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Responses of Trigeminal Ganglion Neurons during Natural Whisking Behaviors in the Awake Rat

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SUMMARY

Rats use their whiskers to locate and discriminate tactile features of their environment. Mechanoreceptors surrounding each whisker encode and transmit sensory information from the environment to the brain via afferents whose cell bodies lie in the trigeminal ganglion (Vg). These afferents are classified as rapidly (RA) or slowly (SA) adapting by their response to stimulation. The activity of these cells in the awake behaving rat is yet unknown. Therefore, we developed a method to chronically record Vg neurons during natural whisking behaviors and found that all cells exhibited (1) no neuronal activity when the whiskers were not in motion, (2) increased activity when the rat whisked, with activity correlated to whisk frequency, and (3) robust increases in activity when the whiskers contacted an object. Moreover, we observed distinct differences in the firing rates between RA and SA cells, suggesting that they encode distinct aspects of stimuli in the awake rat.

INTRODUCTION

Sensory receptors transduce somatosensory information into electrical impulses as animals actively move their tactile organs in space and across objects. In primates, the glabrous skin of hands provides the main cutaneous surface for sensory discrimination. For rats, arrays of whiskers on the face serve as the primary tactile organ. Although there are clear differences in the way these two species actively explore tactile features of their environment, there are similarities in the underlying neural mechanisms used by both. Primates actively move their fingers and palms across surfaces to activate receptors in a spatiotemporal pattern that is transmitted to higher brain centers to discriminate features of the object (Gamzu and Ahissar, 2001; Nicolelis et al., 2003). Likewise, rats actively sweep their whiskers through space and across objects during a whisking cycle to encode features of their environment. These signals convey sufficient information to distinguish between objects of different shape and texture (Carvell and Simons, 1990; Brecht et al., 1997; Ahissar and Arieli, 2001; Sachdev et al., 2001, 2002; Harvey et al., 2001; Arabzadeh et al., 2005; Mehta and Kleinfeld, 2004; Albarracin et al., 2006; Derdikman et al., 2006; Knutsen et al., 2006; Hipp et al., 2006). In both rats and primates, tactile information is transduced via mechanoreceptors to afferents, whose cell bodies lie in somatosensory ganglia (trigeminal ganglia in rat and dorsal root ganglia in primate).

It is critical to understand the responses of these first-order neurons because these constrain all subsequent somatosensory processing. Thus, given the similarities between rats and primates, at the level of the trigeminal ganglion (Vg) the rat is an important model that can be used to study somatosensory information encoding and processing (Szwed et al., 2003, 2006). There are several advantages to studying the transduction of sensory information by the primary afferents in rats as opposed to primates. First, in the rat the cutaneous surface is not continuous but consists of an array of whiskers laid out in specific rows and columns on the rat's face. For each whisker, thousands of mechanoreceptors transduce incoming tactile information into electrical impulses that are then transmitted via primary afferent neurons of the trigeminal nerve (NV) to the brain (Ebara et al., 2002; Rice et al., 1986). Every whisker-responsive cell in the Vg responds to movement of one and only one whisker, and these responses are transmitted through the brain in an orderly representation to the primary somatosensory cortex.

A second advantage to using a rat model is that movements of whiskers in space are constrained compared to movement of primate hands. Because the ultimate goal is to observe the response of these cells under natural exploratory conditions, the whisker system provides a better means to track and identify sensory stimuli. Finally, the rat Vg, located at the base of the brain, is more easily accessed than the primary afferents entering the spinal cord of primates. Nevertheless, if the rodent trigeminal somatosensory system is to be considered a model for studying the transduction of sensory information in primates, it is important to know which properties of the system are the same and which are different across the species.

While there have been several studies of primary afferents in awake primates, there are no studies of the activity

of these afferents in awake rats. Responses of cells in the Vg recorded in the anesthetized rat do not address how these cells respond when recorded during whisker movements or how such responses are related to the rat's behavior. Thus it is obviously critical to know the repertoire of responses of primary afferent neurons during natural active whisking behaviors in order to fully understand the appropriateness of the rat whisker system as a model for somatosensory processing in primates. An important question is whether the different cell types within the ganglion have physiological significance. In rats and primates, primary afferents can be classified into two distinct types based on their response to passive stimulation: rapidly (RA) or slowly (SA) adapting. Studies of awake primates suggest different functional properties for RA and SA cells (Johnson, 2000, 2001; Blake et al., 1997a, 1997b; Friedman et al., 2002; Goodwin and Wheat, 2004; Berryman et al., 2006). A lack of similar awake data from rats makes a direct comparison difficult and limits our understanding of the neuronal response properties elicited by stimuli in the natural environment.

Therefore, a major goal of this study was to address this by characterizing the responses of RA and SA cells in awake naturally behaving rats. We first developed a technique to chronically record from the Vg and evaluated responses of Vg cells during distinct natural whisking behaviors—Rest, Whisking in Air, and Contact. Next, we identified a subset of these cells as either RA or SA and contrasted their responses during these conditions to test whether they exhibit distinguishable response properties. Finally, during periods of Whisking in Air we examined the relationship between whisker position during a whisk cycle and neuronal activity to determine if these cells are more likely to fire during a particular phase of the whisk.

RESULTS

This study used chronic extracellular recordings from trigeminal ganglion (Vg) cells in awake, freely moving rats. Our technique allowed for recordings that lasted on average 25 days (range 1–119 days). Six rats received bilateral implants, one electrode per ganglion, that allowed recording up to four neurons simultaneously (two from each electrode; Figure 1). In total we recorded the activity of 80 cells from 14 rats and compared the activity during three different natural whisking behaviors: Rest, Whisking in Air, and Contact (Figure 2).

Firing Rate Correlated with Whisking Behavior

First, the firing rate of Vg neurons when the rat was sitting quietly and not moving its whiskers (Rest) was examined to determine if Vg cells fired spontaneously. Consistent with most studies of Vg cells under anesthetized conditions, no spontaneous neural activity was observed during this behavior from any cell (n = 80). Qualitatively, when these cells were examined during episodes of Whisking in Air, all 80 neurons fired spikes when the rat whisked despite not touching any surfaces. Therefore, all cells were transmitting some information about movement of the whiskers in the absence of contact. Finally, all cells were observed to increase their firing rate when the whiskers contacted the wall of the behavioral chamber and while they remained in contact with the wall (Contact). These results suggest that all cells are able to convey information about whisker contact as well.

To further our understanding of the relationship between firing rate and whisking behavior, firing rates (mean spike frequency) of a subset of cells (n = 27, based on strict requirements; see Experimental Procedures) were assessed during the different behaviors. We observed a total of 219 epochs (710 s) of Rest, 319 epochs (820 s) of Whisking in Air, and 251 epochs (165 s) of Contact.

For all epochs of Rest, as long as the whiskers were not in motion there was no cellular activity (Figure 3).

Cells had a broad range of firing rates when the whiskers were Whisking in Air (no contact). The average neuronal firing rate during these epochs was 11.2 ± 13.4 Hz (Figure 3A) but mostly less than 10 Hz. Yet, the spike frequency across all epochs ranged from 0 to 69 spikes per second (Figure 3B), demonstrating that there was considerable variability in the response of individual cells. An important example of this variability is that, despite the fact that all cells had the ability to respond during whisking in air, cells do not fire during every whisk. There were a small number of epochs (7.5%; n = 24) of whisking in air that lasted as long as 4.4 s when a cell did not fire (Figure 3B). These nonspiking epochs occurred in nine cells (33%) but never exceeded 20% of a cell's Whisking in Air epochs and altogether constituted only 5.7% of the total epoch time. Such nonspiking epochs in awake rats, although rare, could explain why during previous studies in sedated rats cells were observed not to respond during artificial whisking (Szwed et al., 2003).

When contrasting Whisking in Air to Contact, we found that each cell showed a significant increase in its mean firing rate to Contact ($p < 0.05$, Mann-Whitney U test; n = 27 cells; Figure 3C), confirming that all cells are capable of conveying information about object contact. The average neuronal firing rate during Contact was 108.11 ± 85.70 Hz, significantly greater than that during Whisking in Air ($p < 0.01$, Mann-Whitney U test; Figure 3A). Furthermore, cells with the greatest firing rate during Whisking in Air generally had the greatest firing rate during Contact (Figure 3C).

Moreover, there was a broad range of spike frequencies recorded, ranging from 5 to 380 Hz (Figure 3B). This firing rate variability during Contact could explain why Szwed et al. (2003) showed some that cells do not increase their firing rate upon contact. For example, 4 of 27 cells showed at least one Contact epoch with a firing rate less than an epoch of Whisking in Air (Figure 3D). Therefore, there are behavioral conditions under which cells will not increase their firing rate when the whisking rat contacts an object.

Spike Frequency Correlated with Whisk Frequency

Some variability in spike frequency during periods of Whisking in Air was related to changes in whisk frequency.

