Archival Report

Biological Psychiatry

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

Hirofumi Morishita, Jan-Harry Cabungcal, Ying Chen, Kim Q. Do, and Takao K. Hensch

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

http://dx.doi.org/10.1016/j.biopsych.2014.12.026

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.

© 2015 Society of Biological Psychiatry

ISSN: 0006-3223

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

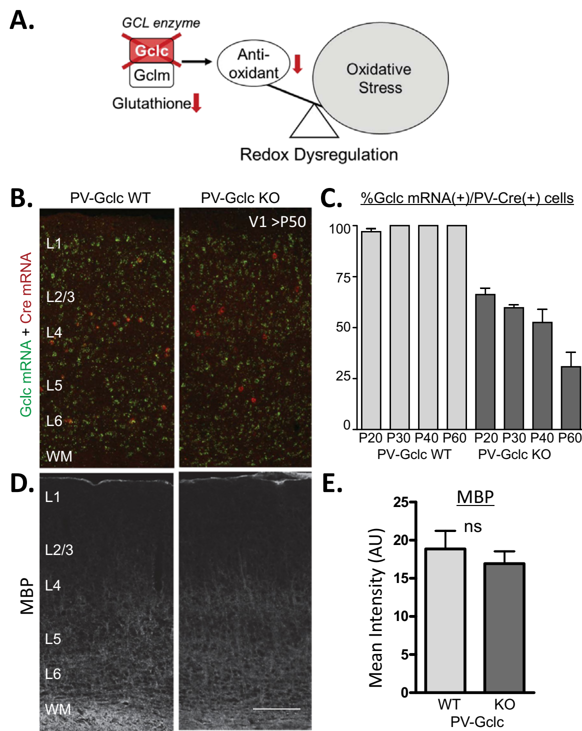

Figure 1. Modeling genetic redox dysregulation within parvalbumin (PV) cells in vivo. **(A)** Gclc normally forms a complex with Gclm as the rate-limiting enzyme for producing the major brain antioxidant and redox regulator, glutathione. Deletion of the *Gclc* gene induces redox dysregulation by lowering glutathione levels. **(B)** Double in situ hybridization for *Gclc* (green) and *Cre* (red) recombinase in mature primary visual cortex (V1) (> postnatal day [P]50) of wild-type (WT) (left) and PV-*Gclc* knockout (KO) (right) mice. **(C)** Quantification of co-localization reveals progressive developmental loss of *Gclc* expression in PV-*Cre* interneurons from 34% (P20) to 70% (P60) in PV-*Gclc* KO mice (dark gray bars) (one-way analysis of variance: $p < .05$), as compared with WT mice in which almost 100% of *Cre*-positive cells constantly express *Gclc*. **(D)** Immunohistochemical visualization of myelin basic protein (MBP) density in mouse V1 (>P50) of WT (left) and PV-*Gclc* KO (right) mice. **(E)** Quantification of mean MBP intensity shows no significant difference between genotypes ($p > .3$, t test). Scale bar, 150 μm. AU, arbitrary unit; GCL, glutamate-cysteine ligase; mRNA, messenger RNA; ns, nonsignificant; WM, white matter.

(Figure 2). The loss of PNNs was quite pronounced (Figure 2C), and the percentage of PV cells bearing PNNs was also reduced (Figure 2D). Compromised PNNs prevent the persistent uptake of Otx2 homeoprotein into the enwrapped PV cells needed for their maturation (including PNN maintenance itself) (27,40,41). Consistent with this, the percentage of Otx2+/PV+ cells was significantly reduced in PV-*Gclc* KO mice, especially in those cells enwrapped with PNNs (Figure S3 in Supplement 1).

PV-*Gclc* KO mice further exhibited a significant loss of perisomatic PV+ puncta surrounding their target pyramidal cell bodies in layer 2/3 of adult visual cortex (Figure S1 in Supplement 1). Instead, there was no indication of neurodegeneration by Fluoro Jade C staining, suggesting that PV cells did not die in PV-*Gclc* KO mice but were rather impaired (Figure S2 in Supplement 1). PNN loss might allow the diffusion of excessive reactive oxygen species outside the PV cell, which might signal microglial cells. In fact, a mild activation of microglia was observed by CD68 staining (Figure 3A,B).

