 μM) did not eliminate the action potential, but only increased action potential threshold. A Na⁺-free solution did eliminate action potential generation in all 8 recorded trigeminal ganglion neurons; therefore sodium-sensitive currents do participate in action potential generation in trigeminal neurons.
designed experiments in the present paper to compare embryonic trigeminal and geniculate neurons when sustained with neurotrophins that would support most neurons and that would best mimic the biological receptive field in vivo. Further experiments to compare properties for either the trigeminal or geniculate ganglion with varying neurotrophin support can be informed by data from the current study.

Use of ganglion explant cultures, and neuron morphology

To study neural properties of developing trigeminal and geniculate ganglia, we used explants of entire or half ganglia instead of dissociated cells to maintain a cellular and molecular environment in ganglion cultures that is more similar to that in vivo. The significance of using ganglion explants is clear from studies of developmental changes in neurotrophin dependence in embryonic trigeminal ganglion, demonstrating emergence of different neurotrophin responses in explant versus dissociated neurons (Enokido et al. 1999). In addition, embryonic DRG neurons maintained in explants have more uniform axon morphology than dissociated cells (Ichinose and Snider 2000). Acute isolation procedures for dissociating neurons also may modify properties of membrane proteins, including ion channels (Molitor and Manis 1999) and can eliminate receptors and channels clustered in distal neurite membranes (Du and Bradley 1996). Furthermore, in dissociated E14 spinal motor neurons grown in culture, sodium currents are only detected in association with neurite outgrowth (Allesandri-Haber et al. 1999).

Neurons in explant cultures of embryonic trigeminal and geniculate ganglia had rounded or ovoid soma and neurite morphologies that included pseudounipolar, bipolar, and multipolar extensions. This varied neuron phenotype is similar to reports of embryonic and neonatal rat dorsal root (Matsuda and Uehara 1984; Matsuda et al. 1996) and nodose ganglia (De Koninck et al. 1994) and chick dorsal root ganglia in vivo and in culture (Matsuda et al. 1996; Riederer and Barakat-Walker 1992). In sensory ganglia in vivo, an initial embryonic, bipolar neurite morphology develops to form the pseudounipolar configuration (Lieberman 1976; Matsuda and Uehara 1984), so embryonic ganglia contain both bipolar and pseudounipolar neurons. Multipolar neurons also are sometimes noted (Lieberman 1976), especially in younger rat embryos (E14–15) (Matsuda and Uehara 1984).

Culture conditions can alter morphology of embryonic neurons. For example, dissociated neonatal rat, nodose neurons all acquire a multipolar morphology when cultured without ganglion satellite cells (De Koninck et al. 1994), and whereas dissociated adult leech ganglion neurons cultured on native substrate have a pseudounipolar morphology, experimental substrates result in neurite retraction and altered patterns (De Miguel and Vargas 2000). Because we studied single neurons...
in whole (geniculate) or half (trigeminal) ganglion explants and used a matrix deposited by living cells (MacCallum et al. 1982), our explant cultures can retain ganglion and matrix properties that are lost in dissociated preparations.

We recorded from neurons that had migrated from the central core of explanted trigeminal or geniculate ganglion. Molecules in the neuronal matrix environment can alter cell cytoskeletal components that in turn affect cell motility (Song and Poo 2001). We know of no studies that directly address effects of migration on basic neurophysiological properties of sensory neurons but cannot exclude the possibility of altered properties with migration.

Passive membrane and action potential properties of trigeminal and geniculate ganglion neurons alter gradually over time in explant culture

In cultured explants of embryonic trigeminal and geniculate ganglia, passive membrane properties altered gradually over several days in culture. Whereas resting membrane potential and time constant did not change, resistance decreased and capacitance increased in association with a small progressive increase in soma size. Several action potential properties also differed with days in culture. Action potential duration decreased and rise and fall slopes increased so that action potentials were shorter and sharper. Amplitude remained constant but threshold current needed to elicit the spike increased slightly in both trigeminal and geniculate neurons.

