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RAPID REPORT

Distinct roles of GABA$_{B1a}$- and GABA$_{B1b}$-containing GABA$_B$ receptors in spontaneous and evoked termination of persistent cortical activity

Michael T. Craig[1,2], Elizabeth W. Mayne[1,2], Bernhard Bettler[3], Ole Paulsen[2,4] and Chris J. McBain[1]

[1] Program in Developmental Neurobiology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[2] Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford OX1 3PT, UK
[3] Department of Biomedicine, Institute of Physiology, University of Basel, CH – 4056 Basel, Switzerland
[4] Department of Physiology, Development and Neuroscience, Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

Key points

- GABA$_B$ receptors containing the GABA$_{B1a}$ subunit contribute to spontaneous termination of UP states.
- GABA$_B$ receptors containing the GABA$_{B1b}$ subunit are essential for afferent-evoked termination of UP states.

Abstract During slow-wave sleep, cortical neurons display synchronous fluctuations between periods of persistent activity (‘UP states’) and periods of relative quiescence (‘DOWN states’). Such UP and DOWN states are also seen in isolated cortical slices. Recently, we reported that both spontaneous and evoked termination of UP states in slices from the rat medial entorhinal cortex (mEC) involves GABA$_B$ receptors. Here, in order to dissociate the roles of GABA$_{B1a}$- and GABA$_{B1b}$-containing receptors in terminating UP states, we used mEC slices from mice in which either the GABA$_{B1a}$ or the GABA$_{B1b}$ subunit had been genetically ablated. Pharmacological blockade of GABA$_B$ receptors using the antagonist CGP55845 prolonged the UP state duration in both wild-type mice and those lacking the GABA$_{B1b}$ subunit, but not in those lacking the GABA$_{B1a}$ subunit. Conversely, electrical stimulation of layer 1 could terminate an ongoing UP state in both wild-type mice and those lacking the GABA$_{B1a}$ subunit, but not in those lacking the GABA$_{B1b}$ subunit. Together with previous reports, indicating a preferential presynaptic location of GABA$_{B1a}$- and postsynaptic location of GABA$_{B1b}$-containing receptors, these results suggest that presynaptic GABA$_B$ receptors contribute to spontaneous DOWN state transitions, whilst postsynaptic GABA$_B$ receptors are essential for the afferent termination of the UP state. Inputs to layer 1 from other brain regions could thus provide a powerful mechanism for synchronizing DOWN state transitions across cortical areas via activation of GABAergic interneurons targeting postsynaptic GABA$_B$ receptors.

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Corresponding authors C. J. McBain: NICHD-LCSN, Section on Cellular & Synaptic Neurophysiology, Porter Neuroscience Center, Bldg 35, Rm 3C903, 35 Lincoln Drive, Bethesda, MD 20892, USA; O. Paulsen, Department of Physiology, Development and Neuroscience, Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK. Email: mcbainc@mail.nih.gov or op210@cam.ac.uk

Abbreviations aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; mEC, medial entorhinal cortex.

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Introduction

During slow-wave sleep, cortical neurons participate in the slow oscillation during which these neurons synchronously fluctuate between periods of persistent activity (‘UP states’) and periods of relative quiescence (‘DOWN states’) (Steriade *et al.* 1993). Such UP and DOWN states can also be observed in brain slices *in vitro*, prepared from a variety of animal species and cortical regions such as the ferret visual cortex (Sanchez-Vives & McCormick, 2000) or, more recently, the rodent medial entorhinal cortex (mEC) (Cunningham *et al.* 2006; Mann *et al.* 2009; Tahvildari *et al.* 2012).

UP states are synaptically driven, with increases in both excitatory and inhibitory transmission relative to DOWN states (Sanchez-Vives & McCormick, 2000; Shu *et al.* 2003). During the UP state, inhibitory conductances dynamically scale to match excitatory conductances (Shu *et al.* 2003). Conversely, during the *in vivo* DOWN state, few inhibitory postsynaptic potentials are seen in intracellular recordings and fast-spiking interneurons appear to be silent (Timofeev *et al.* 2001). The UP state originates within the cortex but transitions between states can be triggered *in vivo* by sensory input (Petersen, 2003) or *in vitro* by electrical stimulation of synaptic inputs arising within (Shu *et al.* 2003) or outwith the cortex (MacLean *et al.* 2005).

Previous work from our group demonstrated that, in the rat mEC, electrical stimulation in layer 3 could evoke a DOWN-to-UP state transition, and subsequent stimulation in layer 1 could terminate this UP state (Mann *et al.* 2009). It was found that $GABA_A$ receptors balanced the UP state and modulated firing frequency, while $GABA_B$ receptors mediated the UP state termination: blockade of $GABA_B$ receptors both prolonged spontaneous UP states and prevented layer 1 stimulation from evoking an UP-to-DOWN state transition (Mann *et al.* 2009).

