Ionotropic glutamate receptor GluA4 and T-type calcium channel Ca\(\text{v}\)3.1 subunits control key aspects of synaptic transmission at the mouse L5B-POm giant synapse

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Abstract
The properties and molecular determinants of synaptic transmission at giant synapses connecting layer 5B (L5B) neurons of the somatosensory cortex (S1) with relay neurons of the posteriomedial nucleus (POm) of the thalamus have not been investigated in mice. We addressed this by using direct electrical stimulation of fluorescently labelled single corticothalamic terminals combined with molecular perturbations and whole-cell recordings from POm relay neurons. Consistent with their function as drivers, we found large-amplitude excitatory postsynaptic currents (EPSCs) and multiple postsynaptic action potentials triggered by a single presynaptic action potential. To study the molecular basis of these two features, ionotropic glutamate receptors and low voltage-gated T-type calcium channels were probed by virus-mediated genetic perturbation. Loss of GluA4 almost abolished the EPSC amplitude, strongly delaying the onset of action potential generation, but maintaining the number of action potentials generated per presynaptic action potential. In contrast, knockdown of the Ca\(\text{v}\)3.1 subunit abrogated the driver function of the synapse at a typical resting membrane potential of \(-70\) mV. However, when depolarizing the membrane potential to \(-60\) mV, the synapse relayed single action potentials. Hence, GluA4 subunits are required to produce an EPSC sufficiently large to trigger postsynaptic action potentials within a defined time window after the presynaptic action potential, while Ca\(\text{v}\)3.1 expression is essential to establish the driver function of L5B-POm synapses at hyperpolarized membrane potentials.

Introduction
The thalamus plays a role not only in processing information ascending from the periphery but also in integrating inputs descending from cortical brain areas. These cortical inputs are relayed back to higher order cortices, thereby forming cortico-thalamo-cortical loops (Deschénes et al., 1998; Killackey & Sherman, 2003). The greater number of projections exist from cortex to thalamus (Sherman & Guillery, 2009), but these corticothalamic synapses have been less well studied, including the question of how they contribute to higher brain functions such as sensorimotor integration.

Barrel cortex layer 5B (L5B) neurons form giant glomerular synapses harbouring several synaptic junctions on dendrites of relay cells in the posteriomedial nucleus (POm) (Hoogland et al., 1991; Jones, 2007). These giant L5B-POm synapses can switch in an activity-dependent manner between two functional modes: driver and coincidence detector (Groh et al., 2008). The functional properties of these synapses will have a strong impact on the spiking activity in relay neurons, and thereby exert an extensive influence on many cortical regions contacted by the axons of these cells (Jones, 2007; Sherman & Guillery, 2009). The morphological and physiological properties of synapses from L5B somatosensory neurons to POm neurons have been described in rats (Hoogland et al., 1991; Veinante et al., 2000; Groh et al., 2008), yet the equivalent synapse has not yet been characterized in mice. Furthermore, the availability of conditional gene deletion in mice allows us to specifically address the molecular underpinnings of functional properties of this giant synapse.

Here, we studied the subunit composition of ionotropic glutamate receptors defining the properties of the excitatory postsynaptic current (EPSC) and on T-type calcium channels producing low-threshold calcium spikes in response to synaptic inputs. AMPA receptors (AMPARs) mediate fast excitatory synaptic transmission and display a range of different functional properties depending on the composition of different subunits (Dingledine et al., 1999). For example, the GluA4 subunit gives rise to currents with the fastest kinetics among all subunit combinations (Mosbacher et al., 1994). In a previous study we found EPSCs with fast rise and decay times in the rat L5B-POm synapse (Groh et al., 2008). Consistent with these considerations, GluA4 subunits are abundantly expressed in...
the thalamus (Mineff & Weinberg, 2000). Hence, we attempted to selectively delete the GluA4 subunit in relay neurons to study its role in synaptic transmission at the LSB-POm synapse.

Our previous study in the rat LSB-POm synapse also revealed that a single presynaptic action potential can trigger several postsynaptic action potentials, hence functioning as a signal amplifier (Groh et al., 2008). This feature may be related to the well-known switch between two modes of firing, tonic or burst (Linás & Jahnsen, 1982; Jahnsen & Linás, 1984), caused by the activation of a low voltage-gated T-type calcium channel (Perez-Reyes, 2003). This led us to selectively interfere with the expression of the T-type calcium channel Ca$_{3.1}$, known to be highly expressed in POm relay neurons (Talley et al., 1999).

Using stereotaxic viral gene delivery (Wimmer et al., 2004) into the target areas, we visualized LSB-originating synaptic boutons and characterized them by direct electrical stimulation (Groh et al., 2008). Taking advantage of mouse models for targeted molecular perturbations, we used the Cre/IoxP-mediated conditional knockout strategy to achieve POm relay cell-specific deletion of the GluA4 subunit. In addition, viral short hairpin RNA (shRNA) expression was used to knock down the T-type calcium channel subunit Ca$_{3.1}$. The physiological consequences of these perturbations were assessed by whole cell recordings from defined postsynaptic thalamic neurons while stimulating fluorescent protein-labelled synaptically coupled presynaptic terminals projecting from cortical LSB neurons. Consistent with previous findings, the mouse LSB-POm synapse is formed by a large functional driver terminal contacting the proximal dendrites of relay neurons. This study demonstrates that GluA4 and Ca$_{3.1}$ are key molecular components shaping the postsynaptic response of the POm neuron to LSB cortical input.

Materials and methods

**Animal model system**

All experiments were carried out in mice (Mus musculus). Control and Ca$_{3.1}$ knockdown experiments were done in the C57BL6/J mouse strain. The ‘floxed’ GluA4 mouse line was described previously and provided by Prof. Hannah Monyer (Fuchs et al., 2007).

Experiments were conducted in accordance with the German animal welfare guidelines and were approved by the responsible authority (Regierungspräsidium Karlsruhe).

**RNAi constructs**

The sequence information for short hairpin RNA (shRNA) to knockdown Ca$_{3.1}$ of the T-type calcium channel and scrambled version of Ca$_{3.1}$ shRNA as a control were obtained from Dr Daesoo Kim, Korea Advanced Institute of Science and Technology (Park et al., 2010). Oligonucleotide sequences of mismatch (MM) control for generating the Ca$_{3.1}$ shRNA (gcagttcgcacatggcaga) construct: 5’-catccGCG TCTGCACTATGCGACAGttgaacacagatgtTCTGCCATGTGCGAAACTG C; 3’-AAAAGCAATTCCTGGCACATGCGGACacatgtggtcaacTCTGCCA GTTGCGGAACTGCA.

