Altered Neocortical Rhythmic Activity States in *Fmr1* KO Mice Are Due to Enhanced mGluR5 Signaling and Involve Changes in Excitatory Circuitry

Seth A. Hays, Kimberly M. Huber, and Jay R. Gibson

Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9111

Despite the pronounced neurological deficits associated with mental retardation and autism, the degree to which neocortical circuit function is altered remains unknown. Here, we study changes in neocortical network function in the form of persistent activity states in the mouse model of fragile X syndrome—the *Fmr1* knock-out (KO). Persistent activity states, or UP states, in the neocortex underlie the slow oscillation which occurs predominantly during slow-wave sleep, but may also play a role during awake states. We show that spontaneously occurring UP states in the primary somatosensory cortex are 38–67% longer in *Fmr1* KO slices. *In vivo*, UP states reoccur with a clear rhythmic component consistent with that of the slow oscillation and are similarly longer in the *Fmr1* KO. Changes in neocortical excitatory circuitry likely play the major role in this alteration as supported by three findings: (1) longer UP states occur in slices of isolated neocortex, (2) pharmacologically isolated excitatory circuits in *Fmr1* KO neocortical slices display prolonged bursting states, and (3) selective deletion of *Fmr1* in cortical excitatory neurons is sufficient to cause prolonged UP states whereas deletion in inhibitory neurons has no effect. Excess signaling mediated by the group 1 glutamate metabotropic receptor, mGluR5, contributes to the longer UP states. Genetic reduction or pharmacological blockade of mGluR5 rescues the prolonged UP state phenotype. Our results reveal an alteration in network function in a mouse model of intellectual disability and autism which may impact both slow-wave sleep and information processing during waking states.

Introduction

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and is caused by loss of function mutations in *FMR1* which encodes the RNA binding protein, FMRP (Verkerk et al., 1991; O'Donnell and Warren, 2002). Many of the impairments in FXS such as altered social responses and hypersensitivity to sensory stimuli are reproduced in the FXS mouse model, the *Fmr1* knock-out (KO) mouse (The Dutch-Belgian Fragile X Consortium, 1994; Miller et al., 1999; Musumeci et al., 2000; Hagerman, 2002; Nielsen et al., 2002; Spencer et al., 2005; Brennan et al., 2006).

It has been hypothesized that altered cortical function mediates the cognitive and behavioral deficits in FXS (Irwin et al., 2001). In support of this idea, many cellular and synaptic alterations have been observed in cortical structures in FXS patients and in *Fmr1* KO mice (for review, see Bassell and Warren, 2008; Pfeiffer and Huber, 2009).

Despite these numerous reported deficits, little electrophysiological evidence of alterations in cortical circuit function exists.

There is a plasticity phenomenon in *Fmr1* KO mice where hippocampal networks become more excitable and epileptic in response to pharmacological blockade of inhibition (Chuang et al., 2005). However, it is unclear how cortical network function under basal conditions is altered in *Fmr1* KO mice. We have reported one such example where persistent activity states, or UP states, are longer in duration in neocortical slices obtained from *Fmr1* KO mice when thalamic stimulation is used to induce the active state (Gibson et al., 2008). UP states are depolarized firing states of neurons that are driven by local recurrent excitation and inhibition, and occur synchronously among all neurons in a cortical region (Haider and McCormick, 2009; Sanchez-Vives et al., 2010). When they occur spontaneously and repeatedly, they underlie the neocortical “slow oscillation,” which is a rhythm (<1 Hz) occurring during slow-wave sleep, but they may also be involved in information processing during awake states (Steriade et al., 2001; Timofeev et al., 2001; Marshall et al., 2006; Haider and McCormick, 2009; Okun et al., 2010). Therefore, UP states are a critical aspect of neocortical circuit function, and understanding how and why they are altered in the *Fmr1* KO mouse would provide important information to how baseline neocortical circuit function is altered in fragile X syndrome. Such data would also provide specific strategies for treatment.

Questions remain about prolonged UP states in *Fmr1* KO mice. First, while prolonged UP states are observed with thalamic stimulation (Gibson et al., 2008), are spontaneous and rhythmic UP states altered and are alterations intrinsic to neocortex? The answer to this is critical for linking prolonged UP states to possible

Received June 21, 2011; revised Aug. 10, 2011; accepted Aug. 12, 2011.

Author contributions: K.M.H. and J.R.G. designed research; S.H. performed research; S.H. and J.R.G. analyzed data; K.M.H. and J.R.G. wrote the paper.

This research was supported by National Institutes of Health Grants HD056370 (J.R.G.), NS045711 (K.M.H.), HD052731 (K.M.H.), and GM008203 (S.A.H.), and by Autism Speaks (K.M.H.). We thank Lorea Ormazabal for technical assistance with the mice. Gifts of mouse models are referenced in Materials and Methods.

Correspondence should be addressed to Jay R. Gibson, University of Texas Southwestern Medical Center, Department of Neuroscience, Box 9111, Dallas, TX 75390-9111. E-mail: Jay.Gibson@UTSouthwestern.edu.

DOI:10.1523/JNEUROSCI.3157-11.2011

Copyright © 2011 the authors 0270-6474/11/3114223-12\$15.00/0

alterations in the slow oscillation rhythm since neocortex has been hypothesized to primarily mediate the slow oscillation (Steriade, 1997; Haider and McCormick, 2009). Second, are UP states longer in the *Fmr1* KO *in vivo* or is this strictly an *in vitro* slice phenomenon? Third, what is the relative role of changes in excitatory versus inhibitory circuitry? Fourth, what cellular processes lead to prolonged UP states?

We find that spontaneously occurring UP states are longer in the *Fmr1* KO—both *in vitro* and *in vivo*—and that this alteration involves changes in mGluR5 (group I metabotropic glutamate receptor) signaling in neocortical excitatory neurons.

Materials and Methods

Mice. Congenic *Fmr1* KO mice on the C57BL/6 background were originally obtained from Dr. Stephen Warren (Emory University, Atlanta, GA) and have been backcrossed onto the C57BL/6J mice from the University of Texas, Southwestern breeding core colony (The Dutch-Belgian Fragile X Consortium, 1994). *Grm5* KO (mGluR5 KO) mice were obtained from Dr. Mark Bear (Massachusetts Institute of Technology, Cambridge, MA) but were made by another group (Lu et al., 1997). *Emx1* Cre mice were obtained from Dr. Takuji Iwasato and Dr. Shigeyoshi Itoharu (Riken BSI, Wako City, Saitama, Japan) (Iwasato et al., 2000, 2008) and *Dlx5/6* Cre mice were obtained from Dr. Marc Ekker and Dr. John Rubinstein (University of California, San Francisco, California) (Monory et al., 2006). Floxed *Fmr1* mice were obtained from Dr. David Nelson (Baylor College of Medicine, Houston, Texas) (Mientjes et al., 2006). All experiments were performed with littermate comparisons. Experimenters were blind to mouse genotype with respect to data depicted in Figures 1, 2, 5, and 6.

Slice preparation. Male mice, “3 weeks” of age [postnatal days 18 (P18)–P24] were deeply anesthetized with Euthasol (pentobarbital sodium and phenytoin sodium solution) and decapitated. The brain was transferred into ice-cold dissection buffer containing (in mM): 87 NaCl, 3 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 7 MgCl_2 , 0.5 CaCl_2 , 20 D-glucose, 75 sucrose, and 1.3 ascorbic acid aerating with 95% O_2 –5% CO_2 . Thalamocortical slices, 400 μm , were made on an angled block (Agmon and Connors, 1991) using a vibratome (Vibratome 1000 Plus). Following cutting, slices were transected parallel to the pia mater to remove the thalamus and midbrain. This transection was not done for the first experiment (see Fig. 1D,E and where indicated in corresponding text). Slices were immediately transferred to an interface recording chamber (Harvard Instruments) and allowed to recover for 1 h in nominal artificial CSF (ACSF) at 32°C containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 2 MgCl_2 , 2 CaCl_2 , and 25 D-glucose. After this, slices were perfused with a modified ACSF that

Figure 1. Spontaneously occurring persistent activity states, or UP states, are longer in layer 4 of *Fmr1* KO somatosensory cortical slices. **A**, Examples of extracellular multiunit recordings from WT and KO slices demonstrate longer UP states in the KO. The rectified and filtered versions of the traces from which the UP states were analyzed are plotted underneath. Dashed lines indicate shorter epochs of the longer traces which are expanded to show more detail. **B**, In some instances, UP states occurred in the form of closely spaced events, or bursts. These were considered one UP state (numbers 2 and 4 in **A** and **B**; see Materials and Methods). **C**, Autocorrelograms from the examples in **A** reveal some rhythmic activity, but in general it was weak (see Materials and Methods for details of autocorrelograms). **D**, Average UP state duration over all slices was higher in KO slices at 3 weeks of age. **E**, The average cumulative distribution of duration in a single slice (normalized for each slice and averaged over slices; same dataset as **C**; see Materials and Methods) was evenly shifted to the right for the KO. Outer dashed lines represent the SE. **F**, Average UP state duration was still longer at 7 weeks of age. **G**, UP state frequency was decreased and percentage of time in the UP state increased. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Calibrations: Vertical (in μV), raw traces, 20; rectified, 0.1. Horizontal (in ms), long traces, 1500; short traces, 300. Numbers inside bars indicate slice number in all figures except Figures 3 and 6D.

better mimics physiological ionic concentrations *in vivo* which contained (in mM): 126 NaCl, 5 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 1 MgCl_2 , 1 CaCl_2 , and 25 D-glucose (based on but modified from Sanchez-Vives and McCormick, 2000; Gibson et al., 2008). We used a slightly higher external K^+ concentration to promote active states (5 mM vs 3.5 mM *in vivo*), but this manipulation was probably unnecessary since the use of 3.5 mM external K^+ still results in spontaneously generated UP states (Rigas and Castro-Alamancos, 2007). Slices remained in this modified ACSF for 45 min and then recordings were performed with the same modified ACSF.

