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Signature morpho-electric, transcriptomic, and dendritic properties of extratelencephalic-projecting human layer 5 neocortical pyramidal neurons

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Abstract

In the neocortex, subcerebral axonal projections originate largely from layer 5 (L5) extratelencephalic-projecting (ET) neurons. The highly distinctive morpho-electric properties of these neurons have mainly been described in rodents, where ET neurons can be labeled by retrograde tracers or transgenic lines. Similar labeling strategies are not possible in the human neocortex, rendering the translational relevance of findings in rodents unclear. We leveraged the recent discovery of a transcriptomically-defined L5 ET neuron type to study the properties of human L5 ET neurons in neocortical brain slices derived from neurosurgeries. Patch-seq recordings, where transcriptome, physiology and morphology are assayed from the same cell, revealed many conserved morpho-electric properties of human and rodent L5 ET neurons. Divergent properties were also apparent but were often smaller than differences between cell types within these two species. These data suggest a conserved function of L5 ET neurons in the neocortical hierarchy, but also highlight marked phenotypic divergence possibly related to functional specialization of human neocortex.

Introduction

Understanding how cellular diversity relates to cell types and circuits remains one of the biggest challenges in modern neuroscience (Zeng and Sanes, 2017). Within the neocortex, excitatory pyramidal neurons display an astonishing diversity in gene expression, morphology, physiology and response to neuromodulation (Dembrow and Johnston, 2014; Gouwens et al., 2019, 2020; Markram et al., 2015; Sugino et al., 2006; Tasic et al., 2018). One seminal discovery shed light on the organizational structure of these excitatory neuron populations by demonstrating that they can be segregated based on their

47 long-range axonal targets (Harris and Shepherd, 2015). This is exemplified in layer 5 where pyramidal
48 neurons can be broadly segregated into two classes based upon whether their long-range axons project
49 only within the telencephalon or both within and outside of the telencephalon (Baker et al., 2018).

50 Both intratelencephalic-projecting (IT) and extratelencephalic-projecting (ET) neurons send
51 axonal projections within the telencephalon (i.e. the cerebral cortex, basal ganglia, etc.), but only ET
52 neurons send long-range axons to subcerebral targets such as the spinal cord, pons and thalamus. In
53 this way, cortical activity that directly affects subcerebral processing/behavior is routed largely through
54 ET neurons. In rodents, L5 ET neurons possess distinctive morpho-electric properties, gene expression,
55 local synaptic connectivity, long-range afferents and response to neuromodulators (Anastasiades et al.,
56 2018; Avesar and Gullledge, 2012; Brown and Hestrin, 2009; Dembrow et al., 2010, 2015; Groh et al.,
57 2010; Guan et al., 2015; Hattox and Nelson, 2007; Kalmbach et al., 2013; Kawaguchi, 2017; Kim et al.,
58 2015; Mao et al., 2011; Sheets et al., 2011; Sorensen et al., 2015; Tasic et al., 2018). These properties
59 distinguish ET neurons from nearly every other neocortical cell type (Gouwens et al., 2019).
60 Furthermore, emerging evidence indicates that ET neurons display unique firing properties *in vivo* and
61 contribute to different aspects of perception and behavior (Economo et al., 2018; Kim et al., 2015; Li et
62 al., 2015; Rojas-Piloni et al., 2017; Saiki et al., 2017).

63 Translating these findings, which have been described primarily in rodents, to the human
64 neocortex is complicated by several factors. First, there are major cross-species differences in the gross-
65 laminar organization of the neocortex, the size of neurons, the laminar expression of genes, and the
66 intrinsic membrane properties of homologous cell types (Beaulieu-Laroche et al., 2018; Berg et al., 2020;
67 Hodge et al., 2019; Kalmbach et al., 2018; Mohan et al., 2015; Zeng et al., 2012). Additionally, there are
68 experimental limitations to studying long-range projections in humans and other primates. In rodents,
69 retrograde tracers or viruses can label ET neurons to target them for patch-clamp physiology or other
70 functional studies (Dembrow et al., 2010; Hattox and Nelson, 2007; Tervo et al., 2016), but these
71 approaches are not possible in humans. In addition, ET neurons appear to be relatively more rare in
72 human compared with rodent neocortex (Hodge et al., 2019, 2020).

73 Recent advances from single cell transcriptomics offer a unique anchor to identify ET neurons in
74 the human neocortex and to study their cellular properties. Here, we leverage these recent advances to
75 address which distinguishing cellular properties of rodent ET neurons are conserved or divergent in the
76 human neocortex.

77 **Results**

78 **Cross species differences in the relative abundance of putative L5 ET neurons**

79 In mice, single-cell transcriptomics readily distinguishes ET versus IT neurons. Combined
80 retrograde labeling and scRNA-seq (i.e. Retro-seq) has validated this target/transcriptome relationship
81 (Economo et al., 2018; Tasic et al., 2018). Alignment of human and mouse neocortical taxonomies
82 based on RNA-sequencing has enabled strong inference of homologous transcriptomic cell types in the
83 human cortex that correspond to L5 ET neuron types in the mouse (Hodge et al., 2019, 2020). This
84 alignment reveals a striking cross-species difference in the relative abundance of transcriptomically-
85 defined ET neurons. Putative ET neurons comprise only 2-6% of excitatory neurons in L5 of the human
86 neocortex compared to 20-30% in mice, depending upon cortical area. It is unclear, however, whether
87 the relative rarity of ET neurons is a unique property of the human neocortex. To address this question,
88 we estimated the relative abundance of ET neurons across mouse, macaque and human temporal
89 cortex by using multiplex fluorescence *in situ* hybridization (FISH) against a conserved L5 ET marker
90 gene (*FAM84B*) and a glutamatergic neuron marker (*SLC17a7*; Figure 1A). In all three species, neurons
91 that expressed both *FAM84B* and *SLC17a7* were characterized by large pyramidal shaped somata
92 (Figure 1B), a hallmark of L5 ET neurons in rodents (Baker et al., 2018; Oswald et al., 2013). Putative ET
93 neurons were most abundant in mice, followed by macaque and then human temporal cortex (Figure

94 1C). Thus, there are differences in the relative abundance of L5 ET neurons in the neocortical column
95 across species, likely related to cortical expansion and dramatic increase in IT neuron abundance and
96 cell type diversity.

97 The identification of a conserved class of transcriptomically defined ET neurons permitted us to
98 identify genes that may contribute to conserved properties of this cell type as well as genes potentially
99 contributing to phenotypic divergence. To identify genes enriched specifically in human ET neurons, we
100 analyzed an existing single nucleus RNA sequencing dataset from human MTG (Hodge et al., 2019).
101 Comparisons across species are complicated by the lack of a clear rodent homologue to human MTG
102 and by evidence that ET neuron gene expression varies greatly between cortical areas in the human and
103 mouse (Hodge et al., 2019; Tasic et al., 2018). Rather than focusing on a single mouse brain region, we
104 therefore utilized a published single cell RNA sequencing dataset derived from two brain regions, the
105 mouse primary visual cortex (VISp) and the anterior lateral motor cortex (ALM). We identified 4,143
106 genes with at least 0.5 log₂ fold enriched expression in transcriptomically-defined ET neurons relative to
107 L5 IT neurons in human MTG. Additionally, 477 DE genes were enriched in mouse ET neurons in both
108 VISp and ALM. This gene set was highly enriched for genes associated with axon guidance and synaptic
109 function (Figure 1D,E; red). A notable example includes *BCL11B*, which is required for subcerebral
110 axonal targeting (Arlotta et al., 2005; Canovas et al., 2015). There were also noteworthy examples of
111 human specific ET genes (Figure 1D,E; blue), including *GRIK1*, which encodes the ionotropic glutamate
112 receptor, GluR5. Nonetheless, these data suggest that known phenotypes of rodent ET neurons, such as
113 subcerebral axonal targeting are broadly conserved in the human L5 ET transcriptomic cell type.

114 In rodents, L5 ET neurons express a unique repertoire of ion channels, which contributes to their
115 specialized physiological properties (Baker et al., 2018). Many of these voltage-gated channels are also
116 targets of neuromodulation. Indeed, the response to various neuromodulators differs between L5 ET and
117 IT neurons in rodents (Dembrow and Johnston, 2014). We therefore identified ion channel and
118 neuromodulator receptor-related genes enriched in transcriptomically-defined L5 ET neurons in the
119 human MTG (Figure 1F-G). Many, but not all, of these genes were enriched in mouse L5 ET neurons in
120 both VISp and ALM. In addition to a major pore forming HCN-channel subunit (*HCN1*), several classes of
121 G-protein-coupled receptors (GPCRs) were also enriched in transcriptomically defined ET neurons in
122 human MTG. Many of these GPCRs are associated with neuromodulators that differentially affect L5 ET
123 versus L5 IT neurons in the rodent. For example, we observed cross-species differences in the
124 expression of genes encoding 5-HT1 and 5-HT2 receptor family subunits (Figure 1G). In mouse L5 ET
125 neurons, *HTR1A* and *HTR1F* were the dominantly expressed subunits whereas in human L5 ET
126 neurons, *HTR1E* (which is absent in the mouse genome) and *HTR1F* were highly expressed, with little
127 *HTR1A* expression. Similarly, *HTR2C* was abundantly expressed in human, but not mouse L5 ET
128 neurons. These data suggest that human and rodent L5 ET neurons might share similar distinctive
129 intrinsic membrane properties and responses to neuromodulation in comparison to neighboring L5 IT
130 neuron types. In contrast, cross-species differences with respect to other human L5 ET enriched genes
131 highlight areas of potential phenotypic divergence.

132 **Transcriptomically defined L5 ET neurons possess distinctive intrinsic membrane** 133 **properties**

134 The transcriptomic classification of cell types has proven remarkably predictive of physiological,
135 morphological, and anatomical properties in both human and mouse neocortex (Bakken et al. 2020; Berg
136 et al., 2020, 2020; Economo et al., 2018; Scala et al., 2020; Tasic et al., 2018). We therefore predicted
137 that in human MTG the electrophysiological properties of transcriptomically defined L5 ET neurons would
138 differ from neighboring IT neurons in a manner consistent with previous observations in rodents (Baker et
139 al., 2018; Dembrow et al., 2010; Gouwens et al., 2019). To test this hypothesis, we performed patch
140 clamp recordings from L5 pyramidal neurons in acute brain slices prepared from neurosurgical
141 resections of human MTG. We utilized current injection protocols designed to detect differences in
142 subthreshold and suprathreshold membrane properties. For a subset of experiments we performed

143 Patch-seq analysis in which the nucleus was extracted after concluding whole cell recording protocols
144 and then processed for snRNA-sequencing. From the resulting RNA sequencing data, the expression
145 levels of thousands of genes were used to assign a transcriptomic cell type identity to the physiologically
146 probed cell by mapping to a reference human MTG transcriptomic cell type taxonomy (Figure 2A, see
147 methods for tree-based mapping details). We grouped Patch-seq sampled neurons and non-Patch-seq
148 sampled neurons into physiologically defined types based on their aggregate neurophysiological
149 signatures. We then asked whether physiologically defined neurons corresponded to genetically defined
150 L5 ET and IT neuron types using Patch-seq mapping.

