Electrophysiological properties of genetically-identified subtypes of layer 5 neocortical pyramidal neurons: Ca\(^{2+}\)-dependence and differential modulation by norepinephrine.

Running Title: Subtypes of Layer 5 Pyramidal Cells

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Abstract

We studied neocortical pyramidal neurons from two lines of BAC mice (etv1 and glt; Gene Expression Nervous System Atlas: GENSAT project), each of which expresses EGFP in a different subpopulation of layer 5 pyramidal neurons. In barrel cortex, etv1 and glt pyramidal cells were previously reported to differ in terms of their laminar distribution, morphology, thalamic inputs, cellular targets, and receptive field size. In this study, we measured the laminar distribution of etv1 and glt cells. On average, glt cells were located more deeply, however the distributions of etv1 and glt cells extensively overlap in layer 5. To test whether these two cell types differed in electrophysiological properties that influence firing behavior, we prepared acute brain slices from 2-4 week old mice where EGFP-positive cells in somatosensory cortex were identified under epifluorescence and then studied using whole-cell current- or voltage-clamp recordings. We studied the details of action potential (AP) parameters and repetitive firing, characterized by the larger slow afterhyperpolarizations (AHPs) in etv1 neurons and larger medium AHPs in glt cells, and compared currents underlying the mAHP and sAHP in etv1 and glt neurons. Etv1 cells exhibited lower dV/dt for spike polarization and repolarization and reduced DC gain (lower f-I slope) for repetitive firing than glt cells. Most importantly, we found (i) that differences in the expression of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} conductances (sK and sAHP channels) determine major functional differences between etv1 and glt cells, and (ii) there is differential modulation of etv1 and glt neurons by norepinephrine (NE).
Keywords

Afterhyperpolarization

Repetitive firing

BAC mice
Introduction

Neocortical pyramidal neurons vary in morphology, physiology, molecular signature, laminar position, inputs, and projection targets and they have been classified using each of these criteria. A focus of much current interest is whether types of pyramidal cells belonging to functional cortical circuits can be identified by other distinguishing characteristics. Two morphological classes of pyramidal cells have been identified in layer 5 (Mason and Larkman, 1990; Kasper et al. 1994; Rumberger et al. 1998). One group includes large cells in deep layer 5 (layer 5B) that have a thick apical dendrite that reaches layer 1, where the dendrite terminates in an apical tuft (thick or thick-tufted cells: Mason and Larkman, 1990; Tsiola et al. 2003). Another group of cells is preferentially located in superficial layer 5 (layer 5A). They have slender dendrites with fewer oblique branches and the apical dendrite often does not reach layer 1 (slender or slender-tufted cells: Kasper et al. 1994). Passive and active electrical properties differ between these morphologically defined subpopulations of pyramidal cells (Kasper et al. 1994).

Pyramidal cells can also be classified by their projection patterns. Thick-tufted pyramidal neurons project subcortically to the pons, tectum, or pyramidal tract (Kasper et al. 1994; Larkman and Mason, 1990; Akintunde and Buxton, 1992; Hattox and Nelson, 2007). Slender-tufted pyramidal neurons are heterogeneous, with corticocortical and corticofugal projecting subgroups (Hattox and Nelson, 2007; Le Be et al. 2007; Mitchell and Macklis, 2005). Slender-tufted neurons project to ipsilateral cortex, through the corpus callosum to contralateral cortex, and to both ipsilateral and contralateral striatum (Kasper et al. 1994; Larkman and Mason,
Electrical properties differ between subpopulations of pyramidal cells defined by their projections. For example, Le Be et al. (2007) found that callosally projecting neurons have a pronounced sAHP and higher input resistance and smaller and broader APs than non-callosally projecting layer 5 cells (see also: Hattox and Nelson, 2007, Dembrow et al. 2010).

Pyramidal cells with projections restricted to intratelencephalic targets have been classified as intratelencephalic (IT-type) neurons and pyramidal neurons with projections beyond the telencephalon (e.g., corticospinal, corticotectal, corticopontine) have been classified as pyramidal tract (PT)-type neurons (Catsman-Berrevoets et al. 1980; Reiner et al. 2003, 2010; Shepherd, 2013; Kress et al. 2013). In rat somatosensory cortex, the vast majority of IT-type neurons are in layers 2/3 and layer 5A (Wilson, 1987; Cowan and Wilson 1994; Reiner et al. 2003, 2010; Molyneaux et al. 2009; Shepherd, 2013). In contrast, ~90% of PT-type cells in somatosensory cortex are in layer 5B (Reiner et al. 2003, 2010). These data suggest a general relationship where slender-tufted pyramidal cells in layer 5A are IT-type and thick-tufted pyramidal cells in layer 5B are PT-type in motor, prefrontal, and somatosensory cortices, although these relationships are far from perfect correlations (e.g., Reiner et al. 2003, 2010).

Pyramidal cells also have unique molecular signatures. Several genes are specific to pyramidal cells in particular layers (Yoneshima et al. 2006; Watakabe et al. 2007; Molyneaux et al. 2009; Greig et al. 2013) or those with defined projection patterns (Hevner et al. 2003; Molyneaux et al. 2009; Molnar and Cheung, 2006, Arlotta et al. 2005). Subsets of pyramidal cells express signature genes including the
transcription factor *etv1*, which is expressed in a subset of layer 5 neurons (Doyle et al. 2008). *Etv1* has been shown to participate in neurogenesis in olfactory bulb (Stenman et al. 2003) and circuit formation in spinal cord (Arber et al. 2000). Another subset of layer 5 cells express *glt25d2* (*glt*), a glycosyl transferase gene (Gong et al. 2003, 2007; Doyle et al. 2008). Differential functional involvement of *glt* cells in mice *in vivo* is exemplified by responses to antidepressants being mediated by IT-type pyramidal cells in layer 5A, whereas *glt* cells are not involved (Schmidt et al. 2012). *Etv1* and *glt* expressing neurons also differentially express many other genes (Doyle et al. 2008; Schmidt et al. 2012). Groh et al. (2010) reported that *glt*-EGFP cells are thick-tufted layer 5 neurons and that *etv1*-GFP cells correspond to slender-tufted layer 5 pyramidal neurons. In cortical barrel (somatosensory) cortex, *glt*-EGFP was reported to be expressed almost exclusively in layer 5B and *etv1*-EGFP in layer 5A but there was more extensive intermingling in visual cortex (Groh et al. 2010). They also found that *glt*-pyramids project to ipsilateral pons and thalamus, but not to contralateral striatum, and thus considered them to be a subset of PT-type neuron. They found that *etv1*-pyramids are IT-type, projecting callosally and to ipsilateral (and to some extent contralateral) striatum, but not to pons or thalamus (Groh et al. 2010).

In the present study, our primary goal was to determine whether there are electrophysiological differences between *etv1* and *glt* pyramidal cells that would shape their firing behavior. We used whole cell current- and voltage-clamp recordings in somatosensory cortex of acute brain slices to examine EGFP-expressing cells from the *etv1* and *glt* mouse lines. We determined the laminar
distribution and soma size of etv1 and glt cells. Also, since etv1 cells are reported to
be a subset of IT-type neurons and glt cells a subset of PT-type neurons, we
examined whether reported electrophysiological differences between PT and IT-
type cells (in input resistance, AP width, SFA, and I_{H}; e.g., Solomon et al. 1993;
Christophe et al.; 2005; Le Be et al. 2007; Hattox and Nelson, 2007; Dembrow et al.
2010; Sheets et al. 2011; Gee et al. 2012) might also be characteristic of glt and etv1
cells.

Our principal finding was the differential expression of Ca^{2+}-dependent K+
conductances responsible for dramatic differences in firing behavior between layer
etv1 and glt pyramidal neurons and the differential modulation of these two cell
types by norepinephrine (NE). In addition, we found that etv1 cells exhibited lower
maximum dV/dt for spike polarization and repolarization, larger slow
afterhyperpolarizations, and reduced DC gain (lower f-I slope) during repetitive
firing than glt cells. We confirmed that etv1 is expressed in pyramidal cells in layer
5A of somatosensory cortex while on average glt cells are found deeper in layer 5
(extend into layer 6). There was, however, considerable overlap between the
distribution of etv1 and glt cells. We found that soma size did not differ between
etv1 and glt cells in somatosensory cortex. We also confirmed the findings of Groh
et al. (2010) that etv1 pyramidal cells had significantly broader action potential (AP)
half-width and greater spike frequency adaptation (SFA), as compared to glt
pyramidal cells.
Materials and Methods

We studied layer 5 neurons from two BAC lines of mice, each of which express EGFP in a different subpopulation of layer 5 pyramidal neurons (Gong et al. 2002, 2003, 2007). We maintain breeding colonies of both mouse lines (Swiss-Webster background), which were originally obtained from the MMRRC (Mutant Mouse Regional Resource Centers) of the GENSAT project. The first line was Tg(Etv1-EGFP)BZ192Gsat/Mmucd (etv1). In the barrel cortex of these mice, EGFP was reported to be expressed in layer 5A pyramidal neurons that have slender apical dendrites, with more extensive intermingling in visual cortex (Groh et al. 2010). The second line was Tg(Glt25d2-EGFP)BN20Gsat/Mmnc (Glt). In somatosensory cortex of these mice, glt-EGFP was reported to be expressed in a subset of thick-tufted layer 5B pyramidal neurons (Groh et al. 2010). In addition, we recorded basic electrical properties from layer 2/3 pyramidal cells from both mouse lines.

These studies were performed on juvenile mice from 2-4 weeks of age. All procedures were approved by the Animal Care and Use Committee, University of Tennessee Health Science Center. The animals were anesthetized with isoflurane until they were areflexive. Briefly, the animal was placed into a sealed plastic container into which gauze soaked with isoflurane was placed under a fiberglass screen floor. After anesthesia with isoflurane, the animal was decapitated and the brain was removed and dropped into ice-cold cutting solution bubbled with O$_2$ for 30-60 s. This solution contained (in mM): 250 sucrose, 2.5 KCl, 1 NaH$_2$PO$_4$, 11 glucose, 4 MgSO$_4$, 0.1 CaCl$_2$, 0.4 ascorbate, 0.6 sodium pyruvate, and 15 HEPES (pH
7.3–7.4; 300 mOsm/L). The brain was then sliced into 300-µm-thick coronal sections using a vibrating tissue slicer (Vibroslice, Campden Instruments).

