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DISTINCT INTRINSIC MEMBRANE PROPERTIES DETERMINE DIFFERENTIAL INFORMATION PROCESSING BETWEEN MAIN AND ACCESSORY OLFACTORY BULB MITRAL CELLS

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Abstract-Most mammals rely on semiochemicals, such as pheromones, to mediate their social interactions. Recent studies found that semiochemicals are perceived by at least two distinct chemosensory systems: the main and accessory olfactory systems, which share many molecular, cellular, and anatomical features. Nevertheless, the division of labor between these systems remained unclear. Previously we suggested that the two olfactory systems differ in the way they process sensory information. In this study we found that mitral cells of the main and accessory olfactory bulbs, the first brain stations of both systems, display markedly different passive and active intrinsic properties which permit distinct types of information processing. Moreover, we found that accessory olfactory bulb mitral cells are divided into three neuronal sub-populations with distinct firing properties. These neuronal sub-populations can be integrated in a simulated neuronal network that neglects episodic stimuli while amplifying reaction to long-lasting signals. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: olfactory system, olfactory bulb, vomeronasal system, intrinsic neuronal properties, information processing, mitral cells.

Many mammalian species rely on molecular communication to mediate their social interactions, including mating, dominance, and kin relationships (Keverne, 2002; Halpern and Martinez-Marcos, 2003; Brennan and Kendrick, 2006; Shah, 2006). Such molecules, which are released by one individual in order to communicate with other individuals, are termed "semiochemicals".

The nasal cavity of most mammals contains several distinct chemosensory structures (Breer et al., 2006), of which the two most studied are the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). These are associated with two distinct chemosensory systems: the main olfactory system (MOS) and the accessory olfactory system (AOS), respectively. The AOS is also known as the

*Corresponding author. Tel: +972-4-8288773; fax: +972-4-8288763. E-mail address: shlomow@research.haifa.ac.il (S. Wagner). vomeronasal system. MOE sensory neurons project to the main olfactory bulb (MOB), where they synapse upon mitral cells, the bulb's principal neurons (Mori et al., 1999). MOB mitral cells then project to various paleocortical areas and to several limbic nuclei known as the olfactory amygdala (Scalia and Winans, 1975; Pro-Sistiaga et al., 2007). Similarly, VNO neurons project to the accessory olfactory bulb (AOB), where they synapse with AOB mitral cells (Meisami and Bhatnagar, 1998). These projection neurons project via several areas of the limbic system, known as the vomeronasal amygdala, to hypothalamic nuclei which are associated with reproduction, aggression, and parental behavior (Scalia and Winans, 1975; Mohedano-Moriano et al., 2007; Pro-Sistiaga et al., 2007).

Traditionally, detection and perception of odors were assigned to the MOS, whereas the AOS was thought to sense semiochemicals (Meredith, 1991). However, multiple studies during the last decade implicated the MOS in semiochemical-elicited responses, throwing this separation into doubt (reviewed by Stowers and Marton, 2005). It was also shown that these systems detect at least partially overlapping sets of stimuli (reviewed by Spehr et al., 2006). Thus, a general and fundamental question remains: what is the functional difference between these two chemosensory systems?

The MOS and AOS are thought to arise from an evolutionarily common origin in fish (Grus and Zhang, 2006). Accordingly, they share many molecular, cellular, and anatomical features (Mombaerts, 2004). In both systems, each sensory neuron expresses only one out of a large store of receptor genes, and all neurons expressing a given receptor project to the same glomerular targets in the olfactory bulb, where they terminate upon mitral cells in a glutamatergic excitatory synapse (Berkowicz et al., 1994; Chen and Shepherd, 1997; Jia et al., 1999). The mitral cells are also innervated by two sets of inhibitory interneurons: juxtaglomerular neurons and granule cells (Meisami and Bhatnagar, 1998; Urban, 2002; Schoppa and Urban, 2003). However, a very significant difference between the MOS and AOS appears in the wiring scheme of sensory neurons onto mitral cells. MOS sensory cells expressing the same receptor typically innervate one pair of glomeruli in the MOB, thus creating a focused and parsimonious representation of this receptor in the MOB sensory map (Mombaerts, 1996). Moreover, MOB mitral cells typically extend only one apical dendritic tuft into a single glomerulus, and also extend several lateral dendrites onto which the inhibitory granule cells synapse, thus creating an analytical information-processing system (Mori et al., 1999;

Abbreviations: AHP, after-hyperpolarization; AOB, accessory olfactory bulb; AOS, accessory olfactory system; IFR, instantaneous firing rate; ISI, inter-spike interval; MOB, main olfactory bulb; MOE, main olfactory epithelium; MOS, main olfactory system; PBS, phospate buffered saline; PFA, paraformaldehyde; SH, spike height; VNO, vomeronasal organ; 4AP, 4-aminopyridine.

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Urban, 2002; Schoppa and Urban, 2003). In sharp contrast, VNO sensory neurons expressing a given receptor innervate a set of 10-30 glomeruli, distributed among various domains in the AOB glomerular layer, thus creating a distributed and complex representation of each receptor in the AOB (Belluscio et al., 1999; Rodriguez et al., 1999). Furthermore, AOB mitral cells typically extend a few apical dendrites to multiple, sometimes remote locations in the glomerular layer, where they contact as many as 12 different glomeruli (Takami and Graziadei, 1991). We have shown previously (Wagner et al., 2006) that at least a subset of AOB mitral cells contact glomeruli of several different types, thus integrating information from several distinct receptors. This observation, albeit contradicting the results of one study (Del Punta et al., 2002), was confirmed recently by two independent works using electrophysiological recordings in vivo (Ben-Shaul et al., 2010; Meeks et al., 2010). Therefore, we hypothesized that the main functional distinction between the MOS and the AOS is computational: each system processes the pheromonal information differently (Dulac and Wagner, 2006). However, whereas MOB information processing is an intensively studied subject (Mori et al., 1999; Lledo et al., 2005; Wilson and Mainen, 2006), hardly any data regarding information processing in the AOB are available to challenge this hypothesis.

In the present study we used whole-cell recordings of electrical activity, in conjunction with morphological analysis based on two-photon microscopy in olfactory bulb slices to show that mitral cells of the main and accessory olfactory bulbs display strikingly diverse passive and active intrinsic properties which permit distinct types of information processing. Moreover, we show that, in contrast to the homogeneous population of MOB mitral cells, AOB mitral cells are divided into three neuronal sub-populations displaying distinct firing properties. As a suggestion for the role of these neuronal sub-populations we simulated a hypothetical neuronal network that integrates their firing responses in a way which neglects episodic stimuli while responding to long-lasting signals. Such a mechanism may help the accessory olfactory system decoding the social context of the animal.

