Fast and Slow Inhibition in the Visual Thalamus Is Influenced by Allocating GABA\(_A\) Receptors with Different \(\gamma\) Subunits

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INTRODUCTION

Cell-type specific differences in the kinetics of inhibitory postsynaptic conductance changes (IPSCs) are believed to impact upon network dynamics throughout the brain. Much attention has focused on how GABA\(_A\) receptor (GABA\(_A\)R) \(\alpha\) and \(\beta\) subunit diversity will influence IPSC kinetics, but less is known about the influence of the \(\gamma\) subunit. We have examined whether GABA\(_A\)R \(\gamma\) subunit heterogeneity influences IPSC properties in the thalamus. The \(\gamma^2\) subunit gene was deleted from GABA\(_A\)Rs selectively in the dorsal lateral geniculate nucleus (dLGN). The removal of the \(\gamma^2\) subunit from the dLGN reduced the overall spontaneous IPSC (sIPSC) frequency across all relay cells and produced an absence of IPSCs in a subset of relay neurons. The remaining slower IPSCs were both insensitive to diazepam and zinc indicating the absence of the \(\gamma^2\) subunit. Because these slower IPSCs were potentiated by methyl-6,7-dimethoxy-4-ethyl-\(\beta\)-carboline-3-carboxylate (DMCM), we propose these IPSCs involve \(\gamma^1\) subunit-containing GABA\(_A\)R activation. Therefore, \(\gamma\) subunit heterogeneity appears to influence the kinetics of GABA\(_A\)R-mediated synaptic transmission in the visual thalamus in a cell-selective manner. We suggest that activation of \(\gamma^1\) subunit-containing GABA\(_A\)Rs give rise to slower IPSCs in general, while faster IPSCs tend to be mediated by \(\gamma^2\) subunit-containing GABA\(_A\)Rs.

Keywords: GABA, synapse, thalamus, inhibition

The dorsal lateral geniculate nucleus (dLGN) transmits visual information from the retina to the visual cortex (Nassi and Callaway, 2009) with a variety of modulatory inputs influencing how this information is processed; including glutamatergic cortical inputs, cholinergic brain stem inputs and GABAergic inputs (Sherman and Guillery, 2002; Saalmann and Kastner, 2011). GABAergic modulation originates from both local interneurons within the dLGN (Rafols and Valverde, 1973; Ohara et al., 1983; Acuna-Goycolea et al., 2008; Seabrook et al., 2013) and external projections from the thalamic reticular nucleus (nRT; Sumitomo et al., 1976; Montero and Scott, 1981). These inputs can shape receptive field properties (Sillito and Kemp, 1983; Norton and Godwin, 1992) and regulate visual attention (Hirsch et al., 2015; Wimmer et al., 2015) through the activation of GABA\(_A\) and GABA\(_B\) receptors.

GABA\(_A\) Receptor (GABA\(_A\)R) heterogeneity is particularly influential in generating the variability in inhibitory postsynaptic conductance (IPSC) kinetics that shapes network behavior in the brain.
Synaptic GABA<sub>A</sub>Rs are assembled from α, β and γ subunits (Olsen and Sieghart, 2009). Each α subunit (α1 to α6) produces a particular kinetics with a decay of only a few milliseconds for α1 subunit-containing GABA<sub>A</sub>Rs (Bartos et al., 2001), tens of milliseconds for α3 subunit-containing GABA<sub>A</sub>Rs (Eyre et al., 2012) and around a 100 ms for the slow component of the IPSC mediated by α6 subunit-containing GABA<sub>A</sub>Rs (Bright et al., 2011). The β subunit (β1 to β3) has a more subtle influence on IPSC kinetics related to the phosphorylation status of the β subunit (Nusser et al., 1998; Houston et al., 2009).