151 Using this approach, we obtained 20 patch-seq samples that mapped with high confidence to one
152 of several infragranular IT types and 5 samples (in green) that mapped to the sole L5 ET cluster in the
153 reference human MTG transcriptomic cell type taxonomy (Figure 2B). As expected, Patch-seq samples
154 mapping to L5 ET and IT types were enriched for multiple established marker genes for the respective
155 types (Figure 2C). Transcriptomically defined L5 ET neurons displayed a higher resonant frequency
156 compared to IT neurons in response to a chirp stimulus (Figure 2D-F), in addition to multiple other
157 pairwise differences in intrinsic properties between transcriptomic cell types (Figure S1). Using
158 unsupervised hierarchical clustering based upon physiology, we observed three distinct types of human
159 L5 neurons (Figure 2G). To visualize the collective differences in the properties of these physiologically
160 defined neuron types we used t-distributed stochastic neighbor embedding (t-SNE) for dimension
161 reduction, where the distance between cells approximates differences in physiological properties and
162 thus cells with similar physiological properties cluster together (Figure 2H). All transcriptomically defined
163 L5 ET neurons belonged to one physiologically defined neuron type whereas all transcriptomically
164 defined L5 IT neurons belonged to one of two different physiologically defined cell types (Figure 2I). For
165 simplicity, we refer to these physiologically defined cell types as ET-like, IT-like 1 and IT-like 2, based on
166 their correspondence to transcriptomic cell types and their broad similarity to rodent L5 ET and IT
167 neurons (see below). Notably, the two IT-like physiologically defined cell types were largely enriched for
168 different L5 IT transcriptomic cell types. Thus, there was a strong correspondence between
169 physiologically and transcriptomically defined cell types.

170 **Electrophysiological differences in human L5 ET and IT neurons**

171 These results demonstrate that transcriptomically defined L5 ET and IT neurons in human MTG
172 possess distinct electrophysiological properties. To address which physiological features contributed to
173 the clustering of genetically defined L5 ET and IT neuron types, we made pairwise comparisons of
174 specific features between the physiologically defined cell types.

175 In rodents, subthreshold properties, especially those related to HCN channel expression, readily
176 distinguish L5 ET from IT neurons across several brain regions (Dembrow et al., 2010; Kalmbach et al.,
177 2013; Sheets et al., 2011). Notably, *HCN1*, which encodes a major HCN channel pore forming subunit
178 (Robinson and Siegelbaum, 2003), was enriched in transcriptomically defined L5 ET neurons relative to
179 IT neurons in both mouse and human (Figure 1F). Thus, we predicted that HCN-channel related
180 properties would distinguish L5 ET from IT neurons in human MTG. HCN channels contribute to the
181 resting conductance of a neuron and thus their presence is associated with a lower input resistance and
182 a shorter membrane time constant, resulting in differences in low-pass filtering properties (Kalmbach et
183 al., 2018; Magee, 1998). Additionally, the slow activation and deactivation kinetics of HCN channels
184 contribute to the characteristic voltage sag induced by hyperpolarization and rebound potential upon
185 release from hyperpolarization (Robinson and Siegelbaum, 2003). These kinetic properties endow
186 neurons with membrane resonance in the 2-7 Hz range as well as inductive properties that enable
187 changes in membrane potential to lead changes in current over certain frequencies (Hutcheon et al.,
188 1996; Narayanan and Johnston, 2008; Vaidya and Johnston, 2013). To extract HCN-dependent
189 properties, we measured the voltage response to a series of hyperpolarizing and depolarizing current
190 steps as well as a chirp stimulus (Figure 2A; Figure 3A,B,E).

191 We observed several differences in HCN channel-related subthreshold membrane properties
192 between putative L5 ET and IT neurons. ET-like neurons had a higher resonant frequency, higher
193 resonant strength, and higher 3 dB cutoff than both IT-like neuron types (Figure 3C,D; Figure S2).
194 Similarly, the voltage response of L5 ET neurons, but not IT neurons, led the chirp current injection for
195 low frequency components (Figure S2). L5 ET-like neurons also had a lower input resistance and more
196 pronounced voltage sag/rebound than IT-like neurons (Figure 3F-G; Figure S2). Notably, compared with
197 IT-like 1 neurons, IT-like 2 neurons had ~2x higher input resistance and a larger rebound potential.
198 Furthermore, as reported in rodents, simply plotting resonance frequency as a function of input
199 resistance segregated the neurons types well (Figure 3H; Dembrow et al., 2010; Kalmbach et al., 2015).
200 Thus, human L5 ET neurons have more pronounced HCN-related properties than L5 IT neurons.

201 In addition to subthreshold differences, rodent L5 ET and IT neurons display several known
202 differences in suprathreshold membrane properties (Dembrow et al., 2010; Guan et al., 2015; Hattox and
203 Nelson, 2007; Kalmbach et al., 2013; Oswald et al., 2013; Otsuka and Kawaguchi, 2008; Suter et al.,
204 2013). Action potential properties were extracted from the voltage response to a series of 1 s step
205 current injections of increasing amplitude (Figure 2A). ET-like neurons, perhaps due in part to their low
206 input resistance, displayed the shallowest input/output curves, followed by IT-like and IT-like 2 neuron
207 types (Figure 4A,B). Thus, for a given amplitude current injection, ET-like neurons responded with the
208 lowest number of action potentials. Additionally, we noticed that ET-like neurons tended to respond to
209 near threshold current injections with a high frequency burst of action potentials (Figure 4A), a
210 phenomenon that has been associated with dendritic Ca^{2+} electrogenesis (Beaulieu-Laroche et al., 2018;
211 Larkum et al., 1999; Shai et al., 2015). To illustrate this behavior, we plotted the first instantaneous firing
212 rate (1/first interspike interval) as a function of the amplitude of the current injection above rheobase
213 (Figure 4C). Putative L5 ET neurons displayed the highest instantaneous firing frequencies for at
214 threshold current injections. As an additional way to quantify these bursts, we calculated the percentage
215 of action potentials that occurred within 50 ms of the first spike during the first current step that produced
216 at least 5 spikes. L5 ET-like neurons had the largest percentage of spikes within 50 ms of the first spike
217 and the highest maximum instantaneous firing rate during this current injection (Figure 4D).

218 There were several additional differences in action potential properties between putative L5 ET
219 and IT neurons in human MTG that mirror differences observed in rodent neocortex. Example single
220 action potentials and phase-plane plots are presented in Figure 4E. Similar to rodent L5 ET neurons
221 (Dembrow et al., 2010; Pathak et al., 2016; Suter et al., 2013) human L5 ET-like neurons had fast,
222 narrow action potentials characterized by a fast depolarization/repolarization rate, low voltage threshold
223 and narrow width at half-maximum amplitude (Figure 4F-I; Figure S2). Notably, IT-like 2 neurons had
224 similarly fast and narrow action potentials compared to IT-like 1 neurons (Figure 4F-I; Figure S2). ET-like
225 neurons also displayed the largest amplitude medium afterhyperpolarization potentials, and the highest
226 variability in spike timing (Figure S2), perhaps due in part to enriched expression of channels contributing
227 to AHPs and their propensity to burst (Guan et al., 2015). Some of these differences may be explained
228 by differences in the expression of voltage gated ion channels (Bishop et al., 2015; Kalmbach et al.,
229 2015; Miller et al., 2008; Pathak et al., 2016).

230 As a second approach to investigating which physiological features were most informative to
231 distinguish between L5 ET-like versus IT-like neurons, we trained a series of random forest classifiers on
232 varying subsets of the dataset (Figure S3A). Classifiers had an average accuracy of ~95% when using
233 40% of the dataset in training and maintained close to this level when using as little as 10% of the
234 dataset. Subthreshold features related to passive membrane properties and HCN conductance had the
235 greatest importance (Figure S3B). The performance of random forest classifiers only marginally
236 decreased when just the top 10 most important features were used in training (Figure S3C).
237 Furthermore, the general shape of t-SNE projections was robust to the removal of several physiological
238 features (Figure S3D,E). Together, these data suggest that subthreshold membrane properties
239 discriminate between L5 ET and IT neurons, but that several other features are sufficient to classify
240 these cell types.

241 **Putative L5 ET neurons in human MTG have thick-tufted apical dendrites**

242 Classically, rodent L5 ET neurons can be distinguished from L5 IT neurons by their thick apical
243 tuft dendrites (Baker et al., 2018; Dembrow et al., 2010; Gao and Zheng, 2004; Gouwens et al., 2019;
244 Hattox and Nelson, 2007; Oswald et al., 2013). To determine whether human L5 ET-like neurons
245 possess an apical tuft, we performed dendritic reconstructions of physiologically defined neurons. For
246 comparative purposes we also performed dendritic reconstructions of L5 IT-like neurons. Compared with
247 IT-like neurons, ET-like neurons possess a definite apical tuft terminating at the pial surface, but with the
248 tuft branches starting at different distances from the soma (Figure 5A,B). The total length of the apical
249 dendrites (ET $9091.5 \pm 559.0 \mu\text{m}$, IT $5090.3 \pm 759.9 \mu\text{m}$; FDR corrected $p = 0.026$, Mann-Whitney U test)
250 was greater in L5 ET neurons compared with IT-like neurons (Figure 5C). Furthermore, for the apical
251 dendrites, L5 ET-like neurons possessed more dendritic branches (ET 68.7 ± 5.7 , IT 34.6 ± 4.8 ; FDR
252 corrected $p = 0.026$, Mann-Whitney U test) that on average had a larger diameter (ET $1.04 \pm 0.05 \mu\text{m}$, IT
253 $0.76 \pm 0.06 \mu\text{m}$; FDR corrected $p = 0.035$, Mann-Whitney U test), resulting in greater total surface area
254 (ET $29179.4 \pm 1456.8 \mu\text{m}^2$, IT $12601.1 \pm 2571.7 \mu\text{m}^2$; FDR corrected $p = 0.022$, Mann-Whitney U test)
255 compared with IT-like neurons (Figure 5D,E,F).