UP state recordings and analysis. Spontaneously generated UP states *in vitro* were extracellularly recorded using 0.5 M Ω tungsten microelectrodes (FHC) placed in layer 4 of primary somatosensory cortex. This extracellular monitoring of UP states is a reliable indicator of the synchronous, depolarized state of neuron populations from which the term “UP state” was originally defined (Sanchez-Vives and McCormick, 2000; Rigas and Castro-Alamancos, 2007). As indicated, some recordings were performed in layer 5. A total of 10 min of spontaneous activity was collected from each slice. For drug wash-on experiments, data were collected for 70 min. Recordings were amplified 10,000-fold, sampled at 2.5 kHz, and filtered on-line between 500 Hz and 3 kHz. All measurements were analyzed off-line using custom Labview software. For visualization and analysis of UP states, traces were offset to zero, rectified, and low-pass filtered with a 0.2 Hz cutoff frequency. Using these processed traces, the threshold for detection was set at $4\times$ the RMS (root mean square) noise ($5\times$ for picrotoxin + CGP55845 experiments; see Fig. 4), and an event was defined as an UP state if its amplitude remained above the threshold for at least 200 ms. The end of the UP state was determined when the amplitude decreased below threshold for >600 ms. Two events occurring within 600 ms of one another were grouped as a single UP state. These criteria best accounted for the simultaneous occurrence and identical durations of UP states in layers 4 and 5 (Fig. 2). Our main finding of longer UP states in *Fmr1* KO mice (Fig. 1) was not strictly dependent on these criteria since the same result was obtained with no grouping of events and with different detection thresholds. UP state amplitude was defined based on the filtered/rectified traces and was unitless since it was normalized to the detection threshold. This amplitude may be considered a coarse indicator of the underlying firing rates of neuronal populations. Direct measures of firing rates were not possible because individual spikes could not be isolated except during the quiet periods (the DOWN states). Data are represented by the mean $\pm$ SEM and values. Significant differences were determined using *t* tests, one-way ANOVA, two-way ANOVA, or three-way ANOVA where appropriate (all performed with GraphPad Prism 5 except for the three-way which was performed with Sigmaplot). Repeated-measures ANOVA was also used when appropriate. Bonferroni *post hoc* tests were performed following ANOVAs. Sample number (*n*) is slice or mouse number—the latter for *in vivo* experiments. For all slice experiments, a minimum of 4 mice were used per condition, and on average, 4 slices were examined per mouse.

For cumulative distributions of UP state durations, a normalized cumulative distribution was obtained for each experiment (data from one slice or for *in vivo* experiments, from one mouse), where *y* values were interpolated for predetermined points of the *x*-axis. This enabled the calculation of an average and SD based on experiment, where sample number refers to either slice number or mouse number (the latter for *in vivo*). A Kolmogorov–Smirnov test was performed for statistical analysis. This method equalizes the contribution to the distribution made by each experiment.

Autocorrelations were performed on rectified and filtered traces for 300 and 60 s epochs for slices and *in vivo* recordings, respectively. Autocorrelations were normalized to the variance resulting in a peak of 1 at time 0. To measure rhythmicity, we measured the side-peaks of the autocorrelogram by averaging the two peak-to-trough distances from each side of the peak.

Group I mGluR antagonist and protein synthesis inhibitor pretreatment. As stated above, slices underwent an initial 1 h incubation in nominal ACSF. Like other experiments, slices were then exposed to the “modified” ACSF for the next 45 min, but in experiments using mGluR antagonists, this latter period included the pretreatment with the antagonist in the ACSF. In experiments inhibiting protein synthesis, this latter period included pretreatment with anisomycin (20 μM). The antagonist or ani-

Figure 2. Spontaneously occurring UP states are longer in layer 5. **A**, Examples of simultaneous multiunit recordings of layers 4 (L4) and 5 (L5) in a WT and a KO slice. **B**, The longer duration phenotype in *Fmr1* KO slices also exists to the same extent in both layers 4 and 5. **C**, Scatter plots depicting UP state durations measured in a single experiment from a WT and a KO slice. Each point represents the duration of a single UP state measured simultaneously in layer 4 and 5. Note the strong correlation. **D**, A picture depicting a slice that was used for recording from deep neocortical layers in isolation. Gray shading indicates regions removed. **E**, UP states recorded in layer 5 of slices containing only deep layers. **F**, Isolated deep layers of neocortex display longer UP states in *Fmr1* KO slices. $*p < 0.05$, $***p < 0.001$. Calibrations: (**A**) 30 μV , 5 s; (**E**) 30 μV , 1 s.

somylin remained in the ACSF for the remainder of the experiment—including the recording periods. The pretreatment experimental design, as opposed to application while recording, enabled us to examine many more slices in a single experiment since slices were stored and recorded from the same chamber (see Slice preparation above). More recordings meant that we obtained more accurate UP state duration values (which varied a few hundred milliseconds from one experiment to the next) and meant that we could more readily detect 35–50% changes in UP state

duration among the 8 groups compared in the mGluR antagonist experiments (see Fig. 7B). The preincubation time was selected because previous studies have shown the mGluR-regulated protein synthesis has electrophysiological effects on this time scale. The mGluR antagonists were the mGluR5-selective antagonist, MPEP (10 μ M), and the mGluR1-selective antagonist, LY367385 (100 μ M).

Recordings in vivo. Three- to 4-week-old mice were anesthetized with urethane (1.5 g/kg, 20% solution in distilled water) supplemented with isoflurane (1.5%, 1.5 L/min O₂ flow) and placed in a stereotaxic apparatus with anesthesia maintained. A scalp incision was performed and a craniotomy performed at 2 mm posterior to bregma and 2.5 mm lateral to midline. An extracellular recording electrode (same as described for *in vitro*) was inserted into the craniotomy and advanced to approximately layer 4 and layer 5 of cortex as confirmed with lesions observed after brain fixation. At this point, isoflurane application was stopped, and recordings were performed for 20–30 min. Health and depth of anesthesia were monitored by body temperature, heart rate, respiratory rate, tail pinch reflex, and by the clear occurrence of cycling between UP and DOWN states. Any indication of a lighter anesthesia plane resulted in additional injection of urethane. Immediately after recording, the mouse was killed with an overdose of Euthasol (pentobarbital sodium and phenytoin sodium solution).

Immunohistochemistry. Immunohistochemistry was performed on progeny of Cre (*Emx1* and *Dlx5/6* Cre) and floxed *Fmr1* mice to confirm cell type-specific deletion of *Fmr1*. Mice (P21–P24) were perfused with cold PBS followed by 4% paraformaldehyde (w/v). The brain was removed and resectioned into 80 μ m slices. Antigen retrieval was performed by placing the slices into warm 200 mM sodium citrate and microwaving for 1 min at low power. The slices were blocked for 1 h at room temperature in PBS with 3% normal goat serum and 0.5% Triton-X. Primary antibodies were dissolved in blocking solution and applied overnight at 4°C. Secondary antibodies were dissolved in blocking solution and applied for 1 h at room temperature. Primary antibodies used were mouse anti-FMRP (2F5, 1:200, gift from Dr. Jennifer Darnell, Rockefeller University, New York, NY) and rabbit anti-GABA (A2052, 1:1000, Sigma). The specificity of 2F5 has been demonstrated previously (Gabel et al., 2004) and by our comparison of labeling in *Fmr1* KO versus WT tissue. Specificity of A2052 has been demonstrated previously (Stevens et al., 2010) and in this study, its labeling was consistent with our use of the *DLX5/6* Cre mouse which is GABAergic neuron specific (see Fig. 5; FMRP was deleted only in GABAergic neurons) (Monory et al., 2006). Secondary antibodies used were goat anti-mouse 555 and goat anti-rabbit 488 (1:250, Sigma).

