Neuroscience 265 (2014) 60–71

TWO FUNCTIONAL INHIBITORY CIRCUITS ARE COMPRISED OF A HETEROGENEOUS POPULATION OF FAST-SPIKING CORTICAL INTERNEURONS

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Abstract—Cortical fast spiking (FS) interneurons possess autaptic, synaptic, and electrical synapses that serve to mediate a fast, coordinated response to their postsynaptic targets. While FS interneurons are known to participate in numerous and diverse actions, functional subgroupings within this multi-functional interneuron class remain to be identified. In the present study, we examined parvalbumin-positive FS interneurons in layer 4 of the primary somatosensory (barrel) cortex – a brain region well-known for specialized inhibitory function. Here we show that FS interneurons fall into two broad categories identified by the onset of the first action potential in a depolarizing train as: ''delayed firing FS interneurons (FS_D) and early onset firing FS interneurons (FS_E). Subtle variations in action potential firing reveal six subtypes within these two categories: delayed non-accommodating ($FS_{D\text{-}NAC}$), delayed stuttering ($FS_{D\text{-}STUT}$), early onset stuttering ($FS_{E\text{-}STUT}$), early onset-late spiking ($FS_{E\text{-}LS}$), early onset early-spiking ($FS_{E\text{-}ES}$), and early onset accommodating ($FS_{E\text{-}AC}$). Using biophysical criteria previously employed to distinguish neuronal cell types, the FS_D and FS_E categories exhibit several shared biophysical and synaptic properties that coincide with the notion of specificity of inhibitory function within the cortical FS interneuron class.

Key words: interneuron, inhibitory neurotransmission, basket cells.

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Abbreviations: aCSF, artificial cerebral spinal fluid; AHP, after hyperpolarization potential; BSA, bovine serum albumin; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; EPSC, excitatory postsynaptic current; FS, fast spiking; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; IPSC, inhibitory postsynaptic current; IR, infrared; ISI, inter-spike-interval; PBS, phosphate-buffered solution.

http://dx.doi.org/10.1016/j.neuroscience.2014.01.033
0306-4522/

INTRODUCTION

The diversity exhibited by cortical interneurons is considered a requisite for specialized function in inhibitory synaptic transmission (Ascoli et al., 2008). Nomenclature for cortical interneurons is based on descriptive characteristics of morphology, biochemistry and action potential firing patterns; however, no singular classification technique is fail-safe as there are often overlapping features. Fast-spiking (FS) interneurons are characterized by high-frequency action potential firing, the expression of the calcium binding protein parvalbumin and fast inhibitory $GABA_A$ receptor-mediated synaptic and autaptic currents (Kawaguchi and Kubota, 1997; Gupta et al., 2000; Bacci et al., 2003a,b; Li et al., 2009). The FS interneuron class is known for forming inhibitory synapses on either the axon initial segment (e.g., chandelier cells) or somatic (e.g., basket cells) regions of their target neurons (Kawaguchi and Kubota, 1998; Wang et al., 2002). These intrinsic and synaptic specializations enable FS interneurons to form complex feedforward and feedback circuits (Thomson and Bannister, 2003; Staiger et al., 2009) to regulate multiple key functions such as rhythmic activity and sensory-evoked responses (Porter et al., 2001; Sohal et al., 2009). Given the numerous and complex functional attributes of FS interneurons they nevertheless remain grouped together in a singular functional category with no clearly defined identification of diversity of function within this interneuron class.

While interneuron classification appears arbitrary, most agree that using multiple criteria in brain regions with diverse and increased populations of interneurons is a means toward understanding specialized circuitry (Beierlein et al., 2003). Layer 4 of the rodent barrel cortex represents a key platform to examine functional heterogeneity because of the high density and diverse population of FS interneurons (Karagiannis et al., 2009; Staiger et al., 2009). Neurons located within the multicellular barrel structures in layer 4 receive thalamic afferents carrying information from the mystacial vibrissae (Woolsey and Van der Loos, 1970). FS interneurons are postsynaptic to whisker-driven thalamic afferents and are highly sensitive to a diverse range of whisker movements (White and Rock, 1981; Swadlow, 2003). *In vitro* studies show that activation of thalamocortical fibers results in robust (Cruikshank et al., 2007) and sometimes variable thalamic-evoked

responses indicating selective activation of layer 4 FS interneurons (Agmon and Connors, 1992; Porter et al., 2001). Emerging evidence indicates that action potential onset may be an identifier of subdivisions within the FS interneuron class (Goldberg et al., 2008; Karagiannis et al., 2009). In the present study, we propose a categorization into two functional populations of FS interneurons identified by action potential onset, and supported by statistical differences in biophysical and synaptic properties. These data suggest a new functional subgrouping and the existence of specialized FS interneuron circuitry germane to barrel cortex function.