256 In addition to dendritic morphology, the somatic morphology of L5 ET and IT neurons is different
257 in rodents. In many cortical areas, L5 ET neuron somata are larger and possess a larger initial apical
258 shaft compared with L5 IT neurons (Gao and Zheng, 2004; Oswald et al., 2013). To quantify somatic
259 shape, we measured the ratio of the somatic height/width as well as the width of the initial apical dendrite
260 shaft of biocytin filled neurons (Figure 5G). In contrast to L5 IT-like neurons, L5 ET-like neuron somata
261 were taller (ET $28.78 \pm 1.89 \mu\text{m}$, IT $23.31 \pm 0.93 \mu\text{m}$; FDR corrected $p = 0.01$, Mann-Whitney U test) than
262 they were wide (ET $16.50 \pm 0.56 \mu\text{m}$, IT $16.23 \pm 1.20 \mu\text{m}$; FDR corrected $p = 0.47$, Mann-Whitney U test;
263 Figure 5H). Similarly, the initial apical shaft was wider in ET-like ($3.80 \pm 0.21 \mu\text{m}$) neurons compared with
264 IT-like neurons ($2.07 \pm 0.60 \mu\text{m}$, FDR corrected $p = 0.0001$, Mann-Whitney U test; Figure 5I). These
265 properties gave L5 ET-like neuron somata a distinctive teardrop shape that was readily visible in both
266 biocytin fills and under IR-DIC optics during patch-clamp experiments.

267 **Human L5 ET-like neurons display strong dendritic electrogenesis**

268 To directly test whether the dendrites of human L5 ET neurons display electrogenesis as
269 suggested by their propensity to fire in bursts, we made whole cell recordings from the dendrites of L5
270 neurons in human MTG (Figure 6A). Example voltage responses to hyperpolarizing and depolarizing
271 current injections measured at different distances from the soma are shown in Figure 6B. For a subset of
272 these dendritic recordings we performed subsequent whole cell recordings from the soma through a
273 second pipette. Projecting the physiological features recorded at the soma onto a tSNE plot revealed that
274 the dendritically probed neurons were L5 ET-like neurons (compare Figure 6C to Figure 2H).

275 We first asked whether the subthreshold membrane properties of human L5 ET-like neurons vary
276 with distance from the soma, as observed in many rodent L5 ET neurons (Dembrow et al., 2015;
277 Kalmbach et al., 2013, 2015, 2017). While input resistance did not change as a function of distance from
278 the soma (Figure 6D), other membrane properties associated with HCN channel expression did. Sag
279 ratio and rebound potential were more prominent at distal recording sites (Figure S4). Furthermore,
280 resonance frequency, 3dB cutoff and total impedance phase increased as a function of recording
281 distance (Figure S4). Thus, as in many rodent L5 ET neurons, HCN related properties increase as a
282 function of distance from soma.

283 In response to depolarizing current injections, we observed prolonged, all-or-nothing plateau
284 potentials in L5 ET-like dendrites (Figure 6B). The properties of these plateau potentials varied
285 significantly with distance from the soma. The width and area under the curve of the spikes increased
286 with distance from the soma, whereas the maximum rate of rise decreased (Figure 6E-G). Intriguingly,
287 the current required to evoke a plateau potential did not vary with distance from the soma and was
288 similar to the current required to elicit a somatic action potential via somatic current injection. Notably,

289 while dendritic properties were fairly consistent across recordings, we cannot rule out the possibility that
290 some of our recordings were from L5 IT neuron dendrites. Nonetheless, these observations suggest that
291 human L5 ET neurons possess active mechanisms to counteract enhanced compartmentalization
292 associated with their extensive dendritic arbors.

293 **Cross-cell type variability is greater than cross-species variability**

294 These data suggest that the defining physiological properties of human L5 ET neurons are
295 broadly similar to those reported in rodent neocortex. To directly compare somatic membrane properties
296 across species, we grouped human and mouse L5 neurons into physiologically defined cell types based
297 upon the same physiological features used in Figure 2 (Figure 7A-left). As previously explored with the
298 human data (Figure 2), we then asked whether the physiologically defined cell types corresponded to
299 genetically defined cell types via Patch-seq or labeling by a L5 ET neuron specific enhancer virus
300 (Graybuck et al., 2019). Similar to clustering human data alone, clustering data from both species
301 revealed at least three distinct types of neurons. For both species, physiologically defined L5 ET neurons
302 corresponded to genetically defined L5 ET neurons (Figure 7A-right). Furthermore, ET-like neurons in
303 both species occupied similar areas of the tSNE plot of physiological features (Figure 7A). These data
304 suggest that the intrinsic membrane properties of L5 ET neurons are broadly conserved in mouse and
305 human neocortex.

306 Nonetheless, these observations do not rule out the possibility of subtler cross-species
307 differences in the somatic membrane properties of L5 ET neurons. We thus also made pairwise
308 comparisons between the somatic membrane properties of mouse and human neurons. For this
309 analysis, we collapsed the two IT neuron types into one IT neuron group. We observed both cell type
310 and cross-species differences in nearly every membrane property examined (Figure 7; Figure S5). To
311 determine whether differences are larger between species or between cell types we calculated the effect
312 size associated with each physiological feature. The cell-type-specific effect size was nearly always
313 larger than the effect size determined across species, with a few notable exceptions. For example, cross-
314 species differences were larger than cross-cell-type differences for some suprathreshold properties,
315 including input/output gain and action potential half-width (Figure 7; Figure S5). Taken together, these
316 observations highlight a general conservation of the core defining features of L5 ET and L5 IT neuron
317 types from mouse to human, pointing to a common cell type diversity and resultant functional
318 architecture of L5 across species. These observations also highlight many points of divergence in
319 specific features of L5 ET and IT neuron types between these two species.

320 **Discussion**

321 In the rodent neocortex, the properties of L5 ET neurons are clearly distinguishable from
322 neighboring L5 IT neurons, which make up the other major class of L5 pyramidal neurons (Baker et al.,
323 2018). Investigation of the distinctive properties of L5 ET neurons in the human neocortex has been
324 hampered by an inability to identify these neurons by their defining feature - long-range subcerebral
325 axonal projections and projection targets. In this study, we circumvent this problem by leveraging
326 transcriptomic definitions of cell types and a mapping of homologous cell types between mouse and
327 human cortical taxonomies. We demonstrate that the morpho-electric properties and gene expression
328 profiles in human L5 ET neurons are as distinctive as their rodent counterparts. Nevertheless, our results
329 reveal substantial divergence and specialization of features.

330 The identification of a transcriptomic cell type in human middle temporal gyrus (MTG) that is
331 homologous to rodent L5 ET neurons permitted us to identify genes that may contribute to conserved
332 phenotypes (Hodge et al., 2019). In mouse VISp/ALM and human MTG, L5 ET neurons were especially
333 enriched for genes associated with axon guidance and synaptic regulation. These genes may be related
334 to the subcerebral axonal targeting and afferent connectivity of L5 ET neurons. Additionally, both mouse
335 and human L5 ET neurons express different complements of genes encoding neuromodulatory receptors
336 relative to neighboring mouse and human L5 IT neurons. This observation is consistent with the

337 differential effect of neuromodulators on L5 ET and L5 IT neurons in rodent neocortex and suggests
338 human ET neurons may also respond uniquely (with respect to human IT cells) to neuromodulation
339 (Baker et al., 2018; Dembrow and Johnston, 2014). Similarly, rodent and human ET neurons shared
340 assorted differentially expressed ion channel related genes that may contribute to conserved intrinsic
341 membrane properties.

342 Indeed, patch-seq/patch-clamp recordings revealed broad physiological similarities between
343 rodent and human L5 ET neurons. In both rodent and human L5 ET neurons, HCN-channel-related
344 membrane properties were enhanced compared with L5 IT neurons. In rodents, HCN-channel
345 expression is enriched in the apical dendrite of L5 ET neurons, where it strongly shapes synaptic
346 integration by narrowing the window whereby inputs can be summed in time (Dembrow et al., 2015;
347 Kalmbach et al., 2013, 2015, 2017). These properties tend to make L5 ET neurons most sensitive to
348 inputs containing frequency components in the theta band (4-12 Hz). Our findings indicate that many of
349 these subthreshold integrative properties are conserved in human L5 ET neurons. Human L5 ET
350 neurons also exhibited a distinctive action potential waveform, similar to rodent ET neurons. For
351 example, in both species, action potentials were faster and narrower in L5 ET neurons compared with L5
352 IT neurons.

353 Human L5 ET neurons, like rodent L5 ET neurons, tended to respond to near-threshold current
354 injections with bursts of action potentials. In rodent L5 ET neurons, these bursts of action potentials are
355 associated with dendritic Ca^{2+} plateau potentials (Larkum et al., 1999; Shai et al., 2015). Using direct
356 dendritic patch-clamp recordings, we showed that plateau potentials can be elicited in human L5 ET
357 neuron dendrites, and that these events were strongly reminiscent of Ca^{2+} spikes in some rodent L5
358 neurons. We did not record from confirmed IT neuron dendrites and thus it remains to be seen whether
359 L5 IT dendrites similarly display electrogenesis. Nonetheless, in rodent L5 neurons, plateau potentials
360 serve as a mechanism for associating bottom-up input arriving at the basal dendrites with top-down input
361 arriving at the apical tuft in L1 (Larkum, 2013). Importantly, human L5 ET neurons possessed a
362 pronounced apical tuft in L1, suggesting that they similarly receive substantial feedback input from higher
363 cortical areas. L5 ET neurons in the human neocortex may thus perform a similar computation as rodent
364 L5 ET neurons of coupling feedback and feedforward input in the neocortical circuit.

365 Our findings confirm some observations from the sole previous report of human L5 dendritic
366 membrane properties (Beaulieu-Laroche et al., 2018), but we also note some key differences. As
367 previously described, HCN-dependent membrane properties varied as a function of distance from the
368 soma, with the exception of R_N . The flattened somatic-dendritic gradient of R_N represents a marked
369 departure from rodent ET neurons (Kalmbach et al., 2013) and may reflect a relative decrease in the
370 density of resting conductance in the dendrite (Beaulieu-Laroche et al., 2018) of human L5 neurons.
371 Unlike (Beaulieu-Laroche et al., 2018), we observed strong electrogenesis in human L5 ET dendrites.
372 While the Beaulieu-Laroche study was performed solely in tissue derived from epileptic patients, we
373 observed dendritic plateau potentials in tissue derived from both epilepsy and tumor patients. Thus, it is
374 unlikely that differences in the underlying medical conditions of patient populations used in these studies
375 contributed to different findings. Aside from methodological differences (pipette solution, external
376 solution, slice preparation, exact surgical sampling etc.), there are several possible explanations for this
377 discrepancy. First, it is unclear exactly which neuronal population(s) were sampled in the previous report.
378 This is especially pertinent given the relative rarity of human L5 ET neurons (2-6% of L5 excitatory
379 neurons in human neocortex versus 20-30% in mouse neocortex (Hodge et al., 2019, 2020; Figure 1).
380 Furthermore, in rodents there is variability in the types of nonlinearities observed in L5 dendrites. For
381 example, the apical trunk of L5 ET neurons in the prefrontal cortex display strong Na^+ mediated spikes
382 but not Ca^{2+} plateaus, whereas in sensory cortical areas Ca^{2+} electrogenesis is ubiquitous (Gulledge and
383 Stuart, 2003; Harnett et al., 2013; Kalmbach et al., 2017; Larkum et al., 1999; Santello and Nevian, 2015;
384 Shai et al., 2015). Even within a single brain region, there are differences in the propensity of the apical
385 trunk of L5 neurons to generate plateau potentials. Some of this variability appears to be related to
386 differences in dendritic architecture (Fletcher and Williams, 2019). Similar variability in dendritic

387 morphology and related electrogenesis may therefore occur in human neocortex. Nonetheless, we find
388 that at least some human L5 ET dendrites display strong dendritic electrogenesis.

