1 **Developmental dynamics of voltage-gated sodium channel isoform expression in the human**
2 **and mouse neocortex**
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30

31   **Abstract**
32
33   **Objective:** Genetic variants in the voltage-gated sodium channels *SCN1A*, *SCN2A*, *SCN3A*, and
34   *SCN8A* are leading causes of epilepsy, developmental delay, and autism spectrum disorder. The
35   mRNA splicing patterns of all four genes vary across development in the rodent brain, including
36   mutually exclusive copies of the fifth protein-coding exon detected in the neonate (5N) and
37   adult (5A). A second pair of mutually exclusive exons is reported in *SCN8A* only (18N and 18A).
38   We aimed to quantify the expression of individual exons in the developing human neocortex.
39   **Methods:** RNA-seq data from 176 human dorsolateral prefrontal cortex samples across
40   development were analyzed to estimate exon-level expression. Developmental changes in exon
41   utilization were validated by assessing intron splicing. Exon expression was also estimated in
42   RNA-seq data from 58 developing mouse neocortical samples.
43   **Results:** In the mature human neocortex, exon 5A is consistently expressed at least 4-fold
44   higher than exon 5N in all four genes. For *SCN2A*, *SCN3A*, and *SCN8A* a synchronized 5N/5A
45   transition occurs between 24 post-conceptual weeks (2nd trimester) and six years of age. In
46   mice, the equivalent 5N/5A transition begins at or before embryonic day 15.5. In *SCN8A,* over
47   90% of transcripts in the mature human cortex include exon 18A. Early in fetal development,
48   most transcripts include 18N or skip both 18N and 18A, with a transition to 18A inclusion
49   occurring from 13 post-conceptual weeks to 6 months of age. No other protein-coding exons
50   showed comparably dynamic developmental trajectories.
51   **Significance:** Splice isoforms, which alter the biophysical properties of the encoded channels,
52   may account for some of the observed phenotypic differences across development and
53   between specific variants. Manipulation of the proportion of splicing isoforms at appropriate
54   stages of development may act as a therapeutic strategy for specific mutations or even epilepsy
55   in general.
56

57   **Keywords**
58   isoform, splicing, epilepsy, neurodevelopmental disorders, SCN1A, SCN2A, SCN3A, SCN8A, 5A,
59   5N, 18A, 18N
60

61 **1. Introduction**

62 Genetic variation in the genes *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* are a major cause of epileptic
63 encephalopathy (EE), autism spectrum disorder (ASD), and developmental delay.[1–3] These four
64 homologous genes encode voltage-gated sodium channels (Na_V1.1, Na_V1.2, Na_V1.3, and Na_V1.6
65 respectively) that are critical for a range of functions in the central nervous system,[4] including
66 axonal action potential initiation and propagation,[5,6] dendritic excitability,[7,8] macroscopic
67 anatomical development,[9] and activity-dependent myelination.[10] The functional role,
68 subcellular location, expression-level, and isoform selection of voltage-gated sodium channels
69 vary across development and understanding this relationship is critical for understanding the
70 etiology of the associated disorders and their therapeutic management.[7,11–19] While some
71 isoform-level differences have been assayed in rodents and mature human brains,[20–22] the
72 trajectories in the developing human cortex have not been described.[23]

73

74 Sodium channel genes are composed of multiple exons, which can be protein-coding (CDS for
75 CoDing Sequence), untranslated regions (UTRs), or non-coding exons (NCEs). Differing
76 combinations of these exons are called isoforms, which can change the amino acid sequence of
77 the encoded proteins (proteoforms). The best-characterized isoform change across these four
78 sodium channels are the two mutually exclusive copies of the fifth protein-coding exon.[17,24] This
79 exon encodes part of the first domain of the Na_V channel, including the end of transmembrane
80 segment S3, most of transmembrane segment S4, and a short extracellular linker connecting
81 these two segments. In humans, each copy of this fifth protein-coding exon is 92 nucleotides in
82 length, encoding 30 amino acids, of which one to three amino acids vary between the two exon
83 copies for each gene (Fig. 1B). 'A' isoforms (5A) include the ancestral and canonical copy, with
84 an aspartic acid residue (Asp/D) encoded at position 7 of 30.[25] 'N' isoforms (5N) use the
85 alternative copy, with an asparagine (Asn/N) residue at position 7 of 30 in *SCN1A*, *SCN2A*, and
86 *SCN8A* and a serine residue (Ser/S) in *SCN3A*. Despite this relatively small change in protein
87 structure, differential inclusion of 5N or 5A can have marked effects on channel function.
88 Indeed, these splice isoforms can alter channel electrophysiological characteristics,[26,27] the
89 functional impacts of variants associated with seizure,[23] neuronal excitability,[28] response to
90 anti-epileptics,[21,22,26] and seizure-susceptibility.[28]

91

92 The utilization of the 5N or 5A varies across development, with 5N generally being expressed at
93 higher levels in the neonatal period while 5A predominates in adults.[27] This switch is defined
94 best in mouse, where the 5N:5A ratio varies by gene and brain region along with
95 developmental stage.[20] For *Scn2a* in mouse neocortex, the 5N:5A ratio is 2:1 at birth (postnatal
96 day 0/P0) and flips to 1:3 by P15. For both *Scn3a* and *Scn8a*, 5A predominates throughout the
97 postnatal period with a 1:2 ratio at P0 increasing to 1:5 by P15.[20] *Scn1a* lacks a functional copy
98 of 5N in the mouse genome. Similar developmental profiles currently have not been reported
99 for humans beyond the of 5N/5A utilization *SCN1A* in adults, in which a 5N:5A ratio of over 1:5
100 was observed in the temporal cortex and hippocampus of adult surgical resections.[21,22]

101

102 In addition to the 5N/5A switch, a similar developmental shift in mutually exclusive exons has
103 been reported for "exons 18N or 18A" in *SCN8A* only, regulated by the RNA-binding protein
104 RBFOX1.[16,29,30] Using GENCODE human v31 gene definitions,[31] 18A maps to the 20th protein-