EXPERIMENTAL PROCEDURES

Preparation of slices for electrophysiology

All animal use procedures were carried out in strict accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Georgetown University and Children's National Medical Center. For all experiments, adult (P25–40) mice (C57BL/6, Jackson Laboratories) of either sex were used. Animals were deeply anaesthetized with brief exposure to CO_2 and decapitated. Brains were removed, blocked, and placed in an ice-cold and oxygenated high-sucrose slicing solution for 2–3 min (in mM): 234 sucrose, 11 glucose, 26 $NaHCO_3$, 2.5 KCl, 1.25 $NaH_2PO_4{*}H_2O$, 10 $MgSO_4$ and 0.5 $CaCl_2$; gassed with 95% O_2/5% CO_2. Slices including the somatosensory cortex were cut in either the tangential or thalamocortical configuration as previously described (Agmon and Connors, 1991; Fleidervish et al., 1998; Li et al., 2009). Tangential slices were employed in order to isolate the columnar and cross-columnar cellular connections within layer 4. Slices were generated by placing the brain ventral side down on a constructed angle indicator and sliced with a sterilized razor blade at simultaneous 10° and 30° from the midline cuts. The blocked brain was glued (cut side down) on a vibratome stage and immersed in cold sucrose-slicing solution. Once the position of the pial surface of the brain was identified, the first two slices at 50 and 250 μm were discarded and a third slice at 270–300 μm was collected. Thalamocortical brain slices were prepared by making simultaneous 10° horizontal and 35° from the midline, with the brain placed ventral side down in a constructed angle indicator. The blocked brain was glued cut side down on a vibratome stage (Leica Microsystems, Wetzlar, Germany) and immersed in cold sucrose-slicing solution. Once the position of the pial surface of the brain was identified, the first 2400 μm were cut and discarded and three consecutive slices of 300 μm were collected. Tangential and thalamocortical slices were incubated in oxygen-saturated artificial cerebral spinal fluid (aCSF) containing the following (in mM): 126 NaCl, 26 $NaHCO_3$, 10 glucose, 2.5 KCl, 1.25 $NaH_2PO_4{*}H_2O$, 2 $MgCl_2{*}6H_2O$, and 2 $CaCl_2{*}2H_2O$; pH 7.4. All slices were incubated at 32 °C for at least one hour prior to recording. Slices were placed in a recording chamber and visualized with a fixed staged, upright microscope (Nikon, E600 FN) equipped with a 4× objective and a 60× insulated objective, infrared (IR) illumination, Nomarski optics, and an IR-sensitive video camera (COHU).

Electrophysiological recordings

Whole-cell patch-clamp recordings from layer 4 FS interneurons in barrel cortex were performed, unless otherwise noted, at room temperature with continuous perfusion (2 ml/min) of aCSF. Glass pipettes (non-filament, Garner Glass Company) were pulled (Model P-97, Sutter Instruments) to obtain electrodes with resistances between 2.5 and 3.5 MΩ when filled with intracellular solution. Two intracellular solutions were used in this study. A high chloride concentration solution was used to enhance $GABA_A$ receptor-mediated inhibitory currents (in mM): 70 K-gluconate, 70 KCl, 2 NaCl, 10 HEPES, 4 EGTA, 2 Na_2-ATP, 0.5 Na_2-GTP (Ecl −16 mV). For thalamic activation of fast glutamatergic currents a solution containing physiological levels of chloride was used (in mM): 130 K-gluconate, 10 KCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 2 Na_2-ATP, 0.5 Na_2-GTP. All electrophysiological recordings were performed in the whole-cell configuration. A gigaohm seal was formed between the cell and glass pipette and a solenoid-controlled vacuum transducer was used to apply brief suction pulses (120 psi at 20–50 ms) to break into the cell. All recordings were performed in either current clamp or voltage clamp mode (Multiclamp 700A, Molecular Devices, Sunnyvale, CA USA) and digitized (DigiData 1322, Molecular Devices) for fast acquisition of raw traces and offline analysis (PClamp 9, Molecular Devices). All cortical FS interneurons were characterized in current clamp mode using a series of hyperpolarizing and depolarizing current injections in order to measure action potential firing patterns and properties for additional characterization such as: rheobase current, action potential threshold, resting membrane potential, input resistance, saturation frequency, accommodation ratio, action potential duration at half-width, action potential amplitude, after hyperpolarization potential (AHP), sag (or I_h current), rise time and time constant. Some of the characterized FS interneurons were selected to study their firing patterns at physiological temperatures (32 °C). Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in voltage clamp mode (V_{Hold} = −60 mV) in the presence of the glutamate receptor blockers, 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM final, Tocris Bioscience, Bristol, UK) and DL-2-amino-5-phosphonopentanoic acid (DL-AP5, 100 μM final, Tocris). $GABA_A$ receptor-mediated autaptic IPSCs ($_{aut}$IPSCs) were obtained in voltage clamp mode using a brief (0.5 ms) depolarization step from −70 mV to +10 mV to elicit a spike followed by an inward $GABA_A$ receptor-mediated IPSC (identified by blocking with the competitive $GABA_A$ receptor antagonist SR 95531 hydrobromide [Gabazine, Tocris]).

For thalamic activation of glutamatergic currents, a 25-μm concentric bipolar stimulating electrode (FHC) was positioned such that it contacted intact fibers projecting from the ventrobasal complex of the