Functional $GABA_B$ receptors exist as heterodimers between $GABA_{B1}$ and $GABA_{B2}$ subunits, with the $GABA_{B1}$ subunit existing in two isoforms, $GABA_{B1a}$ and $GABA_{B1b}$ (Bettler *et al.* 2004). Evidence from both the hippocampus and the neocortex suggests that $GABA_B$ receptors containing $GABA_{B1a}$ subunits are preferentially located presynaptically whilst those containing $GABA_{B1b}$ subunits are preferentially located postsynaptically (Perez-Garci *et al.* 2006; Vigot *et al.* 2006). In this study, we sought to determine whether the location of $GABA_B$ receptors affected their role in terminating the UP state. Using mice in which either the $GABA_{B1a}$ subunit or the $GABA_{B1b}$ subunit had been genetically ablated, we could dissociate the effects of $GABA_B$ receptors containing the different subunits. We found that $GABA_B$ receptors containing the $GABA_{B1a}$ subunit modulate the timing of the spontaneous UP state termination and those containing the $GABA_{B1b}$ subunit are necessary for terminating the UP state by electrical stimulation in layer 1.

Methods

Ethical approval

All experiments were conducted in accordance with the UK Animals Scientific Procedures Act (1986) and in accordance with animal protocols approved by the National Institutes of Health. Transgenic mice lacking either the $GABA_{B1a}$ or the $GABA_{B1b}$ subunit (Vigot *et al.* 2006), and wild-type controls (BALB/c mice; Harlan, Bicester, UK) were used.

Slice preparation and electrophysiology

Horizontal slices (400 μm) containing the mEC were prepared from postnatal day 14–21 mice of both sexes after decapitation under deep isoflurane-induced anaesthesia. Slices were cut in ice-cold (<4°C) standard artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (3–3.5), NaH_2PO_4 (1.25), $MgSO_4$ (2), $CaCl_2$ (2) and $NaHCO_3$ (26), and were incubated at room temperature for 1 h in interface conditions with standard aCSF, before being transferred to modified aCSF with reduced $MgSO_4$ (1 mM) and $CaCl_2$ (1.2 mM). Slices were maintained in interface conditions prior to recording; they were then mounted on a coverslip (coated with 0.1% poly-L-lysine in ultrapure H_2O) and transferred to a submerged-style recording chamber where they were superfused with modified aCSF at 4–5 ml min^{-1} at 32–34°C, conditions that promote spontaneous network activity (Hajos *et al.* 2009).

Whole-cell current-clamp recordings were made from principal cells in layer 3 of mEC, using glass pipettes pulled from standard borosilicate glass containing (in mM): potassium gluconate (110), Hepes (40), ATP-Mg (2), GTP (0.3), NaCl (4) and biocytin (2–4 mg ml^{-1}) (pH 7.2–7.3, osmolarity 275–290 mosmol l^{-1}). Membrane potential values were not corrected for the liquid junction potential. Electrical stimulation was carried out using Digitimer DS3 constant current stimulators with monopolar steel electrodes.

Data acquisition and analysis

Data were recorded using an Axon Multiclamp 700A or 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) and low-pass filtered at 2 kHz. The signal was digitized at 5 kHz using either an Axon Digidata 1322A on a PC running Axon PClamp 9 or an Instrutech

ITC-18 on a PC running Igor Pro using procedures written in-house. Data acquired using PClamp were imported into Igor Pro using Neuromatic (ThinkRandom; http://www.thinkrandom.com/) for further analysis.

UP and DOWN state transitions were monitored automatically with an algorithm that detected changes in DC membrane potential and membrane potential fluctuations using a moving average window method (Craig, 2011). All detected UP states were confirmed by visual inspection. Statistical comparisons were made using analysis of variance (ANOVA) with post-hoc Bonferroni multiple-comparison correction, or Student's two-sample and paired t tests as appropriate. Unless otherwise stated, all values are given as mean ± SEM.

Drugs and chemicals

CGP55845 was purchased from Tocris Bioscience (Bristol, UK). All other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA).

Results

Electrical stimulation in mouse mEC can evoke UP and DOWN state transitions

Whole-cell recording from layer 3 pyramidal cells was used to monitor UP and DOWN states, which occurred spontaneously at a frequency of $3.1 \pm 0.5\ \mathrm{min}^{-1}$ in wild-type BALB/c mice ($n = 5$). As previously reported in the rat (Mann *et al.* 2009), electrical stimulation (100–250 μA for 100–150 μs) in layer 3 of the mEC in BALB/c mice could evoke an UP state (Fig. 1*A* and *B*). UP states evoked by layer 3 stimulation had a similar duration and firing frequency to those occurring spontaneously (UP state duration, spontaneous *vs* L3 stimulation: 1.9 ± 0.15 s *vs* 1.5 ± 0.14 s; $P > 0.05$; UP state firing frequency, $4.6 \pm 1.24\ \mathrm{s}^{-1}$ *vs* $3.7 \pm 0.95\ \mathrm{s}^{-1}$; $P > 0.05$; $n = 11$; Fig. 1*C*). Stimulation in layer 1 (150–250 μA for 100–150 μs) 500 ms after layer 3 stimulation could then terminate the evoked UP state (Fig. 1*A* and *B*). Layer 1 stimulation significantly shortened the duration of the evoked UP state (UP state duration, L3 stimulation *vs* L3 + L1 stimulation: 1.5 ± 0.14 s *vs* 0.8 ± 0.07 s; $P < 0.01$; $n = 11$; one-way ANOVA; Fig. 1*C*). The duration and firing frequency of UP states displayed a large degree of variation within an individual slice. Figure 1*D* and *E* display the UP state duration (Fig. 1*D*) and firing frequency (Fig. 1*E*) for 20 consecutive, spontaneously occurring UP states observed in three different slices. The range of the coefficient of variation (CV) in these examples was 0.36–0.66 for UP state duration, and 0.35–0.86 for firing frequency. These results confirm that the mouse mEC shows UP and DOWN states with properties similar to those of the rat mEC.