EXPERIMENTAL PROCEDURES

Slice preparation

Animals were maintained in the SPF mice facility of the University of Haifa under veterinary supervision, according to NIH standards, with *ad libitum* food and water supply and lights turned on between 7:00–19:00. All experiments were approved by the Animal Care and Use Committee of the University of Haifa. Mice aged 3–6 months (males and females) were anaesthetized (isoflurane Abbott Laboratories, Abbott Park IL, USA) and killed by cervical dislocation. Olfactory bulb slices were prepared as previously described (Wagner et al., 2006). We used coronal, sagittal, or horizontal planes for MOB slices and semi-coronal (Del Punta et al., 2002) or sagittal planes for AOB slices, with no differences in the results. Most experiments were done on semi-coronal AOB and horizontal MOB slices. In a few experiments recordings were made from both MOB and AOB in the same sagittal slice. 300– 400- μ m-thick slices were equilibrated for at least 1 h and up to 5 h in physiological solution containing (mM): 125 NaCl, 25 NaHCO₃, 15 glucose, 3 KCl, 2 CaCl₂, 1.3 NaH₂PO₄, and 1 MgCl₂, bubbled with 95% O₂ and 5% CO₂ gas mixture, pH 7.4. For electrophysiological recordings slices were submerged in bubbled physiological solution within a recording chamber (Warner Inst., Hamden, CT, USA) which was constantly perfused at a rate of 1–3 ml/min. All experiments were done at room temperature in the presence of a blocker of GABA_A neurotransmission (50 μ M bicuculline, 5 μ M gabazine, or 50 μ M picrotoxin, all purchased from (Sigma-Aldrich, St. Louis, MO, USA or Tocris Bioscience, Ellisville, MO, USA). When 4-aminopyridine (4AP, Sigma-Aldrich, St. Louis, MO, USA) was used it was delivered to the recording chamber via the perfusion in a final concentration of 0.5 mM.

Electrophysiology

All recordings were done using Axioskop FS2 microscope (Zeiss, Thornwood, NY, USA) equipped with Nomarski optics and epi-fluorescence. Infrared differential interference contrast (IR-DIC) video-microscopy using IR-sensitive camera (C2400, Hamamatsu, Japan) was used for targeting the neurons by the patch pipette. Mitral cells were identified using a 40× water immersion objective by their cell bodies' strict location in the mitral cell layer for the MOB and by their cell bodies' location in the ventral side of the external plexiform layer for the AOB. Whole-cell patch current-clamp recordings were done using borosilicate pipettes filled with standard intracellular solution containing (mM): K-gluconate, 120; KCl, 14; Na-gluconate, 10; HEPES, 10; EGTA, 5; CaCl₂, 0.5; MgATP, 3; NaGTP, 0.5; phosphocreatine, 10 (10–15 $M\Omega$). When BAPTA was used to block changes in the intracellular calcium levels, we used similar intracellular solution with no CaCl₂ and 5 mM of BAPTA (tetrapotassium salt, Invitrogen Carlsbad, CA, USA) instead of EGTA. Seal resistance was at least 2 G Ω and typically 5–8 G Ω . Electrical signals were amplified and filtered (bandpass, 30 kHz) using Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA, USA). All amplified signals were digitized at 2-10-kHz rate using National Instruments board and analyzed using homemade software written in LabVIEW 7.0 (National Instruments, Austin, TX, USA). Current injections were given at 5 s inter-spike interval (ISI).

Dye filling and morphological analysis

In about 10% of the cases whole-cell recordings were performed with Alexa Fluor 568 (0.5 mg/ml; Invitrogen Carlsbad, CA, USA) in the intracellular solution in order to label the recorded cells. In these cases the slice was fixed immediately after the termination of the recording session using 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 20-40 min at room temperature, followed by overnight incubation in 1% PFA in PBS at 4 °C. The following day the labeled slice was washed three times for 15 min with PBS and imaged using an Ultima two-photon microscope (Prairie Technologies, Middleton, WI, USA) equipped with $40 \times$ objective (0.8 NA). A femtosecond laser (Mai-Tai, Spectra-Physics, Santa Clara, CA, USA) was used to excite the dye at 850 nm. Images (1024×1024) were acquired at 1-µm steps in the zZ dimension. Reconstructions were performed manually from the complete 3D image stacks using Neurolucidae (MBF Bioscience, Williston, VT, USA).

Data analysis

All statistical differences were calculated using either *t*-test (when comparing two populations) or one-way ANOVA (when comparing three populations) after checking for normal distribution (Kolmogo-rov–Smirnov). Excel 2003 (Microsoft, Redmond, WA, USA) and SPSS (IBM, Armonk, NY, USA) 15.0 for Windows were used for statistical analysis. Threshold stimulus was defined as a stimulation level yielding response in about half of the stimuli, while

threshold-surpassing stimulus was defined as the minimal level of stimulus required for consistent response. Membrane time constants were calculated using the pilling method (Rall, 1969). I-V curves were calculated using the peak voltage response to the 0.8-s long current injections, averaged over four to five stimuli. I-F curves were calculated by means of dividing the number of spikes by 0.5 s, which was the length of the current injection, averaged over three stimuli.

Classification of AOB cells was done according to the following rule: for a given cell, the change from first to last instantaneous firing rates (IFR, the inverse of the inter-spike interval), averaged over three identical 0.5-s long current injections, was calculated for each amplitude of the current step. If the changes were universally positive or negative for all amplitudes, the cell was classified as facilitating or accommodating, respectively. Otherwise, the mean change over the three highest amplitudes was calculated. If the mean change was more than 15% of the cell's mean firing rate, the cell was classified as accommodating or facilitating according to the direction of the mean change. Otherwise, the cell was classified as steady.

Time constants for the changes in IFR, after-hyperpolarization (AHP) and spike height (SH) were calculated by fitting a single exponent over the length of the stimulus to the relevant values averaged over three repetitions of any given stimulus amplitude. Curve fitting was done using MatLab's least mean square function lsqcurvefit. In order to avoid outliers when calculating the mean time constant over all cells, curve fits with a mean square error greater than one were excluded.

Neuronal network modeling

Neurons in the network were modeled as integrate-and-fire neurons.

The current equation for all classes of AOB mitral cells is:

$$C\frac{dV}{dh} = -g_{leak}(V - E_{leak}) - g_{sra}(V - E_{k}) + \frac{l_{e}}{A}$$

where g_{sra} is the spike-rate adaptation conductance governed by the equation:

$$\tau_{sra} \frac{dg_{sra}}{dt} = -g_{sra}$$

and I_e/A is the external input in units of nA/mm².

When V reaches V_{th} , the neuron fires a spike, g_{sra} increases by an amount $\Delta g_{sra} (g_{sra} \rightarrow g_{sra} + \Delta g_{sra})$, and V is then reset to V_{rest} .

Parameters shared by all classes of neuron are set as follows: V_{th} =-50 mV; V_{rost} =-60 mV; g_{leak} =0.0012 mS/mm²; E_{leak} =-60 mV; E_{κ} =-75 mV.

The remaining parameters are class specific and set as follows:

Accommodating. C=0.32 nF/mm²; Δg_{sra} =0.00045 mS/ mm²; τ_{sra} =150 ms.