Plasmid cloning

Ca$_{3.1}$ control and MM shRNA oligonucleotides were inserted into NotI/Eagl restriction enzyme sites of an adenovirus-associated virus (AAV) vector containing the U6 promoter. Expression of mOrange was controlled by the CAG promoter, a combination of the cytomegalovirus enhancer element (CMVE) and chicken β-actin (CBA) promoter (Garg et al., 2004). The woodchuck post-transcriptional regulatory element (WPRE) and the bovine growth hormone poly-A (PA) were added to generate pAM-U6-shRNA Ca$_{3.1}$-CAG-mOrange-WPRE-PA (Zufferey et al., 1999). pAM-CAG-iCre-2A-mOrange-WPRE-PA was obtained by fusing codon-improved Cre (iCre) and mOrange by the 2A peptide, resulting in posttranslational cleavage of the two proteins (Shimshak et al., 2002; Tang et al., 2009).

**Recombinant AAV preparation**

The production of AAV-synaptophysin-enhanced green fluorescent protein (EGFP) was as described elsewhere (Schwenger & Kuner, 2010). The recombinant AAV system consists of a vector plasmid (pAM) in which AAV2 inverted terminal repeats flanked the expression cassette. Two helper plasmids, pDP1 and pDP2, carried all genes necessary for packaging the viral DNA into infectious particles. Recombinant AAV chimeric viruses contain a 1 : 1 ratio of AAV1 and AAV2 and the foreign gene (During et al., 2003; Grimm et al., 2003).

**Stereotaxic injection**

Stereotaxic procedures were done as previously described (Wimmer et al., 2004; Groh et al., 2008). Briefly, 2-week-old (postnatal days 12–15) mice were sedated with a 1.5% mix of isoflurane/oxygen. A hole was drilled at the desired position using a dental drill (Osada EXL-40). Virus was loaded into the injection pipette using a capillary (5 µL, BlauBrand, intramARK, Wertheim, Germany) and injected into the brain by applying gentle pressure using a 50-mL syringe connected to the injection pipette. A high titre (approximately 10$^9$ infective particles per mL) AAV-synaptophysin-EGFP virus preparation (1.5 µL) was evenly distributed over five injection sites into S1 of the following x,y coordinates relative to Bregma and midline (in mm) (1) –3, 0; (2) –2.96, –0.2; (3) –2.92, –0.4; (4) –2.88, –0.6; (5) –2.84, –0.8. At each of these positions injection was done at depths (z) from 1.1 to 0.9 from dura. AAV-iCre-mOrange targeting the GluA4 subunit or AAV-shRNAs-mOrange targeted the Ca$_{3.1}$ T-type calcium channel subunits and its mismatch and scramble controls (0.4 µL) were injected into POm (1.7 mm posterior, 1.27 mm lateral, 3.27 mm below from Bregma). The injection pipette was retracted 2 min after injection and the skin was sutured.

**Preparation of acute brain slices**

After 3–4 weeks of virus incubation, acute brain slices were prepared as described previously (Groh et al., 2008). Coronal sections of 200 µm thickness were cut with a vibratome (VT1200S, Leica Microsystems AG, Wetzlar, Germany) in ice-cold artificial cerebrospinal fluid slicing solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 Na$_2$HPO$_4$, 25 NaCO$_3$, 2.5 glucose, 2 MgCl$_2$ and 0.1 CaCl$_2$. The slices were stored in artificial cerebrospinal fluid bath solution. The slices were incubated for 30 min at 37 °C and then kept at room temperature. To maintain the pH at 7.4, solutions were bubbled with a mix 95% O$_2$ and 5% CO$_2$.

**Electrophysiology**

Whole-cell recordings of POm relay cells were performed in acute brain slices at room temperature using an EPC-10 amplifier.
controlled by PatchMaster software (HEKA Electronics, Lambrecht/Pfalz, Germany). Patch pipettes had open tip resistances of 3–5 MΩ and were filled with solution containing (in mM) 130 K-glucuronate, 20 KCl, 10 HEPES, 4 ATP-Mg²⁺, 5 EGTA, 5 Na₂-phosphocreatine and 0.03 Alexa 594 fluor hydrizide. Liquid junction potentials were not corrected. Series resistance was compensated for to 80–90%. Recordings were Bessel filtered at 2.9 kHz and digitized at 40 kHz. Series resistance was compensated for 80–90%. The extracellular solution contained (in mM): 125 NaCl, 2.5 KCl, 1.25 Na₂HPO₄, 25 NaCO₃, 25 glucose, 1 MgCl₂ and 2 CaCl₂, adjusted to pH 7.4 by bubbling with a mix of O₂/CO₂. Drugs (Tocris Bioscience, Ellisville, MO, USA) were added to bath solution comprising 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 50 μM (2R)-amino-5-phosphonovaleric acid (APV) and 3 μM TTA-P2 (Alomone Labs, Jerusalem, Israel). Capillaries for patch pipettes (World Precision Instruments, Sarasota, FL, USA) and double-barrelled theta glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) for the bipolar stimulator were pulled on a P97 horizontal puller (Sutter Instruments, Novato, CA, USA).

**Targeted stimulation of defined presynaptic giant terminals**

Giant terminals were visualized using real-time multi-channel confocal scanning gradient-contrast microscopy (Upright Leica TCS SP5 confocal microscope; Leica Microsystems AG, Wetzlar, Germany) equipped with an additional external photomultiplier tube and SP5 confocal microscope; Leica Microsystems AG, Wetzlar, Germany. Targeted stimulation of defined presynaptic giant terminals

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**Quantification of terminal size and distance from soma**

Labelled synaptic boutons and relay cells were visualized using real-time multichannel confocal imaging of acute brain slices. EGFP-expressing giant terminals were targeted with a bipolar stimulating pipette at the position corresponding to the maximal size of the bouton. The cell body of the thalamic relay cell was visualized either by Alexa 594-filled patch-pipette or AAV-mediated expression of mOrange. All pictures represent two-dimensional image planes. Individual images were imported into ImageJ for quantification and the diameter of boutons was measured. The distances between soma and terminal correspond to the length from the centre of the terminal to the starting branch point of the dendrite. For both measurements the number of observations is lower than the number of recordings because neither bouton diameters nor distances from soma could be reliably extracted from all images.