For comparison, we also examined the brains of mice carrying a *Gclc* gene deletion from principal neurons, which express the calcium/calmodulin-dependent protein kinase type 2 (CaMK2) not expressed in PV cells (27) (Figure 3B). Consistent with a more widespread redox dysregulation across the pyramidal cell population, a massive activation of microglia (Figure 3C) and neurodegeneration (Figure S2 in Supplement 1) was observed, indicating that this is a robust, nonspecific response to cellular stress of any origin. In contrast, immunoreactivity for PV and PNNs remained intact in the adult visual cortex of CaMK2-*Gclc* KO mice (Figures S4 and S5 in Supplement 1). Microglia have recently been implicated as active participants in brain plasticity per se (42–45). We next examined the status of brain plasticity in PV-*Gclc* KO mice, given their PNN loss—a major brake on adult cortical rewiring (38).

Prolonged Critical Period Plasticity upon PV Cell-Specific Redox Dysregulation

The classic loss of input from an eye briefly deprived of vision in the binocular zone of primary visual cortex is typically

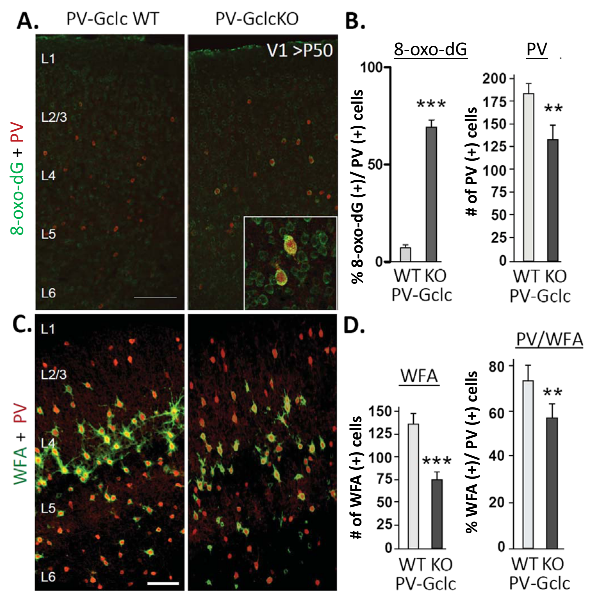

Figure 2. Redox dysregulation and loss of perineuronal nets (PNNs) enwrapping parvalbumin (PV) cells. **(A)** Double-labeling for 8-oxo-7, 8-dihydro-20-deoxyguanine (8-oxo-dG) (green) and PV (red) in mature primary visual cortex (V1) (postnatal day [P]50) of wild-type (WT) (left) and PV-*Gclc* knockout (KO) (right) mice. Higher magnification (inset) confirms elevation of oxidative stress in PV cells of PV-*Gclc* KO mice. **(B)** Left: Quantification (%) of PV+ cells also expressing high levels of 8-oxo-dG in PV-*Gclc* mice (69.3% KO versus 7.2% WT; ***$p < .001$ t test). Right: Total number of PV (+) cells, **$p < .01$ t test. **(C)** Double immunolabeling of PNNs with Wisteria floribunda agglutinin (WFA) (green) and PV (red) in mature V1 (>P50) of WT (left) and PV-*Gclc* KO (right) mice. Scale bar, 100 μm. **(D)** Left: Quantification of the number of WFA+ cells reveals loss of PNNs in PV-*Gclc* KO mice (versus WT; ***$p < .001$, t test). Right: Percentage of PV cells enwrapped by WFA1 PNN also reduced in KO mice (**$p < .01$, t test).

