Title:
Relationship between physiological response type (RA and SA) and vibrissal receptive field of
neurons within the rat trigeminal ganglion.

Authors:
Steven C. Leiser ¹ and Karen A. Moxon ¹,²

¹Dept. Neurobiology & Anatomy, Drexel University College of Medicine,
Philadelphia, PA 19129
²School of Biomedical Engineering, Science & Health Systems, Drexel University,
Philadelphia, PA 19104

Running Head:
Leiser & Moxon △Acute Mapping Study of the Vg

Corresponding Author:
Karen A. Moxon, Ph.D.
Drexel University
School of Biomedical Engineering
3141 Chestnut St
Philadelphia, PA 19104
(215) 895-1959 phone
(215) 895-0570 fax
km57@drexel.edu
[Abstract]:

Cells within the trigeminal ganglion (Vg) encode all the information necessary for the rat to differentiate tactile stimuli, yet it is the least studied component in the rodent trigeminal somatosensory system. For example, extensive anatomical and electrophysiological investigations have shown clear somatotopic organization in the higher levels of this system, including VPM thalamus and SI cortex, yet whether this conserved schemata exists in the Vg is unknown. Moreover although there is recent interest in recording from vibrissae-responsive cells in the Vg it is surprising to note that the locations of these cells have not even been clearly demarcated. To address this we recorded extracellularly from 350 sensory-responsive Vg neurons in 35 Long-Evans rats. First, we determined three-dimensional locations of these cells and found a finer detail of somatotopy than previously reported. Cells innervating dorsal facial features, even within the whisker region, were more dorsal than midline and ventral features. We also show more cells with caudal than rostral whisker receptive fields (RF), similar to that found in VPM and SI. Next, for each vibrissal cell we determined its response type classified as either rapidly (RA) or slowly (SA) adapting. We examined the relationship between vibrissal RF and response type and demonstrate similar proportions of RA and SA cells responding to any whisker. These results suggest that if RA and SA cells encode distinct features of stimuli, as previously suggested, then at the basic physiological level each whisker has similar abilities to encode for such features.
INTRODUCTION

The somatotopic organization of the whisker somatosensory system in the rat brain is well known and has, in part, made it easy to study somatosensory processing in this system. The somatotopic organization that exists in structures from the brainstem trigeminal nuclei to the primary somatosensory cortex mirrors how the whiskers are arranged on the face. On the rat’s face, whiskers are arranged in rows, labeled A (most dorsal) to E (most ventral), and columns, labeled with numbers, the most caudal whiskers labeled Column 1 and increasing in number rostrally. At each level in the brain, cells with the same principal receptive field (whisker) are physically next to each other forming clusters of like-responding cells. Just as the whiskers on the face are arranged in rows and columns, these clusters of cells with the same RF are arranged in rows and columns with relative positions similar to the position of their receptive fields on the face. These clusters of vibrissae-responsive cells are known as barrelettes in the brainstem, barreloids in the thalamus and barrels in the cortex. Moreover, within this somatotopy it has been shown that there is a ‘receptive field gradient’ such that the larger, more caudal whiskers are represented by more cells than the rostral whiskers at each level in the brain including brainstem trigeminal nuclei (Ma and Woolsey, 1984; Ma, 1991), VPM thalamus (Van der Loos, 1976), and layer IV of the somatosensory cortex (Woolsey and Van der Loos, 1970; Chapin and Lin, 1984; Killackey et al., 1995).

However, much less is known about the somatotopic organization of the cells whose axons innervate the receptors on the face, including the whiskers, and whose cell bodies lie outside the brain in the trigeminal ganglion (Vg). For example, the extent or area of the vibrissae responsive cells within the Vg is unknown. While it is likely that the somatotopic organization of the whisker somatosensory system of the brain originates with organization in the Vg, this is
not known. Moreover, knowledge of the somatotopic organization of the Vg will help to better target appropriate groups of cells during physiological studies examining the response properties of these cells. While little information is available about the somatotopy of cells within the ganglion, there is information about the relative position of the three branches of the trigeminal nerve that carry the axons of Vg cells from the nerve endings in the face to the brainstem. The mandibular branch (V3), which innervates the temporomandibular joint, mandibular skin, and lower lip, is situated posterior and lateral to both the ophthalmic branch (V1), which innervates supraorbital vibrissae, eye, and nose, and the maxillary branch (V2), which innervates the cheek, upper lip, and Mystacial vibrissae (Waite and Tracey, 1995, see Figure 1A). Therefore, several important questions arise. Is there somatotopy at the level of the trigeminal ganglion? If so, is the somatotopic organization of cells within the Vg similar to the relative position of the branches of the trigeminal nerve? Or is there an additional level of somatotopy that mimics the arrangement of peripheral receptors on the face similar to that found centrally in the trigeminal system?

It has been suggested previously that a crude somatotopy exists in the ganglion (Beaudreau and Jerge, 1968; Zucker and Welker, 1969; Gregg and Dixon, 1973; Arvidsson, 1982; Dorfl, 1985; Klein et al., 1988; Renehan et al., 1989; Lichtenstein et al., 1990; Rhoades et al., 1990; Erzurumlu and Jhaveri, 1992; Killackey et al., 1995; Waite and Tracey, 1995; Scott and Atkinson, 1999; Pali et al., 2000; Maklad et al., 2004). However these reports are not without discrepancy. For example, cells whose receptive fields are in the eye region of the face were reported to be positioned in the ganglion either dorsomedially (Jacquin et al., 1986) or anteromedially (Waite and Tracey, 1995) or just medially (Renehan et al., 1989). Likewise, cells whose receptive fields are in the lower lip and jaw region of the face have been placed posterolaterally (Waite and Tracey, 1995) and dorsolaterally (Jacquin et al., 1986; Renehan et al.,
1989). In addition to the somatotopic organization, Zucker and Welker (1969) suggested the possibility of a ‘receptive field gradient’ (i.e. the number of cells with receptive fields in a whisker column increases from the smaller, more rostral whiskers, to the larger more caudal whiskers) by demonstrating that there are more cells with caudal whisker receptive fields compared to cells with rostral whisker receptive fields.

It is critical to clarify these findings of somatotopic organization in the Vg. This is important for understanding the relationship between the organization in the Vg and the organization of structures in the rest of the brain. Therefore, the first goal of this study was to perform a complete mapping of the Vg to determine its somatotopic organization and the relative position of vibrissae-responsive cells compared to cells that respond to other facial features.

In addition, since Vg cells can be classified by their response type to passive stimulation as either rapidly adapting (RA) or slowly adapting (SA)-type cells, and because these different response types have been suggested to encode different types of information, it is also important to understand the distribution of these cell types relative to the somatotopic organization of vibrissae-responsive cells in the Vg. For example, it is important for understanding encoding strategies of the rat to know if the ratio of RA and SA-type cells responding to a particular whisker is the same across whiskers on the mystacial pad or if a particular whisker group (row or column) have more SA than RA type cells.