Steady. C=0.38 nF/mm²; Δg_{sra} =0 mS/mm²; τ_{sra} =0 ms.

Facilitating. C=0.7 nF/mm²; Δg_{sra} =-0.00075 mS/mm²; τ_{sra} =150 ms.

The readout neuron receives synaptic inputs of the same strength from all three of AOB mitral cells, the accommodating input being inhibitory, and therefore its current equation is of the form:

$$C\frac{dV}{dt} = -g_{leak}(V - E_{leak}) - g_{sra}(V - E_k) - g_s(-P_a + P_s + P_f)(V - E_s) + \frac{I_e}{A}$$

 P_a , P_s , and P_f are the open probabilities for accommodating, steady, and facilitating receptors, respectively, on the readout

neuron. When an AOB mitral cell fires, the open probability is set to 1 and then decays with a time constant τ_{P} .

The leak current, V_{th} and V_{rest} are equivalent to those of the mitral cells and the other parameters are as follows:

C=0.025 nF/mm²; Δg_{sra} =0.0022 mS/mm²; τ_{sra} =6 ms; g_s =0.001 mS/mm²; the time constants for the open probabilities of the accommodating, steady, and facilitating receptors are 200 ms, 500 ms, and 500 ms, respectively.

Throughout this paper population results are summarized as mean \pm SD values. All error bars reflect SEM values.

RESULTS

Comparison of passive membrane properties between MOB and AOB neurons

The intrinsic properties of AOB and MOB mitral cells were analyzed by means of whole-cell recordings in currentclamp mode, in the presence of GABA_A antagonists (see Experimental procedures) to block inhibitory synaptic feedback. Occasionally, the recorded cells were filled with intracellular dye and reconstructed with two-photon microscopy (see Experimental procedures). Labeled MOB and AOB mitral cells are shown in Fig. 1A, C, and their reconstructed images are shown in Fig. 1B, D, respectively. Unlike the single extensive dendritic tuft characterizing all MOB mitral cells (Fig. 1A, red arrowhead), AOB mitral cells usually show multiple small dendritic tufts projected to the glomerular layer (Fig. 1C, red arrowheads). The passive membrane properties of the neurons were investigated in 35 MOB mitral cells from 13 mice and 63 AOB neurons from 30 mice, by injecting negative current steps (Fig. 1E, F, upper panels) from a holding potential ranging between -50 mV and -60 mV. The voltage values at the peak of the voltage responses (Fig. 1E, F, lower panels) were plotted as functions of the injected currents (I-V curves. Fig. 1G. H). For both neuronal populations the differences in slope between the peak and end I-V curves are, on an average, less than 2%, confirming a lack of any timedependent rectification. Moreover, the resultant linear I-V curves for both types of neurons indicate that both cell types show no voltage-dependent rectification within the membrane potential range of -50 to -100 mV. This relatively rare lack of any rectification in the I-V relationships suggests very similar specific passive membrane properties between the two neuronal populations.

The input resistance (R_{in}) of each cell was calculated from the slope of the peak I-V curve. The distributions of R_{in} values for MOB (Fig. 1I, red bars) and AOB (Fig. 1I, blue bars) mitral cells show a striking difference: on an average, the R_{in} of AOB neurons is ~2.5 times that of MOB neurons (299±135 M Ω and 733±496 M Ω for MOB and AOB, respectively). This highly significant difference ($P < 10^{-8}$) could result from differences in specific membrane properties or it could reflect size differences between the neuronal types. Therefore, we calculated the membrane time constant, τ_m , of each cell (see Experimental procedures). As shown in Fig. 1J, the two populations have similar time constants (P > 0.1): average time constants of 45.3±23.5 and 42.5±16.1 ms were calculated for AOB and MOB, respectively. Thus, the difference in input 54

S. Zibman et al. / Neuroscience 189 (2011) 51-67



Fig. 1. MOB and AOB mitral cells display distinct passive membrane properties. (A, C) Two-photon Z-stack images of dye-filled MOB (A) and AOB (C) mitral cells. Note the different scales. The single tuft of the MOB neuron and four tufts of the AOB neurons are marked with red arrowheads. (B, D) Reconstructions of the cells shown in (A, C). (E, F) Membrane-potential responses (lower panels) to hyperpolarizing current injections (upper panels) applied to MOB (E) and AOB (F) neurons. Note the total lack of time-dependent rectification. (G, H) I-V curves of the cells shown in (E, F). Note the linear relationships in both cells and the significantly higher R_{in} of the AOB cell (H). (I) Distributions of R_{in} values for MOB neurons (red bars, n=35) and AOB neurons, (blue bars, n=63). Note the normal distribution of MOB neurons, suggesting a relatively uniform shape of these cells, in contrast to the multi-peak distribution of AOB neurons, which suggests a much more heterogeneous morphology. The two distributions are statistically different ($P<10^{-8}$, one-tail *t*-test). (J) Distribution of τ_m values for MOB and AOB mitral cells (red and blue bars, respectively). Note the similarity between the two populations (P>0.1, one-tail *t*-test). (K) Membrane area calculated from the electrical membrane properties of the dye-filled cells as a function of the morphologically measured dendritic membrane area. The linear regression (slope=0.64) had an R^2 value of 0.92, indicating a good correlation between the two methods. (L) Mean values (error bars=SD) of the membrane area for MOB and AOB mitral cells, as calculated from the electrical properties and morphological reconstructions. The number of cells in each group is indicated at the bottom of each bar. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

resistance was attributed to size differences. We further examined this conclusion by calculating the membrane area, on the common assumption of a specific capacitance of 1 μ F/cm². Indeed, we found that the average membrane area of MOB mitral cells—16,031±6940 μ m²—is 2.2 times that of AOB mitral cells—7240±3333 μ m². By dividing the cell conductance by the membrane area we calculated the specific membrane conductance. The average specific membrane conductance of MOB mitral cells is 0.27±0.11 μ S/cm², very similar (*P*>0.1) to that of AOB neurons— 0.28±0.20 μ S/cm².

In order to get an independent measure of the size difference between the two neuronal types we calculated the dendritic membrane area from the morphological reconstruction of each of the dye-filled cells (eight AOB and three MOB neurons). As shown in Fig. 1K, the membrane area calculated from the cell morphology is highly correlated (R^2 =0.92) with that calculated from the cell capacitance. The ratio between the morphologically calculated membrane areas of AOB (9139±3638 μ m²) and MOB (24,572±11,747 μ m²) mitral cells is 2.7, similar to the ratio calculated from the electrical capacitances of the cells (Fig. 1L).

Thus, the smaller membrane area of AOB neurons endows them with a much higher input resistance than that of MOB neurons. This feature is expected to make AOB neurons responsive to weaker currents than MOB mitral cells.

