Excitatory But Not Inhibitory Synaptic Transmission Is Reduced in Lethargic (Cacnb4<sup>lh</sup>) and Tottering (Cacna1a<sup>tg</sup>) Mouse Thalami

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Caddick, Sarah J., Chunsheng Wang, Colin F. Fletcher, Nancy A. Jenkins, Neal G. Copeland, and David A. Hosford. Excitatory but not inhibitory synaptic transmission is reduced in lethargic (Cacnb4<sup>lh</sup>) and tottering (Cacna1a<sup>tg</sup>) mouse thalami. J. Neurophysiol. 81: 2066–2074, 1999. Recent studies of the homozygous tottering (Cacna1a<sup>tg</sup>) and lethargic mouse (Cacnb4<sup>lh</sup>) models of absence seizures have identified mutations in the genes encoding the α1A and β4 subunits, respectively, of voltage-gated Ca<sup>2+</sup> channels (VGCCs). β subunits normally regulate Ca<sup>2+</sup> currents via a direct interaction with α1 ( pore-forming) subunits of VGCCs, and VGCCs are known to play a significant role in controlling the release of transmitter from presynaptic nerve terminals in the CNS. Because the gene mutation in Cacnb4<sup>lh</sup> homozygotes results in loss of the β4 subunit’s binding site for α1 subunits, we hypothesized that synaptic transmission would be altered in the CNS of Cacnb4<sup>lh</sup> homozygotes. We tested this hypothesis by using whole cell recordings of single cells in an in vitro slice preparation to investigate synaptic transmission in one of the critical neuronal populations that generate seizure activity in this strain, the somatosensory thalamus. The primary finding reported here is the observation of a significant decrease in glutamatergic synaptic transmission mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors in somatosensory thalamic neurons of Cacnb4<sup>lh</sup> homozygotes compared with matched, nonepileptic mice. In contrast, there was no significant decrease in GABAergic transmission in Cacnb4<sup>lh</sup> homozygotes nor was there any difference in effects mediated by presynaptic GABAA receptors. We found a similar decrease in glutamatergic but not GABAergic responses in Cacna1a<sup>tg</sup> homozygotes, suggesting that the independent mutations in the two strains each affected P/Q channel function by causing defective neurotransmitter release specific to glutamatergic synapses in the somatosensory thalamus. This may be an important factor underlying the generation of seizures in these models.

INTRODUCTION

A variety of genetic models of absence epilepsy exhibit spontaneous spike-wave discharges (SWDs) that share electrophysiological, behavioral and pharmacological properties with absence seizures in humans (Berkovic 1997a,b; Williams 1953). Before the genes causing seizures in these models were identified, phenomenologic approaches were used to delineate three neuronal populations comprising the cellular network that generates SWDs in animal models: neocortical pyramidal neurons, thalamic relay neurons, and GABAergic neurons of the nucleus reticularis thalami (NRT) (Caddick and Hosford 1996a; Cruenelli and Leresche 1991; Huguenard and Prince 1994; Snead 1995; Steriade et al. 1993). SWDs are generated when a physiological (tonic) mode of neuronal firing shifts to a burst-firing mode (see Cruenelli and Leresche 1991; Steriade and Llinás 1988; Steriade et al. 1993). An intrinsic conductance critical in the shift from tonic to burst-firing mode is I<sub>T</sub>, the low-threshold calcium current, (Coulter et al. 1993a,b; Cruenelli and Leresche 1991; Roy et al. 1984; Suzuki and Rogawski 1989; White et al. 1989).