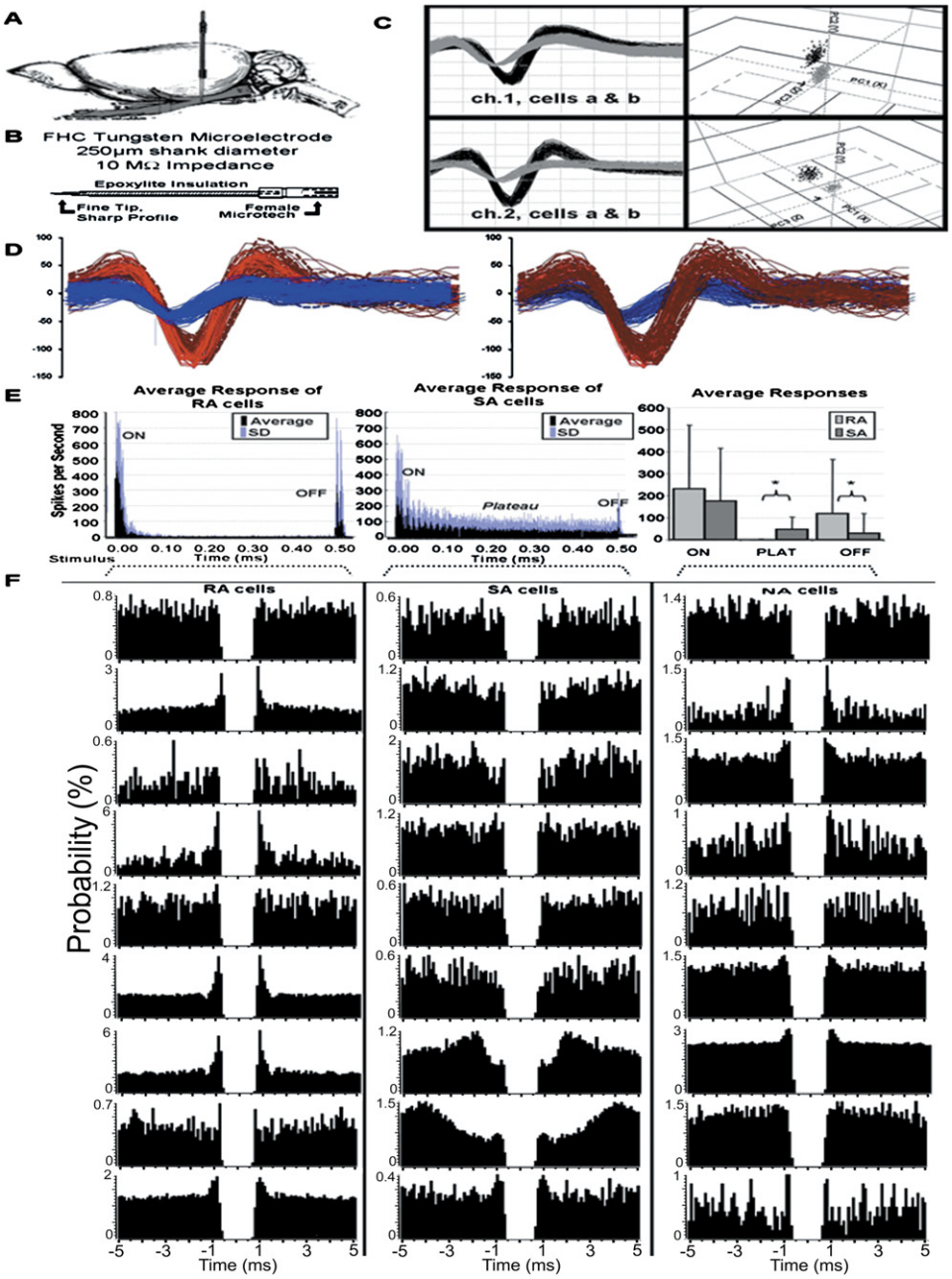


Figure 1. Single Units from Chronically Implanted Vg

(A) Sketch of location of implant.

(B) Electrode design.

(C) Four cells recorded simultaneously from a bilateral implant (two units per electrode) were clearly separated by (left) sampled waveforms (wfm) and (right) 3D plots of the first three principal components of waveform shape (PC1[x], PC2[y], PC3[z]).

(D) Comparison of waveform shape of a single cell recorded during the same awake recording session during epochs of Whisking in Air and Contact. This shows that the shape of the waveform did not change during a recording session or during the different behaviors. Each panel shows the waveform shape from multiple spikes overlaid on top of each other. Light blue (unit 1) and light red (unit 2) waveforms were recorded during Whisking in Air, and dark blue (unit 1) and dark red (unit 2) waveforms were recorded during Contact. The left panel shows whisking in air waveforms (light) on top of the contact waveforms. The right panel shows the same waveforms but with the contact waveform (dark) on top of the whisking in air waveforms.

(E) Average response of RA and SA cells to passive whisker stimulation and comparison of their ON, OFF, and Plateau responses. Shaded lines (left two panels) and error bars (right panel) represent standard deviations. A schematic of the ramp-and-hold stimuli is shown under each trace.

(F) Single units (n = 27) defined by autocorrelations (0.1 ms bins) including spikes from all behavioral states (y axis in probability, x axis ± 5 ms).

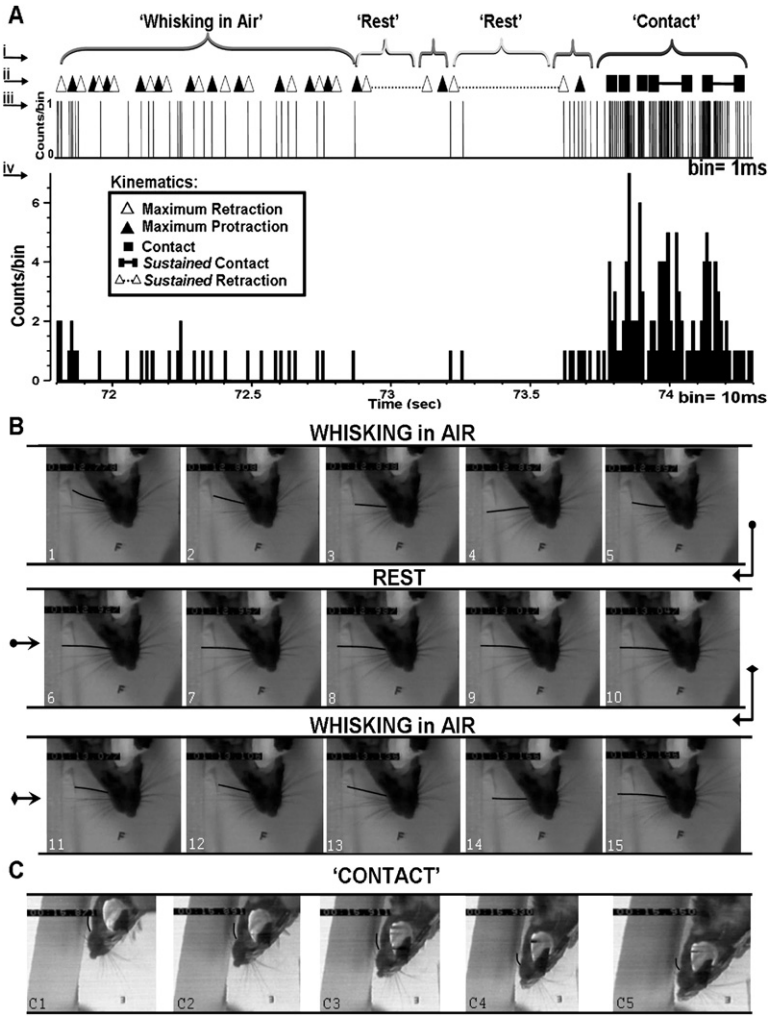


Figure 2. Representation of Whisk Behaviors

(A) Typical responses of a Vg cell included no activity when whiskers were at Rest, activity during Whisking in Air, and robust activity during Contact. Whisk behavior (Ai) and kinematics (Aii) included only maximum retraction (open triangle), maximum protraction (closed triangle), Contact (closed square), and times of sustained Contact or Rest as indicated by connection lines. Rate histograms of the neuronal firing rate are shown as counts per bin (y axis) during this session (time, x axis) in 1 ms bins (Aiii) and for display 10 ms bins (Aiv).

(B and C) Consecutive screen-grabs (5 ms) from high-speed video with synchronized neuronal timestamps (top left). The camera maintained a focus point ("F") as the rat moved through its field. The same whisker is bolded in each frame. (B) Frames show consecutive partial protractions (1–4) and initial retraction (5), followed by five consecutive frames when the whisker does not change position (6–10), then frames of partial whisker protractions (11–15). (C) Frames show the position of the whiskers and mystacial pad during Contact.

Whisk frequency across all epochs varied from 1 to 12 Hz but was predominately between 3 and 8 Hz (mean 5.4 ± 2.7 Hz; Figure 4A). Because these rats were untrained and allowed to naturally explore, their whisk frequency was low compared to possible whisk frequencies but consistent with other studies (see Experimental Procedures).

Neuronal firing rate was positively correlated to whisk frequency ($p < 0.001$; $n = 319$ epochs, 27 cells; Figure 4C). As expected due to this correlation, the average number of spikes per whisk during an epoch was consistent across a broad range of whisk frequencies and average 2.24 ± 0.32 (Figure 4B). Therefore, whisk frequency is encoded by Vg cells.

However, across individual cells there was considerable variability in the relationship of spike frequency to whisk frequency. There was a broad range of spikes per whisk across cells and across epochs, ranging from 0.4 ± 0.4 to 7.0 ± 1.3 spikes per whisk, suggesting that the relationship between spikes and whisks may not be fixed for all cells.

Moreover, there were also differences in the relationship between spike frequency and whisk frequency when individual cells were compared. Although firing rate was correlated to whisk frequency for many cells, this was not the case for all. Individually, approximately half of the cells ($n = 13$ cells) demonstrated a significant positive correlation of spike frequency to whisk frequency, suggesting a relatively consistent increase in spikes for increases in whisks (Figure 4D), but the remaining 14 cells had no consistent relationship (Figure 4E). Taken together, the variability in the relationship between spike frequency and whisk frequency and the number of spikes per whisk across cells likely has several sources in the awake, freely moving rat, including the relative position of the whisker during whisking and its stimulation of any number of receptors in the whisker follicle, in addition to the type of cell (RA or SA) activated.