105 coding exon of major *SCN8A* isoforms (CDS 20, Fig. 1A), while 18N encodes the 8th and last
106 protein-coding exon (CDS 8) of a shorter eight protein-coding exon transcript
107 (ENST00000548086.3, Fig. S1). In the embryonic mouse brain, most *SCN8A* transcripts include
108 18N or skip both 18N and 18A, leading to non-functional channels, while 18A predominates in
109 the adult mouse and human brain.[16]
110
111 Here, we present data on the utilization of GENCODE-annotated protein-coding exons in four
112 seizure-associated voltage-gated sodium channels in the human and mouse neocortex across
113 development. We demonstrate a synchronized transition from 5N to 5A utilization between 24
114 post-conceptual weeks (2nd trimester) and six years-of-age across all four voltage-gated sodium
115 channels and a transition from 18N to 18A in *SCN8A* from 13 post-conceptual weeks to 6
116 months-of-age. These isoform differences can modify the function of the encoded voltage-
117 gated sodium channels, raising the potential that interventions, such as antisense
118 oligonucleotides, could be used to modify the isoform ratio as a potential therapy for disorders
119 caused by variants in sodium channel genes or epilepsy.
120

121 **2. Materials and Methods**

122

123 **2.1 Genomic data**

124 To quantify the relative proportion of protein-coding exon expression across development in

125 the human cortex, we assessed bulk tissue RNA-seq data from 176 *post mortem* dorsolateral

126 prefrontal cortex (DLPFC) samples from the BrainVar cohort.[32] The BrainVar cohort also has

127 corresponding whole-genome sequencing data that were used to derive per sample genotypes,

128 as described previously.[32] To assess corresponding patterns of exon expression in mouse cortex

129 across development, we assessed 58 samples with bulk tissue RNA-seq data in wildtype C57/B6

130 mice. Thirty-four of these were generated as controls for ongoing experiments and 24 were

131 downloaded from GEO.[33]

132

133 **2.2 Exon expression**

134 To assess exon expression in the human cortex, the 100bp paired-read RNA-seq data from

135 BrainVar were aligned to the GRCh38.p12 human genome using STAR aligner[34] and exon-level

136 read counts for GENCODE v31 human gene definitions were calculated with DEXSeq[35] and

137 normalized to counts per million (CPM).[36] Despite the similar amino acid sequence, the

138 nucleotide sequence of 5N and 5A is sufficiently differentiated across the four genes that 100bp

139 reads align unambiguously to one location in the genome.[37] Reads were detected in 5N and 5A

140 for all samples, across all four genes, with the exception of the *SCN1A* for which 31 of 176

141 samples (17.6%) had no detectable 5N reads (Fig. 2A). Along with quantifying the expression of

142 5N and 5A (Fig. 2), we also assessed expression for the surrounding constitutive exons, as a

143 control (Fig. S2). For the mouse cortical data, the same analysis methods were used but with

144 alternative references, specifically the GRCm38/mm10 genome and GENCODE vM25 gene

145 definitions. A similar approach was used to assess the utilization of 18N and 18A in *SCN8A*.

146

147 **2.3 Intron splicing**

148 We applied a complementary approach to detecting 5N and 5A exon usage by assessing intron

149 splicing via reads that map across exon-exon junctions in the same 176 BrainVar samples. Reads

150 were aligned with OLego aligner[38] using the same genome build and gene definitions as for

151 exon expression. Clusters of differential intron splicing were identified with Leafcutter[39] and

152 differences across development were detected by comparing 112 prenatal samples to 60

153 postnatal samples. No cluster was detected for 5N/5A in *SCN1A*, preventing assessment across

154 development, but clusters were identified and assessed for the other three genes and for

155 18N/18A in *SCN8A* (Figs. 3, 5).

156

157 **2.4 Quantitative trait locus (QTL) analysis**

158 Common variants with a minor allele frequency ≥5% in both the prenatal (N = 112) and

159 postnatal (N = 60) samples and Hardy Weinberg equilibrium p value ≥ 1×10^{-12} were identified

160 previously.[32] Variants within one million basepairs of each sodium channel gene were extracted

161 and integrated with the Leafcutter clusters, along with the first five principal components

162 calculated from common variants identified in whole-genome sequencing data from these

163 samples and 3,804 parents from the Simons Simplex Collection[32,40] to predict sQTLs with