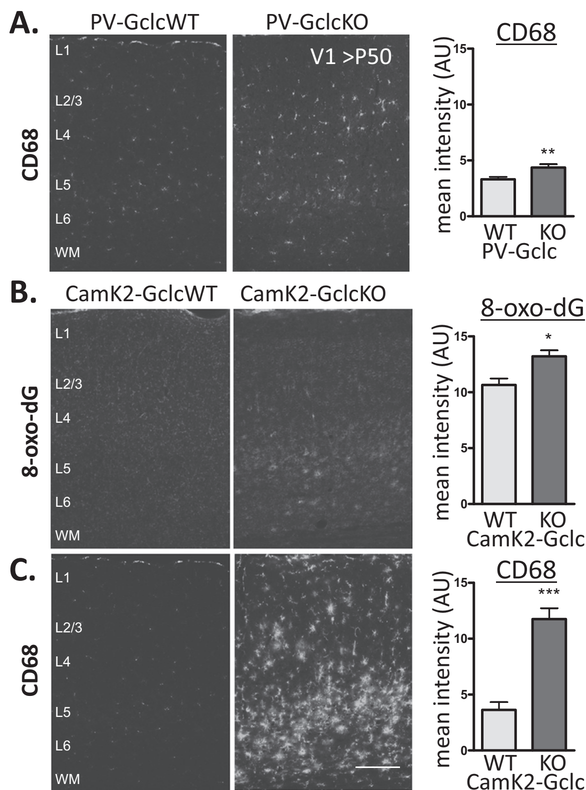

Figure 3. Microglial activation after cell-specific redox dysregulation. **(A)** Immunohistochemistry for CD68, a marker of activated microglia, shows mild elevation in parvalbumin (PV)-*Gclc* knockout (KO) (middle) compared with wild-type (WT) mice (left) in primary visual cortex (V1) (>postnatal day [P]50) ($p < .01$, t test). **(B)** Marked elevation of oxidative stress labeling (8-dihydro-20-deoxyguanine [8-oxo-dG]) in calcium/calmodulin-dependent protein kinase type 2 (CaMK2)-*Gclc* KO (middle) compared with WT mice (left) ($p < .05$, t test). **(C)** Massive activation of microglia (CD68) in CaMK2-*Gclc* KO (middle) compared with WT mice (left) in V1 (>P50) ($p < .01$, t test). Scale bar, 150 μm. AU, arbitrary unit; WM, white matter.

constrained before P50 [reviewed in (46,47)] (Figure 4A). When the majority of PV cells lacked *Gclc* gene expression by this age in our conditional mutant mice (Figure 1A), extracellular single-unit recording continued to yield a significant shift away from the deprived, contralateral eye following short-term (4–day) monocular deprivation (Figure 4B,C,E). The CBI differed significantly between genotypes (.57 in KO versus .68 in WT; age range between P54 and P63, $p < .001$). This enhanced plasticity in PV-*Gclc* KO mice past the peak of the critical period was not corrected by concurrent administration of the benzodiazepine diazepam (Figure S6 in Supplement 1), suggesting an earlier window may correct the plasticity phenotype (18).

Consistent with a limited *Gclc* deletion in younger KO mice (Figure 1C) during the natural critical period, there was no difference from WT plasticity levels at that age (Figure S7 in Supplement 1; CBI after 4-day monocular deprivation = .49 in PV-*Gclc* KO versus .51 in PV-*Gclc* WT; $p > .2$, $n = 3$ mice each). Moreover, in CaMK2-*Gclc* KO mice carrying a more severe redox dysregulation restricted to pyramidal cells, no extended plasticity was seen as in typical adult WT mice (Figure 4D,E; CBI = .69 and .67, respectively; $p > .8$). Given the inverse relationship of microglial activation and the level of cortical plasticity in adult PV-*Gclc* KO and CaMK2-*Gclc* KO mice, the contribution of microglial response to cortical plasticity may be more subtle than expected or reflect an optimal range of activation.

DISCUSSION

Our results reveal a prolonged period of brain plasticity—or a failure to stabilize cortical circuits—in young adult animals under conditions of PV cell-specific glutathione dysregulation in vivo. Mistimed developmental trajectories of brain plasticity may therefore contribute, in part, to the etiology of mental illnesses involving oxidative stress, such as schizophrenia (2,3,18). This reflects a redox-sensitive failure to maintain PV cells with fully enwrapped PNNs, which normally balance plasticity and stability of cortical circuits across development in a twofold manner (38,39).

First, intact PNNs may structurally limit the robust synaptic plasticity directly upon PV cells (48–50). Second, PNN disruption prevents the persistent uptake of Otx2 homeoprotein into the enwrapped PV cells required for their maintenance (27,40,41) (Figure S3 in Supplement 1). Subsequent inability to engage downstream molecular pathways by Otx2 within the PV cell would retain a juvenile plastic state (41). Notably, the major endogenous source of this Otx2 has recently been identified as the choroid plexus (51), which lines the ventricles that are commonly found to be enlarged in schizophrenia.

Two of the primary PNN components, chondroitin sulfate and hyaluronan, are sensitive to excessive free radicals (52), and the relatively late timing of *Gclc* deletion here overlaps with their usual condensation into tight nets around PV cells. Our recent study revealed a protective function of these PNNs as a redox sensor (53). The high metabolic requirements of the fast-spiking PV interneuron (23) may readily generate reactive oxygen species when their antioxidant systems are impaired, leading to the breakdown of PNNs themselves (Figure 2C,D) (53). Our study suggests that cell-autonomous redox dysregulation within PV cells is the primary contributor to a reduction of PNNs observed across mouse models and human patients sharing global redox dysregulation.

