Distinct neural progenitor pools in the ventral telencephalon generate diversity in striatal spiny projection neurons

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Abstract

Heterogeneous populations of neural progenitors in the embryonic lateral ganglionic eminence (LGE) generate all GABAergic spiny projection neurons (SPNs) found in the striatum. Here we investigate how this diversity in neural progenitors relates to diversity of adult striatal neurons and circuits. Using a combination of *in utero* electroporation to fluorescently pulse-label striatal neural progenitors in the LGE, brain slice electrophysiology, electrical and optogenetic circuit mapping and immunohistochemistry, we characterise a population of neural progenitors enriched for apical intermediate progenitors (aIPs) and a distinct population of other progenitors (OPs) and their neural offspring. We find that neural progenitor origin has subtle but significant effects on the properties of striatal SPNs. Although aIP and OP progenitors can both generate D1-expressing direct pathway as well as D2-expressing indirect pathway SPNs found intermingled in the striatum, the aIP derived SPNs are found in more medial aspects of the striatum, exhibit more complex dendritic arbors with higher spine density and differentially sample cortical input. Moreover, optogenetic circuit mapping of the aIP derived neurons show that they further integrate within striatal circuits and innervate both local D1 and D2 SPNs. These results show that it is possible to fluorescently label distinct neural progenitor pools within the LGE and provide the first evidence that neural progenitor heterogeneity can contribute to the diversity of striatal SPNs.

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Introduction

A fundamental question in neuroscience is how neuronal cell types and neural circuits arise and what critically guides their development. Recent studies in the dorsal telencephalon or pallium have highlighted important and distinct roles for embryonic neural progenitors, at both the level of single neural progenitors (Yu *et al.*, 2009; Yu *et al.*, 2012; Cadwell *et al.*, 2019) and distinct pools of neural progenitors (Tyler *et al.*, 2015; Ellender *et al.*, 2018), in shaping neuronal identity and synaptic connectivity. However, much less is known about how embryonic neural progenitors in the ventral telencephalon or subpallium contribute to the cellular diversity and neural circuitry found within ventral brain structures. Neural progenitors in the ventral portions of the embryonic brain can be found in the ganglionic eminences – transitory structures that generate most interneurons of the brain and the spiny projection neurons (SPNs) of the striatum (Wonders & Anderson, 2006; Wamsley & Fishell, 2017). In this study we focused on the neural progenitors found within the lateral ganglionic eminence (LGE) (Graybiel & Ragsdale, 1978; Olsson *et al.*, 1998; Mason *et al.*, 2005; Pilz *et al.*, 2013; Kelly *et al.*, 2018), which give rise to the striatal SPNs, and include radial glial cells, basal radial glial cells, subapical progenitors, basal progenitors and short neural precursors, amongst others (Olsson *et al.*, 1998; Stenman *et al.*, 2003; Pilz *et al.*, 2013). Many of these are not unique to the LGE and have previously been characterised in detail in the proliferative zones of the cortex (Noctor *et al.*, 2001; Noctor *et al.*, 2004; Gal *et al.*, 2006; Kowalczyk *et al.*, 2009; Stancik *et al.*, 2010; Shitamukai *et al.*, 2011; Wang *et al.*, 2011; Franco & Muller, 2013; Taverna *et al.*, 2014). However, the relative abundance as well as key proliferative behaviours of many of these progenitors differ between cortex and the LGE (Pilz *et al.*, 2013). Furthermore, it is unknown to what extent these different pools of neural progenitors contribute to the diversity of striatal projection neurons.

To investigate the relationship between neural progenitor pool and striatal neuron diversity, we used *in utero* electroporation to fluorescently pulse-label two pools of actively dividing neural progenitors in the LGE distinguished by their differential expression of the tubulin alpha1 (Tα1) promoter (Gal *et al.*, 2006; Stancik *et al.*, 2010). We find that Tα1-expressing neural progenitors exhibit many of the characteristics of the previously described short neural precursors and subapical progenitors found within the LGE (Pilz *et al.*, 2013), including division in the

ventricular zone, lack of basal processes during division and relatively fast cell-cycle kinetics. In contrast, the non-Tα1-expressing neural progenitors tend to retain their basal processes during division and exhibit slower cell-cycle kinetics. Overall, our observations suggest that Tα1-expressing neural progenitors consist of intermediate progenitors found in apical regions of the LGE such as short neural precursors and subapical progenitors (Pilz *et al.*, 2013) and we therefore refer to them collectively as apical intermediate progenitors (aIPs) and the non-Tα1-expressing neural progenitors consist of all other neural progenitors that are actively dividing at the same time and we collectively refer to them as other progenitors (OPs). We find that aIP and OP neural progenitors mainly generate striatal neurons, with all the hallmarks of GABAergic spiny projection neurons (SPNs) including expression of neurochemical markers such as CTIP2, but also generate a small number of neurons found within the olfactory bulb. Using the neurochemical marker PPE we find that both aIP and OP neural progenitors generate D1-expressing direct pathway as well as D2-expressing indirect pathway SPNs. Stereological investigation of progenitor derived striatal neurons shows that aIP derived neurons are on average found in more medial aspects of the striatum. Whole-cell patch-clamp recordings of aIP and OP derived neurons in acute striatal slices reveals that their electrical properties are similar and consistent with those of SPNs. Indeed, subsequent anatomical reconstruction of recorded neurons confirms that they exhibit all the hallmarks of SPNs, including radially oriented dendrites with large numbers of spines. Interestingly, we find differences with aIP derived SPNs exhibiting a greater local dendritic complexity as well as a higher density of spines. Lastly, using both electrical and optogenetic circuit mapping we find that both aIP and OP derived SPNs integrate within striatal circuits. Firstly, both receive excitatory glutamatergic input from cortex with aIP derived SPNs exhibiting a significantly prolonged response to cortical activation. Secondly, aIP derived SPNs form local GABAergic inhibitory synaptic connections with neighbouring D1 and D2 SPNs.

In conclusion, this study shows it is possible to label distinct pools of neural progenitors in the LGE and suggests that neural progenitor heterogeneity contributes to striatal diversity in that neural progenitor origin has subtle but significant effects on the spatial distribution of striatal neurons, their morphology as well as their sampling of excitatory inputs.

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Results

The LGE of the ventral telencephalon contains tubulin alpha1 (Tα1) expressing neural progenitors

To investigate the relationship between the diverse pools of neural progenitors in the LGE and their contribution to striatal neuronal identity and connectivity we performed *in utero* electroporation (IUE) to fluorescently label actively dividing progenitors in the ventricular zone (Stancik *et al.*, 2010). Two DNA constructs were electroporated into the LGE: a Tα1-cre construct in which cre recombinase is under the control of a portion of the Tα1 promoter (Stancik *et al.*, 2010), and a CβA-FLEx reporter construct that incorporates a flexible excision (FLEx) cassette where cre recombination permanently switches expression from TdTomato fluorescent protein to enhanced green fluorescent protein (GFP) (Franco *et al.*, 2012).

We find that 24 hours after IUE the LGE contains both GFP and TdTomato expressing cells, consisting of Tα1-expressing ($Tα1^+$) and non-Tα1-expressing ($Tα1^-$) neural progenitors and young migrating neurons (**Figure 1A**). We find that the time of IUE influenced the relative proportion of GFP and TdTomato expressing cells seen 24 hours later, with IUE at E15.5 resulting in a greater number of $Tα1^+/GFP^+$ cells (E12.5: 4.8 ± 4.1% and E15.5: 34.5 ± 3.0%, Mann-Whitney test, p=0.002, n = 7 and 17 embryonic brains, **Figure 1B**), suggesting that, similar to their cortical counterparts (Stancik *et al.*, 2010; Ellender *et al.*, 2018) Tα1-expressing progenitors form a considerable population of actively proliferating cells during later periods of neurogenesis.

Using IUE at E15.5 we next investigated the spatial distribution of $Tα1^+/GFP^+$ and $Tα1^-/TdTomato^+$ cells within the LGE to further characterize these cells and gain insight into their proliferative behaviour. Firstly, we assessed the location of all $Tα1^+/GFP^+$ and $Tα1^-/TdTomato^+$ cells and dividing progenitors relative to the ventricular wall 24 hours after IUE (**Figure 1C** and **D**). $Tα1^+/GFP^+$ and $Tα1^-/TdTomato^+$ cells can be found interspersed in the proliferative zone of the LGE with the $Tα1^+/GFP^+$ labeled cells on average significantly further away from the ventricular wall than the $Tα1^-/TdTomato^+$ labeled cells ($Tα1^+/GFP^+$: 66.24 ± 3.57 μm and $Tα1^-/TdTomato^+$: 56.45 ± 2.90 μm, paired t-test, p=0.0055, n = 30, **Figure 1C**). However, when we limited analysis to only actively dividing progenitor cells, labeled

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172 with the mitotic marker phospho-histone H3 (pH3), no difference was seen in their
173 distance from the ventricle (Tα1^{+}/GFP^{+}: 52.28 ± 6.21 μm and Tα1^{-}/TdTomato^{+}: 48.78
174 ± 3.99 μm, p=0.586, paired t-test, n = 16 brains, **Figure 1D**). These results are
175 consistent with both Tα1^{+}/GFP^{+} and Tα1^{-}/TdTomato^{+} progenitors dividing mainly
176 within the ventricular zone of the LGE (Pilz *et al.*, 2013). Furthermore, our results
177 suggest that 24 hours after IUE Tα1^{+}/GFP^{+} cells, likely consisting of young migrating
178 neurons and non-dividing neural progenitors, inhabit increasingly subapical positions
179 within the LGE. Whereas in the cortex young migrating neurons mostly exhibit radial
180 migration (Noctor *et al.*, 2001) newly born neurons in the striatum undergo tangential,
181 radial and other forms of migration (Halliday & Cepko, 1992; Tan & Breen, 1993;
182 Reid & Walsh, 2002; Tinterri *et al.*, 2018). Indeed, when we look at the average
183 dispersion of all labeled cells in a subset of brain slices along two axis perpendicular
184 to the ventricular wall we find a positive linear correlation between the amount of
185 horizontal and vertical spread of labeled cells (R=0.56, p=0.00004, n=26 slices) which
186 is similar for both Tα1^{+}/GFP^{+} and Tα1^{-}/TdTomato^{+} cells (p>0.05) and is not seen
187 when we limit the analysis to only actively dividing neural progenitors (R=0.24,
188 p=0.18, n=20 slices, **Figure 1E**). Lastly, the LGE has also been shown to consist of
189 several domains depending on differential expression of certain transcription factors
190 (Stenman *et al.*, 2003; Flames *et al.*, 2007; Xu *et al.*, 2018). When we split the LGE in
191 4 different domains we find that both Tα1^{+}/GFP^{+} and Tα1^{-}/TdTomato^{+} cells can be
192 found in all progenitor domains of the LGE (**Supplemental Figure 1**).
193 We next investigated the morphology of actively dividing neural progenitors
194 in the LGE. The distinguishing features of short neural precursors in the dorsal and
195 ventral telencephalon are their division at the ventricular wall and their short
196 morphology during division lacking a prominent basal process contacting pial
197 surfaces (Gal *et al.*, 2006; Stancik *et al.*, 2010; Pilz *et al.*, 2013; Ellender *et al.*, 2018).
198 Indeed, when we look at the morphology of labeled neural progenitors we find that
199 many actively dividing Tα1^{+}/GFP^{+} progenitors exhibit a short rounded morphology
200 during division and could be found at the ventricular wall (**Figure 1F**). However,
201 some Tα1^{+}/GFP^{+} progenitors divide slightly away from the ventricular wall
202 resembling subapical progenitors (Pilz *et al.*, 2013) (**Figure 1F**). In contrast, many
203 actively dividing Tα1^{-}/TdTomato^{+} progenitors retained their basal process during
204 division and could be found either at or away from the ventricular wall (**Figure 1G**).
205 This was confirmed quantitatively by assessment of the number of cells in the VZ that

