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SPONTANEOUS FIELD POTENTIALS IN THE GLOMERULI OF THE OLFACTORY BULB: THE LEADING ROLE OF JUXTAGLOMERULAR CELLS

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Abstract—Field potentials recorded in the olfactory bulb glomerular layer (GL) are thought to result mainly from activation of mitral and tufted cells. The contribution of juxtaglomerular cells (JG) is unknown. We tested the hypothesis that JG are the main driving force to novel spontaneous glomerular layer field potentials (sGLFPs), which were recorded in rat olfactory bulb slices maintained in an interface chamber. We found that sGLFPs have comparable magnitudes, durations and frequencies both in standard horizontal slices, where all layers with all cell types were present, and in isolated GL slices, where only JG cells were preserved. Hence, the impact of mitral and deep/medium tufted cells to sGLFPs turned out to be minor. Therefore, we propose that the main generators of sGLFPs are JG neurons. We further explored the mechanism of generation of sGLFPs using a neuronal ensemble model comprising all types of cells associated with a single glomerulus. Random orientation and homogenous distribution of dendrites in the glomerular neuropil along with surrounding shell of cell bodies of JG neurons resulted in substantial spatial restriction of the generated field potential. The model predicts that less than 20% of sGLFP can spread from one glomerulus to an adjacent one. The contribution of JG cells to the total field in the center of the glomerulus is estimated as ~50% (~34% periglomerular and ~16% external tufted cells), whereas deep/medium tufted cells provide ~39% and mitral cells only ~10%. Occasionally, some sGLFPs recorded in adjacent or remote glomeruli were cross-correlated, suggesting involvement of interglomerular communication in information coding. These results demonstrate a leading role of JG cells in activation of the main olfactory bulb (MOB) functional modules. Finally, we hypothesize that the GL is not a set of independent modules, but it represents a subsystem in the MOB network, which can perform initial processing of odors.

Key words: electrogenesis, slices, glomerular layer, closed field, model.

Field potentials recorded in brain tissue are generated by summation of electrical fields' f neuronal dipoles (Lorente de Nó, 1947; Buresh et al., 1962; Hubbard et al., 1969; Guselnikov, 1976; Karnup, 1980, 1982). Synchronous fluctuations of electrical charges on similarly oriented ends of dipoles, for instance in apical dendrites of pyramidal cells in the hippocampus or neocortex, result in electroencephalographic (EEG) waves. In brain structures, lacking comparable orientation of neuronal dipoles or constructed of quadrupole-like multipolar neurons, a distinctive EEG cannot be recorded (Buresh et al., 1962; Guselnikov, 1976). In layered structures, such as the hippocampus and neocortex, current source density analysis can determine where the majority of synchronously depolarized neuronal compartments are located (for a review, see Mitzdorf, 1985). An active zone (in which neurons are depolarized by ionic current influx, hence the extracellular potential is negative) and a passive zone (with a phase reversal of the extracellular field due to the dipolar properties of cells) are referred to as the sink and source, respectively. In such structures, the sink is readily revealed upon orthodromic stimulation resulting in a synchronized excitatory synaptic input (Lambert et al., 1991; Aroniadou and Keller, 1993; Kandel and Buzsaki, 1997). Similar observations have been made in the main olfactory bulb (MOB) (Aroniadou-Anderjaska et al., 1999). This structure is stratified and has five main layers (from the surface to depth): olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL) and granule cell layer (GCL). The olfactory nerve (ON) establishes synaptic contacts exclusively in the GL, in the neuropil of spherical structures about 100–120 μm in diameter, which are referred to as glomeruli. In rat, each glomerulus *in vivo* comprises the apical dendritic tufts of 13–25 mitral cells, of about 25–50 medium and deep tufted cells and dendrites of 1500–2000 juxtaglomerular (JG) cells (Allison, 1953; Meisami and Safari, 1981; Frazier and Brunjes, 1988; Royet et al., 1988;

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Abbreviations: ACSF, artificial cerebrospinal fluid; avgCCF, averaged cross-correlation function; BIC, bicuculline; CCF, cross-correlation function; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D-(−) 2-amino-5-phosphopentanoic acid; DIC, differential interference contrast; EEG, electroencephalogram/electroencephalographic; eFP, evoked field potential; EPL, external plexiform layer; ET, external tufted; GCL, granule cell layer; GL, glomerular layer; IGL, isolated glomerular layer; JG, juxtaglomerular; LLD, long-lasting depolarization; MCL, mitral cell layer; MOB, main olfactory bulb; M/T, mitral/tufted; ON, olfactory nerve; ONL, olfactory nerve layer; PG, periglomerular; SA, short-axon; sACSF, sucrose-containing artificial cerebrospinal fluid; sGLFP, spontaneous glomerular layer field potential; sLLD, spontaneous long-lasting depolarization.