Comparison of firing responses between MOB and AOB neurons

The firing response of a neuron to a given stimulus is influenced by its passive membrane properties but is determined mainly by its intrinsic firing properties. Therefore, we compared the intrinsic firing properties of AOB and MOB mitral cells. Using depolarizing current steps (0.5-s long) from a holding potential of about -60 mV, we constructed the current-firing frequency (I-F) relationships of the neurons. At this stage the firing frequency was calculated by dividing the number of spikes by the current step duration, and plotted against the current step amplitude. Fig. 2 shows results for typical MOB (Fig. 2A, B) and AOB (Fig. 2C, D) mitral cells. The MOB cell responds to threshold-surpassing current injections with a relatively highfrequency burst of action potentials (Fig. 2A). Increasing the injected current significantly increases the frequency of the action potentials (Fig. 2B) until saturation is reached, usually between 70 and 120 Hz. The results are summarized in Fig. 2E. The initial phase of the I-F curve displays a characteristic step-like function of the injected current, which reflects the absence of low-frequency firing. This characteristic feature is emphasized in Fig. 2G, where the normalized I-F curves of three other MOB mitral cells are superimposed. In contrast, AOB cells show a smoothly monotonic dependence of the firing frequency on the injected current, even at its lower range (Fig. 2F, H). Thresholdsurpassing stimuli (Fig. 2C) elicit only one to three action potentials, which appear after a significant delay. Increasing the stimulus intensity (Fig. 2D) causes a train of spikes that lasts for the duration of the current pulse. It should be noted that despite the two to three times higher input resistance of

AOB mitral cells, the current threshold is only 26% higher in MOB neurons (25.3±17.7 pA and 20.1±10.3 pA for MOB and AOB mitral cells, respectively), which suggests a lower voltage threshold for MOB cells. The distributions of the rates of threshold-surpassing and the maximal firing responses to depolarizing current steps are shown in Fig. 2I, J, respectively. Both parameters are significantly ($P < 10^{-7}$) higher in MOB cells (threshold surpassing-15.9±6.7 Hz; maximal-84.0 \pm 19.8 Hz; n=21) than in AOB neurons (threshold surpassing—4.7±2.2 Hz; maximal—32.3±9.2 Hz; n=65). The almost non-overlapping ranges of firing exhibited by the two neuronal populations are evident in the pairs diagram (Fig. 2K), in which the threshold-surpassing and maximal values for each cell are connected with red and blue lines for MOB and AOB neurons, respectively. Thus, AOB mitral cells seem to be tuned for firing activity within a very different dynamic range than MOB mitral cells.

The dynamics of firing responses of MOB neurons to constant stimuli

Along with the distinct firing rates, we noticed a few more properties of the firing response which markedly differ between AOB and MOB mitral cells, especially for stronger stimuli. As exemplified in Fig. 2B, D, while the firing response of MOB mitral cells reach a steady state in its spike height (SH), after-hyperpolarization (AHP) and instantaneous firing rate (IFR) at a very early stage of the response, many AOB mitral cells display monotonous changes in all three parameters throughout the firing response. We decided to analyze these differences in higher details. First we analyzed these properties in MOB mitral cells. Fig. 3A shows the voltage response of a typical MOB neuron to a depolarizing current step of 100 pA while in Fig. 3B the changes along the response in the IFR (top), AHP (middle), and SH (bottom) are plotted. As manifested, from the first spike to the last spike there is an increase in the IFR, hyperpolarization of the SH and a depolarization of the AHP. Moreover, a steady state in all three parameters is reached with a time constant <150 ms (IFR: 113.0±58.8 s; AHP: 69.4±40.3 s; SH: 117.5±94.0 s). Fig. 3C shows the distribution of differences between the last and first IFRs, along three identical stimuli of 100 pA for each MOB mitral cell. As displayed, the differences are normally distributed around mostly positive values (15.9±24.5 Hz), with only 22% of all traces showing a (mostly slight) reduction of firing rate along the current step. The distribution of differences between the last and first AHP (Fig. 3D) shows a very similar pattern, a normal distribution around relatively small positive values (4.56±2.8 mV). While the difference between the last and first SH (Fig. 3E) show multi-peak distribution, only 18% of the population show (<2 mV) depolarization of SH along the current step (-4.0 ± 4.0) . These results suggest again that MOB mitral cells are relatively homogeneous in their intrinsic membrane properties. Moreover, as seen in Fig. 3F, the IFR (blue circles) and AHP (green triangles) averaged over all MOB responses to 100-pA stimuli show comparable time courses, with time constants of 103±46.6 ms and 59.11±30 ms, respectively, which are close to the time course of the change in SH (105.5 ± 71.0 ,



Fig. 2. MOB neurons are more excitable than AOB neurons. (A, C) Superimposed threshold (red traces) and threshold-surpassing (black traces) responses of MOB (A) and AOB (C) neurons to depolarizing current steps (0.5 s) from a baseline potential of about -60 mV. Note the all-or-none burst of the MOB neuron, in contrast to the graded (one to three spikes) response of the AOB neuron. (B, D) Responses of the same neurons to near-maximal current injections. Note the rapid steady state in IFR, SH, and AHP achieved in the MOB neuron (B), in contrast to the prolonged modulation of all three parameters in the AOB neuron (D). (E, F) I-F relations for the cells shown above. Note the step-like initial phase and high maximal rate (>100 Hz) of the MOB I-F curve (E), in contrast to the monotonic rise and low maximal rate (<50 Hz) of the AOB I-F curve (F). (G, H) Superimposed I-F curves of three other MOB (G) and AOB (H) neurons, normalized to their maximal values. (I, J) Distributions of minimal (I) and maximal (J) firing rates for AOB (blue) and MOB (red) mitral cells. In both parameters a statistically significant difference ($P < 10^{-7}$, one-tail *t*-test) was found between the two neuronal types. (K) A pairs diagram showing the threshold-surpassing and maximal firing rates for each cell connected by red lines for MOB mitral cells and blue lines for AOB neurons. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

not shown) along these stimuli. The rapid steady state achieved with a similar time course in all three parameters suggests a dynamic most likely governed by the transient A-type potassium current previously shown to be active with similar dynamics in MOB mitral cells (Chen and Shepherd, 1997; Balu et al., 2004; Christie and Westbrook, 2006).