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206 retained a basal process. Overall, we find that Tα1^{+}/GFP^{+} cells often lacked a basal
207 process in contrast to Tα1^{-}/TdTomato^{+} cells that retained theirs (Tα1^{+}/GFP^{+}: 20.6 ±
208 4.7% and Tα1^{-}/TdTomato^{+}: 35.9 ± 7.8%, p=0.03, Wilcoxon signed rank test, n = 15
209 brains, **Figure 1H**).

210 A further distinguishing characteristic of both short neural precursors and
211 subapical progenitors found in the LGE is their relatively fast cell cycle kinetics (Pilz
212 *et al.*, 2013). To investigate whether the Tα1^{+}/GFP^{+} progenitor population exhibits
213 fast cell cycle durations we performed IUE at E15.5 and subsequently fixed tissue at
214 varying delays ranging from 12 to 26 hours and labeled dividing neural progenitors
215 with the mitotic marker pH3 (Stancik *et al.*, 2010). Consistent with the fast cell cycle
216 kinetics described for the short neural precursors and subapical progenitors in the
217 LGE we find that the Tα1^{+}/GFP^{+} progenitors return to G1/S phase faster that the Tα1^{-}
218 /TdTomato^{+} progenitors. Indeed, after a 16 hour delay Tα1^{+}/GFP^{+} progenitors are the
219 dominant population of pH3 expressing cells (Tα1^{+}/GFP^{+}: 19.85 ± 7.15% and Tα1^{-}
220 /TdTomato^{+}: 5.18 ± 1.72%, t-test, p = 0.025, n = 6 brains) whereas after a 20 hour
221 delay it is the Tα1^{-}/TdTomato^{+} progenitors which form the dominant population of
222 pH3 expressing cells (Tα1^{+}/GFP^{+}: 4.98 ± 2.11% and Tα1^{-}/TdTomato^{+}: 15.81 ±
223 3.63%, t-test, p = 0.036, n = 8 brains, **Figure 1I**).

224 In conclusion, our results suggest that the LGE in the ventral telencephalon
225 contains a population of Tα1-expressing neural progenitors that share many
226 characteristics with short neural precursors and subapical progenitors previously
227 described (Pilz *et al.*, 2013), including a ventricular location of division, a short round
228 morphology during division and fast cell-cycle kinetics. In contrast we are also able to
229 label a population of neural progenitor that does not express Tα1 that share many
230 characteristics with radial glial cells (Pilz *et al.*, 2013) and which tend to retain its
231 basal process and exhibits slower cell cycle kinetics.
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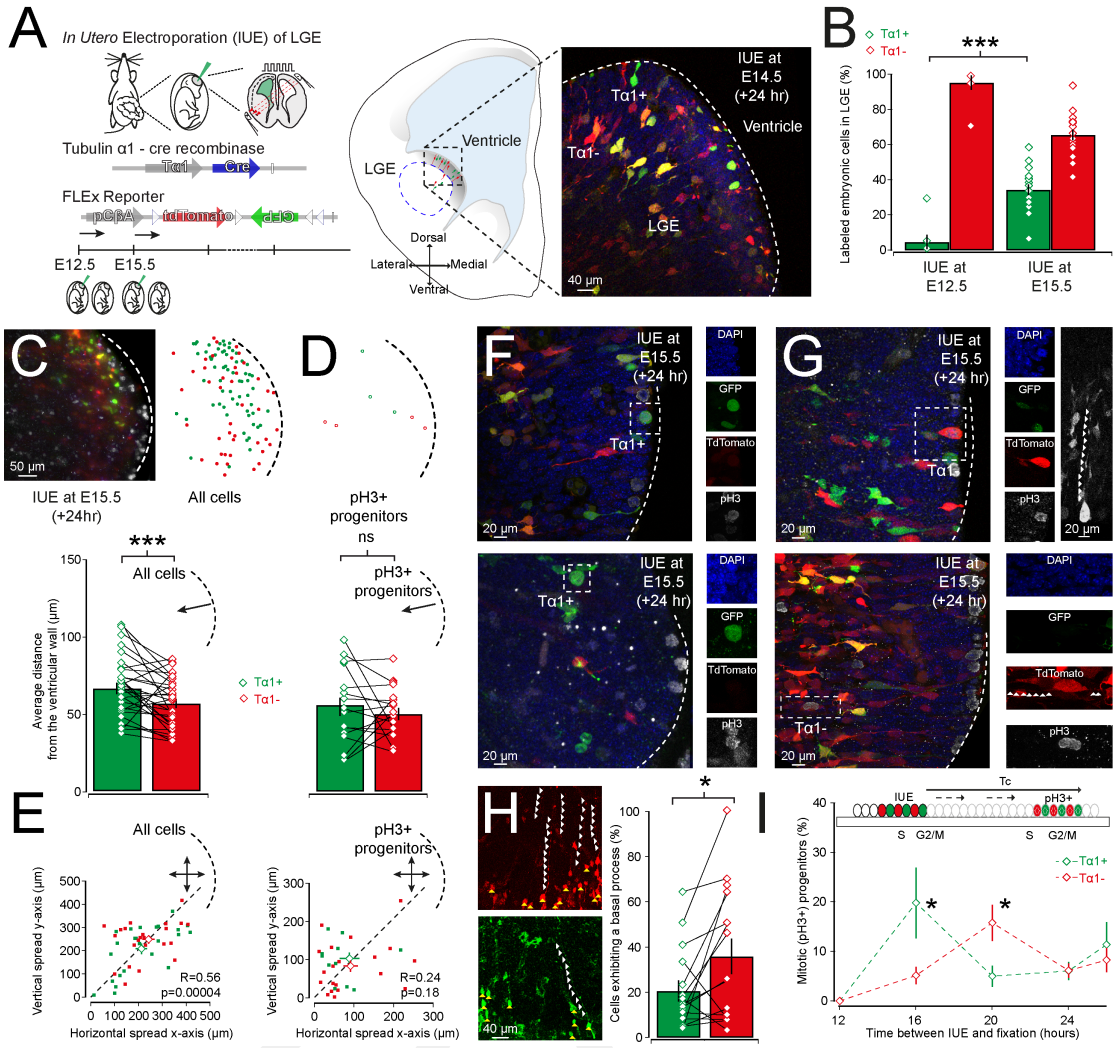

Figure 1: The LGE contains both Tα1-expressing and non-Tα1-expressing neural progenitors. (**A**) *In utero* electroporation (IUE) of Tα1-cre and FLEx reporter plasmids was performed at embryonic day (E)12.5 and E15.5 to label Tα1-positive (Tα1$^+$) and Tα1-negative (Tα1$^-$) neural progenitors lining the ventricular wall of the LGE with respectively the fluorescent markers GFP or TdTomato (left). 24 hours after IUE fluorescently labeled Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ neural progenitors and young neurons can be found in the LGE (right). Yellow cells were were assumed to have undergone recombination relatively recently and counted as GFP$^+$. (**B**) Quantification of Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ cells reveals that the Tα1$^+$/GFP$^+$ cells form a substantial population during late stages of embryogenesis. (**C**) The distance from the ventricle of Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ cells 24 hours after IUE differs in that Tα1$^+$/GFP$^+$ cells are located significantly further away from the ventricular wall. (**D**) A difference in distance from the ventricle is not seen for actively dividing Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ cells. (**E**) Horizontal and vertical migration of all labeled cells exhibits a linear correlation (left) whereas this is not the case for actively dividing progenitors (right). (**F**) Actively dividing Tα1$^+$/GFP$^+$ progenitors exhibiting a short morphology and lacking a basal endfoot can be found

253 dividing at the ventricular wall (top) and at a small distance from ventricle (bottom),
254 corresponding to respectively a short neural precursor and subapical progenitor. (**G**)
255 In contrast, dividing Tα1$^-$/TdTomato$^+$ cells predominantly retain a basal process
256 during division and can be found dividing at both the ventricular wall (top) and at a
257 distance from the ventricle (bottom) corresponding to respectively a radial glial cell
258 and bipolar radial glial cell. (**H**) Quantification of cells in the proliferative zones of
259 the LGE containing a basal process. Note that significantly more Tα1$^-$/TdTomato$^+$
260 cells retain a basal process. (**I**) Estimation of cell cycle duration using embryonic
261 tissue fixed at variable delays after IUE and labeling for the mitotic marker phospho-
262 histone H3 (pH3). A rapid increase in the number of neural progenitors that were
263 positive of the marker pH3 was taken as a return to the mitotic ('M') phase for that
264 population of neural progenitors and a proxy for the cell cycle duration ('Tc').
265
266
267 ***Both progenitor pools can generate striatal D1 and D2 spiny projection neurons***
268 Our *in utero* electroporation labeling experiments suggest that Tα1 expression can
269 distinguish between two pools of neural progenitor in the LGE. The Tα1-expressing
270 neural progenitors exhibit many of the characteristics of described short neural
271 precursors and subapical progenitors whereas, in contrast, the non-Tα1-expressing
272 neural progenitors tend to share many characteristics with the described radial glial
273 cells within the LGE (Pilz *et al.*, 2013). Overall, our observations would suggest the
274 pool of Tα1-expressing neural progenitors is enriched for apically dividing
275 intermediate progenitors and therefore we will refer to them collectively as apical
276 intermediate progenitors (aIPs) and the neurons they generate as aIP derived and the
277 non-Tα1-expressing neural progenitors consist of all other neural progenitors that are
278 actively dividing at the same time and we will refer to them collectively as other
279 progenitors (OPs) and the neurons they generate as OP derived.
280 When we let the electroporated embryos develop postnatally we observe many
281 GFP and TdTomato expressing neurons spread throughout the striatum (**Figure 2B**).
282 The GFP expressing neurons are generated from the population of aIP neural
283 progenitors and are referred to as aIP derived and the TdTomato expressing neurons
284 from the population of OP neural progenitors and are referred to as OP derived
285 (**Figure 2B**). At higher magnifications both pools of labeled neurons exhibit the
286 morphology of striatal spiny projection neurons (SPNs) including dendrites densely
287 covered with spines (**Figure 2B**). The relative proportion of aIP and OP derived
288 neurons seen postnatally is very similar to the ratio of labeled embryonic neural
289 progenitors found 24 hours after IUE at E15.5, with a significantly larger proportion