389 While rodent and human L5 ET neurons had broadly similar genetic and morpho-electric
390 properties, there were nonetheless many cross-species differences that point to potential areas of cross-
391 species divergence and/or specialization. Foremost among these was the striking sparsification of L5 ET
392 neurons from mouse to macaque to human, consistent with previous observations from primary motor
393 cortex (Bakken et al. 2020). This may reflect the dramatic increase in cortical volume relative to
394 subcerebral volume in primates and humans (Heffner and Masterton, 1975; Herculano-Houzel et al.,
395 2015). Additionally, we observed several differences in the expression of genes encoding GPCRs, which
396 yield testable hypotheses concerning cross-species and cross-cell type differences in neuromodulation
397 that could be areas for future study. Genes encoding 5-HT receptors were especially divergent across
398 species, suggesting that human L5 ET neurons may respond differently to serotonin and related agonists
399 as compared to mouse L5 ET neurons. This could be of great clinical significance and underscores the
400 importance of direct investigation of human neuron physiology and neuromodulation.

401 In addition to the cross-species differences that we could observe, there are undoubtedly other
402 differences that were not accessible to the methods used in this study. Chief among these is the somato-
403 dendritic distribution of ion channels. Previous reports have shown that the human L5 dendrites are more
404 electrically compartmentalized compared with rat dendrites (Beaulieu-Laroche et al., 2018). This
405 enhanced compartmentalization appears to be due in part to cross-species differences in the density of
406 ion channels. Additionally, in the supragranular layers, human pyramidal neurons possess dendritic non-
407 linearities not previously observed in rodent neurons (Gidon et al., 2020), that may be due to differences
408 in the somato-dendritic expression of select ion channels. Furthermore, there could be differences in the
409 passive membrane properties of human L5 ET neurons, or in related dendritic morphological properties
410 (Deitcher et al., 2017; Eyal et al., 2016).

411 Finally, our general strategy of investigating L5 ET neurons could serve as a roadmap for
412 studying human and non-human primate L5 ET neuron types across cortical areas. The transcriptome of
413 L5 ET neurons varies greatly across cortical areas in both rodents and humans (Hodge et al., 2020;
414 Tasic et al., 2018), suggesting that areal signatures in functional properties may also greatly vary as a
415 direct consequence. Most strikingly, a few famous morphological types of L5 ET neurons are found in
416 primate but not in rodent neocortex, including several gigantocellular neurons (e.g. the Betz cell of
417 primary motor cortex, the Meynert cell of V1 and the Von Economo neuron of fronto-insular/anterior
418 cingulate cortex; Allman et al., 2010; Jacobs et al., 2017). We recently utilized a similar approach to
419 highlight conserved and divergent features of primate Betz cells and mouse corticospinal neurons
420 (Bakken et al., 2020). Determining how cross-areal and cross-species variability in gene expression
421 translates to phenotypic diversity at the level of cell types promises to deepen our understanding of
422 conserved and divergent aspects of neocortical brain function through evolution and to improve
423 prospects for translational neuroscience.

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466 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

467 **Human surgical specimens**

468 Surgical specimens were obtained from local hospitals (University of Washington Medical Center,
469 Swedish Medical Center and Harborview Medical Center). Hospital institute review boards approved all
470 procedures involving human tissue before commencing the study and all patients provided informed
471 consent. Data included in this study were obtained from neurosurgical tissue resections for the treatment
472 of refractory temporal lobe epilepsy (n=23) or deep brain tumors (n=3) in 21 male and 5 female patients
473 with a mean age of 39.23 ± 3.07 years (Table S1).

474 **Mouse specimens**

475 All procedures involving mice were approved by the Allen Institute's Institutional Care and Use
476 Committee. Mixed strains of male and female transgenic mice, from 60-90 days old were used for

477 experiments. Mice were maintained on a 12-hour light/dark cycle in a temperature and humidity
478 controlled room. Mice were housed 3-5 per cage with free access to food and water.

479 **Macaque specimens**

480 All procedures involving macaque monkeys were approved by the University of Washington's
481 Institutional Care and Use Committee. Male (n=2) and female (n=1) macaques (*Macaca nemestrina*)
482 from 3-17 years old designated for use in the Washington National Primate Research Center's Tissue
483 Distribution Program were used for experiments. Monkeys were housed in individual cages on a 12-hour
484 light/dark cycle in a temperature and humidity controlled room.
485

486 **METHOD DETAILS**

487 **Acute brain slice preparation.** Brain slice preparation was similar for all species. Human
488 neurosurgical specimens deemed not to be of diagnostic value were placed in a sterile, prechilled,
489 carbogenated (95% O₂/5% CO₂) container filled with an artificial cerebrospinal fluid composed of (in
490 mM): 92 N-methyl-D-glucamine (NMDG), 2.5 KCl, 1.25 NaH₂PO₄, 30 NaHCO₃, 20 4-(2-hydroxyethyl)-1-
491 piperazineethanesulfonic acid (HEPES), 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5
492 CaCl₂·4H₂O and 10 MgSO₄·7H₂O. Surgical specimens were quickly transported from the surgical site
493 to the Institute while continuously bubbled with carbogen. Macaques were anesthetized with sevoflurane
494 gas during which the entire cerebrum was extracted and placed in the same aCSF described above.
495 After brain extraction, monkeys were administered intravenous injection of a lethal dose of sodium-
496 pentobarbital. We then dissected the superior temporal gyrus of the temporal lobe for brain slice
497 preparation. Mice were deeply anesthetized by intraperitoneal administration of Avertin (20 mg/kg) and
498 were perfused through the heart with the same NMDG aCSF described above.

499 Brain specimens were sectioned on a Compressstome VF-200 using a zirconium ceramic blade
500 (Precisionary Instruments) at 300 or 350 μm using the protective recovery method (Ting et al., 2014). To
501 ensure that the dendrites of pyramidal neurons were relatively intact, macaque and human specimens
502 were trimmed and mounted such that the angle of slicing was perpendicular to the pial surface. Mouse
503 brains were sectioned in the coronal plane.

504 **Patch clamp recordings.** Brain slices were placed in a submerged, heated recording chamber that
505 was continuously perfused with carbogenated aCSF consisting of (in mM): 119 NaCl, 2.5 KCl, 1.25
506 NaH₂PO₄, 24 NaHCO₃, 12.5 glucose, 2 CaCl₂·4H₂O and 2 MgSO₄·7H₂O (pH 7.3-7.4). Slices were
507 visualized with an Olympus BX51WI microscope and infrared differential interference contrast (IR-DIC)
508 optics and a 40x water immersion objective.

509 Patch pipettes (2-5 MΩ for somatic; 4-8 MΩ for dendritic) were filled with one of two internal
510 solutions. The first solution contained (in mM): 126.0 K-gluconate, 10.0 HEPES, 0.3 EGTA, 4.0 mM KCl,
511 4 Mg-ATP, 0.3 Na₂-GTP, 10.0 phosphocreatine disodium salt hydrate, 0.5% biocytin and .02 Alexa 594
512 or 488. The second pipette solution was used for Patch-seq experiments and contained (in mM): 110.0
513 K-gluconate, 10.0 HEPES, 0.2 EGTA, 4 KCl, 0.3 Na₂-GTP, 10 phosphocreatine disodium salt hydrate, 1
514 Mg-ATP, 20 μg/ml glycogen, 0.5U/μL RNase inhibitor (Takara, 2313A), 0.5% biocytin and 0.02 Alexa
515 594 or 488. The pH of both solutions was adjusted to 7.3 with KOH. Alexa filled neurons were visualized
516 upon termination of recording using a 540/605 nm excitation/emission filter set. The liquid junction
517 potential was calculated to be -13 mV and was not corrected.

518 Whole cell somatic and dendritic recordings were acquired using a Multiclamp 700B amplifier and
519 either PClamp 10 data acquisition software or custom MIES acquisition software
520 (<https://github.com/AllenInstitute/MIES/>) written in Igor Pro. Electrical signals were digitized at 20-50 kHz

521 by a Axon Digidata 1550B (Molecular Devices) or a ITC-18 (HEKA) and were filtered at 2-10 kHz. Upon
522 attaining whole cell current clamp mode, the pipette capacitance was compensated and the bridge was
523 balanced. Access resistance was monitored throughout the recording and was 8-30 M Ω for somatic
524 recordings and 15-40 M Ω for dendritic recordings.

525 **Processing of Patch-seq samples.** Prior to data collection for these experiments, all surfaces were
526 thoroughly cleaned with RNase Zap, and as needed DNase Away. At the end of Patch-seq recordings,
527 negative pressure (~-20 mbar) was applied through the pipette for ~5 minutes after which the nucleus
528 was extracted by very slow pipette withdrawal with higher negative pressure (~-70 to -100 mbar). The
529 pipette was removed from the recording chamber and the contents of the pipette were expelled into a
530 PCR tube containing lysis buffer (Takara, 634894). Patch-seq sample tubes were held on dry ice in a
531 benchtop plexiglass enclosure throughout the recording session to ensure collected samples remained
532 free of RNase and DNase contamination. Sample tubes were then transferred to -80C for storage until
533 further processing. cDNA libraries were produced using the SMART-Seq v4 Ultra Low Input RNA Kit for
534 Sequencing (Takara 634894, Lot 1709695A) according to the manufacturer's instructions, using 20 PCR
535 cycles for cDNA amplification. Samples proceeded through library construction using Nextera XT DNA
536 Library Preparation Kit (Illumina FC-131-1096) according to the manufacturer's instructions except at
537 0.2x reaction size. Samples were sequenced to approximately 1million paired-end 50b reads/sample.

538 **Biocytin histology.** As described previously (Gouwens et al., 2019), a horseradish peroxidase (HRP)
539 enzyme reaction using diaminobenzidine (DAB) as the chromogen was used to visualize biocytin-filled
540 neurons following physiological recordings. 4,6-diamidino-2-phenylindole (DAPI) stain was also used to
541 identify cortical layers.