**Determination of pool size**

We used back-extrapolation of steady-state depression to determine the pool size (Schneeggenburger et al., 1999). Briefly, this approach estimates the cumulative EPSC amplitudes before steady-state depression develops.

**Data analysis**

All the data was analysed with custom written routines using Igor Pro (Wavemetrics Inc., Lake Oswego, OR, USA). Two-sided Student’s t-tests or Mann–Whitney tests were used to determine the statistical significance for comparison of two conditions, and ANOVA or Kruskal–Wallis tests were used for multiple comparisons, depending on normality testing with D’Agostino & Pearson omnibus test using Prism software (GraphPad Software Inc., La Jolla, CA, USA). Results are presented as mean ± SEM unless otherwise noted. P values < 0.05 were considered significant, and P values < 0.01 were considered highly significant.

**Results**

**Labelling and stimulation of corticothalamic giant terminals**

Giant corticothalamic terminals originating from cortical L5B pyramidal neurons were labelled by stereotaxic delivery of AAV-synaptophysin-EGFP to the S1 region of 2-week-old mice (Fig. 1A). The labelled L5B-POm terminals (Fig. 1B) had an average diameter of 3.5 ± 0.1 μm (n = 55). They were typically situated on the primary dendrites of POm relay cells at an average distance from the soma of 18.7 ± 1.2 μm (n = 32) (Fig. 1C and D), suggesting close electrotonic coupling to the soma via the rather thick primary dendrites.

To determine the synaptic properties of the mouse L5B-POm synapse, we electrically stimulated visually identified individual presynaptic terminals with a double-barrel pipette advanced to less than 1 μm from the terminal, while recording from the synaptically connected postsynaptic thalamic relay neuron in the whole-cell configuration (Groh et al., 2008). At a holding potential of −70 mV, the stimulation intensity was gradually increased until an EPSC was evoked (Fig. 1E). Thereafter, the EPSC amplitude did not increase further with greater stimulation intensities (see Methods). This all-or-none behaviour, tested for every recording included in this study, suggests that glutamate release was caused by an action potential triggered in the presynaptic terminal. We performed additional tests to demonstrate that stimulation of the terminal was highly local and exquisitely sensitive to the position of the pipette tip. Displacements larger than 1 μm resulted in a loss of synaptic stimulation (Fig. 1F). First, an L5B-POm terminal connected to the labelled relay neuron was successfully stimulated (Fig. 1F1). When moving the pipette to a structurally unconnected terminal 3 μm away, the postsynaptic response was lost (Fig. 1F2). In less than 10% of the attempted stimulations we recorded multiple different amplitudes at the same site of stimulation, each responding to different stimulation strengths (Fig. 1G). These recordings were not further pursued nor included.

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in the analysis of the data, because selective stimulation of the labelled terminal could not be ascertained.

Properties of evoked EPSCs

Mean EPSC amplitude was 257 ± 33 pA, the 20–80% rise time was 0.8 ± 0.05 ms and the decay time constant was 3.5 ± 0.2 ms (76 synapses obtained from 40 mice). The amplitude of the EPSCs was highly variable (Fig. 1E), possibly reflecting a different number of active zones per giant terminal or differences in postsynaptic receptor number. However, the large amplitudes may also arise from axons forming several giant terminals on the same relay neuron. In this scenario, electrical stimulation of one terminal would elicit release also in the other terminals with delays too short to be resolved in our recordings. The rapid rise and decay kinetics of the evoked EPSCs suggest that most of the synaptic current was mediated by AMPARs. The NMDA receptor antagonist APV showed a tendency to slightly reduce the EPSC (before APV, 327.05 ± 79 pA; after APV, 270 ± 69 pA, n = 4, P = 0.31, t-test). In contrast, the fast component of the EPSC was strongly reduced by the AMPAR antagonist CNQX (70 ± 10 pA, n = 14, P = 0.0002, t-test) (Fig. 1F).
**Synaptic short-term depression**

To determine frequency-dependent properties of transmission, we repetitively stimulated presynaptic terminals ten times at frequencies ranging from 10 to 100 Hz (Fig. 2A). At 10 Hz stimulation, EPSC amplitude had already dropped to 30% that after the first EPSC. Stimulating at 50 Hz or more resulted in a slight accumulation of the postsynaptic current. The extent of depression increased with stimulation frequency (Fig. 2B). The decline in EPSC amplitudes at different frequencies could be described with single exponential functions [time constants: 10 Hz 109 ± 18 ms (n = 17), 20 Hz 35 ± 5 ms (n = 17), 50 Hz 12 ± 2 ms (n = 12), 100 Hz 4 ± 2 ms (n = 9)]. Steady-state depression increased with frequency (Fig. 2C). In summary, these results indicate strong depression of the L5B-POm synapse upon repetitive stimulation.

**Pool size and fraction of the pool released by a single stimulus**

Using the short-term depression data, we estimated the total pool size by back-extrapolation of EPSC amplitudes obtained at 50 Hz stimulation (Fig. 2D) (Schneggenburger et al., 1999; Groh et al., 2008). The pool size was determined to be 448 ± 46 pA (n = 14). A single presynaptic stimulus released 51 ± 7% (n = 14) of the total pool, as determined by dividing the pool size by the EPSC amplitude generated by a single stimulus.

**Recovery from short-term depression**

Next, we determined the time course of recovery from short-term depression using a paired-pulse protocol with varying stimulation time intervals (Fig. 3A). Plotting the relationship between inter-stimulus interval and the EPSC magnitude revealed a doubly exponential time course with a fast time constant of 15 ± 4 ms and a slow time constant of 795 ± 187 ms (n = 7) (Fig. 3B and C). In conclusion, our results predict that the mouse L5B-POm synapse will only work as a relay synapse up to a frequency of approximately 2 Hz (estimated from the recovery time course), but will increasingly fail with higher rates of presynaptic activity. Hence, the L5B-POm terminal functions as a low-pass frequency filter.