Different Response Profiles of RA and SA in Active Whisking

To evaluate the role of RA and SA responses during natural whisking, their neuronal activity ($n = 9$ each; see Experimental Procedures) was contrasted during Whisking in Air and Contact.

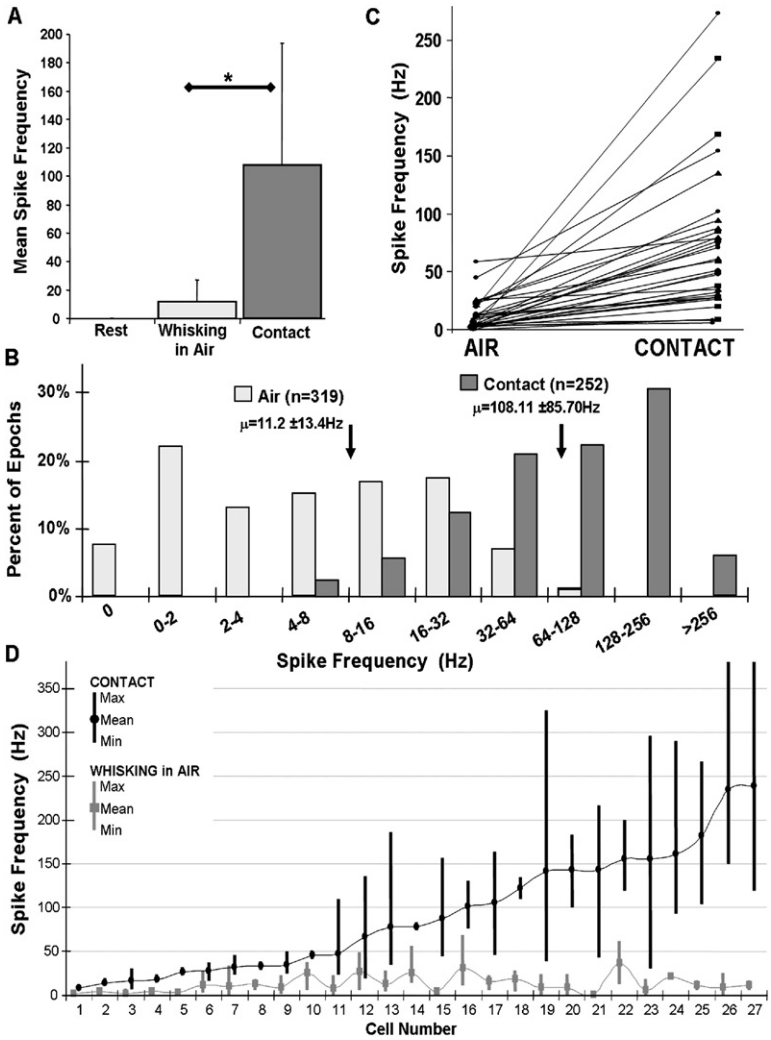


Figure 3. Comparison of Firing Rates during Different Behaviors

(A) The average response of Vg cells (n = 27) to Contact was significantly greater than the response to Whisking in Air (*p < 0.01, Mann-Whitney U test). Because there was no neuronal activity during Rest, no further analyses were done. Error bars represent standard deviation (SD).

(B) Distributions of spike frequencies per epoch for Whisking in Air and Contact represented as a percentage of total epochs for that behavior (y axis). For visualization, mean spike frequency (x axis) is plotted on a binary logarithmic scale (consistent across figures). Arrows indicate mean firing rate.

(C) All cells had a greater mean firing rate during Contact compared to Whisking in Air. Lines connect symbols plotted for mean spike frequency during each behavior for the same cell. All lines have a positive slope.

(D) Distribution of mean firing rates for each cell (arbitrary cell number, x axis) during Contact (black) and Whisking in Air (gray). Error bars denote the maximum and minimum firing rate for each condition for each cell. Most cells showed no overlap between the minimum firing rate during Contact and the maximum firing rate during Whisking in Air; however, for four cells (numbers 3, 6, 7, and 12) the minimum firing rate during Contact was lower than the maximum firing rate during Whisking in Air, demonstrating that a minority of Contact epochs had firing rates lower than a Whisking in Air epoch.

During epochs of Whisking in Air, SA cells consistently fired at higher firing rates than RA cells, suggesting that they may convey different information about the movement of whiskers. The average firing rate for SA cells' Whisking in Air epochs was 15.85 ± 13.7 Hz (n = 114 epochs, 9 cells), significantly greater than RA cells' Whisking in Air epochs (6.16 ± 10.3 Hz, 112 epochs, 9 cells; $p < 0.001$, Mann-Whitney U test; Figure 5A). For these analyses, a full complement of whisk frequencies ranging from 1 to 12 Hz were selected for both cell types, and these samples were not significantly different from each other (Kolmogorov-Smirnov two-sample test, $p = 0.10$). This phenomenon of higher firing rates for SA compared to RA held true for individual cells as well. The firing rate of most SA cells was greater than the firing rate of most RA cells (Figure 5A). Furthermore, as a population SA cells had a higher number of epochs (more time) with greater firing rates than RA cells during Whisking in Air despite the overlap in firing rate between the two cell types (Figure 5B). In fact, RA cells had more epochs with no spikes (n = 15; 13.4%) than SA cells (n = 1; 0.9%; Figure 5B). These results are consistent with anesthetized studies demonstrating tonic responses of SA cells and phasic responses of RA cells to movement.

During epochs of Contact, a slightly different picture emerges. When the mean spike frequency was examined for each epoch, there was no difference in the firing rate of RA cells compared to SA cells (Figure 5C). The average spike frequency across all epochs for RA cells was 113.4 ± 98 (n = 76 epochs), while that for SA cells was 103.35 ± 51 (n = 93 epochs). The fact that there were no differences in the average firing rates between RA and SA cells during Contact is consistent with the findings of Jones et al. (2004a, 2004b) and Szwed et al. (2003).

Yet, contrasting RA and SA cells' responses during Whisking in Air and Contact epochs (Figure 5D) illustrates important differences in the distributions of responses of RA versus SA cells. One immediate difference is that the spike frequency of RA cells during Whisking in Air is much less than the spike frequency of RA cells during Contact, and there is no considerable overlap between these. Conversely, for SA cells, although the firing rates

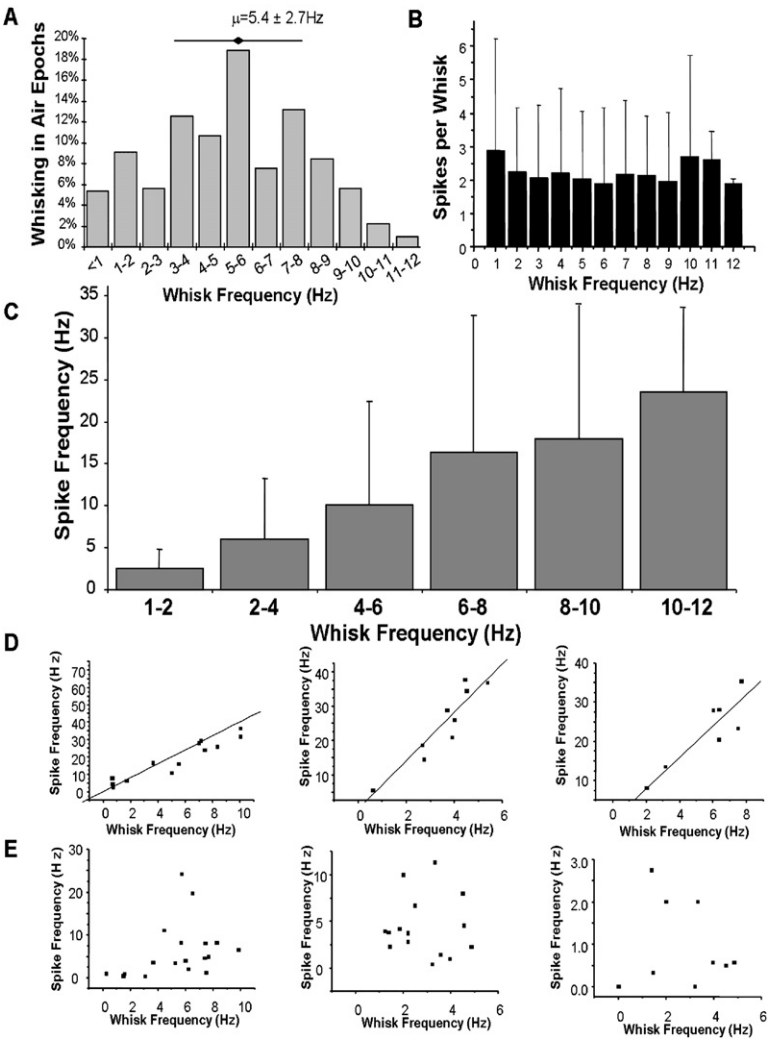


Figure 4. Spike Frequency Correlated with Whisk Frequency

(A) Distribution of whisk frequencies across Whisking in Air epochs (n = 319). (B) The average number of spikes per whisk was constant across whisk frequencies. (C) Spike frequency was highly correlated to whisk frequency (r = 0.99, $p < 0.001$, 27 cells). (B and C) Error bars represent standard deviations. (D and E) Many cells had a positive correlation between spike frequency and whisk frequency, but some did not (three examples shown in [D] and [E], respectively).

during Contact are greater than the firing rates during Whisking in Air, there is overlap between these distributions; this overlap occurs most where the higher firing rates during Whisking in Air converge with the lower firing rates during Contact. Interestingly, these distributions of responses of SA cells are flanked by the distributions of RA cells, such that the spike frequency of SA cells during both Whisking in Air and Contact is greater than the firing rate of RA cells during Whisking in Air but less than the firing rate of RA cells during Contact (Figure 5D). Therefore, RA and SA cells maintain, at some level, discernable characteristics during natural whisking behaviors in the awake rat.