The dynamics of firing responses of AOB neurons to constant stimuli

In contrast to the relatively homogeneous population of MOB neurons, AOB mitral cells show much more heterogeneous responses to depolarizing current steps. The firing rates of some neurons consistently decline along the



Fig. 3. MOB mitral cells uniformly and rapidly reach a steady state in their firing response to constant stimulus. (A) A voltage response (above) of a typical MOB mitral cell to a 100-pA depolarizing current step (below). Note the stable firing following the fourth spike. (B) The IFR (upper panel), AHP (middle panel), and SH (lower panel) values of the response shown in (A), averaged over three identical stimuli, plotted over time. Note that all parameters reach steady state in less than 150 ms. (C) The distribution of the difference between the first and last IFR (Δ IFR) for all MOB cells recorded while responding to 100-pA stimuli. Note the positive values measured in about 80% of traces, manifesting increase in firing real along stimulation. (D) Same for difference between first and last AHP (Δ AHP) values. (E) Same for difference between first and last SH (Δ SH) values. (F) IFR (blue circles, left axis) and AHP (green triangles, right axis) values, averaged over 50-ms time bins for all responses of MOB mitral cells to 100-pA stimuli and plotted against time. Note the comparable time course for both parameters. Error bars represent standard error. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

current steps while others always show progressively increasing firing rate. A third population of neurons has a steady firing response with no consistent change of their firing rates. Thus, we divided the 63 AOB neurons analyzed above and another cohort of 63 AOB mitral cells (altogether 126 cells) according to the modulation of their spiking responses (see Experimental procedures) into three groups: accommodating, steady, and facilitating (40%, 23%, and 37% of the cells, respectively). For comparison, division of MOB mitral cells according to the same criteria gave only 8% accommodating cells and 15% steady neurons while 77% of the cells were classified as facilitating. Fig. 4 shows the voltage responses (Fig. 4A–C) of a typical cell for each group to a 100-pA depolarizing current step and (Fig. 4D–F) the modulation of their IFR (top), AHP (middle) and SH (bottom) along the current step. The distribution of the differences between the first and last IFR values (Δ IFR) for all recorded AOB mitral cells, each represented by three identical stimuli of 100 pA, is shown in Fig. 5A. This distribution exhibits three distinct peaks around the values around -5 Hz, 0 Hz, and +5 Hz, supporting the existence of three distinct neuronal sub-populations of AOB mitral cells. The separate distributions of Δ IFR of the three sub-populations are shown in Fig. 5B, manifesting the highly significant (see legend) differences between the IFR, AHP, and SH appear to be much more complicated in AOB mitral cells than in MOB neurons. For



Fig. 4. AOB mitral cells show various firing patterns in response to identical stimuli. (A–C) Voltage responses (above) of typical accommodating (A), steady (B), and facilitating (C) AOB mitral cells to identical 100-pA depolarizing current steps (below). (D–F) The IFR (upper panel), AHP (middle panel) and SH (lower panel) values of the responses shown in (A–C), respectively, each averaged over three identical stimuli and plotted over time of the current step. Note the similar changes in AHP and SH between the cells, despite the clear differences in IFR dynamics.

example, as manifested in Fig. 5C, while accommodating and steady cells show very similar distributions of differences between last and first SH values (Δ SH), a significantly (see legend) different distribution, biased towards less negative values, is displayed by facilitating cells. Moreover, as exemplified in Fig. 4A, B, even in the case of AOB accommodating and steady cells the AHP gets gradually depolarized along the current step. This is summarized in Fig. 5D where the distributions of the differences between the last and first AHP (Δ AHP) along the responses to 100-pA injected current steps recorded from all three neuronal sub-populations of AOB mitral cells are plotted. As revealed, despite significant (see legend) differences between all three neuronal sub-populations in this property, all the cells show depolarization of the AHP along the stimulation. Therefore, at least for a significant number of AOB neurons, that is the accommodating and steady neurons, the change in AHP along the stimulus is not in accord with the change in firing rate. Indeed, when we plotted Δ IFR as a function of Δ AHP for all traces (not shown), we found a positive correlation in the cases of MOB neurons (R=0.5192) and of AOB facilitating cells (R=0.5038), whereas a negative correlation was found for accommodating cells (R = -0.2354) and no correlation for steady cells (R = -0.0031). These results suggest that of all three categories of AOB mitral cells only in the case of facilitating cells there may be an association between the change in AHP and the change in firing rate along the current step while for the rest of AOB mitral cells, the change in IFR along stimulation seems to be determined

by other intrinsic properties of the cells. This conclusion is supported by comparison of the dynamics of these changes between the three neuronal sub-populations. As seen in Fig. 6A, accommodating and facilitating cells display significantly (P<0.0001) different time courses of the changes in mean IFR along 100-pA current steps, where accommodating cells reach a steady state with a time constant of 49.1 ms while facilitating cells gradually increase their firing rate along the pulse without reaching a clear steady state (time constant=295.1 ms). In contrast, the changes in mean AHP along the 100-pA current step show (Fig. 6B) very similar (P > 0.1) time course between both sub-populations, with time constants of 172.9 ms and 195.4 ms, respectively. Similarly, when the time constants of the changes in these two parameters were separately calculated for all accommodating (blue dots) and facilitating (red triangles) AOB mitral cells for all levels of stimulations it was found that facilitating cells have a much higher time constants in the IFR throughout the stimulation range (Fig. 6C) whereas, as evident from Fig. 6D, a slight (but significant—P < 0.05) difference between these subpopulations as regards to the time constant of the changes in AHP can be seen only for stimuli stronger than 150 pA.

The distinct sub-populations of AOB mitral cells respond differently to changes in intracellular calcium level

Overall these results clearly suggest different intrinsic properties of the three neuronal sub-populations. We checked if there are any differences between them in

58



Fig. 5. Parameters of the firing responses to depolarizing current steps are differentially distributed between the distinct sub-populations of AOB mitral cells. (A) Distribution of Δ IFR values of all the responses of AOB mitral cells (each represented by three responses) to 100-pA current steps. Note the three peaks around the values of -5 Hz, 0 Hz, and +5 Hz, indicating three distinct types of responses. (B) Distributions of the Δ IFR values of the same responses as in (A), divided between the three sub-populations of AOB neurons (blue—accommodating, green—steady, brown—facilitating). All three distributions show statistically significant difference from each other (one-way ANOVA— $F_{(2,220)}$ =79.861, P<0.001; Bonferroni post-hoc analysis, P<0.001). (C) Same (as in B) for Δ SH values. Note the bias of facilitating cells towards less negative values. This difference made facilitating cells statistically different (one-way ANOVA— $F_{(2,220)}$ =16.554, P<0.001; Bonferroni post-hoc analysis, P<0.001) from the other two population which were found statistically indifferent from each other (Bonferroni post-hoc analysis, P<0.5). (D) Same (as in B) for Δ AHP values. Note the bias of accommodating cells towards less negative values. The differences between all three distributions are statistically significant (one-way ANOVA— $F_{(2,220)}$ =17.177, P<0.001; Bonferroni post-hoc analysis, P<0.01). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

terms of passive membrane properties, but found nothing significant (see legend) either in capacitance (Fig. 6E) or input resistance (Fig. 6F). Thus, it is the active properties, most likely voltage- or calcium-activated conductances. which cause the different firing properties of the distinct sub-populations of AOB mitral cells. Indeed, out of 10 neurons recorded with 5 mM of the calcium chelator BAPTA in the pipette solution, none was found to be accommodating (80% of the cells were facilitating and 20% steady), as compared to the 40% accommodating cells found without BAPTA. This BAPTA-induced shift is also manifested in Fig. 7A where ΔIFR values of all the responses of AOB mitral cells to 100-pA current injections in the presence of 5 mM of BAPTA are distributed. As apparent, only <15% of the traces show spike accommodation, as compared to 42% without BAPTA (Fig. 5A). Nevertheless, as regards to facilitating cells the presence of BAPTA did not cause any significant change in their IFR (Fig. 7B, red triangles) along the response to 100-pA current steps, compared to the control condition (Fig. 7B, blue dots). These results suggest that the spiking accommodation exhibited by accommodating cells is caused by activity of a calcium-activated conductance which is not expressed by facilitating cells.