Three γ subunits (γ1 to γ3) exist (Pritchett et al., 1989; Ymer et al., 1990; Herb et al., 1992), but the importance of γ subunit variability for IPSC kinetics has been little considered because γ2 subunit expression dominates in most brain regions (Wisden et al., 1992; Pirker et al., 2000). The global γ2 gene knockout is lethal (Günther et al., 1995), and the γ2 subunit appears essential for targeting of GABA<sub>A</sub>Rs to the synapse and the generation of fast IPSCs (Essrich et al., 1998; Schweizer et al., 2003; Wulff et al., 2007, 2009b), but the absence of IPSCs in the γ2 knockout mice can be rescued with γ3 gene overexpression (Baer et al., 1999). By contrast, the γ1 subunit produces a slower clustering of GABA<sub>A</sub>Rs at synapses and, therefore, results in the generation of slower IPSCs (Dixon et al., 2014).

Genetically deleting the γ2 subunit removes all IPSCs from Purkinje cells (Wulff et al., 2009b), ventrobasal (VB) thalamic relay neurons (Rovó et al., 2014), hippocampal parvalbumin interneurons (Wulff et al., 2009a) and histaminergic hypothalamic neurons (Zecharia et al., 2012), as well as massively reducing IPSC amplitude and frequency in hypothalamic GnRH neurons (Lee et al., 2010). Similarly, in some neocortical neurons γ2 gene ablation reduces IPSC frequency and in this case the γ3 subunit appears to cluster the remaining GABA<sub>A</sub>Rs (Kerti-Szigeti et al., 2014). Here, we report that removal of the γ2 subunit from the dLGN removes IPSCs from only half of the relay neurons and we provide pharmacological evidence that the remaining slower IPSCs are most likely mediated by γ1 subunit-containing GABA<sub>A</sub>Rs.

### MATERIALS AND METHODS

#### Mouse Strains

The HDC-Cre line was generated with homologous recombination with anires-Cre cassette inserted into exon 12 of the hdc gene, between the stop (TAG) codon and the polyadenylation (pA) signal (Zecharia et al., 2012). HDC-Cre mice were crossed with Rosa26-loxP-Stop-loxP-YFP mice (Srinivas et al., 2001) or a floxed γ2 mouse strain (γ2I77lox/lox) separately. The γ2I77lox line was generated with the codon of phenylalanine (F) at position 77 mutated to isoleucine (I) in exon 4 of the γ2 subunit gene, and two loxP sites inserted in intron 3 and intron 4, respectively (Wulff et al., 2007). The F77I mutation resulted in the loss of zolpidem sensitivity from all cells tested (Cope et al., 2004, 2005). Importantly, the physiological properties of the GABA<sub>A</sub>Rs were unchanged in the F77I strain and there was no behavioral phenotype associated with this silent mutation (Cope et al., 2004, 2005). This line has been used to delete IPSCs from a number of cell types (Wulff et al., 2007, 2009a,b; Zecharia et al., 2012; Kerti-Szigeti et al., 2014; Rovó et al., 2014). To generate HDC-γ2 mice and littermate controls, homozygous γ2I77lox/I+ HDC-Cre mice. The γ2I77lox mouse line was genotyped by PCR with the following primers: forward: 5′-GTGATGCTAAATATCCTACAGTG-3′; reverse: 5′-GGATAGTGCATCA-GAGCAATAG-3′ (213 bp wild-type; 250 bp floxed allele) and the HDC-Cre mouse line was genotyped using: forward: 5′-GTTGTCGTCCCTTCTGTGCGG-3′; reverse: 5′-AGGCCACCATGCGCCCCATG-3′ (250bp).

#### Immunohistochemistry

For immunohistochemical localization, mice were deeply anesthetised with sodium pentobarbital (in accordance with UK Home Office guidelines) and transcardially perfused with 4% paraformaldehyde (PBS; Sigma). Coronal slices were cut at a thickness of 30 μm (Leica VT1000S vibratome) and incubated in rabbit anti-GFP (1:1000; Millipore) and mouse anti-NeuN (1:300; Millipore) antibodies overnight. Slices were then incubated for 2 h at room temperature with Alexa Fluor 488 goat anti-rabbit (1:1000; molecular probes) and mouse anti-NeuN (1:300; Millipore) antibodies overnight. Slices were then mounted in Vectashield mounting medium with DAPI (H1200, Vector labs) and the resulting red, green and blue signals were imaged on a Zeiss LSM 510 CLSM microscope (Facility for Imaging by Light Microscopy, FILM, Imperial College).