The functional differences in encoding properties of RA and SA cells have been best clarified in the primate glabrous skin (i.e. fingertips) where research supports the idea that RA cells generally code for object location by detecting low frequency skin motion while SA cells generally discriminate the object, coding for object form and texture (Johnson et al., 1995, 2000; Blake et al., 1997a,b; Dodson et al., 1998; LaMotte et al., 1998; Nishiura et al., 2000; Johnson
In support of this, LaMotte et al., (1998) demonstrated that RA cells’ responses outlined the spatial locations and velocities of the indentations and retractions of skin surrounding the object rather than provide an outline of the shape itself while SA cells provided a consistent spatial rate distribution that represented a third dimension of shape in addition to the outline of the shape in contact with the skin. These data, combined with, evidence from more than three decades of psychophysical and neurophysiological research support the idea that rapidly adapting (RA) and slowly adapting (SA) type cells serve a distinctly different sensory function (for review see, Johnson et al., 2000). The functional differences in encoding properties of RA and SA cells in the rat have not received such attention, despite the fact that studies have demonstrated that the rodent trigeminal somatosensory system is comparable to the primate tactile system. For example, it has been shown that rats utilize their whiskers during active vibrissal palpation in a comparable manner to primates using their hands during active touch, and further that the velocity of rat whisker motion across objects is within the range of velocities for optimal texture discrimination in primates (Carvell and Simons 1990). It has been shown through extracellular recordings of Vg cells in anesthetized rats that SA cells code for whisker position (amplitude) and RA cells code for whisker velocity or acceleration (Shoykhet et al., 2000). These results have prompted speculation that during rats’ awake exploratory behaviors RA and SA cells in the trigeminal ganglion, possibly acting in a comparable manner to RA and SA cells in the primate hand, encode for distinct aspects of the tactile stimuli. Perhaps, RA and SA cells code for object location and object form and texture, respectively. This remains to be tested.

Knowing the relationship between the distribution of RA and SA cells and the somatotopic organization of the Vg is important because it has also been proposed that caudal
whiskers (whiskers in the most caudal columns) encode different tactual features of the environment than rostral whiskers (Carvell and Simons, 1990; Harvey et al., 2001; Sachdev et al., 2002; Neimark et al., 2003) and their suggested roles can be related to the known functions of RA and SA cells. For example, it is possible that the rostral most whiskers gauge the location of the surface while caudal whiskers discriminate its form and texture (Carvell and Simons 1990; Sachdev et al., 2002). Given these theories about whisker and cell type function, one could hypothesize a correlation between a particular receptive field (whisker column) and a particular physiological response type (RA or SA). To fully address this hypothesis, we performed a map of the trigeminal ganglion to determine the extent of the vibrissae-responsive region, its somatotopic organization and, by sampling cells with receptive fields from each whisker, quantitatively assessed the distribution of RA and SA type cells across receptive fields.
EXPERIMENTAL PROCEDURES:

To evaluate the somatotopic organization of the sensory-responsive cells recorded within the trigeminal ganglion, stereotaxic coordinates were recorded for each cell and electrophysiological techniques were used to identify the cells’ receptive field (RF). Using the RF, cells were grouped by their association with one of the three branches of the trigeminal nerves. Between group comparisons of the stereotaxic coordinates along each of the three axes (mediolateral, anteroposterior and dorsoventral) were made and significant differences between the coordinates were evaluated (see Data Analysis, below). If cells were responsive to whisker stimulation, its single whisker RF and its cells type (either RA or SA) were determined. These vibrissae-responsive cells were then grouped into whisker row groups (rows A-E) or whisker column groups (columns 1-6). For the five row groups, between group comparisons of the stereotaxic coordinates were made along each of the three axes. For the six column groups, between group comparisons of the stereotaxic coordinates were made along the same three axes. Significant differences between the coordinates associated with each group were evaluated (see Data Analysis, below). Finally, the presence of a receptive field gradient was determined by comparing the number of cells with caudal whisker RFs to rostral whisker RFs. Finally, the relationship between cell type (RA or SA) and receptive field (whisker) was studied (details below).

Animal preparation and recording: To record the activity from single cells within the ganglion, animals were anesthetized and an electrode was lowered into the ganglion. A total of thirty-five adult, male, Long-Evans rats (230-280g), obtained from Harlan (Indianapolis, IN), were used to complete this study. All procedures were approved by the Institutional Animal Care and Use
Committee at Drexel University and followed NIH Guidelines. Each animal was anesthetized by an i.p. injection of sodium pentobarbital (45mg/Kg) and placed in a stereotaxic frame (Cartesian Research Inc., Sandy, OR). A stereotaxic alignment system was adjusted to ensure the rat’s skull was planar both dorsoventrally and mediolaterally. A large trephination used for the electrode insertions was created in the skull from 0-5 millimeters posterior and 0-4 millimeters lateral from bregma. Appropriate electrode design is critical for stable recordings (Moxon et al, 2004a,b). Because the ganglion is surrounded by dura and the electrode must exit the brain and penetrate a second dural layer surrounding the ganglion (Figure 1B), a high impedance (10MOhm) epoxylite-insulated tungsten microelectrode with shank diameter of 250µm and a sharp tip (FHC#: UEWSGGSE0N1E, FHC, Bowdoinham ME) was used to ensure minimal resistance when penetrating these dural layers. A ground wire was then inserted superficially in a brain region distant from the electrode, secured to a screw on the skull and connected to the electrode headstage. Finally, the electrode was mounted vertically in the stereotaxic electrode manipulator, then digitally aligned to bregma and moved above the craniotomy to predetermined coordinates (based on a grid, defined below). The electrode was lowered to the surface of the dura and all ventral coordinates were considered relative to this position.

To identify the somatotopic organization of the trigeminal ganglion a grid was defined to help select the position of electrode penetrations. The grid extended from 1.0 to 3.0mm lateral from Bregma (2mm mediolaterally) and 0.0 to 4.5mm posterior to Bregma (4.5mm rostrocaudally) (Figure 1E and F). Resolution within the grid was 0.5 mm in the anteroposterior and mediolateral directions and intersections on the grid defined the electrode penetration sites. Serial electrode penetrations were made in each animal at randomly selected sites on the grid. Responses from the neurons recorded by the electrode during each penetration were also used to
define the boundary of the vibrissae-responsive region of the Vg. To identify the somatotopy within the vibrissae responsive region, the resolution of the grid was reduced to 0.25 mm spacing between electrode penetrations within this vibrissae responsive region of the Vg.

Recordings from Trigeminal Ganglion. Extracellular recordings were continuously performed while the electrode was lowered through the brain to the ganglion. Signals were amplified and bandpass filtered (154Hz to 13kHz) by conventional means (Nicolelis and Chapin, 1994; Nicolelis et al., 1995; Chapin et al., 1999) and analog signals were digitized at 40 kHz (MAP System, Plexon Inc., Dallas, TX), displayed on an oscilloscope and played over an audio speaker as the electrode was advanced. The electrode was lowered at approximately 100 μm/min to 9.0mm. In previous studies (Schneider et al., 1981; Leiser and Moxon, 2003), Vg cells were not evident above 9.0mm. The electrode was then lowered more slowly (10μm/min) until the first signs of sensory-responsive cells appeared. Because ganglion neurons are unresponsive unless their receptive field was stimulated, at each interval, the presence of a cell was checked by manually stimulating the whiskers and surrounding facial features with a cotton-tipped probe while monitoring the signal on the oscilloscope and through audio speakers. Facial areas included the eye and supraorbital vibrissae, nose, upper lip, and the lower lip and jaw. Occasionally, odontoreceptive (tooth-responsive) units were encountered but not included. Each area, except the eye, received steady pressure to indent the skin regions. The eye was contacted gently but with sufficient pressure to drive responsive units, however most “eye-responsive” units responded to stimulation of the supraorbital vibrissae. Each vibrissa, including the supraorbital, mystacial, and rostral microvibrissae, received multi-directional stimulation.
When the action potential of a single discriminable neuron was elicited in response to tactile-stimulation, the electrode advancement was halted, the coordinates noted and the receptive field identified in the following way. A wooden probe was moved across the skin and hairs (including whiskers) and the response of the cell was monitored on the oscilloscope and computer screen and through audio-speaker. The location on the face that the cell responded to was determined to be its receptive field. The ventral extent of the Vg was affirmed when either the base of the skull was contacted or cells no longer responded to tactile stimulation. The electrode was always lowered until bone was contacted or to a depth of 11.5mm, even if no sensory-responsive cells were found.