Reagents. Drugs were prepared as stocks and stored at –20°C and used within 2 weeks. The mGluR1-selective antagonist (S)-(+)- α -amino-4-carboxy-2-methylbenzeneacetic acid (LY367385, 100 μ M), the mGluR5-selective antagonist MPEP (10 μ M), the protein translation inhibitor, anisomycin (20 μ M), the GABA_B receptor antagonist, CGP55845 (1 μ M), and the mixed group I mGluR agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG, 10 μ M) were purchased from Tocris Bioscience (Ellisville, MO). The GABA_A receptor antagonist, picrotoxin (100 μ M), was purchased from Sigma. Drugs required 12–18 min upon start of application to reach and perfuse the slice at the steady-state concentration due to delays in the perfusion system.

Results

Spontaneously occurring UP states are longer in layer 4 of *Fmr1* KO slices

Spontaneously generated active states, or UP states, were measured in acute neocortical slices obtained from 3- to 4-week-old WT and *Fmr1* KO mice. UP states were measured with extracellular, multiunit recordings in layer 4, and then analyzed after rectification and low-pass filtering of the recorded traces (Fig. 1A; see Materials and Methods). Activity in both WT and KO slices sometimes occurred in closely associated bursts that we grouped together as a single UP state in our analysis (Fig. 1A₂, B₄; see Materials and Methods). UP states in some slice recordings were not observably rhythmic, but in other recordings, were

weakly rhythmic. This weak rhythmicity was observed by autocorrelograms of the rectified, filtered traces (Fig. 1C).

Similar to thalamically evoked activity states (Gibson et al., 2008), the duration of spontaneously occurring UP states observed in *Fmr1* KO slices was increased by 59% compared with WT (Fig. 1D, E; 777 $\pm$ 39 vs 1236 $\pm$ 85 ms, p < 0.001; WT, KO; n = 18, 18 slices). While this analysis was performed by detecting discrete UP states (see Materials and Methods), the altered spontaneous activity could also be observed by another independent method that did not rely on our detection of UP states—the autocorrelation of the rectified, filtered traces. The width at half-height of the autocorrelation function was 54% larger for traces obtained from KO slices (Fig. 1C; 302 $\pm$ 25 vs 466 $\pm$ 50 ms, p < 0.05; WT, KO), consistent with the longer duration UP states in the KO. Slices in these initial experiments contained the thalamus and its connections with neocortex, and therefore it was possible that the thalamus might be playing a role in the longer UP states. This was not the case since all successive experiments were performed with all subcortical regions removed, and UP states were still longer to the same extent (see controls in Figs. 5–8). The abnormally long UP states persist at later ages (7 weeks) since KO slices obtained from older animals, 7–8 weeks of age, also had longer UP states although the difference was not as pronounced (Fig. 1F; 489 $\pm$ 29 vs 640 $\pm$ 38 ms, p < 0.01; WT, KO; n = 16, 19 slices).

We examined other UP state characteristics in 3-week-old slices by adding control data from another experiment (see controls in Fig. 6; n = 46, 41). In addition to duration being similarly longer in this larger dataset (67% increase; 761 $\pm$ 37 vs 1274 $\pm$ 57 Hz, p < 0.001; WT, KO), we were able to detect an 18% decrease in UP state frequency (Fig. 1G; 0.185 $\pm$ 0.011 vs 0.151 $\pm$ 0.013 Hz, p < 0.05; WT, KO) and a 35% increase in the percentage of time spent in the UP state (13.5 $\pm$ 0.8 vs 18.2 $\pm$ 1.1%, p < 0.001; WT, KO). The number of bursts within an UP state was also increased by 29% (1.68 $\pm$ 0.04 vs 2.16 $\pm$ 0.09 bursts, p < 0.001; WT, KO), but this did not completely account for the 67% increase in duration. We detected no change in the normalized amplitude of UP states as defined by our rectified, filtered traces (5.4 $\pm$ 0.4 vs 6.4 $\pm$ 0.5, p = 0.08; WT, KO; see Materials and Methods) which may reflect no change in the underlying intensity of action potential firing that occurs during UP states. In summary, while a number of characteristics of UP states may be altered in *Fmr1* KO slices, the duration increase was the most salient.

UP states are longer in layer 5

On the scale of seconds, UP states occur approximately simultaneously in all layers in a local neocortical circuit, but on the scale of 10–150 ms, differences in onset and duration across layers are observed (Sanchez-Vives and McCormick, 2000; Chauvette et al., 2010). Therefore, it was not clear whether the longer UP states we observed in layer 4 of *Fmr1* KO slices represented a more general phenomenon across all layers. With all the layer 4 recordings depicted in Figure 1, we simultaneously recorded from layer 5 (Fig. 2A, n = 35, 39). Average UP state duration in layer 5 was not different from that recorded in layer 4 in the same slice. Also, layer 5 UP state duration was longer in *Fmr1* KO compared with WT slices (Fig. 2B, 756 $\pm$ 34 vs 1233 $\pm$ 83 ms, p < 0.001; WT, KO). UP states in a single slice vary in duration, and the length of a single UP state in one layer was strongly correlated with the length in the other layer when examining the interlayer correlation of UP state durations in a single experiment (Fig. 2C; correlation for durations, WT: r = 0.83 $\pm$ 0.07; KO: r = 0.84 $\pm$ 0.03; n = 6, 8). There was spontaneous unit activity during the quiet states (or DOWN states) in layer 5 as previously reported

Figure 3. UP states are longer in *Fmr1* KO mice, *in vivo*. **A**, Examples of multiunit recordings from single WT and KO mice, *in vivo*. **B**, Average duration of UP states is longer in *Fmr1* KO mice (numbers inside bars indicate mouse number). An average cumulative histogram (right) indicates an even shift in durations across all animals (normalized for each mouse and averaged over mice, same dataset as bar graph to the left; see Materials and Methods). Outer dashed lines are SE. **C**, The frequency of UP states was not detectably different. **D**, Autocorrelations of the filtered, rectified traces depicted in **A** reveal a clear rhythmic component of the activity probably reflecting the slow oscillation (see Materials and Methods for details of autocorrelograms). Examples of first and second side-peaks are marked by arrows. * $p < 0.05$. Calibrations: 50 μ V, 3 s.

(Sanchez-Vives and McCormick, 2000), and we observed this in both WT and KO slices with no detected differences in spike frequency (Fig. 2A; 0.92 ± 0.12 vs 0.96 ± 0.09 Hz; WT,KO; $n = 16,16$).

A cross-correlation analysis of the rectified/filtered traces (not durations as in Fig. 2C) was performed on layer 4 and 5 recordings for each slice, and this indicated a high average correlation ($r = 0.88 \pm 0.02$ vs 0.92 ± 0.01 , $p < 0.05$; WT, KO) and a slight delay in the layer 4 signals relative to layer 5 (12.7 ± 1.4 vs 13.7 ± 1.5 ms; WT,KO). The delay is consistent with earlier studies indicating that UP states are initiated in layer 5 (Sanchez-Vives and McCormick, 2000). While these data show an increase in correlation in the KO slices, this difference was small. When examining the raw traces from layer 4 and 5, very little correlation was observed suggesting that while the underlying UP state event is synchronous, the precise firing of neurons in layer 4 and 5 is, in general, not synchronous. In summary, while UP states are longer in both layers 4 and 5 of *Fmr1* KO slices, the relative onset and synchrony of UP states in the two layers appear to be normal.

It has been demonstrated that the deep layers (layers 5 and 6), and not the superficial layers, are sufficient to generate UP states (Sanchez-Vives and McCormick, 2000). To determine whether the longer UP states are intrinsic to particular cortical layers, we

repeated layer 5 recordings from slices containing only the deep layers of neocortex. Slices were resectioned by removal of superficial cortex (layers 1–4, Fig. 2D). UP state duration in both WT and *Fmr1* KO resectioned slices was slightly decreased compared with normal slices but UP state frequency remained unchanged (~ 0.16 Hz) suggesting that these slices were still healthy. Resectioned KO slices still had longer UP states in layer 5 (Fig. 2E,F; 549 ± 27 vs 733 ± 55 ms; WT,KO; $n = 22,22$). This indicates that longer UP states in layer 5 of the *Fmr1* KO are, to a large extent, intrinsic to deep layer circuitry.