GABA$_B$ receptor-mediated inhibition contributes to the spontaneous termination of UP states, as well as afferent stimulation-evoked DOWN state transitions (Mann *et al.* 2009). As GABA$_B$ receptors exist in at least two forms, those containing the GABA$_{B1a}$ subunit and those containing the GABA$_{B1b}$ subunit, respectively (Vigot *et al.* 2006), we sought to determine whether these receptors were differentially involved in terminating the UP state. This was done by comparing the effects of a GABA$_B$ receptor antagonist and layer 1 stimulation in wild-type mice with those in mice genetically engineered to lack either the GABA$_{B1a}$ or the GABA$_{B1b}$ subunit (Vigot *et al.* 2006).

Spontaneous UP states do not differ significantly between wild-type, GABA$_{B1a}{}^{-/-}$ and GABA$_{B1b}{}^{-/-}$ mice

Before examining the role of receptor type in terminating the UP state, we compared the properties of spontaneous UP states between the three genotypes. Representative recordings from wild-type, GABA$_{B1a}{}^{-/-}$ and GABA$_{B1b}{}^{-/-}$ mice are presented in Fig. 2*A*–*C*. We observed no significant differences in the incidence, duration or firing frequency of spontaneous UP states between the wild-type and knockout mice (wildtype ($n = 5$) *vs* GABA$_{B1a}{}^{-/-}$ ($n = 10$) *vs* GABA$_{B1b}{}^{-/-}$ ($n = 5$); UP state incidence: $3.1 \pm 0.5\ \mathrm{min}^{-1}$ *vs* $2.6 \pm 0.3\ \mathrm{min}^{-1}$ *vs* $4.4 \pm 1.2\ \mathrm{min}^{-1}$; $P > 0.05$; one-way ANOVA; Fig. 2*D*; UP state duration: 3.7 ± 0.6 s *vs* 2.1 ± 0.2 s *vs* 3.2 ± 0.7 s; $P > 0.05$; one-way ANOVA; Fig. 2*E*; UP state firing frequency: 3.1 ± 0.5 Hz *vs* 3.0 ± 0.5 Hz *vs* 4.4 ± 1.2 Hz; Fig. 2F).

GABA$_{B1a}$ receptors modulate the duration of the UP state

Pharmacological blockade of GABA$_B$ receptors increases the duration of spontaneous as well as evoked UP states (Mann *et al.* 2009). We therefore investigated the effects on UP state duration of a GABA$_B$ receptor blocker in wild-type as well as GABA$_{B1a}{}^{-/-}$ and GABA$_{B1b}{}^{-/-}$ mice (Fig. 3*A*). As expected, blockade of GABA$_B$ receptors using 1 μM CGP55845, a selective GABA$_B$ receptor antagonist, significantly prolonged the UP state duration in wild-type mice (UP state duration relative to baseline, DMSO *vs* 1 μM CGP55845: $96 \pm 6.7\%$ *vs* $142 \pm 14.9\%$; $P = 0.0217$; Student's t test). Similar to wild-type controls, 1 μM CGP55845 significantly prolonged the UP state duration in GABA$_{B1b}{}^{-/-}$ mice (UP state duration relative to baseline, DMSO *vs* 1 μM CGP55845: $102 \pm 10.0\%$ *vs* $139 \pm 7.7\%$; $P = 0.012$; Student's t test) but not in GABA$_{B1a}{}^{-/-}$ mice (UP state duration relative to baseline, DMSO *vs* 1 μM CGP55845: $104 \pm 8.7\%$ *vs*

$116 \pm 7.4\%$; $P = 0.311$; Student's *t* test). These data are summarized in Fig. 3*B* and indicate that GABA$_\mathrm{B}$ receptors containing the GABA$_\mathrm{B1a}$ but not the GABA$_\mathrm{B1b}$ subunit are responsible for the effect of CGP55845 on the duration of the UP state, suggesting that presynaptic GABA$_\mathrm{B}$ receptors contribute to the spontaneous termination of UP states.

GABA$_\mathrm{B1b}$ receptors are necessary for afferent termination of the UP state

Next, we investigated the effect of layer 1 stimulation in GABA$_\mathrm{B1a}{}^{-/-}$ and GABA$_\mathrm{B1b}{}^{-/-}$ mice, compared to the effect in wild-type animals. As in the rat, stimulation in layer 1 significantly shortened an evoked UP state in wild-type mice and this effect could be blocked by 1 μM CGP55845 (reduction in UP state duration, baseline ($n = 11$) *vs* DMSO ($n = 6$) *vs* 1 μM CGP55845 ($n = 7$): $43 \pm 4.5\%$ *vs* $56 \pm 4.6\%$ *vs* $12 \pm 8.2\%$; $P = 0.0002$; one-way ANOVA, Fig. 4*A*–*C*). Under baseline conditions, layer 1 stimulation also significantly shortened the UP state in GABA$_\mathrm{B1a}{}^{-/-}$ mice, an effect that was also blocked by 1 μM CGP55845 (reduction in UP state duration, baseline ($n = 8$) *vs* DMSO ($n = 5$) *vs* 1 μM CGP55845 ($n = 6$): $59 \pm 4.3\%$ *vs* $61 \pm 4.7\%$ *vs* $4.7 \pm 5.7\%$; $P < 0.0001$; one