**Recording from Vibrissae Responsive Region of the Vg.** When a cell responded to gross stimulation of the whiskers on the mystacial pad, the coordinates were noted, its receptive field (RF) identified and a peristimulus time histogram (PSTH) was generated for later analysis to determine if the cell was an RA or an SA type cell (see Identifying Rapidly Adapting and Slowly Adapting cells, below). To identify the cells RF and preferred direction, each whisker was deflected manually with a hand-held probe (Simons, 1983; Shoykhet et al., 2000). PSTHs provide a robust means to study how neurons code for discrete sensory stimuli even on a single-trial basis (Foffani and Moxon, 2004, Foffani et al., 2004, 2006, Tutunculer et al., 2005). To generate PSTHs, the firing activity during sustained whisker deflection was recorded. First, a template based on the spike amplitude and waveform shape (Figure 1C and D) was used to identify the neuron from background activity (Devilbiss and Waterhouse, 2002, 2004). Sustained whisker deflections were applied by moving the whisker approximately 5 degrees in its preferred direction and maintaining the whisker in its preferred direction for 500 msec using a
precision stepper motor (Gemini GV6) controlled by a servo drive (Parker Hannifin Corporation, Compumotor Division, Rohnert Park, CA). For comparison to other studies, this ramp-and-hold stimulus (rise time < 1 msec) was similar to previous studies (i.e. 5 degrees in preferred direction for 500 msec, Shoykhet et al., 2000). Seventy-five to 100 stimuli were applied at a rate of 0.5 Hz. The spike waveforms were digitized (40 KHz) and, along with the spike times using commercial software (RASPUTIN, Plexon, Inc, Dallas, TX) to generate post-stimulus time histograms (described below). Our goal was to record only from the cell body of these cells and the waveform shape (negatively going) suggest that these recording were indeed made from the cell body (Figure 1C and D). The motor was mounted onto an adapted Kopf stereotaxic frame so that it was held firmly in place but could be precisely positioned to deliver the stimulus to only one whisker. A pulse was sent to the MNAP hardware to indicate the onset of stimulation.

**Histology:** In order to ensure proper electrode placement electrolytic lesions were induced by passing currents (30µA, 20 seconds, unipolar) through the tips of the recording electrodes (see Pabst, 1973). In several preliminary experiments designed to test the accuracy of our recording technique three to four lesions were created in a single dorsoventral penetration. This was repeated for a minimum of two more dorsoventral penetrations in areas of the ganglion either more anterior or posterior or medial and lateral. This yielded approximately nine lesions across each of the planes (anteroposterior, mediolateral, and dorsoventral), which were used to confirm the stereotaxic precision and consistency of the procedure. The ganglions were then removed and fixed. Afterwards they were cut (30 microns) coronally and stained with Nissl to locate the lesions and verify the position of the electrode tips relative to each other. Lesions along the dorsoventral tract were clearly visible, always located in the region of the Vg targeted, and the
distance between the lesions were consistently spaced, matching the relative distances between the stereotaxic coordinates targeted. These data, in addition to the fact that the anatomic location of the ganglion and the highly typical responses of the primary afferent cells precluded a source of recording other than the Vg (Zucker and Welker, 1969; Shoykhet et al., 2000; Szwed et al., 2003) confirm the accuracy of electrode placement.

**Data analyses:** To determine if there was a somatotopic organization of cells within the Vg, cells were grouped by their receptive fields (RFs). Branch groups were generated by grouping cells whose axons were associated with one of the three branches of the trigeminal nerve, the ophthalmic branch (V1), maxillary branch (V2), mandibular branch (V3). Row and column groups were generated for cells with RFs in the vibrissae responsive region by grouping cells with RFs in the same whisker row or column. For example, cells with receptive fields corresponding to the most dorsal whisker row (A1, A2, A3, A4 etc) were grouped into the A-row group. Likewise, when assessing columns, cells with receptive fields corresponding to the most caudal whisker column (A1, B1, C1, D1, and E1) made up the Column 1 group. Refer to Figure 1A for a description of how the whiskers are arranged on the face.

For each group, we first determine whether or not our samples were normally distributed using a Kolmogorov-Smirnov One Sample Test (K-S test) and found that for all analyses the data were not normally distributed. The analysis was applied to the recorded coordinates for all cells (n=350) in each of the three coordinate planes, dorsoventral, mediolateral, and anteroposterior. The analysis was performed for branch groups and for whisker groups (row and columns groups) to determine if the samples were normally distributed. In each case the K-S test
was statistically significant \((p<0.05)\), hence the observed data did not follow the hypothesized (normal) distribution and non-parametric tests were chosen to assess statistical differences.

To determine if there were significant differences in the recorded coordinates between different branch groups or if there were significant differences in the recorded coordinates between the different whisker groups in the dorsoventral, mediolateral, and anteroposterior coordinates the non-parametric Kruskal-Wallis test was used. The test was applied separately for each of the three coordinate axes (dorsoventral, mediolateral, and anteroposterior). When the Kruskal-Wallis test was significant \((p<0.05)\), the Mann-Whitney U test with the Bonferroni correction was used to evaluate which group’s coordinates were significantly different from each other in each of the three coordinate planes. The Bonferroni correction is a highly conservative approach and was applied because multiple Mann-Whitney U tests were used. The idea behind the Bonferroni correction is that if one is testing \(n\) independent hypotheses, one should use a significance level of \(0.05/n\). So, for example, when comparing the locations of cells separated by whisker row (5 rows), there were 10 comparisons between groups (1 axes), thus the \(p\)-value is reduced to \(0.05/10\), or \(p=0.005\), and only comparisons with a \(p\)-value less than this was considered significantly different. In the text, \(p\)-values corresponding to the Mann-Whitney U test are reported unless otherwise stated.

To determine if there was a receptive field gradient (i.e. increasing number of cells with receptive fields in a whisker column (row) as one moves from the more rostral (dorsal) whiskers, to the more caudal (ventral) whiskers), the number of cells with the same whisker receptive fields was assessed by considering the number of cells corresponding to each individual whisker or whisker group (either row group or column group) as a sample and using the Mann-Whitney U test with Bonferroni correction to assess significant differences between groups (see above).
The percentage of cells responding to each whisker was then compared to the innervation of each whisker follicle to determine if there was a correlation. The percentage of axons for each whisker (data from Welker and Van der Loss, 1986) was correlated to the percentage of cells with the same whisker RF determined from this study using the Gamma correlation with a p<0.05 considered significant. The Gamma correlation statistic was preferred to the Spearman R correlation because the fact that we were collecting multiple data points (i.e. sets of coordinates) from a single penetration meant that our coordinates were not completely independent from each other. Therefore our data contained tied observations and the Spearman R would be inappropriate. However in terms of the underlying assumptions and interpretation Gamma is equivalent to Spearman R. All analyses were performed in Statistica (StatSoft, Inc., Tulsa, OK) unless otherwise noted.