#### Electrophysiology and Synaptic Recording

For electrophysiological recording, mice were routinely handled to reduce stress levels and brain slices were then prepared from adult (3–6 months postnatal) mice that were killed by cervical dislocation (in accordance with UK Home Office guidelines). The slicing solution contained (in mM) the following: NaCl 125, KCl 2.5, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 4, NaH<sub>2</sub>PO<sub>4</sub> 1.25, NaHCO<sub>3</sub> 26, glucose 11, 1 kynurenic acid, pH 7.4, when bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Slices were cut using a vibratome tissue slicer (Campden instruments) at a thickness of 250 μm and immediately transferred to a holding chamber containing slicing solution continuously bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Once slicing was complete, slices were then transferred to a 37°C heat block for 10 min, after which the slicing solution was exchanged for recording ACSF (in mM: NaCl 125; KCl 2.5; CaCl<sub>2</sub> 2; MgCl<sub>2</sub> 4; Na<sub>2</sub>HPO<sub>4</sub> 1.25; NaHCO<sub>3</sub> 26; glucose 11; 1 kynurenic acid, pH 7.4, when bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>). The slices were subsequently incubated in the recording ACSF at room temperature for at least another 30 min before electrophysiological recordings.

Slices were visualized using a fixed-stage upright microscope (BX51WI, Olympus) fitted with a high numerical aperture water-immersion objective and a digital camera. Patch pipettes were fabricated from thick-walled borosilicate glass capillaries (1.5 mm o.d., 0.86 mm i.d., Harvard Apparatus) using a two-step vertical puller (Narishige, PC-10). Pipette resistances were typically 3–4 MΩ when back filled with internal solution.
Statistical Tests
All average values represent the mean ± the standard error of the mean (SEM). Data distributions were compared using Origin 8.5 and functions were fitted to data distributions using unconstrained least-squared fitting procedures. The type of statistical test used in each experiment is specified individually.

RESULTS
The Histamine Decarboxylase Gene Drives Cre Expression in the dLGN
The HDC-Cre mouse line was crossed with the LoxSTOPlox Rosa-YFP mouse line (Figure 1A) and YFP expression was examined in the resulting HDC-CRE-YFP line (Figure 1B). As expected from previous studies (Zecharia et al., 2012), the YFP signal was associated with histamine-producing neurons of the Tuberomammillary Nucleus (TMN), ependymal cells lining ventricles and putative macrophages that were sparsely distributed throughout the neocortex. However, the attention of this study was focused on the dLGN where a high proportion of cells were shown to be YFP-positive (Figure 1C). On average we found that 41% of cells were NeuN positive in the adult dLGN (DAPI+: 926 cells, NeuN+:
neurons (379 cells) counted in representative slice sections; consistent with previous estimates of neuronal density in the mammalian dLGN (Wei et al., 2011). Co-fluorescence of YFP signal with the neuronal marker NeuN indicated that YFP proteins are exclusively confined in NueN-positive neurons and ~90% of NueN-positive neurons within the dLGN had undergone recombination and expressed YFP (DAPI+: 926 cells, NeuN+: 379 cells, YFP+: 340 cells), because the hdc-cre gene is transiently expressed during postnatal development of the thalamic relay neurons of the dLGN (Zecharia et al., 2012). In the example volume of tissue analyzed in Figure 1D, 42 out of the 47 NeuN-positive neurons (~89%) expressed YFP (Figure 1E) from a total of 102 cells that were stained with DAPI (Figures 1D–F). These results demonstrate the usefulness of the HDC-Cre mouse as a method for altering gene expression within neurons of the dLGN.

