Neuroscience Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Similar rate of information transfer on stimulus intensity in accessory and main olfactory bulb output neurons

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HIGHLIGHTS

- We compared main and accessory olfactory bulb output neurons.
- The two neurons showed distinct kinetic properties of voltage-gated currents.
- The variants of the Hodgkin–Huxley model reproduced their different firing.
- Mutual information between firing frequency and current amplitude was estimated.
- The rate of the information transfer in the two neurons was almost the same.

ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 15 May 2014
Accepted 29 May 2014
Available online xxx

Keywords:
Olfactory system
Accessory olfactory bulb
Patch clamp
Action potential
Voltage-gated channel
Mutual information

ABSTRACT

Recently, evidence has accumulated that the vomeronasal system cooperates with the main olfactory system to process volatile cues that regulate the animal's behavior. This is contradictory to the traditional view that the vomeronasal system is quite different from the main olfactory system in the time scale of information processing. Particularly, the firing rate of mitral/tufted cells in the accessory olfactory bulb (MT_{AOB}) is known to be significantly lower than that of mitral cells in the main olfactory bulb (MC_{MOB}). To address this question of whether the low-frequency firing in MT_{AOB} carries less information than the high-frequency firing in MC_{MOB} in the early stages of stimulation, we compared MT_{AOB} and MC_{MOB} for their firing mechanisms and information transfer characteristics. A model computation demonstrated that the inherent channel kinetics of MT_{AOB} was responsible for their firing at a lower frequency than MC_{MOB}. Nevertheless, our analysis suggested that MT_{AOB} were comparable to MC_{MOB} in both the amount and speed of information transfer about depolarizing current intensity immediately after current injection onset (<200 ms). Our results support a hypothesis of simultaneous processing of common cues in both systems.

1. Introduction

Mitral/tufted cells (MT_{AOB}) in the accessory olfactory bulb (AOB) and mitral cells (MC_{MOB}) in the main olfactory bulb (MOB) are second-order neurons of the vomeronasal system and the main olfactory system, respectively. The intrinsic frequency of MT_{AOB} firing was shown to be lower than that of MC_{MOB} firing over the range of the direct current injection from the threshold to the saturation level under the whole-cell patch-clamp condition [1]. In addition, Ca^{2+}-activated K^+ current was thought to be involved in the genuine distinction of sub-populations within MT_{AOB}. The sub-populations of MT_{AOB} were considered to be involved in a safety mechanism that attenuates responses in the early stage (<200 ms) of stimulation [1]. Meanwhile, the excitability of MC_{MOB} has been elaborately modeled by integrating various types of currents, including voltage-gated Na^+, K^+, and Ca^{2+} currents and Ca^{2+}-activated K^+ current [2]. Nevertheless, the mechanisms responsible for the difference in the intrinsic frequency of firing between MT_{AOB} and MC_{MOB} remain unclear.

Also, the relationship between the intrinsic firing frequencies and information processing is unknown in both MT_{AOB} and MC_{MOB}. The firing frequency of MT_{AOB} *in vivo* gradually increased until reaching a peak after ~20 s following the initiation of physical contact with the stimulating animal [3]. This is far slower than odor

Abbreviations: AOB, accessory olfactory bulb; MC, mitral cells; MI, mutual information; MOB, main olfactory bulb; MT, mitral/tufted cells.

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http://dx.doi.org/10.1016/j.neulet.2014.05.058
0304-3940/

processing by MC_{MOB}, which is time-locked to sniffing of theta frequency (4–6 Hz) and discriminates odor quality within one cycle (<200 ms) [4]. Traditionally, an often-emphasized characteristic of vomeronasal system functions is that speed is less important than accuracy [5]. However, we recently identified specific pyrazine analogs as volatile constituents of wolf urine. These pyrazines elicit responses in MT_{AOB} and provoke avoidance and freezing behaviors in mice [6]. These findings suggest that predator–prey chemosignalling is processed by not only the main olfactory system but also the vomeronasal system in mice. If the vomeronasal system immediately interacts with the main olfactory system through interconnected olfactory brain regions [7], information processing by the AOB should be less different from that by the MOB in temporal scale than previously thought.

In this study, the channel kinetics and information transfer characteristics of MT_{AOB} were compared with those of MC_{MOB}. Using patch-clamp techniques for brain slices, we revealed the inherent kinetics of voltage-gated Na^+ and K^+ currents of MT_{AOB} firing at a lower frequency than MC_{MOB}. Moreover, information conveyed by firing frequencies about the amplitude of current injections was compared between MT_{AOB} and MC_{MOB}. For this purpose, mutual information (MI) was employed. MI is a measure of the interdependence between two variables and is often regarded as a generalization of correlation coefficient [8]. MI quantified the relationships between firing frequencies and current injection amplitude. MI analysis showed that the amount and speed of information transfer of MT_{AOB} were comparable to those of MC_{MOB} in the early stages of stimulation (<200 ms). This supports the possibility of dual information processing by MT_{AOB} and MC_{MOB}, which allows both olfactory systems to interact with each other without temporal gaps.