In order to further explore this possibility we used a low concentration (0.5 mM) of 4AP, a rather non-selective

blocker of voltage-activated potassium conductances, to induce widening of the spikes in AOB mitral cells. Indeed, as shown in Fig. 7C, D, the spike width at half its amplitude is about twice as large in the presence of 4AP (Fig. 7D) as compared to the control condition (Fig. 7C) for all three neuronal sub-populations. In the presence of 4AP the mean spike width shifts from 3.73 ms, 3.85 ms, and 3.47 ms to 6.63 ms, 6.73 ms and 6.70 ms for accommodating, steady, and facilitating cells, respectively. This doubling of the spike width is expected (Bondy et al., 1987; Cole et al., 1996) to significantly enhance calcium entry into AOB mitral cells during spiking activity through voltage-activated calcium channels (Dong et al., 2009). Hence, it is predicted to slow down the firing of accommodating cells due to increased activation of their assumed calcium-activated potassium conductance, while leaving firing activity of AOB facilitating mitral cells relatively intact. Indeed, as seen in Fig. 7E, the firing rate of accommodating AOB mitral cells along 100-pA current steps in the presence of 4AP is about 50% lower than in the control condition. In contrast, 4AP has only a minor effect on the firing rate of facilitating cells (red empty triangles), as compared to the control condition. Similarly, Fig. 7F shows that while the presence of 4AP causes a significant (>5 mV) hyperpolarization of the AHP at accommodating, as compared to control cells, it has no effect on the AHP of facilitating cells. Altogether these data



Fig. 6. The distinct sub-populations of AOB mitral cells show similar dynamics of some firing parameters and different dynamics of others. (A) Mean IFR values, averaged over 50-ms time bins, for accommodating (blue dots) and facilitating (red triangles) AOB mitral cells, plotted over time of response to 100-pA current steps. Note the rapid steady-state achieved by accommodating cells as opposed to no steady state achieved for facilitating cells. A statistically significant difference (P<0.01, paired *t*-test) was found between both sub-populations. (B) Mean AHP values, averaged over 50-ms time bins, for accommodating (blue dots) and facilitating (red triangles) AOB mitral cells, plotted over to 100-pA current steps. Note the similar dynamics between the two neuronal sub-populations (P>0.1, paired *t*-test). (C) Mean time constants for the changes in IFR during responses of accommodating (blue dots) and facilitating (red triangles) AOB mitral cells to depolarizing current steps, plotted as a function of the injected current level. Note the significant (P<0.01, paired *t*-test) differences between the two neuronal sub-populations. (D) Mean time constants for the changes in AHP during responses of accommodating (blue dots) and facilitating (red triangles) AOB mitral cells to depolarizing current steps, plotted as a function of the injected current level. Note the similarity between sub-populations below 140 pA. Nevertheless, over all values both populations were found to be significantly different (P<0.05, paired *t*-test). (E) The various sub-populations of AOB mitral cells (blue—accommodating, green—steady, brown—facilitating) show similar (one-way ANOVA, $F_{(2,62)}$ =0.379, P>0.5) mean capacitance values. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.



Fig. 7. Firing responses of the distinct sub-populations of AOB mitral cells are differentially affected by manipulations of the intracellular calcium level. (A) Distribution of Δ IFR values of the responses of AOB mitral cells to 100-pA current steps, recorded with 5 mM of the calcium chelator BAPTA in the intracellular solution. Note that in <15% of the cases a negative value, indicating spike accommodation, was found. (B) Mean IFR values recorded from facilitating cells, averaged over 50-ms time bins and plotted over time of response to 100-pA current steps, in the presence (red triangles) and absence (blue dots) of BAPTA in the intracellular solution. Note the similarity (P>0.1, paired *t*-test) between the results in both conditions. (C) The distributions of spike width between the distinct sub-populations of AOB mitral cells in the control condition. Note the doubling of the spike width between the distinct populations of AOB mitral cells (n=16) in the presence of 0.5 mM 4AP in the bath solution. Note the doubling of the spike width as compared to the control condition (C) for all neuronal sub-populations. The widening of the spike in the presence of 4AP is expected to increase calcium entry to the cells during firing. (E) Mean IFR values recorded from accommodating (blue, n=10) and facilitating (red, n=4) cells, averaged over 50-ms time bins and plotted over time of response to 100-pA current steps, in the presence (empty symbols, dashed lines) and absence (filled symbols, continuous lines) of 4AP in the bath solution. Note \sim 50% reduction in IFR of accommodating caused by 4AP presence, as opposed to its negligible effect on facilitating cells. (F) Same as in (E) for AHP values. Again note the significant hyperpolarization of the AHP caused by 4AP presence for accommodating cells, as opposed to the lack of influence of 4AP on facilitating cells. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

suggest that accommodating AOB mitral cells are influenced by calcium-activated conductance which is not functional in facilitating cells. Hence, these neuronal sub-populations are probably genuinely distinct.

A neuronal network of the three sub-populations of AOB mitral cells

Fig. 8A shows the first (blue dots) and last (green triangles) IFRs averaged over all accommodating (left panel), steady (middle panel), and facilitating (right panel) AOB mitral cells, plotted as a function of the level of the injected

current. As manifested, all cells display similarplotted as a function of the level of the injected first and last IFRs till the level of about 50 pA, beyond which accommodating cells show a last IFR which is progressively smaller than the first IFR whereas facilitating cells show the opposite trend. One interpretation of the prolonged modulation of the firing response of AOB mitral cells along a constant stimulus is that these neurons are capable of encoding the stimulus duration. A possible computational function for the AOB mitral cells is indicated by exploring what happens when the output of the three sub-populations are linearly inte-