Paradoxically, redox dysregulation in schizophrenia may then act, in part, by transiently prolonging or reopening critical periods of developmental plasticity. An excessively plastic status may precede the progressive degenerative process as in other brain disorders (29,54). In chronic schizophrenia patients, deficits in visual cortex and long-term potentiation-like plasticity are reported when probed by transcranial direct current stimulation (55–58). First-onset patients instead do not differ significantly from healthy control subjects with a trend toward increased plasticity (59). Individuals at high risk show greater cognitive gains relative to adults with chronic schizophrenia in response to computer-based cognitive training (60). Our study points to the importance of studying the developmental trajectory of cortical plasticity in schizophrenia patients to understand the relation to disease progression, remission, and treatment response (61).

Prolonged developmental plasticity may then provide a diagnostic opportunity for cognitive interventions targeting brain plasticity in the prodromal stage (62–64). Unfortunately, the current study was limited before and after the age range we

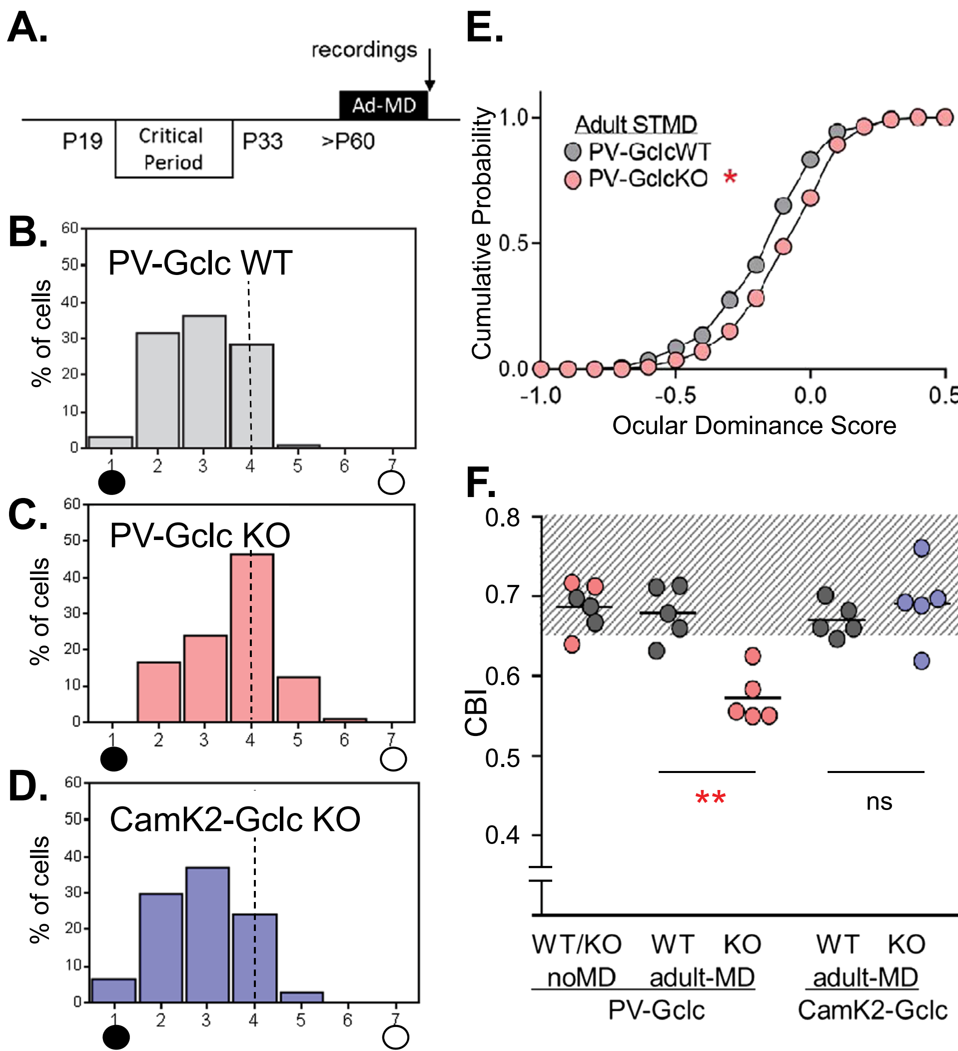

Figure 4. Prolonged cortical plasticity by parvalbumin (PV) cell specific redox dysregulation. **(A)** Contralateral eye bias assessed by single-unit electrophysiology in mouse primary visual cortex after 4 days of short-term monocular deprivation (STMD) in adulthood. **(B–D)** Ocular dominance distribution after STMD (five mice each) in **(B)** wild-type (WT) (127 cells), **(C)** PV-*Gclc* knockout (KO) (121 cells), and **(D)** calcium/calmodulin-dependent protein kinase type 2 (CaMK2)-*Gclc* KO mice (108 cells). Age range from postnatal day (P) 54 to P63. Visual responses classified on a seven-point scale from exclusively contralateral (group 1) to ipsilateral driven (group 7). PV-Gclc KO versus WT: $p < .0005$; PV-Gclc KO vs. CamK2-Gclc KO: $p < .0001$, X2 test. **(E)** Cumulative probability of quantified spike response after adult STMD confirms shifted ocular dominance scores for PV-*Gclc* KO (pink-filled circles) compared with WT (gray-filled circles) (*$p < .05$, Kolmogorov-Smirnov test). **(F)** Mean contralateral bias index (CBI), starting from the same baseline values (.68) in nondeprived mice across genotypes, is shifted only in PV-*Gclc* KO mice by STMD (.57; **$p < .001$ versus WT, adjusted p value, Tukey's multiple comparison test). No adult plasticity in CaMK2-*Gclc* KO mice (mean CBI = .69 versus .67 in WT mice; $p > .8$, adjusted p value, Tukey's multiple comparison test, one-way analysis of variance). Shaded area indicates CBI range of nondeprived (aplastic) mice. Ad-MD, adult monocular deprivation; MD, monocular deprivation; ns, nonsignificant.