**Classification of Rapidly adapting and slowly adapting cells.** All single vibrissae-responsive units were classified as either rapidly adapting (RA) or slowly adapting (SA). The method for classifying each cell as either an SA or RA was based on previous studies (Lichtenstein et al., 1990; Shoykhet et al., 2000, 2003) in which a neuron was classified as slowly adapting if activity during a well defined period of sustained stimulation (the plateau response, defined next) significantly exceeded spontaneous activity of the cell (Mann-Whitney U, p<0.05). All other neurons were classified as rapidly adapting. To evaluate the plateau response, for each cell, a standard peristimulus time histogram (PSTH) was produced by summing the number of spikes recorded during presentation of the 75-100 sustained whisker deflections using 100 1-ms bins before the stimulus and 600 1-ms bins after the stimulus. Time zero represented time of stimulus onset. Spontaneous activity was measured from the standard PSTH during the 100-ms period
immediately preceding stimulus onset. The plateau response was the activity of the cell measured from the standard PSTH between 200 and 300 msec post-stimulus. Significant differences between spontaneous activity and the plateau response were evaluated using the nonparametric Mann-Whitney test because the activity of the cell (during both spontaneous and plateau periods) were not normally distributed. If a significant difference was found, the cell was classified as SA, otherwise it was classified as RA. However, to be consistent with previous studies, significant differences between spontaneous activity and the plateau response were also evaluated using a Student’s t-test (Lichtenstein et al., 1990; Shoykhet et al., 2000, 2003). No difference in the classification of cells (i.e. the number of RA or SA) was found between the two tests.

Cells were then grouped based on their classification as RA or SA and their coordinates compared to determine if these cells were positioned in the ganglion in separate locations or if they were intermingled. The posterior, lateral, and ventral coordinates of the RA cells were separately compared to the corresponding coordinates of the SA cells to evaluate any significant differences using the same procedure as above (Mann-Whitney U with Bonferroni correction, \( p < 0.05 \) as significant). Finally, the relationship between receptive field (whisker row or column) and cell type (RA or SA) was examined. The Two Proportions Test (NCSS, Kaysville, Utah) was used to assess the differences between the ratio of RA to SA-type cells for each whisker group (individual rows and columns) compared to the population ratio. The probability level (group vs. population) determined by the Fisher’s Exact Test was used to test significance with a value of \( p < 0.05 \) considered significant.
RESULTS:

Overall, we found a somatotopic organization within the Vg that extended within the vibrissae responsive region. To examine the somatotopic organization of the trigeminal ganglion, 350 sensory-responsive cells were recorded from the trigeminal ganglion of 35 Long-Evans rats. The whisker region of the trigeminal ganglion was targeted and, therefore, 240 of these cells responded to stimulation of one and only one whisker on the ipsilateral mystacial pad. The remaining 110 cells responded to tactile stimulation of facial regions adjacent to the mystacial pad and included 45 that responded to the region of the lower lip, 31 to the nose, 24 to the eye and supraorbital vibrissae, and 10 to the upper lip and microvibrissae. Three-dimensional stereotaxic coordinates were noted for each cell to assess the somatotopic organization of the Vg and identify the position and areal extent of the vibrissae responsive cells within the Vg (Figure 1F and Figure 2).

Vibrissae-responsive cells (n=240) were found medially in the Vg. The extent of the vibrissae-responsive cells spanned from 1.0-2.5mm lateral (median=2.0) and 0.5-4.5mm posterior (median=1.5) from bregma, with an average location of 2.03mm ±0.44SD lateral and 1.66mm ±0.79SD posterior from bregma (n=240) (Figure 1F). The ventral locations of vibrissae-responsive cells within the Vg were between 9.10-10.95mm (median=10.0) with an average depth of 10.10mm ±0.36SD ventral from the dural surface, yet the average depth of the first and last vibrissae-responsive cell across all penetrations was 9.81 ±0.29mm and 10.27 ±0.38mm ventrally from the dural surface, respectively (Table 1). The dorsal and ventral extent of vibrissae-responsive cells varied slightly in both the anteroposterior and mediolateral axes with the greatest difference between the most ventral and the most dorsal coordinates being in the most lateral locations (Table 1 and Figure 2). These coordinates were verified histologically.
(refer to methods). To better understand the probability of encountering a particular receptive field in a given penetration, the number of times a receptive field was encountered for each penetration is provided in Figure 2. One can see a trend for the dorsal to ventral organization and the medial to lateral separation of the cells associated with the ophthalmic or maxillary branches of the trigeminal nerve, which discussed in detail next.

In summary, we show that the greatest possibility of encountering vibrissae-responsive cells in the Vg lies within an area of approximately 2 mm wide in the anteroposterior axis, 1.25 mm in the mediolateral axis, and as one passes through the ganglion, from dorsal to ventral, vibrissae-responsive cells will be encountered for less than 1.85 mm.

**Somatotopic Organization consistent with Organization of the Branches of the Trigeminal Nerve**

When cells were grouped by the branch of the trigeminal nerve that their axons are associated with, there were significant differences along all three coordinate axes (Kruskal Wallis, p<0.001 for each of the three major axes, medial-lateral, anterior-posterior, and dorsal-ventral). The first major organizational trend was that cells whose axons are within the mandibular branch of the trigeminal nerve (V3 – lower jaw) were situated more posterolaterally than the cells whose axons are within either the ophthalmic (V1 – eye region) or maxillary (V2 – nose, upper jaw, whiskers) branches. The second major organizational trend was that as one passes through the ganglion from dorsal to ventral cells innervating the more dorsal facial features (V1) are encountered first, followed by midline features (V2) and finally ventral facial features (V3).
When examining the position of cells within the Vg along the mediolateral axis and the anteroposterior axis in detail, several of these trends were significant. Cells whose axons are within the mandibular branch of the trigeminal nerve (V3 – lower jaw) were significantly more lateral and more posterior in the Vg than cells whose axons are within either the ophthalmic (V1 – eye region) or maxillary (V2 – nose, upper jaw, whiskers) branches (Figure 3A, Mann-Whitney U, p<0.001, for both). Cells responding to features innervated by the ophthalmic (V1) and maxillary (V2) branches of the trigeminal nerve were found in similar lateral and posterior coordinates (Figure 3A, Mann-Whitney U, p=0.59 and 0.73, respectively). Therefore, along the mediolateral and anteroposterior axes, the somatotopic organization of cells within the Vg was consistent with the relative position of their axons within the trigeminal nerve.

The significant differences observed in the mediolateral direction for cells associated with the different branches of the trigeminal nerve were also observed for subgroups of cells with the same receptive field. For example, cells that responded to stimulation of the lower lip and whose axons reside in the mandibular branch of the trigeminal nerve were significantly more lateral than cells responding to the vibrissae (Mann-Whitney U, p<0.001), whose axons reside in the maxillary branch. Lower lip cells were also significantly more lateral than cells responding to either the eye or nose (Mann-Whitney U, p<0.001) whose axons reside in the ophthalmic branch of the trigeminal nerve (Figure 3B). Thus there is a distinct medial-lateral separation of cells whose axons are in the mandibular branch from cells whose axons are in the ophthalmic or maxillary branches of the trigeminal nerve.

The significant differences observed in the anterior-posterior direction for cells associated with the different branches of the trigeminal nerve were also observed for subgroups of cells with the same receptive field. For example, the lower lip-responsive cells were significantly
more posterior than eye-responsive cells and more posterior than both vibrissae and upper lip-
responsive cells (Mann-Whitney U, p<0.001; Figure 3B). These differences show a division
along the anterior-posterior axis separating cells within the Vg whose axons lie in the mandibular
branch from cells whose axons lie in ophthalmic and maxillary branches.