Positional cloning techniques were used recently to identify the genes causing absence seizures in two models: the gene encoding the α1A subunit of voltage-gated calcium channels (VGCCs) in the homozygous tottering (Cacna1a<sup>tg</sup>) mouse (Fletcher et al. 1996) and the gene encoding the β4 subunit of VGCCs in the homozygous lethargic (Cacnb4<sup>lh</sup>) mouse (Burgess et al. 1997). These findings permit investigations of the molecular mechanisms through which these gene mutations cause the SWDs of absence seizures. The α1A subunit of VGCCs forms the pore of P/Q-type calcium channels (Mori et al. 1991; Randall and Tsiens 1995; Sather et al. 1993; Starr et al. 1991; Sta et al. 1994; Zhang et al. 1993), which subserve a variety of functions including neurotransmitter release at presynaptic sites (reviewed by McCluskey 1994). The β4 subunit is one of at least four subtypes of β subunits, each of which enhances calcium flux by binding to regulatory domains on α1 subunits (Castellano et al. 1993; Josephson and Varadi 1996; Lacerda et al. 1994; Massa et al. 1995; Perez-Garcia et al. 1995; for review, see Castellano and Perez-Reyes 1994; Carterall 1991; McCluskey 1994; Miller 1992).

Because the mutated β4 subunit in Cacnb4<sup>lh</sup> homozygotes lacks the binding domain necessary for regulation of α1 subunits (Pragnell et al. 1994), we hypothesized that β4 subunits in these mice would be rendered nonfunctional, resulting in decreased calcium influx after activation of VGCCs during synaptic transmission. In principle, decreased availability of calcium in the nerve terminal would result in decreased transmitter release with a consequent reduction in postsynaptic responses (for review, see Dolphin 1995, 1996; Tarelius and Breer 1995). In this study, we addressed this hypothesis by examining excitatory and inhibitory synaptic transmission at a synapse that is critical to the generation of absence seizures in Cacnb4<sup>lh</sup> homozygotes, the ventrobasal (VB) thalamic nucleus...
(Caddick and Hosford 1996a; Hosford et al. 1995a). We also tested the related hypothesis that postsynaptic responses would be affected in a similar manner in Cacnala\(^{8}\) homozygotes, by virtue of aberrant P/Q-channel function. The primary finding reported here is a significant decrease in glutamatergic synaptic transmission in both mutant strains in contrast to a lack of effect on GABAergic transmission at presynaptic or postsynaptic sites. Because the decrease in excitatory responses affects both \(N\)-methyl-D-aspartate (NMDA) and non-NMDA responses in Cacnb\(^{4b}\) homozygotes, these findings suggest a selective decrease in calcium flux at excitatory synapses in VB neurons. Preliminary reports of these findings have been presented elsewhere (Caddick and Hosford 1997; Caddick et al. 1997).

METHODS

Mouse colonies

Colonies of Cacnb\(^{4b}\) homozygotes and their nonepileptic background control strain [designated \(+/-\); descended from (C57BL/6 Ji X C3H/HeSnJ) F\(_2\), \(F_2\)] were maintained in the Duke University vivarium. Male lethargic heterozygotes (Cacnb\(^{4b}\)/+) were bred with female lethargic heterozygotes to produce 25\% homozygous Cacnb\(^{4b}\) in the progeny. By 14 days of age, these Cacnb\(^{4b}\)/+ homozygotes were distinguished from their phenotypically normal heterozygous lethargic and \(+/-\) littermates by the presence of an ataxic gait.

Colonies of Cacnala\(^{8}\) homozygotes and their background control strain (C57BL/6) were maintained at the Advanced Biosciences Labs-National Cancer Institute vivarium. Through crosses in which the \(tg\) allele was maintained in repulsion to the semidominant allele Os, which causes oligosyndactylism, Cacnala\(^{8}\) homozygotes could be recognized at birth by verifying the lack of oligosyndactylism. Matched Cacnala\(^{8}\) homozygotes and C57BL/6J mice were shipped to D. A. Hosford’s lab for study.

All mice received food and water ad libitum, and they were maintained on a 12 h/12 h light/dark cycle. Animal care and use adhered to guidelines proposed in Guide for the Care and Use of Laboratory Animals (National Institutes of Health 1985) and to guidelines of the American Physiological Society. Animal use was monitored by the local Institute Animal Care and Use Committees (IACUCs) of Duke University and the Durham Veterans Affairs Medical Center.