164 FastQTL.[41] This analysis was performed on all samples, prenatal-only samples, and postnatal-

165 only samples, with false discovery rate (FDR) estimated from the results of each analysis using
166 the Benjamini-Hochberg procedure.[42] To assess correlation of 5N expression for the SNP
167 rs3812718, genotypes were extracted for chr2:166,053,034 C>T (GRCh38) and compared with
168 5N expression calculated by DEXSeq, as described above.
169
170 **2.5 Statistical analysis**
171 The 5N:5A expression ratio was calculated from normalized exon expression values (CPM).
172 Linear regression was used to assess whether this ratio varied across development by
173 comparing the log-transformed 5N:5A ratio to log-transformed post-conceptual days (Fig. 2).
174 The difference in ratio was also assessed between the mid-late fetal samples (N=112) and
175 childhood/adolescent/young adult samples (N=35) with a two-tailed Wilcoxon test. To compare
176 intron splicing between prenatal and postnatal samples, we used the P-values estimated with a
177 Dirichlet-multinomial generalized linear model, as implemented in Leafcutter.[39]
178
179 **3. Results**
180
181 **3.1 Expression of voltage-gated sodium channels in the human cortex**
182 Gene expression varies dramatically across development for many genes, especially during the
183 late-fetal transition, during which half the genes expressed in the brains undergo a concerted
184 increase or decrease in expression.[12,32,43,44] To assess gene-level developmental trajectories, we
185 analyzed bulk-tissue RNA-seq of the human DLPFC in 176 *post mortem* samples from the
186 BrainVar cohort (104 male, 72 female, spanning 6 post-conceptual weeks to 20 years after
187 birth).[32] The gene-level expression profile of all four voltage-gated sodium channels changes
188 during this late-fetal transition (Fig. 1F-I), with *SCN1A*, *SCN2A*, and *SCN8A* expression rising from
189 mid-fetal development through infancy to early childhood, while *SCN3A* expression falls.
190
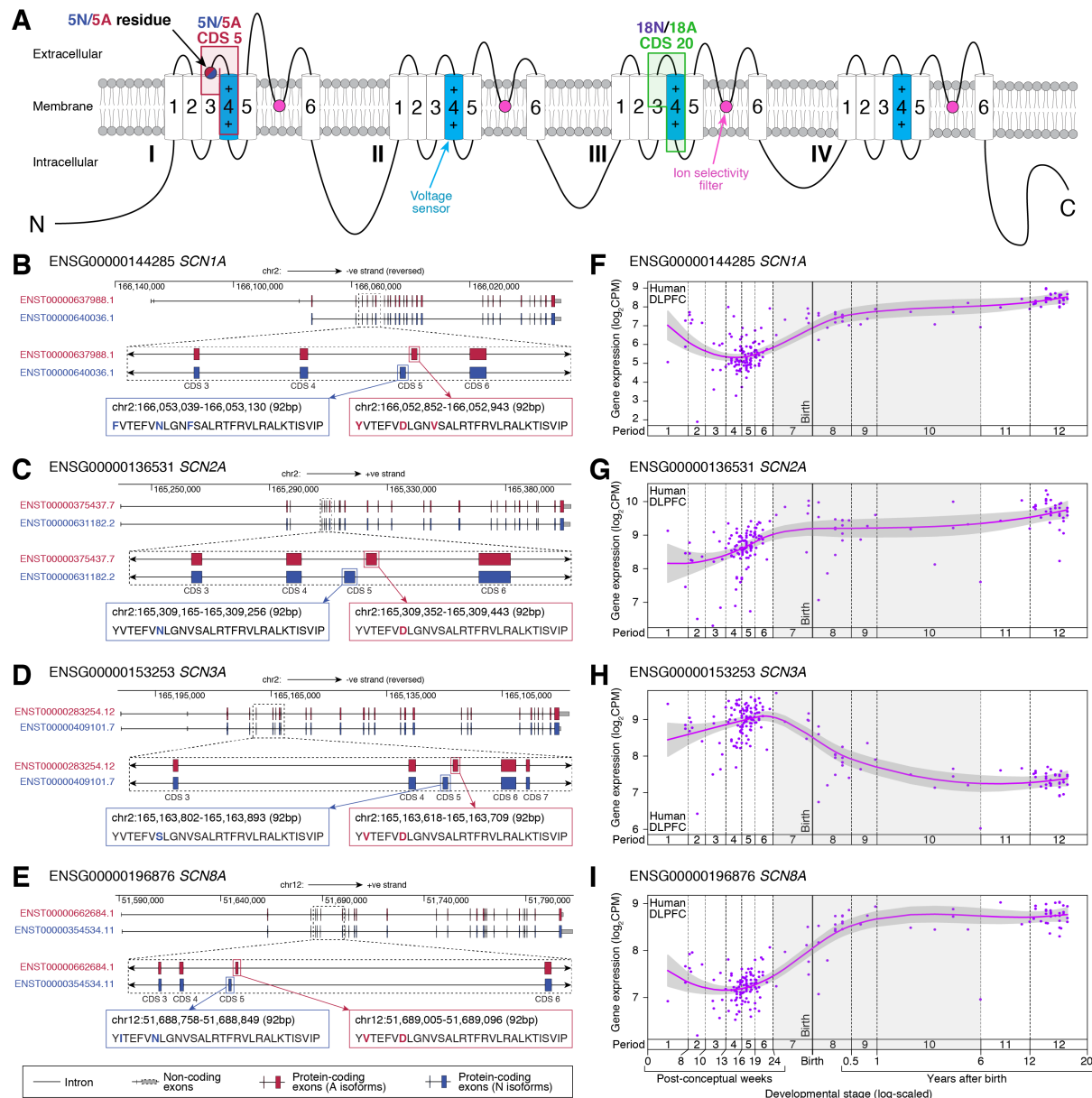
Figure 1. Splicing isoforms in voltage-gated sodium channels. A) Voltage-gated sodium channels are composed of four similar domains (I, II, III, IV), each of which includes six transmembrane segments with extracellular or intracellular linkers. The fourth transmembrane segment (S4) in each domain acts as a voltage sensor. Between the fifth and sixth transmembrane segment (S5, S6) is a pore loop that forms the ion selectivity filter. The fifth protein-coding exon (5N/5A, CDS 5) encodes a portion of the first domain, while the 20th protein-coding exon (18N/18A, CDS 20) encodes a similar portion of the third domain. **B)** Location, genomic coordinates (GRCh38/hg38), and amino acid sequence of the '5A' and '5N' exons four sodium channels. **C)** Patterns of whole-gene expression in the human dorsolateral prefrontal cortex (DLPFC) across prenatal and postnatal development from the BrainVar dataset [32]. CPM: counts per million. Genomic coordinates are based on GRCh38/hg38 using GENCODE v31 gene definitions.

3.2 Developmental trajectories of 5N and 5A expression in the human cortex

The majority of protein-coding exons follow the expression trajectory of their parent gene across development (Fig. S3), however all four sodium channels show dynamic changes in the utilization of 5N/5A (Fig. 2). This is especially marked for *SCN2A* and *SCN8A*, where 5N is

