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PROPERTIES OF EXTERNAL PLEXIFORM LAYER INTERNEURONS IN MOUSE OLFACTORY BULB SLICES

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Abstract—In the external plexiform layer (EPL) of the main olfactory bulb, apical dendrites of inhibitory granule cells form large numbers of synapses with mitral and tufted (M/T) cells, which regulate the spread of activity along the M/T cell dendrites. The EPL also contains intrinsic interneurons, the functions of which are unknown. In the present study, recordings were obtained from cell bodies in the EPL of mouse olfactory bulb slices. Biocytin-filling confirmed that the recorded cells included interneurons, tufted cells, and astrocytes. The interneurons had fine, varicose dendrites, and those located superficially bridged the EPL space below several adjacent glomeruli. Interneuron activity was characterized by high frequency spontaneous excitatory postsynaptic potential/currents that were blocked by the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione and largely eliminated by the voltage-sensitive Na^+ channel blocker, tetrodotoxin. Interneuron activity differed markedly from that of tufted cells, which usually exhibited spontaneous action potential bursts. The interneurons produced few action potentials spontaneously, but often produced them in response to depolarization and/or olfactory nerve (ON) stimulation. The responses to depolarization resembled responses of late- and fast-spiking interneurons found in other cortical regions. The latency and variability of the ON-evoked responses were indicative of polysynaptic input. Interneurons expressing green fluorescent protein under control of the mouse glutamic acid decarboxylase 65 promoter exhibited identical properties, providing evidence that the EPL interneurons are GABAergic. Together, these results suggest that EPL interneurons are excited by M/T cells via AMPA/kainate receptors and may in turn inhibit M/T cells within spatial domains that are topographically related to several adjacent glomeruli. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: AMPA, GABAergic inhibition, fast-spiking interneurons, high-frequency EPSP/EPSCs, autocorrelation, glomerular coding.

The external plexiform layer (EPL) of the olfactory bulb is the second level of synaptic processing in olfaction. This layer lies immediately below the glomeruli, where the axons of the olfactory nerve (ON) converge onto the dendrites of mitral and tufted (M/T) cells and periglomerular (PG) cells. The EPL primarily consists of a dense neuropil formed by the M/T cell dendrites and GABAergic granule cell dendrites, which ascend into the EPL from the deeper, mitral cell and granule cell layers, respectively. Within the EPL, the lateral dendrites of the M/T cells form large numbers of type 1 (ultrastructurally excitatory) synapses with the granule cell dendrites, which return type 2 (ultrastructurally inhibitory) synapses, ~80% of which are paired (Price and Powell, 1970; reviewed in Shepherd et al., 2004). The GABAergic synapses of the EPL have been shown to provide robust, feedback inhibition (see Jahr and Nicoll, 1982) and lateral inhibition (Isaacson and Strowbridge, 1998; Margrie et al., 2001; Lowe, 2002; Xiong and Chen, 2002) of M/T cells. Together with A-type K^+ currents (Christie and Westbrook, 2003), this inhibition regulates the spread of backpropagating action potentials along the M/T cell lateral dendrites, which can extend up to ~1 mm from the cell body (Mori et al., 1983).

In addition to the granule cells, the olfactory bulb contains a variety of other interneurons. The most numerous are the PG cells, which are a heterogeneous cell population (see Kosaka et al., 1998). Many of the PG cells are GABAergic, however, and they appear to inhibit M/T cells at the first level of synaptic processing in the glomerular layer (GL) (Getchell and Shepherd, 1975; Duchamp-Viret et al., 1993). The inhibition can be local, within a glomerulus (Urban and Sakmann, 2002), or disynaptically mediated via short axon (SA) cells over distances of up to 20–30 glomeruli (Aungst et al., 2003).