In vitro slice preparation

The experiments in this study were performed on male, age-matched (P14–28) pairs of either Cacnala\(^{8}\) homozygotes and \(+/-\) mice or Cacnala\(^{8}\) homozygotes and C57BL/6J mice. Briefly, mice were anesthetized under halothane before removing the brains in oxygenated (95\% \(O_2\), 5\% \(CO_2\)) cold (\(4^\circ\)C) artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 25 NaHCO\(_3\), 10 glucose, 1 MgCl\(_2\), 2 CaCl\(_2\), 3.3 KCl, and 1.25 NaH\(_2\)PO\(_4\). Brains were glued, mice or Cacna1a

oxygenated (95\% \(O_2\) -5\% \(CO_2\)) cold (4°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 25 NaHCO\(_3\), 10 glucose, 1 MgCl\(_2\), 2 CaCl\(_2\), 3.3 KCl, and 1.25 NaH\(_2\)PO\(_4\). Brains were glued, mice or Cacna1a

Matched (P14 –28) pairs of either Cacnb4 were pulled on a Narashige PB-7 with resistance of 2–5 M\(_\Omega\). Glass microelectrodes

Intracellular recordings: all mice

Whole cell voltage-clamp recordings of VB cells were made using the blind patch technique (Blanton et al. 1989). Glass microelectrodes (WPI) were pulled on a Narashige PB-7 with resistance of 2–5 M\(_\Omega\). For initial experiments measuring excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs), microelectrodes were filled with solution containing (in mM) 100 N-methyl-D-glucamine, 100 methanesulfonic acid, 40 CsF, 2 MgCl\(_2\), 10 HEPES, and 1 lidocaine N-ethyl-bromide (QX-314; pH 7.2 with CsOH). In later experiments examining NMDA and non-NMDA responses, microelectrodes were filled with solution containing (in mM) 120 cesium gluconate, 10 HEPES, 10 cesium EGTA, 4 QX-314, and 2 MgATP (pH 7.25; osmolality 280–290 mosmol). The QX-314 served not only to occlude postsynaptic GABA\(_B\) responses but also to prevent the generation of action potentials that could contaminate measurements of EPSCs or IPSCs. Recordings were made using a whole cell amplifier (Warner PC 501-A) and stored on-line using the Strathclyde Software acquisition and analysis package (J. Dempster, Strathclyde University). Cells initially were held at \(-60\) mV and input resistance determined by a series of voltage steps (\(I-V\)) from \(-100\) to \(-40\) mV. An adjacent stimulating electrode (monopolar tungsten; WPI) delivered current ranging from 5 to 40 \(\mu\)A (80 \(\mu\)S) every 15 s. The current at which the maximal response was obtained was noted and used for subsequent stimulations (see following text). Series resistance was monitored, and cells were rejected if this increased >15 M\(_\Omega\). Additional pharmacological blockers then were added to the external bathing medium as described in the next section, depending on the responses to be studied.

EPSCs and IPSCs

Cacnb4\(^{th}\) HOMOZYGOTES. In initial experiments EPSCs were isolated by adding bicuculline methiodide (BMI, 5 \(\mu\)M) and CGP 55845A (1 \(\mu\)M) to the bathing medium; along with QX-314 (1 mM) already present in the internal solution, these agents blocked all GABA-mediated responses. Cells were held at \(-60\) mV. In later experiments that were designed to measure the respective contributions of NMDA and non-NMDA responses to the EPSCs, cells were held at +30 mV to remove magnesium block and to minimize rundown, thereby maximizing NMDA responses; 6,7-dinitroquinazoline (DNQX; 20 \(\mu\)M) also was added to the bathing medium to eliminate non-NMDA responses. Stimulus-response (I/O) curves were obtained for all cells, and subsequent stimulation was set to achieve maximal-amplitude events.