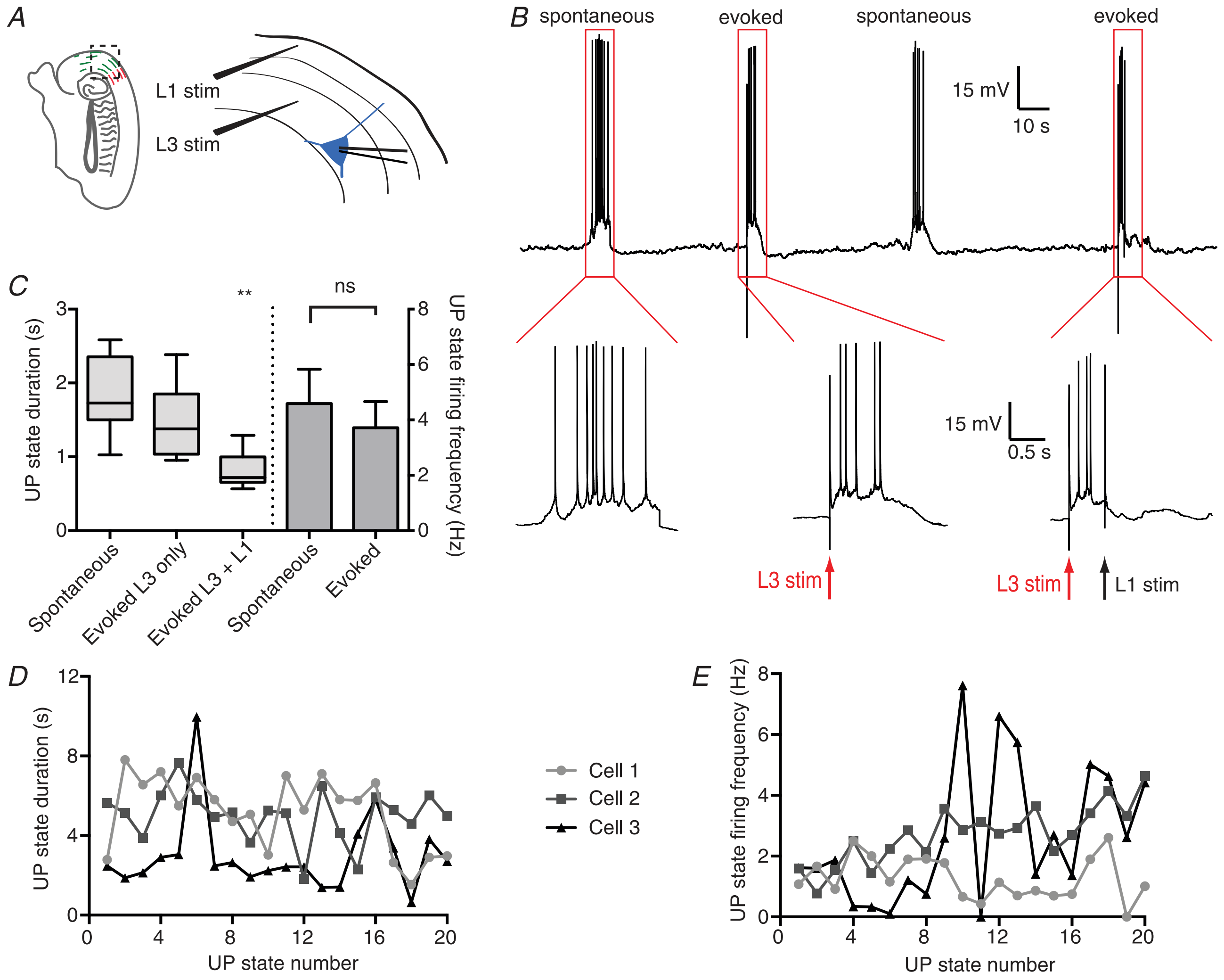

Figure 1. Electrical stimulation in the mouse mEC can turn on and off persistent activity
A, recording schematic. Whole-cell current-clamp recordings were made from principal cells in layer 3 of the mEC. UP states were evoked by stimulating in layer 3 within 200 μm of the principal cell soma, and subsequent stimulation in layer 1 was used to terminate the UP state. *B*, representative trace with expansions showing a spontaneous UP state (lower left), an UP state evoked by layer 3 stimulation (lower middle) and an UP state evoked with layer 3 stimulation and terminated with layer 1 stimulation (lower right). *C*, duration and firing frequency of UP states evoked by layer 3 stimulation were not statistically significant from spontaneous UP states, but layer 1 stimulation significantly shortened the UP state. The whiskers in the boxplots represent the minimum and maximum values *D*, UP state duration plotted for 20 consecutive spontaneous UP states for three different neurons. *E*, UP state firing frequency plotted for the same neurons and UP states as *D*. $^{**}P < 0.01$.

way ANOVA, Fig. 4A–C). In contrast, layer 1 stimulation did not terminate an evoked UP state in GABA$_{\mathrm{B1b}}^{-/-}$ mice in any condition (reduction in UP state duration, baseline ($n = 8$) *vs* DMSO ($n = 5$) *vs* 1 μM CGP55845 ($n = 11$): $-1.8 \pm 3.6\%$ *vs* $-2.2 \pm 7.8\%$ *vs* $5.3 \pm 4.3\%$; $P > 0.05$; one-way ANOVA, Fig. 4A–C). From these results, we conclude that GABA$_{\mathrm{B1a}}$ subunit-containing receptors are not required for the afferent-evoked termination of the UP state and that this effect is mediated via GABA$_{\mathrm{B1b}}$ subunit-containing GABA$_{\mathrm{B}}$ receptors.

