Intrinsically Bursting Olfactory Receptor Neurons

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Bobkov YV, Ache BW. Intrinsically bursting olfactory receptor neurons. J Neurophysiol 97: 1052–1057, 2007. First published November 29, 2006; doi:10.1152/jn.01111.2006. Rhythmically bursting neurons are fundamental to neuronal network function but typically are not considered in the context of primary sensory signaling. We now report intrinsically bursting lobster primary olfactory receptor neurons that respond to odors with a phase-dependent burst of action potentials. Rhythmic odor input as might be generated by sniffing entrains the intrinsic bursting rhythm in a concentration-dependent manner and presumably synchronizes the ensemble of bursting cells. We suggest such intrinsically bursting olfactory receptor cells provide a novel way for encoding odor information.

INTRODUCTION

Rhythmically oscillating or “bursting” neurons are known to be fundamental to neuronal network function in both vertebrates and invertebrates (Buzsáki and Draguhn 2004; Marder et al. 2005; Ramirez et al. 2004), including neural networks associated with sensory processing (Hayar et al. 2004a, b; Krahe and Gabbiani 2004; Zheng et al. 2006). Although there is evidence for repetitive bursting in several types of primary sensory neurons (papers cited in Wiederhold and Carpenter 1982), including thermoreceptors (Iggo 1969), electroreceptors (Braun et al. 1994), and crustacean proprioceptors (Birmingham et al. 1999; Combes et al. 1997), the idea that bursting primary sensory neurons may play a fundamental role in sensory coding has been considerably less well studied, particularly with respect to olfaction. Reports of bursting discharge in primary olfactory receptor neurons (ORNs) have appeared in the literature for many years but have largely been ignored, probably stemming in part from the early association of prolonged bursting discharge with dying or strongly overstimulated cells (Ache, personal knowledge). Yet, more recent evidence for conditional oscillations in amphibian and mammalian ORNs (Frings and Lindemann 1988; Reisert and Matthews 2001a, b) and the rhythmic bursting discharge seen in mammalian vomeronasal receptor cells (e.g., Holy et al. 2000) suggest that the question of rhythmic discharge in primary ORNs be revisited. Is there any evidence, for example, that bursting discharge can be inherent in ORNs and, if so, does bursting discharge at least have the potential for encoding information about odorants? Here, we address this question in lobster ORNs, an established animal model for olfactory research (e.g., Ache and Derby 1985). We show that intrinsically bursting ORNs coexist along with the more traditional tonically discharging ORNs in the lobster olfactory organ and that the spontaneous bursting rhythm of these cells can be modulated by odorants in a concentration-dependent manner. We argue that odor input from bursting ORNs together with input from tonically discharging ORNs provide a novel means for encoding odor information.

METHODS

Preparation

Primary lobster (Panulirus argus) ORNs were studied in situ using single annuli sectioned from the filamentous olfactory organ (antennule) mounted in the bottom of a 35-mm plastic culture dish filled with Panulirus saline (PS; see Solutions and odors). The preparation was arranged such that one of two superfusion streams bathed the somata of the ORNs with PS (about 1 ml/min), whereas the other stream independently bathed the olfactory sensilla (aesthetascs) that contain the outer dendrites of the ORNs with either PS or PS + odorant (about 1 ml/min; see Solutions and odors). The stream bathing the outer dendrites could be switched briefly from PS to PS + odorant using a stepper motor–driven rapid solution changer (RSC-160, Bio-Logic Instruments, Claix, France). The duration of the odorant pulse delivered to the outer dendrites was increased in steps from 40 to 800 ms to change the intensity of the odorant. Stimuli were always applied randomly, independent of any ongoing action potentials or bursts of action potentials. ORNs recorded in this configuration were viable and remained responsive to odorants for many hours with little or no evidence of decline in responsiveness over the recording period.

