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Prolonged Period of Cortical Plasticity upon Redox Dysregulation in Fast-Spiking Interneurons

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ABSTRACT

BACKGROUND: Oxidative stress and the specific impairment of perisomatic gamma-aminobutyric acid circuits are hallmarks of the schizophrenic brain and its animal models. Proper maturation of these fast-spiking inhibitory interneurons normally defines critical periods of experience-dependent cortical plasticity.

METHODS: Here, we linked these processes by genetically inducing a redox dysregulation restricted to such parvalbumin-positive cells and examined the impact on critical period plasticity using the visual system as a model (3–6 mice/group).

RESULTS: Oxidative stress was accompanied by a significant loss of perineuronal nets, which normally enwrap mature fast-spiking cells to limit adult plasticity. Accordingly, the neocortex remained plastic even beyond the peak of its natural critical period. These effects were not seen when redox dysregulation was targeted in excitatory principal cells.

CONCLUSIONS: A cell-specific regulation of redox state thus balances plasticity and stability of cortical networks. Mistimed developmental trajectories of brain plasticity may underlie, in part, the pathophysiology of mental illness. Such prolonged developmental plasticity may, in turn, offer a therapeutic opportunity for cognitive interventions targeting brain plasticity in schizophrenia.

Keywords: GABA, Oxidative stress, Parvalbumin, Perineuronal net, Schizophrenia, Visual cortex

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Schizophrenia is increasingly recognized as a neurodevelopmental disorder that involves alterations in brain circuits, including dysfunction of parvalbumin (PV)-positive, fast-spiking gamma-aminobutyric acid (GABA) neurons (1,2). Mounting evidence also indicates that redox dysregulation, an imbalance between oxidative stress and antioxidant defense systems, may play a role in the pathogenesis [reviewed in (3)]. Reduced antioxidant enzymes are reported in schizophrenic patients (4–8), and several divergent genetic (e.g., *Gclc, Gclm, DISC1, PRODH, G72, NRG1*) (9–17) or external factors (e.g., stress, social isolation, ketamine, neonatal ventral hippocampal lesion) (18–24) collectively lead to redox dysregulation. Animal models of globally disrupted redox state exhibit defects in fast-spiking interneurons (16,23,25) consistent with physiological consequences on excitatory-inhibitory circuit balance during neurodevelopment.

Notably, the postnatal maturation of PV-positive GABA neurons has been found to define critical periods of experience-dependent cortical plasticity (26). These windows in infancy and early childhood reflect heightened circuit rewiring to adaptively match sensory maps and complex behaviors to the surrounding environment and are then gradually consolidated into adulthood. Accordingly, disruption of excitatory-inhibitory balance powerfully shifts the timing and quality of critical period development, as seen in the primary visual cortex (27–29). High metabolic demands render fast-spiking interneurons particularly vulnerable to oxidative stress (23). Here, we examined whether late developing redox dysregulation within PV cells alone would directly impact the profile of cortical plasticity.

METHODS AND MATERIALS

Animals

Wild-type mice (C57BL/6J; Charles River, Wilmington, Massachusetts) and CamK2-Cre transgenic (c/o A. Nagy, Ph.D., University of Toronto, Ontario, Canada) (30), PV-ires-Cre (c/o S. Arber, Ph.D., Friedrich Miescher Institute Basel, Basel, Switzerland) (31), or Gclc-f/f mice (32) were crossed to generate CamK2-Gclc or PV-Gclc knockout (KO)/wild-type (WT) mice raised from birth on a 12-hour light/dark cycle to various postnatal ages. To rule out potential ectopic effects of Cre expression (33,34), we were careful to compare only with homozygous $Cre^{+/+}$ animals as control animals.

In Situ Hybridization

Probes for mouse *Gclc* and *Cre* were synthesized using T3/T7 RNA polymerase (Roche, Indianapolis, Indiana) labeled with digoxigenin or fluorescein and hybridized to frozen sections.

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Gclc probe was generated against a pCMV-SPORT6 plasmid containing the full-length mouse *Gclc* complementary DNA sequence between Not1 and Sal1 restriction enzyme sites (clone ID: 4193582, Invitrogen, Life Technologies, Grand Island, New York). Cre probe was generated using the template pBluescript2SK(-) plasmid including the full sequence of *Cre* subcloned from pCAG-Cre (Addgene plasmid 13775: between EcoR1 and Cla1 restriction enzyme sites). TSA-Plus DNP System (PerkinElmer Life Sciences, PerkinElmer, Branford, Connecticut) in combination with fast red staining was used to amplify the signal for double fluorescent in situ hybridization.