DRAFT

290 of OP derived neurons (aIP derived: 36.7 ± 5.5% and OP derived: 63.3 ± 5.5%,
291 p=0.0017, paired t-test, n = 16 brains, **Figure 2C**). We next investigated the spatial
292 distribution of aIP and OP derived neurons in rostral, central and caudal sections of
293 the striatum in a total of 15 electroporated brains (**Supplemental Figure 2**). In
294 general, across all sections we find that the normalised position (see **Methods**) of all
295 labeled SPNs was biased to the medial aspects (Wilcoxon Sign Rank, p = 0.000005, n
296 = 15 mice / 23 sections, **Figure 2D**), but not to either dorsal or ventral aspects of the
297 striatum (Wilcoxon Sign Rank, p > 0.05). We find that within individual sections the
298 average location of aIP derived and OP derived neurons differ suggesting that they
299 might exhibit different migration patterns (Halliday & Cepko, 1992; Tan & Breen,
300 1993; Reid & Walsh, 2002; Tinterri *et al.*, 2018). Although this likely is not captured
301 fully in coronal slices, we nonetheless find that aIP derived neurons were
302 approximately twice as likely to be statistically biased (Wilcoxon Sign rank, p <
303 0.016) towards the medial aspect of striatum (73% of sections) than OP derived
304 neurons (37% of sections) with the strongest medial bias seen in the central and
305 caudal planes (**Supplemental Figure 2**).
306 We next investigated which types of striatal SPN were labeled. Combining
307 immunocytochemistry for the marker for spiny projection neurons CTIP2 (Arlotta *et*
308 *al.*, 2008) and the marker for D2 SPNs PPE (Gerfen *et al.*, 1990) we investigated the
309 degree of co-localization of these markers with our aIP and OP derived striatal
310 neurons (**Figure 2E**). We find that nearly all labeled neurons co-localize with CTIP2
311 (aIP derived/CTIP2$^+$: 99.6 ± 0.4% and OP derived/CTIP2$^+$: 99.4 ± 0.3%, both n = 9,
312 **Figure 2F**) suggesting the labeled neurons are SPNs. Secondly, labeling for the D2
313 SPN marker PPE reveals that both progenitor pools can generate both PPE$^-$ D1-
314 expressing direct pathway SPNs and PPE$^+$ D2-expressing indirect pathway SPNs (aIP
315 derived/PPE$^+$: 35.2 ± 7.0% and OP derived/PPE$^+$: 48.2 ± 5.3%, n = 9) with a trend for
316 the aIP progenitor pool to generate more PPE$^-$ D1 SPNs (vs. 50%, p<0.071, one-
317 sample t-test, n = 8). Lastly, we asked whether SPNs derived from these distinct
318 progenitor pools end up in different compartments of the striatum. We used antibody
319 staining against the μ-opioid receptor (MOR) to delineate the MOR-rich patches and
320 the MOR-poor matrix compartments of the striatum (**Figure 2G**). We find that on
321 average only 6% of labeled neurons can be found in the striatal patches with the
322 remainder found in the matrix with no difference between aIP and OP derived
323 neurons (aIP/MOR$^+$: 4.8 ± 1.3% and OP/MOR$^+$: 7.8 ± 1.6 %, n = 12, **Figure 2H**). As

different embryonic stages have been shown to differently contribute to the formation of the striatal neurons and compartments (Brand & Rakic, 1979; Graybiel & Hickey, 1982; Marchand & Lajoie, 1986; van der Kooy & Fishell, 1987; Fishell *et al.*, 1990; Newman *et al.*, 2015; Kelly *et al.*, 2018; Tinterri *et al.*, 2018) we repeated these experiment using IUE at E12.5 (**Supplemental Figure 3**). We find broadly similar results in that the proportion of labeled adult neurons reflect the ratio of embryonic progenitors with less aIP derived neurons labeled during this earlier stage of neurogenesis (aIP derived: 6.6 ± 4.0% and OP derived: 93.4 ± 4.0%, n = 10, **Supplemental Figure 3B**), virtually all labeled neurons express the SPN marker CTIP2 (aIP derived/CTIP2$^+$: 100.0 ± 0.0% and OP derived/CTIP2$^+$: 99.2 ± 0.5%, n = 6, **Supplemental Figure 3C**) and both progenitor populations can generate putative D1-expressing direct pathway SPNs and D2-expressing indirect pathway SPNs (aIP derived/PPE$^+$: 19.2 ± 3.6 % and OP-derived/PPE$^+$: 34.0 ± 6.0% n = 8, **Supplemental Figure 3D**). However, labeling at this younger embryonic age seems to generate fewer indirect pathway D2 SPNs (E12.5 vs. E15.5, Mann-Whitney test, p = 0.037; n = 8 and 12) and slightly more labeled neurons in MOR$^+$ patches (E12.5: 12.4 ± 2.4% and E15.5: 7.0 ± 1.3% in MOR$^+$ patches; Mann-Whitney test, p = 0.170, n = 4 and 12, **Supplemental Figure 3E**). Lastly, the LGE has been shown to not only generate striatal SPNs, but has also been shown to generate interneurons found in the olfactory bulb (Stenman *et al.*, 2003). Therefore, we investigated whether we could find labeled neurons also in the olfactory bulb in a small subset of mice that had undergone IUE at E15.5. We did find small numbers of labeled neurons in sections of the olfactory bulb (~40 neurons per section) with the majority being OP derived (aIP derived olfactory bulb interneurons: 3.9 ± 0.7% and OP derived olfactory bulb interneurons: 96.1 ± 0.7, n = 5 brains, **Supplemental Figure 4**).

Overall, these results suggest that aIP and OP neural progenitors in the LGE mainly generate D1 and D2 spiny projection neurons, which can be found intermingled in the striatum as well as in patch and matrix compartments. Furthermore, a small number of olfactory bulb neurons are generated mainly from the population of OP neural progenitors.

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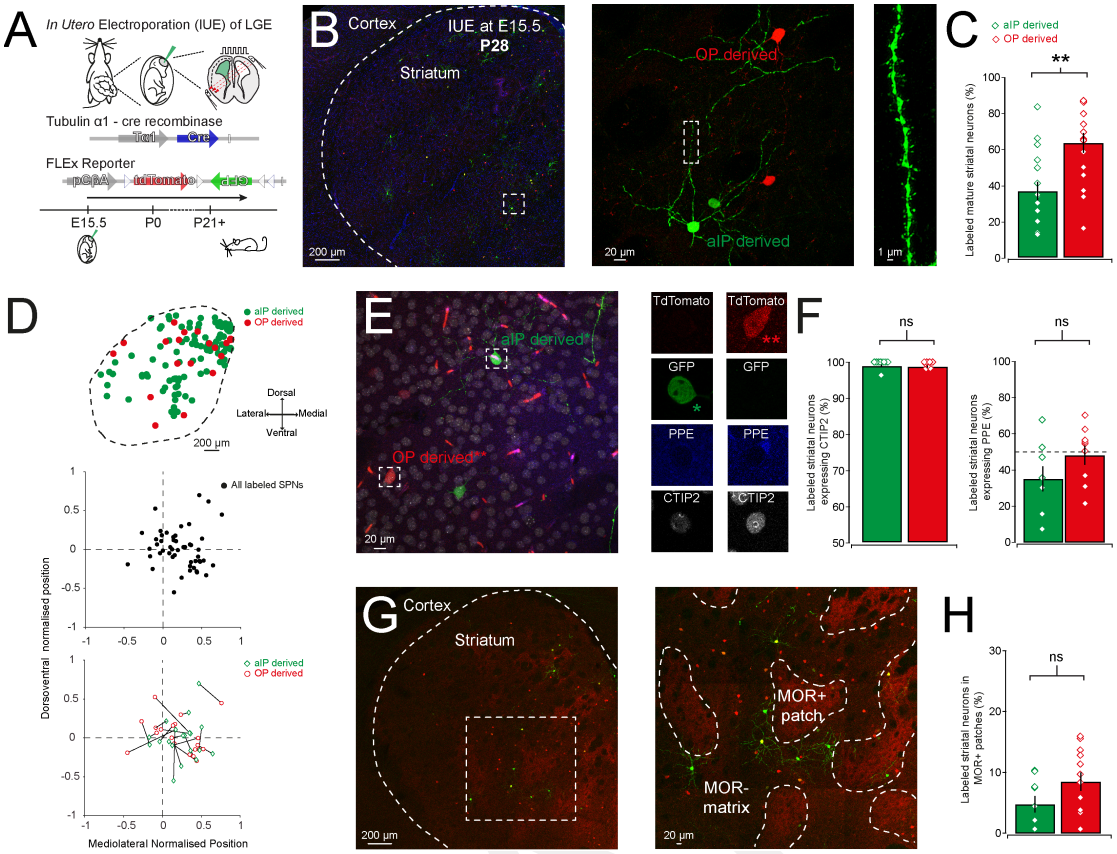