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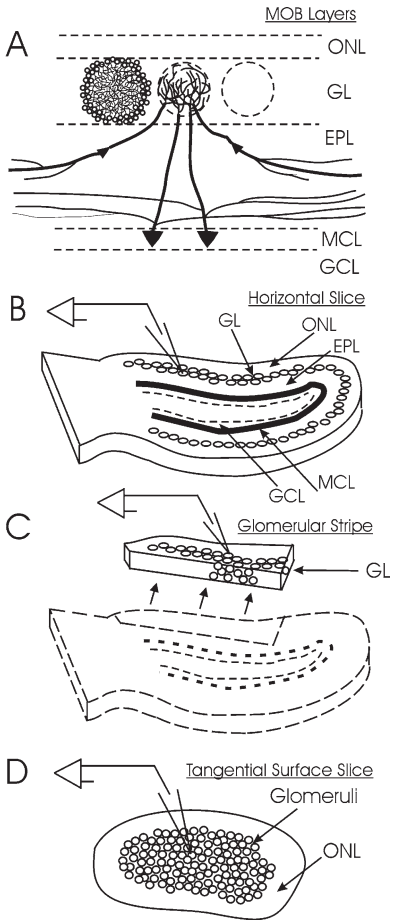

Fig. 1. (A) Scheme of olfactory bulb layers. Dashed circles in the GL indicate the border between the glomerular neuropil and the surrounding shell of JG cell bodies. In the left glomerulus only dendrites of JG cells arborizing within the glomerular core are shown. In the central glomerulus only dendritic arborizations of M/T cells are shown. Secondary dendrites of tufted cells and lateral dendrites of mitral cells in the EPL are curtailed. (B–D) Olfactory bulb slice preparations. (B) Horizontal slice of the MOB. (C) Isolated segment of the GL from a horizontal slice. Recordings were performed from GL sites free of adjacent EPL. (D) Tangential slice of GL+ONL made from the medial blade of the MOB (GL on the upper surface with ONL underneath). Recordings were performed from sites free of EPL remains.

Shipley et al., 1996; Royet et al., 1998). Cell bodies of JG neurons surround the glomerular neuropil, thus constituting a structural and a functional module (Fig. 1A). A conspicuous feature of the MOB is that the dendrites of all neurons that have been described so far, possess both postsynaptic and presynaptic loci; therefore, dendrodendritic synaptic interactions provide the majority of interneuronal communications within the bulb (Pinching and Powell, 1971a; Isaacson, 1999; Isaacson and Strowbridge, 1998; Sassoe-Pognetto and Ottesen, 2000; Schoppa and Urban, 2003). Despite their key position in the pathway of transmission of odor information to the brain, the role of JG cells is poorly understood. JG cells comprise three morphologically different kinds of neurons: periglomerular (PG), external tufted (ET) and short-axon (SA) cells. The primary dendrites of PG and ET cells converge toward the center of the glomerulus, resulting in roughly radial symmetry of the module. In contrast, SA cells do not belong to any particular glomerulus—their dendrites and axons are arbitrary directed and randomly distributed in the extraglomerular space—so they are in position to provide interglomerular interactions (Aungst et al., 2003). The primary dendrites of each PG, ET, tufted and mitral cell are affiliated with a single glomerulus (with few exceptions; Shipley et al., 1996). Dendritic arborizations of such a cellular ensemble constitute the glomerular core (neuropil) with an essentially homogenous density (Pinching and Powell, 1971b). Cell bodies of mitral cells are located in the MCL about 200–300 μm away from their tufts, thus forming large dipole moments. Deep and medium tufted cells have shorter primary dendritic shafts, which are often oriented obliquely in various directions (Macrides and Schneider, 1982). In contrast, mitral/tufted (M/T) cells have long lateral dendrites in the EPL, extending up to 1 mm (Fig. 1A and Fig. 6). Thus, a sub-population of M/T neurons, whose apical dendrites are affiliated with a single glomerulus, creates a very local concentrated sink and a broadly distributed source. When the ON is stimulated a number of glomeruli are simultaneously activated, resulting in a pronounced sink in the GL and a corresponding source in the MCL and the lower EPL (Aroniadou-Anderjaska et al., 1999). Thus, the current viewpoint is that the sink in the GL is generated primarily by synchronous depolarization of mitral and tufted cell tufts, whereas the impact of JG cells is negligible because they do not have large dipole moments and they are not oriented parallel to each other (Mori, 1987; Shipley et al., 1996). Hence, their synaptic currents were considered unlikely to contribute significantly to local field potentials (Rall and Shepherd, 1968).

In the present study, we show that spontaneous field potentials can be recorded without any stimulation in acute MOB slices in normal artificial cerebrospinal fluid (ACSF). Furthermore, spontaneous field potentials of comparable amplitude and duration can also be recorded in slices containing only the GL. Therefore, a substantial part of these field potentials may be generated by the ensemble of JG cells. This hypothesis is further supported by modeling the fields produced by the glomerular module. We assumed that an extracellular field potential in the GL originates from the simultaneous depolarization of the majority of dendrites within the glomerular core. The constructed electrostatic model was based on the basic anatomy of the olfactory bulb, where subsets of mitral, tufted and JG neuron types generated electrical fields in accordance with their known location and morphology. Both experimental and simulated data strongly suggest a preponderant contribution of JG cells to the total glomerular field as compared with mitral and tufted cells.

EXPERIMENTAL PROCEDURES

Sprague–Dawley rats (21–30 days old) of either sex were anesthetized with chloral hydrate and decapitated in accordance with Institutional Animal Care and Use Committee and NIH guidelines. Every effort was made to minimize the number of animals used and their suffering. The olfactory bulbs were removed and immersed in the ice-cold sucrose-containing artificial cerebrospinal fluid (sACSF) sat-