studied (P54–P63), either by premature death of PV-*Gclc* KO mice by P80 (as PV is also expressed in muscle) or insufficient deletion of the *Gclc* gene at younger stages. Also, we cannot fully rule out the possibility that the prolonged plasticity in these mice is mediated by an as yet unknown consequence of *Gclc* deletion not directly related to redox dysregulation. Future studies in other animal models are required to rigorously map the trajectory of brain plasticity across the life span in relation to redox dysregulation and other risk factors.

Altered PNNs have been found in the human postmortem amygdala and entorhinal and prefrontal cortices of patients (65–67), and GABA-related functional deficits are seen even in their visual cortex (68–71). Pathophysiological changes (1–3) are consistent with dematuration or a removal of brakes on plasticity (72,73). Several molecular brakes of relevance to schizophrenia (other than PNNs) have been identified to limit critical period plasticity, including myelin signaling through a NgR-PirB complex (2,74,75) or dampened nicotinic receptor signaling by Lynx1 (29,76). Curiously, all brakes to date converge on PV cell function as a hub of vulnerability (77).

This may offer novel strategies or drug targets, in addition to antioxidants (18,25) or Otx2 upregulation (41,51), for correcting aberrant brain plasticity in mental illness.

ACKNOWLEDGMENTS AND DISCLOSURES

Supported by National Institutes of Health (1DP1OD003699 and 1P50MH094271 to TKH), Swiss National Science Foundation (#31-116689), National Center of Competence in Research SYNAPSY - The Synaptic Bases of Mental Diseases financed by the Swiss National Science Foundation (n° 51AU40_125759), Fondation Damm-Etienne (to KQD), and the Brain & Behavior Research Foundation (to HM).

We thank M. Cuenod (Lausanne) for helpful insights, A. Prochiantz (Paris) and H. Bond for Otx2 immunostaining, and M. Marcotrigiano and M. Nakamura for animal breeding and maintenance.

All authors reported no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the FM Kirby Neurobiology Center (HM, TKH), Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston,

Massachusetts; Department of Psychiatry (J-HC, KQD), Center for Psychiatric Neuroscience, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; School of Pharmacy (YC), University of Colorado, Anschutz Medical Campus, Aurora Colorado; and Center for Brain Science (TKH), Department of Molecular Cellular Biology, Harvard University, Cambridge, Massachusetts.

HM is currently affiliated with the Department of Psychiatry, Department of Neuroscience, Department of Ophthalmology, Mindich Child Health and Development Institute, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York; YC is currently affiliated with the Department of Environmental Health, Yale School of Public Health, New Haven, Connecticut.

Address correspondence to Takao K. Hensch, Ph.D., Harvard University, FM Kirby Neurobiology Center, Department of Neurology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115; E-mail: hensch@mcb.harvard.edu.

Received Nov 21, 2013; revised Dec 29, 2014; accepted Dec 31, 2014.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.12.026.