The second major organizational trend was that cells innervating the more dorsal facial
features (i.e. the eye; V1) were encountered more dorsally, whereas the cells innervating the
ventral facial features (i.e. the lower lip; V3) were found more ventrally and cells innervating the
midline facial features (i.e. the nose, whiskers, and upper lip; V2) were found in between. Eye-
responsive cells (V1 – ophthalmic branch) were found significantly more dorsal than both lower
lip responsive cells (V3-madibular) and vibrissae responsive cells (V2 - maxillary) (Mann-
Whitney U, p<0.001; Figures 3C and 3D). As a group, although only the position of the eye-
responsive cells were significantly more dorsal than cells responding to the lower lip, a
dorsoventral alignment of cells that matches the dorsoventral location of their receptive field on
the face was found. In fact, the first cells typically encountered during an electrode penetration
through the Vg responded to the skin surrounding the eye and supraorbital vibrissae. This dorsal
to ventral somatotopy observed throughout the Vg was consistent with the dorsal to ventral
position of the trigeminal nerves innervating the ganglion and extended to the vibrissae
responsive cells associated with the maxillary branch of the trigeminal nerve, as examined
below.

To show the probability of encountering a particular receptive field for a given set of
penetration coordinates (mediolateral and anteroposterior), the findings above were converted
into a contour plot (Figure 4). Therefore, for example, if one were most interested in locating
vibrissae-responsive cells, penetrations between 1.0 to 2.0 mm posterior and 2.0 to 2.5 mm lateral would be used (Figure 4).

**Cells within the vibrissae-responsive region of the Vg demonstrated a Dorsal-to-Ventral Somatotopy**

To assess the somatotopic organization of cells within the vibrissae-responsive region of the trigeminal ganglion, these cells were grouped into their respective whisker column and row and the stereotaxic coordinates of each group were compared. The main result was that there was no significant difference in the locations of cells along any of the axes when they were grouped by columns but there were significant differences when cells were grouped by rows. When cells were grouped by columns, cells that responded to whiskers in the most rostral columns (Columns 5 and 6) were found anteromedially (Figure 5A) and ventrally (Figure 5B) compared to cells that responded to caudal whiskers (Column 1) but none of these differences were significant (Kruskal-Wallis p=0.30 mediolateral, and p=0.42 anteroposterior, and p=0.06 dorsoventral). To further test the possibility of columnar somatotopic organization, cells were further grouped into rostral and caudal whiskers but no differences were found. Finally, organization within animals was examined. Animals were most likely to demonstrate a trend of transitioning from column 1 to column 5 (caudal to rostral) as the position in the ganglion moved from more anterior to more posterior (four out of six animals, Table 2) and also exhibited a trend of transitioning from column 1 to column 5 as the position in the ganglion moved from more dorsal to more ventral (seven out of sixteen animals, Table 2), but there was no consistent trend along the mediolateral axis. Therefore, a weak somatotopic organization for the whisker columns may exist along the anteroposterior or dorsoventral axis within the ganglion.
There was a substantial somatotopic organization of cells within the ganglion when they were grouped by whisker row. Despite that there was no significant differences between the posterior or lateral locations of the cells (Kruskal-Wallis ANOVA, p=0.18 and p=0.72, respectively), (Figure 5C), even when organization within individual animals was examined (Table 2), there was a significant difference in the ventral locations of the cells (Kruskal-Wallis ANOVA, p<0.05), (Figure 5D). Cells responding to the most dorsal whiskers (rows A and B, n=86) were significantly more dorsal than cells responding to the most ventral whiskers (rows D and E, n=62), (Mann-Whitney U, p=0.007). This was confirmed by examining the organization within individual animals where 14 out of 17 animals showed this same dorsal to ventral organization (Table 2). Thus the dorsoventral somatotopy seen for the gross anatomy above (refer to Figures 3B and 3D) is maintained within the vibrissae-responsive region of the Vg.

More cells responded to larger, caudal whiskers than the smaller rostral whiskers

While each whisker row was equally represented in the sample of cells, there were significantly more cells with caudal whisker receptive fields (RFs) than cells with rostral whisker RFs. This was determined by grouping cells by their receptive field (whisker) into row groups (cells with RFs in the same row) and column groups (cells with RFs in the same column) in our sample of vibrissae-responsive cells (n=240) (Figure 6A and 6B). When cells whose RFs where in the same row were grouped, each row was equally represented and there were no significant difference between the numbers of cells with RFs in on whisker row compared to the number of cells with RFs in any other whisker row (Figure 6C) (Kruskal-Wallis ANOVA, p=0.42). Conversely, when cells were grouped by columns, there were significantly more cells responsive to caudal whiskers than rostral whiskers (Kruskal-Wallis ANOVA, p=0.015) (Figure 6D).
Therefore, the composition of the different trigeminal somatosensory regions of the brain having more cells with caudal whiskers RFs than rostral whisker RFs is also present in the trigeminal ganglion.

Furthermore, a significant trend was observed such that as one moves from the more rostral whiskers towards the more caudal whiskers, each successive whisker column is represented by more cells. On a column by column basis, the number of cells whose RFs were in Column 1, the most caudal column, were significantly greater than the number of cells whose RFs were in Column 4, a column in the middle (Mann-Whitney U, p=0.01), and the number of cells whose RFs were in Columns 5 & 6, combined, the most rostral columns (Mann-Whitney U, p=0.01), (n=5 whiskers for each column group). Likewise, the number of cells with RFs in the straddler vibrissae, more caudal than column 1, were significantly greater than the number of cells with RFs in Columns 5 & 6 combined (Mann-Whitney U, p=0.01, n=4 and 5 whiskers, respectively). We define this phenomenon of increasing number of cells with RFs in a column when progressing from the smaller, more rostral whiskers to the larger, more caudal whiskers as a receptive field gradient. This trend or receptive field gradient was significant ($\Gamma = -0.58$, $p<0.001$), (Figure 6D). Therefore, the larger, more caudal whiskers have more cells available to process information than the smaller, more rostral whiskers.

This receptive field gradient describing the greater representation of caudal whiskers than rostral whiskers by cells in the Vg is consistent with a previous study reporting the number of axons innervating each whisker follicle (Welker and Van der Loos, 1986). When the percentage of cells responding to stimulation of each whisker was compared to the percentage of axons innervating each whisker, the same “pipe-organ” pattern reported by Welker and Van der Loos was observed for the cells in the Vg (Figure 7A). In fact, the number of cells recorded for a
particular whisker observed in our study correlated significantly with the number of axons innervating that whisker ($\Gamma = 0.51$, $p<0.001$, straddler vibrissae not included because axon numbers were not reported), (Figure 7D). The comparison between cells and axons could be seen more clearly when they were grouped by the row or the column that they innervated. Therefore, our data are in agreement with the innervation data that there are no differences in the number of axons innervating each row (Figure 7B) but there are more axons innervating the caudal whiskers than the rostral whiskers (Figure 7C).

**RA and SA cells equally distributed across both rows and columns**

We next studied the location of RA and SA type cells within the vibrissae-responsive region of the Vg and their distribution among cells with different whisker RFs. The response properties of each of the 240 vibrissae-responsive cells to passive whisker deflection were evaluated and each cell was categorized as either slowly adapting (SA) or rapidly adapting (RA) (see Methods). Nearly sixty percent (58.3%, $n=140$) of the vibrissae-responsive cells were classified as slowly adapting (SA) while the remaining 41.7% ($n=100$) were rapidly adapting (RA). This particular ratio of SA to RA cells has been documented before in the Vg yet its functional meaning is not known.