**Voltage-dependent scaling of the input–output function in POm neurons**

We next addressed the input–output function of the POm neuron using postsynaptic current clamp recordings in conjunction with presynaptic stimulation. Only in 21% (16 out of 76) of the synapses

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**Fig. 2.** Strong short-term depression of transmission at the L5B-POm synapse. (A) Raw current traces of synaptic stimulation at different frequencies. (B) Frequency dependence of short-term depression. Data are fitted to single exponential functions (n = 9–17). (C) Frequency dependence shown as a function of stimulus number to allow comparison of steady-state depression. (D) Cumulative plot of EPSCs during a 50-Hz train. Data points between 100 and 200 ms were fitted with a linear function (grey dotted line) whose intersection with the ordinate was taken as the size of the pool.

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that we recorded from did a single presynaptic stimulation trigger postsynaptic spikes (Fig. 4A). Interestingly, the EPSC amplitudes recorded earlier in the experiment were not correlated with the number of postsynaptic spikes generated (Pearson coefficient $-0.34$, $P = 0.1$; see examples in Supporting Information Fig. S1). A typical suprathreshold voltage trace recorded at a membrane potential of $-70$ mV is shown in Fig. 4B. Close inspection of the voltage trace typically reveals an initial steep slope corresponding to the rapid EPSC followed by a hump and subsequent less steep slope leading to action potential initiation. The mean delay from the stimulus to the peak of the first action potential was $12 \pm 4$ ms ($n = 12$). In experiments showing suprathreshold responses, a single presynaptic action potential typically elicited $4 \pm 0.5$ ($n = 12$) action potentials in the relay neurons at a hyperpolarized membrane potential. Given the much shorter time scale of the EPSCs, these findings suggest that activation of a voltage-dependent conductance is required to bring the cell to firing threshold. Low voltage-activated T-type calcium channels, well suited to boost excitatory postsynaptic potentialFig. 3. Fast recovery from short-term depression at the L5B-POm synapse. (A) Superimposed raw traces illustrating the paired-pulse experiment. Only a few representative traces are shown; the intervals tested can be inferred from B. (B) Biphasic time course of recovery from short-term depression determined using a paired-pulse protocol ($n = 7$). (C) B shown at higher magnification from 0 to 550 ms.

Fig. 4. L5B-POm inputs produce different postsynaptic responses depending on postsynaptic membrane potential. (A) Correlation of EPSC amplitude and number of postsynaptic action potentials elicited upon a single presynaptic stimulation at a postsynaptic membrane potential of $-70$ mV. (B) Representative example of a recording at $-70$ mV holding potential chosen from recordings obtained from 13 synapses. (C) As in B but recorded at $-60$ mV.
GluA4 is the dominant AMPAR subunit in POm relay neurons

The kinetic profile of the EPSCs from L5B-POm synapses suggests that postsynaptic POm neurons may contain homeric GluA4 subunits, which form AMPARs with the fastest decay kinetics (Fig. 1D) (Mosbacher et al., 1994; Mineff & Weinberg, 2000; Groh et al., 2008). To investigate the role of GluA4 subunits in corticothalamic synaptic transmission, we used stereotoxic delivery of AAV particles encoding Cre-recombinase and mOrange into the POm of mice harbouring conditional GluA4 alleles (Fuchs et al., 2007). At the same time, we injected AAV-synaptophysin-EGFP into the S1 area to label the giant terminals. Then, electrophysiological recordings were established from mOrange-expressing GluA4-deficient POm cells making synaptic contacts with L5B neurons (Fig. 5A). Consistent with our previous study, clearance of existing glutamate receptors from POm neurons required 3–4 weeks after viral gene transfer (Abraham et al., 2010). Expression of AAV-mOrange without Cre recombinase did not affect the EPSC amplitudes [wild-type (WT) 257 ± 33 vs. mOrange control 190 ± 61, n = 5, P = 0.33, t-test], suggesting that synaptic transmission was not substantially affected by this manipulation.

To test whether thalamic neurons respond differently according to the composition of AMPAR subunits by corticothalamic stimulation, we compared EPSCs between WT and GluA4 knockout (KO) neurons. The response evoked by a single presynaptic stimulation was profoundly changed in GluA4+/− (n = 42 synapses, 19 mice) compared with WT (Fig. 5B–E). EPSC amplitudes were decreased to approximately 35% at L5B-POm synapses lacking GluA4 subunits (Fig. 5D). To test if other GluA subunits contribute to the EPSC, we applied CNQX to WT and GluA4+/− slices. CNQX application decreased the EPSC amplitude to approximately 38%, a value similar to the decrease found in GluA4−/− cells (Fig. 5B and D). Hence, the proportion of the current remaining after CNQX (38%) resembled that of GluA4−/− (35%). Also, CNQX only marginally blocked EPSCs of GluA4−/− synapses (GluA4−/− 79 ± 5 pA vs. GluA4+/− with CNQX 59 ± 16 pA, n = 7, P = 0.3, t-test). Therefore, AMPARs at L5B-POm synapses dominantly comprise homeric GluA4 subunits, but they may nevertheless contain a small fraction of GluA1 and GluA3 subunits as well (Mineff & Weinberg, 2000).

![Image](https://example.com/image.jpg)

**Fig. 5.** Strong reduction of AMPAR-mediated currents in GluA4 KO synapses. (A) Online overlay of three channels for targeted electrophysiological recordings; cell morphology (grey), Cre-infected POm neurons expressing mOrange (red) and EGFP-labelled corticothalamic giant terminals (green). Single confocal frames. Scale bar = 10 μm. (B) Postsynaptic currents in WT, GluA4 KO and after CNQX (10 μm) application. (C) Difference in kinetics illustrated by scaling the GluA4−/− EPSC to the peak of a WT EPSC. Fits to the decaying phase of the EPSC are shown as green lines. (D) Bar diagrams representing the mean ±SEM EPSC amplitudes. WT, 257 ± 33 pA (n = 76); GluA4−/−, 79 ± 5 pA (n = 42); CNQX application, 70 ± 10 pA (n = 15) (P < 0.01, Kruskal–Wallis test). Dunn’s post-hoc test assigned a highly significant difference to WT vs. GluA4−/− and CNQX and no difference between GluA4−/− and CNQX. (E) Mean ±SEM decay time constant. WT, 3.5 ± 0.2 ms (n = 76); GluA4−/−, 6.3 ± 0.5 ms (n = 42); CNQX, 6.5 ± 0.7 ms (n = 15) (P < 0.01, Kruskal–Wallis test). Dunn’s post-hoc test assigned a highly significant difference to WT vs. GluA4−/− and CNQX and no difference between GluA4−/− and CNQX.