The membrane and action potential differences over days in culture are similar to the reported increase in soma size, decreased membrane resistance, and increased capacitance and shorter, more rapidly rising action potentials observed in cultured, embryonic hippocampal (Porter et al. 1997), septal (Thinschmidt et al. 1999), and neocortical neurons (Yamada et al. 1999), and in postnatal brain slice preparations of rat nucleus accumbens (Belneau and Warren 2000). However, the differences for each neuron type, trigeminal or geniculate, are often small, always very gradual, and statistical significance was achieved primarily through contribution of data from days 9 and 10 in culture. Explant cultures fed continuously with the appropriate neurotrophin to maximize survival and neurite growth can provide an optimal environment for neuron maintenance, and our data demonstrate the utility of this system for studying and comparing ganglion properties over a period of several days in culture.

Embryonic trigeminal and geniculate ganglion neurons have different passive membrane properties

Passive membrane and action potential properties of cultured E16 trigeminal and geniculate neurons are significantly and substantially different from each other even at this relatively early embryonic age. Although the mature target fields of trigeminal and geniculate ganglion neurons are in direct proximity within the fungiform papillae, the trigeminal and geniculate neurons innervate distinct specialized receptors in the rat tongue and have different embryonic origins (Graham and Begbie 2000), so neurophysiological differences might be expected. However, we have found that differences in basic electrophysiological properties of neurons in these two sensory ganglia are established well in advance of peripheral maturation of the taste system and do not present a homogeneous neurophysiological profile at E16.

Our data indicate that trigeminal ganglion neurons in the rat embryo have larger average soma size, lower resting membrane potential, lower input resistance, and shorter time constant compared with geniculate neurons. There are some data from postnatal and adult rodent to indicate that the direction of these differences is similar to that in mature ganglia. For example, as reported in RESULTS, embryonic soma diameters overlap with ranges reported in postnatal animals (Janigro et al. 1997; Kim and Chung 1999; King and Bradley 2000; Koga and Bradley 2000; Liu and Simon 1996) and indicate smaller soma in adult geniculate than in trigeminal ganglion. The average resting membrane potential of embryonic trigeminal neurons in our experiments, −59 mV, is between those reported from studies of adult rat trigeminal, −62 mV (Janigro et al. 1997) and −52 mV (Liu at al. 2001). Similarly, the average resting
membrane potential of $-57 \text{ mV}$ from embryonic geniculate neurons in our experiments is between values reported from acutely isolated geniculate neurons from postnatal and adult rat, $-55 \text{ mV}$ (King and Bradley 2000) and $-64 \text{ mV}$ (Koga and Bradley 2000). The average input resistance in E16 geniculate neurons ($525 \text{ M\Ohm}$) also is between values reported postnatally, $336 \text{ M\Ohm}$ (King and Bradley 2000) and $574 \text{ M\Ohm}$ (Koga and Bradley 2000). Reported membrane time constant averages 25 ms in adult (Koga and Bradley 2000) compared with 30 ms in embryonic geniculate.

Different action potential and discharge properties in embryonic trigeminal and geniculate ganglion neurons

Trigeminal and geniculate ganglion neurons also have very different action potential properties at E16. However, without a direct comparison between postnatal trigeminal and geniculate ganglia, it is not clear whether these particular properties are still as substantially different after birth. Average action potential amplitude for our embryonic trigeminal neurons ($105 \text{ mV}$) is lower than averages for various neuron types in adult rat trigeminal (113–128 mV) (Liu et al. 2001) and the threshold in E16 neurons ($-36 \text{ mV}$) is lower than in adult ($-12$ to $-27 \text{ mV}$) (Liu et al. 2001). This suggests that embryonic trigeminal action potential properties will have a considerable postnatal maturation. There are no data for adult geniculate to compare to our embryonic data, but substantial maturation is expected as in trigeminal.

