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IMPAIRED SYNAPTIC PLASTICITY IN THE PREFRONTAL CORTEX OF MICE WITH DEVELOPMENTALLY DECREASED NUMBER OF INTERNEURONS

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Abstract—Interneurons are inhibitory neurons, which protect neural tissue from excessive excitation. They are interconnected with glutamatergic pyramidal neurons in the cerebral cortex and regulate their function. Particularly in the prefrontal cortex (PFC), interneurons have been strongly implicated in regulating pathological states which display deficits in the PFC. The aim of this study is to investigate the adaptations in the adult glutamatergic system, when defects in interneuron development do not allow adequate numbers of interneurons to reach the cerebral cortex. To this end, we used a mouse model that displays ~50% fewer cortical interneurons due to the Rac1 protein loss from Nkx2.1/Cre expressing cells (Rac1 conditional knockout (cKO) mice), to examine how the developmental loss of interneurons may affect basal synaptic transmission, synaptic plasticity and neuronal morphology in the adult PFC. Despite the decrease in the number of interneurons, basal synaptic transmission, as examined by recording field excitatory postsynaptic potentials (fEPSPs) from layer II networks, is not altered in the PFC of Rac1 cKO mice. However, there is decreased paired-pulse ratio (PPR) and decreased long-term potentiation (LTP), in response to tetanic stimulation, in the layer II PFC synapses of Rac1 cKO mice. Furthermore, expression of N-methyl-D-aspartate (NMDA) subunits is decreased and dendritic morphology is altered, changes that could underlie the decrease in LTP in the Rac1 cKO mice. Finally, we find that treating Rac1 cKO mice with diazepam in early postnatal life can reverse changes in dendritic morphology observed in non-treated Rac1 cKO mice. Therefore, our data show that disruption in GABAergic inhibition alters glutamatergic function in the adult PFC, an effect that could be reversed by enhancement of GABAergic function during an early postnatal period.

Key words: synaptic plasticity, NMDA, MGE-derived interneurons, Rac1, dendritic spines, diazepam.

INTRODUCTION

Physiological studies (Haider and McCormick, 2009; Yizhar et al., 2011) and studies applying neurophysiological simulations using neuronal models (Konstantoudaki et al., 2014; Murray et al., 2014) have shown that excitation-inhibition balance is critical for maintaining proper functioning of the cerebral cortex. Excitation is provided by glutamate release from pyramidal neurons, while inhibition is provided by GABA release from several types of interneurons, which can be classified according to their protein expression, electrophysiological profile, and morphology. Two main types of interneurons include: (a) those that express the calcium binding protein parvalbumin (PV), have fast-spiking profile and target the soma and axon of the pyramidal neurons, and (b) those that express the neuropeptide somatostatin (SST), have a regular-spiking electrophysiological profile and primarily target the apical dendrites of the pyramidal neurons (Markram et al., 2004; Yizhar et al., 2011). Both cortical PV^+ and SST^+ interneurons originate from the medial ganglionic eminence (MGE) of the embryonic mouse brain (Wonders and Anderson, 2006).

Many neuropsychiatric disorders, such as epilepsy, anxiety, schizophrenia and autism exhibit an imbalance between excitatory and inhibitory mechanisms, in several brain regions including the prefrontal cortex (PFC) (Lewis et al., 2003; Yizhar et al., 2011; Marín, 2012). Specifically, reduction in interneuronal markers, such as GAD65/67 and PV, or GABA system adaptations have been correlated with several mental diseases, for example, schizophrenia (Lewis et al., 2003; Lodge et al., 2009; Hyde et al., 2011), autism (Fatemi et al., 2008a,b; Blatt and Fatemi, 2011), depression (Markram et al., 2004; Kalueff and Nutt, 2007; Yizhar et al., 2011; Möhler, 2012) and epilepsy (Powell, 2013).

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Abbreviations: aCSF, artificial cerebrospinal fluid; cKO, conditional knockout; EGTA, ethylene glycol tetraacetic acid; fEPSPs, field excitatory postsynaptic potentials; HEPES, 4-(2-hydroxyethyl)-1-piper azineethanesulfonic acid; LTP, long-term potentiation; MGE, medial ganglionic eminence; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; PD, postnatal day; PFC, prefrontal cortex; PPR, paired-pulse ratio; PV, protein parvalbumin; SST, somatostatin; YFP, yellow fluorescent protein.

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Although a reduction in GABAergic markers has been observed in several neuropsychiatric illnesses, it is not known whether these changes are causative or an adaptation of other primary modifications. The enhanced knowledge with regard to the transcription factors and intracellular mediators regulating various aspects of interneuron development has resulted in the generation of transgenic mouse lines with fewer cortical interneurons due to impaired proliferation and/or migration (Lewis et al., 2003; Cobos et al., 2005; Butt et al., 2008; Kerjan et al., 2009; Yizhar et al., 2011; Marín, 2012; Neves et al., 2012; Finlay and Uchiyama, 2015). These mice can be used to determine whether developmental defects in interneuron function would underlie network-wide changes in the brain. In this study, we utilize a transgenic mouse line that is missing the Rac1 gene from Nkx2.1/Cre-expressing cells ($Rac1^{fl/fl}$/$Nkx2.1^{+/Cre}$) of the MGE (referred to as the Rac1 (conditional knockout) cKO mouse hereafter; (Vidaki et al., 2012)). The Nkx1.2 transcription factor controls the generation of distinct interneuron subtypes originating from the MGE (Marin et al., 2000; Anderson et al., 2001). We have shown previously that the Rac1 cKO mice contain about 50% fewer MGE-derived GABAergic interneurons in the postnatal barrel cortex. This decrease results mainly from a longer G1 phase of MGE interneuron progenitors, leading to a delay in cell cycle exit, due to the Rac1 protein loss specifically from these cells (Vidaki et al., 2012). The interneurons found in the postnatal cortex have normal morphology, however the 50% that do not migrate remain aggregated in the ventral telencephalon and exhibit defective growth cones. Many mice die after 3 weeks of age (see the Experimental procedures section).