207 expressed at a higher level than 5A in the mid-fetal brain but this reverses soon after birth.
208 Plotting the 5N:5A ratio allows exon utilization to be assessed independent of changes in gene
209 expression (Fig. 2). All four genes show changes in the N:A ratio across development, with a
210 modest change for *SCN1A* (0.14 fetal to 0.02 childhood/adolescent; p=0.00003, two-sided
211 Wilcoxon test, Figure 2B) and dramatic changes for *SCN2A* (3.7 to 0.09; p=5×10^{-19}, Fig. 2D),
212 *SCN3A* (0.96 to 0.18; p=6×10^{-18}, Fig. 2F), and *SCN8A* (3.7 to 0.09; p=5×10^{-19}, Fig. 2H). As a
213 control, we applied this approach to assess the ratio of CDS 4 and CDS 6 across development.
214 We observed no developmental shift in the 4:6 ratio for *SCN1A*, *SCN2A*, and *SCN3A*, however
215 the exon 4:6 ratio is marginally higher than expected in the prenatal period for *SCN8A* (0.82 vs.
216 0.66; 9×10^{-10}, Fig. S2). This developmental variation in *SCN8A* is not observed for the
217 surrounding protein-coding exons and reflects a modest increase in CDS 4 expression in the
218 prenatal period, based on the expected expression given the exon length (Fig. S4).
219
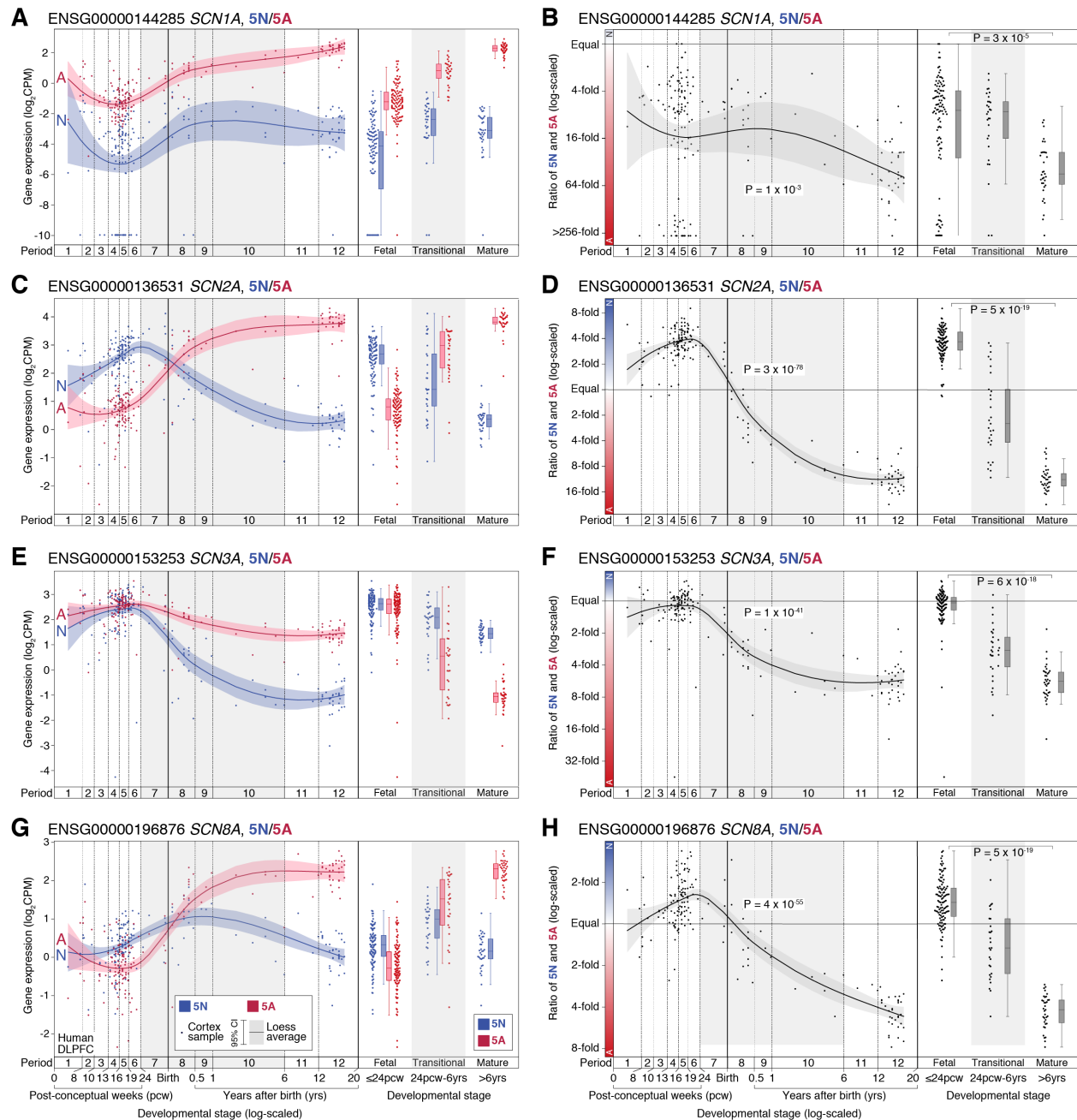
Figure 2. Expression of 5N and 5A in the human cortex across development. A) The expression of 5N (blue) and 5A (red) in *SCN1A* is shown for 176 BrainVar human cortex (DLPFC) samples across development (points). On the left, the colored line shows the Loess smoothed average and 95% confidence interval (shaded region). On the right, boxplots show the median and interquartile range for the same data, binned into fetal, transitional, and mature developmental stages. **B)** The ratio of 5N and 5A expression from panel 'A' is shown across development (left) and in three developmental stages (right). **C-H)** Panels A and B are repeated for the genes *SCN2A*, *SCN3A*, *SCN8A*. For comparison, the same plots for CDS four and six are shown in Figure S2. CPM: Counts per million; DLPFC: Dorsolateral prefrontal cortex. Statistical tests: B, D, F, H) Left panel, linear regression of $\log_2$(5N:5A ratio) and $\log_2$(post-conceptual days). Right panel, two-tailed Wilcoxon test of $\log_2$(5N:5A ratio) values between fetal and mature groups.

3.3 Intron splicing around 5N and 5A in the human cortex

233 To verify that mutually exclusive use of 5N and 5A underlies the observed exon expression
234 changes (Fig. 2), we considered RNA-seq reads that spanned exon-exon junctions to quantify
235 intron splicing. Clusters of differential intron splicing corresponding to 5N/5A usage were
236 identified by Leafcutter for *SCN2A*, *SCN3A*, and *SCN8A* (Fig. 3), but not *SCN1A*, likely due to the
237 consistently low expression of N isoforms (Fig. 2). The splicing patterns for *SCN2A*, *SCN3A*, and
238 *SCN8A* are consistent with the observed exon expression changes (Fig. 2, 3) and at least 99% of
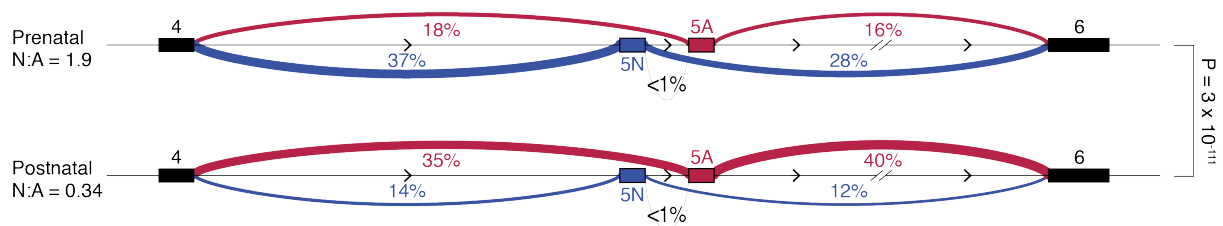239 reads are consistent with mutually exclusive 5N/A utilization.
240

