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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF PYRAMIDAL-LIKE NEURONS IN THE STRATUM ORIENS OF CORNU AMMONIS 1 AND CORNU AMMONIS 2 AREA OF *PROECHIMYS*

C. A. SCORZA,[a] B. H. S. ARAUJO,[a] L. A. LEITE,[a] L. B. TORRES,[a,b] L. F. P. OTALORA,[c] M. S. OLIVEIRA,[d] E. R. GARRIDO-SANABRIA[c] AND E. A. CAVALHEIRO[a]*

[a]*Disciplina de Neurologia Experimental, Universidade Federal de São Paulo/Escola Paulista de Medicina, Rua Botucatu 862, 04023-900 São Paulo, Brazil*

[b]*Instituto Evandro Chagas, Centro Nacional de Primatas, Belém, Brazil*

[c]*Department of Biological Sciences, University of Texas at Brownsville and Center for Biomedical Studies, Brownsville, TX 78520, USA*

[d]*Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil*

Abstract—*Proechimys* (Rodentia: Echimyidae) is a neotropical rodent of the Amazon region that has been successfully colonized in the laboratory and used for experimental medicine. Preliminary studies indicated that *Proechimys* (casiragua) rodents express an atypical resistance to developing a chronic epileptic condition in common models of temporal lobe epilepsy. Moreover, previous investigation of our laboratory described a remarkably different Proechimy's cytoarchitecture organization of the hippocampal CA2 subfield. In the present study, we investigated the intrinsic neuronal properties and morphological characteristics of the *Proechimys*'s hippocampal pyramidal neurons of the CA1 and CA2 areas. A comparative approach was performed using neurons recorded in Wistar rats. A striking finding in *Proechimys* rodents was the presence of large pyramidal-like neurons throughout the stratum oriens from CA2 to CA1 area. In order to confirm such distinctive feature of the *Proechimys*'s hippocampus, we performed Nissl staining and immunohistochemistry for neurofilament protein SM311. CA2 pyramidal neurons in the stratum pyramidale of *Proechimys* exhibited a significantly higher input resistance and lower time constant when compared to corresponding cell groups in the same area of the Wistar rat's. This newly identified population of pyramidal-shaped neurons in stratum oriens of *Proechimys* exhibited distinct electrophysiological and morphological properties. This included larger capacitance, lower input resistance, larger rheobase, long latency to first action potential and slower firing frequency. In addition, the apical dendrites of these neurons were oriented in parallel to apical dendrites of regular pyramidal neurons in stratum pyramidale. Moreover, these neurons were immunoreactive to SM311 as the majority of the neurons of the pyramidal layer. The functional role of these hippocampal neurons of the rodent *Proechimys* deserves further investigation. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

*Corresponding author. Tel: +55-11-5573-9304.
E-mail address: esper@cgee.org.br (E. A. Cavalheiro).

Abbreviations: CA2, cornu ammonis 2; DIC, difference interference contrast; EPSCs, excitatory post-synaptic currents; EPSPs, excitatory postsynaptic potentials; HSD, honest significant difference; PBS, phosphate-buffered saline.

Key words: *Proechimys*, neurobiotin, hippocampus, stratum oriens.

Proechimys (Rodentia: Echimyidae) is a tropical rodent native to the Amazon region that has been successfully colonized in the laboratory and used for experimental medicine since 1964 (Hawking et al., 1964). Although originally utilized as hosts for infectious diseases research, these animals have recently attracted the attention of neuroscience research. *Proechimys* exhibit distinctive characteristics. For instance, immediately after offspring are born (usually one or two) they are capable of initiating locomotive activity and, to some degree, exploring their local environment. This indicates a higher degree of prenatal maturation of the nervous system when compared to other species of laboratory rats (i.e. Wistar and Sprague–Dawley).