Recording

Except where noted otherwise, action potentials (spikes) were recorded from ORNs extracellularly using loose-patch recording. Patch electrodes were pulled from borosilicate capillary glass (Sutter Instrument) and filled with PS. Resistance of the electrodes was 2.07 ± 0.33 MΩ (n = 67). Currents were measured with an Axopatch 200B patch-clamp amplifier (Axon Instruments) using an AD–DA converter (Digidata 1320A, Axon Instruments), low-pass filtered at 5 kHz, sampled at 5–20 kHz. Data were collected and analyzed with pCLAMP 8.1/9.2 software (Axon Instruments) and SigmaPlot 8/9.0 (SPSS). When necessary, multiunit recordings were sorted into individual unit recordings using the template search procedure provided in pCLAMP 9.0 software. The time of occurrence of the spike was taken as the time of peak current deflection, i.e., the peak of the spike. To detect and analyze bursts we used the interval burst analysis protocol provided in pCLAMP 9.0 software. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
BURSTING OLFACTORY NEURONS

Solutions and odor

PS (tryptophan powder/ml of PS).

Solutions and odor

For detailed analysis was obtained from a different animal. All recordings were performed at room temperature (about 21°C).

RESULTS

Of 447 ORNs surveyed, most showed one of two distinct patterns of spontaneous and odorant-evoked discharge. The predominant pattern of responsiveness, which included roughly 70% of the cells surveyed, was for the cells to be tonically active between 0.1 and 8.3 Hz (mean = 2.8 ± 0.1, n = 318) and gradually increase their ongoing rate of discharge in a concentration-dependent manner in response to odorant stimulation. The discharge pattern of one such cell is shown in Fig. 1A to provide a basis of comparison for the other pattern of discharge that is the main focus of the paper (see following text). Because the tonically discharging cells are of the type usually reported for this animal for some years (e.g., Bobkov and Ache 2005) they are not considered further here other than to note that in the present recording configuration these cells showed a striking constancy in their response pattern to any given stimulus concentration (Fig. 1A). Our ability to create a recording configuration that for the first time evoked such highly consistent responses from the ORNs over many hours presumably enhanced our ability to clearly identify cells that deviated from this pattern of responsiveness.

In contrast to the tonically discharging cells, most of the remaining cells showed spontaneous, rhythmic bursts of action potentials, and responded to odorant stimulation with an evoked burst, although the spontaneous bursting frequency and the structure of the bursts varied across different cells. The discharge pattern of two such cells illustrates the diversity inherent among cells of this type (Fig. 1B). The spontaneous bursting frequency of the different cells ranged from 0.02 to 0.9 bursts/s (mean = 0.22 ± 0.02, n = 92). It is important to note that although different cells had different spontaneous bursting frequencies and burst structure, the frequency of spontaneous bursting and the burst structure for any given cell was consistent, including showing little or no decrement over the period of several hours during which many cells were studied (e.g., Fig. 2A). Nor were cells observed to transition to or from the tonic pattern of discharge described above. Cells showing bursting discharge co-localized with those showing tonic discharge because cells showing both patterns of response could be recorded from the same cluster of ORN somata that innervated a single olfactory (aesthetasc) sensillum.

Bursting appeared to be intrinsic in the cells. Spontaneous bursting discharge was observed in acutely dissociated cells. Bursting discharge disappeared within 2 min of breakthrough into the whole cell mode (making whole cell recording imprac-
In addition to changing the parameters of bursting, low 
$[Ca^{2+}]_o$, also reversibly decreased the amplitude and modified the shape of the action potentials (Fig. 2B). This effect could be reasonably, although not exclusively, explained by depolarization after a decrease in extra/intracellular calcium concentration and partial inactivation of voltage-gated sodium channels. However, given the known complex interaction between calcium and many ion channels, understanding of the role of calcium in burst generation in these cells demands more detailed experimental and computational analyses.