In E16 explant cultures, geniculate action potentials are of lower amplitude, shorter duration, and steeper rise time than trigeminal. Furthermore, whereas all trigeminal neurons have one action potential at threshold, a large proportion of geniculate neurons generates multiple spikes. Embryonic geniculate neurons, in comparison to trigeminal, also have a much lower threshold for action potential generation by depolarizing current and a substantially shorter AHP. Lower threshold and shorter AHP are properties that also characterize repetitively firing neurons in the adult DRG, compared with single-spiking neurons (Villeire and McLachlan 1996).

The contrast in embryonic trigeminal and geniculate ganglia in discharge properties is seen also in adult ganglia. Depolarization of trigeminal neurons from adult rat elicited only one or two spikes (Janigro et al. 1997) similar to the single spikes from embryonic neurons in our experiments. In contrast, large proportions of geniculate neurons from adult rat were multiple spiking (King and Bradley 2000; Koga and Bradley 2000); again this is similar to our data on embryonic geniculate neurons. Furthermore, adult trigeminal neurons also required much greater current (Janigro et al. 1997) to elicit an action potential than geniculate (King and Bradley 2000; Koga and Bradley 2000).

In summary, some of the observed, distinctive differences in action potential properties between postnatal and adult geniculate and trigeminal ganglion cells, including discharge properties and threshold of excitation, are present already at E16 in rat. Maturation of geniculate ganglion cell innervation patterns of taste buds in fungiform papillae is a prolonged process, through $\geq 40$ days postnatal in rat (Krimm and Hill 1998, 2000). Furthermore, taste responses from the chorda tympani nerve, which derives from geniculate ganglion cells, alter dramatically during the first postnatal month (Hill 2001). The extent to which these developmental changes in ganglion cell innervation of taste buds and papillae and in afferent response properties relate to electrophysiological properties of the ganglion neurons themselves is not known. However, our experiments have shown that several basic electrophysiological properties of ganglion cells are established well in advance of mature patterns of target receptor organ innervation.

Sodium currents and TTX sensitivity in embryonic ganglia

Distinct ionic currents must contribute to the different passive membrane and action potential properties of trigeminal and geniculate ganglion neurons. Differences in the sodium currents participating in action potential generation are revealed by sensitivity to TTX. Although action potentials of mature sensory neurons can exhibit differing sensitivity to TTX, in general the proportion of neurons with TTX-sensitive compared with TTX-resistant action potentials increases during maturation (Koerber and Mendell 1992; Omri and Meiri 1990; Roy and Narahashi 1992).

However, we observed important differences in sensitivity to TTX between embryonic trigeminal and geniculate neurons. High concentrations of TTX increased the threshold of excitation for E16 trigeminal neurons, but ability to spike was always sustained. In contrast, all geniculate neurons lacked ability to spike after treatment with low concentrations of TTX; that is, all neurons had TTX-sensitive action potentials. In adult rat trigeminal neurons, sodium current recordings demonstrated presence of both TTX-sensitive and -resistant channels (Kim and Chung 1999; Liu et al. 2001), similar to our findings for embryonic trigeminal ganglion where action potentials are resistant to TTX but the threshold to spike is increased.

No other data are published on sodium currents in geniculate neurons. In other cultured cranial sensory ganglia of rat (petrosal and nodose), about 90% of neurons possessed both TTX-sensitive and -resistant components of sodium current (Bossu and Feltz 1984). The complete TTX-sensitivity for action potentials in all of the E16 geniculate neurons that we studied seems distinctive, but we cannot exclude a possible developmental addition of TTX-resistant currents in later embryos or postnatally.