Rhythmically occurring UP states are longer in *Fmr1* KO mice, *in vivo*

To determine whether the longer UP states we observe in *Fmr1* KO slices were a physiologically relevant phenomenon, we recorded UP states in somatosensory cortex *in vivo* in anesthetized mice. As in the acute slices, UP states were indeed longer in *Fmr1* KO mice and had similar durations as those observed in acute slices (Fig. 3A,B; 765 ± 112 vs 1130 ± 112 ms, $p < 0.05$; WT,KO; $n = 9,11$ mice). We observed no change in the frequency of UP states (Fig. 3C; 0.26 ± 0.03 vs 0.25 ± 0.03 Hz; WT,KO) which is consistent with our *in vitro* findings where the frequency change was less salient than the duration change. Other parameters were unchanged in the KO, such as the intensity of UP state neuronal firing as defined by our rectified, filtered traces (16.0 ± 3.3 vs 14.6 ± 2.0 , WT,KO, unitless, see Materials and Methods). These results demonstrate that our observations *in vitro* reflect an existing condition *in vivo*.

The UP states *in vivo* probably occurred as part of the slow oscillation which has been previously been clearly observed in anesthetized cats (Steriade et al., 1993a,b) and mice (Fellin et al., 2009). Our recordings contained a distinct rhythmic component which was < 1 Hz, and therefore reminiscent of the slow oscillation. This can be observed in the autocorrelogram of the rectified, filtered traces (Fig. 3D). When we quantified the consistency of the rhythm in our recordings, we found that UP states *in vivo* were more rhythmic compared with those measured in slices. This was first evident by comparing autocorrelograms of the rectified/filtered traces. The first side-peak of the autocorrelogram was larger from recordings *in vivo* ($0.30 \pm 0.09/0.41 \pm 0.11$; WT/KO; $n = 9,11$) compared with slices ($0.14 \pm 0.07/0.13 \pm 0.07$; WT/KO; $n = 18,19$; two-way ANOVA, $p < 0.001$ for preparation type). The same result was observed for the second, more distant, side-peak as well ($0.30 \pm 0.09/0.38 \pm 0.11$ vs $0.15 \pm 0.05/0.15 \pm 0.05$; *in vivo* vs slice; $p < 0.001$). Side-peaks always occurred in increments > 1 s consistent with the frequency of the slow oscillation. We also measured the ratio of the SD to the average period length for each experiment, and this was lower *in vivo* indicating again that UP state reoccurrence was more regular, and hence more rhythmic ($0.41 \pm 0.14/$

0.35 ± 0.18 vs $0.59 \pm 0.12/0.57 \pm 0.14$; *in vivo* vs slice; $p < 0.05$, two-way ANOVA). Finally, if UP states were occurring randomly and not rhythmically, the period length distribution would be in the form of a decaying exponential (like the classical theory of mEPs) (Johnston and Wu, 1995). *In vivo*, this was not the case since the average distribution of periods was a two-tailed, single peaked function and not a decaying exponential (data not shown). This, again, was less clear in slices where the WT distribution for periods appeared more weakly two-tailed, single-peaked, but for the KO, neither a two-tailed or decaying exponential distribution could be resolved. In summary, our *in vivo* data suggest that the UP state portion of the slow oscillation is lengthened in the *Fmr1* KO mouse.

Changes in excitatory circuitry likely contribute to longer UP states

To begin to understand the locus of change that causes prolonged UP states in *Fmr1* KO mice, we next tested whether neocortical excitatory circuitry alone displays properties that would promote longer activity states. We isolated the role of excitatory circuitry in generating spontaneously active states by pharmacologically blocking GABA_A and GABA_B receptors using the antagonists picrotoxin (100 μ M) and CGP55845 (1 μ M), respectively. Block of these GABAergic receptors resulted in periodic epochs of persistent activity that were different from normal UP states in both duration and shape (Fig. 4). These active states were longer in the *Fmr1* KO (Fig. 4A,B; 5.2 ± 0.5 vs 12.1 ± 1.3 s, $p < 0.01$; WT,KO; $n = 17,16$), suggesting that alterations occurring in excitatory circuitry promote longer activity states. Neither the number of bursts per active state nor the frequency at which active states occurred were detectably different in the KO in the presence of GABA receptor antagonists (bursts/active: 5.4 ± 1.0 vs 9.0 ± 1.5 , $p = 0.06$; frequency: 0.026 ± 0.004 vs 0.022 ± 0.002 Hz, $p = 0.44$; WT,KO), but the antagonist treatment itself resulted in more bursts per active state ($p < 0.01$) and a much lower frequency of occurrence ($\sim 15\%$ of untreated, $p < 0.001$) when compared with untreated slices in Figure 1G. Treatment had no detectable effect on percentage of time in an active state but did increase normalized amplitude for both genotypes ($\sim 30\%$, $p < 0.05$). Additional experiments implementing GABA_A receptor block alone (picrotoxin, 100 μ M) also resulted in longer active states in *Fmr1* KO slices (2.0 ± 0.2 vs 2.8 ± 0.3 s, $p < 0.05$; WT,KO; $n = 15,14$) indicating that changes in GABA_A signaling alone did not play a significant role in longer UP states. In summary, these data support the assertion that alterations in the recurrent excitation process among excitatory neurons play a significant role in prolonged UP state duration in the *Fmr1* KO mouse.

Blocking GABAergic synapses in hippocampal slices causes a slowly occurring induction of very long persistent activity states in the CA3 region—but only in *Fmr1* KO slices and not WT slices (Chuang et al., 2005). This plasticity of cell excitability requires group I mGluR activation. The long activity states in CA3 occur intermittently among very short activity states, and hence, the distribution of durations is bimodal. In a subset of slices in this study, neocortical UP states were measured before and during

Figure 4. Pharmacologically isolated excitatory circuitry displays longer persistent activity bursts in *Fmr1* KO slices. **A**, Traces of prolonged bursts of activity collected during application of the GABA_A antagonist, picrotoxin, and the GABA_B receptor antagonist, CGP55845. **B**, The average duration of the bursts was longer in *Fmr1* KO slices. **C**, The time course of the effect of GABA receptor blockade during 2 individual experiments. Antagonists are actually in the bath solution at time 0, but takes 12–18 min to reach the slice. Therefore, the upper bars depict the estimated time that the antagonists reach the slice. **D**, Average time course of the effects of GABA receptor blockade. The onset of the effects is not as sharp as individual examples in **C** because of variability in the time that the antagonists reach the slice preparation. The sample number in **D** is less than that in **B** because only a subset of the slices were monitored before and after antagonist application. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. In **D**, repeated-measures ANOVA and Bonferroni corrections were used. Calibrations: 50 μ V, 5 s.

picrotoxin + CGP55845 application (Fig. 4C,D; $n = 10,10$) and before and during picrotoxin application alone (data not shown, $n = 7,6$). In both sets of experiments, drug application quickly lengthened UP state duration in both WT and KO slices to the same extent and after 60 min, the distribution of durations in a single experiment were unimodal in distribution. Therefore, our data demonstrate that the effects of inhibitory blockade on network activity states in *Fmr1* KO slices are different in neocortex compared with those observed previously in the hippocampus.

Longer UP states are due to deletion of *Fmr1* in cortical excitatory neurons

Next, we determined the relevant locus of *Fmr1* deletion that causes longer UP states in *Fmr1* KO mice. To address this question, we compared the effects of *Fmr1* deletion in excitatory neurons versus inhibitory neurons.

To perform excitatory neuron-specific deletion, we crossed *Emx1* Cre males with floxed *Fmr1* females. In the *Emx1* Cre mouse line, Cre is expressed only in cortical excitatory neurons (i.e., neocortex and hippocampus) (Iwasato et al., 2000, 2008). Using immunohistochemistry, we confirmed that Cre⁺:*Fmr1*^{flox/Y} progeny had *Fmr1* deleted only in cortical excitatory neurons while other genotypes showed no *Fmr1* deletion (Fig. 5A). In control mice (Cre⁺:*Fmr1*^{+/Y}, Cre⁺:*Fmr1*^{+/Y}, Cre⁺:*Fmr1*^{flox/Y}), 96% of GABAergic neurons were FMRP+ while 15% of FMRP+ neurons were GABAergic. Since GABAergic and glutamatergic neurons comprise 15% and 85% of all neurons in neocortex, respectively (Gonchar and Burkhalter, 1997), these immunohistochemical data are consistent with FMRP expression in both excitatory and inhibitory neurons. In mice undergoing recombination (Cre⁺:*Fmr1*^{flox/Y}), 98% of GABAergic neurons were FMRP+ and 97% of FMRP+ neurons were GABAergic. In other words, with recombination, only the GABAergic neurons were FMRP+ while the neocortical excitatory glutamatergic neurons, by inference, were all FMRP-. FMRP expression in other