As noted earlier (Fig. 1B), bursting cells responded to transient odorant stimulation with an evoked burst. The evoked burst had a similar structure to that of the spontaneous bursts in the same cell (Fig. 3, A and B). The ability to evoke a burst with odorant was phase dependent, i.e., it depended on when the odorant was applied relative to the time of the last spontaneous burst. A phase-response curve (PRC) generated for the same data shown in Fig. 3A quantifies this effect (Fig. 3C). The PRC shows that applying an odorant of a given concentration between phase 0 and 0.3 failed to evoke bursts or produce a measurable phase shift, whereas applying the same odorant later than phase 0.3–0.4 advanced the spontaneous burst period (Fig. 3C). The phase dependency of evoking a burst with an odorant can also be demonstrated in terms of the probability of the cell responding (Fig. 3D, closed circles, left-hand ordinate). Here, the probability is estimated as the number of evoked bursts divided by the number of stimulus applications. Fitting the data with a sigmoid function (solid line through closed circles) gave a time to one-half-maximum of 10.4 s, i.e., that an odorant of that concentration applied 10.4 s after a spontaneous burst (at about 0.3 into the burst cycle) would evoke a burst with a probability of 0.5. The superimposed IBI histogram compares the evoked burst probability with the probability of a spontaneous burst (Fig. 3D, bars, right-hand ordinate). The probability of occurrence of a spontaneous burst even at the mean spontaneous bursting interval of about 30 s, calculated from fitting a Gaussian function to the IBI distribution (solid line over bar histogram), is <0.1. Although shown here in detail for one cell, all 54 rhythmically active ORNs that responded to the odorant (not all cells would necessarily respond to the single odor mixture used in this study) did so by triggering a burst similar to the spontaneous bursts in a phase-dependent manner, even though the cells had different inherent bursting frequencies and burst structure. Thus as in many other rhythmically bursting neurons (e.g., Ramirez et al. 2004), odor inputs can phase-shift the bursting cycle and, in doing so, are effectively amplified in a nonlinear manner by triggering bursts that override (entrain?) the spontaneous bursting rhythm.

The probability of evoking a burst with an odorant was also concentration dependent. More intense stimuli caused the cell to discharge proportionally earlier in the cycle. As shown for one cell in Fig. 4A, applying an odorant at three different concentrations (left three panels, only 40 of 80 responses at each concentration shown for brevity) caused the cell to discharge proportionally earlier in its discharge cycle. This effect can be seen more clearly in the corresponding immediate PRCs (right three panels), which show the relative period change ($\Delta P/P$) at different stimulus phases ($\Delta T/P$). Note the shift of the PRC to an earlier time in the bursting cycle as the concentra-

**FIG. 2.** Effects of extracellular $K^+$ and $Ca^{2+}$ on spontaneous bursting. A: recording (left) of bursts of action potentials showing the effect of $[K^+]_o$ on the bursting pattern and the corresponding interburst interval (IBI) histogram (middle). Bin width, 0.5 s. Right: plots of the basic burst parameters as a function of $[K^+]_o$, including the mean IBI (top), the mean number of spikes per burst (middle), and the mean burst duration (bottom). SE in all instances falls within the symbols. B: recording (left) of bursts of action potentials in another ORN showing the effect of $[Ca^{2+}]_o$ on the bursting pattern in control (top trace) and low $Ca^{2+}$ (bottom trace) conditions (see METHODS). Right: histograms of the corresponding IBI (top, bin width, 1 s), the number of spikes per burst (middle), and burst duration (bin width, 0.1 s). Bursts were detected using specified interval burst analysis at a delimiting interval of 500 ms and a delimiting number of 6 spikes per burst.

tical, given the slow frequency of bursting), suggesting it was dependent on one or more diffusible intracellular factors. Depolarizing the cells increased the bursting frequency, one of the most general characteristics of rhythmically active neurons despite mechanisms of bursting (e.g., Harris-Warrick 2002). Depolarizing one cell by increasing extracellular $[K^+]_o$ from 5 to 14 mM increased the burst frequency from 0.09 ± 0.002 to 0.27 ± 0.01 Hz and decreased the number of action potentials per burst from 7.6 ± 0.07 to 4.5 ± 0.07 (Fig. 2A). A similar result was obtained for both of the two other bursting ORNs tested (data not shown). Bursting discharge also was $Ca^{2+}$-dependent as in many other intrinsically bursting neurons, suggesting that burst termination could involve activation of a $Ca^{2+}$-sensitive, voltage-dependent $K^+$ conductance (e.g., Harris-Warrick 2002). Lowering $[Ca^{2+}]_o$ from 16 mM to 100 $\mu$M increased the frequency of bursting in one such cell from 0.07 ± 0.01 to 0.1 ± 0.003 Hz, increased the burst duration from 0.54 ± 0.03 to 1 ± 0.03 s, and increased the number of action potentials per burst from 7.9 ± 0.1 to 31.4 ± 0.6 (Fig. 2B). A similar result was obtained for both of the two other ORNs tested (data not shown).
tion of the odorant was increased, showing the efficacy of entrainment increased with increasing odorant concentration. As done for a single odorant concentration in Fig. 3D, the data from the cell shown in Fig. 4A can also be expressed in terms of the probability of generating an evoked burst in response to odorant stimulation (Fig. 4B). Note the progressive left shift of the average evoked burst probability with increasing odorant concentration (Fig. 4D, left-hand ordinate, open circles vs. light solid circles vs. dark solid circles). Fitting a sigmoid function to the evoked burst probability at each concentration indicates that a twofold increase in odorant concentration extended the time within which the cell could trigger evoked bursts by about 6 s, i.e., about 0.5 of a burst cycle. The maximum stimulus concentration used applied 3.2 s after a spontaneous burst (i.e., about 0.27 into the burst cycle) would evoke a burst with a probability of 0.5. In comparison, the probability of occurrence of a spontaneous burst even at the mean spontaneous bursting interval for this particular cell of about 11 s, calculated from fitting a Gaussian function to the IBI distribution (solid line over bar histogram), is about 0.2. Although shown here in detail for one cell, all of the 18