GABA\(_A\)-receptor-mediated IPSCs were isolated by adding DNQX and \(\alpha\)-amino-5-phosphonovaleric acid (\(\alpha\)-APV; both 20 \(\mu\)M); internal QX-314 and external CGP 55845A assured the absence of postsynaptic GABA\(_B\) responses. Cells were held at 0 mV to increase the driving force for chloride and to optimize IPSC amplitude. Stimulus-response (I/O) curves were obtained for all cells and subsequent stimulation was set to achieve maximal-amplitude events.

The contribution of presynaptic GABA\(_A\) receptors to IPSC amplitudes was examined by delivering paired stimuli at the maximal stimulus (assessed as in preceding text), using a 200-ms interpulse interval. For each pair of pulses, the ratio of response amplitudes was calculated. The amount of paired-pulsed depression stemming from activation of presynaptic GABA\(_A\) receptors then was assessed by delivering a second set of paired pulses using the same parameters, but in the presence of CGP 55845A (1 \(\mu\)M).

Cacnala\(^{8}\) HOMOZYGOTES. EPSCs and IPSCs were measured at the same time; GABA\(_A\) responses were occluded by including QX-314 (1 mM) in the internal solution. Cells were held at \(-60\) mV. Stimulus-response (I/O) curves were obtained for all cells and subsequent stimulation was set to achieve maximal-amplitude events.

Analysis/statistics

Data were averaged and compared using an unpaired Student’s \(t\)-test. All numerical means and all error bars are expressed as means ± SE.
RESULTS

Recordings were obtained from 58+/+ and 60 homozygous Cacnb4<sup>lh</sup> VB cells (Fig. 1) and from 6 C57BL/6J and 5 homozygous Cacna1a<sup>tg</sup> VB cells. On breakthrough, cells were held in voltage clamp at −60 mV and subjected to a series of voltage steps from −100 to −40 mV. Input resistance of each cell then was calculated. Input resistance was not significantly different between Cacnb4<sup>lh</sup> homozygotes and +/- nor between Cacna1a<sup>tg</sup> homozygotes and C57Bl/6J (Table 1), similar to observations in a previous study (Caddick and Hosford 1996a).

Evaluation of glutamatergic synaptic transmission: Cacnb4<sup>lh</sup> homozygotes

In the initial set of experiments using slices from Cacnb4<sup>lh</sup> homozygotes, VB cells were clamped at −60 mV in the presence of BMI (5 μM). Synaptically evoked EPSCs were recorded by applying current locally through an adjacent stimulating electrode. Increasing intensities of stimulus were given to assess the stimulus necessary to produce a maximal response amplitude. This stimulus then was applied every 15 s, and the responses were collected. Under these conditions, the peak amplitude and latency to peak were measured and compared between +/- and Cacnb4<sup>lh</sup> homozygotes. There was no significant difference in the latency to peak (+/+: 5 ± 0.2 ms, n = 10; Cacnb4<sup>lh</sup> homozygotes: 6 ± 0.5 ms, n = 11). However, there was a significant increase in the peak amplitude of the EPSC in +/- versus Cacnb4<sup>lh</sup> homozygotes, (+/+: 1.1 ± 0.1 nA; Cacnb4<sup>lh</sup> homozygotes: 0.4 ± 0.08 nA; P < 0.001; Fig. 1). The reduction in EPSC amplitude in Cacnb4<sup>lh</sup> homozygotes was evident at all stimulus intensities tested (Fig. 2) and can be seen as a significant shift in the input/output curve. This result indicates that the difference in amplitude can be found regardless of the strength of synaptic stimulation that may be present under physiological or pathological conditions and regardless of the number of synaptic inputs stimulated.

The inclusion of QX-314 in the recording electrode assured the lack of action potential generation, which if present could contaminate EPSC measurements. To verify that the EPSCs were not contaminated by other voltage-gated currents, we examined the reversal potential of the EPSCs and found that it was 0 mV. Because a considerably more positive reversal potential would be expected if the EPSCs were contaminated by other voltage-gated currents such as $I_{Na}$, these data indicate that the EPSCs were uncontaminated.

