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Energy deficit in parvalbumin neurons leads to circuit dysfunction, impaired sensory gating and social disability

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ABSTRACT

Parvalbumin-expressing, fast spiking interneurons have high-energy demands, which make them particularly susceptible to energy impairment. Recent evidence suggests a link between mitochondrial dysfunction in fast spiking cortical interneurons and neuropsychiatric disorders. However, the effect of mitochondrial dysfunction restricted to parvalbumin interneurons has not been directly addressed in vivo. To investigate the consequences of mitochondrial dysfunction in parvalbumin interneurons in vivo, we generated conditional knockout mice with a progressive decline in oxidative phosphorylation by deleting *cox10* gene selectively in parvalbumin neurons (PV-Cox10 CKO). *Cox10* ablation results in defective assembly of cytochrome oxidase, the terminal enzyme of the electron transfer chain, and leads to mitochondrial bioenergetic dysfunction. PV-Cox10 CKO mice showed a progressive loss of cytochrome oxidase in cortical parvalbumin interneurons. Cytochrome oxidase protein levels were significantly reduced starting at postnatal day 60, and this was not associated with a change in parvalbumin interneuron density. Analyses of intrinsic electrophysiological properties in layer 5 primary somatosensory cortex revealed that parvalbumin interneurons could not sustain their typical high frequency firing, and their overall excitability was enhanced. An increase in both excitatory and inhibitory input onto parvalbumin interneurons was observed in PV-Cox10 CKO mice, resulting in a disinhibited network with an imbalance of excitation/inhibition. Investigation of network oscillations in PV-Cox10 CKO mice, using local field potential recordings in anesthetized mice, revealed significantly increased gamma and theta frequency oscillation power in both medial prefrontal cortex and hippocampus. PV-Cox10 CKO mice did not exhibit muscle strength or gross motor activity deficits in the time frame of the experiments, but displayed impaired sensory gating and sociability. Taken together, these data reveal that mitochondrial dysfunction in parvalbumin interneurons can alter their intrinsic physiology and network connectivity, resulting in behavioral alterations similar to those observed in neuropsychiatric disorders, such as schizophrenia and autism.

1. Introduction

GABAergic parvalbumin interneurons (PV INs) display fast-spiking action potentials and innervate the somata and axonal initial segments of their postsynaptic targets (Ascoli et al., 2008). These properties make them potent regulators of neuronal circuits (Woodruff and Yuste, 2008), and PV cortical INs (cINs) are vital for the balance of excitation and inhibition in the cortex (Inan et al., 2013; Rubenstein and Merzenich, 2003). Aberrant excitation/inhibition (E/I) balance in cortical microcircuits has been linked to neuropsychiatric disorders such as autism spectrum disorders (ASDs) and schizophrenia (Lisman, 2012; Uhlhaas and Singer, 2015; Zikopoulos and Barbas, 2013). Disruption of the E/I balance via solely enhancing excitation in the medial prefrontal cortex (mPFC) by optogenetic stimulation in freely behaving mice impaired their sociability (Yizhar et al., 2011), a phenotypic hallmark of both ASD and schizophrenia (Couture et al., 2010; Kaidanovich-Beilin et al., 2011; Sugranyes et al., 2011). This impairment in sociability was rescued (at least partially) by the concurrent optogenetic activation of PV cINs (Yizhar et al., 2011). Additional studies have demonstrated that deficits in PV IN function are associated with behavioral defects of both ASD and

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schizophrenia, including abnormal sociability and sensory gating (Barnes et al., 2015; Cho et al., 2015; Gogolla et al., 2009; Lewis et al., 2012). These results emphasize the importance of E/I balance in establishing social behavior, and shed light onto the pathophysiology underlying neuropsychiatric disorders.

Behavioral impairments in neuropsychiatric disorders and in animal models of these disorders have also been associated with altered network oscillations in the gamma range (30–80 Hz) (Kikuchi et al., 2011; Kogan et al., 2015; Lodge et al., 2009; Orekhova et al., 2007; Rubenstein, 2010; Spencer, 2008; Spencer, 2011; Spencer et al., 2003; Spencer et al., 2004; Wilson et al., 2007). GABAergic transmission is required for the coordination of network oscillations through synchronization of cortical circuits by generating a narrow window for effective excitation (Szabadics et al., 2001; Támas et al., 2000; Uhlhaas and Singer, 2010). In particular, hippocampal and neocortical PV INs are required for synchronization of the high frequency gamma oscillations (Lewis et al., 2012; Sohal et al., 2009; Uhlhaas and Singer, 2010), and loss of PV cINs in the mPFC correlates with reduced gamma-band response in a mouse model of schizophrenia (Lodge et al., 2009).

Both gamma oscillations and the fast-spiking firing properties of PV INs have high energy demands (Carter and Bean, 2009; Carter and Bean, 2011). The fast-spiking property of PV INs, but not the firing of excitatory pyramidal neurons, was compromised in the presence of mitochondrial respiratory chain inhibitors (Whittaker et al., 2011). Additionally, hippocampal PV INs were reported to have higher mitochondrial content compared to other neurons (Gulyas et al., 2006). Together, these observations suggest that PV INs rely heavily on mitochondrial energy metabolism, and it has been suggested that mitochondrial dysfunction in PV INs could result in abnormalities of gamma oscillations and consequently in impairment of complex information processing (Kann et al., 2014). However, the in vivo cellular and behavioral effects of mitochondrial dysfunction specifically in PV cINs are still unknown.