Discussion

Here we have dissociated the contributions of GABA$_{\mathrm{B1a}}$- and GABA$_{\mathrm{B1b}}$-containing GABA$_{\mathrm{B}}$ receptors to the termination of UP states in the mEC *in vitro*. For all excitatory synapses that have been analysed for the location of GABA$_{\mathrm{B1a}}$ and GABA$_{\mathrm{B1b}}$ subunits (hippocampal CA3–CA1, hippocampal mossy fibre – CA3, thalamic and cortical inputs to the lateral amygdala, thalamus and neocortex), the GABA$_{\mathrm{B1a}}$ subunit has predominantly been found to be presynaptic and the GABA$_{\mathrm{B1b}}$ subunit predominantly postsynaptic (Gassman & Bettler, 2012). While the synaptic location of these subunits in the entorhinal cortex has not been studied in detail, we may assume that the distribution will be similar, although we cannot rule out that either receptor may exist in both locations. Hence we conclude that receptors containing the GABA$_{\mathrm{B1a}}$ subunit, presumably presynaptic, help control the UP state duration by modulating spontaneous UP-to-DOWN state transitions, whereas receptors containing the GABA$_{\mathrm{B1b}}$ subunit, most likely

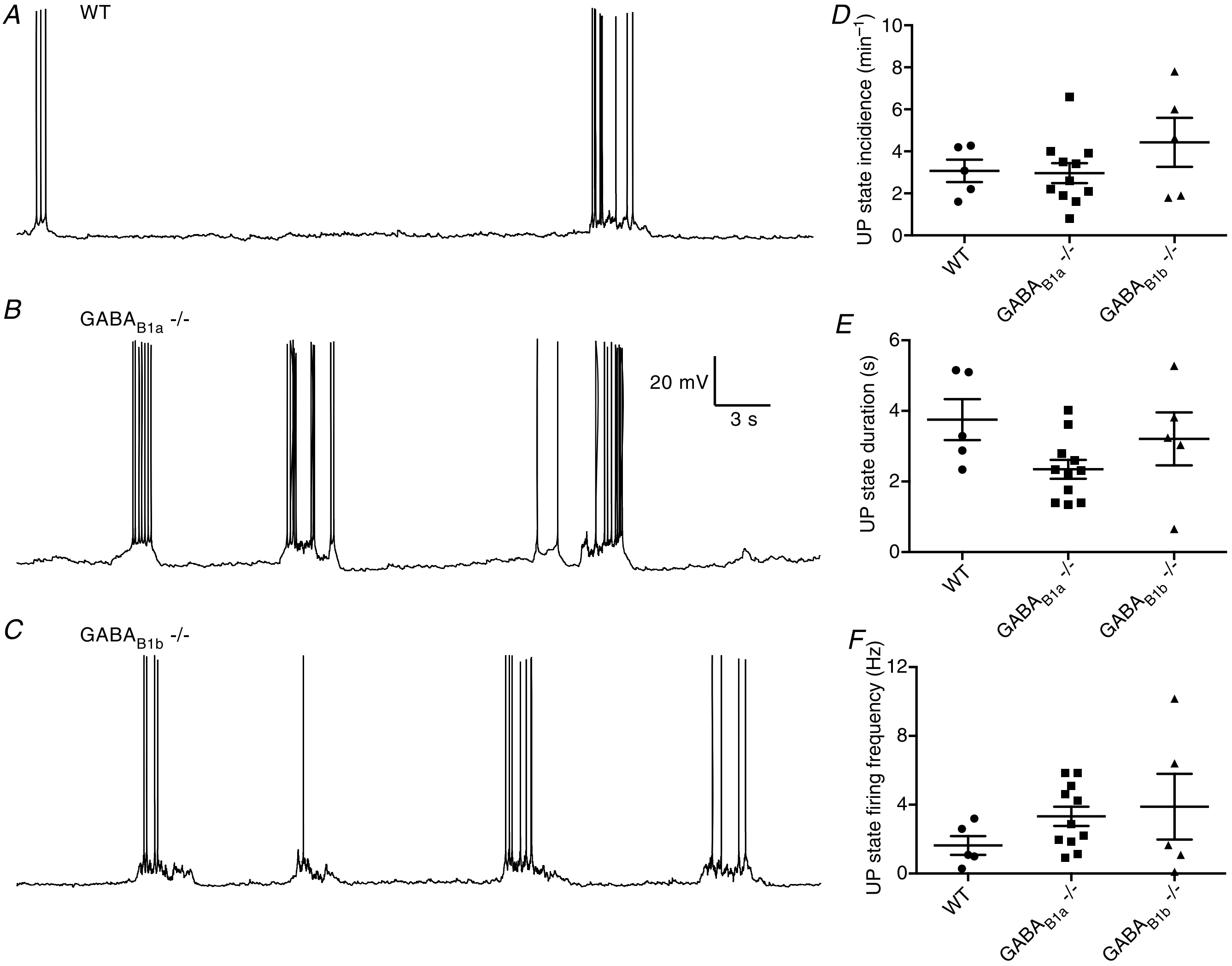

Figure 2. Recordings from layer 3 principal cells
A–C, representative recordings made from layer 3 principal cells for 60 s for wild-type (WT) mice (*A*), GABA$_{\mathrm{B1a}}^{-/-}$ mice (*B*) and GABA$_{\mathrm{B1b}}^{-/-}$ mice (*C*). *D–F*, overall, no significant differences in UP state incidence (*D*), duration (*E*) or firing frequency (*F*) were observed between the three groups.

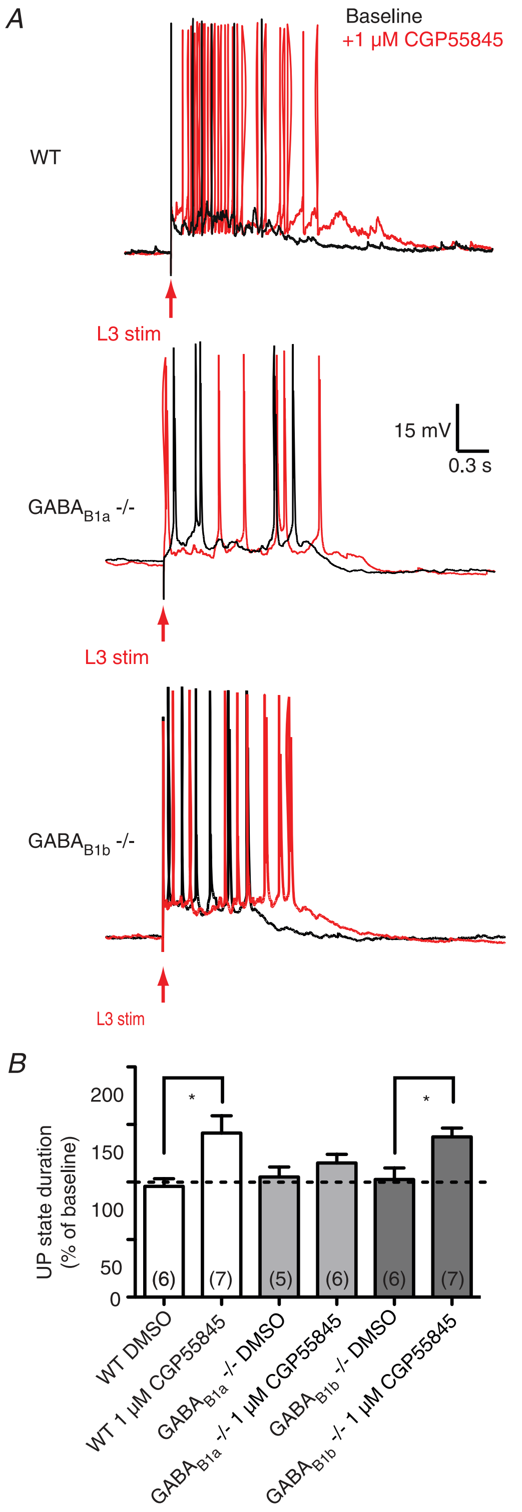

Figure 3. GABA$_{B1a}$-containing receptors contribute to the spontaneous termination of UP states