Figure 5. UP states are longer due to deletion of *Fmr1* in neocortical excitatory neurons. **A**, Immunohistochemistry for GABA (green) and FMRP (red) shows that FMRP is expressed in both inhibitory and excitatory neurons in control animals (top row), FMRP is deleted only in excitatory neurons when recombination occurs in *Emx1* Cre^{+/+}:floxed *Fmr1* mice (middle row), and FMRP is deleted only in inhibitory neurons when recombination occurs in *Dlx5/6* Cre^{+/+}:floxed *Fmr1* mice (bottom row). **B**, UP state duration is longer in slices where FMRP was deleted only in cortical excitatory neurons (black bar), thereby recapitulating the phenotype of the constitutive *Fmr1* KO. The large triangular bar indicates that the “recombined” genotype is different from all of the 3 nonrecombinant controls. **C**, Deletion of FMRP in inhibitory neurons did not affect UP state duration (black bar). ****p* < 0.001. n.s., not significant. Calibrations: (**B**, **C**) 50 μ V, 1 s.

noncortical structures, such as striatum and thalamus, was normal. When we examined UP states among the 4 possible genotypic combinations, we found that slices obtained from mice with deletion of *Fmr1* in cortical excitatory neurons was sufficient to mimic the long UP states of *Fmr1* KO mice (Fig. 5B; Cre^{-/-}:*Fmr1*^{+/Y} = 986 $\pm$ 56, Cre^{+/-}:*Fmr1*^{+/Y} = 939 $\pm$ 39, Cre^{-/-}:

Fmr1^{flox/Y} = 776 $\pm$ 74, Cre^{+/+}:*Fmr1*^{flox/Y} = 1398 $\pm$ 83 ms; *p* < 0.001 comparing Cre^{+/+}:*Fmr1*^{flox/Y} with any other genotype; *n* = 20, 22, 19, 19). No change in UP state frequency was detected in any genotype.

Next we examined the role of *Fmr1* deletion from inhibitory neurons. We crossed *Dlx5/6* Cre males with floxed *Fmr1* females. In the *Dlx5/6* Cre mouse line, Cre is expressed in GABAergic neurons in the forebrain, and occasionally, in some excitatory neurons in some regions of more caudoventral neocortex (Dr. John Rubenstein, personal communication; Monory et al., 2006). But somatosensory cortex is dorsal, and therefore Cre expression should be restricted to GABAergic neurons. This was confirmed by immunohistochemistry. In control mice (Cre^{-/-}:*Fmr1*^{+/Y}, Cre^{+/+}:*Fmr1*^{+/Y}, Cre^{-/-}:*Fmr1*^{flox/Y}), 99% of GABAergic neurons were FMRP+ while 14% of FMRP+ neurons were GABAergic—again consistent with expression of FMRP in both excitatory and inhibitory neurons. In mice undergoing recombination (Cre^{+/+}:*Fmr1*^{flox/Y}), 0% of GABAergic neurons were FMRP+ and 0% of FMRP+ neurons were GABAergic, confirming that FMRP was deleted from all GABAergic neurons (Fig. 5A). Unlike that found for deletion of *Fmr1* in cortical excitatory neurons, we found that deletion of *Fmr1* in inhibitory neurons had no effect on UP state duration (Fig. 5C; Cre^{-/-}:*Fmr1*^{+/Y} = 729 $\pm$ 50, Cre^{+/+}:*Fmr1*^{+/Y} = 776 $\pm$ 32, Cre^{-/-}:*Fmr1*^{flox/Y} = 811 $\pm$ 49, Cre^{+/+}:*Fmr1*^{flox/Y} = 791 $\pm$ 56 ms; *n* = 20, 19, 21, 22). Therefore, the longer UP states in the *Fmr1* KO are induced by deletion of *Fmr1* in cortical excitatory neurons.

Longer UP states are rescued by genetic reduction mGluR5 signaling

Ample evidence suggests that many phenotypes in *Fmr1* KO mice are due to excess group 1 metabotropic glutamate receptor (mGluR1 or mGluR5) signaling—specifically that mediated by mGluR5 (Huber et al., 2002; McBride et al., 2005; Yan et al., 2005; Dölen et al., 2007; Meredith et al., 2011). To test whether excessive activation of mGluR5 contributes to the longer UP states, we bred mGluR5 (*Grm5*) KO mice with *Fmr1* KO mice to produce *Grm5* heterozygous progeny, which has previously been shown to decrease mGluR5 protein to 60% of normal levels, and thereby presumably decreasing the signaling mediated by this receptor (Dölen et al., 2007). Using this strategy, we compared UP state duration between WT and *Fmr1* KO mice on both a *Grm5* WT background and on a *Grm5* heterozygous background. UP states measured on the *Grm5* heterozygous background (*Fmr1* KO/*Grm5* Het mice) had normal duration in KO slices (Fig.

Figure 6. Genetic reduction of mGluR5 protein rescues UP state duration in *Fmr1* KO slices. **A, B**, Examples of traces from WT and *Fmr1* KO slices (**A**) and mice (**B**, *in vivo*) on a WT *Grm5* background (top two traces) and on a heterozygous *Grm5* background (bottom two traces). **C, D**, Combination of *Fmr1* KO and *Grm5* heterozygosity (gray) restores UP state duration to WT levels. *Grm5* heterozygosity on a WT background has no effect on UP state duration. The large triangular bar indicates that the KO duration is different from that of both WT and KO on the *Grm5* heterozygous background. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Calibrations: 50 μ V, 1 s.

6A,C; 683 ± 57 vs 1310 ± 64 ms, $p < 0.001$, $n = 29,23$ for WT,KO; *Fmr1*KO/*Grm5*Het = 785 ± 67 ms, $p < 0.01$, $n = 21$, when compared with *Fmr1* KO) and in KO mice, *in vivo* (Fig. 6B,D; 579 ± 74 vs 1205 ± 232 ms, $p < 0.01$, $n = 6,4$ for WT,KO; *Fmr1*KO/*Grm5*Het = 785 ± 67 ms, $p < 0.05$, $n = 7$, when compared with *Fmr1* KO). This indicated that a reduction of mGluR5 levels in the *Fmr1* KO normalizes, or rescues, UP state duration. The reduction of mGluR5 dosage on a WT background does not cause any change in UP state duration in both slices (*Grm5*het: 828 ± 45 ms, $n = 16$) and *in vivo* (*Grm5*het: 822 ± 46 ms, $n = 10$), indicating that a reduction of mGluR5 levels alone does not affect UP states. No differences in amplitude or frequency of UP states were detected across genotypes. Together, these findings implicate enhanced mGluR5 activation in contributing to longer UP states in neocortical circuits of *Fmr1* KO mice.

Longer UP states are rescued by pharmacological blockade of mGluR5 signaling

To determine whether group I mGluRs play an acute, as opposed to a developmental, role in determining UP state duration, we examined the effects of specific group I mGluR antagonists on UP states in WT and *Fmr1* KO slices. Our strategy incorporated a 3 factor design based on 1) genotype, 2) pretreatment with the mGluR5-selective antagonist, MPEP (10 μ M), and 3) pretreatment with mGluR1-selective antagonist, LY367385 (100 μ M). This resulted in a comparison of UP state duration among 8 different groups (Fig. 7A,B). We measured UP states after 45 min of pretreatment and with drug still present. We found that MPEP, but not LY367385, had a differential effect on UP state duration which indicated that mGluR5 signaling, but not mGluR1 signaling, was acutely altered in *Fmr1* KO slices. This was observed by three-

Figure 7. Pretreatment with an mGluR5 antagonist rescues UP state duration in *Fmr1* KO slices. **A**, Examples of multiunit recordings from untreated slices and from slices after 45 min preincubation with an mGluR5 selective antagonist (MPEP, 10 μ M) or an mGluR1 selective antagonist (LY367385, 100 μ M). **B**, Summary of the effects of pretreatment on UP state duration. MPEP rescued the duration phenotype by equalizing the WT and KO UP state durations to normal WT levels. In contrast, while LY367385 significantly decreased duration in both WT and KO slices, UP states were still longer in the KO. The large triangular bar indicates that the untreated KO duration is different from that of both MPEP-treated genotypes. ** $p < 0.01$, *** $p < 0.001$. n.s., not significant. Calibrations: 50 μ V, 1 s.

way ANOVA analysis of duration data, which revealed a significant interaction between MPEP treatment and genotype ($p < 0.05$) while no interaction was detected between LY treatment and genotype.

Comparisons between individual groups (Bonferroni *post hoc* tests) also supported differential acute mGluR5 signaling. While untreated WT and KO slices had different durations (Fig. 7B; 1038 ± 59 vs 1428 ± 63 ms, $p < 0.001$, $n = 42,38$), no difference was observed for MPEP-treated WT and KO slices (935 ± 54 vs 1076 ± 63 ms, $n = 32,44$). These same data indicated that MPEP had no detectable effect on UP state duration in WT slices, but decreased UP state duration in KO slices ($p < 0.001$). No changes in frequency of UP states were detected with MPEP treatment. Therefore, while mGluR5 signaling does not contribute to UP state duration in WT slices, it does contribute to prolonged duration in KO slices. In summary, these data show that the longer UP state duration phenotype in KO slices is “rescued” to normal WT durations by acutely blocking mGluR5.