FIG. 3. Bursting ORNs respond to odors in a phase-dependent manner. A: raster display of individual action potentials from a single bursting ORN before and after repetitive stimulation with an odorant pulse (vertical line). The trials were aligned relative to the time between the presentation of the odor pulse and the preceding spontaneous burst. Time interval increases from the bottom to the top of the display. Each trial contains a second odorant pulse applied randomly (not marked). B: histograms comparing the structure of spontaneous (top) and evoked (bottom) bursts for the same ORN as in A, aligned relative to the greatest instantaneous frequency within each burst. Histograms were normalized to the total number of bursts included in the analysis. Bin width = 5 ms. C: plot of the immediate phase-response curve (PRC) showing that odorants applied at phases later than 0.3–0.4 advanced the spontaneous burst period (see calculation details in METHODS). D: plot of the same data showing the probability of eliciting a burst in response to an odorant (left ordinate) as a function of time since the last burst and odorant pulse. Probability estimated over 1-s intervals (n = 223). Solid line, sigmoid function fit to the points with $T_o = 10.4$ s, showing that a stimulus of that intensity applied 10.4 s after a spontaneous burst would evoke a burst with a probability of 0.5. Superimposed normalized IBI histogram (bin width, 2 s) of the spontaneous activity of the cell (right ordinate). Solid line, Gaussian distribution with a mean 30.4 s. $[K^+]_o$, 5 mM.

FIG. 4. Effect of stimulus intensity on the evoked bursting of another ORN. A, left: raster displays of individual action potentials evoked by odor pulses of 3 different intensities (between blocks, lowest concentration at top). Stimulus intensity was regulated by changing the stimulus pulse duration (150, 250, and 300 ms) and denoted by the intensity of the background. Segments of the trials aligned relative to the time between spontaneous bursts and odor pulses to more clearly demonstrate the effect of concentration. Corresponding plots of the immediate PRCs (right) showing that the phase shift (efficacy of entrainment) increases with odorant intensity. B: plot of the probability of eliciting a burst in response to an odorant of increasing concentration (left ordinate) for the same data shown in A. Gray colored circles and lines correspond to A. Superimposed is the normalized IBI histogram (bin width, 1 s) of the spontaneous activity (right ordinate). Note that a 2-fold increase in odorant concentration extends the cycle time within which the cell can trigger an evoked burst by about 5 s ($T_o$ values from 9.1 to 3.7 s). $[K^+]_o$, 8 mM. Each burst contains 4.4 ± 0.09 spikes on average. PRC (A, right panels) and evoked burst probability histograms (B) were each generated from 80 applications of each stimulus concentration.
rhythmically active ORNs tested with different odorant concentrations responded to the odorant by triggering a burst similar to the spontaneous bursts in a concentration-dependent manner, even though the relative odor sensitivity and therefore the corresponding magnitude of the phase shift varied across different ORNs. Again, as in many other rhythmically bursting neurons (e.g., Prinz et al. 2003), increasing the strength of the input—in this case odorant concentration—produces a greater phase shift in the bursting cycle.

