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ALTERATIONS IN MEMBRANE AND FIRING PROPERTIES OF LAYER 2/3 PYRAMIDAL NEURONS FOLLOWING FOCAL LASER LESIONS IN RAT VISUAL CORTEX

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Abstract—Focal cortical injuries are well known to cause changes in function and excitability of the surviving cortical areas but the cellular correlates of these physiological alterations are not fully understood. In the present study we employed a well established ex vivo–*in vitro* model of focal laser lesions in the rat visual cortex and we studied membrane and firing properties of the surviving layer 2/3 pyramidal neurons. Patch-clamp recordings, performed in the first week post-injury, revealed an increased input resistance, a depolarized spike threshold as well as alterations in the firing pattern of neurons in the cortex ipsilateral to the lesion. Notably, the reported lesion-induced alterations emerged or became more evident when an exciting perfusing solution, known as modified artificial cerebrospinal fluid, was used to increase the ongoing synaptic activity in cortical slices. Conversely, application of glutamatergic or $GABA_A$ receptor blockers reduced the observed alterations and $GABA_B$ receptor blockers abolished the differences completely. All together the present findings suggest that changes in synaptic receptors function, following focal cortical injuries, can modulate membrane and firing properties of layer 2/3 pyramidal neurons. This previously unknown functional interplay between synaptic and membrane properties may constitute a novel cellular mechanism to explain alterations in neuronal network function and excitability following focal cortical injuries.

Key words: cortical lesions, patch clamp recordings, input resistance, spike threshold, mACSF, $GABA_B$ receptors.

INTRODUCTION

Brain injuries are a leading cause of disability worldwide. Traditionally, the loss of function following these pathological events was attributed to the irreversible damage of neuronal tissue. Accumulating lines of evidence, however, suggest that functional alterations, occurring in the structurally intact brain areas adjacent to the lesion, could also contribute to the observed neurological deficits and to processes of functional recovery. These physiological disturbances, spreading from the site of primary damage, include metabolic changes (Witte et al., 2000; Alves et al., 2005) and alterations in neuronal activity. In particular, several electrophysiological *in vivo* studies revealed a period of hyperexcitability strongly expressed in the first week post-lesion. In a heat-lesion model in the cat visual cortex, few days after the lesion induction, neurons located between 1 to 2.5 mm from the border of the injury exhibited an increased spontaneous and evoked activity (Eysel and Schmidt-Kastner, 1991). Similarly, a significant increase in the spontaneous firing frequency was found in the tissue adjacent to a photochemically induced infarction in the rat sensorimotor cortex (Schiene et al., 1996). Furthermore, in a model of focal cortical compression, recordings of multi-unit activity from the rat barrel cortex showed that an increase in neuronal responsiveness, upon whisker stimulation, was already visible two hours after the injury onset (Ding et al., 2011). At the cellular level an unbalance between excitatory and inhibitory synaptic transmission has so far been considered the most plausible explanation for the hyperexcitability observed *in vivo* (Domann et al., 1993; Mittmann et al., 1994; Neumann-Haefelin et al., 1995; Buchkremer-Ratzmann et al., 1996; Schiene et al., 1996; Reinecke et al., 1999; Neumann-Haefelin and Witte, 2000). Indeed, a reduced inhibition, coupled with an enhanced excitation, will inevitability increase the synaptically driven depolarization and thereby the firing activity of neurons. Although this scenario is a quite realistic one, it may not tell the whole story. Changes in ongoing synaptic activity are likely to influence neuronal excitability in a more complex fashion. For instance, several lines of evidence suggest that background synaptic transmission can exert a powerful influence on neuronal membrane and firing properties (Destexhe and Paré, 1999; Chance et al., 2002; Léger et al., 2005; Bar-Yehuda and Korngreen, 2007). Alterations in neuronal membrane properties are

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Abbreviations: ADP, afterdepolarization; AHP, afterhyperpolarization; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; $GABA_AR$, $GABA_A$ receptor; $GABA_BR$, $GABA_B$ receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ISI, interspike interval; (m)ACSF, (modified) artificial cerebrospinal fluid; PTX, picrotoxin; resting Vm, resting membrane potential; Ri, input resistance; sEPSCs, spontaneous EPSCs; sIPSCs, spontaneous IPSCs; SFA, spike frequency adaptation.