A ENSG00000136531 *SCN2A*
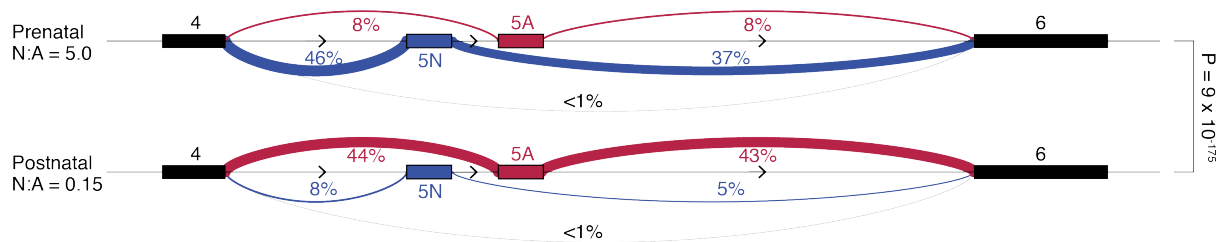
**B** ENSG00000153253 *SCN3A* (reversed)
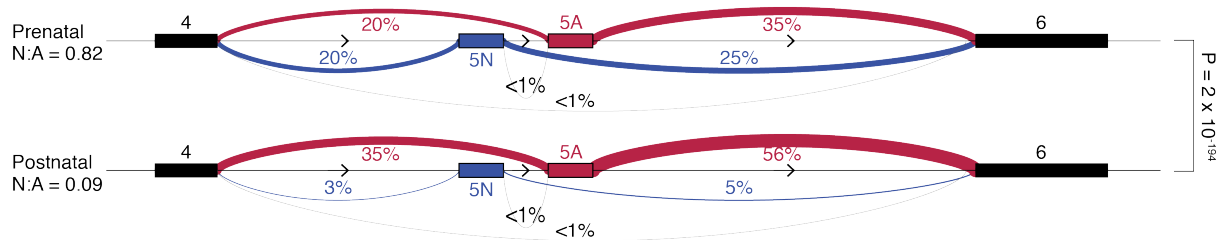
C ENSG00000196876 *SCN8A*

241
242
243 **Figure 3. Intron splicing of sodium channel genes in the developing human cortex. A)** Sashimi plot of splicing in the prenatal
244 (top, N=112 samples) and postnatal (bottom, N=60 samples) DLPFC for *SCN2A*. Linewidth is proportional to percentage of split
245 reads observed for each intron and this value is given as a percentage. Introns related to 5N inclusion are shown in blue, those
246 related to 5A inclusion are shown in red, and others are in grey. **B-C)** Equivalent plots for *SCN3A* (a negative strand gene with
247 the orientation reversed to facilitate comparison to the other two genes) and *SCN8A*. P-values compare the prenatal and
248 postnatal cluster using a Dirichlet-multinomial generalized linear model, as implemented in Leafcutter.[39]
249

3.4 Developmental trajectories of 5N and 5A expression in the mouse cortex

251 We repeated the analysis of sodium channel 5N/5A expression using bulk tissue RNA-seq data
252 from the mouse cortex across development (N=58; E15.5 to P75). Our data are consistent with
253 the N:A ratios described previously.[20] We observe more substantial differences at the extremes
254 of development: *SCN2A* (3.3 fetal to 0.06 mature; p=0.00003, two-sided Wilcoxon test, Fig. 4C),

255 *SCN3A* (2.4 to 0.14; p=0.00008, Fig. 4E), and *SCN8A* (1.8 to 0.22; p=0.00003, Fig. 4G). Mice lack a
256 functional 5N exon in *SCN1A*.

257
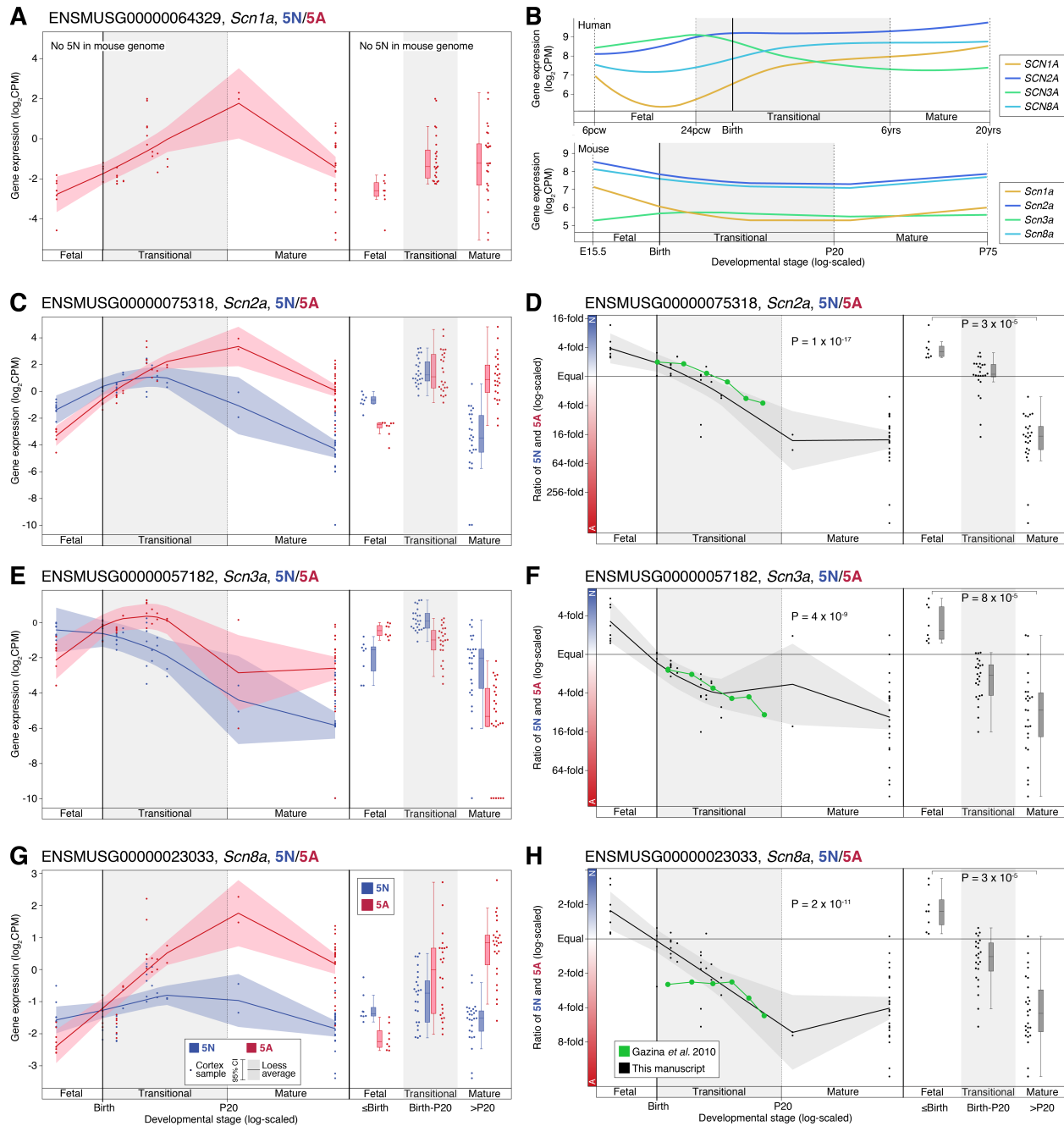
258
259 **Figure 4. Expression of 5N and 5A in the mouse cortex across development. A)** The expression of 5A (red) in *Scn1a* is shown
260 for 58 mouse cortex samples across development (points); no functional 5N equivalent is present in the mouse genome. On the
261 left, the colored line shows the Loess smoothed average and 95% confidence interval (shaded region). On the right, boxplots
262 show the median and interquartile range for the same data, binned into fetal, transitional, and mature developmental stages.
263 **B)** The Loess smoothed average expression of the four voltage-gated sodium channels in human cortex (top, Fig. 1) and mouse
264 cortex (bottom). **C)** Panel 'A' is repeated for *Scn2a* with the inclusion of 5N (blue). **D)** The ratio of 5N and 5A expression from
265 panel 'C' is shown across development (left) and in three developmental stages (right). Values reported previously in mouse
266 cortex are shown in the same scale in green for comparison [20]. **E-H)** Panels 'C' and 'D' are repeated for the genes *Scn3a*, *Scn8a*.