**Histology and Microscopy**

For anatomical studies, 4 animals of each type were deeply anesthetized with sodium-pentobarbital (50 mg / kg ip) and then perfused through the heart with 4% paraformaldehyde-0.1% picric acid in 0.15 M sodium phosphate buffer (pH 7.2–7.4). The brains were removed and stored in 4% paraformaldehyde-0.1%picric acid in 0.15 M sodium phosphate buffer for 48 hours and then sectioned at 50 µM on a vibratome (Leica VT1000). Sections were matched for animal age (P18-22) and the anterior-posterior level of section and were counterstained with NEUROTRACE 530/615 (Life Technologies) to indicate the distribution of cells and allow the determination of cortical layers and sublayers. Sections were matched for animal age (P18-22) and the anterior-posterior level of section and were counterstained with NEUROTRACE 530/615 (Life Technologies) to indicate the distribution of cells and allow the determination of cortical layers and sublayers. We rehydrated the sections for 40 minutes in 0.1M phosphate-buffered saline (PBS) and then washed the sections for 10 min in PBS plus 0.1% Triton X-100. After washing the sections two times (5 min each) in PBS, we stained with 200 µL NEUROTRACE 530/615 at 1:200 for 20 minutes. We then washed the excess stain for 10 min in PBS plus 0.1% Triton X-100, followed by two 5 min washes in PBS. The sections were mounted and cover-slipped with a solution containing 2.5% of the anti-fade reagent 1,4 diazabicyclo[2.2.2]octane that was dissolved in a 0.1 M Tris buffer (pH 8-8.5) containing 10% polyvinyl alcohol and 25% glycerin. All chemicals were obtained from Sigma-
For imaging and analysis, we used a Zeiss 710 confocal microscope attached to a Zeiss AxioObserver inverted microscope (University of Tennessee Neuroscience Institute Imaging Center) for the confocal images shown in **Figure 1**. For the low power images depicting expression pattern, images were taken with a 10x objective and were tiled and stitched from several fields of view. Images shown are from individual optic sections (1 μm optical thickness) at the depth of clearest GFP expression. We examined the laminar distribution of cells from 4 animals of each type using higher magnification images (20x objective, 0.8 n.a.) and Zeiss Zen software by measuring the intensity of fluorescence with a series of 1 μm thick lines (1000 μm in length, 20-50 lines per animal), orthogonal to the pia and sampled through cells with EGFP fluorescence. We imported the data into IGOR, where each line was represented by a single wave. We determined an average wave for each group, smoothed the data (25 point rolling average), and fit the result with a single Gaussian function representing fluorescent fluorescence intensity as a function of distance from the pia. The fitting curves were scaled to the same baseline and maximum intensity to more easily compare the relative distribution of glt and etv1 cells. For measurements of soma area, we used a 63x oil immersion lens (1.4 n.a.) and obtained a Z stack (1 μm optical sections) through 5-7 fields of view (in somatosensory cortex) per slice, one slice per animal (n= 4 etv1 and 4 glt animals). The Z stack was read into Neurolucida (MBF Bioscience, Williston, VT) and soma perimeters were outlined at the depth of greatest diameter for each cell.
Slice recordings

Slices were placed in a recording chamber on the stage of an Olympus BX50WI upright microscope. Slices were bathed in artificial cerebrospinal fluid (aCSF) bubbled with 95% $\text{O}_2$/5% $\text{CO}_2$, delivered at 2 ml / min, and heated with an in-line heater (Warner Instruments, Hamden, CT) to 33 ± 1°C (measured with a thermistor in the bath adjacent to the slice). The aCSF contained (in mM) 125 NaCl, 3 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, and 20 glucose (pH 7.4, 310 mOsm). Pharmacological agents were added directly to the aCSF. All slice recordings of repetitive firing were done in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 $\mu$M) to block $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazoleproipionic acid (AMPA) receptors, 2-amino-5-phosphonovaleric acid (APV, 50 $\mu$M), or (α)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 5 $\mu$M) to block N-methyl-D-aspartate (NMDA) receptors, and picrotoxin [100 $\mu$M; to block $\gamma$-aminobutyric acid type A (GABA$_A$) receptors] to ensure that studied effects of the pharmacological agents were exerted directly on the postsynaptic cell. Norepinephrine was made up as a 20 mM stock in Millipore-filtered, distilled H$_2$O containing 20 mM ascorbic acid on the day of the experiment and then diluted to the final dose (10 $\mu$M) in aCSF. A 50 $\mu$M stock of the sK channel blocker apamin was dissolved in 5% acetic acid, frozen, and then thawed and diluted to 100 nM in aCSF on the day of the experiment.

Pyramidal neurons in layers 2/3 and 5 were visualized with infrared/ differential
interference contrast (IR/DIC) video-microscopy (Dodt and Zieglgansberger, 1990; Stuart et al. 1993) using a 40x (0.8 n.a.) Olympus water-immersion objective and an IR-sensitive camera (Olympus OLY-150 or DAGE-MTI). Etv1 or glt pyramidal cells were visually identified by the presence of EGFP epifluorescence using an FITC filter. There was typically a main band of EGFP+ cells in layer 5 in each animal. Recordings were directed within this main band. We switched between IR-DIC and epifluorescence to determine cell type and to obtain a GΩ seal. Electrode position was controlled with Sutter ROE-200 manipulators and PC-200 controller or Luigs-Neumann manipulators and controller. Whole cell patch-clamp recordings were acquired using either an Axon Multiclamp 700A or Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) and PClamp 9 or 10 software. For current clamp recordings, the data were digitized at 20-50 kHz and filtered at 10 kHz. Voltage clamp recordings of tail currents were digitized at 10 kHz and filtered at 2 kHz. We recorded with borosilicate electrodes (Warner G150F: 3–8MΩ in the bath) produced with a horizontal electrode puller (Flaming-Brown P-87; Sutter Instruments, Novato, CA, USA). For current- and voltage-clamp recordings, electrodes were filled with an internal solution containing (in mM): 130.5 KMeSO$_4$, 10 KCl, 7.5 NaCl, 2 MgCl$_2$, 10 HEPES, 2 adenosine 5-triphosphate (ATP), 0.2 guanosine 5-triphosphate (GTP). 100 μM EGTA was added to the intracellular solution. Data were collected only from cells forming a 1-GΩ or tighter seal. All reported voltages were corrected by subtracting the measured liquid junction potential (8 mV).

An adaptation index was calculated for repetitive firing, as defined in Groh et al.
We measured the time interval between successive spikes elicited using a 2s current injection at 300 pA. We excluded the first two interspike intervals (ISIs) and then normalized each interval to the third ISI as a function of the ISI number. We then fitted the data with linear regression and 100 * (the slope of the regression line) was the adaptation index. An adaptation index of “10,” means that on average, the time interval increases by ten percent between successive spikes.

Statistics

Prism (GraphPad Software, San Diego, CA) software was used to perform statistical tests. Student’s paired t-test was used to compare sample population data between etv1 and glt cells and summary data are presented as means +/- SEM, unless noted otherwise. Paired t-tests were used to compare control (Ctl) vs. drug effects. We used a one-way ANOVA to compare multiple experimental groups (etv1, glt, layer 2/3), with post-hoc Tukey’s multiple comparisons tests to determine which individual means differed. For all tests, P values <0.05 were considered to be significantly different. Sample population data are represented as scatterplots, histograms of mean ± SEM, or as box plots (Tukey, 1977). Box plots indicate the upper and lower quartiles as edges of the box, with the median represented as a line crossing the box. The stems indicate the largest and smallest non-outlying values. Outlying values are 1.5 times the quartile boundaries.

Results

Anatomical Measurements (Figure 1). The detailed study of Groh et al. (2010)
characterized etv1 and glt mice in terms of cell size, dendritic morphology, laminar position, proportion of excitatory cells accounted for by EGFP+ cells, and projection patterns (from retrograde labeling). In the present study, we concentrated on somatosensory cortex since Groh et al. (2010) found little overlap in distribution between these two cell lines in the somatosensory (barrel) cortex (in contrast to the substantial overlap in visual cortex). Groh et al. (2010) also reported that etv1 cells are a subset of slender pyramidal cells in layer 5A (~55% of excitatory neurons in this layer: Groh et al. 2010) and that glt cells comprise a subset of thick layer 5B neurons (~55% of excitatory neurons in this layer). Figure 1 shows our findings for the relative laminar position of these two cell types in somatosensory cortex (aged P18-22: n= 4 animals for both etv1 and glt). On average, etv1 cells were located more superficially within layer 5 than glt cells (EGFP distribution was centered at ~625 μm from the pial surface in etv1 cells and ~695 μm in glt cells). Counterstaining with a cell marker (NEUROTRACE 530/615: Life Technologies) indicated that the etv1 cells are found within superficial layer 5 (layer 5A). We found that glt cells were distributed more deeply on average, however there was considerable overlap with depths where etv1 cells were expressed and glt cell distribution extended into layer 6 (Figure 1). Our measurements also indicate that on average soma size did not differ between glt and etv1 cells (Figure 1).

**Electrical Recordings.** Our primary goal was to determine whether there are electrophysiological differences between etv1 and glt pyramidal cells that would shape their firing behavior. All recordings were from etv1-expressing or glt-expressing pyramidal neurons from 2-4 week old BAC mice. We used these
relatively young ages because at > 4 weeks, we were unable to consistently detect the fluorescent cells. Initial comparisons were made between etv1 and glt neurons in layer 5. We also recorded basic electrical properties from layer 2/3 pyramidal neurons from both mouse lines.

**Passive properties, “sag”, and rheobase (Table 1, Figure 2).** We first examined resting membrane potential (RMP) to compare these cell types in the absence of significant synaptic input, similar to “down states” observed during slow wave sleep in vivo (Steriade et al. 1993; Stern et al. 1997). RMP was measured immediately after entering whole cell mode. We found that the average RMP of layer 2/3 pyramidal cells was significantly more negative than either of the layer 5 subtypes (see also Larkman and Mason, 1990; Higgs et al. 2006, Breton and Stuart, 2009; Etherington and Williams, 2011) and etv1 cells had significantly more negative RMPs than glt cells (Figure 1A inset, 2C). We also measured input resistance ($R_{in}$: measured at peak voltage deflection to a -50 pA current injection) as an index of how the cells would passively respond to current inputs. $R_{in}$ did not differ significantly between the two subtypes of layer 5 pyramidal cells or for layer 5 cells vs. layer 2/3 pyramidal cells (Figure 2D).

Many pyramidal cells exhibit a time-dependent relaxation of the voltage response to hyperpolarizing current injections (“sag”: measured in response to a -150 pA current injection). This sag is considered an indicator of the kinetics and amplitude of $I_H$ current, relative to the membrane time constant (Schwindt et al. 1988c). We measured % sag as $100 \times \frac{(\text{peak response}) - (\text{steady state response})}{(\text{peak response})}$. All three types of pyramidal neurons had % sag that differed
significantly from each other, with glt neurons having the greatest sag (Table 1, Figure 2E). The time to the peak negative voltage deflection in the sag protocol was significantly shorter for glt neurons vs. etv1 or layer 2/3, suggesting more rapid kinetics of I_H in glt neurons (Table 1). We tested the response of several cells of each type to the blocker of H current, ZD-7288 (ZD: 50 μM). Application of ZD 7288 significantly reduced voltage sag in layer 2/3 pyramidal cells (p < 0.004: 5.7 ± 1.1 control vs. 1.26 ± 0.7% ZD; n = 8), etv1 cells (p < 0.0001: 21.1 ± 1.9 control vs. 0.3 ± 0.2% ZD; n = 6), and glt cells (p < 0.0001: 28.5 ± 1.4 control vs. 1.4 ± 0.7% ZD; n = 6) cells (data not shown).

We also measured rheobase, the current required to reach AP threshold during a long current injection (500 ms), as an indicator of neuronal excitability (lower rheobase = greater excitability). We found that rheobase was significantly lower in glt vs. etv1 cells (Table 1, Figure 2F), indicating that glt cells are more easily brought to threshold by DC input. The rheobase of layer 2/3 pyramidal cells was significantly higher than that of glt cells, but was not significantly different from etv1 cells (Table 1, Figure 2F).