Figure 2: Both aIP and OP neural progenitors in the LGE can generate D1 and D2 striatal spiny projection neurons. (**A**) Mice that underwent IUE at E15.5 with Tα1-cre and FLEx reporter plasmids were left to grow up untill young adulthood (postnatal day 21 and over). (**B**) The striatum of these mice contain both GFP^{+} and TdTomato^{+} neurons derived from respectively a pool of apical intermediate progenitors (aIPs) or other progenitors (OPs). The adult neurons derived from both progenitor pools display the radial morphology and spine studded dendrites (right) characteristic of striatal spiny projection neurons (SPNs). (**C**) Quantification of the relative proportion of aIP and OP derived striatal neurons reveals a similar distribution as the respective embryonic neural progenitors at E15.5. Note the significantly larger proportion of OP derived neurons. (**D**) Example diagram of location of aIP and OP derived neurons in a central section of the striatum (top). Normalised mediolateral and dorsoventral positions of all labeled neurons across all counted sections reveals that labeled neurons inhabit more medial aspects of the striatum (middle). Normalised mediolateral and dorsoventral positions of aIP and OP derived neurons within individual sections, linked with lines, shows that in many sections they reside in different striatal regions (bottom). (**E**) An example image of striatum containing aIP and OP derived neurons and labeled for the SPN marker CTIP2 (white) and the D2-SPN marker PPE (blue). The red streaks result from auto-fluorescence of blood vessels. (**F**) Virtually all labeled neurons are positive for the SPN marker CTIP2 and both aIP and OP derived neurons consist of PPE positive

380 (D2) and PPE negative (putative D1) SPNs. (**G**) Labeling for the μ-opioid receptor
381 (MOR) reveals that aIP and OP derived neurons can be found in both MOR^+ patch
382 and MOR^- matrix compartments. (**H**) Quantification of aIP and OP derived neurons
383 labeled for MOR reveals both can be found within MOR^+ patches in equal numbers.
384
385
386 ***Both aIP and OP derived neurons have electrical and morphological properties***
387 ***consistent with SPNs.***
388 So far we have shown that we can pulse-label two different pools of neural
389 progenitors in the LGE and that both pools can generate D1 and D2 striatal SPNs
390 based on their neurochemical profile. We next investigated in more detail whether the
391 labeled neurons exhibit the electrophysiological and morphological properties of
392 SPNs. To do this we performed whole-cell patch-clamp recordings of aIP and OP
393 derived neurons as well as unlabeled neurons in dorsal striatum (**Figure 3A**). The
394 inclusion of biocytin in the intracellular solution allowed for post-hoc labeling and
395 reconstruction of recorded cells (**Figure 3B** and **C**). We investigated a variety of
396 electrophysiological properties, including membrane potential (aIP derived: -80.3 ±
397 1.2 mV, OP derived: -78.9 ± 0.7 mV and unlabeled: -78.3 ± 1.2 mV; $p>0.05$, t-test, n
398 = 15, 34 and 18 neurons), input resistance (aIP derived: 88.1 ± 8.4 MΩ, OP derived:
399 81.0 ± 4.7 MΩ and unlabeled: 80.3 ± 7.5 MΩ; $p>0.05$, t-test), action potential
400 threshold (aIP derived: -41.6 ± 1.8 mV, OP derived: -39.8 ± 0.9 mV and unlabeled: -
401 39.5 ± 1.2 mV; $p>0.05$, t-test) as well as action potential frequency, which all
402 suggested that labeled neurons exhibited electrical properties consistent with those of
403 SPNs. However, we did not observe significant differences in these or other
404 electrophysiological properties between aIP and OP derived neurons (**Figure 3D** and
405 **Table 1**).
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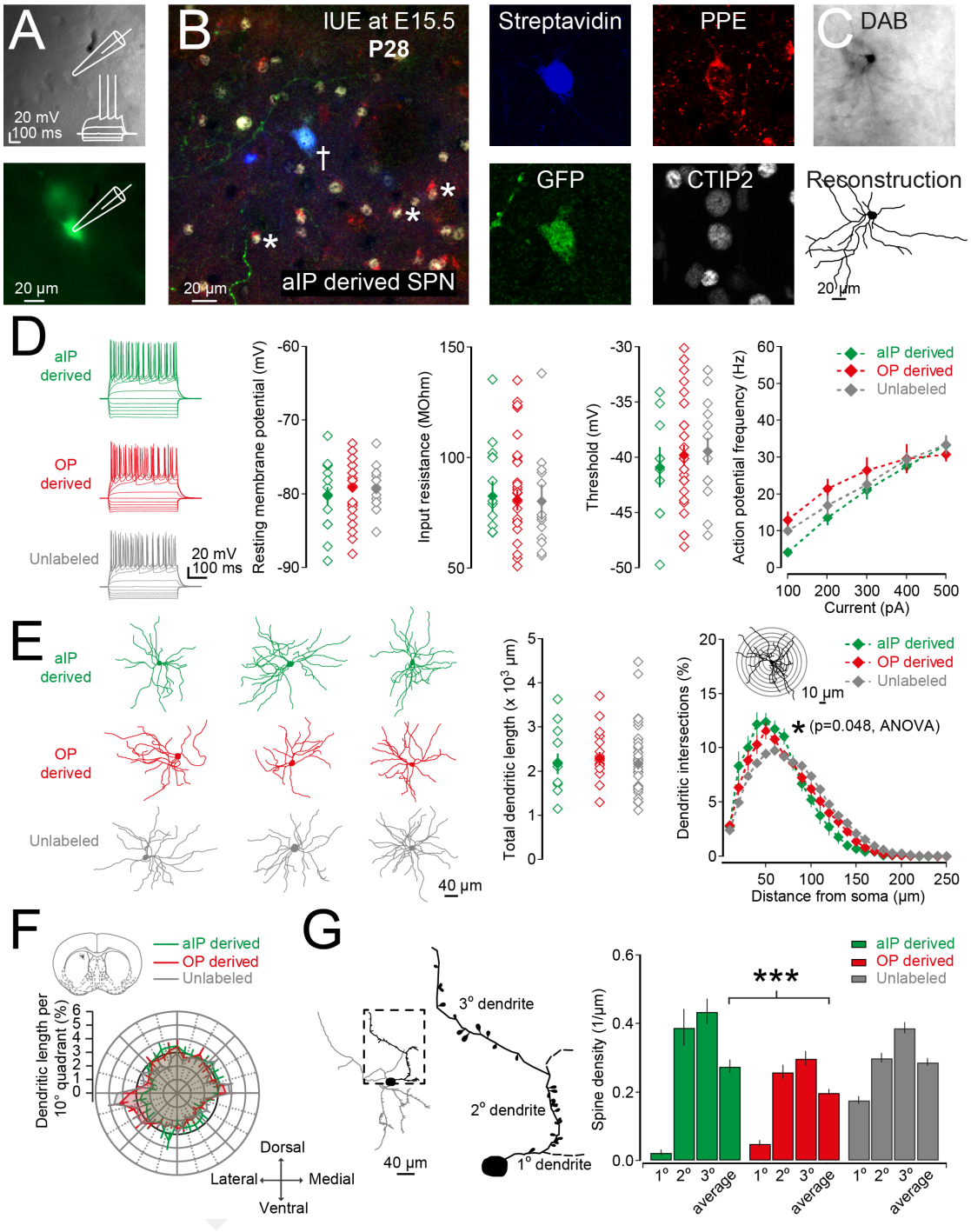

Figure 3: aIP derived SPNs exhibit greater dendritic complexity and spine density. (**A**) Electrophysiological properties of progenitor-derived striatal neurons were assessed using whole-cell patch-clamp recordings in acute brain slices. Example Dodt contrast image (top) and fluorescence image (bottom) of a recorded aIP derived GFP^{+} neuron. Inset: response of the neuron to hyperpolarizing and depolarizing current steps consistent with that of a SPN. (**B**) Recorded cells were labeled with biocytin during recordings and revealed using streptavidin-Alexafluor405 conjugated antibodies in fixed tissue (indicated with a cross) and tested for the expression of the SPN marker CTIP2 and the D2 SPN marker PPE. Indicated with white asterisks are

neighboring CTIP2$^+$ and PPE$^+$ neurons. (**C**) Recorded cells were further processed for DAB immunohistochemistry allowing for reconstruction of their dendritic arbors as well as spine counts. (**D**) Hyperpolarizing and depolarizing current steps were used to characterise the electrophysiological properties of aIP derived, OP derived and unlabeled striatal neurons. The electrophysiological properties were consistent with those of SPNs and no significant differences were found between either group in their resting membrane potential, input resistance, spike threshold or action potential frequency. (**E**) Example reconstructions of DAB processed aIP derived (top), OP derived (middle) as well as unlabeled SPNs (bottom). The total dendritic length of the different SPNs did not differ. Assessment of dendritic complexity using Scholl analysis reveals that aIP derived neurons exhibit a subtle but significant larger dendritic complexity close to the soma. (**F**) Dendritic polarity analysis reveals that all SPNs exhibit a similar radial morphology. (**G**) Quantification of the number of spines on the primary (1°), secondary (2°) and tertiary (3°) dendrites reveals that aIP derived neurons exhibit an overall higher density of spines as compared to OP derived neurons.

We next investigated the morphological properties of the SPNs derived from aIP and OP progenitors. Recorded cells were post hoc processed for DAB immunohistochemistry allowing for reconstruction of their dendritic arbors as well as quantification of dendritic spines (**Figure 3E - G**). Comparing the total dendritic length we did not find significant differences in the length of their dendrites (aIP derived: 2184.7 ± 228.8 µm, OP derived: 2299.3 ± 147.2 µm and unlabeled neurons: 2182.6 ± 129.3 µm; p=0.626, Kruskal-Wallis H test, n = 11, 16 and 35 neurons, **Figure 3E**). We next investigated the dendritic complexity using Scholl analysis and find that all neurons exhibit the greatest dendritic complexity close to the soma (~50 µm distance) with a small by significant greater complexity for aIP derived over OP derived neurons (p=0.048, ANOVA, n = 11 and 16, **Figure 3E**). Polarity analysis shows that the orientation of the dendrites is not significantly different between aIP derived, OP derived or unlabeled neurons and that all exhibit a radial morphology (**Figure 3F**). Quantification of dendritic spines on the primary, secondary and tertiary dendritic segments reveal that the aIP derived SPNs have a higher density of spines than the OP derived SPNs (average aIP derived: 0.28 ± 0.02 spines/µm and average OP derived: 0.20 ± 0.01 spines/µm, t-test, p=0.0058, n = 13 and 28, **Figure 3G**).