In contrast to MPEP, the selective blockade of mGluR1 signaling with LY367385 had a strong but similar effect on both WT and KO slices. For both genotypes, UP state duration, UP state frequency, and the incidence of slices displaying UP states were reduced by ~40–60% ($p < 0.05$ for all). However, duration was still longer in the KO (Fig. 7B; LY367385 results: 456 ± 26 and 720 ± 72 ms, $p < 0.05$, $n = 21,22$). Therefore, mGluR1 function strongly regulates UP states, but does so similarly in both WT and *Fmr1* KO slices.

Longer UP states do not depend on recent protein translation

One function of FMRP is to suppress translation of its mRNA targets (Bassell and Warren, 2008). Therefore, it has been pro-

Figure 8. Pretreatment with a protein translation inhibitor has no effect on WT or *Fmr1* KO UP state duration. **A**, Examples of multiunit recordings from untreated slices and from slices after 45 min preincubation with anisomycin (20 μ M). **B**, While UP state duration was longer in KO slices, durations were not affected by anisomycin treatment. $**p < 0.01$. Calibrations: 50 μ V, 1 s.

posed that without FMRP, as in fragile X syndrome, there is excess mGluR5 driven translation which leads to phenotypes of the disease (Bear et al., 2004; Dölen and Bear, 2008). In support of this idea, mGluR5- and translation-dependent plasticity is up-regulated in *Fmr1* KO mice (Huber et al., 2002; Bear et al., 2004; Chuang et al., 2004). Therefore, it is possible that mGluR5 signaling causes longer UP states in KO slices through signaling to translation. If so, inhibiting protein translation would be expected equalize UP state duration among WT and KO slices similar to MPEP. We preincubated slices in the translational inhibitor, anisomycin (20 μ M) for 45 min before and during recording. This treatment had no effect on UP state duration in either WT or KO slices (Fig. 8), suggesting that at the time scale studied here, longer UP states in *Fmr1* KO mice are not due to mGluR5-dependent protein synthesis, but instead due to a translation independent signaling function of mGluR5.

Group I mGluR activation increases UP state duration nondifferentially

In support of the mGluR antagonist studies above, pharmacological enhancement of group I mGluR activity was sufficient to prolong UP state duration in WT and *Fmr1* KO slices. Application of the group I agonist, DHPG (10 μ M), increased the duration of UP states in WT slices (Fig. 9A,B; WT untreated: 671.7 ± 103.2 ms; WT+DHPG 826.8 ± 131.4 ms; $p < 0.01$; $n = 6$) and

Figure 9. A group I mGluR agonist lengthens UP state duration in both WT and KO slices. **A**, UP state duration plotted as a function of time for 1 WT and 1 KO experiment where DHPG was applied. DHPG is added to ACSF at the 5 min time point, but the bars above the graphs depict the estimated time that DHPG reaches the slice (same applies to **B**). Trendline indicates time course average. **B**, Average time course for DHPG effect on UP state duration over all experiments plotted with raw durations (left) and durations normalized to the 0 min time point (right). $*p < 0.05$, $**p < 0.01$. p -values refer to an effect on duration by time (repeated-measures ANOVA).

Fmr1 KO slices (Fig. 9A,B; *Fmr1* KO untreated: 1361 ± 141.1 ms; WT+DHPG: 1639 ± 134.4 ms; $p < 0.05$; $n = 7$). The increase in duration for both genotypes were proportionally identical (Fig. 9B), and based on the nondifferential affect of mGluR1 antagonists in Figure 7, these similar effects of DHPG may be acting through mGluR1 receptors.

We monitored UP states before and after DHPG application in these experiments since, in previous studies, DHPG caused normally quiet hippocampal CA3 region slices to display very long activity bursts interspersed with shorter bursts (bimodally distributed peaks at 0.5 s and at 4–10 s) (Taylor et al., 1995; Chuang et al., 2005). We did not observe this type of change in our neocortical slice preparation (Fig. 9A) indicating that different group I mGluR-regulated processes are occurring in neocortex.

Discussion

Little is known about circuit dysfunction in *Fmr1* KO mice, or for that matter, in any mouse model of intellectual disability or autism. In this study, we find that spontaneously occurring UP states are longer in the mouse model of fragile X syndrome—the *Fmr1* KO mouse. UP states were 62% longer in slices (combining data from Figs. 1C, 6–8) and 67% longer *in vivo* (combining data from Figs. 3, 6)—the latter supporting the physiological relevance of this network functional change. Neocortical excitatory neurons are probably the most significant locus of these changes since, (1) electrophysiological changes causing the longer UP states are intrinsic to neocortex, (2) activity bursts mediated by excitatory circuitry alone are longer in *Fmr1* KO slices, and (3) the specific locus for *Fmr1* deletion that induces longer UP states is neocortical excit-

atory neurons. Finally, we find that enhanced mGluR5 signaling underlies the longer UP states in *Fmr1* KO slices.

Longer spontaneously occurring UP states in *Fmr1* KO mice

We have previously demonstrated that UP states in response to thalamic stimulation were longer in *Fmr1* KO mice (Gibson et al., 2008). However, it was not clear to what extent this resulted from alterations in thalamic circuitry since interactions between the thalamus and neocortex can affect UP states (Rigas and Castro-Alamancos, 2007; Crunelli and Hughes, 2010). Here we show that spontaneously occurring and rhythmic UP states are longer in the *Fmr1* KO mouse. In slices, these UP states are mediated by neocortical circuits, independent of any thalamic pathway stimulation, and independent of the thalamus itself. The results also indicate that longer thalamically evoked UP states in *Fmr1* KO slices are mostly likely due to changes in neocortex and not to changes in thalamic circuitry. Because spontaneously occurring UP states are thought to be the cellular process underlying the slow oscillation during sleep (Steriade et al., 1993c), our data more directly suggest that the increase in UP state duration and time spent in the UP state (Fig. 1) may impact processes involving the slow oscillation rhythm in fragile X patients. This possible link is strengthened by our observations of longer UP states *in vivo* which were observed with a clear rhythmic component reminiscent of the slow oscillation.

The synchrony of UP states among different neocortical areas relies on long distance projections, but the actual generation of UP states depends on “local” network function (~1 mm radius) since they directly rely on recurrent synaptic excitation and inhibition among neurons in a local neocortical region (Haider and McCormick, 2009). Therefore, the longer UP states in *Fmr1* KO mice are probably caused by network changes on this local scale, and more specifically, our study indicates that changes in local excitatory circuits likely play a significant role.

Comparison with prolonged epileptic bursts in CA3 of *Fmr1* KO mice

Properties of persistent activity states observed in the CA3 region of the hippocampus are also altered in *Fmr1* KO slices (Chuang et al., 2005). These activity states are not UP states, but instead are prolonged epileptic bursts which are induced experimentally by group I mGluR agonists in both WT and *Fmr1* KO slices (Taylor et al., 1995). Interestingly, they can also be induced with the application of a GABAergic antagonist which, in turn, activates group I mGluRs, but this method of induction only occurs in *Fmr1* KO slices, and not in WT slices (Chuang et al., 2005). Our observations of prolonged UP states may have a common link with prolonged bursts in CA3 since both involve prolonged activity states, both depend on changes in group I mGluR signaling, and both appear to be mediated by changes in excitatory neurons. Moreover, longer UP states in the neocortex (Fig. 7), as well as epileptic bursts in the hippocampal slices obtained from *Fmr1* KO mice, are both reduced to normal durations with mGluR5 antagonism (Chuang et al., 2005).

But our findings differ in some key aspects. First, while persistent activity states in hippocampal CA3 slices permit the effective study of the plasticity of neuronal excitability, they are epileptic in nature and require intense pharmacological manipulations for their induction. On the other hand, UP states are widely thought to underlie aspects of neocortical function under normal conditions. Also, they do not require any special induction protocol, but instead, occur spontaneously as a baseline process. Bursts in CA3 can be induced by GABAergic receptor blockade, but only in

Fmr1 KO slices, while GABAergic receptor blockade similarly increases UP state duration in both WT and KO neocortical slices (Fig. 4). Finally, CA3 bursts require new protein synthesis, whereas prolonged neocortical UP states persist independent of new protein synthesis (Fig. 8).

Together, our data and those of Chuang et al. (2005) reveal an mGluR5-mediated hyperexcitability of circuit function in *Fmr1* KO mice that is common to both hippocampus and neocortex. However, such circuit dysfunction is manifest differently in the two brain regions, perhaps due to the anatomy and physiology of the circuit or distinct function or localization of mGluR5 within each brain region.