Reduced GABAergic Drive in the γ2 Knockout

The HDC-Cre mouse was crossed with the γ2I77lox mouse to produce HDC-Δγ2 mice and littermate controls. Whole-cell voltage-clamp recordings were then made from identified thalamic relay neurons of the dLGN (Figure 2A). In littermate control mice, 46 out of 47 neurons exhibited sIPSCs whereas just under half of HDC-Δγ2 cells (31 out of 66) were devoid of sIPSCs (Figure 2B). Across all cells recorded, the average sIPSC frequency was 6.27 ± 0.66 Hz (n = 47) in control cells compared to 2.55 ± 0.4 Hz (n = 66) in HDC-Δγ2 cells resulting in a significant reduction (K-S test, P = 1 × 10^{-7}) in synaptic drive following γ2 subunit removal. In those cells containing sIPSCs the average frequency was 6.41 ± 0.66 Hz (n = 46) in control cells compared to 4.80 ± 0.51 Hz (n = 31) in HDC-Δγ2 cells (K-S test, P = 0.21). Therefore, the main impact of γ2 removal is the loss of sIPSCs in a subset of thalamic relay neurons. As shown in Figure 2C, the remaining sIPSCs in HDC-Δγ2 cells were blocked by the GABA_A antagonist picrotoxin (30 μM). This blocking action was associated with a reduction in the holding current and this tonic current was observed in the control and HDC-Δγ2 neurons irrespective of the presence or absence of sIPSCs (Figure 2C). To assay any change in the contribution of 6 subunit-containing GABA_A Rs to thalamic relay neuron excitability in the HDC-Δγ2 cells, we took advantage of the allosteric modulator DS-2 (Wafford et al., 2009; Ye et al., 2013). As expected the tonic current recorded from thalamic relay neurons was enhanced by DS-2 with little action on sIPSCs (Figure 2D). The DS-2 induced change in holding current was 83.7 ± 20.1 pA (n = 6 cells) in control cells compared to 100.7 ± 12.9 pA (n = 6 cells) in HDC-Δγ2 cells with no significant difference (two-tailed t-test, P = 0.49; Figure 2E). We also did not observe any change in IPSC kinetics or amplitude during DS-2 application (Figure 2F). Consistent with no change in the tonic conductance following γ2 subunit removal, the average input conductance from the cells used in different aspects of this study was 4.03 ± 1.01 nS in the control (n = 47 cells from 27 mice) compared to 3.35 ± 0.89 nS (n = 35 cells from 16 mice) in the HDC-Δγ2 that exhibited sIPSCs and 4.14 ± 0.88 nS (n = 31 cells from 14 mice) in the HDC-Δγ2 that did not exhibit sIPSCs (two-tailed t-test, P > 0.44 in all cases). There was also no significant difference of membrane capacitance between these cell groups (two-tailed t-test, P > 0.12 in all cases; control cells: 99 ± 7 pF, n = 47; HDC-Δγ2 cells with no sIPSCs: 93 ± 5 pF, n = 31; HDC-Δγ2 cells with sIPSCs: 108 ± 8 pF, n = 35), indicating the resting membrane excitability and the cell shape had not dramatically altered. Therefore, crossing the HDC-Cre mouse line with the γ2I77lox mouse line has resulted in the complete removal of sIPSCs from only 50% of thalamic relay neurons raising the possibility that alternative synaptic GABA_A R types contribute to phasic inhibition within the remaining cells of the dLGN.