Combined these results suggest that both aIP and OP derived neurons exhibit all the electrical and morphological hallmarks of striatal SPNs. Furthermore, they

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455 show that subtle differences can be detected in the morphological properties of
456 neurons derived from different progenitor pools (Guillamon-Vivancos *et al.*, 2019) in
457 that aIP derived neurons exhibit greater local dendritic complexity and a higher
458 density of dendritic spines.
459
460
461 ***Integration of progenitor derived striatal SPNs into striatal neural circuits***
462 We next asked to what extent aIP and OP derived neurons are integrated within the
463 circuits of the basal ganglia and striatum. To do this we performed IUE at E15.5 to
464 label both aIP derived and OP derived SPNs and examined both the synaptic inputs
465 and outputs of labeled SPNs. We first investigated to what extent aIP and OP derived
466 SPNs sample cortical afferents. Cortical afferents were activated using a stimulating
467 electrode placed in the external capsule while recording from both aIP and OP derived
468 neurons in the striatum (**Figure 4B**). All experiments were performed in the presence
469 of GABA receptor antagonists to avoid erroneous activation of GABAergic afferents
470 (see **Methods**). We find that aIP derived and OP derived SPNs exhibit similar
471 amplitude EPSPs (aIP derived: 1.70 ± 0.44 mV and OP derived: 2.08 ± 0.62 mV,
472 p=0.974, Mann Whitney test, n = 12 and 10, **Figure 4D**) and that these do not differ
473 from amplitudes found in unlabeled SPNs (unlabeled: 1.50 ± 0.33 mV, aIP vs
474 unlabeled: p=0.648 and OP vs unlabeled: p=0.650). However, we find that the
475 duration and decay time of the EPSPs are significantly longer in the aIP derived
476 neurons (**Table 2**) suggesting they might contain different configurations of glutamate
477 receptors.
478 We next asked to what extent these progenitor derived neurons innervate
479 neighbouring SPNs and in particular whether aIP derived neurons similarly innervate
480 the D1-expressing direct pathway and D2-expressing indirect pathway SPNs. We
481 used electroporation of Tα1-cre and creON-mCherry plasmids (Saunders *et al.*, 2012)
482 at E15.5 to selectively express the light-activatable channel ChR2 in aIP derived
483 neurons in C57Bl/6 and D1 or D2-GFP transgenic mice with the latter facilitating the
484 classification of patched neurons as either D1 or D2 SPNs **(Figure 5A**).
485
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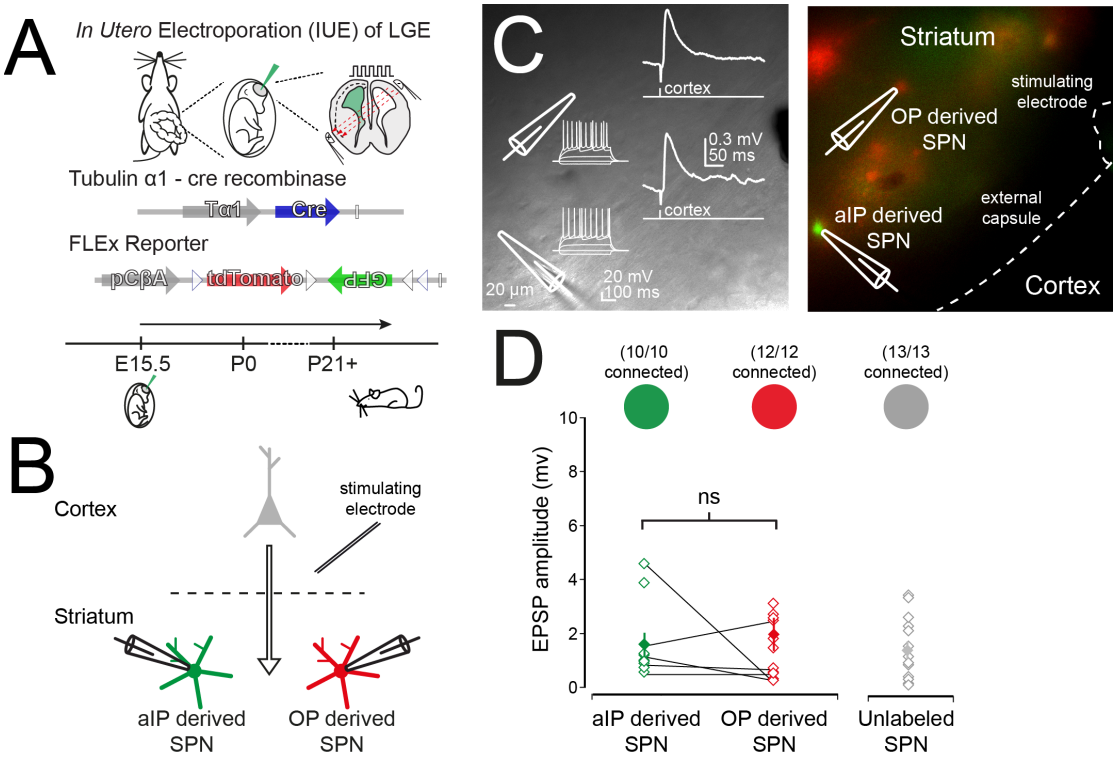


Figure 4: Both aIP and OP derived SPNs receive cortical input. (**A**) IUE of Tα1-Cre and FLEx reporter plasmids was performed at E15.5 to label both aIP and OP derived striatal neurons. (**B**) Diagram of the experimental setup consisting of electrical stimulation of cortical afferents while recording from both aIP and OP derived neurons in the striatum. All recordings were performed in the presence of GABA receptor antagonists. (**C**) Example Dodt contrast (left) and fluorescence (right) image of the experimental setup consisting of a Tungsten electrode placed in the external capsule and the simultaneous recording of a GFP^+ aIP derived SPN and a $TdTomato^+$ OP derived SPN. (**D**) Scatter plot of the amplitudes of the excitatory postsynaptic responses for aIP derived, OP derived and unlabeled SPNs upon stimulation of the cortical afferents. Note that all three classes of SPN receive similar amplitude cortically evoked EPSPs.

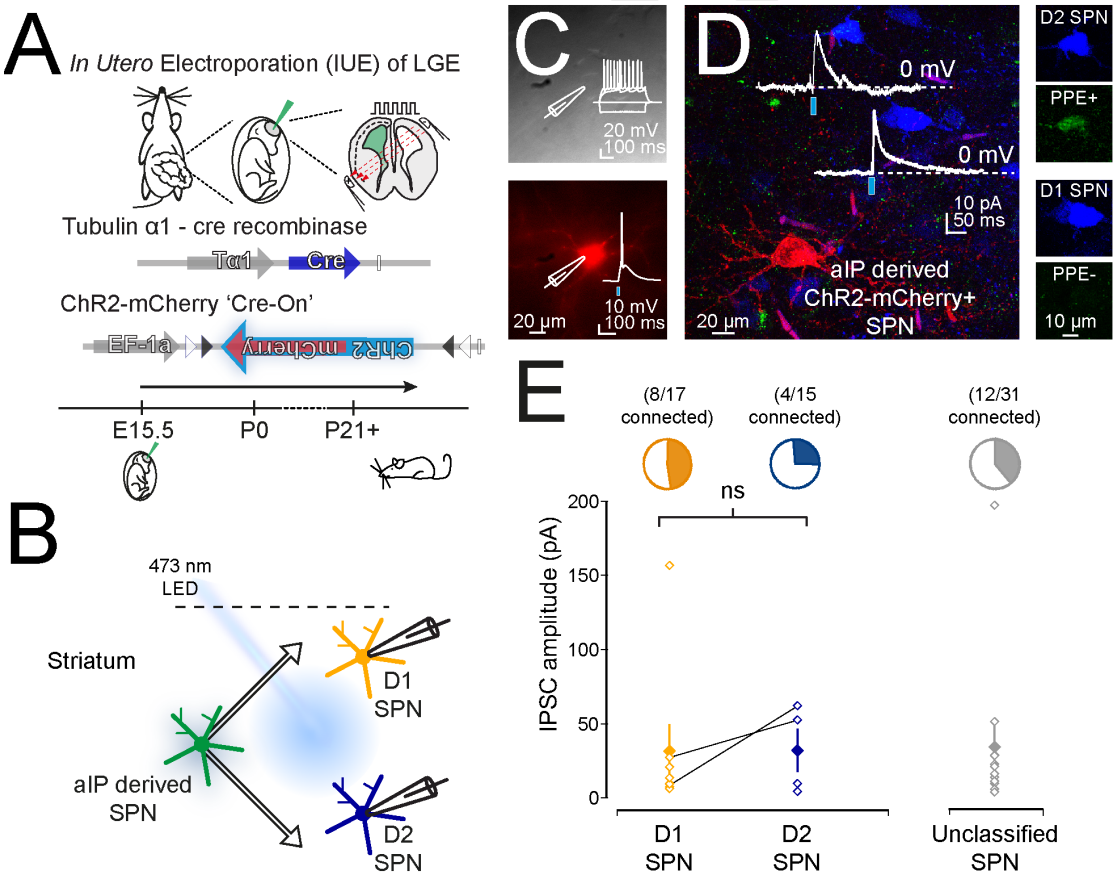
In postnatal acute brain slices we were now able to deliver short pulses of blue light to activate ChR2-mCherry expressing aIP derived neurons (**Figure 5C**) while performing whole-cell voltage-clamp recordings of single or pairs of D1 and D2 SPNs at a holding potential of 0 mV to facilitate detection of inhibitory events (**Figure 5B** and **D**). We recorded from a total of 63 SPNs of which 17 were confirmed D1 SPNs, 15 were confirmed D2 SPNs and 31 were unclassified. Although we find that the incidence of finding a connected SPN is slightly higher for the D1 SPNs than the D2 SPNs (D1 SPN: 47.1% and D2 SPN: 26.7%) this does not reach statistical

DRAFT

511 significance (p=0.517; Fisher's exact test). For the neurons that received inputs from
512 the aIP derived neurons we find no significant difference in the amplitude of the light
513 evoked inhibitory postsynaptic current (IPSC) as recorded at a holding potential of 0
514 mV (D1: 31.23 ± 18.11 pA, D2: 31.97 ± 14.69 pA and unclassified: 34.15 ± 15.30
515 pA, p=0.797, Kruskall Wallis test, n = 8, 4 and 12, **Figure 5E**).

516 In conclusion, these results suggest that both aIP and OP derived SPNs are
517 embedded within the neural circuits of the striatum. Indeed, both aIP and OP derived
518 striatal SPNs receive excitatory glutamatergic cortical input and aIP derived neurons
519 send inhibitory GABAergic outputs to neighbouring D1 and D2 SPNs. Interestingly,
520 we find that aIP and OP derived neurons differentially respond to cortical inputs, with
521 aIP derived SPNs exhibiting prolonged EPSP kinetics, suggesting that they might
522 sample excitatory inputs differently.