542 Slices were mounted and imaged on an upright AxioImager Z2 microscope (Zeiss, Germany)
543 equipped with an AxioCam 506 monochrome camera and 0.63x optivar. High-resolution image stacks
544 were captured with a 63X objective at 0.44 μ m increments along the Z axis. ZEN software was used to
545 stitch tiled images.

546 **Multiplex fluorescence in situ hybridization**

547 Fresh-frozen human (postmortem and surgical) MTG, macaque (*M. nemestrina*) MTG, and mouse TEa
548 tissues were sectioned at 14-16 μ m onto Superfrost Plus glass slides (Fisher Scientific). Sections were
549 prepared from at least two donors per species. Sections were dried for 20 minutes at -20°C and then
550 vacuum sealed and stored at -80°C until use. The RNAscope multiplex fluorescent v1 kit was used per
551 the manufacturer's instructions for fresh-frozen tissue sections (ACD Bio), except that fixation was
552 performed for 60 minutes in 4% paraformaldehyde in 1X PBS at 4°C, post-dehydration drying was done
553 for 15 minutes at 37°C and protease treatment was shortened to 10 minutes. Sections were imaged
554 using a 60X oil immersion lens on a Nikon TiE fluorescence microscope equipped with NIS-Elements
555 Advanced Research imaging software (version 4.20). For all RNAscope mFISH experiments, positive
556 cells were called by manually counting RNA spots for each gene. Cells were called positive for a gene if
557 they contained ≥ 3 RNA spots for that gene. Lipofuscin autofluorescence was distinguished from RNA
558 spot signal based on the larger size of lipofuscin granules and broad fluorescence spectrum of lipofuscin.

559
560 To quantify the fraction of putative ET cells (defined as *FAM84B* and *SLC17A7* double positive cells) in
561 layer 5 in each species, the boundary of layer 5 was first delineated using DAPI to identify cortical layers.
562 The total number of *SLC17A7*⁺ cells within layer 5 was quantified and then the total number of
563 *SLC17A7*⁺,*FAM84B*⁺ cells in layer 5 was quantified. The percentage of putative ET cells (*SLC17A7*⁺,
564 *FAM84B*⁺) was then calculated as a fraction of the total number of *SLC17A7*⁺ cells in layer 5. Counts
565 were repeated on at least 2 donors per species and at least 2 sections per donor.
566

567

QUANTIFICATION AND STATISTICAL ANALYSIS

568

Neurophysiology. We used three basic current stimuli to probe the intrinsic membrane properties of L5 neurons. In the first protocol, we measured the voltage response to a series of 1s steps from -150 pA to +50 pA in +20 pA increments. Maximum and steady-state input resistance (R_N) were calculated from the linear portion of the current-maximum or steady state voltage relationship generated in response to these current injections. Voltage sag was defined as the ratio of maximum to steady-state R_N . Rebound slope was calculated from the slope of the rebound amplitude as a function of steady-state membrane potential.

575

The second stimulus was a chirp stimulus that increased in frequency either linearly from 1-15 Hz over 15 s or logarithmically from 0.2-40 Hz over 20s. The amplitude of the chirp was adjusted for each neuron to produce a peak-to-peak voltage deflection of ~10 mV. Impedance amplitude (ZAP) was derived as the ratio of the Fourier transform of the voltage response to the Fourier transform of the chirp:

579

$|Z(f)| = \sqrt{\text{Re}(Z(f))^2 + (\text{Im}(Z(f)))^2}$, where $\text{Im}(Z(f))$ and $\text{Re}(Z(f))$ are the imaginary and real parts of the impedance $Z(f)$, respectively. The frequency at which the maximum impedance occurred was the resonant frequency (f_R). Resonance strength (Q) was measured as the ratio of the maximum impedance amplitude to the impedance amplitude at 1 Hz. The 3dB cutoff was defined as the frequency at which the ZAP profile attenuated to a value of $\sqrt{(1/2)Z_{max}}$. Impedance phase (ZPP) was derived as: $\phi(f) =$

584

$\tan^{-1} \frac{\text{Im}(Z(f))}{\text{Re}(Z(f))}$. Total inductive phase (Z Φ) was defined as the area under the inductive part of the ZPP.

585

Synchrony phase was the frequency at which the ZPP was 0.

586

The third stimulus was a series of 1 second depolarizing current injections increasing in amplitude by +50 pA/step. We measured the number of spikes and the first instantaneous firing rate in response to each current injection. Gain was defined as the linear slope of this action potential frequency-current relationship. Single action potential properties were measured from voltage responses to the lowest current that produced a spike. Action potential threshold was defined as the voltage at which the first derivative of the voltage response exceeded 20 V/s. AP width was measured at half the amplitude between threshold and the peak voltage. Fast AHP was defined relative to threshold. Spike frequency accommodation (SFA) and medium afterhyperpolarization (mAHP) were calculated from current injections producing ~10 spikes during the 1 s step. SFA was defined as the ratio of the second to the last interspike interval. The mAHP was defined as the minimum voltage after the spike train. For dendritic recordings we also quantified the area and width of plateau potentials. The threshold for a plateau was defined as the voltage where the first derivative reached 2 V/s. Plateau width at half maximum amplitude and plateau area were calculated relative to this threshold.

599

We grouped neurons based on physiological properties derived from these current injections using hierarchical clustering. First we performed principal component analysis on all physiological features. Principal components explaining at least 1% of the variance were then used to cluster neurons into groups using Ward's algorithm. The number of clusters was determined using the sigClust package in R which generates a p value for the null hypothesis that data points are drawn from a single Gaussian as opposed to two Gaussian distributions. The p value for this analysis was set at < 0.01, which yielded three clusters.

606

Random forest classifiers were constructed using the randomForest package in R (Liaw and Wiener, 2002). We varied the percentage of cells included in the training data set from 10-50%. For each training set, we constructed 100 forests consisting of 100 trees. Mean accuracy and importance values were calculated from each set of forests on the data held out of the training sets.

610

Comparisons across groups are presented as geometric box plots unless otherwise denoted. Statistical significance was assayed using ANOVAs, t-tests, Mann-Whitney U tests, or Person's correlation where appropriate. P values were FDR adjusted for multiple comparisons. Effect sizes are reported as eta-squared values.

613

614 **Morphological reconstruction.** Dendritic reconstructions were generated based on a digital 3D
615 image stack that was run through a Vaa3D-based image processing and reconstruction pipeline, as
616 previously described (Gouwens et al., 2019). Somatic morphology was quantified directly via a maximum
617 projection of a series of 63x images of biocytin and DAB reacted neurons. The initial apical shaft width
618 was measured 50 μm from the center of the soma.

619

620 **Patch-Seq sample mapping.** To determine the corresponding transcriptomic cell type for each
621 Patch-seq sample, we utilized a tree-based mapping strategy. For each neuron, we computed its
622 correlation with each branch of the reference cell type taxonomy, starting from the root and working
623 towards the leaves. Marker genes associated with each branch of the taxonomy were used for
624 correlations. The confidence of the mapping was determined by applying 100 bootstrapping iterations of
625 the process. For each iteration 70% of the reference cells and 70% of maker genes were randomly
626 sampled for mapping. The percentage of times a given cell mapped to a given transcriptomic cell types
627 was the mapping probability. Only neurons with a mapping probability greater than 50% to a given
628 terminal leaf were included. As an additional quality control measure, only Patch-seq samples with a
629 normalized summed expression of “on”-type marker genes (NMS; (Tripathy et al., 2018) were included.
630 We mapped mouse Patch-seq samples to a published mouse VISp scRNA-seq cell type taxonomy
631 (Tasic et al., 2018) and human samples to a published human MTG snRNA-seq cell type taxonomy
632 (Hodge et al., 2019).

633 **Differential Gene Expression analysis**

634 To identify differentially expressed genes between L5 ET and L5 IT neurons for each species,
635 expression matrices were trimmed to only include genes with one-to-one orthologs in human and mouse
636 (downloaded from NCBI Homologene in November, 2019). Raw expression matrices for each brain
637 region were CPM normalized then Log2 transformed. Average expression was determined for each
638 subclass (i.e. L5 ET and L5 IT), then log fold change was determined between subclasses for each brain
639 region. The following clusters from human MTG were used for L5 IT subclass input; Exc L5-6 RORB
640 TTC12, Exc L4-5 RORB FOLH1B, Exc L5-6 THEMIS C1QL3, Exc L4-6 RORB SEMA3E, and Exc L4-6
641 RORB C1R; and the Exc L4-5 FEZF2 SCN4B cluster was used as input for L5 ET. For mouse, all
642 previously defined L5 IT and L5 PT clusters were used as input. Initial GO analysis of conserved L5 ET
643 DE genes (> 0.5 Log2FC across all three brain regions) with PANTHER Classification System (Mi et al.,
644 2019) revealed numerous significant GO biological process categories for axonogenesis, axon guidance,
645 axon development, etc. Additionally, numerous synaptic-related categories were enriched (i.e synaptic
646 membrane adhesion, synaptic organization, synaptic structure, etc.). Respective terms were aggregated
647 into an Axon Guidance gene list, and a Synaptic Regulation gene list for visualization purposes in Figure
648 1, with aggregated gene lists shown in Table S2. Human L5 ET-enriched (> 1 Log2FC) DE genes from
649 each GO category were plotted as line plots and colored red if L5 ET neurons had > 1 Log2FC
650 expression over L5 IT in all brain regions, indicating conserved L5 ET DE genes. Lines were colored blue
651 if L5 ET expression was > 1 Log2FC in human MTG and < 0 in both mouse regions, indicating human-
652 specific L5 ET DE genes.