A, representative traces taken from wild-type (WT), GABA$_{B1a}$$^{-/-}$ and GABA$_{B1b}$$^{-/-}$ mice. *B*, the selective GABA$_B$ receptor antagonist CGP55845 (1 μM) significantly prolonged the UP state in wild-type and GABA$_{B1b}$$^{-/-}$ mice, but not in GABA$_{B1a}$$^{-/-}$ mice. Error bars are SEM; number of slices in parentheses; *$P < 0.05$; Student's *t* test.

located postsynaptically, are necessary for afferent-evoked DOWN state transitions.

Presynaptic GABA$_B$ receptor activation can inhibit the release of both excitatory and inhibitory neurotransmitters (e.g. Pérez-Garci *et al.* 2006; Olah *et al.* 2009). As UP states are characterized by a balanced increase in both synaptic excitation and inhibition (Shu *et al.* 2003), it is possible that a gradual build up of extracellular GABA during the UP state progressively inhibits transmitter release via GABA$_B$ receptors at both excitatory and inhibitory synapses, and that the blockade of presynaptic GABA$_B$ receptors prolongs the UP state by preventing this presynaptic inhibition. As the blockade of GABA$_B$ receptors can prolong UP states not only in mEC but also in other cortical areas (Wang *et al.* 2010), this might imply that GABA$_B$ receptor modulation of spontaneous termination of the UP state is a shared mechanism across cortical areas. Given our current findings, one might have expected to see a prolongation in spontaneous UP state duration in the GABA$_{B1a}$$^{-/-}$ mice compared to GABA$_{B1b}$$^{-/-}$ and wild-type mice. However, the large degree of variation of UP state properties observed within individual slices (Fig. 1) and between slices from the same genotype (Fig. 2) could have occluded these differences, necessitating the use of GABA$_B$ receptor antagonists to unmask the contribution of receptor location to spontaneous termination, or compensatory mechanisms might have developed in GABA$_{B1a}$$^{-/-}$ mice.