**Action Potentials.** We next examined the response of etv1 and glt neurons to a single 10 ms, suprathreshold current injection. To characterize the action potential (AP), we measured AP amplitude, voltage threshold (V_{th}: measured as the voltage corresponding to the abrupt increase in dV/dt), width at half amplitude (HW: relative to RMP), and the maximum rate of change (dV/dt) for both the upstroke and downstroke (Table 2, Figure 3). AP amplitude was significantly larger for layer 2/3 PCs than either of the layer 5 subtypes. V_{th} was lower in glt vs. etv1 cells
suggesting that \textit{glt} neurons may reach threshold more readily than \textit{etv1} neurons.

We found that spikes were narrower in \textit{glt} cells vs. \textit{etv1} cells (c.f., Groh et al. 2010) and both layer 5 subtypes have narrower spikes than layer 2/3 cells (Figure 3D). Narrow spikes often correlate with high firing rates in various cell types (Bean, 2007). The maximal rates (dV/dt) of AP depolarization and repolarization (indicators of Na\textsuperscript{+} current density and repolarizing current density, respectively) were both significantly greater in \textit{glt} pyramidal neurons than in \textit{etv1} cells (Figure 3E). Layer 2/3 PCs had lower maximum dV/dt for spike repolarization than \textit{glt} or \textit{etv1} cells.

**AHPs.** Afterhyperpolarizations (AHPs) reflect conductances activated during spiking. Three AHPs have been described in neocortical pyramidal neurons (Schwindt et al. 1988a,b; Lorenzon and Foehring, 1992, 1993; Abel et al. 2004; Andrade et al. 2012). The fast AHP (fAHP) reflects repolarization mechanisms for the AP, as well as residual inward currents and electrotonic redistribution of charge (Schwindt et al. 1988b). The medium AHP (mAHP) can also be activated by a single AP and is partially mediated by Ca\textsuperscript{2+}-dependent and apamin-sensitive sK channels (Schwindt et al. 1988b; Spain et al. 1991; Lorenzon and Foehring, 1993; Pineda et al. 1998; Abel et al. 2004). The mAHP is also influenced by voltage-gated K\textsuperscript{*} conductances, as well as I\textsubscript{H} (Schwindt et al. 1988b,c; Storm, 1989; Gulledge et al. 2013). The slow AHP (sAHP) has Ca\textsuperscript{2+}- and Na\textsuperscript{+}-dependent parts (Schwindt et al. 1988b; Foehring et al. 1989; Gulledge et al. 2013), requires multiple APs to activate, and is insensitive to apamin. The initial Ca\textsuperscript{2+}- sensitive portion of the sAHP lasts ~1-2 s and is mediated by unknown channels that are negatively modulated by
transmitters that activate PKA (e.g., norepinephrine through β-receptors; Foehring et al. 1989; Andrade et al. 2012).

We found that the mAHP after a single AP (Figure 3F) was significantly larger in glt cells ($4.1 \pm 0.3$ mV, $n = 56$) compared to etv1 cells ($3.1 \pm 0.23$ mV, $n = 70$) or layer 2/3 cells ($1.3 \pm 0.2$, $n = 23$; which is also significantly different vs. etv1). The amplitude of the peak AHP and sAHP are known to depend on the number and frequency of APs used to elicit them (Schwindt et al. 1988a; Abel et al. 2004). Therefore, to compare the amplitude of the peak AHP and sAHP between cell types, we used a standard protocol with ten, suprathreshold current injections (5 ms), repeated at 50 Hz. Under these conditions, etv1 cells had a significantly larger sAHP (measured at 500 ms after the last AP) and larger peak AHP than glt cells (Table 3, Figure 4). (The peak AHP after multiple APs includes a contribution from the sAHP conductance as well as that from the mAHP conductance: Abel et al. 2004; Kaczorowski et al. 2007). Layer 2/3 cells had smaller peak AHPs than either layer 5 cell subtype and smaller sAHP vs. etv1 cells, although the RMP when this protocol was run was significantly more negative in the layer 2/3 cells ($-78 \pm 1$ mV) vs. the layer 5 cells ($-74 \pm 0.5$ mV for etv1 and $-74 \pm 0.4$ mV for glt), which would have reduced the driving force and influenced AHP amplitude.

Repetitive firing. Most neocortical pyramidal neurons fire rhythmically in response to a DC stimulus and are referred to as regular spiking (RS) cells (McCormick et al. 1985; Agmon and Connors, 1992; Chagnac-Amitai et al. 1990; Mason and Larkman, 1990). RS cells initially fire at a higher rate and exhibit spike frequency adaptation (SFA) to a constant stimulus (McCormick et al. 1985; Chagnac-
All of the pyramidal cells in this study were of the RS type (Figure 5A; McCormick et al. 1985), although some glt cells exhibited an initial spike doublet before RS firing (6/63 cells = 13%; e.g., Figure 2C). RS firing can be characterized by the relationship between average firing frequency and the injected current (f-I curve) and the relationship between instantaneous firing rate and time (f-t plot).

The overall f-I relationship was more linear in etv1 cells and bilinear or curvilinear in glt neurons (Figure 5). The f-I slopes for the first 100-200 ms of firing were similar for all three pyramidal cell subtypes (Table 4, Figure 5) but the relationships were significantly different between layer 5 cell types for 500 ms or 2 s firing (Figure 5). Glt cells have a steeper initial f-I slope than etv1 cells (i.e., higher gain: Table 4, Figure 5). In both cell types, f-I slopes were higher for the initial 100-200 ms of firing compared to 500 ms or 2 s of firing, although this was much more dramatic for etv1 neurons (Table 4, Figure 5). For the full 2 s, the f-I slope of L2/3 cells was intermediate between etv1 and glt cells and was significantly greater than etv1 cells (Table 4).

The greater divergence in f-I slopes after the first 200 ms of firing suggests greater SFA in etv1 neurons. This is shown directly by the f-t relationships of these two cell types (in response to a 2 s, 300 pA current injection: Figure 6A vs. B). All of the cell types differed in % adaptation [100 * (frequency of 3 rd ISI) - (frequency of final ISI)] / (frequency of 3 rd ISI)], with adaptation greatest in etv1 cells, almost nonexistent in glt cells, and intermediate in layer 2/3 cells (Figure 6D). We also found a significant difference in the adaptation index (as defined by Groh et al. 2010: see...
Methods. *Etv1* neurons (39 ± 7, n = 21) had a much greater adaptation index than *glt* neurons (1.1 ± 0.3, n = 27), with layer 2/3 pyramidal cells intermediate (24 ± 14; Figure 6D, Table 4). The 3rd ISI was chosen for normalization (for both adaptation index and % adaptation) to emphasize the prolonged time-dependent changes evident after the steep decline in firing rate in the first two intervals in both cell types and the presence of an initial AP doublet in some *glt* cells (Guan et al. 2013; Hattox and Nelson, 2007). *Etv1* neurons exhibit a clear biexponential decay of firing rate during a 2s current injection, with fast (τ ~ 18 ms: 89% of decay) and slow (τ ~ 240 ms: Figure 6A) components. In contrast, *glt* cells exhibit very little slow adaptation (~5% of total: Figure 6B). Layer 2/3 pyramidal cells also exhibited two time constants for adaptation, with one τ ~ 16 ms (95%) and the other τ ~ 435 ms (5%: Figure 6C). We did not observe a time-dependent acceleration of firing during any of the DC current injections, as previously shown in a subset of layer 5 pyramidal cells in mouse motor cortex (Miller et al. 2008) and prefrontal cortex (Dembrow et al. 2010), but not somatosensory cortex (Miller et al. 2008).

Voltage clamp of AHP currents. The dramatic differences in firing behavior (f-I relationships and SFA) between *etv1* and *glt* cells suggested that these two populations of layer 5 cells may have differential expression of the Ca^{2+}-dependent conductances that underlie the I_{mAHP} and I_{sAHP} (Schwindt et al. 1988a; Abel et al. 2004). As noted above, the peak AHP after multiple APs is influenced by both the mAHP and sAHP conductances (Schwindt et al. 1988a; Abel et al. 2004). Thus, to obtain clearer separation between the underlying conductances, we used whole cell voltage-clamp to reveal the currents underlying the mAHP (I_{mAHP}) and sAHP.
(I_{SAHP}). We elicited the AHP currents with a voltage step from -70 mV to +30 mV (for 200 ms). Space-clamp is poor in these dendritic neurons but we focused on the underlying small and very slowly deactivating tail currents upon return to -60 mV. TTX was not present so typically a few unclamped APs occurred in all cell types at the beginning of the step. Tail currents were obtained from 14 etv1 neurons and 14 glt neurons (Figure 7). Most of the tail currents were well fit as the sum of two exponentials, corresponding to I_{mAHP} and I_{SAHP}, respectively. In etv1 neurons both I_{mAHP} and I_{SAHP} were usually of similar amplitude (estimated by extrapolation of the exponential fit of the tail current to time zero: Table 5, Figure 7D), although in some cells a large slow component (I_{SAHP}) obscured I_{mAHP}. The slower I_{SAHP} had a $\tau$ of ~900 ms and the faster I_{mAHP} had a $\tau$ of ~66 ms (Table 5). In contrast, while similar fast and slow components could be seen in glt neurons, the amplitude of I_{mAHP} was much larger than in etv1 neurons and much larger than I_{SAHP}, which only made up ~18% of the tail current amplitude in glt neurons (Table 5). Two time constants were also evident in layer 2/3 pyramidal neurons (n=21), although the time constants for both I_{mAHP} and I_{SAHP} were significantly slower in layer 2/3 cells vs. layer 5 cells (Table 5). The proportion of the current due to I_{SAHP} was similar in layer 2/3 and glt cells.

**Calcium dependence.** To further test for cell-type differences in Ca^{2+}-dependent mechanisms, we compared cells before and after bath application of the non-specific inorganic Ca^{2+} channel blocker Cd^{2+} (400 $\mu$M). In those experiments, we did not add NaH_{2}PO_{4} to the aCSF (to prevent precipitation). Cd^{2+} did not cause significant changes in RMP or rheobase in etv1 (n = 4) or glt cells (n = 5; data not shown). We
also found no statistical differences for any parameters of a single AP in either cell type (data not shown). We also elicited AHPs with 10 APs at 50 Hz to test for Ca\(^{2+}\)-dependence of the AHPs. In etv1 cells (n = 5), both the peak AHP (control 6.4 ± 1.9 mV; Cd\(^{2+}\): 2.9 ± 1.1 mV) and the sAHP (at 500 ms after the last AP; control 2.6 ± 1.7 mV; Cd\(^{2+}\): 0.6 ± 0.5 mV) were significantly reduced by Cd\(^{2+}\). In glt cells (n = 5) both the peak (control 4.8 ± 0.7 mV; Cd\(^{2+}\): 2.4 ± 1.0 mV) and sAHP (control 0.6 ± 0.4 mV; Cd\(^{2+}\): 0.2 ± 0.2 mV) were reduced, although this was only significant for the peak AHP. In voltage-clamped etv1 cells (n = 3), both I\(_{mAHP}\) and I\(_{sAHP}\) were reduced by Cd\(^{2+}\), indicating that they were Ca\(^{2+}\)-dependent (Figure 7C). For the four glt cells tested, there were no clear slow tail components present initially but Cd\(^{2+}\) reduced the remaining faster time constant in all cases (data not shown). Our data indicate differential expression of Ca\(^{2+}\)-dependent mechanisms between etv1 and glt cells.