Proechimys rodents live in complex environments in their natural habitats. Moreover, living in the wildness of rainforests may have put considerable selection pressures on these animals, especially on their nervous system, to adapt and survive predators. Certainly, their natural habitats offered more complex cues to their sensory system than any "artificial" environment can provide in the traditional vivarium conditions of experimental rats (i.e. Wistar, Sprague–Dawley). Compelling data indicate that domestication modifies (reduce brain's size) in several species (Ebinger, 1975; Kruska, 1975a,b; Kruska and Schott, 1977; Rohrs and Ebinger, 1999; Rehkamper et al., 2003; Kruska, 2005; Stuermer and Wetzel, 2006). For instance, several brain areas are smaller in laboratory rats versus wild Norway rats (Kruska, 1975a,b; Kruska and Schott, 1977). In this regard, *Proechimys* also offers a unique opportunity to investigate the anatomy and physiology of rodent nervous system structures, such as the hippocampus's involvement in cognitive mapping, sensory coding, memory, and so forth at different stages of evolution.

Previous study of our laboratory described a remarkably different Proechimy's cytoarchitecture organization of the hippocampal Cornu ammonis 2 (CA2) subfield (Scorza et al., 2010). We identified a very distinctive Proechimy's CA2 sector exhibiting disorganized cell presentation of the pyramidal layer and atypical dispersion of the pyramidal-like cells to the stratum oriens, strongly contrasting to the densely packed CA2 cells in the Wistar rats. Furthermore, *Proechimys*'s CA2 subfield presented significant higher density of calcium-binding proteins when compared to Wistar rats (Scorza et al., 2010). In addition, previous investigations of our group showed that *Proechimys* ro-

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dents expressed an atypical resistance to developing a chronic epileptic condition in common models of temporal lobe epilepsy (Arida et al., 2005). In this line of evidence, *Proechimys* has been proposed as an animal model of resistance to epilepsy. Fabene at al. (2001) showed that the number of parvalbumin- or nitric oxide synthase-containing interneurons and their staining intensity were significantly increased in animals 30 days after status epilepticus. In addition, the number of glutamic acid decarboxylase (67)-immunoreactive interneurons was found to be markedly increased in the hilus and decreased in the CA1 pyramidal layer (Fabene et al., 2001). In general, due to *Proechimys*'s hippocampal intriguing characteristics, these rodents represent an extraordinary tool for neuroscience research.

The electrophysiological properties and individual morphological characteristics of principal neurons in the *Proechimys* hippocampus have yet to be explored. This information is critical to a better understanding of the *Proechimys* hippocampal network under normal conditions. Intrinsic neuronal properties are the electrophysiological signature of neurons critical for the functioning and operation of neuronal circuits. Therefore, it is critical to determine if these properties are different in Wistar versus *Proechimys* animals. In order to study these properties, we performed patch-clamp recordings from visually identified neurons in the hippocampus. These properties can be subdivided into passive neuronal properties, properties of the action potential (active), properties of afterpotentials—including afterdepolarizing potential (ADP) and afterhyperpolarizing potentials (AHP), firing phenotype (i.e. regular firing, fast spiking, and burst firing), firing frequency and accommodation.

During the development of the experiments, we noticed, by chance, that pyramidal-like cells appeared in the stratum oriens in brain slices of *Proechimys* rodents. The majority of the cells in the stratum oriens of Wistar rats are GABAergic interneurons and do not exhibit a pyramidal cell morphological phenotype. Therefore, we were highly intrigued by this observation. In this line of evidence, we decided to investigate these cells by performing electrophysiological (patch-clamp) recordings and by injecting the morphological tracer neurobiotin. In addition, we performed Cresyl Violet staining and immunohistochemistry (SMI311). SMI311 is a neuronal filament marker that preferentially labels neurons with pyramidal cell phenotypes in the hippocampus and cortex (Shetty and Turner, 1995; Sanabria et al., 2002). Electrophysiological experiments

Table 1. Intrinsic properties of pyramidal cells recorded in CA1 and CA2 area of hippocampus in Wistar rats versus *Proechimys*