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The decay time constant, but not rise time of EPSCs in GluA4−/− synapses, was greatly decreased compared with WT synapses [Fig. 5C and E; rise time: WT 0.8 ± 0.1 ms (n = 76), GluA4−/− 0.9 ± 0.1 ms (n = 42), CNQX 0.8 ± 0.1 ms (n = 15), P = 0.5, Kruskal–Wallis test; decay time WT 3.5 ± 0.2 ms, GluA4−/− 6.3 ± 0.5 ms, CNQX 6.4 ± 0.7 ms, P < 0.05], consistent with the absence of fast AMPAR subunits.

Collectively, these results demonstrate that GluA4 is the main subunit mediating AMPAR currents at the L5B-POm synapse (Fig. 5). The small remaining current may be mediated by kainate receptors or other ionotropic receptors (see Discussion). The strategy employed here resulted in a POm neuron-specific deletion of the GluA4 subunit, a perturbation that cannot be achieved with existing pharmacological tools.

Changes in the input–output function in synapses lacking GluA4

We next investigated how the strongly decreased EPSC amplitudes in GluA4-deficient POm neurons would affect the input–output function of the neuron. Current-clamp recordings from GluA4-deficient POm neurons near −70 mV revealed that a single L5B input could drive postsynaptic action potentials in 14% of the synapses recorded from. The latency from the stimulus onset to the peak of the first action potential was markedly prolonged [WT 12 ± 6 ms (n = 12) vs. GluA4−/− 88 ± 17 ms (n = 5), P < 0.01, Mann–Whitney] (Fig. 6A). However, the average number of spikes in GluA4−/− neurons was not significantly different from WT [WT 4 ± 0.5 (n = 12) vs. GluA4−/− 3.3 ± 0.3 (n = 4), P = 0.6, t-test].

When the membrane potential was held at −60 mV, both WT and KO cells generated a single spike, but the latency between stimulus and spike was again prolonged in GluA4−/− neurons [WT 2 ± 0.4 ms (n = 5) vs. GluA4−/− 5.9 ± 0.8 ms (n = 4), P < 0.05, Mann–Whitney] (Fig. 6B). Our EPSC analysis described above suggested that most of the current was carried by AMPAR containing the GluA4 subunit, and hence block of all AMPARs with CNQX should have the same effect on the input–output properties as deletion of the GluA4 subunit. At −70 mV, CNQX application showed a latency of 90 ± 2.7 ms (n = 3) while at −60 mV it was 3.9 ± 0.2 ms (n = 3). The latencies determined from these preliminary measurements tended to be similar to those found in GluA4−/−. Hence, these observations are consistent with the idea that in WT most of the current is passed by AMPARs predominantly consisting of GluA4 subunits. Surprisingly, the remaining current of unknown origin was able to drive postsynaptic action potentials.

Together, the lack of GluA4 in POm relay neurons decreased the temporal precision of signal transfer from L5B neurons to POm relay neurons.

Knockdown of T-type calcium channel Ca3.1 subunit in defined POm relay neurons

To elucidate the molecular basis of the voltage-dependent current elicited by the EPSC in POm neurons, we targeted Ca3.1, a T-type channel subunit prominently expressed in the POm (Talley et al., 1999). Expression of the Ca3.1 subunit was selectively suppressed in the POm nucleus by viral expression of shRNA (Park et al., 2010). Three to 4 weeks after viral injection, we recorded responses from labelled POm relay neurons to depolarizing current injections (Fig. 7A). Knockdown of Ca3.1 eliminated rebound depolarization in all 33 POm relay neurons examined, consistent with the pioneering work performed with Ca3.1-specific shRNA and in global Ca3.1 null mice (Kim et al., 2001; Park et al., 2010). As expected, Ca3.1 knockdown relay neurons consistently displayed

![Fig. 6. Prolonged delay to the first action potential in the absence of GluA4. (A) Representative traces recorded from WT (black) and GluA4 KO (blue) at −70 mV in response to a single presynaptic stimulus are superimposed. (B) Representative traces recorded at a membrane potential of −60 mV. Colour code as in A. Downward deflection of the stimulus artefact is truncated in both panels. Delays were determined as outlined by double-headed arrows. *P < 0.05, **P < 0.01.](image-url)
intact tonic spiking \((n = 33\) cells). Using the specific T-type channel blocker TTA-P2 (Shipe et al., 2008) in WT neurons produced identical results, consistent with the shRNA knockdown of Ca\(_{3.1}\) \((n = 5,\) Fig. 7B). To control for non-specific effects of the shRNAs, we defined two control groups: MM \((n = 23\) cells) and scrambled (SC) \((n = 5\) cells) versions of Ca\(_{3.1}\) shRNA (Park et al., 2010). Both controls consistently retained the ability to initiate low-threshold spikes subsequent to a hyperpolarizing prepulse as observed in both control groups. POm neurons of both groups reproducibly exhibited rebound burst spikes known to occur in WT (Fig. 7C and D), suggesting that no off-target effects or unspecific effects due to the high expression levels of shRNA occurred in the relay cells.

To test whether the Ca\(_{3.1}\) knockdown affected the EPSCs generated by the L5B-POm synapse we compared EPSCs of knockdown with the two controls. Neither EPSC amplitudes \([Ca_{3.1} \text{ knockdown}: 124 \pm 9 \text{ pA} (n = 37), \text{MM}: 134 \pm 27 \text{ pA} (n = 11), \text{SC}: 108 \pm 14 \text{ pA}, n = 4, P > 0.05, \text{ANOVA}])\) nor decay times \([Ca_{3.1} \text{ knockdown}: 0.9 \pm 0.1 \text{ ms} (n = 37), \text{MM}: 0.9 \pm 0.2 \text{ ms} (n = 11), \text{SC}: 0.99 \pm 0.23 \text{ ms} (n = 4), P > 0.05, \text{ANOVA}])\) were statistically discriminable, suggesting that the Ca\(_{3.1}\) knockdown does not affect the EPSC.

In conclusion, the shRNA-mediated knockdown of Ca\(_{3.1}\) specifically abolished rebound-firing without exerting unspecific effects on synaptic transmission.