We first assessed whether SA and RA cells were found in different regions of the Vg. SA cells were found more anterior than the RA cells ($p=0.02$), but there was no clear anatomical separation in the mediolateral ($p=0.08$) or dorsoventral ($p=0.12$) planes ($p$-values from Mann-Whitney U). Since there was no difference in the anteroposterior direction of the distribution of cells with different RFs, it is not clear what the functional significance of this distribution of SA cells is.
Our second result shows that the RA and SA cells were equally distributed across both rows and columns. The ratio of SA to RA cells within each whisker group was compared to the population as a whole to determine if cells within a particular whisker group were more likely to be RA or SA type cells. There was no significant difference between the ratio of SA to RA cells within any whisker row (Figure 6C) or column group (Figure 6D) compared to the population ratio (Two Proportions test, p>0.05 for each), although there was some variability in the ratio of SA to RA-type cells innervating each whisker row or column. Therefore, it is unlikely that any computational differences associated with RA and SA cells can be exclusively associated with either caudal or rostral whiskers.
DISCUSSION:

There were three major results of this study. First, the Vg has a finer detail of somatotopy than previously reported and the orientation of cells within the Vg replicates not only the innervation pattern of the trigeminal nerve branches but also the gross arrangement of facial features on the face. Second, there were more cells with caudal whisker receptive fields than cells with rostral whisker receptive fields and a trend of increasing numbers of cells with receptive fields in the same column along the rostrocaudal axis within the vibrissae responsive region of the Vg, corresponding to the size of the whisker (which also increases from the smaller, more rostral whiskers to the large more caudal whiskers). Finally, there was a similar distribution of RA and SA cells across every whisker row and column, suggesting that each whisker is represented by a population of cells able to support the information encoded by both RA and SA cells.

Somatotopic Organization of Vg

This study demonstrated a dorsoventral somatotopy of cells within the Vg consistent with previous studies (Beaudreau and Jerge, 1968; Gregg and Dixon, 1973; Erzurumlu and Jhaveri, 1992; Waite and Tracey, 1995; Borsook et al., 2003; Kerem et al., 2005) and a distinct orientation of cells in the Vg that replicates the arrangement of facial features on face. Cells innervating the more dorsal facial features (eye) were encountered more dorsally, ventral features (lower lip) more ventrally, and midline features (nose, whiskers, and upper lip) in between. Collectively our findings show that the rostral pole of the face is pointing posteromedially and tilted ventrally in the Vg. To illustrate this somatotopy we provide a figure with an outline of a rats’ face overlaid onto the mean locations of our recorded cells (Figure 8).
and this figure superimposed onto a known grid of the rodent trigeminal ganglion (modified from Schneider et al., 1981).

Our results clarify earlier contradictions regarding the location of eye responsive cells and cells responding to lower lip and jaw. For example, earlier studies agreed that cells whose receptive fields are in the eye region of the face are positioned medially but Jacquin et al., (1986) suggested they were dorsomedially and Waite and Tracey (1995) suggested they were positioned anteromedially. From our results, it is now clear that both are correct and these cells are positioned dorsally in the anteromedial ganglion. Furthermore, our data are in agreement with Waite and Tracey (1995) regarding cells whose receptive fields are in the lower lip or jaw region of the face, associated with the mandibular branch of the trigeminal nerve, and demonstrate that they are positioned in the posterolateral region of the ganglion. However, we found these cells to be in the most ventral portion of the posterolateral region of the ganglion, not dorsolaterally as suggested by Jacquin et al., (1986) and Renehan et al., (1989).

The dorsoventral somatotopy of facial features found in the Vg as a whole extended to the vibrissae-responsive cells whose axons lie in the maxillary branch of the trigeminal nerve (V2). These vibrissae responsive cells were found to be clustered in a clearly demarcated region medially within the Vg. Although, it has been suggested that the cells innervating the more dorsal whiskers (i.e. A and B rows) are situated medially in the Vg while cells of ventral whiskers (i.e. D and E rows) lie laterally (Zucker and Welker, 1969) and other authors have not disagreed with this statement (Arvidsson, 1982; Jacquin et al., 1986; Rhoades et al., 1990; Renehan et al., 1989; Lichtenstein et al., 1990), this pattern was not observed in our study. Animals in our study were equally likely to show the more dorsal rows more medially (2 animals) as they were to show the more dorsal rows more laterally (2 animals). We therefore
think the sampling protocol used by Zucker and Welker (1969) may have contributed to their conclusion. Interestingly, however our data do support the conclusions that caudal whiskers tended to be more dorsal (Zucker and Welker, 1969; Jacquin et al., 1986; Renehan et al., 1989). We further show that cells innervating dorsal whiskers were more dorsal than cells innervating ventral whiskers. Therefore, somatotopic organization within the vibrissae responsive region of the Vg mirrors the somatotopic organization of the Vg as a whole and grossly represents the arrangement of receptive fields on the face.

Our results support developmental theories suggesting that the arrangement of peripheral neurons may be a template for the design of central nuclei (Erzurumlu and Jhaveri, 1992; Killackey et al., 1995; Scott and Atkinson, 1999). We conclude this on two reasons. First, the dorsal to ventral somatotopic organization of the Vg observed in this study is inverted compared to the somatotopic organization of the trigeminal region of the brainstem and second, the Vg and the brainstem rotate 180° relative to each other during development (Waite and Tracey, 1995). During development, the somatotopy in the Vg is established before outgrowing axons have contacted their peripheral or central targets. At this time, the ganglion appears to rest on the brainstem in early head morphogenesis when the distance to travel out to the periphery and centrally to the brainstem is very small. These Vg cells then extend neurites known as pioneers both peripherally and centrally (Scott and Atkinson, 1999). Finally, these structures rotate 180° relative to each other producing, we suggest, the inverted relationship between the periphery and brainstem. Our results show that the somatotopic position of cells in the Vg grouped by trigeminal nerve branch is inverted from that found in the principle trigeminal nucleus (PrV), where cells whose receptive fields are innervated by the mandibular branch of the trigeminal nerve are situated dorsally, cells whose receptive fields are innervated by ophthalmic branch are
situated ventrally, and, finally, cells whose receptive field are innervated by the maxillary branch are situated in between (Waite and Tracey, 1995). Given these developmental steps, if the Vg is a template for the organization of central structures, then the ganglion and brainstem should have similar, albeit inverted, somatotopy, which is clearly what we have found.