REFERENCES

1. Lewis DA, Hashimoto T, Volk DW (2005): Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312–324.
2. Insel TR (2010): Rethinking schizophrenia. Nature 468:187–193.
3. Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M (2009): Redox dysregulation, neurodevelopment, and schizophrenia. Curr Opin Neurobiol 19:220–230.
4. Do KQ, Trabesinger AH, Kirsten-Kruger M, Lauer CJ, Dydak U, Hell D, *et al*. (2000): Schizophrenia: Glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. Eur J Neurosci 12:3721–3728.
5. Andreazza AC, Kauer-Sant'anna M, Frey BN, Bond DJ, Kapczinski F, Young LT, Yatham LN (2008): Oxidative stress markers in bipolar disorder: A meta-analysis. J Affect Disord 111:135–144.
6. Yao JK, Keshavan MS (2011): Antioxidants, redox signaling, and pathophysiology in schizophrenia: An integrative view. Antioxid Redox Signal 15:2011–2035.
7. Wang JF, Shao L, Sun X, Young LT (2009): Increased oxidative stress in the anterior cingulate cortex of subjects with bipolar disorder and schizophrenia. Bipolar Disord 11:523–529.
8. Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT (2011): Decreased levels of glutathione, the major brain antioxidant, in postmortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol 14:123–130.
9. Gysin R, Kraftsik R, Sandell J, Bovet P, Chappuis C, Conus P, *et al*. (2007): Impaired glutathione synthesis in schizophrenia: Convergent genetic and functional evidence. Proc Natl Acad Sci U S A 104:16621–16626.
10. Tosic M, Ott J, Barral S, Bovet P, Deppen P, Gheorghita F, *et al*. (2006): Schizophrenia and oxidative stress: Glutamate cysteine ligase modifier as a susceptibility gene. Am J Hum Genet 79:586–592.
11. Park YU, Jeong J, Lee H, Mun JY, Kim JH, Lee JS, *et al*. (2010): Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. Proc Natl Acad Sci U S A 107: 17785–17790.
12. Gokhale A, Larimore J, Werner E, So L, Moreno-De-Luca A, Lese-Martin C, *et al*. (2012): Quantitative proteomic and genetic analyses of the schizophrenia susceptibility factor dysbindin identify novel roles of the biogenesis of lysosome-related organelles complex 1. J Neurosci 32:3697–3711.
13. Goldshmit Y, Erlich S, Pinkas-Kramarski R (2001): Neuregulin rescues PC12-ErbB4 cells from cell death induced by H(2)O(2). Regulation of reactive oxygen species levels by phosphatidylinositol 3-kinase. J Biol Chem 276:46379–46385.
14. Otte DM, Sommersberg B, Kudin A, Guerrero C, Albayram O, Filiou MD, *et al*. (2011): N-acetyl cysteine treatment rescues cognitive deficits induced by mitochondrial dysfunction in G72/G30 transgenic mice. Neuropsychopharmacology 36:2233–2243.
15. Krishnan N, Dickman MB, Becker DF (2008): Proline modulates the intracellular redox environment and protects mammalian cells against oxidative stress. Free Radic Biol Med 44:671–681.
16. Steullet P, Cabungcal JH, Kulak A, Kraftsik R, Chen Y, Dalton TP, *et al*. (2010): Redox dysregulation affects the ventral but not dorsal hippocampus: Impairment of parvalbumin neurons, gamma oscillations, and related behaviors. J Neurosci 30:2547–2558.
17. Kulak A, Steullet P, Cabungcal JH, Werge T, Ingason A, Cuenod M, Do KQ (2013): Redox dysregulation in the pathophysiology of schizophrenia and bipolar disorder: Insights from animal models. Antioxid Redox Signal 18:1428–1443.
18. Cabungcal JH, Counotte DS, Lewis EM, Tejeda HA, Piantadosi P, Pollock C, *et al*. (2014): Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. Neuron 83: 1073–1084.
19. Hu W, Zhang M, Czeh B, Flugge G, Zhang W (2010): Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacology 35:1693–1707.
20. Grillo CA, Piroli GG, Rosell DR, Hoskin EK, McEwen BS, Reagan LP (2003): Region specific increases in oxidative stress and superoxide dismutase in the hippocampus of diabetic rats subjected to stress. Neuroscience 121:133–140.
21. Jiang Z, Rompala GR, Zhang S, Cowell RM, Nakazawa K (2013): Social isolation exacerbates schizophrenia-like phenotypes via oxidative stress in cortical interneurons. Biol Psychiatry 73:1024–1034.
22. Powell SB, Sejnowski TJ, Behrens MM (2012): Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia. Neuropharmacology 62:1322–1331.
23. Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, Dugan LL (2007): Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. Science 318: 1645–1647.
24. Schiavone S, Sorce S, Dubois-Dauphin M, Jaquet V, Colaianna M, Zotti M, *et al*. (2009): Involvement of NOX2 in the development of behavioral and pathologic alterations in isolated rats. Biol Psychiatry 66:384–392.
25. Cabungcal JH, Steullet P, Kraftsik R, Cuenod M, Do KQ (2013): Early-life insults impair parvalbumin interneurons via oxidative stress: Reversal by N-acetylcysteine. Biol Psychiatry 73:574–582.
26. Hensch TK (2005): Critical period plasticity in local cortical circuits. Nat Rev Neurosci 6:877–888.
27. Sugiyama S, Di Nardo AA, Aizawa S, Matsuo I, Volovitch M, Prochiantz A, Hensch TK (2008): Experience-dependent transfer of Otx2 homeoprotein into the visual cortex activates postnatal plasticity. Cell 134:508–520.
28. Fagiolini M, Hensch TK (2000): Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183–186.
29. Morishita H, Miwa JM, Heintz N, Hensch TK (2010): Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. Science 330: 1238–1240.
30. Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, *et al*. (1999): Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401–414.
31. Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S (2005): A developmental switch in the response of DRG neurons to ETS transcription factor signaling. PLoS Biol 3:e159.
32. Chen Y, Yang Y, Miller ML, Shen D, Shertzer HG, Stringer KF, *et al*. (2007): Hepatocyte-specific Gclc deletion leads to rapid onset of steatosis with mitochondrial injury and liver failure. Hepatology 45:1118–1128.
33. Kobayashi Y, Hensch TK (2013): Germline recombination by conditional gene targeting with Parvalbumin-Cre lines. Front Neural Circuits 7:168.
34. Harno E, Cottrell EC, White A (2013): Metabolic pitfalls of CNS Cre-based technology. Cell Metab 18:21–28.