To further investigate these differences, we contrasted responses during both Whisking in Air and Contact (Figures 5E–5G). For each individual RA (Figure 5E) and SA (Figure 5F) cell, the mean spike frequencies during Contact were always significantly greater than during Whisking in Air ($p < 0.01$, Mann-Whitney U test on individual cells; n = 9 RA, 9 SA). Consistently, both RA and SA cells exhibited significantly shorter interspike intervals (ISI) during Contact than during Whisking in Air ($p < 0.001$, Mann-Whitney U test; data not shown). During Whisking in Air, RA cells had an average ISI of 129 ± 106 ms, ranging from 38 to 280 ms, while during Contact they had 30 ± 32 ms ISIs with a range from 1 to 50 ms. Likewise, during Whisking in Air, SA cells had an average ISI of 72 ± 62 ms, ranging from 36 to 232 ms, while during Contact they had 19 ± 10 ms ISIs with a range from 7 to 36 ms. These data show that during Contact both RA and SA cells are responding at a much faster rate than during Whisking in Air.

To compare the change in response from Whisking in Air to Contact for RA to that of SA cells, the mean spike frequency was calculated by averaging, on a single-cell basis, the spike frequency for all epochs of the same behavior. As a population, RA cells produced nearly a 20-fold greater response to Contact (109.6 ± 75.8) than to Whisking in Air (5.6 ± 8.4; Figure 5G). Conversely, SA cells produced only a 6.2 times greater response to Contact (99.4 ± 48.3) than to Whisking in Air (16.0 ± 13.7; Figure 5G). The percent change in spike frequency when

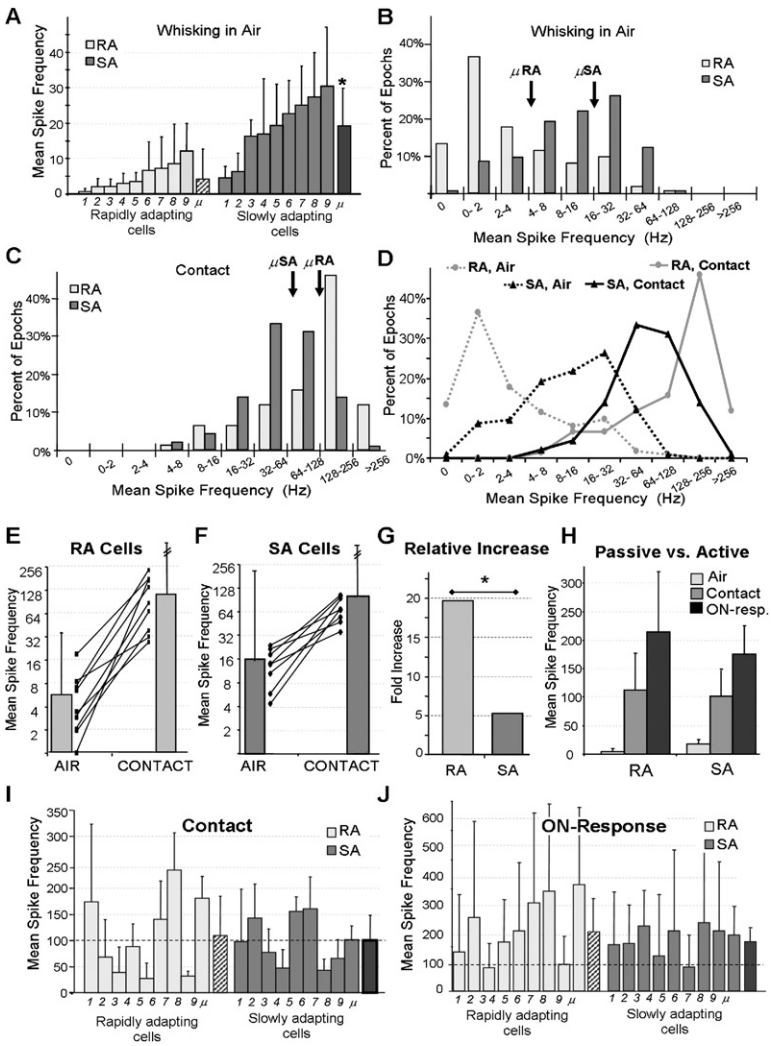


Figure 5. Comparison of Rapidly and Slowly Adapting Cell Activity during Different Behaviors

(A) The firing rate of most slowly adapting (SA) cells was greater than the firing rate of most rapidly adapting (RA) cells during Whisking in Air. The x axis shows cell number in order of increasing firing rate for RA (left) and SA (right). The columns marked μ show that SAs had a significantly greater firing rate than RAs during Whisking in Air. Distributions of firing rates during Whisking in Air (B) show RA cells centered on low firing rates while SA cells were broader, but during Contact (C) firing rates were similar for both. (D) Unique distributions of firing rates (x axis) emerge for RA (gray) and SA (black) cells during Whisking in Air (dashed) and Contact (solid) epochs. (E–G) Contact always elicited a significant increase in firing rates for both RA (E) and SA (F) compared to Whisking in Air. Lines connect mean spike frequencies during each behavior for the same cell. (G) RA cells had a 20-fold increase in firing rate, which was significantly greater than the 5-fold increase for SA cells. (H–J) Comparison of the firing rates during Whisking in Air, Contact, and the ON response during passive whisker stimulation in anesthetized rats. Each cell was capable of firing at a greater firing rate during the ON response (J) than during Contact (I). Note the different scales in (I) and (J). Note the different scales in (I) and (J). Error bars represent standard deviations.

transitioning from Whisking in Air to Contact for RA cells was significantly greater than the increase for SA cells ($p = 0.01$, Mann-Whitney U test; Figure 5G). This is not surprising given that during Whisking in Air the firing rate of SA cells is more than four times that of RA cells. These results suggest that RA and SA cells convey different information about Contact.

Finally to ensure that our stimulus during Contact did not produce an upper limit in the firing rate of any of the cells tested, for every cell the firing rates during its ON response to passive whisker deflection (sedated) and to Contact were compared (Figures 5H–5J). For each RA and SA cell, the firing rate during the ON response (Figure 5J) was significantly greater than the response during Contact (Figure 5I; paired t test, $p < 0.005$ for both), suggesting that the firing rates for Contact were not near the upper limit of the response capabilities of any of the cells.

Furthermore, the ON response of RA cells to passive whisker deflection was not significantly different than the ON response of SA cells to passive whisker deflection (Figure 5J). This result is consistent with our finding that there was no difference between the firing rate of RA and SA cells during Contact. In addition, because the ON response is a measure of passive contact of the whisker with an object under anesthesia, this measure is a good predictor of activity during the awake state.

In summary, both RA and SA cells significantly increased their firing rate during Contact compared to Whisking in Air, yet their firing rate during Contact was indistinguishable. However, because SA cells have a significantly greater firing rate during Whisking in Air, RA cells increased their firing more than SA cells when transitioning from Whisking in Air to Contact.

SA Fire More Spikes per Whisk Than RA

There were both similarities and differences between the activity of RA and SA cells ($n = 9$ each) during Whisking in Air. As a population, the firing rates of both were significantly correlated with whisk frequency ($r = 0.61$; $p < 0.001$; Figure 6A). Furthermore, their average number of spikes per whisk did not change with whisk frequency

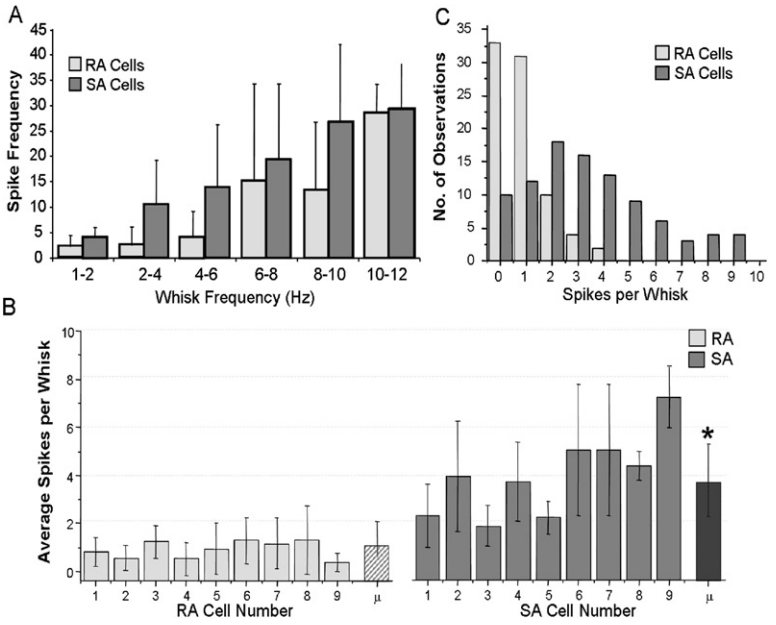


Figure 6. RA and SA Cell Responses during Whisking in Air

(A) There was a significant positive correlation of spike frequency to whisk frequency for RA ($r = 0.52$, $p < 0.001$) and SA ($r = 0.52$, $p < 0.001$) cells.