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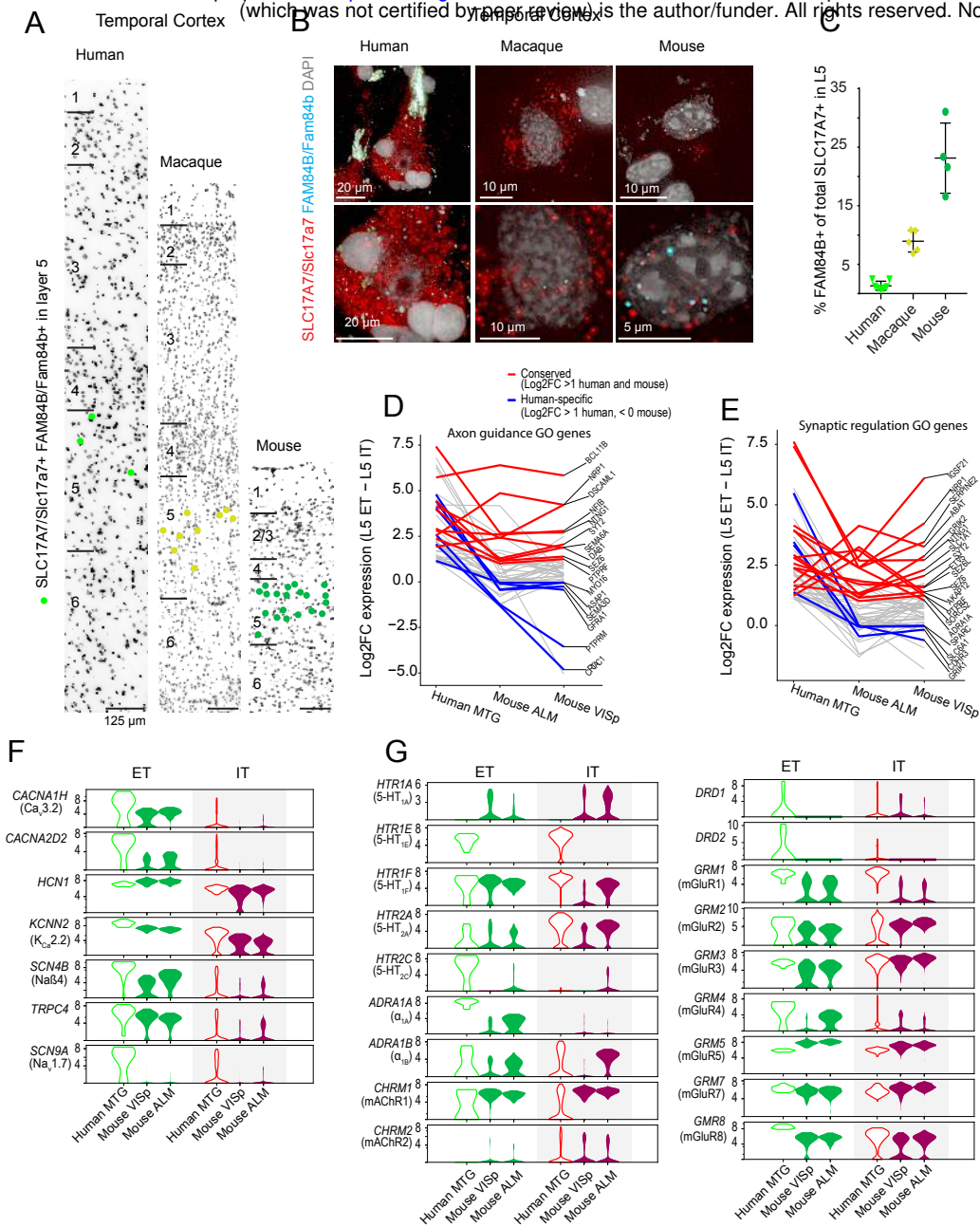


Figure 1

Figure 1- ET neurons are sparse in human MTG, but share many genes with mouse ET neurons A) Representative inverted images of DAPI-stained sections of human, macaque and mouse temporal cortex. Dots denote the location of cells labeled using multiplex fluorescent in situ hybridization (mFISH) for FAM84B and SLC17A7. Horizontal bars denote putative layer boundaries. B) Example mFISH images of FAM84B and SLC17A7 labeling in L5 pyramidal neurons in human, macaque and mouse temporal cortex. C) Quantification of the proportion of SLC17A7+ cells expressing the ET marker FAM84B in temporal cortex of mouse, macaque and human expressed as a fraction of the total number of excitatory cells in L5. Individual data points are denoted by symbols. Line graphs of D) axon guidance and E) synaptic regulation related genes with expression enrichment in L5 ET versus IT neurons in human MTG (> 1 log₂ fold-difference) and their respective enrichment in L5 ET neurons in mouse VISp. Notable conserved (red) and human specific (blue) genes are highlighted F) Human L5 ET neuron enriched ion channel genes and their expression in the mouse VISp and ALM. G) Neuromodulator receptor gene expression in human MTG and mouse VISp/ALM.

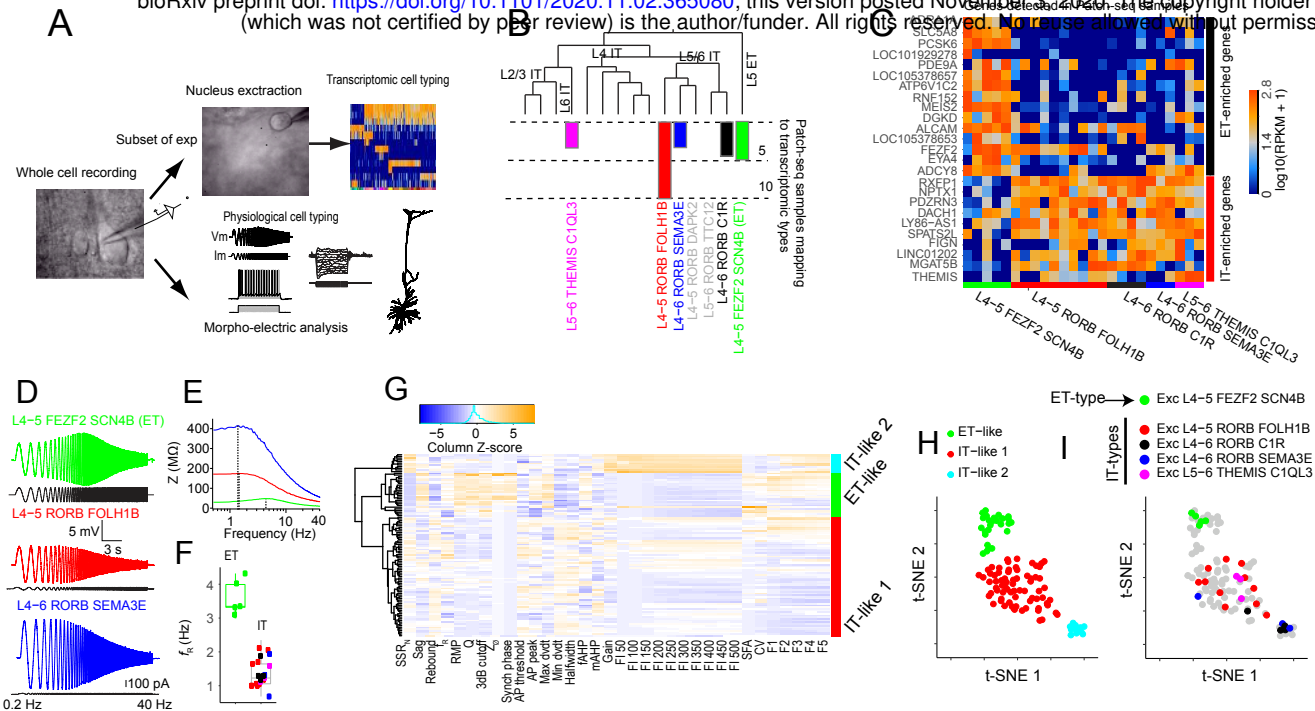


Figure 2

Figure 2- Patch-seq analysis reveals distinctive membrane properties of transcriptomically defined L5 ET neurons. A) We obtained whole cell patch clamp recordings from L5 pyramidal neurons in human brain slices prepared from neurosurgical specimens. For a subset of experiments, Patch-seq analysis was performed in which the RNA content of extracted nuclei were sequenced for the post-hoc assignment of a transcriptomic cell type to the physiologically probed neuron. Several physiological features were extracted from all recordings. B) Glutamatergic transcriptomic cell types in human MTG are shown, with the number of patch-seq samples that mapped with high confidence to each type indicated by the bar plots. C) Heat map of ET and IT enriched genes detected in patch-seq samples. D) Example voltage response of three different transcriptomically-defined cell types to a chirp stimulus and E) Corresponding impedance amplitude profiles (ZAP). Dashed lines denote resonant frequencies. F) Transcriptomically-defined ET neurons displayed a higher resonant frequency than IT neurons ($p < 0.001$, FDR corrected Mann-Whitney U test). G) Heat map of physiological features of human L5 pyramidal neurons. These features were used to cluster cells into physiologically defined types using Ward's algorithm. The dendrogram represents the outcome of this clustering. tSNE projection of the features shown in G) color-coded by H) physiologically-defined cell type and I) transcriptomically defined cell type.

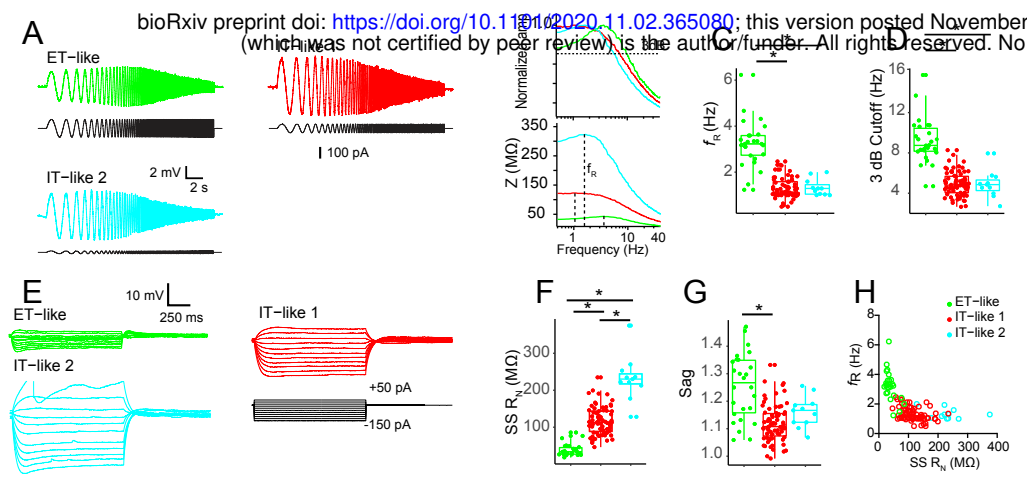


Figure 3

Figure 3-Subthreshold membrane properties of L5 neuron types in human MTG. A) Example voltage response of L5 neuron types to a chirp stimulus. B) ZAP (bottom) and normalized frequency response (top) constructed from the voltage responses in A). Dashed lines indicate resonant frequency (ZAP) and 3dB cutoff (normalized curve). Pairwise comparisons of C) resonant frequency and D) 3 dB cutoff. E) Example voltage responses to a series of hyperpolarizing and depolarizing current injections. Pairwise comparisons of F) Input resistance and G) Sag ratio. H) Resonant frequency as a function of input resistance. * $p < .05$, FDR corrected Mann-Whitney U test.