**Receptive Field Gradient**

Our result that there is a trend of increasing number of cells with vibrissal receptive fields along the rostrocaudal axis of the mystacial pad such that there are more cells with caudal whisker receptive fields than cells with rostral whisker receptive fields is consistent with the somatotopic organization of the central nuclei of the brain. This greater representation of cells with caudal whisker receptive fields compared to cells with rostral whisker receptive fields has been well-defined in layer IV of the somatosensory cortex (Woolsey and Van der Loos, 1970; Killackey et al., 1995), VPM thalamus (Van der Loos, 1976), brainstem trigeminal nuclei (Ma and Woolsey, 1984; Ma, 1991) and demonstrated previously in the Vg with in a sample of cells (Zucker and Welker, 1969). Moreover, the number of cells with receptive fields for each whisker follows a “pipe-organ” pattern similar to that found for the increasing numbers of axons innervating the caudal versus the rostral whisker follicles (Lee and Woolsey, 1975; Welker and Van der Loos, 1986). Finally, the size of the follicles surrounding caudal whiskers is greater than the size of the follicles around rostral whiskers (Haidarliu and Ahissar, 2001). We suggest that since the larger caudal whiskers have a greater follicular size, they have more receptors, and these receptors are supported by more cells, not strictly more axon collaterals per cell.
Structure-Function Relationship: correlation between receptive field and physiological response type

Our data suggest that there are more SA cells than RA cells within the Vg. While many studies have reported the ratio of rapidly adapting (RA) and slowly adapting (SA)-type cells in the Vg (Zucker and Welker, 1969; Gibson and Welker, 1983a,b; Jacquin et al., 1986; Renehan et al., 1989; Lichtenstein et al., 1990; Waite and Jacquin, 1992; Chiaia et al., 1993, 1997; Kyriazi et al., 1994; Waite and Tracey, 1995; Shoykhet et al., 2000, 2003; Minnery et al., 2003) the proportions of cells identified in those previous studies cannot be taken as the ratio of each type to the total population because of sampling biases in their recording techniques. For example, certain studies have reported relatively low numbers of RA-type cells (Lichtenstein et al., 1990) by excluding cells having large amplitude, high-velocity thresholds, which are typically RA, or by deliberately recording equal numbers to characterize both populations evenly (Kyriazi et al., 1994). Our goal was to record an unbiased sample from across the extent of the vibrissae-responsive region of the ganglion. Our findings were consistent with studies by Zucker and Welker (1969) and Chiaia et al. (1997) reporting approximately 60% of the cells as SA and the remaining 40% as RA, despite the differences in rat species.

To date, it is not known if this greater number of SA-type cells compared to the number of RA-type cells has any functional consequences. Cells in the primate associated with the glabrous skin of the hands can also be defined as either RA or SA type cells by their response to tactile stimuli. We suggest it may be possible to extend what is known about the function of these cells in the primate to better understand their function in the rat. In the primate glabrous skin, SA-type cells are likely encoding information about object discrimination (for review see Johnson, 2001). Therefore, it may be possible that SA-type cells in the rat whisker follicle also
function primarily for object discrimination. Therefore, the larger proportion of SA-type cells may be necessary to support the complex array of discrimination tasks performed by the rat’s whiskers. This remains to be tested.

Since it is clear that rats use their mystacial vibrissae to locate and discriminate objects, an interesting question that arises is whether there is a correlation between the cells vibrissal receptive field and the cells’ response type. Namely could SA-type cells be more likely to have caudal whisker receptive fields, given that both have been implicated in object discrimination, while RA-type cells be more likely to have rostral whisker receptive fields since both have been implicated in object localization. This, however, does not appear to be the case. In fact, although there was an increase in cells innervating the more caudal whiskers, the increase was not specific to one cell type (RA or SA) but rather consisted of a similar, proportional increase for both RA- and SA-type cells. Although different whiskers could be specialized for different tasks, this distribution of RA and SA cells across the vibrissal receptive fields suggests that each whisker has the ability to support a variety of functions, inherent to both RA and SA cells. Recent studies seem to support this idea (Harvey et al., 2001; Krupa et al., 2001; Hartmann et al., 2003; Neimark et al., 2003; Szwed et al., 2003; Jones et al., 2004a,b).

In conclusion, the somatotopic organization of cells within the Vg, including the Vibrissae-responsive cells, mirrors the arrangement of features on the face and is reflective of the somatotopic organization in the brain. Since the Vg develops first it is likely the template for subsequent somatotopic organization throughout the trigeminal somatosensory system. This study provides the most current map of positions of cells within the rodent trigeminal ganglion with respect to their receptive field on the rats’ face. Vibrissae-responsive cells can be found medially within the ganglion from approximately 9.82 mm ventral from the surface of the brain.
for about 1.85 mm of depth. Therein, the larger, more caudal whiskers are represented by more
cells than the smaller rostral whiskers. Although there are more SA-type cells in the Vg, the
distribution of RA and SA type cells is the same regardless of the cell’s receptive field,
suggesting that all whiskers are capable of encoding information encoded by both types of cells.
The functional differences of vibrissae, if any, must then be context- or behavior- dependant.
ACKNOWLEDGEMENTS:
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DISCLOSURES:
N/A
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Table 1. Dorsal to ventral extent of vibrissae-responsive cells within the trigeminal ganglion.

The dorsal to ventral extent of the vibrissae-responsive cells are shown for each penetration on the grid (see methods). A. The most dorsal coordinate for which vibrissae-responsive cells were identified. B. The most ventral coordinate for which vibrissae-responsive cells were identified. C. The distance that vibrissae-responsive cells can be found along the dorsoventral axis, derived by subtracting, for each lateral and posterior coordinate the most dorsal coordinate from the most ventral coordinate.
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Table 2. Within animal trends of somatotopic organization for the vibrissae-responsive cells.

Trends within each animal were examined to determine if there was any somatotopic organization that was lost by averaging across animals. The first column lists whether cells where grouped by row or column. The second column lists the number of animals for which vibrissae-responsive cells were found in more than one penetration along the axis studied. The third column lists the number of animals that had trends along the direction indicated for that group (A. anterior to posterior, B. medial to lateral and C. dorsal to ventral). Column four list the number of animals that had a trend in the opposite direction. The last column lists the number of animals that showed no trend.
The trigeminal somatosensory system has at each level in the neuraxis identifiable groups of neurons, related in a one-to-one fashion to each whisker on the rat’s face that are somatotopically organized in a manner that directly reflects the arrangement of the whiskers on the face (A). To identify the somatotopic organization of the trigeminal ganglion (Vg), single tungsten microelectrodes were lowered through the brain and into the ganglion (B) at selected coordinates defined by a grid (E–F). (A) V1, the ophthalmic branch of the trigeminal nerve (in white) innervates dorsal facial features (eye and nose), V2, the maxillary branch innervates the medial face (mystacial vibrissae and upper lip), and V3, the mandibular branch innervated ventral facial features (lower lip). (B) The electrode was lowered through the brain and into the ganglion, which lies just below the brain at the base of the skull. (C-D) To ensure single units were recorded from somata the polarity for each cell was checked (refer to Methods for details). Scale bars show 500μV (y) and 100μsec (x). All cells in our sample had negative going waveforms. (C) Multiple waveforms from a single cell are superimposed on each other. (D) The average and standard deviation of the waveforms sampled from all the neurons are presented. (E-F) A recording grid that covered the likely extent of the trigeminal ganglion based on preliminary findings was defined for electrode penetrations (refer to Methods for details). (E) This diagram, modified from Schneider et al., (1981), illustrates the trigeminal ganglion with the
region sampled in this study outlined (dotted rectangle). (F) Sampling results of our study providing the total number of vibrissae-response cells over the total number of sensory-responsive cells recorded for that site and, in parentheses the total number of times that site was sampled. Dark grey shaded regions indicate the presence of vibrissae-responsive neurons, while light grey shaded regions indicate the presence of only other sensory-responsive cells. Non-shaded regions indicate that either the site was not sampled (no numbers) or the cells that were sampled were not sensory-responsive to the modalities tested in this study (0/0).
Figure 2. Distribution of responsive cells recorded within the trigeminal ganglion.