(B) Individually and as a group, SA cells had significantly greater number of spikes per whisk than RA cells (*$p < 0.001$, Mann-Whitney U test). Error bars are SD.

(C) Distributions show RA cells centered on few spikes per whisk, while SA cells were greater.

(Kolmogorov-Smirnov, $p = 0.9$; data not shown). However, SA cells always maintained a greater spike frequency than RA cells across whisk frequencies (Figure 6A). In fact, SA cells had on average significantly more spikes per whisk (3.5 ± 2.5) than RA cells (0.87 ± 0.3; $p < 0.001$, Mann-Whitney U test, Figures 6B–6C). Therefore, during episodes of Whisking in Air SA cells could potentially convey almost five times more information about the position or movement of the whisker than RA cells.

There was yet another difference between RA and SA cells. When the distribution of firing rates was examined on a per epoch basis, RA cells consistently fired at low frequencies, always less than four spikes per whisk but typically less than two, while the distribution of SA cell firing covered a broader range of spike frequencies, from very low, about one spike per whisk, to over seven (Figure 6D). Consequently, RA cells may have a consistent role when conveying information about whisking position, perhaps only firing to changes in direction or at a particular threshold of movement or position, while SA cells seem to have more diverse roles during Whisking in Air.

Further supporting this, the responses of individual cells were similar to the results of the population. Individual SA cells fired at a broad range of whisk frequencies, while all RA cells fired close to one spike per whisk. For example, the average number of spikes per whisk for RA cells ranged from 0.4 to 1.3 spikes per whisk, while for SA cells it was 1.4–7.0 (Figure 6B). On an individual cell basis, the firing rates of eight SA cells were well correlated to whisk frequency ($p < 0.05$) while spike frequency was well correlated to whisk frequency for only four RA cells. Therefore, the differences between RA and SA cell types may account for the distinction between populations of cells that are well correlated to whisk frequency and those cells whose firing rate is not well correlated.

Cells Time-Locked to a Phase of the Whisk

To evaluate the variability in the neuronal responses within individual whisks, neuronal activity was correlated to the position of the whisk during natural whisking for 435 whisks from ten cells (Table 1), whose kinematics (from full retraction to full protraction and back to retraction) could be clearly seen using frame-by-frame video analysis (5 ms frames). Whisks lasted on average 142 ± 44 ms, consisting of a longer protraction (81 ± 23 ms) than retraction (61 ± 22 ms). As expected in natural whisking, there was considerable variation in the duration of each individual whisk. Perievent rasters were created with the trials sorted on the duration of the whisk, from shortest to longest (Figures 7 and 8), to examine the timing of each spike relative to the position of the whisker.

All cells showed considerable variability in their firing patterns during individual whisks, likely due to changes in whisk speed and direction that occur during natural whisking behaviors in addition to the position or trajectory of the whisker that could not be accounted for in this study. Every cell exhibited at least one whisk when it did not fire a spike, and it was possible to identify epochs of whisking during which a cell fired to less than 90% of individual whisks (Figure 8E). Furthermore, while each cell could be observed to fire in a burst, bursts were not consistent across trials, and no systematic bursting activity was observed.

Because of differences in individual whisk durations, all spikes were aligned by whisker position. Perievent position histograms were generated around full retraction or full protraction to evaluate whether a cell was more likely to fire during particular phases of the whisk. A subpopulation of both RA and SA cells were well time-locked to a particular phase (Table 1), suggesting that they encode the position of the whisker in space. Also, the timing of their response corresponded to their preferred direction in

Table 1. Summary of Whisk Kinematic Data

Cell	Number of Whisks	Number of Whisks with No Spikes (% Total)		Time-Locked	Type	PW
7A	32	15	46.9%	yes	RA	B4
7B	96	63	65.6%	yes	SA	A2
7C	17	5	29.4%	yes	RA	A4
7D	17	1	5.9%	yes	SA	B3
8A	96	66	68.8%	yes	—	—
8B	96	9	9.4%	yes	SA	A4
8C	31	17	54.8%		RA	A4
8D	17	8	47.1%		SA	C4
8E	19	17	89.5%		RA	D5
8F	14	8	57.1%		—	—

Data for Figures 7 and 8. The number of individual whisks that did not elicit a spike ranged from 6% to 90% of the total number of whisks analyzed for that particular cell, illustrating a per whisk variability in the spike frequency for each cell that is independent of cell type. Time-locking indicates that these cells had a significant increase in spike probability during a particular phase of the whisk (Kolmogorov-Smirnov test, $p < 0.05$).

passive stimulation. For example, most cells were activated during passive stimulation to movement of the whisker in the rostral plane, delivering the stimulus to the caudal aspect of the follicle, which would occur in natural whisking when the whisker began to retract from a protracted state. This phenomenon was commonplace in our data set and is consistent with the notion that the firing of a particular cell to its preferred stimulus reliably encodes for that stimulus (Kyriazi et al., 1994; Shoykhet et al., 2000; Minnery and Simons, 2003).

Conversely, cells exhibited other response profiles during whisk cycles, including sporadic firing throughout and across individual whisks (Figures 8C–8F), time-locking to initial protraction (Figure 8A), and tonic responses during retraction (Figure 8B). Interestingly, during artificial whisking Szwed et al. (2003) also saw a minority of cells that had tonic responses. Therefore, it is likely that, while most cells respond phasically to whisker stimulation, some cells respond with more tonic activity.

In summary, during awake, natural whisking behaviors certain RA and SA cells are better time-locked to a particular phase of the whisk. Nevertheless, despite the fact that significant phasic or tonic responses could be identified for these cells, the consistency of their response was much less than that seen under anesthetized conditions or artificial whisking. It is likely that, during natural whisking, the rat employs several whisking strategies that modulate the responses of single cells to optimally encode information regarding its surroundings.

DISCUSSION

We chronically recorded Vg neurons in awake, freely moving rats and correlated these signals to natural whisking behaviors to determine the information being encoded and if the traditional classification of cell types (RA and SA) is relevant in awake rats. We conclude that (1) no Vg cells had activity when the whiskers were at Rest, (2) all cells had significant activity during Whisking in Air that was correlated with whisk frequency, (3) all cells increased firing rate when whiskers contacted an object, (4) no cells fired only during whisking or contact but rather all responded to both, (5) spatial information is conveyed by certain neurons firing to a particular phase of the whisk, and (6) the response properties of RA and SA cells are distinctly different during awake behaviors. These results suggest that at the primary level of processing all cells provide information about the movement of whiskers in air and are capable of encoding information regarding contact with an object. Information processing at the Vg and how it is modulated during natural whisking behaviors is important because this information influences all subsequent processing in the trigeminal system.

Vg Cells Encode Features of Whisker Movement

Vg cells encode for the frequency and phase of movement, providing important insight into understanding neuronal activity in higher brain centers (e.g., VPM thalamus and whisker barrel cortex). For example, that there is no activity from Vg cells when the whiskers are not moving clarifies the origin of baseline neuronal activity in trigeminal brain nuclei of freely moving rats in the absence of whisker movement. This activity does not arise from spontaneous activity of primary afferents and must, therefore, be firing of cells within the trigeminal brain nuclei or arise from other brain centers outside the trigeminal system.

Another insight arises from the fact that all Vg cells are capable of significant neuronal activity when the whiskers are moving in air. Despite the likelihood of changes in whisk parameters and physiological mechanisms, the number of spikes per whisk is relatively constant, and spike frequency tends to increase as whisk frequency