267 CPM: Counts per million. Statistical tests: D, F, H) Left panel, linear regression of $\log_2$(5N:5A ratio) and $\log_2$(post-conceptual
268 days). Right panel, two-tailed Wilcoxon test of $\log_2$(5N:5A ratio) values between fetal and mature groups.
269

270 **3.5 No evidence of common polymorphisms regulating 5N or 5A utilization**
271 A common polymorphism (rs3812718, GRCh38 chr2:166,053,034 C>T, IVS5N+5G>A) has
272 previously been associated with epilepsy, seizures, and response to anti-epileptics,[21,22,26,45,46]
273 though this variant did not reach genome-wide significance in a mega-analysis of epilepsy.[47]
274 Prior analyses of expression in the adult human temporal cortex showed evidence that the
275 homozygous variant allele (TT in DNA, AA in cDNA) was associated with reduced utilization of
276 5N.[21,48] We do not observe evidence for such a relationship in the prenatal or postnatal
277 prefrontal cortex (Fig. S5) and this polymorphism is not identified as a splicing quantitative trait
278 locus (QTL) in GTEx.[49] Furthermore, this variant is not predicted to alter splicing behavior using
279 the SpliceAI algorithm.[50] The TT genotype is associated with increased expression of *SCN1A* in
280 the adult human basal ganglia with (p=1x10^{-10}).[49]
281

282 **3.6 Developmental trajectories of 18N and 18A expression in *SCN8A***
283 We next considered the developmental timing of the transition between 18N and 18A in *SCN8A*
284 (Fig. 1A, 5A). Intron splicing shows a robust difference between prenatal and postnatal human
285 dorsolateral prefrontal cortex (P = 4 x 10^{-185}, Fig. 5B), with the prenatal period characterized by
286 high frequencies of transcripts excluding 18A, either including 18N or skipping both 18N and
287 18A, while in the postnatal cortex 18A is included in 93% of reads. Considering exon expression
288 (Fig. 5C, 5D), the expression of 18A increases markedly over development and this is distinct
289 from other protein-coding exons for *SCN8A* (Fig. S3). The 18N/18A transition begins around 13
290 post-conceptual weeks and continues till six months-of-age, with both timepoints being earlier
291 than the equivalents for 5N/5A in *SCN8A* and the other genes.
292
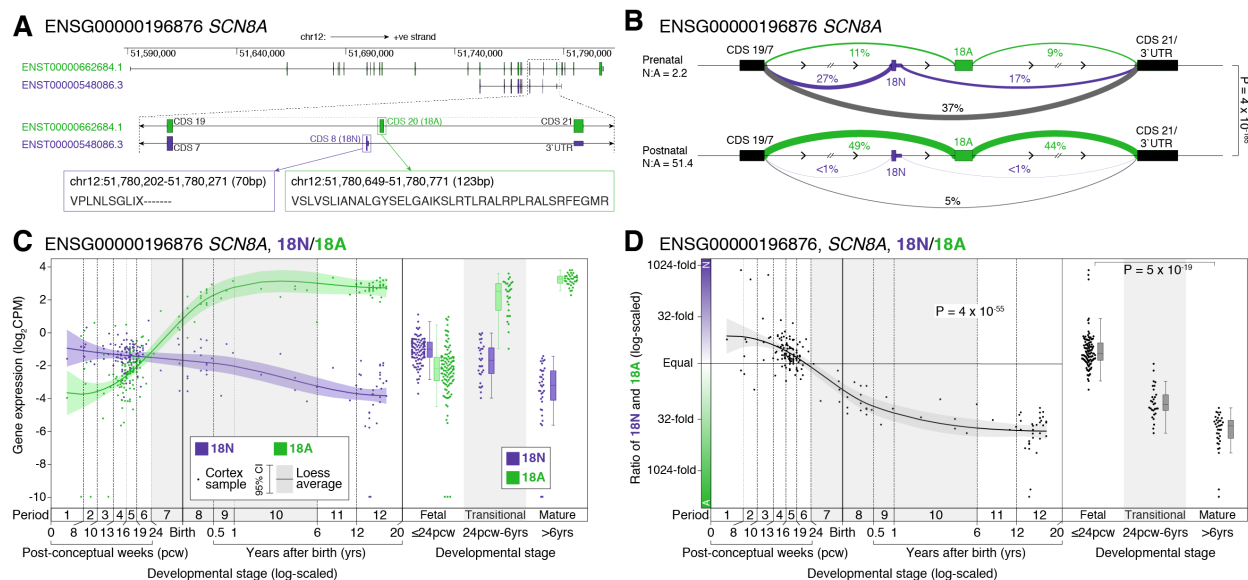
293
294 **Figure 5. Developmental trajectories of CDS 20 (18N/18A) in human cortex in *SCN8A*. A)** Location, genomic coordinates
295 (GRCh38/hg38), and amino acid sequence of the 18N and 18A exons in *SCN8A*. **B)** Sashimi plot of intron splicing in the prenatal
296 (top, N=112 samples) and postnatal (bottom, N=60 samples) dorsolateral prefrontal cortex. Linewidth is proportional to
297 percentage of reads observed for each exon-exon junction and this value is also shown as a percentage. Introns related to 18N
298 exon inclusion are shown in purple, those related to 18A exon inclusion are shown in green, and others are in grey. **C)**