Mitochondrial dysfunction has also been linked to ASD and schizophrenia (Hjelm et al., 2015; Legido et al., 2013; Rajasekaran et al., 2015; Rossignol and Frye, 2014; Toker and Agam, 2015). Therefore, we hypothesized that a mitochondrial defect that is restricted to PV INs in mice may result in phenotypes that resemble abnormalities seen in individuals with ASD and schizophrenia. To investigate the consequences of mitochondrial dysfunction in PV cINs in vivo, we generated conditional knockout (CKO) mice by eliminating *cox10* expression in PV cells (PV-Cox10 CKO). Cox10 is a heme farnesyl transferase required for the assembly of cytochrome oxidase (COX), the terminal enzyme of the mitochondrial electron transfer chain. In earlier studies, conditional deletion of *cox10* in forebrain excitatory neurons resulted in a slowly progressive neurodegenerative phenotype (Diaz et al., 2012), suggesting that this is a viable approach to induce a progressive decline of oxidative phosphorylation (OXPHOS) in neurons. Using PV-Cox10 CKO mice, we examined how mitochondrial impairment in PV cINs affects their intrinsic physiology, connectivity at single-cell as well as network levels, and mouse behavior. Since impaired sociability is a hallmark of ASDs and schizophrenia, this behavioral aspect was explicitly tested using the three-chamber sociability task. Furthermore, pre-pulse inhibition (PPI) of startle reflex was used as a model to study sensorimotor gating and its deficits in neuropsychiatric disorders, such as schizophrenia.

2. Materials and methods

2.1. Mice

Knock-in mice carrying floxed alleles of *cox10* ($cox10^{f/f}$) were a generous gift from Dr. Carlos Moraes at the University of Miami. These mice were crossed with the $pvalb^{Cre/+}$ knock-in line (Jackson Laboratories Stock no: 008069). The progeny of this mating were then intercrossed to generate $pvalb^{Cre/+}$::$cox10^{f/f}$ (PV-Cox10 conditional KO, CKO) mice. Wild-type ($pvalb^{+/+}$::$cox10^{f/f}$) littermates were used as controls in order to avoid any confounding effect due to the loss of one copy of *pvalb* or *cox10*. For whole-cell patch clamp experiments, a reporter line expressing tdTomato specifically in PV cells was generated by crossing PV-Cox10 CKO mice to the Ai9 line (Jackson Laboratories Stock no: 007909). Since Cre expression was necessary for reporter expression, $PV^{Cre/+}$::$cox10^{+/+}$ littermates were used as controls.

PV-Cox10 CKO mice gained weight normally up to postnatal day (P) 75. A significant reduction in the weight of both female and male PV-Cox10 CKO mice compared to littermate controls was observed starting at P80, and the difference in weight increased as these mice aged further (Supplementary Fig. 1). All animal procedures were conducted in accordance with protocols approved by the Cornell University Research Animals Resource Center (RARC).

2.2. Immunohistochemistry

Mice at P45, P60, P80 and P100 were perfused (intracardiac) with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were then removed and post-fixed in fresh 4% PFA overnight. After rinsing the brains in PBS, 50 μm-thick coronal sections were obtained using a vibrating microtome. Primary antibodies used for immunofluorescence labeling included mouse anti-Cox1 (MitoSciences, 1:1000), rabbit anti-parvalbumin (Swant, 1:5000), rat anti-somatostatin (SST) (Chemicon, 1:500), and rabbit anti-calretinin (CR) (Millipore, 1:1000). These primary antibodies have been extensively used over decades of studies and their specificity has been established through many publications for anti-COX1 (D'Aurelio et al., 2004; D'Aurelio et al., 2001), anti-parvalbumin, somatostatin, and calretinin (Inan et al., 2012; Xu et al., 2008). Fluorescent secondary antibodies were labeled with Cy2 or Cy3 (Jackson laboratories, 1:500). The nuclear marker 4′,6-diamidino-2-phenylindole (DAPI, 300 nM) was applied together with the secondary antibodies.

Sections were first blocked with 5% heat-inactivated goat serum in PBS with 0.1% triton X-100 (PBST) for one hour, at room temperature, and then incubated in blocking solution containing primary antibodies overnight at 4 °C. Following the overnight incubation, sections were washed in PBST six times for 10 min followed by corresponding secondary immunolabeling for 2 h at room temperature. Sections were then washed three times for 10 min and mounted with ProLong Gold Antifade reagent (Molecular Probes).

Cox1 (subunit 1 of COX) content of different cIN subgroups (as defined by labeling for PV, SST and CR) was compared in all layers of the mPFC and in layer 5 of the primary somatosensory cortex (S1). PV, SST and CR cINs were identified by their corresponding immunostaining and were selected for analysis, blind to Cox1 expression. Cox1 content analysis of different cIN subgroups was performed as explained below for PV cINs. We measured percent (%) area of Cox1 content within the cell soma rather than fluorescent intensity because fluorescent intensity can easily be affected by experimental conditions while % area is a more conservative analysis and any difference detected is likely to be an underestimation of the actual difference in the immunofluorescence labeling.

Analyses of Cox1 content in PV cINs of PV-Cox10 CKO mice and controls were carried out blind to the genotype in two cortical areas, mPFC (including all layers of anterior cingulate and prelimbic areas) and layer 5 of S1 (cortical area where whole-cell patch clamp recordings were performed). 4 mice per genotype and per age, and 40 cells per cortical area were analyzed. mPFC was studied in sections that are within +1.6 mm to +1.8 mm with respect to the Bregma. S1 was identified by the visualization of barrels using DAPI staining. PV-expressing cells that are in sections within −0.94 mm to −1.46 mm with respect to the Bregma and within 50–200 μm ventral to the barrels were analyzed as layer 5 PV cINs. 0.5 μM-thick Z-stack images of PV and Cox1 labeling were collected from the area under investigation using a 60× objective. cINs that had complete somata immunolabeling within the Z-stack