Role of group I mGluRs

Our study indicates that enhanced mGluR5 signaling acutely leads to longer UP states in *Fmr1* KO slices. Enhanced mGluR5 signaling is not caused by increased mGluR5 protein in *Fmr1* KO mice since protein level is unchanged (Huber et al., 2002; Dölen et al., 2007). Instead it appears that signaling downstream of mGluR5 activation is enhanced, and while studies have focused on enhanced mGluR-induced protein translation in the *Fmr1* KO (Bear et al., 2004; Osterweil et al., 2010), our data support enhanced signaling independent of protein translation as well (Fig. 8). According to this interpretation of our results, the genetic reduction of mGluR5 protein levels restores normal UP state duration (Fig. 6) by offsetting the increased signaling of individual mGluR5 receptors. Unlike mGluR5 antagonism, mGluR1 antagonism robustly suppressed the duration and frequency of UP states to a similar extent in both WT and *Fmr1* KO slices (Fig. 7B). These data suggest that mGluR1 regulation of UP states is normal in *Fmr1* KO mice. mGluR1 and mGluR5 may regulate UP state duration through a number of mechanisms including modulation of the intrinsic excitability of neocortical neurons or synaptic function within the circuit (Kim et al., 2003; Young et al., 2004; Bianchi et al., 2009; Niswender and Conn, 2010). To our knowledge, this is the first indication for dysfunction of a specific group I mGluR (mGluR5, but not mGluR1) in *Fmr1* KO mice.

What currents mediate the longer UP states?

The detailed mechanism of longer UP states in the *Fmr1* KO mice is unknown. Changes in inhibitory and excitatory circuitry may be involved, but the fact that we saw longer active states when inhibitory synapses were blocked by a GABAergic antagonist (Fig. 4) suggests that a significant component underlying longer UP states in the *Fmr1* KO resides in excitatory circuitry. Measurements of monosynaptic excitatory transmission in acute slices indicate that synaptic excitation among neocortical excitatory neurons is either slightly decreased or unchanged (Desai et al., 2006; Bureau et al., 2008; Gibson et al., 2008), but other studies point to increased spine number in neocortical excitatory neurons suggesting increased excitation (Bagni and Greenough, 2005). Therefore, it is unclear the role excitatory synaptic currents play in promoting longer UP states in the *Fmr1* KO mouse. It is also possible that there is an alteration in a nonsynaptic current in excitatory neurons that promotes longer UP states (Brown et al., 2010; Strumbos et al., 2010). Based on our findings of differential regulation of UP states by mGluR5 (Figs. 6, 7), any underlying cellular current will be modulated differentially by mGluR5 when comparing WT and KO neurons. Studies examining single-cell persistent firing in cortical neurons have reported that mGluR5 activation promotes persistent firing through a nonsynaptic membrane current (Yoshida et al., 2008; Zhang and Séguéla, 2010).

Implications of longer UP states in *Fmr1* KO mice

Because *Fmr1* KO slices spend 35% more time in the UP state (Fig. 1D), they may be considered to be more active. This implies that the neocortical circuitry may be more excitable in general, thereby affecting neocortical function in many behavioral states. This is consistent with symptoms in fragile X patients such as overt epilepsy, EEG abnormalities suggestive of epilepsy, and hyper-responsiveness to sensory stimuli (Hagerman et al., 1991; Miller et al., 1999; Musumeci et al., 1999; Rojas et al., 2001; Berry-Kravis, 2002; Incorpora et al., 2002; Castrén et al., 2003; Frankland et al., 2004). It is also consistent with *Fmr1* KO mice having an increased propensity for audiogenic seizures (Chen and Toth, 2001; Nielsen et al., 2002; Spencer et al., 2006). Therefore, it is possible that the prolonged UP states themselves or the cellular mechanism that causes the longer UP states mediates the hyper-excitability in fragile X syndrome.

Thirty percent of children with FXS are diagnosed with autism and 1–2% of autistic children have FXS, making *Fmr1* one of the leading genetic causes of autism (Kaufmann et al., 2004; Hagerman et al., 2005). Because of this strong link to autism, it is intriguing that our findings are consistent with the hypothesis that hyperexcitability in neocortical circuits underlie autism (Rubenstein and Merzenich, 2003).

UP states have been demonstrated to underlie the slow oscillation, which is a neocortical rhythm (<1 Hz) that occurs during the deeper stages of slow-wave sleep and anesthesia (Steriade et al., 1993b; Steriade, 1997; Amzica and Steriade, 1998). The slow oscillation during slow-wave sleep has been proposed to be involved in long-term memory consolidation in neocortex (Marshall and Born, 2007; Crunelli and Hughes, 2010). In a previous study, we have also demonstrated that inhibition during UP states is less synchronous (Gibson et al., 2008). Therefore, it is possible that the prolonged UP states with less synchronous inhibition may modify the slow oscillation in fragile X syndrome which, in turn, could impact memory consolidation. Our study suggests closer scrutiny of the slow oscillation in fragile X patients is warranted, but to date, while circadian rhythm and sleep problems have been reported in both fragile X patients and *Fmr1* KO mice (Musumeci et al., 1991; Miano et al., 2008; Zhang et al., 2008; Kronk et al., 2010), no such data exist.

References

- Agmon A, Connors BW (1991) Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience* 41:365–379.
- Amzica F, Steriade M (1998) Cellular substrates and laminar profile of sleep K-complex. *Neuroscience* 82:671–686.
- Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci* 6:376–387.
- Bassell GJ, Warren ST (2008) Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron* 60:201–214.
- Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. *Trends Neurosci* 27:370–377.
- Berry-Kravis E (2002) Epilepsy in fragile X syndrome. *Dev Med Child Neurol* 44:724–728.
- Bianchi R, Chuang SC, Zhao W, Young SR, Wong RK (2009) Cellular plasticity for group I mGluR-mediated epileptogenesis. *J Neurosci* 29:3497–3507.
- Brennan FX, Albeck DS, Paylor R (2006) *Fmr1* knockout mice are impaired in a leverpress escape/avoidance task. *Genes Brain Behav* 5:467–471.
- Brown MR, Kronengold J, Gazula VR, Chen Y, Strumbos JG, Sigworth FJ, Navaratnam D, Kaczmarek LK (2010) Fragile X mental retardation protein controls gating of the sodium-activated potassium channel Slack. *Nat Neurosci* 13:819–821.
- Bureau I, Shepherd GM, Svoboda K (2008) Circuit and plasticity defects in the developing somatosensory cortex of FMR1 knock-out mice. *J Neurosci* 28:5178–5188.
- Castrén M, Pääkkönen A, Tarkka IM, Ryynänen M, Partanen J (2003) Augmentation of auditory N1 in children with fragile X syndrome. *Brain Topogr* 15:165–171.
- Chauvette S, Volgushev M, Timofeev I (2010) Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb Cortex* 20:2660–2674.
- Chen L, Toth M (2001) Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience* 103:1043–1050.
- Chuang S, Yan Q, Bauchwitz RP, Wong RK (2004) Synaptic activation of Gp I mGluRs is epileptogenic in hippocampal slices of FMRP knock-out mice. *Soc Neurosci Abstr* 30:228.5.
- Chuang SC, Zhao W, Bauchwitz R, Yan Q, Bianchi R, Wong RK (2005) Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. *J Neurosci* 25:8048–8055.
- Crunelli V, Hughes SW (2010) The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat Neurosci* 13:9–17.
- Desai NS, Casimiro TM, Gruber SM, Vanderklish PW (2006) Early postnatal plasticity in neocortex of FMR1 knockout mice. *J Neurophysiol* 96:1734–1745.
- Dölen G, Bear MF (2008) Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. *J Physiol* 586:1503–1508.
- Dölen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, Bear MF (2007) Correction of fragile X syndrome in mice. *Neuron* 56:955–962.
- The Dutch-Belgian Fragile X Consortium (1994) *Fmr1* knockout mice: a model to study fragile X mental retardation. *Cell* 78:23–33.
- Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG (2009) Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci U S A* 106:15037–15042.
- Frankland PW, Wang Y, Rosner B, Shimizu T, Balleine BW, Dykens EM, Ornitz EM, Silva AJ (2004) Sensorimotor gating abnormalities in young males with fragile X syndrome and *Fmr1*-knockout mice. *Mol Psychiatry* 9:417–425.
- Gabel LA, Won S, Kawai H, McKinney M, Tartakoff AM, Fallon JR (2004) Visual experience regulates transient expression and dendritic localization of fragile X mental retardation protein. *J Neurosci* 24:10579–10583.
- Gibson JR, Bartley AF, Hays SA, Huber KM (2008) Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. *J Neurophysiol* 100:2615–2626.
- Gonchar Y, Burkhalter A (1997) Three distinct families of GABAergic neurons in rat visual cortex. *Cereb Cortex* 7:347–358.
- Hagerman R (2002) The physical and behavioral phenotype. In: *Fragile X syndrome: diagnosis, treatment, and research* (Hagerman R, Hagerman P, eds), pp 3–109. Baltimore: Johns Hopkins UP.
- Hagerman RJ, Amiri K, Cronister A (1991) Fragile X checklist. *Am J Med Genet* 38:283–287.
- Hagerman RJ, Ono MY, Hagerman PJ (2005) Recent advances in fragile X: a model for autism and neurodegeneration. *Curr Opin Psychiatry* 18:490–496.
- Haider B, McCormick DA (2009) Rapid neocortical dynamics: cellular and network mechanisms. *Neuron* 62:171–189.
- Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A* 99:7746–7750.
- Incorpora G, Sorge G, Sorge A, Pavone L (2002) Epilepsy in fragile X syndrome. *Brain Dev* 24:766–769.
- Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler IJ, Greenough WT (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet* 98:161–167.
- Iwasato T, Datwani A, Wolf AM, Nishiyama H, Taguchi Y, Tonegawa S, Knöpfel T, Erzurumlu RS, Itohara S (2000) Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature* 406:726–731.
- Iwasato T, Inan M, Kanki H, Erzurumlu RS, Itohara S, Crair MC (2008) Cortical adenylyl cyclase 1 is required for thalamocortical synapse maturation and aspects of layer IV barrel development. *J Neurosci* 28:5931–5943.
- Johnston D, Wu S (1995) *Foundations of cellular neurophysiology*. Cambridge, MA: MIT.
- Kaufmann WE, Cortell R, Kau AS, Bukelis I, Tierney E, Gray RM, Cox C,