In adult DRG neurons, large-diameter cells have only TTX-sensitive currents, whereas TTX-resistant currents are found only in small diameter neurons (Caffrey et al. 1992; Elliot and Elliott 1993; McLean et al. 1988; Strassman and Raymond 1999). However, some small cells can also contain TTX-sensitive currents (Villiere and McLachlan 1996). Recently a direct role for the specifically characterized TTX-resistant sodium channel, Na$_{\text{1.8}}$, has been demonstrated in action potential generation in small, C-type, mouse DRG neurons (Renganathan et al. 2001). Furthermore, the Na$_{\text{1.8}}$ channel is expressed as early as E15 in rat, reaches adult levels postnatally, and is distributed mainly in DRG neurons that have unmyelinated C fibers (Benn et al. 2001). Because nociceptive neurons comprise a major population of small sensory neurons, the distinctive TTX-resistant sodium currents that are particularly characteristic of small diameter sensory neurons are thought to be important in nociception. Furthermore, polymodal nociceptive, C type neurons in adult mouse cornea have broad, TTX-resistant action potentials with a hump on the repolarization phase (De Armentia et al. 2000). These are
characteristics of E16 trigeminal neurons. One of the principal sensory functions of the trigeminal ganglion is transmission of nociceptive information, so the potential functional role of TTX-resistant sodium currents in trigeminal should be further explored.

**Calcium currents in embryonic trigeminal and geniculate neurons**

Calcium plays an important role in development of neurons. For example, neurite outgrowth, growth cone motility and synaptogenesis all depend on changes in calcium conductances, and voltage-gated calcium channels provide the major pathway for calcium ions into the cell (Mattson and Kater 1987; Turrigiano et al. 1995). Our experiments demonstrate that embryonic trigeminal and geniculate cells have multiple types of calcium channels and suggest further studies to determine whether Ca channels participate in various functional differences between E16 trigeminal and geniculate ganglia.

A general observation for immature sensory neurons is production of broad somatic spikes with inflections or “shoulders” in the falling phase of the action potential (Fulton 1987; Koeber et al. 1988). According to many investigators (Gallego 1983; Schild et al. 1994), currents through voltage-gated calcium channels contribute to this inflection. In both trigeminal and geniculate neurons at E16, there were obvious inflections on the repolarizing phase of the action potential; therefore we used calcium channel blockers to examine effects on the shape of the inflection and to begin an initial identification of the types of calcium channels in these embryonic neurons.

In geniculate neurons, currents through L-, N-, and P/Q-type calcium channels all participate substantially in the action potential inflection, with the greatest contribution through N-type channels. Various types of calcium currents also were observed in E16 trigeminal neurons, and as in geniculate neurons, the high-threshold, conotoxin-sensitive, N-type calcium current had a dominant effect on the inflection in the action potential falling phase. Voltage-clamp studies of calcium currents in acutely isolated, adult rat trigeminal ganglion neurons also showed that about 65% of the whole cell high-voltage-activated calcium current was suppressed by blocking the N-type calcium current (Kim and Chung 1999). Blocking the L-type current had a minimal effect on the shape of action potential in embryonic trigeminal neurons in contrast to geniculate.

In DRG neurons, also, N-type calcium channels are major calcium current carriers at embryonic and postnatal stages (Diochot et al. 1995). However, developmental changes in channel types have been reported. Whereas E13 mouse DRG neurons have L-, N-, P-, and Q-type channels, by E18 there is a developmental switch from expression of P- and Q-type currents to the Q-type current alone (Hilaire et al. 1996). The increase in Q-type current coincides with the period of synaptogenesis. In rat retinal ganglion cells, shifts in calcium currents coincide with a postnatal period of dendritic remodeling (Schmid and Guenther 1999). Comparable developmental changes in calcium currents, which are so pronounced in various aspects of neuron function and interactions, may occur in trigeminal and geniculate neurons as well.

In summary, our data suggest that the L-type calcium current has a greater effect on the action potential inflection in E16 geniculate compared with trigeminal ganglion neurons; this would indicate that different types or properties of calcium current are expressed between neuronal types already at this embryonic period of development. In addition, several types of calcium current participate in calcium entry during action potentials in cultured E16 neurons in both trigeminal and geniculate ganglia.