Area Stratum	Wistar		*Proechimys*			ANOVA	
	CA1 Pyramidale *n*=8	CA2 Pyramidale *n*=7	CA1 Pyramidale *n*=7	CA2 Pyramidale *n*=11	CA1 and CA2 Oriens *n*=15	*F*	*P*-level
Passive membrane properties							
Capacitance, pF	60.2±28.3	51.6±23.8	36.6±14.1	45.5±16.2	151.4±56.2	12.81	0.0001
Resting membrane potential, mV	−61.8±3.7	−61.9±1.7	−65.1±3.1	−65.0±2.7	−62.0±2.5	1.75	0.15
Input resistance, MΩ	265.9±45.5	258.7±58.2	262.4±49.4	383.2±95.1	134.7±31.6	8.35	0.00005
Membrane time constant, ms	21.5±4.5	27.0±12.1	16.5±6.1	15.6±4.7	13.5±7.3	2.61	0.04
Active membrane properties							
Action Potential (AP) properties amplitude of AP, mV	113.0±11.2	100.1±11.3	104.3±11.2	103.3±10.8	107.7±11.1	1.05	0.41
AP threshold, mV	−46.1±2.3	−48.1±6	−50.7±5.3	−50.8±4.7	−49.0±4	0.92	0.46
Rheobase, pA	23.8±11.2	15.7±6.1	18.6±2.2	19.1±6.6	38.7±13.1	5.84	0.0007
AP half-height width, ms	2.2±0.4	2.2±0.3	2.6±0.2	2.7±0.4	2.2±0.2	1.98	0.09
Peak AP rise slope, mV/ms	141.3±24.5	108.5±15.5	101.6±29.1	104.2±33.2	127.7±30.3	1.50	0.21
Peak AP decay slope, mV/ms	−47.1±6.9	−44.2±4.4	−41.7±5.6	−37.9±9.3	−47.7±8.1	1.91	0.12
First AP latency, ms	53.8±5.5	47.1±4.5	60.0±10.5	120.9±20.9	232.7±90.5	5.5	0.001
Afterpotentials							
Afterdepolarization potential, mV	8.8±1.2	8.9±2.5	8.9±5.3	2.4±1.8*	6.8±1.5	5.53	0.001
Fast AHP, mV	−1.8±0.8	−1.9±0.6	−1.6±0.7	−2.3±1.1	−2.1±1.1	1.4	0.24
Medium AHP, mV	−2.1±0.5	−1.9±0.4	−3.0±0.9	−2.7±1.3	−1.7±0.7	0.21	0.92
Slow AHP, mV	−1.0±0.3	−0.8±0.25	−0.7±0.4	−0.7±0.4	−0.8±0.4	1.77	0.15
Firing frequency properties							
Interspike interval 1st spike, ms	37.9±5.5	21.9±4.6	33.1±5.6	32.8±8.8	51.0±15.5	0.45	0.76
Interspike interval last spike, ms	77.5±6.9	82.0±10.5	82.3±12.2	68.9±22.5	105.5±40.5	3.62	0.01
Maximal firing frequency, Hz	26.4±3.9	45.8±15.5	30.2±8.5	30.5±12.8	19.6±6.8	1.7	0.06
Steady-state frequency, Hz	12.9±4.3	12.2±4.2	12.9±5.6	14.5±6.3	9.5±4.5	4.5	0.04
Adaptation index	0.5±0.08	0.3±0.09	0.4±0.05	0.5±0.1	0.5±0.12	1.48	0.22
Depolarizing "sag"							
Sag ratio	0.93±0.2	0.95±0.2	0.91±0.2	0.91±0.1	0.97±0.3	4.18	0.01
Sag amplitude, mV	4.9±1.4	3.7±2.5	10.3±3.1	10.0±3.8	2.8±1.1	3.1	0.02

Data presented as mean±standard deviation. AHP, afterhyperpolarization.

* Only two out of 11 cells presented active afterdepolarization (ADP).