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$_{B1b}$ subunit, but not in those lacking the GABA$_{B1a}$ 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.
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 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 Intrutech
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 ± 0.5 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. 1A 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 ± 1.24 s⁻¹ vs 3.7 ± 0.95 s⁻¹; P > 0.05; n = 11; Fig. 1C). 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. 1A 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. 1C). The duration and firing frequency of UP states displayed a large degree of variation within an individual slice. Figure 1D and E display the UP state duration (Fig. 1D) and firing frequency (Fig. 1E) 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. 2A–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 ± 0.5 min⁻¹ vs 2.6 ± 0.3 min⁻¹ vs 4.4 ± 1.2 min⁻¹; P > 0.05; one-way ANOVA; Fig. 2D; 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. 2E; 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. 3A). 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 ± 6.7% vs 142 ± 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 ± 10.0% vs 139 ± 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 ± 8.7% vs
116 ± 7.4%; \( P = 0.311 \); Student’s \( t \) test). These data are summarized in Fig. 3B and indicate that GABA\(_B\) receptors containing the GABA\(_{B1a}\) but not the GABA\(_{B1b}\) subunit are responsible for the effect of CGP55845 on the duration of the UP state, suggesting that presynaptic GABA\(_B\) receptors contribute to the spontaneous termination of UP states.

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

Next, we investigated the effect of layer 1 stimulation in GABA\(_{B1a}^{-/-}\) and GABA\(_{B1b}^{-/-}\) 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 \( \mu M \) CGP55845 (reduction in UP state duration, baseline \((n = 11)\) vs DMSO \((n = 6)\) vs 1 \( \mu M \) CGP55845 \((n = 7)\): 43 ± 4.5% vs 56 ± 4.6% vs 12 ± 8.2%; \( P = 0.0002 \); one-way ANOVA, Fig. 4A–C). Under baseline conditions, layer 1 stimulation also significantly shortened the UP state in GABA\(_{B1a}^{-/-}\) mice, an effect that was also blocked by 1 \( \mu M \) CGP55845 (reduction in UP state duration, baseline \((n = 8)\) vs DMSO \((n = 5)\) vs 1 \( \mu M \) CGP55845 \((n = 6)\): 59 ± 4.3% vs 61 ± 4.7% vs 4.7 ± 5.7%; \( P < 0.0001 \); one-way ANOVA, Fig. 4A–C).
UP-to-DOWN state transitions and GABA<sub>B</sub> receptors

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

Discussion

Here we have dissociated the contributions of GABA<sub>B1a</sub>- and GABA<sub>B1b</sub>-containing GABA<sub>B</sub> 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<sub>B1a</sub> and GABA<sub>B1b</sub> subunits (hippocampal CA3–CA1, hippocampal mossy fibre – CA3, thalamic and cortical inputs to the lateral amygdala, thalamus and neocortex), the GABA<sub>B1a</sub> subunit has predominantly been found to be presynaptic and the GABA<sub>B1b</sub> 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<sub>B1a</sub> subunit, presumably presynaptic, help control the UP state duration by modulating spontaneous UP-to-DOWN state transitions, whereas receptors containing the GABA<sub>B1b</sub> subunit, most likely

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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<sub>B1a</sub><sup>−/−</sup> mice (B) and GABA<sub>B1b</sub><sup>−/−</sup> 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.

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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-Garcí 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<sub>B1b</sub>-containing receptors are necessary for afferent-evoked termination of the UP state

A, representative traces taken from wild-type (WT), GABA<sub>B1a</sub>−/− and GABA<sub>B1b</sub>−/− 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<sub>B1a</sub>−/− mice but not in GABA<sub>B1b</sub>−/− mice. C, the selective GABA<sub>B</sub> 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 GABAA receptors responsible for afferent-evoked termination of the UP state, it is likely that the GABAA receptors mediate the UP state through activation of inwardly rectifying K+ (GIRK or Kir3) channels (Bettler et al. 2004) and/or by inhibiting dendritic Ca2+ channels of pyramidal cells (Perez-Garcia 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 GABAAergic interneurons acting on postsynaptic GABAA 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 GABAA 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 GABAA receptors may play a powerful role in regulating persistent network activity, and show that receptors containing GABAA1a and GABAA1b subunits have different roles in this regulation.

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**Author contributions**

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