Neuroscience 189 (2011) 51–67

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

S. ZIBMAN,[a] G. SHPAK[b] AND S. WAGNER[b]*

[a]*Institute for Life Sciences and Interdisciplinary Center for Neural Computation, Hebrew University, Jerusalem, Israel*

[b]*Department of Neurobiology and Ethology and Department of Biology, University of Haifa, Haifa, Israel*

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.

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 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;

*Corresponding author. Tel: +972-4-8288773; fax: +972-4-8288763.
E-mail address: shlomow@research.haifa.ac.il (S. Wagner).
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.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.05.039

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-μm-thick slices were equilibrated for at least 1 h and up to 5 h in physiological solution containing (mM): 125 NaCl, 25 $NaHCO_3$, 15 glucose, 3 KCl, 2 $CaCl_2$, 1.3 NaH_2PO_4, and 1 $MgCl_2$, bubbled with 95% O_2 and 5% CO_2 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 μM bicuculline, 5 μM gabazine, or 50 μM 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; $CaCl_2$, 0.5; MgATP, 3; NaGTP, 0.5; phosphocreatine, 10 (10–15 MΩ). When BAPTA was used to block changes in the intracellular calcium levels, we used similar intracellular solution with no $CaCl_2$ and 5 mM of BAPTA (tetrapotassium salt, Invitrogen Carlsbad, CA, USA) instead of EGTA. Seal resistance was at least 2 GΩ and typically 5–8 GΩ. 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× 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 (Kolmogorov–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