Fast IPSCs Are Less Prevalent in dLGN Relay Neurons of HDC-Δγ2 Mice

Kinetic analysis of sIPSCs revealed a small number of neurons in control mice (8 out of 47) that contained a single population of fast rising and fast decaying sIPSCs (Figure 3A). In two of these eight cells, we recovered fills with clear Y-like morphology similar to that reported previously for dLGN relay neurons with predominantly fast IPSCs (Bright et al., 2011). As expected from our previous studies, the majority of thalamic relay neurons (39 out of 47) exhibited a high proportion of slow rising and slow decaying sIPSCs (Figure 3B). As shown in Figure 3D, a clear reduction in sIPSC frequency was apparent across the entire population of cells with no overlap between the distributions of IPSCs in control cells and remaining IPSCs in HDC-Δγ2 cells. In order to estimate the proportion of fast sIPSCs (P_{fast-sIPSC}) present in any given cell, we defined a cut-off criterion (t_{crit}) for fast sIPSCs based upon data obtained from cells that exhibited a single population of fast rising and fast decaying sIPSCs. A single Gaussian fit was used to define a t_{crit} at which fast sIPSCs could be identified at a 95% confidence level. The average t_{crit} based upon Gaussian fits to the data obtained from all eight fast IPSCs-only cells (termed T_{crit}, to differentiate with t_{crit} from individual cells) was 1.7 ± 0.2 ms for the rise-time and 7.9 ± 1.0 ms for the decay. Figure 3C illustrates data from an HDC-Δγ2 relay neuron that contained both fast and slow IPSCs. Using the T_{crit} values obtained from the wild-type population, the P_{fast-IPSC} was 0.1 in this cell. To determine whether γ2 deletion has reduced the prevalence of fast IPSCs across all cells, the distribution of P_{fast-IPSC} was also compared (Figure 3E). There was a reduction in the prevalence of fast IPSCs with only one recording from the HDC-Δγ2 mice giving a P_{fast-IPSC} >0.5. Indeed, γ2 subunit removal was associated with a reduction in P_{fast-IPSC} (K-S Test, P < 0.001) with an average P_{fast-IPSC} of 0.27 ± 0.03 in recordings from the control mice compared to 0.11 ± 0.02 in recordings from the HDC-Δγ2 dLGN. The loss of these fast sIPSCs in the knockout may reflect the loss of γ2-containing GABA_A Rs in a mixed GABA_A R population in dLGN thalamic relay neurons. To test this hypothesis the pharmacological data associated with our whole-cell voltage-clamp recordings was analyzed.
All Relay Neurons Are Affected by the \( \gamma_2 \) Knockout

To distinguish between GABA\(_A\)R heterogeneity or partial recombination in dLGN neurons, we assayed the diazepam sensitivity of the remaining sIPSCs recorded from HDC-\( \Delta \gamma_2 \) neurons as the \( \gamma_2 \)F77I point mutation abolishes zolpidem sensitivity but diazepam sensitivity persists (Buhr et al., 1997; Cope et al., 2004). In the littermate control cells, 3 \( \mu \)M diazepam caused the average sIPSC weighed decay time to increase from 10.94 ± 0.37 ms to 15.39 ± 0.38 ms (\( n = 4 \); paired \( t \)-test, \( P < 0.001 \)) with little change in the average 10%–90% rise-time (paired \( t \)-test, \( P = 0.26 \)) or peak amplitude (paired \( t \)-test, \( P = 0.4 \)) of sIPSCs. The average sIPSC waveform constructed from a control neuron before and during 3 \( \mu \)M diazepam was then applied to those HDC-\( \Delta \gamma_2 \) dLGN relay neurons that contained sIPSCs, recorded in control ACSF (gray trace) and in the presence of 10 \( \mu \)M DS-2 (black trace). The similarity of the two superimposed average waveforms illustrates how sIPSC properties (10%–90% rise-time, peak amplitude and weighted decay) are little affected by the application of DS-2.
FIGURE 3 | The prevalence of fast sIPSCs is reduced in thalamic relay neurons recorded from the γ2 knockout dLGN. (A) The top traces are superimposed individual sIPSCs recorded from a control dLGN relay neuron. The scatter plot below the traces describes the relationship between 10%–90% rise-time and weighted decay time for all sIPSCs recorded from this cell. The all-point histograms on the two axes illustrate the frequency distribution for each of these parameters. In this cell both of these distributions can be described with a single Gaussian function (solid line). The dashed lines superimposed upon the scatter plot were obtained from this Gaussian fit indicating the 95% confidence limit that defines the $t_{\text{crit}}$ for these two parameters. These boundaries were used to define a $P_{\text{fast-sIPSC}}$ in this cell of 0.95. (B) The top traces are superimposed individual sIPSCs recorded from another control dLGN relay neuron. In this example, we could identify fast rising and fast decaying sIPSCs (black traces) similar to those in (A) as well as slow rising and slow decaying sIPSCs (gray traces). The scatter plot below the traces in panel (B) describes the relationship between 10%–90% rise-time and weighted decay time for all sIPSCs recorded from this cell. The all-point histograms on the two axes illustrate the frequency distribution for each of these parameters. In this cell both of these distributions could be adequately described with a single Gaussian function. Therefore, the averaged $t_{\text{crit}}$ values ($T_{\text{crit}}$) obtained from the cell population illustrated in (A) was used to define a $P_{\text{fast-sIPSC}}$ of 0.05 for this cell. (C) Similar conventions to (B) but the data from this cell was obtained from a HDC-γ2 dLGN relay neuron. (D) Cumulative probability distribution for all sIPSC frequency estimates for control and HDC-γ2 dLGN relay neurons. (E) Cumulative probability distribution for all $P_{\text{fast-sIPSC}}$ estimates for control and HDC-γ2 dLGN relay neurons.