TABLE 1. Intrinsic membrane properties in VB neurons in Cacnb4<sup>lh</sup> homozygotes and +/- controls

<table>
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<tr>
<th></th>
<th>Cacnb4&lt;sup&gt;lh&lt;/sup&gt; Homozygotes</th>
<th>+/- Mice</th>
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<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>−58.8 ± 0.4</td>
<td>−59.5 ± 0.4</td>
</tr>
<tr>
<td>Input resistance</td>
<td>132 ± 14</td>
<td>138 ± 20</td>
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</table>

Values are means ± SE.
Cacnb4lh homozygotes: 22 (addition of 20 under conditions that would occlude non-NMDA responses magnesium block (see METHODS). Under these conditions, we

amplitudes of NMDA EPSCs (difference between the amplitudes of total and NMDA EPSCs. The amplitudes of non-NMDA EPSCs were calculated as the dif-

ference between the amplitudes of total and NMDA EPSCs (bottom; 353 ± 6

11; Cacnb4lh homozygotes: 152 ± 30 pA; *P < 0.001; Fig. 3). Cells then were examined under conditions that would occlude non-NMDA responses (addition of 20 μM DNXQ to the bathing medium). The amplitudes of non-NMDA EPSCs were calculated as the difference between the amplitudes of total and NMDA EPSCs. The amplitudes of NMDA EPSCs (+/+: 73 ± 12 pA, n = 8; Cacnb4lh homozygotes: 22 ± 14 pA, n = 9; P < 0.05; Fig. 4) and non-NMDA EPSCs (+/+: 280 ± 58 pA;

Evaluation of GABAergic synaptic transmission: Cacnb4lh homozygotes

VB cells in slices from Cacnb4lh homozygotes were clamped at 0 mV in the presence of N-APV and DNXQ (20 μM). Synaptically evoked IPSCs were recorded by applying current locally through an adjacent stimulating electrode, and stimuli of increasing intensities were given to assess maximal response amplitude. The stimulus then was applied every 15 s and the responses collected (Fig. 5). Under these conditions, the peak amplitude and latency to peak were measured and compared between +/+ and Cacnb4lh homozygotes. There was no significant difference in the latency to peak (+/+: 6 ± 0.2 ms; Cacnb4lh homozygotes: 6 ± 0.1 ms) nor was there a significant difference in peak amplitude of the IPSC in +/+ versus Cacnb4lh homozygotes (+/+: 2.7 ± 0.4 nA; Cacnb4lh homozygotes: 1.8 ± 0.3 nA).

The contribution of presynaptic GABA receptors to GABAergic transmission was assessed by comparing paired-pulse depression (PPD) of IPSC amplitudes using a 200-ms interpulse interval. There was no significant difference in the percent of PPD between strains (+/+: 29 ± 3%; Cacnb4lh homozygotes: 31 ± 3%; Cacnb4lh homozygotes: 1.8 ± 0.3 nA).