As the electrode is lowered perpendicular to the surface of the brain at each of the anteroposterior or mediolateral positions on the grid (refer to methods), the number and dorsoventral location of the cells is noted. A-H represent different stereotaxic coordinates posterior to bregma: A. 0.0 mm, B. 0.5 mm, C. 1.0 mm, D. 1.5 mm, E. 2.0 mm, F. 2.5 mm, G. 3.0 mm, and H. 3.5, 4.0, and 4.5 mm. Within each plot are columns representing the stereotaxic coordinate lateral to bregma. Only penetrations where a sensory responsive cell was found are shown. For each column, the ventral coordinate from the surface of the brain is presented from 9.1 mm to 11.5 mm. If a cell was found, the receptive field is noted and, in parenthesis, the number of cells with that receptive field.
Figure 3. Somatotopic organization of sensory-responsive cells within the trigeminal ganglion.

Two trends of somatotopy of cells within the Vg were identified. The locations of sensory-responsive cells were consistent with the relative position of the branches of the trigeminal nerve (V-nerve), but also showed a dorsoventral organization that reflected the position of the receptive fields on the face. (A) Cells responding to features innervated by the ophthalmic (V1) and maxillary (V2) branches of the trigeminal nerve were found in similar medial (A, y-axis) and anterior coordinates (A and C, x-axis), while cells responding to features innervated by the mandibular (V3) branch of the trigeminal nerve, were significantly more lateral and posterior than both V1- and V2-branch cells. (B) These differences could also be seen when cells were grouped by their receptive field. Cells that responded to the stimulation of the lower lip (LL) were significantly more lateral (B, y-axis) than cells responding to the vibrissae (V), eye
(E) and nose (N), as well as significantly more posterior (B and D, x-axis) than cells responding to the vibrissae, eye, and upper lip (UL). (C) Cells associated with V1 were found significantly more dorsal (C, y-axis) than cells associated with either V2 or V3 while cells associated with V3 are significantly more posterior (C, x-axis) than cells associated with V1 or V2. (D) Eye-responsive cells were found significantly more dorsal than both vibrissae (V) and lower lip (LL)-responsive cells (D, y-axis). As one progresses from dorsal to ventral in the Vg, eye-responsive cells were encountered first, followed typically by nose-, then vibrissae- and upper lip- cells, and finally lower lip-responsive cells. This grossly resembles the arrangement of their receptive fields on the face. Plotted are the mean locations of cells (center boxes) and standard deviations (radial arms). In A-D, labeled black lines with asterisks in identify groups that were significantly different from each other (Mann-Whitney U tests) at the p-values noted. The black lines stretch from the means of the two values tested.
Figure 4. Probability of encountering a receptive field within the trigeminal ganglion.

Contour plots showing the probability of finding cells with a particular receptive field. For all plots, the posterior position relative to bregma is along the x-axis (0-4.5 mm posterior) and the lateral position relative to bregma is along the y-axis (1.0 to 3.5 mm posterior). The color coding is unique for each plot and presents the probability distribution of finding the receptive field at any particular location. For example, vibrissae-responsive cells (C) are most...
likely to be found between 1.0 and 2.5 mm posterior and 1.5 and 3.0 mm lateral to bregma. A. Eye responsive cells, B. Nose responsive cells, C. Vibrissae responsive cells, D. Upper lip responsive cells, E. Lower lip responsive cells. Dorsal to ventral coordinates are not shown.
A dorsoventral somatotopy of vibrissae-responsive cells that matches the placement of their receptive field on the face was found. (A and B) There was no significant difference in the locations of cells grouped by column in either the lateral (A, y-axis) or posterior (A and B, x-axis) coordinate or along the dorsoventral axis (B, y-axis). There was also no significant differences in the locations of cells grouped by row in either the lateral (C, y-axis) or posterior (C and D, x-axis) coordinate. However, there was a significant difference between the ventral locations (D, y-axis) of the cells and their respective row. When cells were grouped by their responses to dorsal whiskers (rows A and B, n=86) and ventral whiskers (rows D and E, n=62), dorsal whisker cells were significantly more dorsal than ventral whisker cells (black line with asterisk, Mann-Whitney U, p=.007). Plotted are the mean locations of cells (center boxes) and standard deviations (radial arms). Straddler vibrissae, not included on this graph (see text), had a mean location of 1.54mm ±0.7SD posterior, 1.94mm ±0.3SD lateral and 10.06mm ±0.32SD ventral from dural surface.
FIGURE 6:

Figure 6. Rostrocaudal innervation gradient of vibrissae-responsive cells in the Vg.

We recorded 240 vibrissae-responsive cells and at least two cells from each whisker on the mystacial pad (A and B). (B) The number of recorded units for each whisker is given as a percent relative to the total number of vibrissae-responsive cells. This chart allows visualization of the receptive field gradient (see text). Cells are arranged by row (color coded) and presented clockwise in orientation from dorsal, A-row, to ventral, E-row. Numbers inside the pinwheel are column numbers and are presented clockwise in orientation from caudal, Column 1, to rostral, Column 5. Straddler vibrissae are placed in between rows. (C and D) The total number of cells for each whisker row (C) was relatively the same, however a significant difference between the numbers of cells in each whisker column was seen (D). The number of cells for each whisker in Column 1 was significantly greater than the number of cells for each whisker in Column 4 and Columns 5-6, and the number of cells for the Straddler vibrissae was significantly greater than the number of cells in Columns 5-6 (asterisked black lines, Mann-Whitney U, p<0.01). Although, there was no other significant differences, a trend of increasing cell numbers can be seen progressing from the smaller, more rostral whiskers to the larger, more caudal whiskers. Also presented are the ratio of SA (light gray) to RA (dark gray) cells innervating each whisker row (C) or column (D). Although there was some variability in the ratio of SA to RA-type cells innervating each whisker row or column there was no significant difference between the ratio of SA to RA-type cells of any whisker row or column compared to the population ratio (58% SA, 42% RA, Two Proportions test, p>0.05). Black lines with asterisks stretch between groups that were significantly different from each other (Mann-Whitney U test, p<0.01 as stated above).
Figure 7. Receptive field gradient of vibrissae-responsive cells correlate to axonal innervation.

The data reporting the number of axons innervating each whisker follicle (light gray, △, axon numbers from Welker and Van der Loos, 1986) was compared to the distribution of cells in our sample (dark gray) that responded to movement of that whisker. (A) Axon and cell numbers are plotted as a percentage of the total number of axons and cells observed, respectively. Note
the distinct “pipe-organ” pattern first described by Welker and Van der Loos, 1986. (B and C) This trend is not noted for whisker rows (B) but is clear for whisker columns (C). The larger, more caudal whiskers had more axons innervating their follicles and more cells recorded than the smaller, more rostral whiskers. (D) The number of cells recorded for a particular whisker, given as a percent (# cells/total n) observed in our study correlated significantly with the relative axon numbers innervating that whisker (solid line, R=0.72, Gamma (R^2)=0.51, p<0.001). Individual whisker names replace points on the graph.
Figure 8. Dorsoventral somatotopy of cells within the Vg.

Our findings show a dorsoventral somatotopy of cells within the Vg. Cells innervating the more dorsal facial features (e.g. eye) were encountered more dorsally, ventral features (e.g. lower lip) more ventrally, and midline features (e.g. nose, whiskers, and upper lip) in between. Collectively our findings also show that the rostral pole of the face is pointing posteromedially and tilted ventrally in the Vg. To further illustrate our findings an outline of a rats’ face is overlaid onto the mean locations of our recorded cells. (C-E) To relate our findings we have also superimposed the position of this image onto a known grid of the rodent trigeminal ganglion (C and E, grid of Vg modified from Schneider et al., 1981; D, schematic of rats’ face illustrating vibrissal receptive fields used to create image).