523



524

525 **Figure 5: aIP derived SPNs connect to both D1 and D2 SPNs** (**A**) IUE of Tα1-Cre
526 and DIO-ChR2-mCherry plasmids allowed for the expression of ChR2-mCherry in
527 aIP derived neurons. (**B**) Diagram of the experimental setup consisting of recordings
528 of D1 and D2 SPNs while stimulating aIP derived neurons with a 473 nm wide-field
529 LED. All recordings were performed in the presence of glutamate receptor

530 antagonists. (**C**) Whole-cell patch-clamp recordings of aIP derived ChR2-mCherry
531 expressing striatal neurons revealed that ~3 ms flashes of light could elicit single
532 action potentials. Inset top: response of the neuron to hyperpolarising and depolarising
533 current steps. (**D**) To investigate striatal inhibitory synaptic output from aIP derived
534 neurons, simultaneous whole-cell patch-clamp recordings were performed from D1
535 and D2 SPNs, as determined during recordings in D1 or D2-GFP transgenic mice or
536 posthoc using immunocytochemistry for PPE. Example image of streptavidin labeled
537 PPE^+ (upper neuron) and PPE^- SPN (lower neuron) in close apposition to an aIP
538 derived-ChR2-$mCherry^+$ SPN. Traces correspond to light evoked IPSCs. (**E**) aIP
539 derived striatal neurons exhibited a slight preference to connect to D1 SPNs (47%)
540 and optical activation of aIP derived neurons results in similar amplitude IPSCs in all
541 tested D1, D2 and unclassified SPNs.
542
543
544
545 **Discussion**
546 In this study we examined to what extent neural progenitor pool diversity in the lateral
547 ganglionic eminence (LGE) contributes to cellular and circuit diversity in the
548 striatum. Using *in utero* electroporation we are able to label two distinct pools of
549 dividing neural progenitors in the LGE, which are active during later stages of
550 neurogenesis. Using differential expression of the tubulin alpha1 (Tα1) promoter we
551 are able to label a Tα1-expressing pool of neural progenitors we refer to as apical
552 intermediate progenitors (aIPs) and a non-Tα1-expressing pool we refer to as other
553 progenitors (OPs). We find that both progenitor types are actively dividing in the
554 ventricular zone of the LGE around embryonic days (E)15.5-16.5, but exhibit distinct
555 proliferative behaviours. Whereas aIP progenitors exhibit a short round morphology
556 during division, lack a basal process and exhibit fast cell cycle kinetics, OP
557 progenitors tend to retain a basal process and exhibit slower cell cycle kinetics. We
558 show that both can generate D1-expressing direct pathway spiny projection neurons
559 (SPNs) as well as D2-expressing indirect pathway SPNs found in both patch and
560 matrix compartments of the striatum. Whereas aIP and OP derived SPNs were found
561 intermingled throughout the striatum on average the aIP derived population of SPNs
562 inhabited more medial aspects of the striatum. Whole-cell patch-clamp recordings and
563 morphological reconstruction of aIP and OP derived neurons confirmed they were
564 SPNs with aIP derived SPNs exhibiting a more complex local dendritic tree and an
565 increased dendritic spine density. Electrical circuit mapping shows that both aIP and
566 OP derived SPNs sample input from cortical excitatory afferents with aIP derived

DRAFT

567 SPNs exhibiting differential sampling and longer duration responses. Lastly,
568 optogenetic circuit mapping reveals that aIP derived SPNs send local GABAergic
569 outputs to both D1 and D2 SPNs. Overall, we show that it is possible to label diverse
570 pools of neural progenitors in the LGE and their progeny in the striatum. Diversity in
571 the pool of progenitors can generate diversity in adult striatal neurons, which
572 manifests itself in different spatial distributions, morphological properties and
573 sampling of excitatory afferents.
574
575 *Different pools of embryonic neural progenitors in the LGE*
576 The neural progenitors in the LGE of the embryonic mouse brain (Smart, 1976)
577 generate most of the SPNs of the striatum as well as some of the interneurons of the
578 olfactory bulb (Olsson *et al.*, 1998; Stenman *et al.*, 2003) with postnatal generation of
579 striatal neurons also contributing (Stopczynski *et al.*, 2008). Recent studies have
580 highlighted that the LGE contains as much heterogeneity in neural progenitors (Pilz *et*
581 *al.*, 2013) as pallial structures (Franco & Muller, 2013). Indeed, in the LGE these
582 progenitors include radial glial cells, basal radial glial cells, basal progenitors,
583 subapical progenitors as well as short neural precursors, amongst others (Pilz *et al.*,
584 2013). It is currently unknown how this heterogeneity at the progenitor level relates to
585 the cellular and circuit diversity found in the striatum.
586 Several lines of evidence suggest that that the population of progenitors
587 labeled using the promoter sequence for Ta1 correspond to the short neural precursors
588 and subapical progenitors recently described in the LGE (Gal *et al.*, 2006; Stancik *et*
589 *al.*, 2010; Pilz *et al.*, 2013; Ellender *et al.*, 2018). We find that the population of Tα1-
590 expressing neural progenitors divides at some distance from the ventricular wall but
591 mostly with the ventricular zone, their cell cycle kinetics are fast and correspond to
592 about 17 hours, they form a large population of dividing progenitors during later
593 stages of neurogenesis (E15.5-E16.5) and lastly they lack a basal process during
594 division. These criteria are consistent to those described for short neural precursors
595 and the subapical progenitors that are often generated from the short neural precursors
596 (Pilz *et al.*, 2013). Conversely, even though we find that our population of non-Tα1-
597 expressing neural progenitors also divides in the ventricular zone, it is more dominant
598 during early stages of neurogenesis (E12.5/E13.5), has slower cell cycle kinetics
599 corresponding to about 20 hours (Pilz *et al.*, 2013), and many progenitors retain a
600 basal process during division. These criteria are consistent with the described

DRAFT

601 morphology of radial glial cells in the LGE (Pilz *et al.*, 2013) and the cortex (Gal *et*
602 *al.*, 2006; Kriegstein & Alvarez-Buylla, 2009).
603 As the Tα1-expressing neural progenitors could be found actively dividing in
604 the apical aspects of the ventricular zone and had many properties of both short neural
605 precursors and subapical progenitors (Pilz *et al.*, 2013) we proposed to refer to them
606 collectively as apical intermediate progenitors (aIPs) and our non-Tα1-expressing
607 neural progenitors as other progenitors (OPs). Therefore, at the point of labeling, our
608 *in utero* strategy marked a progenitor population enriched for aIPs and a population of
609 concurrently dividing OPs. Although the method allowed us to distinguish neurons
610 derived from different progenitor pools, it provides limited information on the lineage
611 pathways taken by the neurons. Moreover, neural progenitor lineage in the LGE is
612 complex with extended proliferative divisions and likely both progenitor pools
613 generate neurons via other progenitors (Pilz *et al.*, 2013).
614
615 *Progenitor derived striatal spiny projection neurons and associated circuits*
616 We find that both aIP and OP neural progenitors generate neurons that can be found
617 intermingled in the striatum. By varying the embryonic time at which we perform our
618 IUE we confirm that aIP progenitors are mainly active at later stages of neurogenesis
619 as we find more aIP derived neurons when we electroporate at E15.5 as compared to
620 E12.5. Immunohistochemistry for the SPN marker CTIP2 (Arlotta *et al.*, 2008)
621 reveals that most if not all labeled neurons are SPNs independent of whether IUE was
622 performed at E12.5 or E15.5. Furthermore, immunohistochemistry for the D2 SPN
623 marker PPE (Gerfen *et al.*, 1990) reveals that both aIP and OP progenitor pools can
624 generate D1 and D2 SPNs at both E12.5 and E15.5. These observations are consistent
625 with the idea that the generation of D1 and D2 SPNs is controlled by other genetic
626 factors, environmental factors or molecular clocks (Lobo *et al.*, 2006; Franco *et al.*,
627 2012; Cepko, 2014; Kelly *et al.*, 2018). Indeed, similar observations have been made
628 in the cortex where both layer 4 stellate neurons and layer 2/3 pyramidal neurons can
629 be generated from Tα1-expressing as well as GLAST-expressing neural progenitors
630 (Stancik *et al.*, 2010) and indeed a single cortical progenitor can generate clones with
631 layer-specific characteristics in all layers of cortex (Yu *et al.*, 2009). Whereas the
632 neural progenitor pool of origin did not seem to affect SPN type or location in patch
633 or matrix compartments (Pert *et al.*, 1976; Gerfen, 1984; Herkenham *et al.*, 1984;
634 Gerfen *et al.*, 1985; Jimenez-Castellanos & Graybiel, 1989; Gerfen, 1992; Crittenden

DRAFT

635 & Graybiel, 2011), we find that the earlier time of electroporation resulted in both
636 pools of progenitors generating a higher proportion of D1 SPNs (Marchand & Lajoie,
637 1986; van der Kooy & Fishell, 1987; Kelly *et al.*, 2018) as well as generating more
638 SPNs in patch compartments (Brand & Rakic, 1979; Graybiel & Hickey, 1982; van
639 der Kooy & Fishell, 1987; Fishell *et al.*, 1990; Newman *et al.*, 2015; Tinterri *et al.*,
640 2018).

641 Interestingly, we find that the different pools of neural progenitors generate
642 D1 and D2 SPNs found in different aspects of the striatum, with aIP derived SPNs on
643 average found in more medial aspects of the striatum. Already at embryonic ages the
644 aIPs and related young neurons are found at slightly more distal locations relative to
645 the ventricle, which might suggest that the location of aIP derived neurons in the adult
646 brain results from such early differences. However, migration in the striatum is
647 complex in that newly born neurons undergo tangential, radial and other forms of
648 migration (Halliday & Cepko, 1992; Tan & Breen, 1993; Reid & Walsh, 2002;
649 Tinterri *et al.*, 2018), suggesting other factors could also contribute to the ultimate
650 location of striatal neurons (Franco *et al.*, 2012; Kelly *et al.*, 2018). Interestingly,
651 detailed studies of the inputs to the striatum have revealed that dorsomedial aspects of
652 the striatum exhibit a high degree of input heterogeneity (Pan *et al.*, 2010; Guo *et al.*,
653 2015; Hunnicutt *et al.*, 2016) suggesting these different progenitor derived SPNs
654 might process distinct inputs.

655 Electrophysiological and morphological study of single neurons reveals that
656 both aIP and OP derived neurons mostly exhibit similar properties consistent with
657 those of SPNs (Day *et al.*, 2008; Gertler *et al.*, 2008; Krajeski *et al.*, 2018).
658 Interestingly, we find that aIP derived neurons exhibit a greater local dendritic
659 complexity and a higher dendritic spine density than OP derived neurons. This
660 difference might suggest that they differentially sample excitatory inputs. Indeed,
661 whereas the overall strength of cortical inputs to both aIP and OP derived neurons
662 seems similar, the aIP derived neurons exhibit comparatively long duration EPSPs.
663 This might well result from the expression of a different complement of glutamate
664 receptors postsynaptically.