**Changes in the input–output function of Ca\(_{3.1}\) knockdown relay neurons**

After establishing the functionality of the shRNA-mediated Ca\(_{3.1}\) knockdown, we examined the input–output function of the POm relay neuron in response to synaptic input. A single presynaptic action potential evoked a postsynaptic EPSP \((8.1 \pm 2.4 \text{ mV}, n = 13)\) but consistently failed to evoke burst firing in the Ca\(_{3.1}\)-deficient POm neuron at a membrane potential of \(-70 \text{ mV} (n = 13 \text{ synapses, 13 mice})\) (Fig. 8A, red).

Consistent with the intact tonic discharge observed in Ca\(_{3.1}\)-lacking neurons upon depolarizing current injection (Fig. 7A), we found that synaptic stimulation at a postsynaptic membrane potential of \(-60 \text{ mV}\) consistently produced a single postsynaptic action potential (Fig. 8B, \(n = 5\) synapses). The latency from stimulus to the peak of the action potential was indistinguishable from WT \([Ca_{3.1} \text{ knockdown: 1.1} \pm 0.2 \text{ ms} (n = 5)\) vs. WT \(2 \pm 0.4 \text{ ms} (n = 5), P = 0.2, \text{Mann–Whitney}]\).

These results suggest that T-type calcium channels contribute only marginally, if at all, to synaptic transmission at depolarized membrane potentials, but are essential to produce burst firing at more hyperpolarized potentials in response to a single presynaptic action potential.

**Discussion**

We characterized structural and functional properties of a mouse corticothalamic synapse connecting L5B neurons of the somatosensory cortex to relay neurons of the thalamic POm nucleus. This L5B-POm giant synapse drives excitation of POm relay neurons in a strongly frequency-dependent manner. Individual presynaptic action potentials can trigger multiple postsynaptic action potentials, constituting a synaptic signal amplifier. Deletion of the GluA4 subunit strongly reduced the synaptic current and resulted in a marked delay from the presynaptic action potential to the first postsynaptic action potential, while maintaining the number of postsynaptic action potentials triggered by a single stimulus. Knockdown of the Ca\(_{3.1}\) subunit in POm relay neurons abolished the amplifier function at hyperpolarized potentials, essentially silencing the synapse. Only at depolarized postsynaptic membrane potentials could a one-to-one spike transfer be achieved.

These perturbations demonstrate that the GluA4 subunit provides a major portion of the glutamatergic current required to activate the synaptic amplifier within a short latency after the presynaptic action potential, while the Ca\(_{3.1}\) subunit is essential to establish the

**Fig. 7.** Rebound burst firing abolished in Ca\(_{3.1}\) knockdown but not in shRNA controls. (A) No rebound burst firing in the absence of T-type channel Ca\(_{3.1}\) but preservation of tonic spikes \((n = 33)\). Somatic current injections of \(-300 \text{ pA} \) and \(400 \text{ pA}\) applied for 300 ms. (B) T-type blocker TTA-P2 (3 \(\mu\)M, grey dotted line, \(n = 5\)) selectively blocks burst firing. (C) Mismatch (blue, \(n = 23\)) and (D) scrambled controls (green, \(n = 5\)) exhibit burst and tonic firing.
Single action potential elicited in Ca the physiological signification characterized here will help in future experiments to uncover synaptic amplification of this giant synapse. The two perturbations characterized here will help in future experiments to uncover the physiological significance of membrane potential-dependent synaptic amplification at this remarkable giant synapse.

Structure-function relationship at the L5B-POm synapse and comparison with other giant synapses

The relatively large size (average diameter 3.3 μm, Fig. 1B) of L5B-POm terminals provides sufficient space to accommodate multiple active zones functioning in parallel, similar to other giant terminals such as the calyx of Held or hippocampal mossy fibre terminals (Rollenhagen & Lubke, 2006). Our functional analysis suggests that L5B-POm terminals may contain approximately 22 active zones. In contrast to the calyx of Held, the parallel active zone design does not result in a reliable relay function for the L5B-POm synapse. However, based on the lack of an effect of CNQX after deletion of GluA4 receptors, the role of the thalamus and cortex in initiation of up-states remains of debate, a relatively small change of the resting membrane potential may bring about distinctive differences in POm spiking, thereby possibly reflecting the momentary brain state (Crunelli & Hughes, 2010).

Physiological impact of strong short-term depression at the L5B-POm synapse

The mouse L5B-POm synapse undergoes strong short-term depression at frequencies exceeding a few hertz (Fig. 2), similar to the situation found in the rat L5B-POm synapse (Groh et al., 2008). In the line with this finding, consecutive EPSCs of similar magnitude were induced only for intervals that were longer than about 400 ms (Fig. 3B). In addition to the replenishment of releasable vesicles on the presynaptic side, it is conceivable also that the recovery of T-type calcium channels from inactivation on the postsynaptic side may occur during this time window, with both mechanisms optimizing the strength of synaptic transmission after a period of quiescence. Indeed, this time window correlates well with the period of hyperpolarization that is needed for de-inactivation of T-type calcium channels (Perez-Reyes, 2003; Groh et al., 2008). Hence, a burst response in the POm neurons can be preferentially triggered by low-frequency stimuli (Swadlow & Gusev, 2001; Groh et al., 2008; Rothman et al., 2009). In addition, hyperpolarization of the POm relay neuron by GABAergic and M2 cholinergic inputs will boost the response to subsequent depolarizing L5B inputs through de-inactivation of T-type calcium channels (Barthó et al., 2002; Bokor et al., 2005; Urbain & Deschênes, 2007).

Voltage-dependent switch of synaptic transfer mode

At hyperpolarized membrane potentials, the L5B-POm synapse can function as a driver and amplifier synapse. Essential for this function are T-type calcium channels expressed in POm relay neurons. The EPSP generated by a single L5B-POm synapse suffices to activate T-type calcium channels, resulting in a delayed and long-lasting depolarization that ultimately yields a burst of action potentials (Fig. 4B). Hence, presynaptic spikes will become translated into multiple postsynaptic spikes. Only at more depolarized potentials will the L5B-POm synapse switch to a one-to-one transmission mode because of the inactivation of T-type calcium channels. Cortical activity depolarizes thalamic neurons in the range between −70 and −60 mV, resulting in markedly different firing patterns of POm neurons in response to L5B excitation (Fig. 4) (Dossi et al., 1992). Also, subcortical inputs may adjust the resting membrane potential of POm relay neurons (Lee & Dan, 2012; Poulet et al., 2012). Thus, although the role of thalamus and cortex in initiation of up-states remains of debate, a relatively small change of the resting membrane potential may bring about distinctive differences in POm spiking, thereby possibly reflecting the momentary brain state (Crunelli & Hughes, 2010).