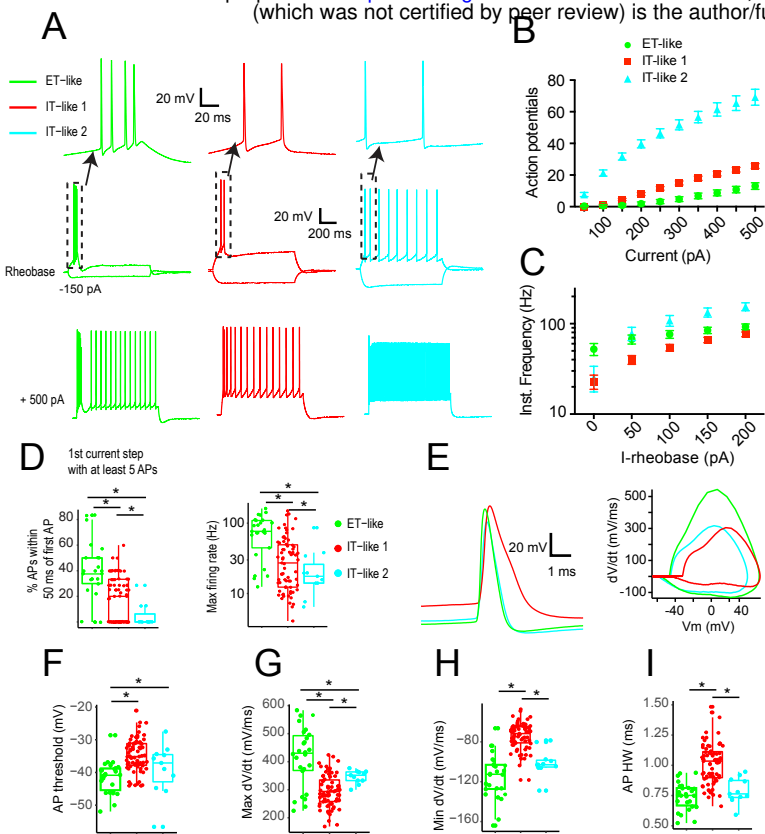


Figure 4

Figure 4-Suprathreshold membrane properties of L5 neuron types in human MTG. A) Example voltage responses to near threshold current injection (middle) and +500 pA (bottom). (top)- expanded view of spikes at near rheobase current injection. B) The number of action potentials evoked as a function of current injection amplitude. C) First instantaneous frequency plotted as a function of current injection amplitude above rheobase. D) (left) The percentage of action potentials occurring within 50 ms of the first spike and (right) maximum instantaneous firing rate for the first current injection producing at least 5 spikes. E) Example action potentials (left) and corresponding phase-plane plots (right). Differences in F) action potential threshold, G) maximum dV/dt H) minimum dV/dt and I) action potential width at half-maximum amplitude. * $p < .05$, FDR corrected Mann-Whitney U test.

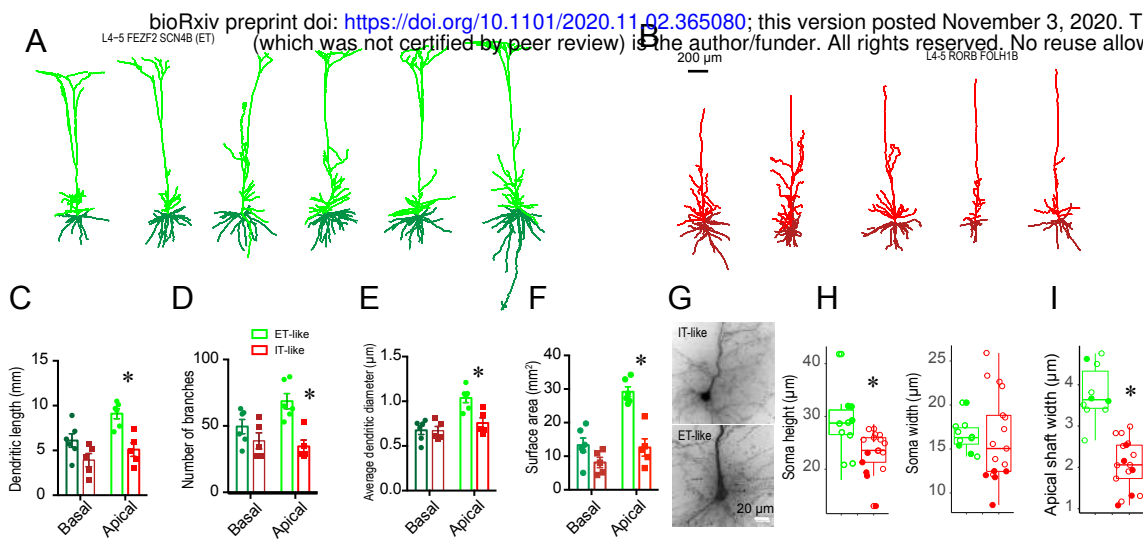


Figure 5

Figure 5 - Morphological features of L5 ET- and IT- like neurons in human MTG. Dendritic reconstructions of A) ET-like and B) IT-like neurons in human MTG. Apical and basal dendrites are denoted by different shades of green or red. C) Comparison of basal and apical dendritic length between ET and IT neurons. D) Comparison of the number of basal and apical dendritic branches between ET and IT neurons. E) Comparison of average dendrite diameter between ET and IT neurons. F) Comparison of basal and apical total dendritic surface area between ET and IT neurons. G) Example biocytin fills of perisomatic regions for ET and IT-like neurons. Comparison of H) soma height/width and I) initial apical shaft width. For H and I, filled symbols correspond to transcriptomically-defined cells. * $p < 0.05$, FDR corrected Mann-Whitney U test.

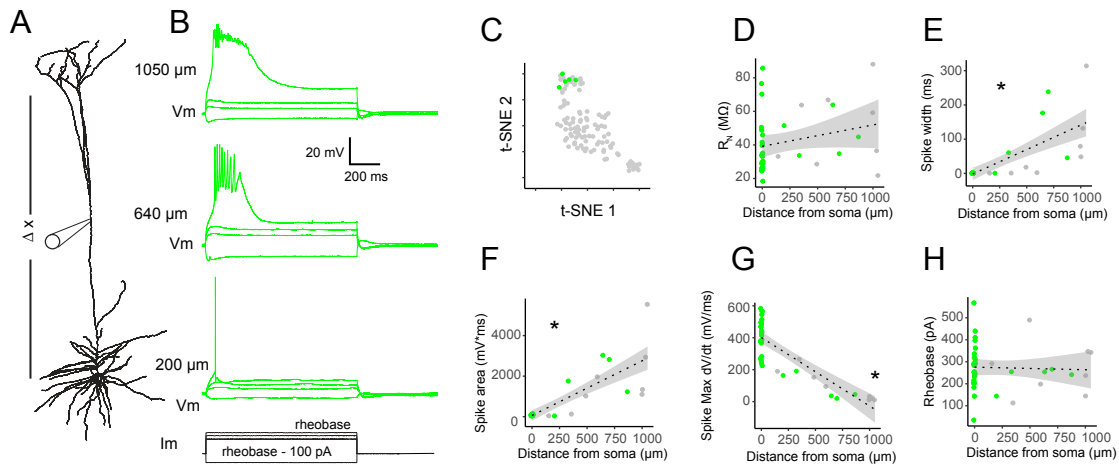


Figure 6

Figure 6- Putative ET neurons display strong dendritic electrogenesis. A) Direct electrical recordings of dendritic membrane properties were performed at various distances from the soma. For a subset of experiments, the soma was subsequently patched with a separate electrode. B) Example voltage responses to hyperpolarizing and depolarizing current injections. Depolarizing current injections were capable of eliciting an all-or-none plateau potential. C) tSNE projection of somatic membrane properties of cells in which the dendritic properties were probed are shaded in green. D) Input resistance ($p = 0.13$), E) Spike width ($p < 0.001$), F) plateau potential area ($p < 0.001$), G) Maximum dV/dt ($p < 0.001$) and H) Rheobase ($p = 0.80$) plotted as a function of distance from soma. * $p < 0.05$, FDR corrected Pearson's correlation. Shaded region corresponds to SEM. For D-H, green dots denote cells ($n = 5$) in which the somatic properties were also probed.

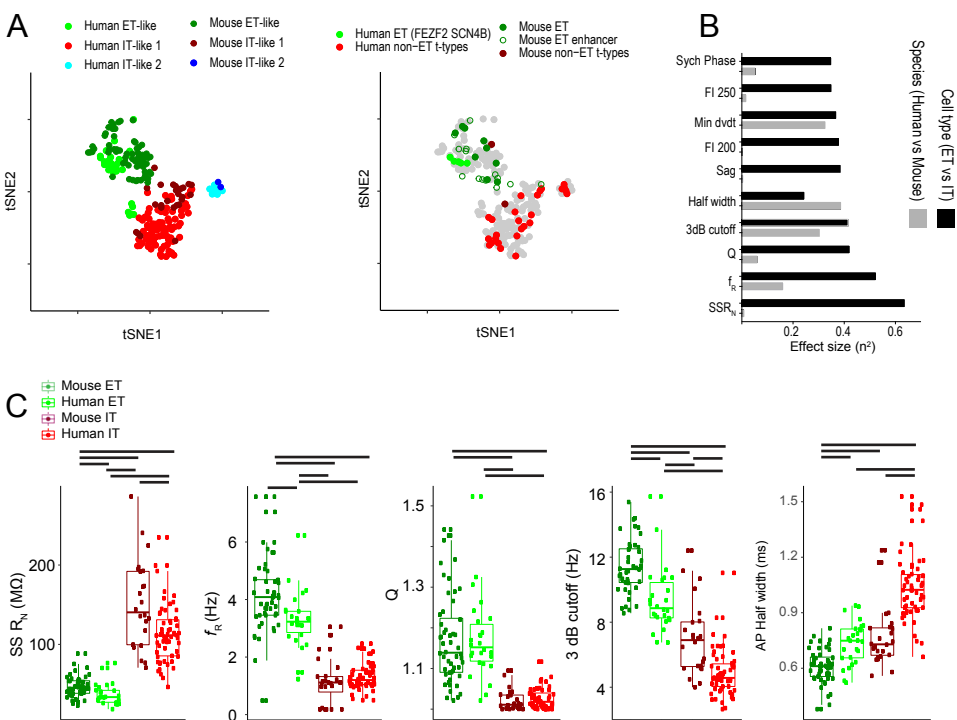


Figure 7

Figure 7 - Cross-species comparison reveals conserved and divergent ET neuron somatic membrane properties. A) tSNE of intrinsic membrane properties for human and mouse L5 pyramidal neurons color coded by (left) physiologically-defined cell type and (right) transcriptomic cell type or labeling by an ET-enhancer virus. B) The top 10 largest effect sizes resulting from ANOVA with cell type (ET versus IT) and species (human versus mouse) as the factors. C) Physiological features plotted as a function of species and cell type for the five features with the largest effect sizes. Gray bars denote * $p < 0.05$, FDR corrected Mann-Whitney U test.