Calcium current types in DRG vary with soma size and age of animal (Kostyuk et al. 1993; Scroggs and Fox 1992). Our initial studies in embryonic trigeminal and geniculate neurons are not at this level of detail but potential differences in both younger and more mature ganglion neurons can be tested. Because calcium entry into the neuron alters action potential shape, it has electrophysiological effects. For example, calcium entry through L-type channels can activate Ca$^{2+}$-dependent potassium channels that contribute to repolarization (Davies et al. 1999). With entry through N-type channels, a small conductance is activated. Calcium entry through other channels can prolong the AHP. Therefore differences in calcium channel types will directly alter action potential discharge and presumably relate to functional differences between different ganglion neurons. Study of calcium currents at various developmental stages should clarify the role of calcium in contributing to neurophysiological differences in trigeminal and geniculate neurons.

**Conclusions**

This study is the first to directly compare neurophysiological properties in embryonic trigeminal and geniculate neurons, which provide innervation to contiguous receptor territories in the fungiform papillae on the mammalian tongue. Information from developing lingual temperature, touch, and nociceptive receptors and lingual taste organs, will be conveyed to the brain by discharge patterns of neurons in the trigeminal and geniculate ganglia. Therefore knowledge of the intrinsic electrophysiological characteristics of trigeminal and geniculate neurons is essential for a complete understanding of the formation of lingual somatosensory and taste circuits.

We have demonstrated that in ganglion explant cultures over several days, supported with optimal neurotrophin for either the trigeminal or geniculate, neurons extend neurites with complex growth cones and have gradually altering neurophysiological properties as in other in vitro systems of central neurons. When E16 trigeminal and geniculate neural properties are compared, there are differences in passive membrane, action potential, and discharge characteristics and types or distributions of ion channels. Why are trigeminal and geniculate ganglia so different in neural properties? Clearly these ganglia will have distinctive functional roles in the mature nervous system: primarily nociceptive and somatosensory for the trigeminal and primarily gustatory for the geniculate. However, at E16, mature peripheral target receptors are not yet formed to provide differentiated neural discharge patterns through the innervating ganglion neurites. Although neurites from the trigeminal ganglion do indeed innervate fungiform papillae and surrounding tongue epithelium at E16, published data about functional nerve endings or receptors are not available. Because considerable morphological development occurs after mid-embryonic stages, target tissue/nerve interactions still have a lengthy maturation period. Geniculate ganglion neurites also innervate the fungiform papillae at E16, in a relatively circumscribed apical region, but no taste buds dif-
differentiate until the end of gestation. Therefore at E16, trigeminal and geniculate neurites reach peripheral target organs but do not have mature functional interactions with receptor elements. Thus the major differences that we observed in passive membrane, action potential, and ion channel properties between trigeminal and geniculate neurons emerge without functional input or neural activity deriving from differentiated receptors.

Whereas peripheral target organs are not yet mature at E16, the trigeminal and geniculate neurites do encounter very different neurotrophin territories in the developing papilla and tongue (Nosrat et al. 2001). Indeed, localized sources of neurotrophins at high concentrations might attract growing neurites that come into the tissue environment (Tucker et al. 2001).

Geniculate neurites grow to a region with intense expression of BDNF mRNA in the developing fungiform papilla apex, and trigeminal neurites to a region in papilla walls with more intense neurotrophin-3 mRNA expression (Nosrat et al. 1996). Furthermore, neurotrophin dependence for cell survival and neurite extension is well differentiated by E16 (Al Hadlaq et al. 2001a; Mistretta 1998). Different neurotrophins in target fields may have a role in regulating emergence of distinctive neural properties in E16 trigeminal and geniculate ganglia. At embryonic ages, the molecular environment in the target region of these ganglia may have more influence on the emerging electrophysiological properties of the neurons than any neural activity that derives from target fields. Postnatally, ganglion properties might be more responsive to neural discharge patterns from developing receptive fields.

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Present address of A. Grigalunas: Institute for Biomedical Research, Kaunas Medical University, Kaunas, Lithuania.

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