We next used a pharmacological approach to examine specific Ca\(^{2+}\)-dependent conductances.

**Role of sK channels.** In neocortical pyramidal cells, a large proportion of the mAHP and underlying current is mediated by apamin-sensitive, sK-type K\(^+\) channels (Schwindt et al. 1988a, 1992a; Lorenzon and Foehring, 1993; Pineda et al. 1998; Abel et al. 2004; Jones and Stuart, 2013). We tested whether sK channels differentially contribute to the properties of etv1 vs. glt cells by application of the sK channel blocker, apamin (100 nM). We examined AHP currents in voltage clamp mode, the AHPs after a single AP and after 10 APs (at 50 Hz), and repetitive firing in response to 2s current injections.

In voltage clamp, we elicited tail currents (at -60 mV) with a 200 ms step from -70
mV to +30 mV. In etv1 cells (n = 9), the tail current was dominated by a single
exponential component with a $\tau_{\text{decay}}$ of 1258 ± 184 ms and amplitude of 228 ± 32 pA
(extrapolated from the exponential fit). Apamin (100 nM) blocked current in 5 of 9
etv1 cells (with no effect in the remaining 4 cells). The apamin-sensitive component
in the 5 apamin-sensitive cells (obtained by subtraction: 84 ± 31 pA) had a decay $\tau$
of 200 ± 65 ms. In glt cells the tail current exhibited only a single decay time
constant in 3 of 6 cells tested (Figure 8D, inset). The other three cells had an
additional slow component (decay $\tau$ of 1071 ± 307 ms). The apamin-sensitive
current in these six cells had a decay $\tau$ of 158 ± 71 ms and an amplitude of 344 ± 124
pA. Thus apamin blocked a current consistent with I_{mAHP} in both cell types but this
current was much larger in glt cells. Given the microdomain interaction between
intracellular Ca$^{2+}$ and sK channels (Abel et al. 2004; Fakler and Adelman, 2008;
Andrade et al. 2012; Jones and Stuart, 2013), it is likely that either Ca$^{2+}$ entry is
greater or there are more sK channels expressed in glt cells vs. etv1 cells.

Apamin had no effect on resting membrane potential, peak or steady-state $R_{\text{m}}$, or
rheobase in etv1 (n = 15) or glt (n = 11) neurons. Apamin also caused no significant
change in the AHP following a single AP in etv1 neurons (control: 2.4 ± 0.5 mV;
apamin: 1.5 ± 0.3 mV; n = 13; Figure 8A). In contrast, in glt neurons apamin
significantly reduced the single AP mAHP (2.3 ± 0.3; apamin: 1.1 ± 0.2; n = 13;
Figure 8B). Following 10 APs at 50 Hz, apamin again significantly reduced the peak
AHP in glt neurons (control: 4.8 ± 0.7 mV; apamin: 3.2 ± 0.7 mV; n = 10; Figure 8D)
but not etv1 neurons (control: 6.2± 0.8 mV; apamin: 5.2 ± 0.8 mV; n = 14; Figure
8C). The sAHP (measured at 500 ms after the last AP) was not affected by apamin in
etv1 (control: 3.6 ± 0.6 mV; apamin: 3.2 ± 0.6 mV; n = 14) or glt (control: 1.5 ± 0.7 mV; apamin: 1.3 ± 0.6 mV; n = 10) cells (Figure 8). Together with the tail current data, these data suggest relatively greater expression of sK channels (or greater Ca\(^{2+}\) entry or Ca\(^{2+}\) coupling to sK) in glt vs. etv1 cells and a much greater sAHP in etv1 cells.

To examine the functional consequences of differential expression of sK channels, we studied the effects of apamin on repetitive firing (at three times rheobase) in eight etv1 cells and eight glt cells (Figure 9). Neither firing rate nor f-I slope (for 200 ms or 2s) was significantly affected by apamin in etv1 cells (Table 6, Figure 9A). Further, apamin did not affect slow SFA (Table 6, Figure 9C) and the instantaneous firing frequency for the first ISI did not change significantly in etv1 cells (% of control: 93 ± 15 Hz; apamin: 101 ± 14 Hz)(Figure 9C). Percent fast adaptation [100 * ((frequency of first interval) – (frequency of third interval))/(frequency of first interval)] was not significantly decreased by apamin in etv1 cells (% of control: 45 ± 3%; apamin: 40 ± 4%; p < 0.06).

In contrast, apamin did cause a significant increase in firing rate in glt cells (Table 6). This was due to a parallel (additive) shift in the f-I relationships (Figure 9B), with no statistically significant effect on f-I slopes for either the first 200 ms or for the entire 2s of firing (Table 6). Adaptation index and % adaptation were also unchanged in glt cells by apamin (Table 6, Figure 9D). The firing frequency for the first ISI was significantly increased by apamin in glt cells (control: 70 ± 20 Hz; apamin: 91 ± 22 Hz)(Figure 9D). Two of the eight glt cells fired with an initial doublet after apamin. Per cent fast adaptation was not significantly decreased by
apamin in glt cells (control: 37 ± 11%; apamin: 40 ± 7%). Thus, apamin increased firing rate in glt cells starting from the first ISI but did not alter fast or slow SFA.

Role of sAHP conductance. The sAHP (and I_sAHP) in neocortical PCs is Ca^{2+}-
dependent and K*-selective but the underlying channel type is not known (Schwindt et al. 1988a; Abel et al. 2004; Andrade et al. 2012). In many cell types, the sAHP is negatively modulated by neurotransmitters that activate PKA (reviewed in Andrade et al. 2012). A classical example is the sAHP reduction by norepinephrine, acting through β receptors (Madison and Nicoll, 1982; Foehring et al. 1989). We applied norepinephrine (NE: 10 μM) in the bath and examined input resistance, sag, rheobase, AHPs, and repetitive firing.

The effects of NE were dramatically different between etv1 and glt cells for AHPs, AHP currents, and repetitive firing. Peak and slow AHPs were elicited by ten, 5 ms current injections at 50 Hz. We tested 12 glt cells with 10 μM NE (Figure 10B) and found that the effects of NE application were not statistically significant for the sAHP (control 0.9 ± 0.4 mV; NE: 0.4 ± 0.1 mV) or the peak AHP (control 6.1 ± 0.6 mV; NE: 5.8 ± 0.7 mV; n= 12 cells; Figure 10D,E). The effects of NE on the sAHP were much greater in etv1 cells, consistent with the greater expression of the sAHP (and I_sAHP) in this cell type. For all etv1 cells tested (n = 21), application of 10 μM NE greatly reduced the sAHP (measured at 500 ms after the last AP: Figure 10A). The block was 40-100% (mean: 74 ± 4.5 %) from 3.5 ± 0.3 mV in control and 0.9 ± 0.2 mV in NE (p < 0.001; Figure 10E). The peak AHP was also significantly reduced by 10 μM NE in etv1 cells (~20% on average; control: 6.5 ± 0.5 mV; NE: 5.4 ± 0.5 mV; Figure
10D), reflecting the contribution of the sAHP conductance to the peak AHP after 10
spikes (see above). The effects of NE on the sAHP and repetitive firing were
mimicked by 100 μM isoproterenol (a β-agonist) in three etv1 cells.

In voltage clamp, NE was tested on AHP tail currents (Figure 10C). In etv1 cells (n =
6), the tail current could be well fit by a sum of two exponential components: 79 +
27 ms (43% of total) and 1058 ± 181 ms (57% of total). NE (10 μM) selectively
reduced the slow component: the NE-sensitive current (obtained by subtraction)
had a decay τ of 718 ± 173 ms (amplitude: 152 ± 31 pA, 54% of control: Figure
10C,F). Glt cells (n = 4) exhibited only a single component to the tail current, with a
decay τ of 75 ± 15 ms (n = 4; data not shown). NE did not significantly affect the
amplitude of this tail current in glt cells (Figure 10F).

To examine the functional consequences of differential expression of the sAHP, we
next examined the effects of NE on repetitive firing of etv1 and glt cells (at 3 times
rheobase: Figure 11). NE is known to reduce SFA in pyramidal cells, as well as
increase f-I slope (Madison and Nicoll, 1982; Schwindt et al. 1988a; Foehring et al.
1989). Firing (at 3x rheobase) was faster in NE for etv1 cells (Table 6). The f-I
slopes for the first 200 ms of firing, as well as for the entire 2s firing, were
significantly increased in etv1 cells by NE (n = 9; Figure 11A, Table 6). In contrast,
in glt cells (n =9), NE did not significantly affect firing rate (Table 6) or f-I gain over
the first 200 ms or at 2s (Table 6, Figure 11B). NE also had dramatically different
effects on SFA in etv1 and glt cells. NE greatly reduced SFA measured as %
adaptation (n =9) or adaptation index (n = 8) in etv1 cells but not glt cells (Figure
11, Table 6: both at 3x rheobase). This confirms the previously determined mechanistic link between the sAHP conductance and slow SFA in pyramidal neurons (Madison and Nicoll, 1982; Schwindt et al. 1988b; Foehring et al. 1989).

**Other effects of NE.** In addition to its effects on the sAHP and repetitive firing, NE elicited a depolarization that lasted several minutes in both etv1 (n = 10) and glt cells (n = 9; Figure 10A,B). $R_{in}$ was not significantly changed by NE in etv1 cells (n = 10; ctl: 119 ± 8 MΩ; NE: 125 ± 10 MΩ) or glt cells (n = 9; ctl: 135 ± 13 MΩ; NE: 145 ± 14 MΩ). Sag was not changed by NE in etv1 cells (ctl: 16 ± 2%; NE: 14 ± 2%; n = 9), but was significantly reduced in glt cells (ctl: 26 ± 2.%; NE: 22 ± 2%; n = 8). Rheobase was unchanged in glt neurons by NE (ctl: 65 ± 12 pA; NE: 65 ± 19 pA; n = 10) but was significantly reduced in etv1 cells (179 ± 23 pA; NE: 138 ± 21 pA; n = 12).

**Discussion**

We used whole cell recordings from mouse neocortical pyramidal cells in acute brain slices from BAC mice to test for electrophysiological differences between etv1 and glt cells. We found clear differences between etv1 and glt cells in APs, AHPs, and firing properties. Compared to glt cells, etv1 cells were generally less excitable in that they had higher rheobase and $V_{th}$. Etv1 cells also had broader APs, less sag, larger sAHPs, and fired slower, with lower gain and greater SFA. In contrast, the more excitable Glt cells had lower $V_{th}$, narrower APs, greater sag, larger mAHPs, and very little sAHP. The narrower APs in glt cells reflected the higher peak $dV/dt$ for
both spike polarization and repolarization, suggesting differences between layer
etv1 and glt cells in both depolarizing Na\(^+\) currents and repolarizing K\(^+\) currents
(Sheets et al. 2011). Glt cells also fired faster and with higher gain and exhibited
almost no SFA. We confirmed the findings of Groh et al. (2010) that etv1 pyramidal
cells differed from glt pyramidal cells in AP width and SFA. A notable difference in
our study compared to Groh et al. (2010) was that they found R\(_{in}\) to be lower in glt
cells but R\(_{in}\) did not differ significantly between pyramidal cell types in our sample.