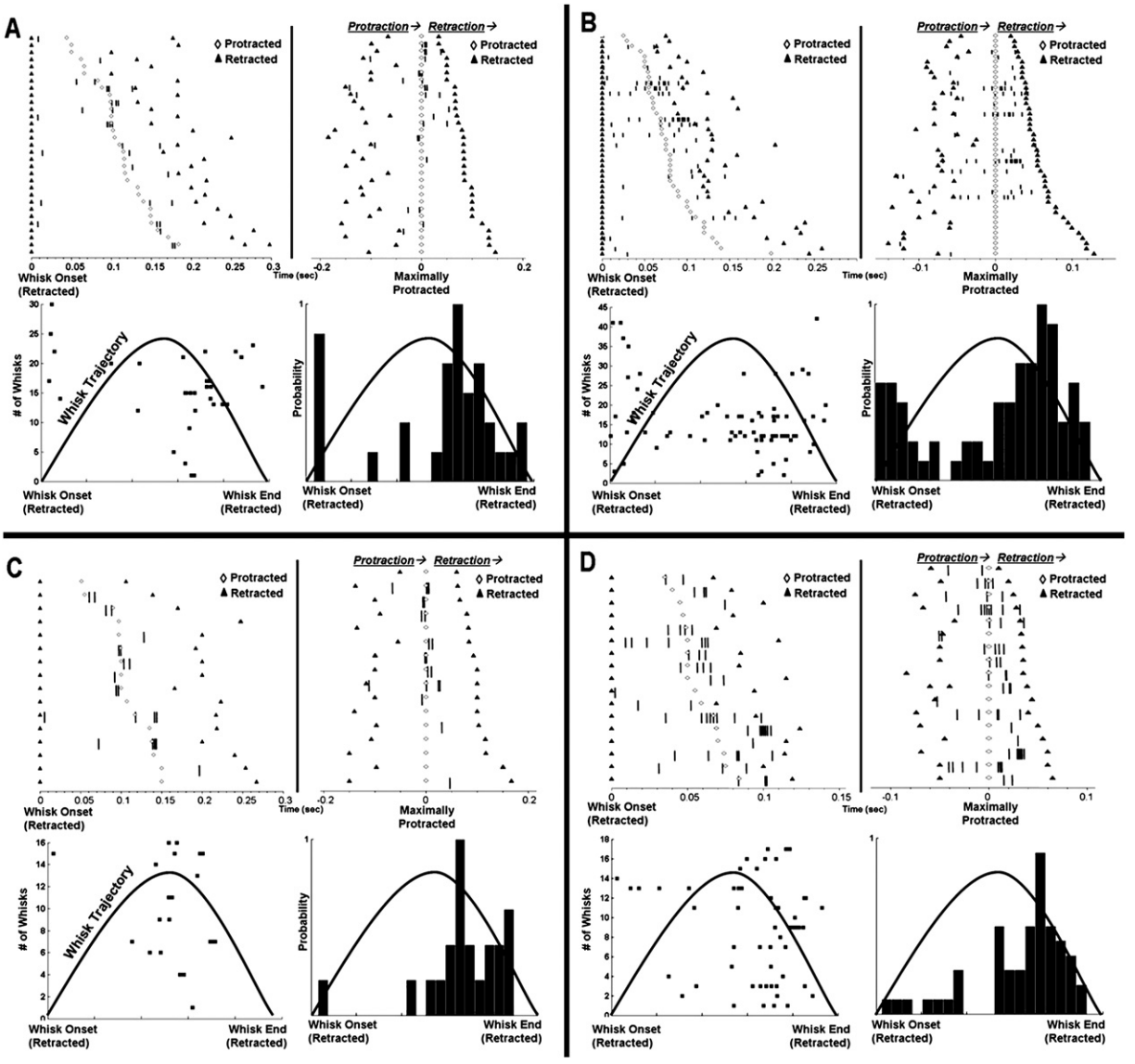


Figure 7. Time-Locked Neuronal Activity across Individual Whisks

Kinematics were used to evaluate activity during different phases of the whisk and across whisks. Each panel represents one cell and shows raster plots of spike firing during individual whisks (y axis) aligned by whisk onset (top left) or centered on full protraction (top right). Closed triangles represent full retraction (whisk onset and end). Open diamonds represent full protraction (middle of whisk). Plots are ordered based on protraction (top left) or retraction (top right) time. Bottom panels are raster plots (left) or perievent position histograms (right) of each whisk (y axis) to a normalized timescale from whisk onset to end represented by the curved line. Most whisks elicited spikes, but every cell had at least one whisk that did not (see Table 1). Both RA (A and C) and SA (B and D) cells were well time-locked to retraction-from-protracted phase of the whisk, suggesting that they encode the position of the whisker in space.

increases. Whether from the force against the opposing air or by the underlying muscles, the whisker moves against nerve endings with sufficient force to generate action potentials. Therefore, the increase in neuronal activity known to occur in the VPM when the rat transitions from rest to whisking (Fanselow et al., 2001) is likely due to increased activation of the trigeminal system originating in the Vg.

Finally, our data show that Vg cells can encode for the position of the whisker in space. This knowledge is important for the rat to predict the relative position of its vibrissa and to transform information about an object in head-centered coordinates. Yet, our data imply that the ability to encode whisker position is state dependent and that small changes in whisk kinematics produce different responses from Vg cells.

Our findings support the theory that at the neural ensemble level precise spike timing conveys sufficient information for perception of complex tactile features by

downstream neuronal circuits (deCharms and Merzenich, 1996; Nicolelis et al., 1995, 2003; Ghazanfar et al., 2000; Foffani and Moxon, 2004; Jones et al., 2004a, 2004b; Foffani et al., 2004). In fact, each cell, RA and SA, even if activated under different contexts, can encode and convey several types of information (spatial reference, whisk frequency, contact), which implies a level of information processing that is rich considering these are primary sensory afferents. These ideas are also supported by the conclusion of Zucker and Welker (1969) and Gibson and Welker (1983a, 1983b), which state that a population of Vg neurons is capable of encoding many parameters of the stimulus delivered to the vibrissae.

RA and SA Cells

There are several important differences between RA and SA cells' responses. (1) During Whisking in Air, SA cells fire nearly three times more spikes per whisk than RA cells, consistent with data from anesthetized rats showing that RA cells fire to onset of whisker movement and SA cells fire during the entire movement period. (2) Although all cells increase their firing rate during contact compared to whisking in air, RA cells increased more than five times SA cells. (3) During Contact, RA and SA cells had similar firing rates, consistent with Jones et al. (2004a, 2004b), who found no differences between their responses to contact under anesthesia and that there are no differences between their ON responses during passive whisker deflection.

These results suggest that the distinction between RA and SA cells is important in understanding the neuronal responses from the Vg and their influence on higher brain regions and further that these important differences can only be viewed when the cells were recorded during natural whisking conditions and active touch.

Classifying Vg Cells by Response Properties

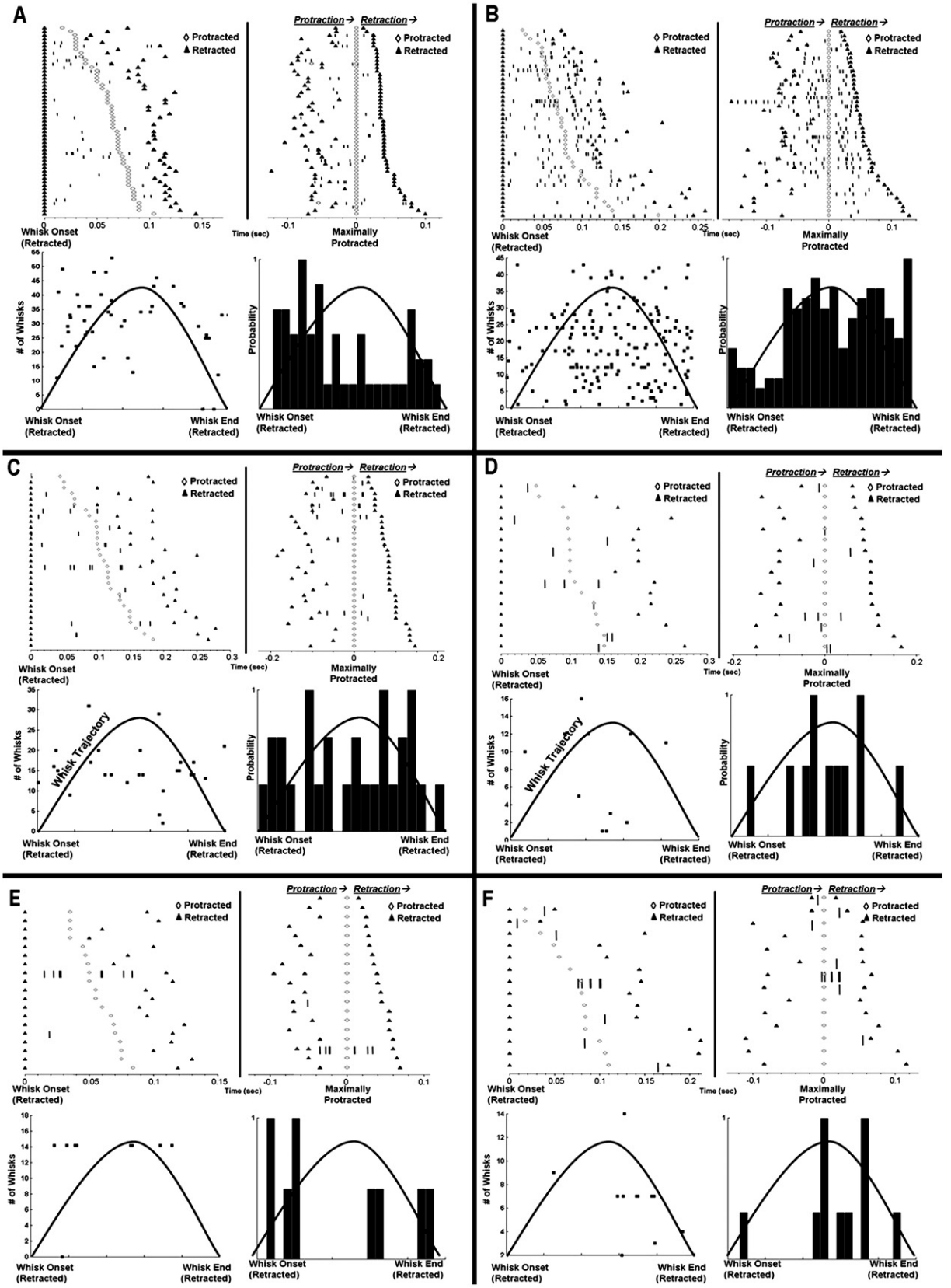
Recording from freely moving rats allows examination of neuronal activity under a broader and potentially more functionally relevant behavior than recording during anesthetized conditions. We intentionally did not restrain the rat nor induce artificial stimuli to control whisk kinematics. As a result most cells recorded here exhibited a broad range of response patterns. For example every cell fired during Whisking in Air but some cells demonstrated periods with no spikes lasting up to 4.5 s. Therefore, there are conditions under which Vg cells can demonstrate no activity during Whisking in Air followed by an increase in firing during Contact. In fact, this was described by Szwed et al. (2003) using fictive whisking and contact with a fixed object in sedated rats. They classified these cells as "Touch Cells" defined by not responding during whisks but responding only when the whisker touched an object.