2. Materials and methods

2.1. Slice preparation

All experiments were carried out in accordance with the Guidelines for the Use of Laboratory Animals of Asahikawa Medical University and approved by the committee of Asahikawa Medical University for Laboratory Animal Care and Use (approval ID: 13002). BALB/c mice (3 months or older, 50 males and 3 females) were deeply anesthetized with pentobarbital sodium (150 mg/kg) and treated by cardiac perfusion with ice-cold sucrose-based Ringer's solution (in mM: 234 sucrose, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 $MgCl_2$, 0.5 $CaCl_2$, 11 glucose, pH 7.4) oxygenated with 95%-O_2 and 5%-CO_2 mixed gas. Forebrains including both the AOB and MOB were dissected out and cut into parasagittal slices at a thickness of 200 μm with a microslicer. The slices were incubated in normal Ringer's solution (in mM: 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $MgCl_2$, 1 $CaCl_2$, 11 glucose, pH 7.4) saturated with the O_2/CO_2 gas at 37 °C for 30 min and equilibrated in the same solution at room temperature (24–26 °C) until use.

2.2. Patch-clamp recordings

Electrophysiological experiments were performed at room temperature. The forebrain slice was placed in a homemade recording chamber filled with normal Ringer's solution saturated with the O_2/CO_2 gas. Individual cells were observed by infrared differential interference contrast video microscopy through an E600FN microscope (Nikon, Tokyo, Japan) with a water-immersion 40× objective. Glass electrodes were filled with K-gluconate intracellular solution (in mM: 140 K-gluconate, 2 $MgCl_2$, 2 Na_2ATP, 0.5 EGTA/KOH, 10 HEPES, pH 7.2/KOH) to measure action potentials and voltage-gated K^+ current (I_K). To measure voltage-gated Na^+ currents (I_{Na}), CsCl intracellular solution (in mM: 115 CsCl, 15, NaCl, 2 $MgCl_2$, 2 Na_2ATP, 0.5 EGTA/CsOH, 10 HEPES, 10 tetraethylammonium chloride, pH 7.2/CsOH) was used. The electrode resistance was 8.6 ± 0.20 MΩ ($n = 157$) for K-gluconate intracellular solution and 6.2 ± 0.87 MΩ ($n = 12$) for CsCl intracellular solution. The seal resistance achieved with the tips of the glass electrodes on cell surfaces was 11 ± 1.2 GΩ ($n = 142$) for K-gluconate intracellular solution and 14 ± 4.3 GΩ ($n = 12$) for CsCl intracellular solution. The series resistance that occurred by the formation of a whole-cell patch configuration was 43 ± 2.2 MΩ ($n = 157$) for K-gluconate intracellular solution and 34 ± 8.6 MΩ ($n = 12$) for CsCl intracellular solution. Membrane potential and currents were acquired through an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) with a 10-kHz low-pass filter, and were digitized by a Digidata 1320A digitizer (Molecular Devices) controlled on pClamp software (Molecular Devices) at a sampling frequency of 20 kHz. Subtraction of the capacitive current and compensation of the series resistance were performed manually using the built-in circuit of the amplifier in the range without overcompensation. Liquid junction potentials were estimated at 15 mV for using K-gluconate intracellular solution and at 3 mV for using CsCl intracellular solution. They were adjusted in calculations for the kinetic parameters of voltage-gated currents. Electrophysiological data were analyzed offline using Excel software (Microsoft, Redmond, WA, USA) with Visual Basic-based macro programs and curve-fitted using KaleidaGraph software (Synergy Software, Reading, PA, USA). Data values are shown as mean ± 95% confidence intervals unless otherwise noted.

2.3. Morphology

Cells in AOB ($n = 54$) and MOB ($n = 46$) were recorded with K-gluconate intracellular solution containing 1% biocytin for labeling. After patch-clamp recording, the slices were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C overnight and stored in a long-term protectant (30% sucrose, 30% ethylene glycol, 1% polyvinylpyrrolidone in 0.1 M phosphate buffer, pH 7.4) at 4 °C until use. For staining, the fixed slices were washed in PBS with 0.4% triton X-100 (PBSx) and treated with 0.6% H_2O_2 in PBSx for 15 min followed by washing in PBSx. The slices incubated in PBSx containing avidin–biotin conjugated with horseradish peroxidase (HRP) from the ABC kit (Vector Laboratories, Burlingame, CA, USA) for 1 h followed by washing in PBSx. The recorded cells were visualized by diaminobenzidine (0.5 mg/ml in 50 mM Tris–HCl, pH 7.6) reacting with HRP that was specifically linked to the biocytin label, in the presence of H_2O_2 (4 mM solution/ml). Pictures were taken using a BX51 microscope (Olympus, Tokyo, Japan) to confirm cellular shape and location.

2.4. Confirmation of cell types

MT_{AOB} and MC_{MOB} were distinguished from other types of cells on the basis of their morphology and electrical excitability. In morphology, MT_{AOB} have the largest soma within the mitral/tufted cell layer of AOB and extend multiple primary dendrites to different glomeruli. MC_{MOB} locate their soma in the mitral cell layer of MOB. They have a single primary dendrite that forms a glomerulus, and some long secondary dendrites that extend laterally in the external plexiform layer [9]. Typical images of MT_{AOB} and MC_{MOB} are shown in Fig. 1A. In addition, MT_{AOB} are known to be regionalized into the rostral and caudal regions differently in response to gender-specific urinary pheromones [10]. We chose MT_{AOB} in the rostral region of AOB. In electrical excitability, MT_{AOB} ($n = 42$) showed a maximum firing rate ($rate_{max}$) of 22 ± 3.2 Hz and a coefficient of variance of interspike intervals (CV_{ISI}) of 0.22 ± 0.058. MC_{MOB} ($n = 42$) showed $rate_{max}$ of 33 ± 4.8 Hz and CV_{ISI} of 0.32 ± 0.093. Interneuron-like

Please cite this article in press as: T. Noguchi, et al., Similar rate of information transfer on stimulus intensity in accessory and main olfactory bulb output neurons, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.05.058