Immunohistochemistry

Mice were perfused transcardially with .9% saline and 4% paraformaldehyde, then brains were removed into 30% sucrose and transferred to ethylene glycol solution for cryoprotection. Brains were cut in coronal section (30 or 40 μm) on a freezing microtome. Sections were rinsed in phosphate buffered saline, then incubated overnight at 4°C in monoclonal antibody against parvalbumin (rabbit,1:500, Swant, Marly, Switzerland), 8-oxo-7,8-dihydro-20-deoxyguanine (8-oxo-dG) (mouse, 1:350, Trevigen, Gaithersberg, Maryland), NeuN (mouse, 1:400, EMD Millipore, Billerica, Massachusetts), CD68 (rat, 1:400, Serotec, AbD Serotec, Raleigh, North Carolina), biotin-conjugated lectin Wisteria floribunda agglutinin (WFA) (1:400 in phosphate buffered saline, Vector Laboratories, Burlingame, California), Otx2 (CD4, 1:50 gift from A. Prochiantz, College de France, Paris, France) and secondary antibodies (anti-IgG-Alexa-488, 594, 633/streptavidin-Alexa 488; 1:400, Invitrogen, Life Technologies). Fluoro-Jade C (Millipore, EMD Millipore) staining was performed according to previously described procedure (35).

Quantification of PV+ cells with strong 8-oxo-dG immunoreactivity was performed by combining threshold (between the values of 20 and 256) and particle analysis (particle size above 60 pixel^2) modules of ImageJ software (National Institutes of Health, Bethesda, Maryland) to distinguish strongly labeled cells from background signal. PV+/WFA+ cell counts were quantified using the spot module of Imaris 7.1 software (Bitplane AG, Zurich, Switzerland) as described previously (25). Briefly, we analyzed the amount of co-localization of immunostained voxels, using all immunostained voxels with fluorescence intensity above .03 (arbitrary unit) and the Coloc module of Imaris software. Immunostained voxels with fluorescence intensity below .03 (arbitrary unit) were neglected, as these were within the background fluorescence intensity level. Three to five mice were used per experimental condition.

Monocular Deprivation Procedure

Eyelid margins were trimmed by iris scissor and eyes sutured shut under isoflurane anesthesia. Eyes were closed for 4 to 5 days for short-term monocular deprivation from postnatal day (P)25 (during the critical period) or after (P50).

Extracellular Recording In Vivo

Electrophysiological recordings were performed under Nembutal/chlorprothixene anesthesia using standard techniques in mice (28). Ocular dominance (OD) in the binocular zone from each mouse was calculated as a contralateral bias index (CBI): [(n1 − n7) + 2/3(n2 − n6) + 1/3(n3 − n5) + N]/2N, where N = total number of cells and n = number of cells corresponding to ocular dominance score of x. The CBI approaches 1.0 as contralateral (contra) eye input dominates and is reduced when the ipsilateral (ipsi) eye becomes stronger. OD score was computed on cells with a complete peristimulus time histogram analysis of peak and baseline spiking activity, by alternately covering either eye. OD score was defined as {[Peak(ipsi) − baseline(ipsi)] − [Peak(contra) − baseline(contra)]} / {[Peak(ipsi) − baseline(ipsi)] + [Peak(contra) − baseline(contra)]} (29). For statistical comparison of OD distributions, normalized OD scores of individual neurons were computed and plotted as a cumulative distribution for each experimental group. CBI values across groups of mice were compared by one-way analysis of variance or Student *t* test.

RESULTS

Modeling Genetic Redox Dysregulation Within PV Cells In Vivo

To perturb redox balance specifically within PV interneurons, we conditionally deleted the *Gclc* (glutamate cysteine ligase catalytic subunit) gene—the rate-limiting enzyme that produces the primary endogenous antioxidant and redox regulator, glutathione (3) (Figure 1A). Importantly, the activity of glutathione is lower in schizophrenic patients, and the GAG-repeat of the *Gclc* promoter is associated with the illness (9). As systemic *Gclc* deletion is lethal (36), we crossed mice with the *Gclc* gene flanked by *loxP* sites (32) to animals expressing Cre recombinase under control of the PV promoter (31). To rule out potential ectopic effects of Cre expression (33,34), we were careful to compare PV-Cre$^{+/+}$; Gclc$^{f/f}$ (PV-*Gclc* KO) mice with homozygous PV-Cre$^{+/+}$ (PV-*Gclc* WT) control animals.

This yielded a progressive *Gclc* deletion in cortical PV cells from 34% at P20 to 70% in adulthood (>P50), as evaluated by double in situ hybridization for *Gclc* and *Cre* messenger RNA (Figure 1B,C). The cell-specific manipulation was subtle and did not cause myelin deficits (Figure 1D,E) typically observed upon global redox dysregulation (3,37). Instead, a cell-autonomous enhancement of oxidative stress was observed in the majority (69%) of PV cells when staining for a product of mitochondrial DNA oxidation, 8-oxo-dG (Figure 2A,B). This allowed us to further test the impact of PV cell-specific oxidative stress on postadolescent cortical plasticity. Note that we cannot rule out the possibility of mild noncell autonomous increase of oxidative stress below detection of our 8-oxo-dG staining threshold set to capture only those cells with enhanced oxidative stress beyond the basal levels of physiological stress.

Redox Dysregulation Decreases Perineuronal Nets Enwrapping PV Cells

As PV cells mature through their critical period, they are increasingly enwrapped by a perineuronal net (PNN) of extracellular matrix. Enzymatic removal of PNNs can reopen plasticity in adulthood (38,39). In the primary visual cortex of PV-*Gclc* KO mice, we observed significantly fewer PV-immunoreactive cells and PNNs, as revealed by WFA staining