However, in our study every cell demonstrated an ability to fire during whisking in air. The natural variability of whisker movements employed by awake rats, likely unavailable in fictive whisking, and the variability of stimulus parameters and thresholds required for activating each unit could explain the presence of pure "Touch Cells" under anesthetized, fictive whisking conditions and some of our data during natural whisking. Possible systematic errors derived from artificial whisking as opposed to smooth protraction in "normal" whisking may be a source of such discrepancy. Therefore, there are behavioral conditions under which cells temporarily act like "Touch Cells," not responding during whisking in air, but the fact that all cells respond during whisking in air and contact in the awake rat precludes the possibility of classifying Vg neurons as "Touch Cells."

It is possible that Szwed et al. (2003) identified "Touch Cells" because the stimuli during the whisking phase may have been under the required threshold to drive the activity of those cells while the stimulus during contact was sufficient. Vg cells have a broad range of deflection thresholds required for activation (Zucker and Welker, 1969; Gibson and Welker, 1983a, 1983b) defined by stimulus parameters including direction, amplitude, and velocity (Lichtenstein et al., 1990; Carvell and Simons, 1990, 1995; Shoykhet et al., 2000; Sachdev et al., 2001, 2002; Bermejo et al., 2002; Berg and Kleinfeld, 2003a, 2003b; Minnery and Simons, 2003; Arabzadeh et al., 2005; Kleinfeld et al., 2006). This is likely the reason why Szwed et al. (2003) identified "High-Threshold Cells," which responded to passively applied rapid deflections but not to contact or whisking.

A second example of variability in responses is seen in the different firing patterns of cells during Contact. Our data demonstrate that all cells increase firing from Whisking in Air to Contact, consistent with Jones et al. (2004a, 2004b) who found that every Vg cell reliably fired to noisy stimuli applied to single whiskers that simulated complex active touch in sedated rats. Yet, we show that during natural whisking four cells had few epochs of Whisking in Air that were greater than the firing rate during Contact. This is an important property previously documented by Szwed et al. (2003), who classified cells that fired during whisking but not during contact as "Whisking Cells." However, during natural whisking conditions, we show that every cell had greater responses in most of their epochs of Contact than during Whisking in Air. Therefore, we were unable to isolate pure "Whisking Cells."

There are a few reasons why under their experimental conditions Szwed et al. (2003) were able to identify "Whisking Cells" and we were not. (1) We selected robust epochs of contact (see Experimental Procedures) so that all whiskers were stimulated sufficiently to meet the threshold for activation for all cells. (2) We observed a greater range of behaviors during contact, including changes in head angle relative to the object and sustained or repeated contact of whiskers on surfaces as the rat moved. (3) The awake rat has several physiological mechanisms that may modify the threshold needed for activating receptors within the whisker follicle. Because the magnitude and direction of the stimulus parameters dictates the probability of activating a receptor, the conditions of fictive whisking past a stationary object may have been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Time (sec)
Maximally Protracted
# of Whisks
Probability
Whisk End (Retracted)
Whisk Trajectory

insufficient to further activate certain cells. These factors contribute to the variability of the response we observed and the fact that we could observe cells that temporarily behaved like "Whisking Cells" but were able to respond to contact under certain conditions.

Finally, while it is possible that we did not record from cells that would be classified as "Whisking Cells," this is unlikely. Because our electrode placement was determined by whisker stimulation, it is possible that there was a preferential selection for neurons that responded to stimuli that mimic Contact. However, many cells were identified for the first time in the days following the implant, and none of these demonstrated firing patterns that were consistent with those of "Whisking Cells."

It should be mentioned that Szwed et al. (2003) observed "Whisking/Touch Cells" that responded when the whisker touched an object and to whisking itself. All cells recorded here during natural whisking had this property.

Role of Response Variability in Encoding Sensory Information

An important insight gained from these data is that during awake freely moving conditions physiological mechanisms can continuously modulate the responsiveness of Vg cells, offering greater response variability than conditions under anesthesia. For example, changing blood flow to the sinuses within whisker follicles (Ebara et al., 2002) can modulate biomechanical parameters that affect damping of the vibrissae and thus the response from receptors within the follicle. This can be in a time course that varies with the phase of a whisk (Hartmann et al., 2003) and dependent on parameters of movement (i.e., decreasing receptor isolation for increased acceleration; Szwed et al., 2003). Also, the type of receptor and its location in the whisker follicle complex modulate the responsiveness of Vg neurons (Rice et al., 1986; Waite and Jacquin, 1992; Mosconi et al., 1993; Ebara et al., 2002). We could not identify the receptor type or position within the whisker follicle, thereby adding variability to our study.

Another source of variability arises from recent studies that suggest that neuropeptides are released from Merkel cells during mechanical reception and that these modulate neural actions of the sensory neurons (Diamond et al., 1988; Haeberle et al., 2004; Hitchcock et al., 2004; Tachibana and Nawa, 2005; Cahusac and Senok, 2006). Given these findings, perhaps mechanosensory signals are modified prior to the Vg. If so, this could account for some of the variability seen in our data and differences in our data compared to activity of Vg cells recorded during anesthetized conditions.

Finally, the mechanisms listed above can change the parameters needed to reach the threshold required for activation. Under the conditions here, it was not possible to control for these, and whiskers moved with varying amplitude, velocity, and direction often simultaneously. Because the rat continuously moved its head, we could not measure whisk angle, distance traveled, or speed of the whisker and were thus unable to explore the effects of these parameters on neuronal responsiveness.

Thus, in awake freely moving rats we observe a range of responses that is broader than those seen in anesthetized studies that only examine a limited, controlled set of movements and conditions that are not identical to natural whisking. We conclude that the differences we see in neuronal activity are likely related to the variability of whisk parameters and the physiological state of the whisker follicle in awake rats. To know specifically how this variability affects Vg responses, one must in the future control whisking parameters independently (Knutsen et al., 2005; Arabzadeh et al., 2005; Bermejo et al., 2005; Stuttgen et al., 2006; Ferezou et al., 2006; Rajan et al., 2006) because it is obvious that freely moving rats employ a broad range of whisker movements during natural whisking to maximize the probability of activating many cells and thus optimize the ability to encode the features of the environment.

EXPERIMENTAL PROCEDURES

Chronic Implants

Detailed methodology regarding surgical preparation, recording strategies, and data analyses have been described previously (Leiser and Moxon, 2006; Devilbiss and Waterhouse, 2002). Briefly, adult male Long-Evans rats (230–280 g) were obtained from Harlan (Indianapolis, IN) and prepped for implantation of one or two microelectrodes in the trigeminal ganglion (Vg) as follows. All procedures followed NIH and Drexel University IACUC guidelines. Rats were anesthetized using an i.p. injection of sodium pentobarbital (45 mg/Kg), and an electrode was implanted in the vibrissae-responsive region of the Vg (0.5–2.5 mm posterior and 1.5–2.5 mm lateral from Bregma and 9–10 mm ventral from dura; Leiser and Moxon, 2006) (Figure 1A).

Employing the appropriate type of electrode is critical for the length and reliability of recording single-neuron activity from chronic implants (Hubel, 1957; Moxon and Chapin, 2000; Moxon et al., 2004). Because the electrode must pass through the brain and penetrate a thick dural layer surrounding the ganglion, a high-impedance (10 MΩ) epoxylite-insulated tungsten microelectrode with 250 μm shank diameter and sharp tip (UEWSGGSE0N1E, FHC, Bowdoinham ME) was used (Figure 1B). Extracellular recordings were continuously performed, while the electrode was lowered through the brain. Signals were amplified and band-pass filtered (154 Hz to 13 kHz) by conventional means (Nicolelis et al., 1995; Chapin et al., 1999), and analog signals were digitized at 40 kHz (MNAP System, Plexon Inc., Dallas, TX). When spikes of a single discriminable neuron were elicited in response to vibrissal stimulation, the electrode was secured in place using methyl methacrylate. This procedure was repeated for bilateral implants.

Behavioral Chamber and Video

For all recordings, the rat was placed in a 2′ × 3′ chamber based on previous studies (Kao et al., 2006) in an isolated room (5′ × 5′) to prevent changes in air flow. The chamber was constructed of white

Figure 8. Variable Neuronal Activity across Individual Whisks

Activity during different phases of the whisk and across whisks as in Figure 7 shows that a cell (A) was time-locked to initial protraction, an SA cell (B) fired tonically over part of protraction and during retraction, and both RA and SA cells (C–F) did not exhibit phase-dependent activity but rather fired sporadically during the whisk cycle.

melamine and coated in a nonreflective, textured, white absorbent liner (Fisherbrand 14-127-47) with raised crosshatches (2 × 2 cm) every 2 cm to provide a rough surface for whisker contact. Secondary walls of the same material were placed inside the chamber before the rat to increase novelty and encourage contact epochs. These were not moved until the rat was removed. No other objects were placed in the chamber at any time. A high-speed CCD video camera (HSC-250x2, JC Labs, Mtn View, CA) was positioned aloft to capture at high resolution (765 × 246 pixels) 10% of the chamber at 200 pictures per second.

Neural signals and synchronized high-speed video were recorded simultaneously. A time text inserter (GL-250) overlaid time in milliseconds on the stored video (HSR-200 VCR). This clock was reset by the Plexon system to synchronize the video with neural data. Each recording session lasted less than 25 min (average 6.5 min). Because the drift of the MNAP clock and VCR's time stamp is less than 1 min per year (JC Labs), no drift in our video and neuronal times occurred.