Role of GluA4 at L5B-POm synapse

Our results show that GluA4 plays a predominant role in L5B-POm synapses (Fig. 5), consistent with previous studies demonstrating that GluA4 receptors are linked to the strength of corticothalamic synapses involving synchrony between cortex and thalamus (Golshani et al., 2001; Beyer et al., 2008; Paz et al., 2011) and are abundantly expressed in the thalamus (Mineff & Weinberg, 2000). Loss of GluA4 at L5B-POm synapses results in a strong reduction of the EPSC amplitude and slower kinetics, causing a prolonged spike onset (Fig. 6). The latter may have a strong influence on spike-timing-dependent plasticity and may disrupt synchrony in the cortico-thalamo-cortical system (Abbott & Nelson, 2000; Paz et al., 2011). Both may disrupt the timing of sensorimotor activity.

Gene deletions often result in compensatory changes in the expression levels of other proteins (e.g. upregulation of AMPAR). However, based on the lack of an effect of CNQX after deletion of GluA4, we conclude that such compensatory changes are unlikely. By contrast, the remaining currents in GluA4 KO mice and in the presence of CNQX prompted the question of whether other
ionotropic glutamate receptors, or even other synaptic transmitters, are involved in L5B-POm synaptic transmission (Fig. 5). Kainate receptors are known to be blocked at CNQX concentrations of 10 μM such as used here, suggesting a minor contribution in agreement with previous work (Jones, 2007; Miyata & Imoto, 2009; Traynelis et al., 2010). NMDA receptors are equally unlikely to be involved because they are blocked by Mg2+ at hyperpolarized potentials and a small residual current remained in the presence of APV and CNQX (Fig. 1F). However, NMDAR containing the NR3 subunit may contribute as these are insensitive to APV and Mg2+ block (Pachernegg et al., 2012). The only other glutamate receptor subunits that could be considered are the delta subunits, although their functional properties have remained poorly understood (Lomeli et al., 1993). In principle, also other synaptic transmitters targeting ionotropic receptors, such as acetylcholine, serotonin or ATP, could be co-released with glutamate and generate an EPSC. Apart from these biological considerations, possible technical explanations need to be considered too. Stimulation with the double-barrel pipette may activate other synapses targeting the same postsynaptic neuron or may directly stimulate it. This is unlikely, because recordings showing EPSCs with smaller current amplitudes before reaching the threshold of the main EPSC occurred only in rare cases and were not considered further. Furthermore, recordings showing a postsynaptic response scaling with stimulus intensity were not included in the analysis. Hence, direct activation of postsynaptic voltage-dependent conductances is highly unlikely. Another potential mechanism to consider is electroporation of the postsynaptic dendrite by the high voltage pulse, although such electroporation-mediated currents would mostly flow during application of the pulse until the membrane re-seals, producing a current response entirely different from the one we observed. In conclusion, further studies may reveal the underpinnings of the remaining current and its contribution to synaptic transmission.

Given that T-type conductances mediate between the glutamatergic EPSP and the action potential at hyperpolarized membrane potentials (Fig. 4B), it remains unclear why GluA4 in particular, the ionotropic glutamate receptor subunit conferring the fastest kinetics (Mosbacher et al., 1994), mediates the EPSC in POm relay neurons. We suggest that its rapid kinetics may only be important for precise timing of single action potential transfer at depolarized membrane potentials (Fig. 4C).

**Ca3.1 activates synaptic amplifier at hyperpolarized membrane potentials**

This study shows that the membrane potential-dependent synaptic amplifier function of L5B-POm synapses requires the presence of T-type calcium channel Ca3.1 subunits (Fig. 8). This is consistent with results obtained from relay neurons in Ca3.1 global KO mice (Tschetter et al., 2011) and that knockdown of the Ca3.1 gene abolished burst firing in POm neurons, demonstrating that Ca3.1 contributes most of the T-type Ca2+ current in POm relay neurons (Fig. 8A) (Park et al., 2010).

Bursts of action potentials in POm neurons have been implicated in neuronal oscillations, thereby recruiting large-scale networks (Crnelli & Hughes, 2010). Given the widespread outputs from the POm nucleus via cortico-thalamo-cortical loops, temporally linked synchronization is predicted to be disrupted by the lack of T-type calcium channels, which in turn may lead to reduced or impaired transmission from S1 to other cortical areas during sensorimotor processing (Wang et al., 2010). Furthermore, the brief bursts generated by synaptic amplification could facilitate cortical synaptic terminals of relay neurons, thereby increasing the strength of synaptic transmission onto cortical neurons. This mechanism could be used to gate thalamocortical information transfer.

**Supporting Information**

Additional supporting information can be found in the online version of this article:

Fig. S1. Examples of EPSCs and corresponding voltage responses.

**Conflict of interest**

The authors state no conflict of interest.

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**Abbreviations**

AAV, adeno-associated virus; AMPAR, AMPA receptor; APV, (2R)-amino-5-phosphonovaleric acid; CBA, chicken ß-actin; CMVE, cytomegalovirus-adenovirus; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EGF, enhanced green fluorescent protein; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; KO, knockout; L5B, layer 5B; MM, mismatch; PA, poly-A; POm, posteriomedial nucleus; SC, scrambled; shRNA, short hairpin RNA; WPRE, woodchuck post-transcriptional regulatory element; WT, wild-type.

**References**

Abbott, L.F. & Nelson, S.B. (2000) Synaptic plasticity: taming the beast. *Nat. Neurosci.*, 3, 1178–1183.

Abraham, N.M., Egger, V., Shimshek, D.R., Renden, R., Fukunaga, I., Sprengel, R., Seeburg, P.H., Klugmann, M., Margrie, T.W., Schaefer, A.T. & Kuner, T. (2010) Synaptic inhibition in the olfactory bulb accelerates odor discrimination in mice. *Neuron*, 65, 399–411.

Barthó, P.P., Freund, T.F.T. & Ascady, L.L. (2002) Selective GABAergic innervation of thalamic nuclei from zona incerta. *Eur. J. Neurosci.*, 16, 999–1014.

Beyer, B., Deleuze, C., Letts, V.A., Mahaffey, C.L., Boumil, R.M., Lew, T.A., Huguenard, J.R. & Frankel, W.N. (2008) Absence seizures in C3H/HeJ and knockout mice caused by mutation of the AMPA receptor subunit GluA4. *Glia*, 56, 1738–1749.