**Ca\(^{2+}\)-dependent AHPs and associated currents.** The most dramatic differences
between etv1 and glt neurons were in the expression of Ca\(^{2+}\)-dependent
mechanisms: the mAHP, sAHP, and repetitive firing (especially SFA). Whereas glt
neurons had larger apamin-sensitive mAHP and I\(_{mAHP}\), etv1 neurons had larger sAHP
and I\(_{sAHP}\). This suggests differential expression of channel types, with greater
relative importance of sK channels in glt cells and greater relative importance of
sAHP channels in etv1 and layer 2/3 pyramids. In CA1 pyramidal cells (Storm,
1987), Ca\(^{2+}\)-dependent mechanisms also contribute to spike repolarization. In
contrast, only the last phase of spike repolarization is Ca\(^{2+}\)-dependent in neocortical
pyramidal cells (Sun et al. 2003; Traub et al. 2003; Schwindt et al. 1988a; Pineda et
al. 1998; Abel et al. 2004; Higgs et al. 2006; Andrade et al. 2012). Our Cd\(^{2+}\) data
suggest that the more rapid repolarization of the AP in glt neurons is unlikely to be
due to differential expression of somatic BK channels compared to etv1 cells. Rather,
these cell types may differ in voltage-gated potassium channel expression (c.f.,
Toledo-Rodriguez et al. 2004; Sugino et al. 2006).

**Differences in repetitive firing.** The dramatic differences in the relative
importance of sK and sAHP channels underlie distinctive patterns of repetitive firing in etv1 and glt cells. Glt cells had greater expression of sK conductance. These channels had a subtractive effect on firing such that apamin significantly increased the instantaneous firing rate starting from the first ISI in glt cells but had no effects on firing rate in etv1 cells. Our apamin data also indicated that sK channels do not affect gain of firing or SFA in either etv1 or glt neurons.

In contrast, etv1 cells had greater expression of the sAHP conductance. This conductance regulates f-I slope and SFA in neocortical pyramidal neurons (Schwindt et al. 1988b; Foehring et al. 1989; Lorenzon and Foehring, 1993). Consequently, the most dramatic firing difference between pyramidal cell types was for SFA. Whereas most glt cells did not display SFA after the 3rd spike, etv1 neurons exhibited a strong slow phase of SFA. Also consistent with the greater sAHP expression in etv1 neurons, we found that firing rate to a given current injection and the gain of firing (f-I slope) in response to DC inputs were lower in etv1 neurons vs. glt neurons. Thus firing rate would be expected to change less with increased synaptic drive in etv1 cells compared to glt cells.

Also striking was the differential modulation of firing in etv1 and glt neurons by NE. NE selectively reduced the sAHP conductance. Since most glt neurons had very small sAHPs (and $I_{\text{sAHP}}$), NE had little effect on sAHP amplitude in these cells. The more substantial sAHP expression in etv1 cells provides a greater substrate for modulation by NE acting through β receptors (Madison and Nicoll, 1982; Foehring et al. 1989; Lorenzon and Foehring, 1993). Consequently, NE had much greater effects on repetitive firing behavior in etv1 cells than glt cells. By blocking the
conductance underlying the sAHP, NE increased gain and decreased SFA in \textit{etv1} cells, eliminating the most dramatic differences in firing behavior between \textit{etv1} and \textit{glt} neurons. Thus, firing in \textit{etv1} cells has a temporal structure (larger SFA) that is highly sensitive to transmitter modulation. In contrast, NE had no significant effects on f-I slope or SFA in \textit{glt} cells.

**Morphological characterization of \textit{etv1} and \textit{glt} cells.** The morphology, laminar location, and projections of EGFP-positive cells in \textit{etv1} and \textit{glt} mice were characterized in detail by Groh et al. (2010). They found that in barrel cortex, \textit{etv1} cells were restricted to layer 5A and \textit{glt} cells to layer 5B (see also Sharifullina, 2011). GFP-positive cells accounted for \textasciitilde 55\% of excitatory neurons in layers 5A (\textit{etv1}) and 5B (\textit{glt}). Further, \textit{etv1} cells were reported to be slender-tufted (Larkman and Mason 1990), having significantly smaller soma and apical dendrite diameters, as well as narrower apical tufts in layer 1, compared to \textit{glt} cells (Groh et al. 2010). Groh et al. (2010) also used retrograde labeling to show that \textit{glt}-pyramids project to ipsilateral pons and thalamus, but not to contralateral striatum and thus they are a subset of PT-type cells (c.f., Catsman-Berrevoets et al. 1980; Reiner et al. 2003, 2010; Shepherd, 2013). \textit{Etv1}-pyramidal cells were found to be a subset of IT-type neurons: they project callosally and to ipsilateral striatum, and to some extent contralateral striatum but not to pons or thalamus (Groh et al. 2010). In our study, we did not examine projection patterns, however we confirmed the findings of Groh et al. (2010) that \textit{etv1} cells were mostly located in superficial layer 5 in somatosensory cortex and that on average \textit{glt} cells had a deeper distribution. In contrast to their findings, we found extensive overlap between the distribution of \textit{glt}...
and etv1 cells in layer 5 and no differences in their soma areas. One possible reason
for these differences between studies is that we examined S1 somatosensory cortex
in general and they restricted their study to barrel cortex. Another possibility is
genetic differences in our glt animals vs. those used by Groh et al. (2010), despite
both being derived from the same BAC lines.

Comparisons with the electrical properties of pyramidal cells identified by
other means. Layer 5 pyramidal cells have been classified many ways, including
dendritic morphology, laminar location, projections to specific targets, projection
pattern (IT- vs. PT-type), or possession of specific electrophysiological
characteristics. Numerous studies have revealed correlations between classification
schemes, but their concordance is not perfect. There have also been several
attempts to correlate gene expression with pyramidal cell projection pattern
(Christophe et al. 2005; Sugino et al. 2006; Groh et al. 2010) but it is not clear how
precisely these groupings correspond to other types of classification.

In somatosensory (but not visual) cortex, etv1 cells were reported to be a subset of
IT-type, slender-tufted pyramidal cells and glt neurons a subset of the PT-type,
thick-tufted pyramidal cells (Groh et al. 2010). How do our results for etv1 and glt
cells compare to the electrophysiological properties of pyramidal cells defined by
dendritic morphology (slender- vs. thick-tufted), laminar location (layer 5A vs. 5B),
or projection pattern (IT- vs. PT-type)?

Slender- vs. thick-tufted cells. Sharp electrode recordings from rat cortex were
originally used to correlate morphology with electrophysiology in layer 5 (Mason
and Larkman, 1990; Larkman and Mason, 1990). They found that slender-tufted layer 5 cells had higher $R_{\text{in}}$, less sag, lower rate of rise and fall of the AP, broader APs, and greater SFA vs. thick-tufted cells (Larkman and Mason, 1990; Kasper et al. 1994). AP thresholds were similar between these cell types. The mAHP after a single AP was larger in the slender-tufted L5 cells vs. the thick-tufted cells. Our findings that $etv1$ cells had less sag, lower rate of rise and fall or the AP, broader APs, and greater SFA than $glt$ cells are similar to differences between thin- and thick-tufted cells, respectively. Our data differed in that AP thresholds were higher and the mAHP smaller in $etv1$ cells compared to thin-tufted cells.

**Firing pattern.** In the original sharp electrode recordings, (Mason and Larkman, 1990; Kasper et al. 1994), layer 2/3 pyramidal cells, slender-tufted layer 5 cells, and ~1/3 of the thick-tufted cells fired in a regular spiking pattern (RS: McCormick et al. 1985; Connors et al. 1982; but see Schubert et al. 2006). The rest of the thick-tufted cells in layer 5 fired in an intrinsic bursting (IB) pattern, with 3-5 APs of descending amplitude and high frequency in response to a just suprathreshold current injection (c.f. McCormick et al. 1985; Connors et al. 1982). Burst firing in neocortical pyramidal cells is labile, however. Layer 5B pyramidal cells have a higher propensity for burst firing in response to dendritic stimulation vs. somatic current injection (Larkum et al. 1999, 2001, 2004, 2007). Also, prolonged anesthesia with ketamine or pentobarbital elicits burst firing *in vivo* in layer 5 neurons projecting to superior colliculus (PT-type) but not in cells projecting to the contralateral hemisphere (IT-type: Christophe et al. 2005). All of the cells we tested fired in a RS pattern, i.e., no true burst firing cells were observed. True burst firing (as defined
by McCormick et al. 1985) is rarely seen in our hands with somatic whole cell recordings from rodent cortex (with somatic current injection). Some of the burst firing observed in sharp electrode studies may reflect dendritic recordings and dendritic current injection (Schwindt et al. 1997). Burst firing is sensitive to animal age (Franceschetti et al. 1998; Kasper et al. 1994), bath temperature (Hedrick and Waters, 2012), internal Ca\(^{2+}\) chelation (Lorenzon and Foehring, 1995; Schwindt et al. 1992b), ionic composition of internal and external solutions, and perhaps species and strain differences.

Most neocortical neurons are however, capable of two-spike “bursts” and Groh et al. (2010) observed that ~50% of glt cells fired with two-spike intrinsic bursts. Under our recording conditions, two spike bursts were restricted to glt cells (~13%).

Bursts, by means of synaptic facilitation, are thought to make signaling more reliable compared to single APs (Lisman, 1997) and can be regulated by K\(^+\) currents with properties similar to sK channels (Kepecs et al. 2002). Perhaps the relatively large sK conductance in glt cells (vs. etv1) is related to the need for regulation and termination of facultative burst firing in these cells (in response to dendritic excitation).

Thick- and slender-tufted cells vs. projection pattern. Projection pattern and slender- vs. thick-tufted morphology are strongly correlated in several cortical areas (Morishma et al. 2011; Hattox and Nelson, 2007; Dembrow et al. 2010; Gee et al. 2012; Avesar and Gulledge, 2012). Whereas thick-tufted pyramidal cells projecting to brainstem (PT-type) are restricted to deep layer 5 (layer 5B), slender-tufted neurons projecting callosally (IT-type) are more widespread but are more
frequently observed in the more superficial layer 5A (Larkman and Mason, 1990; Mason and Larkman, 1990; Christophe et al. 2005; Wise and Jones, 1976). Connectivity between pyramidal cells also correlates with subcortical projection pattern (Brown and Hestrin, 2009; Morishima et al. 2011; Ueta et al. 2013).

Several studies have examined differences in electrophysiological properties depending upon projection targets. These data can be summarized as follows: thick-tufted neurons projecting to the brainstem or spinal cord (PT-type) have low $R_{in}$ and $V_{th}$, narrow APs, small sAHPs, rapid firing with high gain, and little SFA (Schwindt et al. 1997; Le Be et al. 2007; Hattox and Nelson, 2007; Dembrow et al. 2010; Brown and Hestrin, 2009; Sheets et al. 2011; Avesar and Gulledge, 2012; Suter et al. 2013). Slender-tufted callosal-projecting neurons (IT-type) have high $R_{in}$ and $V_{th}$, broad APs, pronounced sAHP, slower firing with lower gain, and pronounced SFA (Schwindt et al. 1997; Le Be et al. 2007; Hattox and Nelson, 2007; Dembrow et al. 2010; Suter et al. 2013; Brown and Hestrin, 2009; Sheets et al. 2011; Avesar and Gulledge, 2012).