While GABA$_B$ receptors containing the GABA$_{B1a}$ subunit contribute to the spontaneous termination of UP states, those containing the GABA$_{B1b}$ subunit are necessary for afferent-evoked DOWN state transitions. It might seem surprising that, whilst essential for afferent-evoked DOWN state transition, GABA$_{B1b}$ subunit-containing receptors do not appear to contribute to spontaneous DOWN state transition. A parsimonious explanation would be that those interneurons that target these GABA$_B$ receptors are not activated to a large degree by the local circuitry during an UP state, but are rather activated by external afferents. Indeed, it was recently reported that neuropeptide-Y-positive interneurons in layer 2/3 are silent during mEC UP states *in vitro* (Tahvildari *et al.* 2012). Neurogliaform cells are immunoreactive for neuropeptide-Y (Price *et al.* 2005), making them an attractive candidate for mediating afferent termination of the UP state. Neurogliaform cells can elicit combined GABA$_A$ and GABA$_B$ receptor-mediated responses from single action potentials (Tamas *et al.* 2003), and, even at a low density, they can exert a large inhibitory influence by acting via volume transmission on extrasynaptic GABA$_B$ receptors (Olah *et al.* 2009). Neurogliaform cells are present in layer 1 of the neocortex (Hestrin & Armstrong, 1996), where they receive little or no input from superficial pyramidal cells but can exert an inhibitory influence over both excitatory (Wozny & Williams, 2011) and inhibitory