- Capone GT, Stanard P (2004) Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. *Am J Med Genet A* 129A:225–234.
- Kim SJ, Kim YS, Yuan JP, Petralia RS, Worley PF, Linden DJ (2003) Activation of the TRPC1 cation channel by metabotropic glutamate receptor mGluR1. *Nature* 426:285–291.
- Kronk R, Bishop EE, Raspa M, Bickel JO, Mandel DA, Bailey DB Jr (2010) Prevalence, nature, and correlates of sleep problems among children with fragile X syndrome based on a large scale parent survey. *Sleep* 33:679–687.
- Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci* 17:5196–5205.
- Marshall L, Born J (2007) The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci* 11:442–450.
- Marshall L, Helgadóttir H, Mölle M, Born J (2006) Boosting slow oscillations during sleep potentiates memory. *Nature* 444:610–613.
- McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, Sehgal A, Siwicki KK, Dockendorff TC, Nguyen HT, McDonald TV, Jongens TA (2005) Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a *Drosophila* model of fragile X syndrome. *Neuron* 45:753–764.
- Meredith RM, de Jong R, Mansvelder HD (2011) Functional rescue of excitatory synaptic transmission in the developing hippocampus in *Fmr1*-KO mouse. *Neurobiol Dis* 41:104–110.
- Miano S, Bruni O, Elia M, Scifo L, Smerieri A, Trovato A, Verrillo E, Terzano MG, Ferri R (2008) Sleep phenotypes of intellectual disability: a polysomnographic evaluation in subjects with Down syndrome and Fragile-X syndrome. *Clin Neurophysiol* 119:1242–1247.
- Mientjes EJ, Nieuwenhuizen I, Kirkpatrick L, Zu T, Hoogeveen-Westerveld M, Severijnen L, Rifé M, Willemsen R, Nelson DL, Oostra BA (2006) The generation of a conditional *Fmr1* knock out mouse model to study Fmrp function in vivo. *Neurobiol Dis* 21:549–555.
- Miller LJ, McIntosh DN, McGrath J, Shyu V, Lampe M, Taylor AK, Tassone F, Neitzel K, Stackhouse T, Hagerman RJ (1999) Electrodermal responses to sensory stimuli in individuals with fragile X syndrome: a preliminary report. *Am J Med Genet* 83:268–279.
- Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wölfel B, Dodt HU, Zieglgänsberger W, Wotjak CT, et al. (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51:455–466.
- Musumeci SA, Ferri R, Elia M, Colognola RM, Bergonzi P, Tassinari CA (1991) Epilepsy and fragile X syndrome: a follow-up study. *Am J Med Genet* 38:511–513.
- Musumeci SA, Hagerman RJ, Ferri R, Bosco P, Dalla Bernardina B, Tassinari CA, De Sarro GB, Elia M (1999) Epilepsy and EEG findings in males with fragile X syndrome. *Epilepsia* 40:1092–1099.
- Musumeci SA, Bosco P, Calabrese G, Bakker C, De Sarro GB, Elia M, Ferri R, Oostra BA (2000) Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. *Epilepsia* 41:19–23.
- Nielsen DM, Derber WJ, McClellan DA, Crnic LS (2002) Alterations in the auditory startle response in *Fmr1* targeted mutant mouse models of fragile X syndrome. *Brain Res* 927:8–17.
- Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50:295–322.
- O'Donnell WT, Warren ST (2002) A decade of molecular studies of fragile X syndrome. *Annu Rev Neurosci* 25:315–338.
- Okun M, Naim A, Lampl I (2010) The subthreshold relation between cortical local field potential and neuronal firing unveiled by intracellular recordings in awake rats. *J Neurosci* 30:4440–4448.
- Osterweil EK, Krueger DD, Reinhold K, Bear MF (2010) Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *J Neurosci* 30:15616–15627.
- Pfeiffer BE, Huber KM (2009) The state of synapses in fragile X syndrome. *Neuroscientist* 15:549–567.
- Rigas P, Castro-Alamancos MA (2007) Thalamocortical UP states: differential effects of intrinsic and extrinsic cortical inputs on persistent activity. *J Neurosci* 27:4261–4272.
- Rojas DC, Benkers TL, Rogers SJ, Teale PD, Reite ML, Hagerman RJ (2001) Auditory evoked magnetic fields in adults with fragile X syndrome. *Neuroreport* 12:2573–2576.
- Rubenstein JL, Merzenich MM (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav* 2:255–267.
- Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci* 3:1027–1034.
- Sanchez-Vives MV, Mattia M, Compte A, Perez-Zabalza M, Winograd M, Descalzo VF, Reig R (2010) Inhibitory modulation of cortical up states. *J Neurophysiol* 104:1314–1324.
- Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R (2005) Altered anxiety-related and social behaviors in the *Fmr1* knockout mouse model of fragile X syndrome. *Genes Brain Behav* 4:420–430.
- Spencer CM, Serysheva E, Yuva-Paylor LA, Oostra BA, Nelson DL, Paylor R (2006) Exaggerated behavioral phenotypes in *Fmr1/Fxr2* double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. *Hum Mol Genet* 15:1984–1994.
- Steriade M (1997) Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cereb Cortex* 7:583–604.
- Steriade M, Nuñez A, Amzica F (1993a) A novel slow (<1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. *J Neurosci* 13:3252–3265.
- Steriade M, Nuñez A, Amzica F (1993b) Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J Neurosci* 13:3266–3283.
- Steriade M, McCormick DA, Sejnowski TJ (1993c) Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262:679–685.
- Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol* 85:1969–1985.
- Stevens HE, Smith KM, Maragnoli ME, Fagel D, Borok E, Shanabrough M, Horvath TL, Vaccarino FM (2010) *Fgfr2* is required for the development of the medial prefrontal cortex and its connections with limbic circuits. *J Neurosci* 30:5590–5602.
- Strumbos JG, Brown MR, Kronengold J, Polley DB, Kaczmarek LK (2010) Fragile X mental retardation protein is required for rapid experience-dependent regulation of the potassium channel Kv3.1b. *J Neurosci* 30:10263–10271.
- Taylor GW, Merlin LR, Wong RK (1995) Synchronized oscillations in hippocampal CA3 neurons induced by metabotropic glutamate receptor activation. *J Neurosci* 15:8039–8052.
- Timofeev I, Grenier F, Steriade M (2001) Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proc Natl Acad Sci U S A* 98:1924–1929.
- Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, Eussen BE, van Ommen GJB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, et al. (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–914.
- Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP (2005) Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology* 49:1053–1066.
- Yoshida M, Fransén E, Hasselmo ME (2008) mGluR-dependent persistent firing in entorhinal cortex layer III neurons. *Eur J Neurosci* 28:1116–1126.
- Young SR, Chuang SC, Wong RK (2004) Modulation of afterpotentials and firing pattern in guinea pig CA3 neurones by group I metabotropic glutamate receptors. *J Physiol* 554:371–385.
- Zhang J, Fang Z, Jud C, Vansteensel MJ, Kaasik K, Lee CC, Albrecht U, Tamanini F, Meijer JH, Oostra BA, Nelson DL (2008) Fragile X-related proteins regulate mammalian circadian behavioral rhythms. *Am J Hum Genet* 83:43–52.
- Zhang Z, Séguéla P (2010) Metabotropic induction of persistent activity in layers II/III of anterior cingulate cortex. *Cereb Cortex* 20:2948–2957.