Thick-tufted neurons projecting to the brainstem or spinal cord also have larger and faster $I_H$ (and correspondingly more pronounced voltage sag in current clamp) than callosal-projecting (or contralateral striatal-projecting) neurons (Solomon et al. 1993; Christophe et al. 2005; Brown and Hestrin, 2009; Dembrow et al. 2010; Sheets et al. 2011; Gee et al. 2012).

Despite the lack of clear differences in laminar distribution or soma size, our findings for the electrical properties of $etv1$ vs. $glt$ cells were compatible with their representing subsets of IT- vs. PT-type neurons, respectively (as reported by Groh et al. 2010). Consistent with the IT-type pattern described above, $etv1$ cells had
higher Vth, broader APs, less sag, larger sAHPs, and fired slower, with lower gain
and greater SFA. Glt cells were like PT-type neurons in that they had lower Vth,
narrower APs, greater sag, larger mAHPs with very little sAHP. Glt cells fired faster
and with higher gain and exhibited almost no SFA. Glt neurons also had greater sag
in response to hyperpolarization, consistent with increased I_H in these cells
compared to etv1 neurons. Compared to layer 5 cells, L2/3 pyramidal cells were
more hyperpolarized, with intermediate R_in, almost no sag, intermediate SFA, RS,
smaller mAHP, similar V_th, intermediate spike width, faster rate of rise, and
intermediate rate of fall.

**Differences between studies.** In addition to the overlapping laminar distribution
of etv1 and glt cells, some of our electrophysiological measurements were not
consistent with studies of IT- vs. PT-type neurons. In motor cortex, IT-type
corticostriatal neurons had lower rheobase than corticospinal neurons (Sheets et al.
2011), whereas we found that rheobase was lower in glt neurons compared to etv1.
Although our data showed that etv1 cells had less sag than glt cells, our sag values
for etv1 cells were higher than typically seen for callosal-projecting IT-type cells
(Dembrow et al. 2010; Sheets et al. 2011; Avesar and Gulledge, 2012). Possible
reasons for these discrepancies include diversity within PT-type and IT-type
pyramidal cells, species and age differences, differences in internal and external
solutions, differences in protocols between labs, statistical differences between
small data samples, or genetic drift within mouse lines. A notable difference in our
study compared to Groh et al. (2010) was that they found R_in to be lower in glt cells
but R_in did not differ between pyramidal cell types in our sample. This difference is
consistent with our finding that etv1 and glt cells did not differ in soma area, in contrast to differences in cell size reported by Groh et al. (2010).

**Functional consequences.** In summary, the following suite of characters appears to be generally characteristic of etv1 cells (and IT-type cells): higher $V_{th}$, broader APs, less sag, larger sAHPs, and slower firing with lower gain and greater SFA. It has been suggested that spike frequency adaptation in IT-type neurons may be required to stabilize cortical networks (Shepherd, 2013). Glt cells (and PT-type cells) had the following characteristics: lower $V_{th}$, narrower APs, greater sag, very little sAHP, and faster firing with higher gain and almost no SFA. With these properties, glt cells (and PT-type cells) may be specialized for reliable coding of inputs, especially at higher frequencies (Schwindt et al. 1997; Shepherd, 2013). Pyramidal cells with small sAHPs (e.g., glt) are the closest pyramidal cell type to being ideal temporal integrators (Higgs et al. 2006). On the other hand, adapting cells (e.g., like etv1) can accurately follow rapidly changing input at any amplitude (Schwindt et al. 1997).

Consistent with possible roles in processing temporal aspects of sensory input, layer 2/3 pyramidal cells and layer 5 cells with large sAHPs (of which etv1 cells are an example) would have more sensitivity to fluctuations in voltage and greater capabilities for coincidence detection and help to maintain the signal-to-background ratio during strong background excitation (Higgs et al. 2006). In vivo, pyramidal cells are embedded in the cortical network and subjected to synaptic bombardment that has temporal structure. Such noisy “background” synaptic input may alter the sensitivity (modulate gain) of cortical neurons (Chance et al. 2002). Similar gain changes have been proposed as a potential mechanism for multiplicative gain.
control, which occurs during cognitive processes such as focused attention (McAdams and Maunsell, 1999; Treue and Martinez-Trujillo, 1999; Salinas and Thier, 2000). In slice recordings from pyramidal cells with a large sAHP (as found in etv1 cells), suprathreshold coincidence detection is promoted and gain is selectively increased by injected background noise that mimics in vivo synaptic input (Higgs et al. 2006). In contrast, pyramidal cells with little sAHP (like glt cells) show decreased gain in the presence of noise (Higgs et al. 2006). These results predict that large gain increases would occur with synaptic bombardment in etv1 cells in an awake behaving animal, but not in glt cells.

**Effects of NE.** Noradrenergic projections from the locus coeruleus (LC) are thought to control vigilance via cortical actions (Berridge and Waterhouse, 2003; Aston Jones et al. 1999): Low LC activity is correlated with low arousal and drowsiness, optimal performance is associated with intermediate levels of LC activity that may promote focused or selective attention, and high LC activity may produce a state of high behavioral flexibility or scanning attentiveness that leads to loss of attention and reduced performance on tasks requiring attention or vigilance (Aston Jones et al. 1999).

In vivo there is a continuum of effects of NE on pyramidal cells in layer 5 (Waterhouse et al. 2000). In slices, NE depolarized PT-type layer 5 pyramidal neurons, via α1–adrenergic action (Wang and McCormick, 1993). In the present study, we observed depolarizations in both etv1 and glt neurons in response to NE application. We also found that NE induced a reduction in voltage sag to hyperpolarization in glt cells but not etv1 cells. This finding is similar to previous
studies where NE was found to modulate $I_{H}$ through $\alpha$-2 inhibition of PKA (Wang et al. 2007) selectively in PT-type, layer 5B pyramidal cells, resulting in increased excitability (Dembrow et al. 2010; Sheets et al. 2011; Shepherd, 2013). Our findings suggest that NE also has additional profound effects on the gain and time-dependent properties of repetitive firing specifically in $etv1$ neurons but had little effect on repetitive firing of $glt$ neurons. The effects of NE on the sAHP and firing in pyramidal neurons are mediated by $\beta$-receptor activation of PKA (Madison and Nicoll, 1982; Foehring et al. 1989; Lorenzon and Foehring, 1993). Thus, different affects of NE are exerted on $glt$ (or PT-type) cells vs. $etv1$ (or IT-type) cells. It is currently unknown how these mechanisms interact at the level of neocortical circuits.

**Summary.** We found that although $glt$ cells were found deeper than $etv1$ cells on average, there was extensive overlap in the distribution of these cell types in layer 5 of somatosensory cortex and that soma size did not differ between them. There were several electrophysiological differences between $etv1$ and $glt$ pyramidal neurons. The repetitive firing behavior of these two cell types was distinctive, with $etv1$ cells firing slower, with less gain and strong SFA. $Etv1$ cells also have higher AP threshold, broader APs and higher rheobase. These electrophysiological properties are similar to those of IT-type pyramidal neurons in other studies. In contrast, $glt$ cells fired faster, with higher gain and almost no SFA. $Glt$ neurons also have lower AP threshold, narrower APs, and lower rheobase. These properties are similar to those of PT-type pyramidal neurons. A prominent difference was that differential expression of $Ca^{2+}$-dependent sK and sAHP conductances underlie much of the
differences in firing behavior between etv1 and glt cells. The low gain and high SFA of etv1 cells is controlled by a much larger sAHP conductance, which also manifests as larger sAHPs and larger peak AHPs in response to multiple APs at high frequency. The large sAHP would be predicted to increase the capacity of etv1 neurons to detect coincidence between inputs, especially in the face of noisy background inputs. In contrast, glt neurons have very little sAHP or I_{sAHP} and fire with high gain and almost no SFA. In glt neurons, the effective sK expression is greater and these channels have a much greater effect controlling firing rate in glt than etv1 cells. Glt neurons may more reliably code inputs and respond to noisy background inputs with reduced gain. The larger sAHP mechanism in etv1 cells provides a greater substrate for NE modulation of firing behavior. In the presence of NE, etv1 cells fire faster and SFA is reduced, eliminating much of the difference in firing behavior with glt cells.

Acknowledgements

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Figure Legends

Figure 1. Expression of EGFP in select populations of layer 5 pyramidal neurons in two BAC mouse lines: Tg(Etv1-EGFP)BZ192Gsat/Mmucd (etv1), and Tg(Glt25d2-EGFP)BN20Gsat/Mmnc (glt). Data were obtained from 4 animals from each mouse line. A. Tiled and stitched low power image of EGFP expression in superficial layer 5 of an etv1 animal. The slice was counterstained with NEUROTRACE 530/615 (Life Technologies) to reveal cells and laminae. B. Tiled and stitched low power image of EGFP expression in layers 5 and 6 in glt animal. The slice was counterstained with NEUROTRACE 530/615 (Life Technologies) to reveal cells and laminae. C. Histogram indicating no differences in soma area between etv1 (n = 122 cells from 4 animals) and glt cells (n = 60 cells from 4 animals). Soma areas were measured with Neurolucida from high power (63x) sections of cells at the level of the cell nucleus. D. Comparison of laminar positions of EGFP+ cells in primary somatosensory cortex in etv1 and glt animals. The sections were counterstained with NEUROTRACE 530/615 (Life Technologies). Dashed lines indicate upper and lower boundaries of layer 5. Left: Section of S1 cortex from A (etv1). Center: Section of S1 cortex from B (glt) rotated and aligned to show laminar expression of EGFP in the two animals. Right: Neurotrace stain alone is shown (same glt section as center) to reveal cells and cortical laminae (indicated in white). E. Depth profile for average EGFP fluorescence from 4 animals each from etv1 (blue) and glt (red). Data were smoothed (rolling average, 25 points) and fit with a single Gaussian in IGOR. F. Gaussian fits as in E, scaled to the same maximum intensity to show the relative depth distribution (peaks ~625 μm for etv1 and 695 μm for glt).
Figure 2. Comparison of passive properties, voltage sag in response to hyperpolarization, and rheobase among three subpopulations of pyramidal neurons. In all Figures, * = significant difference from etv1 neurons. # = significant difference from layer 2/3 neurons. A. Representative traces for an etv1 pyramidal neuron in response to a -50 pA hyperpolarizing current injection and a +200 pA depolarizing current injection that elicited a single action potential (~rheobase). Inset: Population summary for resting membrane potential (RMP) in layer 2/3, etv1, and glt neurons. Layer 2/3 neurons had significantly more negative RMPs than layer 5 cells. B. Representative traces for a glt pyramidal neuron in response to a -50 pA hyperpolarizing current injection and a +150 pA depolarizing current injection that elicited a several action potentials. Note the spike doublet at the onset of firing. Similar initial doublets were observed in ~13% of glt neurons. Inset: current injection protocol for A-C. C. Representative traces for a layer 3 pyramidal neuron in response to a -50 pA hyperpolarizing current injection (used to determine input resistance) and a +150 pA depolarizing current injection that elicited 3 action potentials. D. Summary population data for peak input resistance (R_{in}) in layer 2/3, etv1, and glt neurons. Peak input resistance was determined as peak voltage change / injected current (-50 pA). E. Summary population data for percent sag in layer 2/3, etv1, and glt neurons. Percent (%) sag was determined as 100*(peak change – steady state voltage change)/ (peak change). Sag was measured from a -150 pA current injection from RMP. Both layer 5 subtypes had significantly larger sag than layer 2/3 neurons and glt neurons had significantly larger sag than etv1 neurons. F. Summary population data for
rheobase, the minimal current (500 ms current injection) required to elicit a single action potential. Both layer 5 subtypes had significantly lower rheobase than layer 2/3 neurons and glt neurons had significantly lower rheobase than etv1 neurons.