35. Schmued LC, Stowers CC, Scallet AC, Xu L (2005): Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. Brain Res 1035:24–31.
36. Dalton TP, Dieter MZ, Yang Y, Shertzer HG, Nebert DW (2000): Knockout of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: Embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous. Biochem Biophys Res Commun 279:324–329.
37. Do KQ, Monin A, Klaey M, Butticaz C, Cabungcal JH, Steullet P, *et al*. (2012): Redox dysregulation affects proliferation, differentiation of oligodendrocyte progenitors and myelination: Relevance to dysconnectivity in schizophrenia. Biol Psychiatry 71:4S.
38. Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002): Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248–1251.
39. Gogolla N, Caroni P, Luthi A, Herry C (2009): Perineuronal nets protect fear memories from erasure. Science 325:1258–1261.
40. Miyata S, Komatsu Y, Yoshimura Y, Taya C, Kitagawa H (2012): Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. Nat Neurosci 15:414–422; S1–S2.
41. Beurdeley M, Spatazza J, Lee HH, Sugiyama S, Bernard C, Di Nardo AA, *et al*. (2012): Otx2 binding to perineuronal nets persistently regulates plasticity in the mature visual cortex. J Neurosci 32:9429–9437.
42. Tremblay ME, Lowery RL, Majewska AK (2010): Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527.
43. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, *et al*. (2011): Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456–1458.
44. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, *et al*. (2012): Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705.
45. Tremblay ME, Majewska AK (2011): A role for microglia in synaptic plasticity? Commun Integr Biol 4:220–222.
46. Hensch TK, Fagiolini M (2005): Excitatory-inhibitory balance and critical period plasticity in developing visual cortex. Prog Brain Res 147:115–124.
47. Morishita H, Hensch TK (2008): Critical period revisited: Impact on vision. Curr Opin Neurobiol 18:101–107.
48. Yazaki-Sugiyama Y, Kang S, Cateau H, Fukai T, Hensch TK (2009): Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462:218–221.
49. Kuhlman SJ, Olivas ND, Tring E, Ikrar T, Xu X, Trachtenberg JT (2013): A disinhibitory microcircuit initiates critical-period plasticity in the visual cortex. Nature 501:543–546.
50. Aton SJ, Broussard C, Dumoulin M, Seibt J, Watson A, Coleman T, Frank MG (2013): Visual experience and subsequent sleep induce sequential plastic changes in putative inhibitory and excitatory cortical neurons. Proc Natl Acad Sci U S A 110:3101–3106.
51. Spatazza J, Lee HH, Di Nardo AA, Tibaldi L, Joliot A, Hensch TK, Prochiantz A (2013): Choroid-plexus-derived otx2 homeoprotein constrains adult cortical plasticity. Cell Rep 3:1815–1823.
52. Rees MD, Hawkins CL, Davies MJ (2004): Hypochlorite and superoxide radicals can act synergistically to induce fragmentation of hyaluronan and chondroitin sulphates. Biochem J 381:175–184.
53. Cabungcal JH, Steullet P, Morishita H, Kraftsik R, Cuenod M, Hensch TK, Do KQ (2013): Perineuronal nets protect fast-spiking interneurons against oxidative stress. Proc Natl Acad Sci U S A 110:9130–9135.
54. Beste C, Wascher E, Dinse HR, Saft C (2012): Faster perceptual learning through excitotoxic neurodegeneration. Curr Biol 22:1914–1917.
55. Daskalakis ZJ, Christensen BK, Fitzgerald PB, Chen R (2008): Dysfunctional neural plasticity in patients with schizophrenia. Arch Gen Psychiatry 65:378–385.
56. McClintock SM, Freitas C, Oberman L, Lisanby SH, Pascual-Leone A (2011): Transcranial magnetic stimulation: A neuroscientific probe of cortical function in schizophrenia. Biol Psychiatry 70:19–27.