Further experiments were carried out to determine the glutamate subtype(s) responsible for reduced EPSC amplitudes in VB neurons from Cacnb4lh homozygotes. The rationale for these experiments was to provide information that would help determine whether the reduction in EPSC amplitudes stemmed from presynaptic or postsynaptic mechanisms. Conditions were optimized for NMDA components by holding cells at a sufficiently depolarized potential (+30 mV) to remove the magnesium block (see METHODS). Under these conditions, we reproducibly observed reduced amplitudes of EPSCs in VB neurons (n = 11) (Fig. 3). EPSCs then were examined under conditions that would occlude non-NMDA responses (addition of 20 μM DNXQ to the bathing medium). The amplitudes of non-NMDA EPSCs were calculated as the difference between the amplitudes of total and NMDA EPSCs. The amplitudes of NMDA EPSCs (+/+: 353 ± 75 pA, n = 11; Cacnb4lh homozygotes: 152 ± 37 pA, n = 11; P < 0.001; Fig. 3). Cells then were examined under conditions that would occlude non-NMDA responses (addition of 20 μM DNXQ to the bathing medium). The amplitudes of non-NMDA EPSCs were calculated as the difference between the amplitudes of total and NMDA EPSCs. The amplitudes of NMDA EPSCs (+/+: 73 ± 12 pA, n = 8; Cacnb4lh homozygotes: 22 ± 14 pA, n = 9; P < 0.05; Fig. 4) and non-NMDA EPSCs (+/+: 280 ± 58 pA;
homozygotes: 35 ± 15%; n = 9 pairs; Fig. 6). Likewise, there was no significant difference in the portion of PPD that was blocked by CGP 55845A and hence GABA B receptor dependent (1/1: 14 ± 5%; Cacnb4 lh homozygotes: 18 ± 15%; n = 9 pairs).

Comparison of EPSC/IPSC A and EPSP/IPSP A pairs in Cacna1a tg and C57BL/6J mice

There was no difference in resting membrane potentials, input resistances, or membrane time constants in Cacna1a tg homozygotes (n = 5) compared with C57BL/6J (n = 6) cells (Table 2). This paralleled the lack of differences in these measures in VB cells from Cacnb4 lh homozygotes and +/+ mice (Table 1). Maximal stimulus-evoked EPSCs were significantly smaller in Cacna1a tg homozygotes than C57BL/6J VB cells (Cacna1a tg homozygotes: 0.36 ± 0.08 nA; C57BL/6J: 0.61 ± 0.26 nA; P < 0.05; Fig. 7). In contrast, there was no significant difference in maximal evoked IPSC A s (Cacna1a tg homozygotes: 0.32 ± 0.12 nA; C57BL/6J: 0.33 ± 0.09 nA; Fig. 7).

To further compare excitatory and inhibitory responses in VB neurons from Cacna1a tg homozygotes and C57BL/6J, locally evoked synaptic potentials also were recorded in these neurons under current clamp. The maximal amplitude of the EPSP recorded in VB neurons in Cacna1a tg homozygotes (6.3 ± 1.3 mV) was significantly reduced (P < 0.05) compared with C57BL/6J (11.7 ± 1.3 mV; Fig. 8). In contrast, there was no significant difference in maximal amplitude of the IPSP A between the strains (Cacna1a tg: 6.7 ± 1.1 mV; C57BL/6J: 4.2 ± 2.3 mV; Fig. 8).

**DISCUSSION**

The principal finding emerging from this study was a significant decrease in the amplitude of EPSCs but not IPSCs in VB neurons of both Cacnb4 lh and Cacna1a tg homozygotes compared with their respective nonepileptic controls. The observed decrease in EPSC amplitudes was apparent in both NMDA and non-NMDA components of Cacnb4 lh homozygotes, strongly suggesting that the decrease stemmed primarily from a presynaptic defect in transmitter release from glutamatergic terminals. A second finding was a lack of any difference in effects mediated by presynaptic GABA B receptors on inhibitory afferents to VB neurons in Cacnb4 lh homozygotes, underscoring the selectivity of altered excitatory transmission at this synapse.

<table>
<thead>
<tr>
<th>TABLE 2. Intrinsic membrane properties in VB neurons in Cacna1a tg homozygotes and C57BL/6J controls</th>
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<tr>
<td>Cacna1a tg Homozygotes</td>
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<tr>
<td>Resting membrane potential, mV</td>
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<td>Input resistance</td>
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Values are means ±SE.
two possible explanations for this result: glutamate but not GABA release from terminals in VB synapses is modulated by VGCCs that are affected by the mutated β4 subunit or glutamate release (significant, 54% decrease in EPSCs) is affected to a much greater extent than GABA release (nonsignificant but detectable 33% decrease in IPSCs).