Neural Recordings and Single-Unit Separation

The rat was connected to the MNAP system, and single neurons were discriminated and then recorded while the rat moved freely. Real-time spike-sorting software (SortClient, Plexon Inc, Dallas, TX) captured action-potential waveform segments around a voltage threshold crossing and sorted them by shape. Template matching and principal components guaranteed clear separation between units before recording started (Figure 1C) (Chapin and Nicolelis, 1999; Devilbiss and Waterhouse, 2002; Foffani and Moxon, 2004; Leiser and Moxon, 2006). All waveforms were saved for offline analyses.

To ensure single-unit separation (i.e., prevent activity of neighboring cells from mixing with the activity of the principal neuron), the saved waveforms for the duration of each cells' recording session were analyzed. First, single units were aligned at the point of threshold crossing and tested for changes in waveform shape and clustering of principle components (Offline Sorter v2.8 and WaveTracker, Plexon Inc, Dallas, TX). There were no changes in waveform shape during different behaviors in the same awake recording session (Figure 1D). Only cells with no significant changes (F-statistic, WaveTracker) in their waveform shape or PCA collected during Whisking in Air and Contact were included in analyses. Secondly, autocorrelations or interspike interval (ISI) histograms were generated for each cell to ensure that no spikes occurred within the cell's refractory period (Figure 1F). Vg cells have been shown to follow stimuli up to 1500 Hz (Gottschaldt and Vahle-Hinz, 1981). Consistent with this, no cells fired within 0.67 ms. In fact, during Whisking in Air few cells (<2% of the intervals recorded) had an ISI less than 1 ms. This ensured reliability in our unit isolation and single-unit separation (Nicolelis et al., 2003).

Classification of Cells

At the end of recording sessions, rats were anesthetized and each whisker was stimulated to identify the cell's principle whisker (PW) and preferred direction (Shoykhet et al., 2000; Shetty et al., 2003; Leiser and Moxon, 2006). Cells were rediscriminated after each recording. To ensure that all cells were unique, cells were defined by their PW and waveform shape, and cells from the same rat were considered different cells only if they had a different PW.

A subset of cells (n = 18) were classified as either RA or SA (n = 9 each) (Figure 1E). For this, each whisker was deflected with a precision stepper motor (Gemini GV6) controlled by a servo drive (Compumotor, Rohnert Park, CA). Stimulation parameters were kept similar to previous studies (Shoykhet et al., 2000). Whiskers were held deflected 500 ms (ramp-and-hold) at a rate of 0.5 Hz in the units' preferred direction 75 to 100 times. A TTL pulse was sent to the MNAP hardware to indicate the onset of stimulation. Spike times of each cell were converted to peristimulus time histograms (PSTHs) with 1 ms bins. Spontaneous firing was measured over 100 ms prior to deflection and responses to sustained whisker deflection (PLATEAU) during the middle 100 ms of the response. Neurons were included in the analyses if their ON responses (first 25 ms after stimulus) exceeded spontaneous activity. A neuron was classified as SA (n = 9) if the PLATEAU response significantly exceeded spontaneous firing (Mann-Whitney U test, $p < 0.05$). All other neurons were classified as RA (n = 9). Note that using the Student's t test with parameters identical to those used in previous studies (Lichtenstein et al., 1990; Shoykhet et al., 2000) yielded no differences in classification.

Active Behaviors

Three common behaviors of the rat were selected for analyses: Rest, Whisking in Air, and Contact. First, we emphasize the following prescripts: (1) only at times when a clear high-resolution image of the whiskers could be seen on the video were the data subsequently analyzed, (2) an epoch was identified as a continuous period of time containing a single behavior, (3) the behavior of the rat did not change during an epoch, (4) any epoch of behavior that was not completely identifiable into the three categories was removed, (5) micromovements or twitching of the vibrissae were not included in any analysis, and (6) the minimum detectable whisker movement was 10° (1/3 normal whisk) but depended on the angle of the rat's head. Therefore, only a fraction of recording times were analyzed, limiting the number of cells for quantitative analyses to 27.

Rest was defined by any time the rat was not moving and not whisking. Whisking in Air was defined as any time the rat actively moved its whiskers in the air, whether sitting still or actively exploring, and no part of the whiskers contacted any surfaces of the chamber or floor (Figure 2B). Contact was defined as any time the whiskers came in contact with walls *and* the mystacial pad was within 10 mm of but not touching the wall, ensuring gross bending of the whiskers likely sufficient for activation of each cell (Figure 2C). We did not include times of touch that occurred with only whisker tips. It was not possible to identify whisks during Contact. Contact epochs included when the rat whisked the wall from a stationary position and while locomoting. There was no significant difference ($p > 0.05$, Kolmogorov-Smirnov test) in the firing rates of each cell to these two types of Contact, so they were grouped. Contact was only compared to Whisking in Air and, due to the limitation of this study, was not used to detect differences in contact types or texture discrimination.

Active Whisking

Whisk frequency (WF) was defined as the number of whisks divided by the time of each epoch. Similar to Berg and Kleinfeld (2003a, 2003b), who found that WF does not change during a bout of whisking, we observed that WF did not change by more than 1 Hz during any Whisking in Air epoch.

Despite that whisking parameters were not controlled but rather the rat was allowed to whisk naturally, the frequency of whisking observed in our study was consistent with others. A review of the literature shows that active WF ranges from 1 to 20 Hz (Carvell and Simons, 1990) (3–20 Hz; Gao et al., 2001) but is typically 5–12 Hz (Welker, 1964; Garabedian et al., 2003) (6–9 Hz; Carvell and Simons, 1990, 1995; Ahissar et al., 2000) (5–10 Hz; Berg and Kleinfeld, 2003a, 2003b).

Moreover, it has been observed that exploratory whisking consists of large-amplitude, low-frequency whisking around 4–6 Hz, and small-amplitude, high-frequency whisker-twitching is around 7–12 Hz (Fanselow et al., 2001) (7 Hz and 9 Hz, respectively; Semba and Egger, 1986). Yet, WF can be modified as the result of learning (Carvell and Simons, 1990; Harvey et al., 2001), can vary during sampling epochs (Berg and Kleinfeld, 2003a, 2003b), and can change during contact (Sachdev et al., 2001, 2002).

Data Analyses

First, every cell (n = 80) was tested to determine if it increased its firing rate as it transitioned from Rest to Whisking in Air to Contact. Then, to quantitatively assess how behavior modulated the firing rate of these cells, we selected only those cells that exhibited (1) epochs of Whisking in Air that provided a WF range of >5 Hz across epochs within a range

of 1–12 Hz, (2) a minimum of five epochs of Rest, (3) a minimum of five epochs of Contact, and (4) all of these requirements within the same awake recording session. Only 27 cells of the 80 met these criteria.

Three analyses were performed on this subset. First, the average firing rate of cells was calculated by averaging the spike frequency of each cell during all epochs of the same behavior. Spike frequency was defined as the total number of spikes divided by the total time of an epoch. Comparisons of the average firing rate were made across different behaviors, between different cell types (RA or SA), and between cells during the same behavior. A Mann-Whitney U test was used to evaluate significant difference at a $p < 0.05$ level (Statistica, StatSoft).

Next, we tested whether spike frequency was correlated to whisk frequency and was performed for Whisking in Air epochs only. A regression analysis with fit through zero was used to evaluate the significance of the correlation (Origin, Microcal). The fit through zero was chosen because no spikes occurred when the rat did not whisk. Note there was no significant difference in the number of epochs for each whisk frequency category (Hz) for RA and SA cells (2×7 contingency table, Chi-square test, $p > 0.05$).

Finally, we tested whether cells were more likely to fire during a particular phase of the whisk. For this analysis we used whisks that occurred under the following conditions: (1) the rat performed whisks within view of the camera, (2) the rat had little or no head movements, and (3) the position of the individual whisks could be seen for a full whisk cycle. This limited our data set to 435 whisks (n = 10 cells). These whisks were not necessarily consecutive or taken from the same epoch. Whisker position was compared to spike times. Kinematics of individual whisks were evaluated by frame-by-frame video analysis with 5 ms resolution (Figure 2B). An individual whisk was defined by two phases: (1) protraction from an actively retracted position to a maximally protracted position and (2) retraction from that fully extended position back to a retracted position. Three time points for each whisk were identified and consisted of (1) the onset of a whisk from its retracted position, (2) the middle of the whisk (maximum protraction), and (3) the termination of the whisk (retracted). PSTHs and perievent rasters were then sorted on the duration of the whisk to examine the relationship between spike times and whisker position. Due to the variability of whisk duration and because we wanted to compare neural activity from many whisks, we normalized the position of each whisk on a scale from onset (0) to termination (1), where time 0.5 equaled maximum protraction, and generated peristimulus position histograms and raster plots for visualization of spike activity for each whisk (Figures 7 and 8). To identify cells that had a significant increase in spike probability during a particular phase of the whisk, a Kolmogorov-Smirnov test for uniformity was performed on the frequency of spikes (normalized histogram, 20 bins) and tested against a uniform distribution. A significant p value ($p < 0.05$) meant that the data was not uniform, indicating that the cell had a particular period of the whisk that had more spikes than others; these cells were considered to be phase locked.

Histology

As described previously (Leiser and Moxon, 2006), recording sites were marked by electrolytic lesions (unipolar 30 μA, 20 s) and verified by Nissl stain in fixed ganglions (30 μm coronal sections). Lesions were clearly visible and always located in the region of the Vg targeted.

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