57. Mears RP, Spencer KM (2012): Electrophysiological assessment of auditory stimulus-specific plasticity in schizophrenia. Biol Psychiatry 71:503–511.
58. Cavus I, Reinhart RM, Roach BJ, Gueorguieva R, Teyler TJ, Clapp WC, *et al*. (2012): Impaired visual cortical plasticity in schizophrenia. Biol Psychiatry 71:512–520.
59. Hasan A, Nitsche MA, Rein B, Schneider-Axmann T, Guse B, Gruber O, *et al*. (2011): Dysfunctional long-term potentiation-like plasticity in schizophrenia revealed by transcranial direct current stimulation. Behav Brain Res 224:15–22.
60. Rauchensteiner S, Kawohl W, Ozgurdal S, Littmann E, Gudlowski Y, Witthaus H, *et al*. (2011): Test-performance after cognitive training in persons at risk mental state of schizophrenia and patients with schizophrenia. Psychiatry Res 185:334–339.
61. Ehlers MD (2012): Hijacking hebb: Noninvasive methods to probe plasticity in psychiatric disease. Biol Psychiatry 71:484–486.
62. Vinogradov S, Fisher M, de Villers-Sidani E (2012): de Villers-Sidani E. Cognitive training for impaired neural systems in neuropsychiatric illness. Neuropsychopharmacology 37:43–76.
63. Kaneko Y, Keshavan M (2012): Cognitive remediation in schizophrenia. Clin Psychopharmacol Neurosci 10:125–135.
64. Fisher M, Loewy R, Hardy K, Schlosser D, Vinogradov S (2013): Cognitive interventions targeting brain plasticity in the prodromal and early phases of schizophrenia. Annu Rev Clin Psychol 9:435–463.
65. Berretta S (2012): Extracellular matrix abnormalities in schizophrenia. Neuropharmacology 62:1584–1597.
66. Pantazopoulos H, Woo TU, Lim MP, Lange N, Berretta S (2010): Extracellular matrix-glial abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia. Arch Gen Psychiatry 67:155–166.
67. Mauney SA, Athanas KM, Pantazopoulos H, Shaskan N, Passeri E, Berretta S, Woo TU (2013): Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. Biol Psychiatry 74:427–435.
68. Rokem A, Yoon JH, Ooms RE, Maddock RJ, Minzenberg MJ, Silver MA (2011): Broader visual orientation tuning in patients with schizophrenia. Front Hum Neurosci 5:127.
69. Yoon JH, Maddock RJ, Rokem A, Silver MA, Minzenberg MJ, Ragland JD, Carter CS (2010): GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. J Neurosci 30:3777–3781.
70. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA (2008): Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. Am J Psychiatry 165:479–489.
71. Kim D, Zemon V, Saperstein A, Butler PD, Javitt DC (2005): Dysfunction of early-stage visual processing in schizophrenia: Harmonic analysis. Schizophr Res 76:55–65.
72. Bavelier D, Levi DM, Li RW, Dan Y, Hensch TK (2010): Removing brakes on adult brain plasticity: From molecular to behavioral interventions. J Neurosci 30:14964–14971.
73. Hagihara H, Ohira K, Takao K, Miyakawa T (2014): Transcriptomic evidence for immaturity of the prefrontal cortex in patients with schizophrenia. Mol Brain 7:41.
74. McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM (2005): Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science 309:2222–2226.
75. Syken J, Grandpre T, Kanold PO, Shatz CJ (2006): PirB restricts ocular-dominance plasticity in visual cortex. Science 313:1795–1800.
76. Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, *et al*. (2008): Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat Genet 40:751–760.
77. Takesian AE, Hensch TK (2013): Balancing plasticity/stability across brain development. Prog Brain Res 207:3–34.