299   Expression of the 18N (purple) and 18A (green) for 176 BrainVar human dorsolateral prefrontal cortex samples across
300   development (points). On the left, the colored line shows the Loess smoothed average with the shaded area showing the 95%
301   confidence interval. On the right, boxplots show the median and interquartile range for the same data, binned into fetal,
302   transitional, and mature developmental stages. **D)** The 18N:18A ratio is shown for each sample from panel 'C' across
303   development (left) and binned into three groups (right). CPM: Counts per million; Statistical analyses: B) Dirichlet-multinomial
304   generalized linear model, as implemented in Leafcutter,[39] D) Left panel, linear regression of $\log_2$(18N:18A ratio) and $\log_2$(post-
305   conceptual days). Right panel, two-tailed Wilcoxon test of $\log_2$(18N:18A ratio) values between fetal and mature groups.
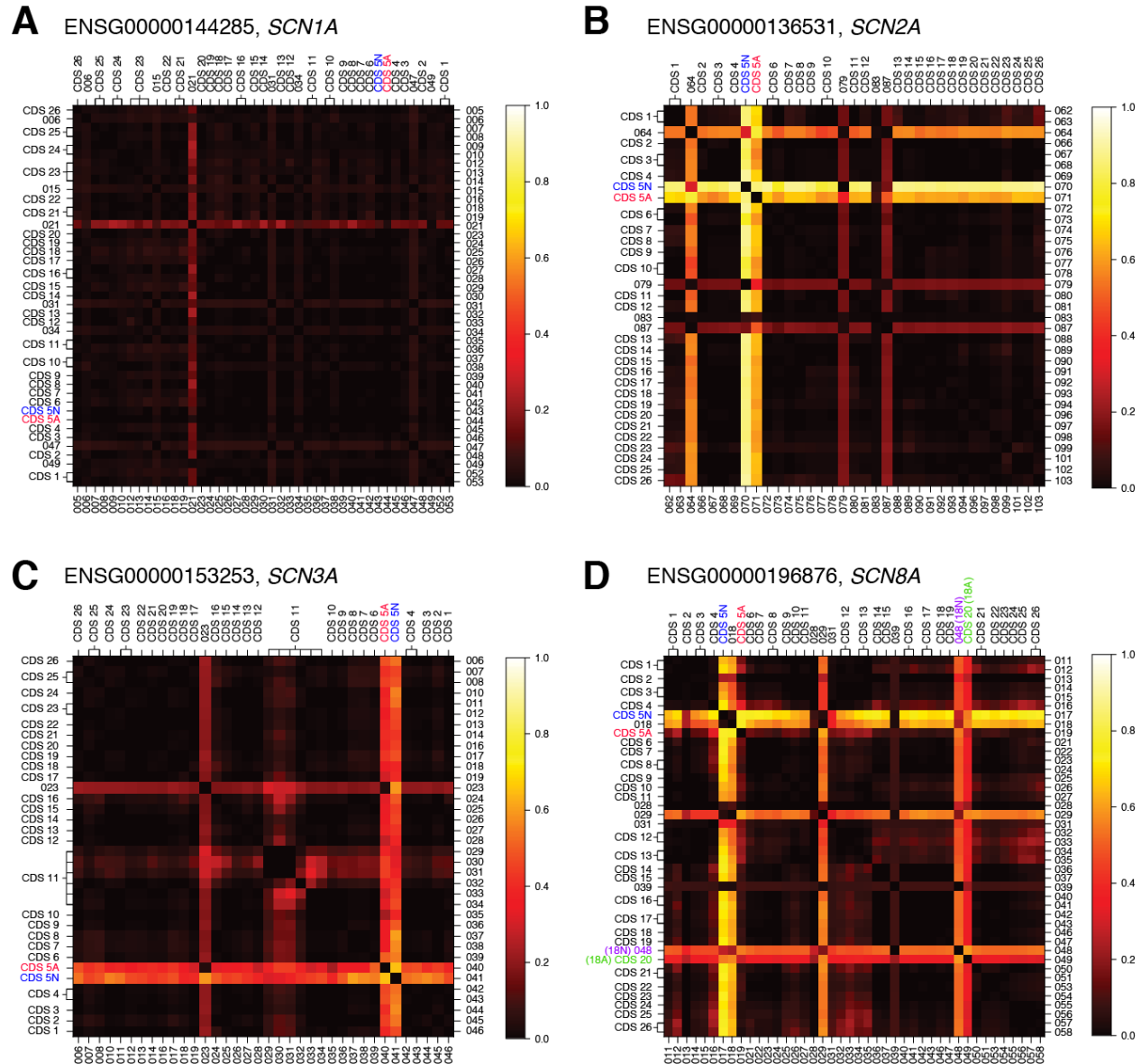
306

307 **3.7 Other annotated protein-coding exons with distinct developmental trajectories**

308 To assess whether other protein-coding exons undergo distinct developmental transitions (Fig.

309 S3), we calculated the ratios of all pairs of protein-coding exons within each for the four sodium

310 channel genes and assessed whether the ratio was correlated with development stage using

311 linear regression. This is the same calculation used to quantify the 5N/5A and 18N/18A

312 transitions (Fig. 2, 5D) and distinguishes exons with expression profiles that differ from the rest

313 of the gene (e.g. 5A in *SCN2A*), rather than simply being expressed at reduced levels, suggesting

314 alternative regulatory processes (Fig. S3). Visualizing the R^2 values of these correlations

315 provides simple method to identify the such distinct trajectories (Fig. 6). Aside from 5N/5A and,

316 in *SCN8A*, 18N/18A, no protein-coding exons common to most isoforms (consistent CDS in Fig.

317 S3) show differential expression, but a few weakly expressed protein-coding exons specific to a

318 small number of isoforms (variable CDS in Fig. S3) do vary across development (Fig. 6).

319

320 GENCODE defines seven variable CDS exons for *SCN1A* (DEXSeq divisions: 006, 015, 021, 031,

321 034, 047, 049; Table S2, Fig. 6A). Of these, only 021 shows a distinct developmental trajectory

322 (Fig. 6A), with reduced postnatal expression relative to other *SCN1A* exons (Fig. S3). This result

323 is verified by the intron splicing data (p = 6 x 10^{-91}, Leafcutter).