10.62 ± 1.03 ms ($n = 5$) in normal ACSF vs. 11.27 ± 1.02 ms in the presence of 3 µM diazepam. The lack of diazepam sensitivity observed in the HDC-Δγ2 neurons (Figure 3B) clearly indicates that the remaining GABA$_A$Rs, responsible for generating the sIPSCs, do not contain the γ2 subunit.

It is also possible that the remaining sIPSCs are mediated by αβ assemblies that lack γ subunits. However, this GABA$_A$R type should be potently blocked by Zn$^{2+}$ ions (Draguhn et al., 1990), a feature not observed in either control or HDC-Δγ2 cells (see Figure 4C). For example, in HDC-Δγ2 cells, the sIPSC peak amplitude was 93.03 ± 41.82 pA ($n = 9$) in normal ACSF compared to 90.35 ± 41.82 pA in the presence of 10 µM Zn$^{2+}$ (paired t-test, $P = 0.63$) and, on average, the IPSC weighted decay time did not significantly change (+8.4 ± 6.3%, $n = 9$; paired t-test, $P = 0.93$). As was the case for all pharmacological manipulations described so far in this study, the sIPSC frequency remained stable at 6.2 ± 1.2 Hz in control ACSF compared to 8.1 ± 1.8 Hz in the presence of Zn$^{2+}$ (two-tailed t-test, $P = 0.2$).

To test the possibility that other γ subunit-containing GABA$_A$Rs contribute to the remaining IPSCs, we examined the actions of DMCM. As well as removing zolpidem sensitivity, the γ2F77I point mutation results in DMCM insensitivity (Buhr
FIGURE 4 | Pharmacological evidence that the remaining sIPSCs in the γ2 knockout do not contain the γ2 subunit. (A) Left panel: superimposed average waveforms obtained in the presence (red trace) and absence (black trace) of 3 μM diazepam in the extracellular solution. The top two waveforms were obtained from the dLGN of control mice and the bottom traces were obtained from HDC-Δγ2 mice. Right panel: scatter plot of the change in weighted decay time estimated for each cell in the control and HDC-1γ2 dLGN. The decay of all four cells in the control dLGN was enhanced by diazepam (n = 4; paired t-test, P < 0.001), but there was no significant change in the decay of sIPSCs in the knockout HDC-1γ2 dLGN (n = 5; paired t-test, P = 0.11). (B) Scatter plot of IPSC weighted decay time against 10%–90% rise-time in an example control dLGN neuron (left panel) and an HDC-1γ2 dLGN neuron. (C) Similar conventions to (A) showing the lack of actions of 10 μM ZnCl2 on control and HDC-1γ2 sIPSCs. (D) Similar conventions to (A) showing the similar actions of 10 μM methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) on control and HDC-Δγ2 sIPSCs. DMCM potentiated the IPSC charge transfer in both control neurons and HDC-Δγ2 dLGN neurons (Control neurons: 39.8 ± 15.59%, paired t-test, P = 0.004; Figure 4D). A similar action of DMCM was observed in HDC-1γ2 cells with a 21.57 ± 6.36% (n = 7) increase in charge transfer (paired t-test, P = 0.03; Figure 4D). These results suggest that the γ2 subunit is absent from all cells recorded from the HDC-Δγ2 dLGN, and γ1 subunit-containing GABAARs contribute to IPSCs in the dLGN.

DISCUSSION

We still do not fully understand the significance of GABAAR heterogeneity for brain function. One possibility is that the distinct kinetics conferred by different GABAAR subunit combinations confers flexibility to neuronal circuits that process different types of information. What is clear from this study is that the γ2 subunit is associated with fast rising and fast decaying IPSCs, whereas synaptic γ1 subunit-containing GABAARs, contribute to the slow rising and slow decaying sIPSCs within the dLGN.