The first of these explanations would imply that VGCC subtypes with differing requirements for a functional β4 subunit mediate glutamate and GABA release at terminals in VB synapses. A precedent for this idea is provided by findings that suggest that selected VGCC subtypes mediate glutamate as opposed to GABA release. In hippocampal CA1 synapses, glutamate release was more dependent on P/Q-type than N-type VGCCs (Burke et al. 1993; Potier et al. 1993), whereas GABA release was more dependent on N-type than P/Q-type VGCCs (Potier et al. 1993). In cultured, synaptically connected thalamic neurons, glutamate release was mediated by non-N- and non-L-type high-threshold calcium currents (Pfrieger et al. 1992), suggesting the possibility that P/Q-type VGCCs were among the population of high-threshold VGCCs that mediated glutamate release. Likewise, in fibers synapsing on Purkinje cells, P- and Q-type VGCCs mediated excitatory transmission, whereas non-P/Q -type VGCCs mediated GABAAergic transmission (Doroshenko et al. 1997).

Alternatively, a second explanation for our findings is that both glutamate and GABA release are affected by mutated β4 subunits in Cacnb4h homozygotes but that the effect is more pronounced on glutamate release. If so, then increasing the sample size of neurons studied might enable a smaller reduction of the GABA A IPSC to be measured at the 0.05 level of significance. However, this scenario is actually a subset of the first possibility discussed earlier because in either case EPSC amplitudes are affected to a greater extent than IPSC amplitudes. Therefore a plausible molecular explanation for the substantially greater reduction in glutamate than GABA release is that VGCC subtypes with differing requirements for a func-

Possible links between mutated β4 subunit and decreased glutamate release from nerve terminals in Cacnb4h homozygotes

There are a number of plausible mechanisms that could link a mutated β4 subunit with a presynaptic defect in glutamate release. For example, VGCCs play a critical role in the cascade of events that occur between the arrival of an action potential at a nerve terminal and neurotransmitter release (see Kelly 1993). In principle, decreased calcium influx through a VGCC composed of defective subunits could result in decreased neurotransmitter release (see Dolphin 1995, 1996; Tareilus and Breer 1995). Thus it is possible that defective β4 subunits, lacking the binding domain required for modulation of calcium flux, could contribute to decreased neurotransmitter release in synapses in Cacnb4h homozygotes.

Supporting the idea that the mutation of VGCC subunits in Cacnb4h homozygotes causes decreased calcium influx, earlier findings from our lab showed a significant reduction in KCl-induced 45Ca\(^{+2}\) uptake in thalamic and neocortical synaptosomes in Cacnb4h homozygotes compared with matched, nonepileptic mice (Lin et al. 1995). This finding supports the premise that mutated β4 subunits can lead to reduced calcium entry into depolarized nerve terminals in Cacnb4h homozygotes, resulting in decreased neurotransmitter release.

However, if presynaptic VGCCs lead to reduced neurotransmitter release in synapses of Cacnb4h homozygotes, then it is surprising that glutamate but not GABA release is reduced significantly at synapses of VB thalamic neurons. There are
Evidence that β4 mutations in Cacnb4β4 homozygotes selectively affect P/Q channels: evidence from Cacna1ag8 homozygotes

Given the idea that glutamate neurotransmission is subserved primarily by P/Q channels, is it possible that the P/Q channel subtype is affected selectively by the defective β4 subunit in Cacnb4β4 homozygotes? Indirect evidence supporting this possibility emerges from the similar phenotypic features of seizures in Cacna1ag8 and Cacnb4β4 homozygotes (Hosford et al. 1992; Noebels 1986; Sidman et al. 1965). The gene mutation that causes seizures in homozygous Cacna1ag8 and its variant, leaner (Cacna1ag8-lb), affects the α1A subunit of VGCCs (Fletcher et al. 1996), which comprises the pore of P/Q-type VGCC (see Catterall 1991; Dolphin 1995; McCleskey 1994; Miller 1992). Moreover, whole cell recordings of dissociated Purkinje cells in homozygous Cacna1ag8-lb mice showed a reduction in P-type currents (Lorenzon et al. 1998). Hence it is possible that mutations affecting P/Q-type VGCCs in Cacna1ag8 and Cacna1ag8-lb homozygotes produce absence seizures that are phenotypically similar to those in Cacnb4β4 homozygotes because of independent mutations that affect the function of P/Q-type VGCCs.