665 Lastly, it is known that SPNs in the striatum form strong lateral inhibitory
666 connections with each other with which they can regulate each other's activity
667 (Taverna *et al.*, 2008; Planert *et al.*, 2010; Chuhma *et al.*, 2011; Krajeski *et al.*, 2018)
668 and these are not uniform but depend on the type of pre- and postsynaptic SPN

669 (Taverna *et al.*, 2008; Planert *et al.*, 2010; Krajeski *et al.*, 2018). For example, the D2
670 SPNs form a large number of strong reciprocal connections with each other (Planert *et*
671 *al.*, 2010; Krajeski *et al.*, 2018). Although our current results suggest that aIP derived
672 SPNs innervate both local D1 and D2 SPNs, future work will further inform us on
673 whether and how diverse neural progenitor pools contribute to the establishment of
674 further fine-scale neural circuits in the striatum.

675 In conclusion, we show that we are able to fluorescently pulse-label two different
676 pools of neural progenitors in the LGE and their offspring. Using this method we
677 reveal that heterogeneity in neural progenitor pools in the LGE can contribute to
678 diversity in the spiny projection neurons and circuits of the striatum. In particular, we
679 find that aspects of spatial location and morphology of neurons, as well as their
680 sampling of excitatory afferents seem particularly sensitive to neural progenitor
681 origin. Future investigations will be informative as to what extent they contribute to
682 other aspects of striatal diversity (Grillner & Robertson, 2016) and whether neural
683 progenitor dysregulation plays a role in early onset neurodevelopmental disorders
684 such as OCD, autism and Tourette's syndrome (Graybiel & Rauch, 2000; Del Campo
685 *et al.*, 2011; Langen *et al.*, 2011; McNaught & Mink, 2011; Shepherd, 2013; Albin,
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1019 **Methods & Materials**
1020
1021 Animals
1022 All experiments were carried out on C57/BL6 wildtype and heterozygous D1-GFP or
1023 D2-GFP mice of both sexes with *ad libitum* access to food and water. The D1-GFP or
1024 D2-GFP BAC transgenic mice report subtypes of the dopamine receptor, either D1 or
1025 D2, by the presence of GFP (Mutant Mouse Regional Resource Centers, MMRRC).
1026 Details of the mice and the methods of BAC mice production have been published
1027 (Gong *et al.*, 2003) and can be found on the GENSAT website [GENSAT (2009) The
1028 Gene Expression Nervous System Atlas (GENSAT) Project. In: NINDS, Contracts
1029 N01NS02331 and HHSN271200723701C, The Rockefeller University (New York),
1030 http://www.gensat.org/index.html]. The BAC transgenic mice were backcrossed to a
1031 C57/BL6 background over 20+ generations prior to use and kept as a heterozygous
1032 mouse line to avoid published issues using these transgenic lines (Bagetta *et al.*, 2011;
1033 Kramer *et al.*, 2011; Chan *et al.*, 2012; Nelson *et al.*, 2012). All mice were bred, IVC
1034 housed in a temperature controlled animal facility (normal 12:12 h light/dark cycles)
1035 and used in accordance with the UK Animals (Scientific Procedures) Act (1986).
1036 Females were checked for plugs daily; the day of the plug was considered embryonic
1037 day (E)0.5 and injection of plasmids and *in utero* electroporation (IUE) was
1038 performed at E12.5 - E16.5.
1039
1040 Electroporation
1041 *In utero* electroporation (IUE) was performed using standard procedures. In short,
1042 pregnant females were anaesthetized using isoflurane and their uterine horns were
1043 exposed by midline laparotomy. A mixture of plasmid DNA (~1.5 µg/µl) and 0.03%
1044 fast green dye was injected intraventricularly using pulled micropipettes through the
1045 uterine wall and amniotic sac. Plasmid DNA included: (i) 'Tα1-Cre', in which the
1046 gene for Cre recombinase is under the control of a portion of the Tα1 promoter
1047 (Stancik *et al.*, 2010); (ii) 'CβA-FLEx' which uses the chicken β-*actin* promoter to
1048 control a flexible excision (FLEx) cassette in which Cre recombination switches
1049 expression from TdTomato fluorescent protein to enhanced green fluorescent protein
1050 (Franco *et al.*, 2012); and (iii) 'DIO-ChR2-mCherry' (pAAV-EF1a-doublefloxed-
1051 hChR2(H134R)-mCherry-WPRE-HGHpA; Addgene #20297), in which Cre
1052 recombination turns on the expression of channelrhodopsin-2 (ChR2) under the
1053 control of the human elongation factor-1a promoter (Saunders *et al.*, 2012). Total
1054 volume injected per pup was ~1 µl. Tα1-Cre and CβA-FLEx constructs (and other
1055 combinations of constructs) were injected as a 1:1 ratio of plasmid DNA (each 3
1056 µg/µl, so final concentration of both constructs was 1.5 µg/µl). The cathode of the
1057 Tweezertrode (Genetronics) was placed just above the eye outside the uterine muscle
1058 and the anode was placed slightly lower at the contralateral cheek region (Baumgart
1059 & Baumgart, 2016). Five pulses (50 ms duration separated by 200 - 950 ms) at 42-
1060 60V with a BTX ECM 830 pulse generator (Genetronics). Typically around 80% of
1061 the pups underwent electroporation. Afterwards the uterine horns were placed back
1062 inside the abdomen, the cavity filled with warm physiological saline and the

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1063 abdominal muscle and skin incisions were closed with vicryl and prolene sutures,
1064 respectively. Dams were placed back in a clean cage and monitored closely until the
1065 birth of the pups.
1066
1067 Slice preparation and recording conditions
1068 Acute striatal slices were made from postnatal animals at 3-5 weeks of age. Animals
1069 were anaesthetized with isoflurane and then decapitated. Coronal 350-400 µm slices
1070 were cut using a vibrating microtome (Microm HM650V). Slices were prepared in
1071 artificial cerebrospinal fluid (aCSF) containing (in mM): 65 Sucrose, 85 NaCl, 2.5
1072 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 25 $NaHCO_3$ and 10 glucose, pH 7.2-7.4,
1073 bubbled with carbogen gas (95% O_2 / 5% CO_2). Slices were immediately transferred
1074 to a storage chamber containing aCSF (in mM): 130 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 2
1075 $MgCl_2$, 2 $CaCl_2$, 24 $NaHCO_3$ and 10 glucose, pH 7.2 - 7.4, at 32 ºC and bubbled with
1076 carbogen gas until used for recording. Striatal slices were transferred to a recording
1077 chamber and continuously superfused with aCSF bubbled with carbogen gas with the
1078 same composition as the storage solution (32 °C and perfusion speed of 2 ml/min).
1079 Whole-cell recordings were performed using glass pipettes (~6MΩ), pulled from
1080 standard wall borosilicate glass capillaries and containing for whole-cell current-
1081 clamp (in mM): 110 potassium gluconate, 40 HEPES, 2 ATP-Mg, 0.3 Na-GTP, 4
1082 NaCl and 4 mg/ml biocytin (pH 7.2-7.3; osmolarity, 290-300 mosmol/l) and for
1083 whole-cell voltage-clamp (in mM): 120 cesium gluconate, 40 HEPES, 4 NaCl, 2
1084 ATP-Mg, 0.3 Na-GTP, 0.2 QX-314 and 4 mg/ml biocytin (pH 7.2–7.3; osmolarity,
1085 290-300 mosmol/L). Recordings were made using Multiclamp 700B amplifiers and
1086 filtered at 4kHz and acquired using an InstruTECH ITC-18 analog/digital board and
1087 WinWCP software (University of Strathclyde, RRID:SCR_014713) at 10 kHz.
1088
1089 Stimulation and recording protocols
1090 Hyperpolarizing and depolarizing current steps were used to assess the intrinsic
1091 properties of the recorded SPNs including input resistance and spike threshold (using
1092 small incremental current steps) as well as the properties of action potentials
1093 (amplitude, frequency and duration). Properties were assessed immediately on break-
1094 in. Activation of excitatory cortical afferents was performed via a bipolar stimulating
1095 electrode (FHC Inc., USA) placed in the external capsule, and in the presence of
1096 blockers of inhibitory GABAergic transmission including the $GABA_A$-receptor
1097 antagonist SR95531 (1 µM) and the $GABA_B$-receptor antagonist CGP52432 (2 µM).
1098 Afferents were activated every 5s with up to 20 repetitions and excitatory
1099 postsynaptic potentials (EPSPs) were recorded from the patched SPNs.
1100 Photoactivation of ChR2 was achieved using widefield 2-3 ms duration light pulses of
1101 ~1 mW via a TTL triggered CoolLED pE-300 system (CoolLED, Andover, UK)
1102
1103 Analysis of recordings
1104 Data were analyzed offline using custom written programs in Igor Pro (Wavemetrics,
1105 RRID:SCR_000325). The input resistance was calculated from the observed
1106 membrane potential change after hyperpolarizing the membrane potential with a set

current injection. The spike threshold was the membrane voltage at which the SPN generated an action potential. The action potential amplitude was taken from the peak amplitude of the individual action potentials relative to the average steady-state membrane depolarization during positive current injection. Action potential duration was taken as the duration between the upward and downward stroke of the action potential at 25% of the peak amplitude. Evoked EPSPs and IPSCs were defined as upward or downward deflections of more than 2 standard deviations (SD) on average synaptic responses generated after filtering and averaging original traces (0.1 Hz high-pass filter and 500 Hz low-pass filter) and used for analysis of synaptic properties. Synaptic properties include measurements of peak amplitude, duration (measured from the start of the upward/downward stroke of the event until its return to the pre-event baseline), rise time (time between 20% and 80% of the peak amplitude) and decay time (measured as the time from peak amplitude until the event returned to 50% of peak amplitude).