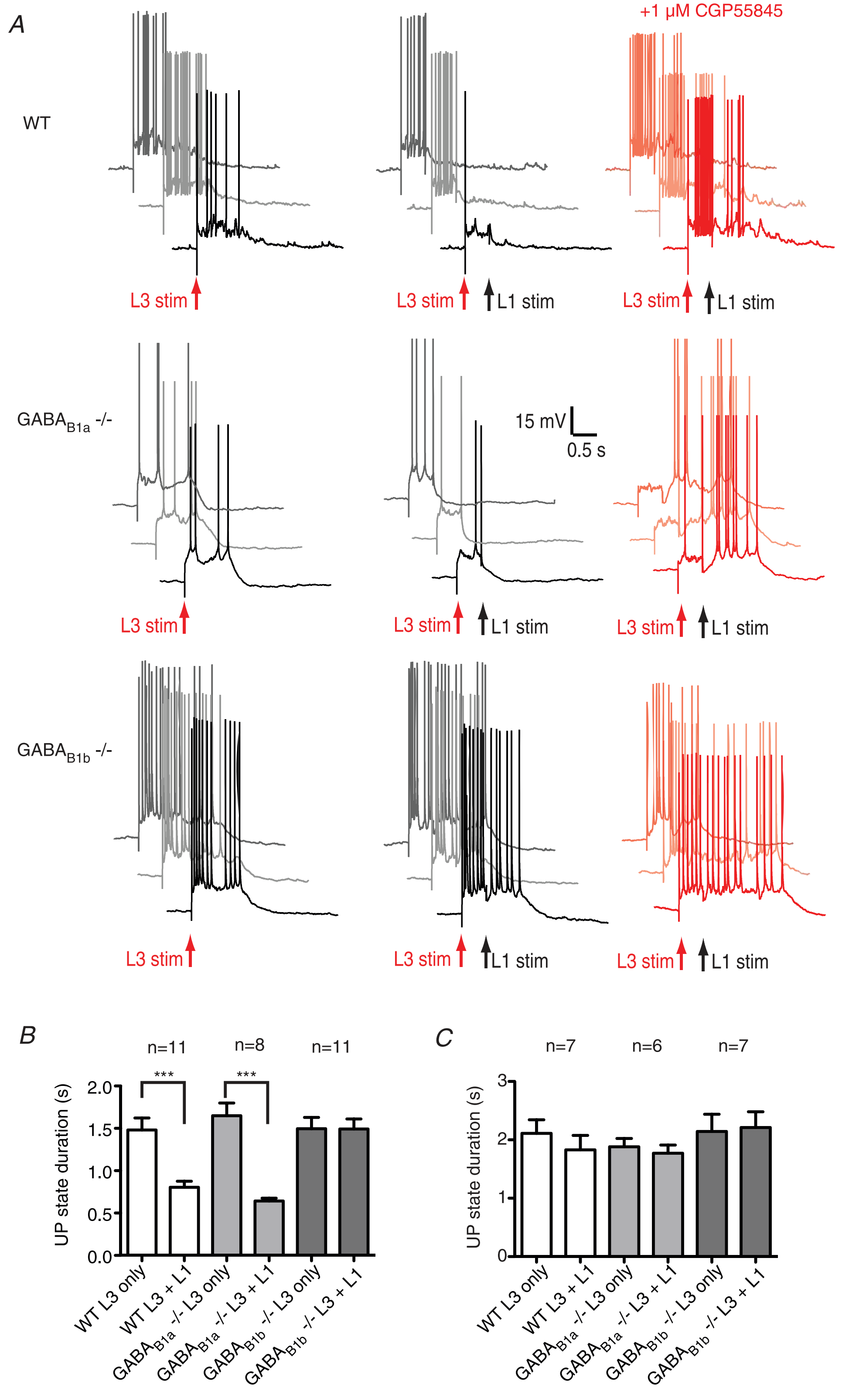

Figure 4. GABA$_{B1b}$-containing receptors are necessary for afferent-evoked termination of the UP state
A, representative traces taken from wild-type (WT), GABA$_{B1a}$$^{-/-}$ and GABA$_{B1b}$$^{-/-}$ mice. Three trials taken from the same neuron are presented for each condition. *B*, layer 1 stimulation shortened the UP state in wild-type and GABA$_{B1a}$$^{-/-}$ mice but not in GABA$_{B1b}$$^{-/-}$ mice. *C*, the selective GABA$_B$ receptor antagonist CGP55845 (1 μM) prevented layer 1 stimulation from shortening the UP state. Error bars are SEM. ***$P < 0.001$; paired *t* test.

cells (Christophe *et al.* 2002). While further work is needed to determine the source of the $GABA_B$ receptor-mediated inhibition responsible for afferent-evoked termination of the UP state, it is likely that the $GABA_B$ receptors terminate the UP state through activation of inwardly rectifying K^+ (GIRK or Kir3) channels (Bettler *et al.* 2004) and/or by inhibiting dendritic Ca^{2+} channels of pyramidal cells (Perez-Garci *et al.* 2006).

Several mechanisms have been suggested to contribute to the spontaneous DOWN state transitions, including disfacilitation of the network (Contreras *et al.* 1996) or a build up of intrinsic activity-dependent K^+ conductances (Sanchez-Vives & McCormick, 2000; Cunningham *et al.* 2006). However, more recent *in vivo* studies suggest that the UP state can be actively terminated: it has been reported that UP-to-DOWN state transitions occur more synchronously than DOWN-to-UP state transitions (Volgushev *et al.* 2006), and another study examining the electroencephalogram in human patients suggested that a DOWN state transition could occur independently of a preceding UP state (Cash *et al.* 2009).

If the UP state is actively terminated, then our results suggest that one mechanism could be through inputs arriving in layer 1 activating GABAergic interneurons acting on postsynaptic $GABA_B$ receptors. The question of where these inputs arrive from has yet to be addressed. *In vivo*, UP state propagation is fast, in the order of 1.5–7 m s^{-1} (Massimini *et al.* 2004), which is faster than the reported local spread of the oscillation through cortical tissue, which approaches 100 mm s^{-1} *in vivo* (Amzica & Steriade, 1995) and 11 mm s^{-1} *in vitro* (Sanchez-Vives & McCormick, 2000). This suggests that local propagation is inconsistent with the synchrony of UP and DOWN state transitions observed *in vivo*. The thalamus could play a role in synchronizing the slow oscillation *in vivo* (Crunelli & Hughes, 2010): the slow oscillation can be spontaneously generated in thalamocortical neurons and also neurons of the nucleus reticularis thalami (Crunelli & Hughes, 2010), and stimulation of the thalamus *in vitro* has been shown to trigger UP states that are indistinguishable from those generated spontaneously (MacLean *et al.* 2005). Applying muscimol to the thalamus of the rat greatly reduced the incidence of UP states (Doi *et al.* 2007), and an early *in vivo* study demonstrated that electrical stimulation of the thalamus could evoke a DOWN state transition in cortical neurons (Contreras & Steriade, 1995). Together, these results suggest that the thalamus may be able to synchronize cortical state transitions. As the thalamus projects extensively to layer 1 of most neocortical regions (Rubio-Garrido *et al.* 2009) as well as the mEC (Herkenham, 1978), thalamic activation of layer 1 interneurons could provide a plausible mechanism for the active termination of the UP state. Other studies have shown that cortico-cortical inputs also converge on layer 1 cells (e.g. Anderson & Martin, 2006), and interhemispheric projections to layer 1 are capable of mediating a long-lasting inhibition of cortical neuron firing, in a mechanism dependent on $GABA_B$ receptors on apical dendrites activated via layer 1 interneurons (Palmer *et al.* 2012).

While further work is needed to determine both the origin of the input to layer 1 and the cell type(s) mediating the effect, the present results provide further evidence that $GABA_B$ receptors may play a powerful role in regulating persistent network activity, and show that receptors containing $GABA_{B1a}$ and $GABA_{B1b}$ subunits have different roles in this regulation.

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Author contributions

The experiments were carried out in the Laboratory of Cellular and Synaptic Neurophysiology at the National Institutes of Health, Bethesda, MD, and in the Department of Physiology, Development and Neuroscience at the University of Cambridge. M.T.C.: conception and design of experments, collection, analysis and interpretation of data, drafting and revising the manuscript. E.W.M.: conception and design of experiments, collection of data. B.B.: critically revising manuscript for important intellectual content. O.P.: conception and design of experiments, interpretation of data, drafting and revising manuscript. C.J.M.: conception and design of experiments, intepretation of data, drafting and revising manuscript. All authors approved the final version of the manuscript.

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