Neuroscience 248 (2013) 165–179

PHYSIOLOGY AND MORPHOLOGY OF INVERTED PYRAMIDAL NEURONS IN THE RODENT NEOCORTEX

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Abstract—An increasing number of studies indicate that there exists greater diversity of cortical neurons than previously appreciated. In the present report, we use a combination of physiological and morphological methods to characterize cortical neurons in infragranular layers with apical dendrites pointing toward the white-matter compared to those neurons with apical dendrites pointing toward the pia in both mouse and rat neocortex. Several features of the dendritic morphology and intrinsic and synaptic physiology of these ''inverted'' neurons revealed numerous differences among this cell type between species. We also found differences between the different cell types within the same species. These data reveal that similar cell types in the rat and mouse may not always share similar physiological and morphological properties. These data are relevant to models of information processing through micro- and larger neocortical circuits and indicate that different cell types found within similar lamina can have different functional properties.

Key words: pyramidal neurons, neocortex, inverted neurons.

INTRODUCTION

Complex cortical functions emerge from the diversity of cortical neurons and the dynamic and plastic properties of their synaptic connections (reviewed in Jones, 1984; White, 1989). Not surprisingly, deficits in cortical function due to developmental disruption, injury, or genetic mutation underlie many neurological disorders such as epilepsy and cognitive impairment. Greater knowledge of cortical neurons and their connections is therefore critical toward the understanding of the mechanisms of cortical function in the normal and diseased brain.

Recent years have seen an explosion of anatomical and physiological studies detailing the diversity of cortical cell-types including GABAergic interneurons (Ma et al., 2006; Xu et al., 2006; Ascoli et al., 2008) and pyramidal cells (Brumberg et al., 2003; Staiger et al., 2004; Hattox and Nelson, 2007; Ramos et al., 2008). Gene and protein expression studies have also revealed previously unknown cell-types (Hevner et al., 2003; Nelson et al., 2006; Yamamori and Rockland, 2006; Hevner, 2007; Watakabe et al., 2007). Continued discovery of novel cortical cell populations and subpopulations emphasizes the need for further quantitative studies examining individual cortical cells and their interconnected neuronal circuits.

Radially oriented apical dendrites pointing toward the pial surface are a characteristic feature of nearly all pyramidal neurons. However, cortical pyramidal neurons with atypically oriented apical dendrites pointing toward the cortical white matter have been recognized since the time of Cajal and in every mammalian species examined. Nevertheless the physiology and anatomy of these ''inverted'' pyramidal cells (IPCs) remains poorly understood (reviewed in Mendizabal-Zubiaga et al., 2007). Found almost exclusively in the infragranular layers (V and VI) of the cortex, IPCs are known to form intracortical and callosal projections but lack the corticofugal projections to subcortical targets such as those made by other infragranular cells (Bueno-López et al., 1991; Reblet et al., 1992,1993,1996; reviewed in Mendizabal-Zubiaga et al., 2007). Thus, despite only representing a small percentage of cells in the cortex (depending on species and area examined: 1–8.5%; Globus and Scheibel, 1967; Parnavelas et al., 1977; Bueno-López et al., 1991; Qi et al., 1999), IPCs are capable of participating in important cortical functions via local as well as interhemispheric projections.

In the present report we quantitatively examined the intrinsic electrophysiological properties of IPCs and pyramidal neurons with dendrites pointing toward the pia whose somata were found in infragranular layers of the mouse and rat somatosensory cortex. Additionally, we utilized biocytin reconstructions in order to quantitatively analyze and compare the dendritic morphology of IPCs.

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Abbreviations: 3D, three-dimensional; ACSF, artificial cerebral spinal fluid; ANOVA, analysis of variance; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IBI, inter-burst intervals; IPC, ''inverted'' pyramidal cell; NA, numerical aperture; IR-DIC, infra-red differential interference contrast; P, postnatal day; UPC, upright pyramidal cells.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.06.004

Finally, we used perfusion of artificial cerebral spinal fluid (ACSF) lacking extracellular magnesium in order to test the role of IPC during periods of increased spontaneous synaptic activity. We observed both morphological and intrinsic physiological differences in IPCs between species as well as differences indicative that IPCs are integrated in distinct synaptic networks in rat versus mouse. Our results provide important data on the intrinsic properties of IPCs, reveal novel species differences in IPCs previously assumed to be homogeneous, and are relevant to models of information processing through micro- and larger neocortical circuits.