More direct evidence is provided by our findings from Cacna1ag8 homozygotes in this study, showing that excitatory but not GABAergic responses are reduced in VB neurons compared with C57BL/6J controls. Together with the phenotypic similarity in seizures in Cacnb4β4 and Cacna1ag8 homozygotes, the similar reduction in excitatory responses in the two strains strongly suggests that P/Q channels are selectively affected by these independent gene mutations.

Our observation of reduced excitatory responses in VB thalamic neurons in Cacna1ag8 homozygotes provides the first evidence supporting reduced glutamatergic transmission in neuronal populations critical to generation of absence seizures in this model. However, our findings accord with observations by Helekar and Noebels (1994), who found reduced non-NMDA conductances during paroxysmal depolarizing shifts in CA3 pyramidal neurons in Cacna1ag8 homozygotes compared with C57BL/6J controls. It is likely that the mutation of α1A subunits of VGCCs in Cacna1ag8 homozygotes affects P/Q-channel function in multiple neuronal populations, irrespective of their theoretical role in the generation of absence SWDs.

Possible links between VGCC function and generation of absence seizures

It has been thought for some time that an imbalance between excitation and inhibition within the thalamocortical loop might be critical for the genesis of absence seizures (Gloor et al. 1990). Indeed the preservation of GABAergic inhibition within this network distinguishes absence seizures from generalized convulsive seizures (Gloor and Fariello 1988). It is possible to speculate then that the pronounced decrease in EPSCs and smaller reduction in IPSCs may tip the balance in favor of GABAergic inhibition of VB neurons in Cacnb4β4 and Cacna1ag8 homozygotes. As a number of research groups have demonstrated, enhanced GABAergic input in thalamocortical neurons can synchronize these neurons into a burst-firing mode (see Steriade and Lance 1988; also Crunelli and Leresche 1991; Huguenard and Prince 1994; Steriade et al. 1993; von Krosigk et al. 1993). A plausible underlying mechanism is that enhanced GABAergic transmission at thalamocortical neurons can deinnervate T-type VGCCs, enabling them to generate an I_T during the decay from the GABA-induced IPSC. Synchronized generation of I_T by a population of thalamocortical neurons then may generate the burst activity of the SWD, producing an absence seizure (Crunelli and Leresche 1991).

Thus in principle, the generation of absence seizures in Cacnb4β4 or Cacna1ag8 homozygotes may stem from synchronization provided by a net enhanced GABAergic input to thalamocortical neurons. This has been suggested as a general mechanism for absence seizures in both genetic and pharmacological models (Banerjee and Snead 1995; Caddick and Hosford 1996b; Crunelli and Leresche 1991; Gloor 1990; Snead 1995).

Alternative mechanisms may underlie the connection between reduced excitatory drive onto VB neurons and the generation of thalamocortical oscillations. For example, it is possible that the reduced excitatory drive is caused in some manner by the mutated β4 subunit of VGCCs, and yet the reduced excitatory drive may be unrelated entirely to generation of oscillations. Likewise other mechanisms besides those given in the preceding text may link thalamic responses to absence seizures. To help distinguish between the many speculative possibilities, further experiments are underway to characterize the extent to which net enhanced GABAergic transmission predominates in synapses critical to the generation of absence seizures, both in these mutant mice and other models, and the mechanisms linking decreased function of P/Q-type VGCCs to the generation of absence seizures in these models. It is hoped that the results forthcoming may facilitate the development of new approaches in the treatment of patients with absence epilepsy.

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