324

A ENSG00000144285, *SCN1A*

B ENSG00000136531, *SCN2A*

C ENSG00000153253, *SCN3A*

D ENSG00000196876, *SCN8A*

325
326
327 **Figure 6. Identification of protein-coding exons with complex developmental trajectories. A)** The correlation between the
328 ratio of CPM expression between pairs of exons (log-scaled) and developmental stage (post conceptual days, log-scaled) for
329 *SCN1A* was assessed with a linear model (e.g. Fig. 2B). The R^2 value of each exon pair is show as a heat map with 'hot' colors
330 representing exon-pairs with high R^2 values for which variation in the ratio is correlated with developmental age, i.e. pairs of
331 exons that show substantially different expression across development. Exon numbers from DEXSeq (Table S2) are shown on
332 the bottom and right and equivalent CDS number on the top and left (see Table S2). **B-D)** The analysis is repeated for *SCN2A*,
333 *SCN3A*, and *SCN8A*.
334
335 In *SCN2A*, the 5N/5A trajectories stand out clearly (Fig. 6B). There are four variable CDS exons
336 (DEXSeq divisions: 064, 079, 083, 087; Table S2, Fig. 6B), three of which have distinct
337 developmental trajectories (Fig. 6B, S3): 064 (Fig. S3, P = 2 x 10^{-12}, Leafcutter), 079 (Fig. S3, P = 7
338 x 10^{-33}, Leafcutter), 087 (Fig. S3, P = 2 x 10^{-20}, Leafcutter). The single variable CDS exon in
339 *SCN3A*, 023 (Table S2, Fig. 6C), varies across development (Fig. S3, P = 3 x 10^{-80}, Leafcutter).
340 Finally, aside from 18N, there are five variable CDS exons in *SCN8A* (DEXSeq divisions: 018, 028,

341   029, 031, 039; Table S2, Fig. 6D) of which 018 and 029 vary across development (Fig. 6D), but
342   neither of these are validated by Leafcutter.

343

344   **Discussion**
345   Using transcriptomic data from 176 human dorsolateral prefrontal cortex samples, we
346   characterized the developmental patterns for all protein-coding exons in *SCN1A*, *SCN2A*,
347   *SCN3A*, and *SCN8A* (Fig. 6, S3). We observed a coordinated decrease in the 5N:5A ratio between
348   24 post-conceptual weeks (2nd trimester) and six years-of-age that is synchronized with
349   widespread transcriptomic changes in the brain during the late-fetal transition.[32,44] This is
350   preceded by a similar decrease in the 18N:18A ratio in *SCN8A* from 13 post-conceptual weeks
351   to 6 months-of-age, which is regulated by *RBFOX1*. By analyzing a wider developmental window
352   than prior analyses[20,21,48] we observed more dynamic changes and larger disparities in exon
353   expression.

354

355   Recent advances have shown that differential splicing patterns can be effective therapeutic
356   targets in humans, for example through intrathecal antisense oligonucleotides.[51,52] Since the
357   electrophysiological consequences of some epileptic encephalopathy associated variants differ
358   between 5N and 5A, manipulating this ratio may offer therapeutic benefit in individuals
359   carrying these variants. We consider three therapeutic scenarios.

360

361   First, for individuals with disorder-associated genetic variants within the 30 amino acids
362   encoded by the 5th exon, expressing the other copy of the 5th exon could skip the variant.
363   Theoretically, this approach could benefit individuals with both loss-of-function (protein-
364   truncating variants, missense, splice site) and gain-of-function (missense) variants in the 5th
365   exon. At present, ten such cases have been identified, all with epileptic encephalopathy
366   variants identified in the 5A exon of *SCN2A* and *SCN8A*.[53,54] Since the total transcript level
367   would be unchanged, this strategy may provide a wider therapeutic window than simply
368   decreasing expression levels. The success of the therapy would depend upon the proportion of
369   transcripts expressing the alternate 5th exon and the ability of this exon to functionally replace
370   the original 5th exon.

371

372   Second, splice isoforms can also have an effect on the biophysical effects of variants outside the
373   5th exon. For example, three recently characterized epileptic encephalopathy associated
374   variants in *SCN2A*—T236S, E999K, and S1336Y—all exhibit more pronounced alterations in their
375   electrophysiological properties in 5N Na$_v$1.2 isoforms.[23] Two other epileptic encephalopathy-
376   associated variants—M252V and L1563V—exhibit biophysical changes only when expressed on
377   5N isoform.[14,55] For individuals with these mutations, tilting expression towards the 5A isoform
378   could provide some symptomatic improvement, especially during infancy. Including both the
379   5N and 5A isoforms in functional characterization of variant impact may identify many more
380   such variants.[23]

381

382   Finally, modifying splicing might aid seizure control in older children and adults. At this age, the
383   5A isoform is predominantly utilized in both *SCN2A* or *SCN8A*, which are mainly expressed in

384 glutamatergic neurons.[11] Reverting expression to the fetal/neonatal state by encouraging 5N
385 utilization could reduce the excitability of cortical glutamatergic neurons, potentially
386 limiting seizures. Since this would require repeated intrathecal administration, it would likely be
387 limited to the most severe cases of epilepsy. Furthermore, it remains to be seen whether this
388 approach could offer therapeutic benefits above and beyond existing antiepileptic drugs.
389
390 Our analysis was limited by the use of short-read transcriptomic data, leading us to focus on
391 quantifying exon-level expression (Fig. 2) and splice junction usage (Fig. 3), rather than relying
392 on estimates of isoform utilization (Fig. S1). We also elected to focus on protein-coding
393 transcripts and exons defined by GENCODE (v31) rather than attempting *de novo* transcriptome
394 assembly. Emerging long-read transcriptomic technology may substantially expand estimates of
395 isoform and exon diversity but these technologies have not been applied to the developing
396 human brain at scale.[56,57] We also note that transcriptomic data is only partially predictive of
397 protein levels and other factors, including channel transport and degradation, may influence
398 the impact of isoforms on neuronal function. Comparing the human and mouse cortex data
399 (Figs. 2, 4), it is possible that more substantial differences in gene and exon expression may be
400 observed at earlier embryonic times in the mouse or with larger sample sizes. In addition, the
401 use of bulk-tissue transcriptomic data limits our ability to assess how individual cell-types or
402 cell-states contribute to the observed isoform trajectories. Technological and methodological
403 advances may provide insights at cell-level resolution in the future.[58]
404
405 **Conclusion**
406 Dramatic differences in exon usage of *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* observed in rodent
407 brains also occur in the human developing cortex, beginning in mid-fetal development and
408 continuing through childhood. These changes in splicing affect the biophysical properties of the
409 encoded channels and are likely to contribute to differences in phenotype observed between
410 individuals with different variants and across development.
411

421 **Author Contributions**
422 Experimental design, S.J.S.; Data generation, L.L., S.F.D., S.P., F.O.G., A.S., J.Y.A., and J.L.R.R.;
423 Data processing, L.L., M.C.G., B.K.S., and D.M.W.; Data analysis, L.L., D.M.W., and S.J.S.;
424 Statistical analysis, S.J.S.; Manuscript preparation, L.L., K.J.B., and S.J.S.
425

426 **Declaration of interests**

427   J.L.R.R. is cofounder, stockholder, and currently on the scientific board of *Neurona*, a company
428   studying the potential therapeutic use of interneuron transplantation.

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