In comparison with the other olfactory bulb layers, the EPL has few cell bodies. In the rat and hamster, which have been most thoroughly studied, it nevertheless contains significant numbers of interneurons (Schneider and Macrides, 1978; Kosaka et al., 1994), tufted cells of several types (Macrides and Schneider, 1982), and astrocytes (Bailey and Shipley, 1993; Mirich et al., 2002). Electrophysiological recordings have shown that the responses of different M/T cell types to ON stimulation are correlated

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EPL, external plexiform layer; EPSP/C, excitatory postsynaptic potential/current; ET, external tufted; GAD, glutamic acid decarboxylase; GFP, green fluorescent protein; GL, glomerular layer; GluR1, glutamate receptor 1; IR, immunoreactive; MCL, mitral cell layer; M/T, mitral/tufted; ON, olfactory nerve; PG, periglomerular; SA, short axon; TTX, tetrodotoxin; VG, Van Gehuchten.

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with the depth of the lateral dendrites in the EPL (Ezeh et al., 1993). No comparable recordings have been obtained from the interneurons of the EPL, the electrophysiological properties and functions of which are unknown.

The EPL interneurons are multipolar neurons that were originally described in the cat by Van Gehuchten and Martin (1891). Schneider and Macrides (1978) subsequently proposed that in the hamster EPL, Van Gehuchten (VG) cells interact with granule cell dendrites. More recently, multipolar neurons with morphological features of VG cells and SA cells have been identified in the rat EPL using staining methods for neuropeptides (Gall et al., 1986), NADPH diaphorase (Scott et al., 1987), and Ca^{2+}-binding proteins (Briñón et al., 1992; Kosaka et al., 1994). Both the VG and SA cells typically have varicose dendrites that branch within either the superficial or deep half of the EPL. The VG cells have more elaborate dendritic arborizations near the cell body, however, and the SA cells have axons. With parvalbumin immunostaining methods, multipolar neurons with more widely branching dendrites that are either varicose or relatively smooth have also been observed throughout the EPL. Moreover, neurons with intermediate branching patterns between the VG, SA, and multipolar types have been observed, suggesting that EPL interneurons may comprise a single morphological continuum (Kosaka et al., 1994).

At the ultrastructural level, the parvalbumin-immunoreactive (IR) interneurons of the rat EPL appear to receive type 1 synapses from M/T cells and to form type 2 synapses onto the M/T cells, ~30–50% of which are reciprocal (Toida et al., 1996). At least some of the parvalbumin-IR interneurons are also GABA-IR (Kosaka et al., 1987). In addition, EPL interneurons are strongly IR for the glutamate receptor 1 (GluR1) α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit (Petralia and Wenthold, 1992; Giustetto et al., 1997; Montague and Greer, 1999). Immunoreactivity for GluR1 (Hamilton and Coppola, 2003) and for parvalbumin (Philpot et al., 1997) is greatly reduced following neonatal naris occlusion, a procedure that deprives the olfactory system of airborne odor stimulation and alters olfactory bulb development.

Together, these observations suggest that the interneurons of the EPL are responsive to the afferent sensory input via M/T cells and might in turn inhibit the M/T cells. To investigate these possibilities, we have initiated electrophysiological studies of EPL interneurons in mouse olfactory bulb slices. Here, we show that interneurons resembling the VG and parvalbumin-IR multipolar interneurons of the rat have dendritic arbors that are spatially related to several adjacent glomeruli, are excited spontaneously via AMPA/kainate receptors, and receive polysynaptic inputs from the ON.