**Figure 3.** Single action potentials (APs). A. *Etv1* neuron (scale bars apply to A-C). Inset: same AP at expanded time base to show spike width. Lower trace: current stimulus protocol. * = significant difference from *etv1* neurons. # = significant difference from layer 2/3 neurons. B. *Glt* neuron. Inset: same AP at expanded time base to show spike width. Lower trace: current stimulus protocol (gain for insets shown in B). C. Layer 2/3 neuron. Inset: same AP at expanded time base to show spike width. Lower trace: current stimulus protocol. D. Summary data for half-width (width at half-amplitude from RMP). *Glt* neurons had narrower APs than *etv1* neurons. Layer 2/3 neurons had significantly broader APs than both layer 5 cells. E. Summary data for maximum dV/dt for AP polarization (up-stroke). *Glt* neurons had significantly greater peak dV/dt than *etv1* cells. F. Summary data for the medium afterhyperpolarization (mAHP) following a single AP. Layer 2/3 neurons had significantly smaller mAHPs than layer 5 cells (although layer 2/3 RMP was 5 mV hyperpolarized on average). The mAHP was significantly larger in *glt* cells than *etv1* cells.

**Figure 4.** Comparison of AHPs after 10 APs elicited by suprathreshold, 5 ms current injections. * = significant difference from *etv1* neurons. # = significant difference from layer 2/3 neurons. *Etv1* cell. Note presence of large sAHP as well as large peak AHP. B. *Glt* cell. Note lack of a sAHP (the total AHP duration is <500 ms). Inset: stimulation protocol was 10, 5 ms suprathreshold current injections at
C. Summary data for peak AHP. Both layer 5 cell types had larger peak AHPs than layer 2/3 cells, however layer 2/3 cells were ~ 5 mV hyperpolarized on average from the layer 5 cells. D. Summary data for the sAHP (measured 500 ms after the last AP). *Etv1* neurons had larger sAHPs than layer 2/3 cells, however layer 2/3 cells were ~ 5 mV hyperpolarized on average from the layer 5 cells. *Glt* neurons had significantly smaller sAHP vs. *etv1* cells.

**Figure 5.** Average firing frequency vs. current relationships (f-I curves) for pyramidal cell subtypes. Data are presented as mean ± SEM for the first 100 ms of firing (black), the first 200 ms (red), the first 500 ms (green), and the entire 2 s firing epoch (blue). * = significant difference from *etv1* neurons. # = significant difference from layer 2/3 neurons. A. Representative repetitive firing traces in response to 2 s current injections at 100 pA. A1. *Etv1* neuron. A2. *Glt* neuron. A3. Layer 2/3 neuron. B. *Etv1* cells. Average frequency vs. current (f-I) plots are shown for averaged data from 22 cells. C. *Glt* cells. Average f-I plots are shown for averaged data from 32 cells. Note the more curved relationship and steeper initial slope compared to that of *etv1* cells in B. D. Layer 2/3 cells. Average f-I plots are shown for averaged data from 16 cells. E. Summary data for all cell populations for the first 200 ms and entire 2 s of firing (mean ± SEM). F-I slopes were obtained by fitting the initial slope (currents up to ~3 * rheobase) where f-I relationships were relatively linear. *Glt* neurons had significantly steeper f-I slope for the 2 s data than *etv1* neurons. * = significant difference vs. *etv1* cells. # = significant difference vs. layer 2/3 cells.

**Figure 6.** Relationships for instantaneous firing frequency (1/ISI, where ISI = inter-
spike-interval) vs. time (f-t curves). * = significant difference from etv1 neurons. # = significant difference from layer 2/3 neurons. A. Etv1 cells exhibited fast and slow phases of spike frequency adaptation (SFA). The slow phase made up ~ 11% of the total SFA. B. Glt cells also exhibited fast and slow phases of spike frequency adaptation (SFA) but the slow phase made up only ~ 5% of the total SFA. C. Layer 2/3 cells exhibited fast and slow phases of spike frequency adaptation (SFA). On average, the slow phase made up ~ 5% of the total SFA. D. Summary data for percent adaptation [% Adaptation = 100 * ((frequency of 3rd ISI)-(frequency of final ISI))/(frequency of 3rd ISI)]. Etv1 cells had significantly greater % adaptation than glt cells and layer 2/3 pyramidal cells.

Figure 7. Voltage clamp of I_AHPs. Currents were elicited by a step from -70 mV to +30 mV. We measured tail currents upon return to -60 mV (see inset in C). * = significant difference from etv1 neurons. # = significant difference from layer 2/3 neurons. A. Example from etv1 neuron. The tail current was fit as the sum of two exponential functions (I_{mAHP} = fast and I_{sAHP} = slow). Inset = summary data for time constants (τs) for 14 etv1 cells. The mean is indicated by a horizontal line. B. Example from glt neuron. The tail current was fit as the sum of two exponential functions. Inset = summary data for τs for 14 glt cells. The mean is indicated by a horizontal line. C. Representative traces from an etv1 neuron, showing the Ca^{2+}-dependence of both the slow and fast decay phase. D. Summary data for tail current amplitude. Etv1 cells had significantly larger I_{sAHP} than layer 2/3 cells or glt cells.
Figure 8. Actions of sK channels were tested by their sensitivity to 100 nM apamin. * = significant difference from etv1 neurons. A. Single AP (10 ms current injection) in etv1 neuron. Apamin blocked the AHP after the spike (mAHP) in this cell but this did not reach statistical significance across cells. Insets: scale bars and box plot of AHP amplitude (n = 8 cells). B. Single AP in glt neuron (10 ms current injection). Apamin significantly reduced the mAHP (inset: n= 8). C. Ten APs at 50 Hz in etv1 neuron. Apamin had no significant effect on the peak AHP. Inset: Summary data for peak AHP amplitude (n = 8 etv1 cells and 8 glt neurons). D. Ten APs at 50 Hz in glt neuron. Apamin reduced the peak AHP in glt neurons only (n = 8 etv1 cells and 8 glt neurons). Inset: Apamin-sensitive tail current had fast decay (τ ~ 200 ms) in voltage clamp (protocol as in Figure 7). Control and apamin-sensitive traces are the average of 8-12 individual traces.

Figure 9. Effects of apamin (100 nM) on repetitive firing. * = significant difference from etv1 neurons. A. Plot of average firing frequency vs. injected current (f-I curve) in etv1 neurons (n = 8). Apamin had no effect on f-I relationships in etv1 cells. B. F-I curve for glt neurons (n = 8). Glt cells fired faster in the presence of apamin but apamin did not significantly affect f-I slope. C. Plot of instantaneous firing frequency vs. time (f-t) for an exemplar etv1 cell (at 3 times rheobase). Apamin had no effect on spike frequency adaptation (SFA) in etv1 cells. Inset: same data expanded to emphasize the first few ISIs. In this cell, firing rate was increased for the first ISI, resulting in a slightly higher firing rate for the rest of the 2s. On average, this effect was not statistically significant in etv1 cells. D. F-t plot for an exemplar glt cell (at 3 times rheobase). Apamin increased
firing rate in glt cells but had no effect on SFA. *Inset: same data expanded to emphasize the first few ISIs. In this cell, firing rate was increased for the first ISI, resulting in a slightly higher firing rate for the rest of the 2s. Similar data were obtained in 8/8 glt cells.

Figure 10. Effects of norepinephrine (NE: 10 µM) on AHPs and tail currents. * = significant difference from etv1 neurons.  

A. 10 AP protocol (50 Hz) for etv1 neuron. Note large sAHP in control trace (black) and reduction of peak and sAHP (measured at 500 ms) by NE (red trace). Upper inset: average depolarizing RMP response of 10 cells to NE as a function of time. Lower inset: scale bar for insets in A and B.  

B. 10 AP protocol for glt neuron. Note lack of sAHP in control trace (black) and lack of effect of NE on the AHP (red trace). Upper inset: average depolarizing RMP response of 10 cells to NE as a function of time. Lower inset: scale bars for voltage traces in A and B.  

C. Tail currents measured at -60 mV after step from -70 mV to +30 mV in an etv1 neuron. The black trace is the control current. Note selective block of the slow component of decay in NE (blue trace). The NE-sensitive current (green trace) rose slowly to its peak and then decayed slowly.  

D. Summary data for peak AHP. NE significantly reduced the peak AHP in etv1 cells (n = 21) but not glt cells (n= 9). Note: the sAHP conductance also contributes to the peak AHP.  

E. NE significantly reduced the sAHP (measured at 500 ms) in etv1 neurons (n = 21) but not glt neurons (n = 9).  

F. NE significantly reduced $I_{sAHP}$ in etv1 cells (n = 6) but not glt neurons (n = 4).
Figure 11. Effects of 10 μM NE on repetitive firing of etv1 and glt neurons. * = significant difference from etv1 neurons. A. Average firing frequency (over 2 s) vs. injected current (f-I curve) in etv1 neurons (n = 10). Data are presented as mean ± SEM for 10 cells tested before and after NE. NE significantly increased firing rate and f-I slope in etv1 neurons. B. Average f-I plot for glt neurons (n = 9). Data are presented as mean ± SEM for 9 cells tested before and after NE. NE did not significantly affect firing rate or f-I slope in glt neurons. C. Instantaneous firing frequency (1 / ISI) vs. time (f-t) in an etv1 neuron (I = 3 times rheobase). NE increased firing rate by blocking spike frequency adaptation (SFA) in this cell. Inset: Box plot showing significant reduction in % adaptation by NE in etv1 cells (n = 10). D. F-t plot for a glt neuron (I = 3 times rheobase). NE did not affect SFA in this cell. Inset: Box plot showing no change in % adaptation by NE in glt cells (n = 9).
A etv1

B glt

5 ms step

10 mV

15 ms ISI

80 ms

Peak AHP sAHP

-74 mV

-75 mV

10 mV

80 ms

Peak AHP

C

Peak AHP

D

sAHP

n = 21

n = 77

n = 62

Peak AHP (mV)

n = 21

n = 77

n = 62

sAHP (mV)

layer 2/3 etv1 glt

layer 2/3 etv1 glt
A. *etv1 f-t (I = 300pA)*

- Fit to two exponentials
  - $\tau_1 = 17.7$ ms (89%)
  - $\tau_2 = 244$ ms (11%)
- Plateau = 6.6 Hz

B. *glt f-t (I = 300pA)*

- Fit to two exponentials
  - $\tau_1 = 21.7$ ms (95%)
  - $\tau_2 = 698$ ms (5%)
- Plateau = 25 Hz

C. *L2/3 f-t (I = 300pA)*

- Fit to two exponentials
  - $\tau_1 = 15.8$ ms (95%)
  - $\tau_2 = 434.8$ ms (5%)
- Plateau = 16.4 Hz

D. % Adaptation

- Layer 2/3
  - n = 21
- ETV
  - #
  - n = 15
- GLT
  - #
  - *
  - n = 27
Table 1. Resting membrane potential (RMP), peak input resistance ($R_{in}$), and rheobase. Data are presented as mean ± SEM (number of cells). Sag TTP = time to peak voltage deflection. * = glt significantly different from etv1 (P< 0.05, unpaired t test). # = significantly different from L2/3 (ANOVA with Tukey post-hoc test).