EXPERIMENTAL PROCEDURES

Preparation of slices

Coronal slices of primary somatosensory cortex (300-μm thick) were prepared from postnatal day (P)14–21, CD1 mice or Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) of either sex on a vibratome (VT1000S, Leica) in accordance with Queens College of the City University of New York and the National Institutes of Health guidelines for the use of laboratory animals and as described previously (Brumberg et al., 2003; Ramos et al., 2008). Animals were anesthetized with an intraperitoneal injection of Euthasol (Virbac AH Inc., Fort Worth, TX) until unresponsive to noxious stimulation (toe-pinch). Following decapitation, the brain was quickly removed, blocked, and placed into ice-cold (4 °C) oxygenated ACSF. ACSF contained (in mM) 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 25 $NaHCO_3$, and 25 D-glucose and was aerated with 95% O_2–5%CO_2 to a final pH of 7.4. Where indicated, slices were perfused with modified ASCF containing zero extracellular Mg in order to elicit spontaneous bursting according to the protocol of Flint and colleagues (Flint and Connors, 1996; Flint et al., 1997).

Electrophysiological recordings

Neurons were visualized with infra-red differential interference contrast (IR-DIC) microscopy (Olympus BX51WI, Center Valley, PA). Patch pipettes (4–7 MΩ tip resistance) were pulled on a Flaming/Brown microelectrode puller (P-97, Sutter Instruments, Novato, CA). Pipettes were filled with (in mM) 120 KGlu, 10 NaCl, 20 KCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, 0.5 EGTA, and 0.3–1% biocytin (wt/vol) for subsequent visualization of the neurons (see following text). Once a stable recording was obtained (resting V_m of < -55 mV, overshooting action potentials, ability to generate repetitive action potentials to a depolarizing current pulse), neurons were classified according to discharge pattern in response to a constant depolarizing current pulse (1000 ms) as intrinsically bursting, regular spiking, etc. (McCormick et al., 1985; Brumberg et al., 2000; Ramos et al., 2008). Injection of depolarizing and hyperpolarizing current steps of increasing amplitudes (10-pA increments) were used to measure intrinsic membrane properties. Off-line analysis of action potential and passive membrane properties was performed using Clampfit9 (Molecular Devices, Sunnyvale, CA).

Histology and neuronal reconstruction

Following recordings slices were placed in cold fixative (4% paraformaldehyde in 0.1 M phosphate buffer) and kept at 4 °C for no more than 2 weeks. Biotin–avidin–HRP histochemistry was preformed as described previously (Ramos et al., 2008). Slices were not re-sectioned. For three-dimensional (3D) morphological reconstructions, the Neurolucida system (MicroBrightfield Inc., Williston, VT) was used in conjunction with an Olympus BX51 microscope using 4×(0.1 numerical aperture (NA)), 10×(0.4 NA) and 60×(1.4 NA, oil) objectives. Digital images were taken using an Optronics Microfire camera attached to a dedicated PC. Morphological measurements of neuronal dendrites and somata were made using the associated NeuroExplorer software package (MicroBrightfield Inc.). Cells were classified as inverted if its principal dendrite was descending toward the cortical white matter. The principal dendrite was determined for both upright and inverted cells by examining dendrite diameter. The thickest dendrite to emerge from the soma was considered to be the principal, or apical, dendrite. These measurements were made by using the ‘‘quick measure line’’ tool within the Neurolucida program and placing a line across the dendrite as it emerged from the soma, values for the four cell types were averaged for subsequent analyses (see Table 1).

Golgi staining and quantification of dendritic spines

Animals (CD1 mice, $N = 13$) of either sex at p80–90 were randomly selected. Brains were immediately removed and rinsed in 0.1 M phosphate buffer (pH 7.13) for 3 min. After the rinse, retrieved brains were immersed in a Golgi-Cox solution (FD Rapid Golgi Stain Kit, FD Neurotechnologies Inc., Columbia, MD) comprising potassium dichromate, mercuric chloride, and potassium chromate. This mixture of solutions was replaced once after 12 h of initial immersion, with storage at room temperature in darkness for 2–3 weeks.

After the immersion period in the Golgi-Cox solution, the embedded brains were transferred to a cryoprotectant solution (FD Rapid Golgi Stain Kit) and stored at 4 °C for at least 1 week in the dark before cutting. The brain slices were sectioned in the coronal plane at approximately 200–250-μm thickness on a freezing cryostat (approximately −25 °C). To prevent ice crystal damage, tissues were rapidly frozen with dry ice and quickly embedded in optimal cutting temperature (OCT) medium. Sliced tissues were transferred onto triple-dipped gelatin slides and were coated with an additional cryoprotectant solution. Cut sections were air dried at room temperature in the dark for at least 2–3 weeks before further processing. After drying, sections were rinsed with distilled water and were subsequently stained in a developing solution (FD Rapid