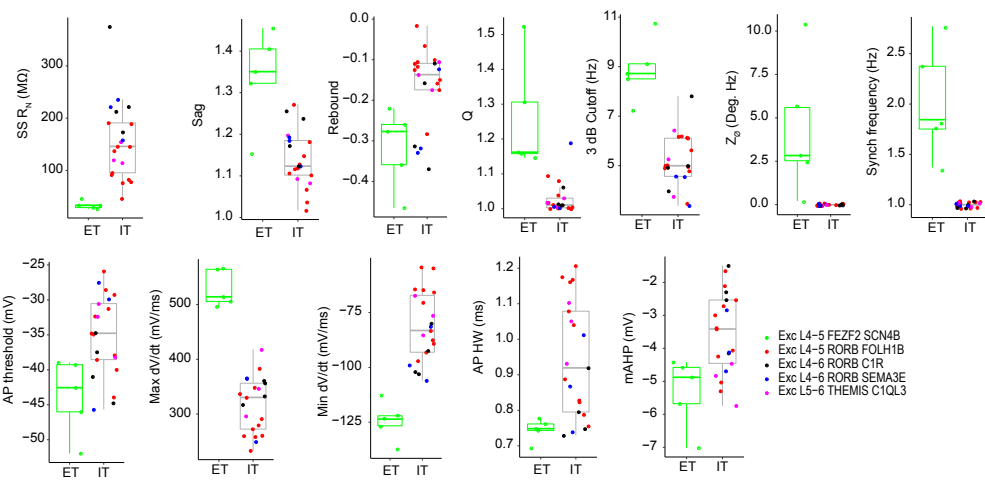


Figure S1

Figure S1- Additional pairwise differences in the intrinsic membrane properties of transcriptomically defined cell types. All comparisons were * $p < 0.05$, FDR corrected Mann-Whitney U test.

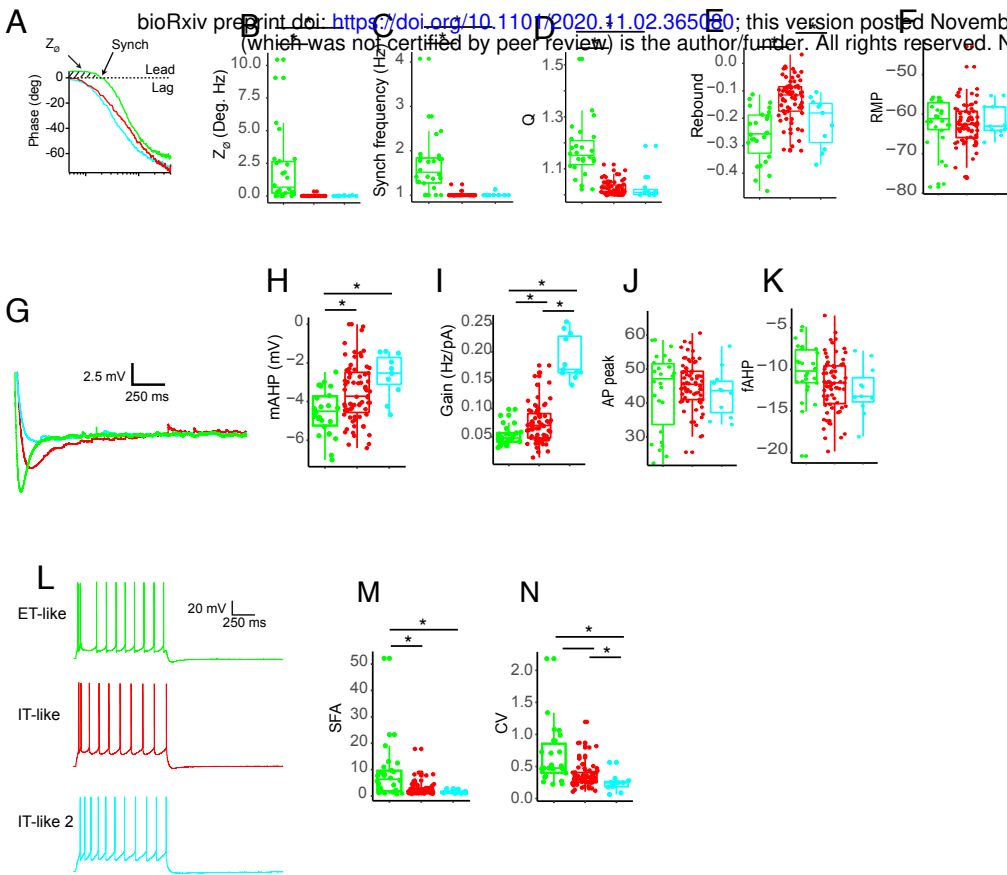


Figure S2

Figure S2-Additional pairwise comparisons of intrinsic membrane properties of physiologically defined L5 pyramidal neurons in human middle temporal gyrus. A) Example impedance phase plot for ET-like, IT-like 1 and IT-like 2 neurons. Dashed line indicates the frequency at which the current and voltage are in phase with one another (ZPP). B-F) Additional pairwise comparisons of subthreshold properties. G) Example medium afterhyperpolarization produced by ~10 Hz firing. H-K) Additional pairwise comparisons of suprathreshold properties. L) Example ~10 Hz firing. Pairwise comparison of M) spike frequency accommodation and N) Coefficient of variation. * $p < 0.05$, FDR corrected Mann-Whitney U test.

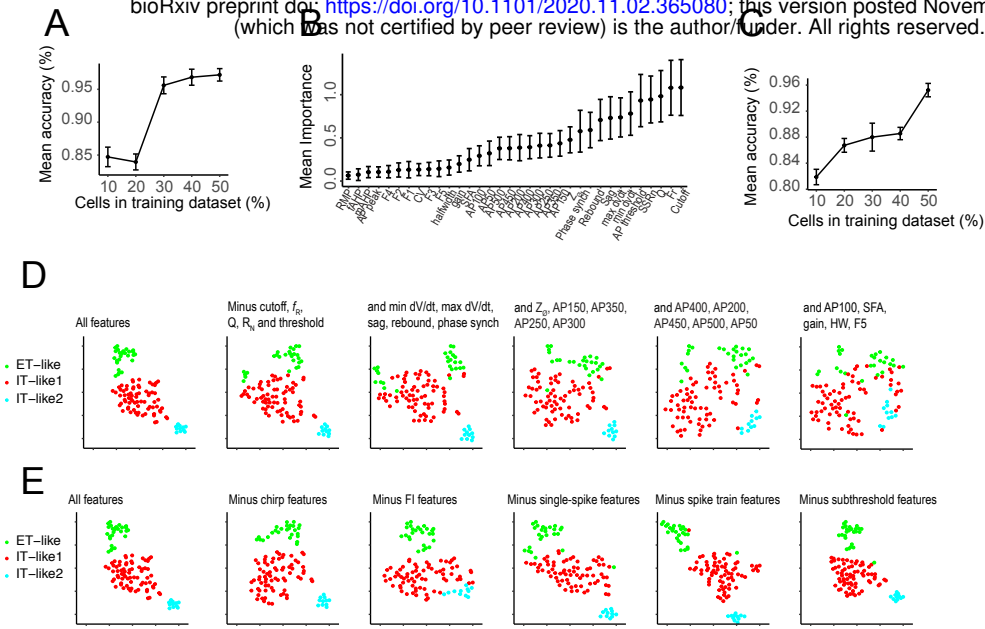


Figure S3

Figure S3-The distinctiveness of L5 neuron properties is determined by an aggregate of physiological features. A) Performance of a random forest classifier as a function of the percentage of cells included in the training dataset. B) Mean importance of physiological features as determined by random forest classifier. C) Performance of random forest classifier using only the top 10 important features. D) tSNE projections based on all physiological features (left) and after subsets of features are successively removed. Features were removed in order based on their mean importance determined by the random forest classifier. E) tSNE projections based on all features (left) and where specific features are removed as indicated.

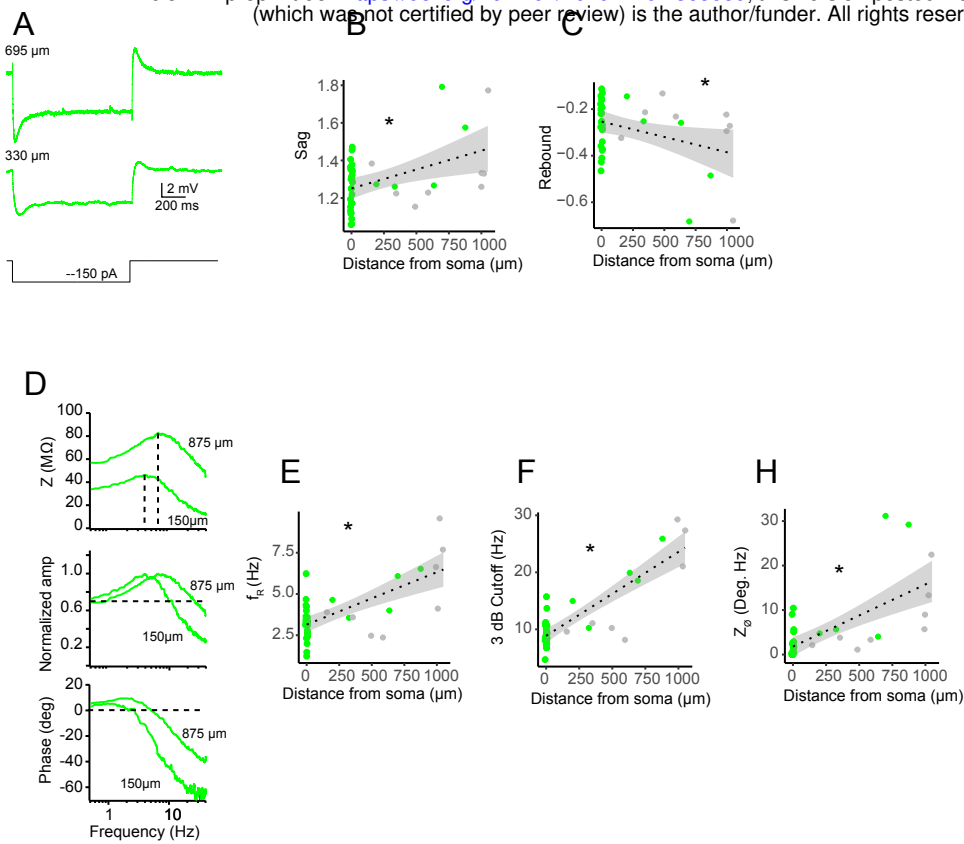


Figure S4

Figure S4- Intrinsic membrane properties of putative L5 ET neuron dendrites. A) Voltage response to hyperpolarizing current injection recorded at two different dendritic recording sites. B) Sag ($p = 0.008$) and C) rebound potentials ($p = 0.04$) as a function of distance from soma. D) Example ZAP (top), normalized frequency response (middle) and ZPP (bottom) for dendritic recordings. E) Resonance frequency, F) 3dB cutoff ($p < 0.001$) and H) total inductive phase ($p < 0.001$) as a function of distance from soma. p values are for FDR corrected Pearson's correlation.

