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STATISTICAL MODELS SUGGEST PRESENCE OF TWO DISTINCT SUBPOPULATIONS OF MINIATURE EPSCs IN FAST-SPIKING INTERNEURONS OF RAT PREFRONTAL CORTEX

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Abstract—Properties of excitatory synaptic responses in fast-spiking interneurons (FSIs) and pyramidal neurons (PNs) are different; however, the mechanisms and determinants of this diversity have not been fully investigated. In the present study, voltage-clamp recording of miniature excitatory post-synaptic currents (mEPSCs) was performed of layer 2–3 FSIs and PNs in the medial prefrontal cortex of rats aged 19–22 days. The average mEPSCs in the FSIs exhibited amplitudes that were two times larger than those of the PNs and with much faster rise and decay. The mEPSC amplitude distributions in both cell types were asymmetric and in FSIs, the distributions were more skewed and had two-times larger coefficients of variation than in the PNs. In PNs but not in FSIs, the amplitude distributions were fitted well by different skewed unimodal functions that have been used previously for this purpose. In the FSIs, the distributions were well approximated only by a sum of two such functions, suggesting the presence of at least two subpopulations of events with different modal amplitudes. According to our estimates, two-thirds of the mEPSCs in FSIs belong to the high-amplitude subpopulation, and the modal amplitude in this subpopulation is approximately two times larger than that in the low-amplitude subpopulation. Using different statistical models, varying binning size, and data subsets, we confirmed the robustness and consistency of these findings.

Key words: fast-spiking interneuron, excitatory postsynaptic current, quantal response, prefrontal cortex.

INTRODUCTION

The mammalian cortex is composed of two major classes of cells: glutamatergic excitatory pyramidal neurons (PNs) and GABAergic inhibitory interneurons (Somogyi et al., 1998). Among different types of cortical interneurons (Kawaguchi and Kubota, 1997; Markram et al., 2004), parvalbumin-containing fast-spiking basket cells are the most common type of GABAergic interneurons (Uematsu et al., 2008). Interactions between fast-spiking interneurons (FSIs) and PNs contribute to the fundamental properties of the cortical networks, and an investigation of the properties of the excitatory inputs in these cell types is required to understand how neural circuitry works. Multiple studies have demonstrated that FSIs typically have a faster time course and larger amplitude of excitatory responses than PNs (Thomson, 1997; Holmgren et al., 2003; Cruikshank et al., 2007; Hull et al., 2009; Kloc and Maffei, 2014). One of the main sources of these differences is the distinct properties of quantal responses (Povysheva et al., 2006). In many studies, it was shown that pyramidal cells and interneurons have skewed amplitude distributions of quantal responses (Bekkers et al., 1990; Zhou and Hablitz, 1998; Hanse and Gustafsson, 2001; Malkin et al., 2014); in addition, it was found that interneurons have a larger proportion of high-amplitude miniature events than pyramidal cells (Povysheva et al., 2006). The determinants of this diversity in the properties of miniature post-synaptic responses between FSIs and PNs have not fully been investigated and the origin of this asymmetry in amplitude distributions is still unclear.

In the present study, we performed a systematic analysis of miniature excitatory post-synaptic current (mEPSC) amplitude distributions in FSIs and PNs. Using different statistical models, we found that in PNs but not in FSIs, the mEPSC distributions are well described by single-skewed unimodal functions. The mEPSC distributions in interneurons can be described only by a sum of two functions, suggesting mEPSCs are composed of two distinct subpopulations with different amplitudes.

EXPERIMENTAL PROCEDURES

Slice preparation and electrophysiological recordings

The experiments were conducted in male Wistar rats (19–22 days old) in agreement with the Rules of Animal

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Abbreviations: ACSF, artificial cerebrospinal fluid; CP-AMPARs, Ca^{2+}-permeable GluA2-lacking AMPA receptors; CV, coefficient of variation; EGTA, ethylene glycol tetraacetic acid; FSI, fast-spiking interneuron; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; mEPSC, miniature excitatory post-synaptic current; PN, pyramidal neuron; TTX, tetrodotoxin.