The γ1 Subunit Contributes to sIPSCs in the dLGN

The main GABAAR receptor genes expressed in the thalamus are α1, α4, β2 and δ (Wisden et al., 1992; Pirker et al., 2000); little γ1–3 expression is detected by either in situ hybridization or immunohistochemistry. Indeed, extrasynaptic GABAAR mediated tonic inhibition dominates in the thalamus (Jia et al., 2005; Bright et al., 2007). Nevertheless, the sensitivity of whole-cell recording is clearly able to demonstrate the presence of phasic inhibition mediated by αβγ-type synaptic receptors (Jia et al., 2005; Bright et al., 2007). We now present evidence that γ2 removal from the dLGN resulted in the complete removal of sIPSCs from half of all relay neurons and the γ2 subunit is in fact absent from the synaptic GABAARs that give rise to IPSCs in the remaining cells. This conclusion is based upon the observation that the remaining IPSCs in the γ2 knockout dLGN
were diazepam insensitive (see Figures 3A,B). The diazepam induced potentiation of γ1- and γ3-containing GABA_ARs is much less pronounced than that known to occur at γ2-containing GABA_ARs (Puia et al., 1991; Herb et al., 1992; Wafford et al., 1993). The point mutation in the γ2F77Ilox line also results in DMCM insensitivity of γ2 subunit-containing GABA_ARs (Buhr et al., 1997). However, DMCM is an inverse agonist at γ3 subunit-containing GABA_ARs (Herb et al., 1992), and will potentiate currents generated by γ1 subunit-containing GABA_ARs (Puia et al., 1991). Therefore, the enhancement of sIPSCs we observe with DMCM (see Figure 3C) is consistent with the presence of γ1 subunit-containing GABA_ARs and offers a simple explanation for the sIPSCs that remain in the γ2 knockout (see Figures 4C,D). In contrast, an inhibitory action of DMCM in IPSCs of the neocortex was used to suggest that γ3 subunits are present in the synaptic GABA_ARs that remain following γ2 removal (Kerti-Szigeti et al., 2014). Given that only one γ subunit is present within the pentameric assembly (Olsen and Sieghart, 2009), we propose that the dLGN can express at least three distinct types of GABA_ARs. An α1, α4, β2 and δ subunit combination contributes to extrasynaptic GABA_ARs that mediate the tonic conductance. The α1, α4, β2 and γ2 subunit combinations will contribute to fast synaptic inhibition and we now suggest that the α1, α4, β2 and γ1 subunit combination will generate a slower form of synaptic inhibition within the dLGN.

A simple relationship between γ subunit identity and IPSC kinetics is, however, unlikely given that fast rising and fast decaying IPSCs remain in the HDC-Δγ2 dLGN neurons, possibly as a result of different GABA_AR proximity to GABA release sites. However, our results clearly demonstrate that deletion of γ2 subunit reduced the proportion of fast rising and fast decaying IPSCs across all cells. The γ1 subunit influences GABA_ARs clustering at central synapses (Dixon et al., 2014), giving rise to slow IPSCs in neurons of the central amygdala (Esmaeili et al., 2009). Macroscopic and single channel behavior of γ1 and γ2 subunit-containing GABA_ARs indicates little difference in activation and deactivation, but inclusion of the γ1 subunit was reported to slow both the rise and decay of sIPSCs and this was interpreted in terms of “loose clustering” of synaptic GABA_ARs (Dixon et al., 2014). We have previously concluded that spillover of GABA from local dLGN interneurons did not result in the activation of high-affinity δ subunit-containing extrasynaptic GABA_ARs within the dLGN in spontaneous activity recordings (Bright et al., 2011; Ye et al., 2013). Consistently, we have no evidence that the sIPSCs remaining in the HDC-Δγ2 dLGN neurons involve activation of these particular receptors as the δ subunit selective drug DS-2 (Wafford et al., 2009) has no action on IPSC properties even though the tonic conductance was clearly enhanced by this allosteric modulator (see Figures 2C,D). However, spillover of GABA onto these δ subunit-containing extrasynaptic GABA_ARs occurs onto VB relay neurons in response to stimulated burst firing of the nRT (Herd et al., 2013). Similarly, we recently reported that DS-2 application resulted in a slowing of ChR2-evoked IPSCs that are driven by optogenetic GABA release from dLGN interneurons (Jager et al., 2016). These results are not contradictory if the magnitude of the GABA transient associated with spontaneous release were much less than the GABA transient associated with evoked release.