Fig. 8. A simulated neuronal network containing the various sub-population of AOB mitral cells show the capacity to attenuate its response to episodic stimuli while fully responding to continuous stimuli. (A) First (blue dots) and last (green triangles) IFRs of the responses of accommodating (left), steady (middle), and facilitating (right) AOB mitral cells to depolarizing current steps, plotted as a function of the injected current level. (B) Linear summation of the I-F curves shown in (A), with a negative value given to the responses of accommodating cells. Note the shallow rise of the resultant first IFR (blue dots) with stimulus level, as opposed to the steep rise of the last IFR (green triangles) with stimulus strength. (C) A scheme describing the simulated neuronal network composed of three integrate-and-fire input neurons (accommodating, steady, and facilitating) and an integrate-and-fire readout neuron. Note the direct inhibition from the accommodating neuron as opposed to the direct excitation from the steady and facilitating neurons. (D) The firing response of the readout neuron of the simulated network plotted as a function of time for three levels of stimulus strength, as marked in the legend. Note that for all three stimulation levels, there is no response earlier than 250 ms from the beginning of stimulation. The transition to firing response starts earlier for stronger stimuli and the final steady state is proportional to the stimulus level. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

grated into a single output cell. Since accommodating neurons slow their firing rate along the stimulation while facilitating cells accelerate it, such integration would weaken or even abolish the information regarding the stimulus duration. However, if one of these neuronal subpopulations is connected to the output cell through an inhibitory synapse the information regarding stimulus duration should be enhanced. Accordingly, a simple overlay of the I-F curves of the three sub-populations of AOB mitral cells shown in Fig. 8A, in which the mean outputs of the facilitating and steady cells are summed and the mean output of the accommodating cells is subtracted, reveals (Fig. 8B) a steady, low, first IFR (blue dots) which is almost independent of stimulus amplitude and a higher last IFR (green triangles) that increases linearly with the amplitude of the stimulus. In order to further check this possibility we simulated a network which is schematically illustrated in Fig. 8C. The network consists of four integrate-and-fire neurons: an accommodating neuron, a steady neuron, a facilitating neuron in the AOB, and a hypothetical readout neuron that receives synaptic input from the three AOB neurons. The three AOB neurons were modeled to match the mean I-F curves of the three sub-populations of AOB mitral cells. For simplicity sake, the accommodating cell directly inhibits the readout cell although in reality any such inhibition would require an intermediary inhibitory interneuron since all AOB mitral cells are glutamatergic (Quaglino et al., 1999).

The model AOB cells are stimulated by 800-ms current steps between 50 pA and 150 pA, parallel to those used in the experiments described above, and the response of the readout neuron is calculated by averaging the instantaneous firing rates over a 100-ms time window, shifted over the length of the stimulus in 50-ms increments. As shown in Fig. 8D, where the firing rate over time is plotted for 50-pA, 100-pA, and 150-pA stimuli, the firing responses have a sigmoidal shape, with the transition occurring earlier for stronger stimuli, although only after 200 ms even for 150-pA stimulation. Moreover, the firing rate of the readout neuron is kept below 10 Hz over the initial 300 ms across the whole range of inputs while over the last 300 ms the firing rate increases as a function of the input strength from 10 Hz to 30 Hz. This type of neuronal network, therefore, attenuates its response to short stimuli whereas the response to persistent stimuli is enhanced.

DISCUSSION

In this study we have shown that MOB and AOB mitral cells in acute brain slices differ markedly in their membrane and firing properties. In terms of their intrinsic properties MOB mitral cells comprise a relatively homogeneous population which is highly excitable, despite their moderate input resistance. Given a constant stimulus the firing response of these cells seems to rapidly reach a steady state proportional to the stimulus amplitude, regardless of its duration. In contrast, AOB mitral cells display much higher input resistance but significantly lower excitability and are a lot more divergent in their firing response that either accelerates, slows, or remains steady along a constant stimulus.

The electrophysiological properties of MOB mitral cells were intensively studied both in vitro (Chen and Shepherd, 1997; Carlson et al., 2000; Heyward et al., 2001; Debarbieux et al., 2003; Rubin and Cleland, 2006; Balu and Strowbridge, 2007; Padmanabhan and Urban, 2010) and in vivo (Cang and Isaacson, 2003; Rinberg et al., 2006) in multiple studies. In contrast, electrophysiological studies of AOB mitral cells are rare and usually concentrated on their synaptic properties (Jia et al., 1999; Taniguchi and Kaba, 2001; Ma and Lowe, 2004; Sugai et al., 2005; Araneda and Firestein, 2006; Castro and Urban, 2009). Here we show for the first time that despite the many similarities between the two bulbs, MOB and AOB mitral cells differ markedly in their intrinsic properties. One very important difference between these neuronal populations is the input resistance, which is almost three times higher in AOB than in MOB mitral cells. This difference exists despite the similar specific passive membrane properties, which includes the common lack of time-dependent rectification, as reflected by the similar membrane time constant. The mean values of input resistance and membrane time constant of MOB mitral cells found by us are comparable to values found in other in vitro studies (Balu and Strowbridge, 2007) but are higher than those found using in vivo recordings (Cang and Isaacson, 2003). We show by both electrophysiological and morphological analyses that it is the much larger size of their membrane area which underlies the smaller input resistance of MOB mitral cells. The size difference is probably due to the much more elaborated dendritic tuft of MOB mitral cells, as compared to the simpler and smaller tufts of AOB mitral cells (Larriva-Sahd, 2008).

One would expect that the much higher input resistance of AOB mitral cells would make them more excitable by current injection than MOB mitral cells. Our findings, however, show a different reality. The minimal level of current injection needed to excite MOB mitral cells (held around -60 mV) is similar to that needed to cause firing in AOB cells. Moreover, MOB mitral cells usually respond even for threshold stimuli with a burst of spikes while AOB threshold responses comprise a single or few spikes only. These firing properties of MOB neurons are probably due to activation of a ramp potential, most likely mediated by persistent sodium conductance, which underlie their firing responses (Rubin and Cleland, 2006; Balu and Strowbridge, 2007). The higher excitability of MOB neurons is also reflected by their much higher maximal mean firing response which in many cases surpasses 100 Hz, whereas AOB neurons are limited in their mean firing response to significantly lower values, usually not more than 50 Hz. Thus, these neuronal types seem capable of responding to stimuli of similar magnitudes in different dynamic ranges.

The internal dynamics of the firing response is another important aspect that differs markedly between MOB and AOB mitral cells. Whereas MOB neurons rapidly (<200 ms) reach a steady state in their firing rate, the firing

response of many AOB neurons is consistently modulated throughout the 0.5-s long current step. Thus, the response of MOB neurons to constant stimuli contains information in the time domain only at early stages (<200 ms) of stimulation. This characteristic of MOB mitral cells fits the sniffing behavior underlying mammalian olfaction, which apparently limits the stimulus duration according to sniffing rate which in behaving mice was found to be in the range of 5-8 Hz (Kepecs et al., 2006; Schoenfeld and Cleland, 2006; Scott, 2006). The tight correlation between the dynamics of the IFR, SH, and AHP in MOB cells suggests that transient A-type potassium currents are involved in the early modulation of their firing response, in agreement with multiple studies that showed an important contribution of this type of current to firing activity of MOB mitral cells (Wang et al., 1996; Chen and Shepherd, 1997; Balu et al., 2004; Christie and Westbrook, 2006; Balu and Strowbridge, 2007).

We found relatively uniform dynamics in the responses of MOB mitral cells to constant stimuli. These results do not contradict the recent work by Padmanabhan and Urban (Padmanabhan and Urban, 2010), whose findings, in some cells, of bursting responses to constant stimuli may reflect the lack of EGTA in the internal solution. Additionally, the observation of more heterogeneous responses to complex stimuli than to fixed stimuli is not surprising (Kretzberg et al., 2001) given the affect on the membrane capacitance which continuously fluctuates with the complex form of the current. In contrast, in response to a fixed stimulus, the membrane capacitance is allowed to fully charge leading to steadier firing. Significantly, a principal component analysis of spike trains resulting from complex stimuli showed no contribution of slow co-varying elements indicating the absence of slow, spike-frequency adaptation. This is in agreement with our finding of a rapidly achieved steady state.