Using the Rac1 cKO mice, our goal is to determine how the developmental decrease in the number of interneurons affects the glutamatergic transmission properties of PFC neurons in adult mice. We demonstrate that PFC neurons of Rac1 cKO mice exhibit: (a) decreased paired-pulse facilitation at 20-Hz frequency, (b) decreased long-term potentiation (LTP), (c) reduced NR2A and NR2B subunits of the N-methyl-D-aspartate (NMDA) receptors, and (d) reduced number of mushroom-type spines. In addition, we find that Rac1cKO mice treated with diazepam, a GABA-A receptor agonist, during the early postnatal period, do not develop the defect in dendritic morphology, suggesting that enhancement of GABA-A receptor function during that period reverses the detrimental effects of decreased number of interneurons in these mice.

EXPERIMENTAL PROCEDURES

Animals and housing

Adult male mice, 60–80 days of age, were used for all experiments. Mice were housed in groups (3–4 per cage) and provided with standard mouse chow and water *ad libitum*, under a 12-h light/dark cycle (light on at 7:00 am) with controlled temperature (21 ± 1 °C). The following genotypes were used for analysis: $Rac1^{(fl/fl)}$;$Nkx2.1^{(+/Cre)}$ (referred to as Rac1 cKO mice) and $Rac1^{(+/fl)}$;$Nkx2.1^{(+/Cre)}$, referred to as heterozygous mice. The heterozygous mice were used as the control group to Rac1 cKO. Animals carrying a floxed allele of Rac1 ($Rac1^{fl/fl}$;$Nkx2.1^{+/Cre}$) were previously described (Vidaki et al., 2012). Specifically, animals carrying a floxed allele of Rac1 ($Rac1^{fl/fl}$) (the fourth and fifth exons of the Rac1 gene are flanked with loxP sites, (Walmsley et al., 2003) were crossed with Nkx2.1Tg(Cre) mice (Nkx2.1 transgenic Cre, (Fogarty et al., 2007)), in order to generate the $Rac1^{fl/fl}$;Nkx2.1Tg(Cre) genotype. The $ROSA26^{fl\text{-}STOP\text{-}fl\text{-}YFP}$ allele was also inserted as an independent marker, to allow visualization of the MGE-derived interneurons in which the Rac1 protein is deleted and Rac1 heterozygous MGE-derived interneurons, via yellow fluorescent protein (YFP) expression (Srinivas et al., 2001). Mice used in these experiments were taken from crossing $Rac1^{fl/fl}$;$Nkx2.1^{+/+}$ with $Rac1^{+/fl}$;$Nkx2.1^{+/Cre}$ genotypes. At least 80% of Rac1 heterozygous and Rac1 cKO animals came from the same litters. Fifty percent of the $Rac1^{fl/fl}$;$Nkx2.1^{+/cre}$ (Rac1 cKO) die within 3 weeks after birth. Our experiments were conducted only in mice that survived at least until postnatal day (PD) 60. All procedures were performed according to the European Union ethical standards and the IMBB and University of Crete ethical rules.

Electrophysiology

Electrophysiological experiments were performed using the *in vitro* slice preparation. Mice were decapitated under halothane anesthesia. The brain was removed immediately and placed in ice cold, oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 1 $MgCl_2$ and 10 glucose (pH = 7.4, 315 mOsm/l). The brain was blocked and glued onto the stage of a vibratome (Leica, VT1000S, Leica Biosystems GmbH, Wetzlar, Germany). 400-μm-thick brain slices containing the PFC were taken and were transferred to a submerged chamber, which was continuously superfused with oxygenated (95% O_2/5% CO_2) aCSF containing (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$ and 10 glucose (pH = 7.4, 315 mOsm/l) at room temperature. The slices were allowed to equilibrate for at least one hour in this chamber before experiments began. Slices were then transferred to a submerged recording chamber, which was continuously superfused with oxygenated (95% O_2/5% CO_2) aCSF (same constitution as the one used for maintenance of brain slices) at room temperature.

Extracellular recording electrodes filled with NaCl (2 M) were placed in layers II/III of PFC. Platinum/iridium metal microelectrodes (Harvard apparatus UK, Cambridge, UK) were placed on layer II of the PFC, about 300 μm away from the recording electrode, and were used to evoke field excitatory postsynaptic potentials (fEPSPs). Responses were amplified using a Dagan BVC-700A amplifier (Dagan Corporation, Minneapolis, MN, USA), digitized using the ITC-18 board (Instrutech, Inc, Longmont, CO, USA) on a PC using custom-made procedures in IgorPro