<table>
<thead>
<tr>
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<th>Etv1</th>
<th>glt</th>
<th>L2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>-75 ± 0.4 (102)#</td>
<td>-73 ± 0.4 (67)#</td>
<td>-82 ± 0.8 (35)</td>
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<tr>
<td>$R_{in}$ (MΩ)</td>
<td>138 ± 6 (102)</td>
<td>138 ± 5.6 (68)</td>
<td>139 ± 13 (35)</td>
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<td>Sag (%)</td>
<td>19 ± 1.0 (69)#</td>
<td>25 ± 1.2 (37)*#</td>
<td>6.5 ± 0.9 (28)*</td>
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<tr>
<td>Sag TTP (ms)</td>
<td>58 ± 2.0 (59)</td>
<td>44 ± 1.9 (37)*#</td>
<td>57 ± 5.4 (20)</td>
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<tr>
<td>Rheobase (pA)</td>
<td>172 ± 12 (76)</td>
<td>97 ± 8 (62)*#</td>
<td>246 ± 28 (25)</td>
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Table 2. Single action potential (AP) parameters. AP = action potential amplitude. $V_{th}$ = voltage threshold. HW = AP width at half amplitude (from resting potential). $dV/dt$ up = maximum rate of AP rise. $dV/dt$ down = maximum rate of AP repolarization. * = glt significantly different from etv1 ($p < 0.05$, unpaired t-test). Data are presented as mean $\pm$ SEM (number of cells). # = significantly different from L2/3 (ANOVA plus post hoc test).

<table>
<thead>
<tr>
<th></th>
<th>AP (mV)</th>
<th>$V_{th}$ (mV)</th>
<th>HW (ms)</th>
<th>dV/dt up (V/s)</th>
<th>dV/dt down (V/s)</th>
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<tr>
<td><strong>Etv1</strong></td>
<td>100 $\pm$ 2 (71)#</td>
<td>-49 $\pm$ 1 (69)</td>
<td>1.0 $\pm$ 0.03 (71)#</td>
<td>310 $\pm$ 10 (71)</td>
<td>105 $\pm$ 3 (71)#</td>
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<td><strong>Glt25</strong></td>
<td>98 $\pm$ 2 (57)#</td>
<td>-52 $\pm$ 1 (57)*</td>
<td>0.9 $\pm$ 0.02 (57)*#</td>
<td>352 $\pm$ 12 (57)*</td>
<td>125 $\pm$ 7 (35)*#</td>
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<tr>
<td><strong>L2/3</strong></td>
<td>112 $\pm$ 2 (23)</td>
<td>-52 $\pm$ 1 (23)</td>
<td>1.2 $\pm$ 0.04 (23)</td>
<td>331 $\pm$ 13 (23)</td>
<td>89 $\pm$ 4 (23)</td>
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Table 3. Peak and slow afterhyperpolarizations (AHPs). AHPs were elicited in response to ten suprathreshold, 5 ms current injections at 50 Hz. AHP = afterhyperpolarization. * = significant difference, glt from etv1 (p < 0.05, unpaired t-test). Data are presented as mean ± SEM (number of cells). # = significant difference vs. L2/3 (ANOVA with Tukey post hoc test). Note that the RMP when this protocol was run was significantly more negative in the layer 2/3 cells (-78 ± 1 mV) vs. the layer 5 cells (-74 ± 0.5 for etv1 and -74 ± 0.4 mV for glt), which likely contributed to the smaller AHP amplitudes in these cells.

<table>
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<th>Peak AHP (mV)</th>
<th>sAHP (mV)</th>
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<td><strong>Etv1</strong></td>
<td>6.6 ± 0.3 (77)#</td>
<td>3.2 ± 0.2 (77)#</td>
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<tr>
<td><strong>Glt</strong></td>
<td>5.5 ± 0.2 (62)*#</td>
<td>0.8 ± 0.2 (62)*</td>
</tr>
<tr>
<td><strong>L2/3</strong></td>
<td>3.7 ± 0.4 (21)</td>
<td>1.3 ± 0.2 (21)</td>
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Table 4. Repetitive firing: slopes of the relationship between average firing frequency vs. current (f-I) and spike frequency adaptation (SFA). Firing was elicited in response to 2s current injections at different amplitudes. ISI = interspike interval. Percent Adaptation (% Adaptation) = 100 * (frequency of 3rd ISI)-(frequency of final ISI) / (frequency of 3rd ISI). Adaptation Index = 100* the slope of a linear regression line fit to 1/ISI vs. ISI number (Groh et al. 2010). Percent Adaptation and Adaptation Index were for firing at 300 pA. * = significant difference, glt from etv1 (p < 0.05, t-test). @ = significant difference from same cell type (etv1 or glt) vs. 200 ms f-I slope (p < 0.05, t-test). # = significant difference vs. L2/3 (ANOVA with Tukey post hoc test). Data are presented as mean ± SEM (number of cells).

<table>
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<th>f-I slope: 200 ms (Hz/nA)</th>
<th>f-I slope: 2s (Hz/nA)</th>
<th>% Adaptation</th>
<th>Adaptation Index</th>
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<td>79 ± 1 (21)</td>
<td>30 ± 1 (22)@#</td>
<td>76 ± 2 (21)#</td>
<td>39 ± 7 (21)</td>
</tr>
<tr>
<td><strong>Glt</strong></td>
<td>89 ± 1 (30)</td>
<td>72 ± 1 (30)*</td>
<td>17 ± 2(27)*#</td>
<td>1.1 ± 0.3 (27)*</td>
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<td><strong>L2/3</strong></td>
<td>92 ± 10 (15)</td>
<td>55 ± 7 (15) @</td>
<td>52 ± 4 (15)</td>
<td>24 ± 14 (15)</td>
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</tbody>
</table>
Table 5. Voltage clamp of the mAHP and sAHP currents. Currents were elicited by a 200 ms voltage step from -70 mV to +30 mV. Tail Currents were measured upon a step to -60 mV. Tail = the difference between the initial and final amplitude of the tail current at -60 mV. mAHP = medium AHP. sAHP = slow AHP. Tail currents were fit with the sum of two time constants ($\tau_{\text{medium}}$ and $\tau_{\text{slow}}$). $\tau$ = time constant. $\tau_{\text{medium}}$ = the faster $\tau$ component corresponding to the mAHP current ($I_{\text{mAHP}}$). $\tau_{\text{slow}}$ = $\tau$ for the the slow AHP current ($I_{\text{sAHP}}$). % slow $\tau$ = the percentage of tail amplitude accounted for by $\tau_{\text{slow}}$. * = significant difference glt from etv1 (p < 0.05, t-test). # = significant difference vs. L2/3 (ANOVA with Tukey post hoc test). Data are presented as mean ± SEM (number of cells).

<table>
<thead>
<tr>
<th></th>
<th>Tail (pA)</th>
<th>$\tau_{\text{medium}}$</th>
<th>$\tau_{\text{slow}}$</th>
<th>% slow $\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Etv1</strong></td>
<td>259 ± 17 (14)</td>
<td>66 ± 10 (14)#</td>
<td>933 ± 101 (14)#</td>
<td>52 ± 4 (14)#</td>
</tr>
<tr>
<td><strong>Glt</strong></td>
<td>372 ± 30 (14)*#</td>
<td>74 ± 11 (14)#</td>
<td>791 ± 75 (14)#</td>
<td>18 ± 4 (14)*</td>
</tr>
<tr>
<td><strong>L2/3</strong></td>
<td>227 ± 26 (21)</td>
<td>149 ± 18 (21)</td>
<td>2611 ± 550 (21)</td>
<td>16 ± 2 (21)</td>
</tr>
</tbody>
</table>
Table 6. Effects of apamin and norepinephrine (NE) on repetitive firing. Firing was elicited in response to 2s current injections at different amplitudes. Firing rate (2s) is the average firing frequency over the entire 2s current injection (in Hz). ISI = interspike interval. Percent Adaptation (\% Adaptation = 100 * (frequency at 3rd ISI - frequency at Steady state ISI / frequency at 3rd ISI) and Adaptation Index (see Methods) were for firing at 3 * rheobase. Data are presented as mean ± SEM (number of cells). @ = significant difference vs. control (p < 0.05, paired t-test).

<table>
<thead>
<tr>
<th></th>
<th>Firing rate (2s) (Hz)</th>
<th>f-I slope: 200 ms (Hz/nA)</th>
<th>f-I slope: 2s (Hz/nA)</th>
<th>% Adaptation</th>
<th>Adaptation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Etv1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15 ± 3 (8)</td>
<td>101 ± 9 (10)</td>
<td>40 ± 12 (10)</td>
<td>82 ± 3 (10)</td>
<td>35 ± 11 (10)</td>
</tr>
<tr>
<td>Apamin</td>
<td>12 ± 2 (8)</td>
<td>108 ± 14 (10)</td>
<td>45 ± 13 (10)</td>
<td>82 ± 5 (10)</td>
<td>39 ± 7 (10)</td>
</tr>
<tr>
<td>Control</td>
<td>9 ± 2 (8)</td>
<td>49 ± 8 (8)</td>
<td>15 ± 5 (8)</td>
<td>83 ± 2 (8)</td>
<td>55 ± 11 (8)</td>
</tr>
<tr>
<td>NE</td>
<td>26 ± 4 (8)@</td>
<td>81 ± 5 (8)@</td>
<td>47 ± 3 (8)@</td>
<td>59 ± 5 (8)@</td>
<td>5 ± 2 (8)@</td>
</tr>
<tr>
<td><strong>Glt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26 ± 3 (8)</td>
<td>160 ± 20 (10)</td>
<td>140 ± 20 (10)</td>
<td>18 ± 5 (10)</td>
<td>0.6 ± 0.2 (10)</td>
</tr>
<tr>
<td>Apamin</td>
<td>37 ± 16 (8)@</td>
<td>170 ± 20 (10)</td>
<td>150 ± 20 (10)</td>
<td>26 ± 5 (10)</td>
<td>0.5 ± 0.1 (10)</td>
</tr>
<tr>
<td>Control</td>
<td>23 ± 2 (9)</td>
<td>150 ± 7 (9)</td>
<td>120 ± 10 (9)</td>
<td>17 ± 3 (9)</td>
<td>0.6 ± 0.2 (9)</td>
</tr>
<tr>
<td>NE</td>
<td>24 ± 2 (9)</td>
<td>160 ± 7 (9)</td>
<td>130 ± 7 (9)</td>
<td>23 ± 5 (9)</td>
<td>0.7 ± 0.2 (9)</td>
</tr>
</tbody>
</table>