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Care and Use Committee of the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, which is fully compatible with European Community Council directives 86/609/EEC. Coronal 300-μm-thick slices of the medial prefrontal cortex were prepared as previously described (Zaitsev et al., 2011). Through all steps, artificial cerebrospinal fluid (ACSF) with the following composition was used (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 24 $NaHCO_3$, and 20 dextrose. ACSF was aerated with carbogen (95% O_2/5% CO_2). Neurons in layer 2/3 were visualized using a BX51WI microscope (Olympus, Japan) equipped with differential interference contrast optics and a Watec video camera (model WAT-127LH, USA) for contrast enhancement. PNs were recognized by their apical dendrites and triangular somata. Interneurons were identified based on their round or oval cell body and lack of apical dendrites; interneurons with relatively large cell bodies were preferentially selected. Patch electrodes (2–4 MΩ) were filled with the internal solution that contained the following (in mm): 114 K-gluconate, 6 KCl, 0.2 EGTA, 10 HEPES, 4 ATP-Mg, and 0.3 GTP (pH was adjusted to 7.25 with KOH). Recordings were done at room temperature (25 °C). Intrinsic membrane properties were assessed from the voltage responses to the series of 500-ms hyperpolarizing and depolarizing current steps with 10-pA increments at 0.5 Hz. FSIs were identified based on their distinctive electrophysiological properties (Kawaguchi and Kubota, 1997; Povysheva et al., 2008, 2013; Zaitsev and Lewis, 2013) (Fig. 1). In response to a depolarizing current injection, FSIs produce trains of narrow action potentials (AP half-width, FSIs: 0.6 ± 0.1 ms, $n = 12$; PNs: 1.7 ± 0.1 ms, $n = 8$, $p < 0.01$) with little frequency adaptation (FSIs: 100 ± 10%, PNs: 46 ± 4%, $p < 0.05$). FSIs also have a shorter membrane time constant (FSIs: 7.5 ± 0.5 ms, PNs: 11 ± 0.5 ms, $p < 0.05$), although the input resistance of the two cell types was similar (FSIs: 204 ± 15 MΩ, PNs: 183 ± 16 MΩ, p = n.s.).

Recordings of mEPSCs were done in the presence of tetrodotoxin (TTX, 0.5 μM; Sigma) and were started after 5–10 min of TTX application. Access resistance was typically 15–20 MΩ and remained stable during the experiments (⩽30% increase) for the cells included in the analysis. Miniature events were detected and were analyzed using Clampfit 10 software (Molecular Devices Corporation, USA). The amplitudes of the miniature responses were determined from the baseline to the peak. For average responses, the time constants of the single exponential fit were used to describe the decay time. The rise time was estimated as the time necessary to rise between 10% and 90% of the peak response.

To define the kinetics of individual events we first fit each event with the template function (Clements and Bekkers, 1997):

$$\text{Template} = A\left(1 - \exp\left(-\frac{t}{\text{Rise}}\right)\right)\exp\left(-\frac{t}{\text{Decay}}\right),$$

where t is the time from onset of the synaptic event, A is the scaling factor used to normalize the peak amplitude,

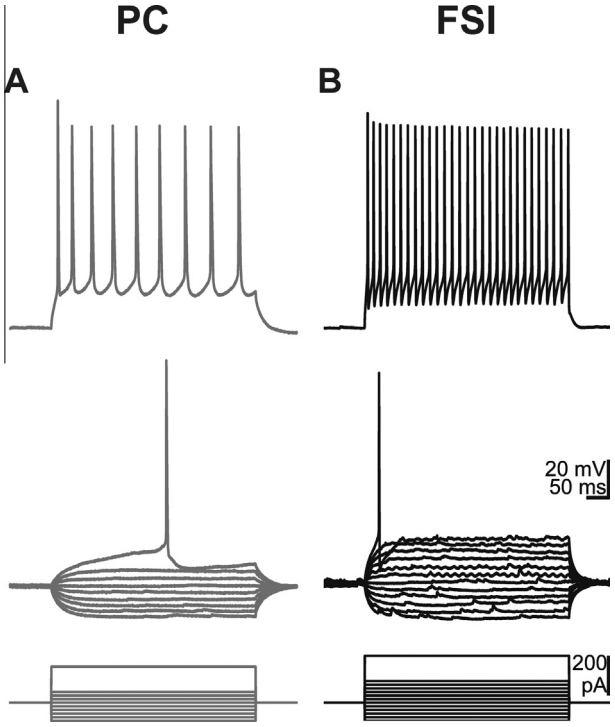

Fig. 1. Intrinsic membrane properties of PNs and FSIs. Representative examples of current responses to hyper- and depolarizing current steps in PN (A) and FSI (B).

Rise is the time constant of the rising phase of the template, and Decay is the time constant of the falling phase of the template. For correlation analysis of rise and amplitude relationship we included only events which were fitted by template function with $r^2 > 0.8$. Rise of 20–80% and decay time were estimated using the shape of the template.

Analysis of the mEPSC amplitude distributions

Further analysis of the mEPSC amplitude distributions was performed using open-source SciPy and NumPy libraries (http://scipy.org) for the Python programing language. Fitting of the distributions was performed using SciPy realization of the non-linear least-squares optimization algorithm.

Along with a Gaussian function, we used for fitting of response distributions several skewed unimodal functions that have been suggested previously. The first function was introduced by Bekkers et al. (1990) to approximate the mEPSC amplitude distribution in cultured hippocampal neurons; the underlying assumption is that a variability of the EPSC may arise from the variation in the amount of the transmitter released from vesicles of different sizes.

$$P_x = w \cdot \exp\left[\frac{\left(-x^{1/3} - \mu\right)^2}{2\sigma^2}\right] x^{-2/3}, \quad (1)$$

where P_x is the probability that an mEPSC will have amplitude x, μ and σ^2 are the mean and variance, respectively, of $x^{1/3}$, and w is a normalizing coefficient.