**DISCUSSION**

Cells showing the two patterns of responsiveness presumably do not represent different developmental stages of a single type of ORN because cells with both patterns of responsiveness always co-localized to the same cluster of ORNs innervating a single olfactory sensillum. Lobster ORNs mature along the length of the olfactory organ, i.e., across clusters of ORNs in different annuli, so that cells of a given cluster presumably are of the same age (Steullet et al. 2000). That, together with the fact that we never saw any of the over 400 cells surveyed transition between tonic and bursting activity, sometimes during recording periods lasting several hours, argue that cells showing tonic and bursting patterns of responsiveness may represent distinct subpopulation of ORNs in the lobster olfactory organ. However, the utility of having the two types of input to the olfactory CNS would not necessarily require that the inputs come from distinct subpopulations of ORNs.

We assume that the periodicity is not imposed and that the bursting is intrinsic to the cells. Bursting behavior was recorded with nondisruptive extracellular recording. It persisted in acutely dissociated cells. The structure of the bursts and the frequency of bursting were consistent for any given cell over hours. As is typical of intrinsically rhythmic neurons (e.g., Harris-Warrick 2002), bursting was sensitive to the imposed membrane potential of the cell and was Ca$^{2+}$-dependent. Intrinsic bursting would be consistent with the understanding that lobster ORNs (Grünew and Ache 1988), as with ORNs in most animals (Ache and Young 2005), do not make peripheral synapses and project independently to the CNS. It would also be consistent with recent evidence that the discharge pattern of insect ORNs is an inherent property of the cell in question (Hallem et al. 2004). Intrinsic bursting of course does not exclude the possibility that the periodicity could be initiated or modulated by one or more activity-dependent factors, such as blood-borne factors that could be delivered to the olfactory organ from neurohemal organs potentially associated with the circulation of the olfactory organ or elsewhere in the animal.

Given that odorants activate bursting ORNs by triggering a concentration-independent, all-or-nothing burst of action potentials rather than a concentration-dependent train of action potentials as in the tonically discharging ORNs, detection of weak stimuli would be selectively enhanced in bursting ORNs. That is to say, even a near-threshold stimulus would elicit an intrinsic, “stereotyped” burst if it arrived at the correct phase of the bursting cycle of the ORN. Although the evoked bursts themselves were independent of stimulus concentration, it is important in the context of having a population of parallel bursting cells that the probability of eliciting a burst was concentration dependent. This would allow odor concentration to be encoded across the population of bursting cells because the more intense the stimulus, the earlier in its bursting cycle the cell would discharge in response to the odorant. As a result, the number of ORNs bursting synchronously would increase as the stimulus intensity increases, which presumably could be read by downstream circuitry in the olfactory lobe and beyond. Stimulus acquisition in the lobster olfactory system is periodic as a result of antennular flicking, a reflex shown to be functionally equivalent to sniffing in mammals (Schmitt and Ache 1979). Flicking intermittently and rapidly exposes the ORNs to odorants and could be expected to synchronize the exposure of all ORNs in the population to odorants within several tens of milliseconds (Koehl et al. 2001; Schmitt and Ache 1997), thereby enhancing burst synchrony across the population. Thus the all-or-none nature of burst-dependent coding, together with synchronization of the bursting neurons, could be expected to selectively enhance the detection and amplification of weak signals, generally assumed to be one of the hallmarks of olfaction.

Bursts are well known to be transmitted across synapses more reliably than isolated action potentials (Lisman 1997). Although speculative in the context of the present study, the synchronized input from bursting cells could also potentially activate olfactory projection neurons and/or local interneurons, the central targets of ORNs, and prepare them to receive more linear input from the tonically active cells, as proposed for single neurons in the lateral geniculate (Sherman 2001). Also, bursts of action potentials potentially could provide an effective mechanism for selective communication with specific subsets of projection neurons or local interneurons (Izhikevich et al. 2003), although it remains to be determined whether ORNs showing the two patterns of responsiveness work ensemble or whether each codes selective subsets of odor information.

Overall, our findings strongly imply that intrinsically oscillatory ORNs occur in the lobster olfactory system. They also show for the first time that rhythmically bursting ORNs at least have the potential to play a fundamental role in coding odor information. The presence of receptor cells with rhythmic bursting discharge in the mammalian vomeronasal organ (Holy et al. 2000) as well as the conditional oscillations seen in amphibian and mammalian ORNs induced by prolonged odorant stimulation (Frings and Lindemann 1988; Reisert and Matthews 2001a,b) suggest our finding may be of general utility and help further our understanding of coding in other olfactory systems.

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