EXPERIMENTAL PROCEDURES

Recording methods

Juvenile and mature 22–34 day old C57BL/6J mice from Jackson Laboratories (Bar Harbor, ME, USA) and transgenic mice reared at the University of Maryland, Baltimore, MD, USA, were used. The transgenic mice expressed enhanced green fluorescent protein (GFP) under control of the regulatory region of the 65 kDa mouse glutamic acid decarboxylase (GAD) 65 gene (Erdélyi et al., 2002; Galarreta et al., 2004). Horizontal slices (400 μm thick) were obtained using a vibratome (Series 1000, Ted Pella Inc., Redding, CA, USA) as previously described (Heyward et al., 2001). The slices were sectioned in cold artificial cerebrospinal fluid (ACSF), immersed for 30–60 min in ACSF at 30 °C, and stored in ACSF at room temperature prior to recording. For recording, the slices were superfused with 30 °C ACSF at the rate of 1.5–3.0 ml/min. The ACSF solutions contained (in mM) NaCl 120, KCl 3, $MgCl_2$ 1.3, $CaCl_2$ 1.3, glucose 10, $NaHCO_2$ 25, BES 5 (300 mOsm), and were pH 7.27 with saturated 95% O_2+5% CO_2. To study excitatory postsynaptic potentials and currents (EPSP/Cs), the normal ACSF was switched to ACSF containing the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM, Tocris Cookson, Ltd., Ellisville, MO, USA) or 1 μM tetrodotoxin (TTX). Unless otherwise specified, chemicals were obtained from Sigma (St. Louis, MO, USA).

Pipettes were pulled from 1.5 mm O.D. borosilicate glass with internal filament using a Flaming-Brown P-97 puller. They were filled with K^+-gluconate 144, NaCl 2, $MgCl_2$ 2, EGTA 0.2, Mg_2ATP 2, Na_3GTP 0.2, HEPES 10, pH 7.04 (280 mOsM), which contained 0.1% biocytin (Molecular Probes or Sigma). Cells were visually identified in the EPL using a microscope equipped with near-infrared differential interference contrast and/or epifluorescence optics (Olympus Optical, Tokyo, Japan). Cell body recordings were then obtained using a Multiclamp 700A or an Axopatch 200B amplifier, Digidata 1322A or 1200 data acquisition system, and pClamp software (Axon Instruments, Foster City, CA, USA). The recordings were low-pass Bessel filtered at 2 kHz and digitized at 10 kHz for subsequent analysis. The electrode resistance (5–15 MΩ) was compensated in the bath in current clamp mode before approaching cells. Series resistance was compensated ~60–70%. Recordings were discontinued if there was a sudden increase in the series resistance (e.g. >30 MΩ). Measurements were not corrected for the liquid junction potential (9–10 mV).

Resting and spontaneous activity

Resting membrane voltage was generally measured immediately after establishing whole-cell configuration. For cells that exhibited spontaneous action potentials, the resting potential was measured immediately after an action potential or action potential burst. Spontaneous synaptic activity was analyzed in both current and voltage clamp modes. The high spontaneous EPSP frequency, EPSP summation, and voltage fluctuations of the interneurons (see Fig. 2A) precluded error-free use of automated template- and threshold-based counting routines in current clamp mode. Therefore, the maximum EPSP frequency of each interneuron was estimated by counting manually the EPSP peaks in five 0.1 s high-frequency intervals, taken from a 20–30 s recording. The same method was used to estimate the maximum action potential frequency of tufted cells. The effects of TTX on the spontaneous synaptic activity of the interneurons were analyzed in voltage clamp mode as follows. The Clampfit threshold event detection routine (Axon Instruments) was used to compare the last 50 EPSCs that occurred before and during TTX superfusion. With this method, EPSCs were visually inspected and those with amplitudes two times the baseline noise level were accepted. The inter-event intervals were then calculated and imported into SigmaStat 2.0 (SPSS, Inc., Chicago, IL, USA) to determine the mean EPSP frequency. SigmaStat was also used for statistical comparisons. The mean (±S.E.M.) is reported throughout the text.

Autocorrelation analyses were used to determine if the spontaneous EPSCs of the interneurons occurred rhythmically, as previously reported (Hayar et al., 2004a). Briefly, 1–2 min segments of voltage-clamp recordings obtained from 16 of the interneurons were first analyzed using Mini Analysis Program (Synap-