Compared to MOB mitral cells, AOB neurons are much more heterogeneous in the modulation of their firing response in time which divides them into three neuronal sub-populations: accommodating, facilitating, and steady. By analyzing the various features of their firing responses to depolarizing current steps we have shown that while these sub-populations exhibit similar passive membrane characteristics, they significantly differ in their intrinsic active properties. Specifically, we have shown that accommodating responses depend on free intracellular calcium, hence are probably mediated by calcium-activated conductance that may characterize accommodating cells, as opposed to the sub-population of facilitating cells. This possibility is further supported by the markedly different reactions of accommodating and facilitating neurons to the presence of 4AP, which strongly decrease the IFR and hyperpolarize the AHP during responses of accommodating cells, while exerting no effect on facilitating cells. Since the 4AP-induced increase of spike width is expected to enhance calcium-activated responses, the different reactions of accommodating and facilitating AOB mitral cells to its presence support a differential expression of calciumactivated conductances between these sub-populations.

Nevertheless, the question of whether these neuronal subpopulations represent genuine, intrinsically distinct neuronal categories of AOB mitral cells or just a differential expression of specific conductance remains open. Regardless of their category, however, the prolonged modulation of the firing responses of most AOB neurons suggests that they are capable of coding the stimulus duration in their firing response.

Whereas the role of the mammalian AOS in the detection of pheromones and other semiochemicals was established long ago (Wysocki, 1979; Halpern, 1987; Meredith, 1991), a growing number of recent studies found a central role for the MOS in responses of mammals to pheromones (see Introduction). These studies have raised an essential question: what is the functional difference between these systems with regard to pheromone detection? This question has been debated in several recent review articles (Restrepo et al., 2004; Baxi et al., 2006; Spehr et al., 2006; Baum and Kelliher, 2009).

We previously proposed (Dulac and Wagner, 2006) that although the two systems may be activated by at least partially overlapping sets of pheromonal cues, they process this information in different ways and thereby address different aspects of it. These distinct informational aspects may converge in brain areas (such as the amygdala) that form a common target for the two systems (Pro-Sistiaga et al., 2007). This proposition was initially based on several differences between the MOS and the AOS with regard to their connectivity. First, while the MOB projects to multiple cortical areas which may serve most of the processing load in the MOS, the AOB projections bypass the cortex and project to effector areas such as the hypothalamus either directly or via the "vomeronasal amygdala" (Halpern and Martinez-Marcos, 2003). This connectivity scheme suggests a more prominent role for the AOB in sensory information processing by the AOS. Second, whereas MOE sensory neurons that express the same receptor usually project to only two MOB glomeruli (Mombaerts, 1996; Zufall and Munger, 2001), VNO sensory neurons expressing a given receptor were shown to innervate many (10-30) glomeruli in the AOB, thus creating a distributed and complex sensory map (Belluscio et al., 1999; Rodriguez et al., 1999; Wagner et al., 2006). Third, in the MOB each mitral cell receives direct excitatory sensory information from only one receptor type, through its single dendritic tuft which innervates a single glomerulus (Chen and Shepherd, 2005; Lledo et al., 2005; Wilson and Mainen, 2006). This wiring scheme seems to support an analytical information flow in which the information elicited in a given "line", by activation of a certain receptor, is processed discretely, with the exception of lateral inhibitory interactions (Mori et al., 1999; Urban, 2002; Lowe, 2003; Schoppa and Urban, 2003; Arevian et al., 2008). This scheme makes the MOB best suited for dissecting the pheromonal blend into its individual components and analyzing each of them separately. In contrast, we showed that AOB mitral cells, with their multiple dendritic tufts, receive input from several types of receptors, and thereby integrate excitatory sensory information arriving from various "lines" (Wagner

et al., 2006). This observation contradicts previous work (Del Punta et al., 2002) but was recently confirmed by *in vivo* electrophysiological recordings for at least some of the AOB mitral cells (Ben-Shaul et al., 2010; Meeks et al., 2010). The integrative scheme suggested by us (Dulac and Wagner, 2006) makes the AOB more suited for processing the more complex qualities of the pheromonal blend, such as co-existence of several given components, which may indicate a proper social context rather than trigger a specific response.

Another dimension in which sensory information may be integrated is time. In contrast to sensory responses in the MOE which are dominated by the discrete sniffing events (Kepecs et al., 2006), the time course of sensory responses in the vomeronasal organ is thought to be determined by slower pumping activity (Meredith et al., 1980) which allows sensory integration along an extended time window (Meredith, 1994). Indeed, recordings made from AOB mitral cells of behaving mice investigating conspecifics revealed responses that were delayed but extended for 10-30 s after the termination of physical contact with the stimulus (Luo et al., 2003). Here we show that the firing responses of AOB mitral cells to a constant stimulus are modulated along at least several hundreds of milliseconds, hence may encode information regarding the duration of prolonged stimuli. Moreover, we have constructed a hypothetical neuronal network, comprised of the three types of AOB mitral cells found by us and a readout neuron targeted by all of them, that integrates its input in time such that its response to a constant stimulus is strongly attenuated at the early (<200 ms) stage of the stimulus but is later fully compliant with input strength. It is important to keep in mind that this model lacks the various types of AOB interneurons, hence cannot be considered as a realistic simulation of AOB activity. Instead, the model serves as a suggestion for the type of information processing that may take place between the three neuronal sub-populations of AOB neurons, according to their intrinsic properties. This type of neuronal network may support a safety mechanism in the AOS that prevents the animal from responding to transient events, but enhances its response when the proper social context occurs. Such a social context could be the presence of a conspecific which would provide a rich source of pheromonal cues for a sufficiently long time.

CONCLUSIONS

MOB and AOB mitral cells differ markedly in their passive and active intrinsic properties. Regarding passive properties, AOB mitral cells display higher input resistance than MOB mitral cells, mainly reflecting their smaller membrane area. Regarding active properties, the two types of mitral cells show very distinct dynamic range of firing responses, with MOB neurons displaying much higher values of minimal and maximal firing rates. Thus, MOB mitral cells are much more excitable than AOB mitral cells. The two populations of neurons significantly differ in their internal dynamics of firing responses to constant stimuli. While MOB mitral cells rapidly reach a steady state, the firing responses of many AOB neurons are modulated over extended time window. AOB mitral cells may be categorized into three neuronal sub-populations: accommodating, facilitating, and steady, according to the dynamics of their firing responses. These neuronal sub-populations differ in their intrinsic firing properties but not in their passive membrane properties. A neuronal network which integrates these neuronal sub-populations may serve as a safety mechanism attenuating the response to episodic stimuli while fully compliant with prolonged stimulation.

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66

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