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also expected following activation of extrasynaptic and metabotropic receptors (Wang et al., 2010; Juuri et al., 2010). Changes in background synaptic activity might therefore produce subtle but at the same time robust effects on what is normally referred as the ''intrinsic'' behavior of neurons. In the present study we employed a well established ex vivo–*in vitro* infrared-laser lesion model in the rat visual cortex and we investigated, in the first week post-lesion, potential changes in passive and active membrane properties of the surviving layer 2/3 pyramidal neurons. To better study the influence of network activity on these neuronal properties, brain slices were activated using an artificial cerebrospinal fluid (ACSF) slightly different in ionic composition from a standard bath solution (Bar-Yehuda and Korngreen, 2007). Similar ''modified'' ACSFs (mACSFs) have been reported to enhance synaptic and neuronal activity in cortical slices and have often been implemented to re-create, at least to some extent, the high ongoing activity found *in vivo* (Sanchez-Vives and McCormick, 2000; Silberberg et al., 2004; Reig and Sanchez-Vives, 2007). Interestingly, in the presence of this exciting medium we observed robust lesion-induced alterations in both input resistance (Ri) and neuronal firing properties. More moderate alterations were present when slices were perfused with a standard solution. Possibly, the reported alterations might have been underestimated so far due to the common use of ''standard'' ACSFs which dampen neuronal activity in brain slices (Bar-Yehuda and Korngreen, 2007). Synaptic transmission was found to play an important role in the observed lesion-mediated effects. Exciting cortical slices may have therefore unmasked these changes by increasing the level of ongoing synaptic activity and as a consequence the contribution of synaptic receptors in shaping the ''intrinsic'' behavior of neurons. All together our findings underscore that focal cortical lesions can cause robust changes in neuronal membrane and firing properties which may contribute, more than previously thought, to the pathophysiological processes following brain injuries.

EXPERIMENTAL PROCEDURES

Ethical statement

This study was carried out in strict accordance with the German regulations for experimentation with vertebrate animals. The protocol was approved by the Ethics committee of the University of Mainz (G11-1-016). The number of animals was kept to a minimum and all efforts were made to minimize suffering.

Cortical lesion induction

Long–Evans rats (n = 59) at the age of 21 days were anesthetized by an intraperitoneal injection of a mixture of Ketamine (100 mg/kg) and Xylazine (8 mg/kg). The animals were subsequently fixated in a stereotaxic apparatus, the skull was exposed and cautiously drilled above the right visual cortex parallel to the midline in a rectangular area of 1-mm width beginning right anterior to the lambda suture and extending 3 mm toward the Bregma without penetrating the dura mater. Cortical lesions were made under visual control with an 810-nm infrared diode laser (OcuLight SLx, Iris Medical, USA) attached to a binocular operating microscope. Multiple, confluent lesions were induced about 2 to 2.5 mm lateral from the midline in order to form an elongated lesion of 1 mm mediolateral width and 3 mm anteroposterior length starting anterior to the lambda suture in the visual cortex (areas V1M, V2ML V2MM) (Paxinos and Watson, 1998). Age-matched siblings were used as sham-operated controls. They underwent the same surgical procedure however after drilling the skull no laser-lesions were induced.

Electrophysiology

Slice preparation. After a survival time of 2–5 days the animals were deeply anesthetized with isoflurane and decapitated. Coronal slices containing the visual cortex (300 μm) were prepared from the lesioned hemisphere by use of a vibratome (LEICA, VT-1000-S, Germany). The tissue was kept at room temperature and incubated for 1 h in a standard ACSF containing (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.5 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, and 25 D-glucose (pH 7.4) and bubbled with 95% O_2 and 5% CO_2. Single slices were transferred into a submerged recording chamber. Slices were superfused (perfusion rate: 3.5 ml/min) with either the standard ACSF or two different ''modified'' ACSFs. The ''modified'' ACSF used for most of the experiments will be referred as K^+ 5 mM and contained (in mM): 126 NaCl, 25 $NaHCO_3$, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 1.25 NaH_2PO_4, and 25 D-glucose. A second ''modified'' ACSF was used for a subset of experiments. This solution will be referred as K^+ 3.5 mM since it had the same ionic composition as K^+ 5 mM but the KCl concentration was decreased from 5 to 3.5 mM. The perfusing solutions were always bubbled with 95% O_2 and 5% CO_2. During the experiments the ACSF temperature was kept at 31 ± 1 °C. The recording chamber was mounted on an upright microscope (Olympus-BX50WI, Olympus, Japan) equipped with 2.5× and 40× water immersion type objectives.

Whole-cell patch-clamp recordings. Current clamp whole-cell patch-clamp recordings were performed from layers 2/3 pyramidal neurons under visual control using DIC optics. Patch pipettes were pulled from borosilicate glass capillaries (GB 150F-8P, Science Products, Germany) and their resistance ranged from 4 to 6 MΩ when filled with intracellular solution. The intracellular solution contained (in mM): 140 K-gluconate, 8 KCl, 2 MgCl2, 4 Na2-ATP, 0.3 Na2-GTP, 10 Na-phosphocreatin and 10 HEPES. The pH was set to 7.3 with KOH. Bridge balance was applied throughout current-clamp experiments. The membrane and firing properties of pyramidal neurons were studied by applying a series of 1-s-lasting square pulses of hyperpolarizing and depolarizing currents through the