Bokor, H., Frere, S.G., Eyer, M.D., Slezia, A., Ulbert, L., Luthi, A. & Ascady, L. (2005) Selective GABAergic control of higher-order thalamic relays. *Neuron*, 45, 929–940.

Crunelli, V. & Hughes, S.W. (2010) The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat. Neurosci.*, 13, 9–17.

Deleuze, C., David, F., Béhuret, S., Sadoc, G., Shin, H.-S., Uebele, V.N., Renger, J.J., Lambert, R.C., Leresche, N. & Bal, T. (2012) T-type calcium channels consolidate tonic action potential output of thalamic neurons to neocortex. *J. Neurosci.*, 32, 12228–12236.

Deschênes, M., Vieimara, P. & Zhang, Z.-W. (1998) The organization of corticothalamic projections: reciprocity versus parity. *Brain Res. Brain Res. Rev.*, 28, 286–308.

Dingleline, R., Borges, K., Bowie, D. & Traynelis, S.F. (1999) The glutamate receptor ion channels. *Pharmacol. Rev.*, 51, 7–61.

Dossi, R.C., Nunez, A. & Steriade, M. (1992) Electrophysiology of a slow (0.5–4 Hz) intrinsic oscillation of cat thalamocortical neurons in vivo. *J. Physiol.*, 447, 215–234.

During, M.J., Young, D., Baer, K., Lawlor, P. & Klugmann, M. (2003) Development and optimization of adeno-associated virus vector transfer into the central nervous system. *Meth. Mol. Med.*, 76, 221–236.
Perez-Reyes, E. (2003) Molecular physiology of low-voltage-activated calcium channels. *Physiol. Rev.*, **83**, 117–161.

Poulet, J.F.A., Fernandez, L.M.J., Crochet, S. & Petersen, C.C.H. (2012) Thalamic control of cortical states. *Nat. Neurosci.*, **15**, 370–372.

Rollenhagen, A. & Lubke, J.H. (2006) The morphology of excitatory central synapses: from structure to function. *Cell Tissue Res.*, **326**, 221–237.

Rothman, J.S., Cathala, L., Steuber, V. & Silver, R.A. (2009) Synaptic depression enables neuronal gain control. *Nature*, **457**, 1015–1018.

Schneggenburger, R., Meyer, A.C. & Neher, E. (1999) Released fraction and total size of a pool of immediately available transmitter quanta at a calyx synapse. *Neuron*, **23**, 399–409.

Schwenger, D.B. & Kuner, T. (2010) Acute genetic perturbation of exocyst function in the rat calyx of Held impedes structural maturation, but spares synaptic transmission. *Eur. J. Neurosci.*, **32**, 974–984.

Sherman, S.M. & Guillery, R.W. (2009) *Exploring the Thalamus and its Role in Cortical Function*. MIT Press, Cambridge, MA.

Shimshek, D.R., Kim, J., Hubner, M.R., Spergel, D.J., Buchholz, F., Casa nova, E., Stewart, A.F., Seeburg, P.H. & Sprengel, R. (2002) Codon improved Cre recombinase (iCre) expression in the mouse. *Genesis*, **32**, 19–26.

Shipe, W.D., Barrow, J.C., Yang, Z.Q., Lindsley, C.W., Yang, F.V., Schlegel, K.A., Shu, Y., Rittle, K.E., Bock, M.G., Hartman, G.D., Tang, C., Ballard, J.E., Kuo, Y., Adarayan, E.D., Prueksaritanont, T., Zrada, M.M., Uebele, V.N., Nuss, C.E., Connolly, T.M., Doran, S.M., Fox, S.V., Kraus, R.L., Marino, M.J., Graufelds, V.K., Vargas, H.M., Bunting, P.B., Has bun-Manning, M., Evans, R.M., Koblan, K.S. & Renger, J.J. (2008) Design, synthesis, and evaluation of a novel 4-aminomethyl-4-fluoropiperidine as a T-type Ca2+ channel antagonist. *J. Med. Chl.*, **51**, 3692–3695.

Swadlow, H.A. & Gusev, A.G. (2001) The impact of ‘bursting’ thalamic impulses at a neocortical synapse. *Nat. Neurosci.*, **4**, 402–408.

Talley, E.M., Criibs, L.L., Lee, J.H., Daud, A., Perez-Reyes, E. & Bayliss, D.A. (1999) Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. *J. Neurosci.*, **19**, 1895–1911.

Tang, W., Ebhich, I., Woff, S.B.E., Michalski, A.-M., Woff, S., Hasan, M.T., Lithi, A. & Sprengel, R. (2009) Faithful expression of multiple proteins via 2A-peptide self-processing: a versatile and reliable method for manipulating brain circuits. *J. Neurosci.*, **29**, 8621–8629.

Traynelis, S.F., Wollmuth, L.P., McBay, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J. & Dingledine, R. (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.*, **62**, 405–496.

Tschetter, A., David, F., Ivanova, T., Deleuze, C., Renger, J.J., Uebele, V.N., Shin, H.S., Bal, T., Leresche, N. & Lambert, R.C. (2011) Minimal alterations in T-type calcium channel gating markedly modify physiological firing dynamics. *J. Physiol.*, **589**, 1707–1724.

Ulrich, D. & Huguenard, J.R. (1997) GABA A-receptor-mediated rebound burst firing and bursting in thalamus. *J. Neurophysiol.*, **78**, 1748–1751.

Urbain, N. & Deschênes, M. (2007) Motor cortex gates vibrisse responses in a thalamocortical projection pathway. *Neuron*, **56**, 714–725.

Veinante, P., Lavallée, P. & Deschénes, M. (2000) Corticothalamic projections from layer 5 of the vibrissal barrel cortex in the rat. *J. Comp. Neurol.*, **424**, 197–204.

Wang, H.-P., Spencer, D., Fellous, J.-M. & Sejnowski, T.J. (2010) Synchrony of thalamocortical inputs maximizes cortical reliability. *Science*, **328**, 106–109.

Wimmer, V.C., Nevan, T. & Kuner, T. (2004) Targeted in vivo expression of proteins in the calyx of Held. *Pflüg. Arch.*, **449**, 319–333.

Zufferey, R., Donello, J.E., Trono, D. & Hope, T.J. (1999) Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.*, **73**, 2886–2892.