### Local dLGN Interneurons and the Slow sIPSC Reticular Inputs

Uniquely, GABA release within the rodent dLGN reflects afferent input from both the nRT and release from local dLGN interneurons. Other nearby first order thalamic nuclei such as the VB do not contain local interneurons, and GABA release in these nuclei is more restricted to the nRT input (Herd et al., 2013). Indeed, γ2 gene deletion from the VB nucleus resulted in a loss of IPSCs from all relay neurons examined (Rovó et al., 2014), which raises the possibility that the remaining IPSCs in the dLGN following γ2 deletion in our study are mediated by local interneurons not present in the VB. We do not exclude the possibility that the observed prevalence of the γ1 subunit can be a compensatory effect of γ2 deletion. Nonetheless, the presence of γ1-containing GABA_ARs following γ2 deletion in our study highlight the importance of γ subunit-containing GABA_ARs in the dLGN, compared to similar γ2 deletion studies mentioned above. Infrequent GABA_AR-mediated responses did remain in some cells in Rovó et al. (2014), but the extremely slow activation/deactivation of these events was interpreted in relation to extrasynaptic δ subunit-containing GABA_AR activation following GABA spillover. Indeed, simultaneous paired recording experiments have demonstrated that nRT burst firing can generate these slow GABA_AR-mediated responses within VB relay neurons (Herd et al., 2013). Importantly, this particular spillover response was absent when extrasynaptic GABA_ARs were genetically removed. Rhythmic activity in the neocortex was little altered following γ2 deletion in VB (Rovó et al., 2014), suggesting that these spillover currents can entrain thalamocortical oscillations in the absence of fast IPSCs (Rovó et al., 2014). Previously, we have also shown that global oscillatory activity across the neocortex was not affected in the HDC-Δγ2 mice during sleep/wake cycle (Zecharia et al., 2012). The IPSCs remaining in the dLGN may well be sufficient to maintain rhythmic activity, but the presence of δ subunit-containing GABA_ARs may also enable spillover-mediated inhibition to occur following nRT related burst firing in a similar manner to that suggested for VB (Rovó et al., 2014).

Comparing these results with similar studies highlights the complexity of synaptic GABA_AR targeting that is present in the mammalian brain. Purkinje cells (Wulff et al., 2009b), hippocampal parvalbumin interneurons (Wulff et al., 2009a) and VB neurons (Rovó et al., 2014) exclusively use γ2 subunit-containing GABA_ARs to generate fast IPSCs while some neocortical neurons make additional use of γ3 subunit-containing GABA_ARs to generate slow decaying IPSCs (Kerti-Szigeti et al., 2014). By combining quantitative analysis with pharmacological data in HDC-Δγ2 neurons, we have now demonstrated that deletion of γ2 subunit-containing GABA_ARs in the dLGN only results in complete deletion of IPSCs in half of dLGN neurons.
The remaining slow rising and slow decaying IPSCs are not mediated by γ2 subunit-containing GABA<sub>A</sub>Rs, and they appear to involve activation of γ1 subunit-containing GABA<sub>A</sub>Rs. This highlights a possible requirement for distinct types of inhibitory control within the different pathways of the visual thalamus.

**ETHICS STATEMENT**

This study was carried out in accordance with the recommendations of the UK Home Office and all experimental procedures have received internal approval by the Imperial College Ethical Committee and are covered by a UK Home Office License.

**AUTHOR CONTRIBUTIONS**

ZY performed electrophysiological experiments, analyzed data, prepared figures and co-wrote the manuscript. XY supervised mouse crossings and performed genotyping. CMH performed electrophysiological experiments and analyzed data. ZA performed the immunohistochemistry. NPF contributed to the writing of the manuscript. WW contributed to the writing of the manuscript. SGB analyzed data, prepared figures and wrote the manuscript.

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