Histological analyses

Following whole-cell patch-clamp recordings the brain slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Biocytin-filled neurons were visualized by incubating sections in 1:10,000 streptavidin AlexaFluor405-conjugated antibodies (ThermoFisher Scientific, Cat#:S32351). Visualized neurons were labeled for chicken ovalbumin upstream promoter transcription-factor interacting protein-2 (CTIP2, 1:1000, rat, Abcam, Cat#:ab14865, RRID:AB_2064130) and pre-proenkephalin (PPE, 1:1000, rabbit, LifeSpan Biosciences, Cat#:LS-C23084, RRID:AB_902714) in PBS containing 0.3% Triton X-100 (PBS-Tx) overnight at 4°C followed by incubation with goat-anti-rat AlexaFluor647 (1:500; ThermoFisher Scientific, CAT#:A-21247, RRID:AB_141778) and goat-anti-rabbit AlexaFluor555 (1:500; ThermoFisher Scientific, CAT#:A-21429, RRID:AB_2535850) or goat-anti-rabbit AlexaFluor488 (1:500; ThermoFisher Scientific, CAT#:A32731, RRID:AB_2633280) secondary antibodies in 0.3% PBS-Tx for 2 h at RT for D1 or D2 SPN classification. Occasionally the endogenous fluorescence would be boosted with antibodies against GFP (1:1000, chicken, Aves Labs, CAT# GFP-1020, RRID:AB_10000240) or TdTomato (1:1000; rat; anti-RFP; Chromotek, CAT# 5f8-100, RRID:AB_2336064) or slices were co-stained with the nuclear marker 4′,6-diamidino-2-phenylindole (DAPI) in PBS (1:100,000) to facilitate the delineation of brain structures. CTIP2 is expressed by SPNs and not interneurons (Arlotta *et al.*, 2008) and PPE reliably labels indirect pathway D2 SPNs (Lee *et al.*, 1997; Sharott *et al.*, 2017). PPE antibody staining was facilitated through antigen retrieval by heating sections at 80° C in 10 mM sodium citrate (pH 6.0) for approximately 30-60 min prior to incubation with PPE primary antibody. After classification of SPNs the slices were washed 3 times in PBS and processed for DAB immunohistochemistry using standard procedures.

Whole-brain fixation of embryonic and adult brains was performed by rapid decapitation of the head and submersion in oxygenated sucrose cutting solution before

1150 submersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The
1151 brains were fixed for 24 – 48 hours, after which they were washed in PBS. Whole-
1152 brain tissue was directly, or in the case of embryonic tissue after embedding in 5%
1153 agar, sectioned at 50 µm on a vibrating microtome (VT1000S; Leica Microsystems).
1154 All sections were pre-incubated in 10-20% normal donkey serum (NDS; Vector
1155 Laboratories) or normal goat serum (NGS; Vector Laboratories) in PBS for more than
1156 1h at RT. GFP^+ ($T\alpha1^+$) and $TdTomato^+$ ($T\alpha1^-$) progenitors and neurons were often
1157 visualized without antibody-mediated augmentation of fluorescence but in rare cases
1158 the endogenous fluorescence was boosted with antibodies against GFP (1:1000,
1159 chicken, Aves Labs, CAT#:GFP-1020, RRID:AB_10000240) or TdTomato (1:1000;
1160 rat; anti-RFP; Chromotek, CAT#:5f8-100, RRID:AB_2336064) and Goat-Anti-
1161 chicken AlexaFluor488 (1:500; Life Technologies, CAT#:A11039,
1162 RRID:AB_142924) and Goat-Anti-rat AlexaFluor555 (1:500; Life Technologies,
1163 CAT#:A-21429, RRID:AB_2535850). In embryonic tissue antibody labeling was
1164 used to label pH3 in neural progenitors (1:500; rabbit; Millipore, CAT#:06-570,
1165 RRID:AB_310177). Adult tissue was either co-stained in 1:100,000 DAPI in PBS to
1166 facilitate the delineation of brain structures or was labeled for MOR (1:3000, goat,
1167 ImmunoStar CAT#:24216, RRID:AB_572251) or CTIP2 (1:1000, rat: Abcam
1168 Cat#:ab14865, RRID:AB_2064130) and PPE (1:1000, rabbit, LifeSpan Biosciences
1169 Cat#:LS-C23084, RRID:AB_902714). PPE staining was facilitated through antigen
1170 retrieval by heating sections at 80° C in 10 mM sodium citrate (pH 6.0) for
1171 approximately 30 min prior to incubation with 1:1000 rabbit anti-PPE in PBS-Tx and
1172 1% NDS overnight at 4°C, after which the reaction was revealed by incubating with
1173 1:500 donkey-anti-rabbit AlexaFluor647 (1:500, Life Technologies CAT#:A31573,
1174 RRID:AB_2536183) in PBS-Tx for 2 h at RT.
1175
1176 Stereology and analysis of tissue
1177 Fluorescence images were captured with a LSM 710 confocal microscope using ZEN
1178 software (Zeiss, RRID:SCR_013672) or Leica DM5000B epifluorescence microscope
1179 using Openlab software (PerkinElmer, RRID:SCR_012158). Counting of labeled
1180 GFP^+ and $TdTomato^+$ progenitors and young neurons and assessing their location
1181 within the embryonic brain was performed using ImageJ (RRID:SCR_003070) on z-
1182 stacks of ~40 µm thickness. In embryonic tissue occasionally yellow cells could be
1183 seen which were counted as GFP^+ and were assumed to have undergone
1184 recombination relatively recently. Positive cells had a fluorescence signal that was at
1185 least twice the background fluorescence (measured from randomly selected regions of
1186 the tissue). X- and y-coordinates labeled cells were used to calculate the distance from
1187 the ventricle and spread. Counting of progenitor cell basal processes was performed in
1188 z-stack projections of confocal stacks of ~40 µm thickness. All clearly delineated
1189 processes above the SVZ and extending towards the pial surface were counted. M-
1190 phase reentry after IUE for aIP and OP was estimated from co-labeling of cells with
1191 the mitotic marker pH3 in tissue fixed with varying time-delays after IUE of Tα1-Cre
1192 and CβA-FLEx plasmids (Stancik *et al.*, 2010). Localizing GFP^+ and $TdTomato^+$

progenitors and young neurons in various sub regions of the LGE was performed using a combination of anatomical landmarks (Schambra & Schambra, 2008) and previous delineations (Flames *et al.*, 2007). Olfactory bulb analysis was performed using a total of 5 brains and all GFP or TdTomato positive cells were counted in z-stacks of ~40 µm thickness.

Progenitor derived neuron counting and analysis was performed similar to (Garas *et al.*, 2016; Garas *et al.*, 2018). In brief, a version of design-based stereology, the 'modified optical fractionator' was used to generate unbiased cell counts and map distributions of striatal neurons in rostral, middle and caudal sections (Franklin & Paxinos, 2008). Once the chosen striatal coronal planes were identified and the immunofluorescence protocol carried out, the dorsal striatum was delineated using a Zeiss Imager M2 epifluorescence microscope (Carl Zeiss, AxioImager.M2) equipped with a 20X (Numerical Aperture = 0.8) objective and StereoInvestigator v9.0 software (MBF Biosciences). Imaging was subsequently performed by capturing a series of completely tessellated, z-stacked images (each 1 µm thick) at depths from 2 to 12 µm from the upper surface of each section at the level of the striatum (thereby defining a 10 µm-thick optical disector). As counts were performed across the entirety of the dorsal striatum within a given rostro-caudal plane, the grid size and counting frame were set to the same size of 420 x 320 mm. To minimize confounds arising from surface irregularities, neuropil within a 2 µm 'guard zone' at the upper surface was not imaged. A neuron was counted if the top of its nucleus came into focus within the disector. If the nucleus was already in focus at the top of the 10 µm -thick optical disector the neuron was excluded. Normalised positions were calculated as described in (Garas *et al.*, 2016; Garas *et al.*, 2018). Mediolateral and dorsoventral bias within each individual section was assessed by computing a Wilcoxon Sign rank test on the positions of all neurons across or within groups to test whether they significantly differed from zero (minimum 8 neurons for a given section). Mediolateral and dorsoventral positions of red and green neurons across animals were compared by computing a Wilcoxon sign rank test on the normalised position in each direction for each section, when there were a minimum of 8 neurons of each type in a single section (n = 20 sections).

DAB-immunoreactive neurons were visualized on a brightfield microscope and were reconstructed and analyzed using Neurolucida and Neuroexplorer software (MBF Bioscience, RRID:SCR_001775). Only labeled neurons that exhibited a full dendritic arbor were included for analysis e.g. cells with clear truncations were not included in the dataset. Scholl analysis and polarity analysis was performed using standard procedures. In brief, both Scholl and polarity plots were generated for individual SPNs by calculating the total dendritic length located within 10° segments with increasing distance from the soma. The dendritic lengths were subsequently normalised for an individual SPN and averaging the normalised plots of individual neurons generated final plots.

<u>Statistics</u>

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All data are presented as means ± SEM. The 'n' refers to the number of brains (Figure 1 and 2) or neurons (Figure 3 – 5) tested. Statistical tests were all two-tailed and performed using SPSS 17.0 (IBM SPSS statistics, RRID:SCR_002865) or GraphPad Prism version 5.0 (GraphPad software, RRID:SCR_002798). Synaptic connectivity ratios were compared with Fisher's Exact test. Continuous data were assessed for normality and appropriate parametric (ANOVA, paired t-test and unpaired t-test) or non-parametric (Mann-Whitney U) statistical tests were applied (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

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1246 **Tables**

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Table 1: Intrinsic membrane properties of progenitor derived SPNs

	aIP derived	OP derived	p-value	Unlabeled
Resting membrane potential (mV)	-80.25 ± 1.21	-79.10 ± 0.64	0.28	-79.35 ± 0.69
Input resistance (MΩ)	88.12 ± 6.77	80.96 ± 4.72	0.36	80.27 ± 7.53
Spike threshold (mV)	-40.93 ± 1.66	-39.98 ± 0.92	0.32	-39.47 ± 1.22
Spike rate (500pA) (Hz)	34.17 ± 2.09	30.80 ± 1.95	0.15	31.46 ± 2.42
Spike rate (400pA) (Hz)	28.58 ± 1.75	28.25 ± 1.76	0.91	27.57 ± 2.14
Spike rate (300pA) (Hz)	22.31 ± 2.71	23.23 ± 1.68	0.77	22.50 ± 2.13
Spike rate (200pA) (Hz)	14.75 ± 2.19	19.70 ± 2.20	0.20	17.78 ± 2.65
Spike rate (100pA) (Hz)	4.17 ± 0.83	13.00 ± 2.29	0.06	9.17 ± 0.83
First ISI (ms)	26.02 ± 2.99	26.92 ± 5.04	0.88	31.46 ± 4.97
Second ISI (ms)	28.08 ± 2.22	27.64 ± 1.88	0.89	32.92 ± 3.89
Third ISI (ms)	32.74 ± 2.63	32.85 ± 2.26	0.98	33.50 ± 2.90
Fourth ISI (ms)	34.09 ± 2.56	35.17 ± 2.36	0.78	34.98 ± 2.61
First spike amplitude (mV)	87.11 ± 3.81	85.76 ± 2.76	0.78	94.13 ± 2.53
Second spike amplitude (mV)	73.13 ± 4.79	65.54 ± 3.42	0.22	81.33 ± 3.34
First spike duration (ms)	2.17 ± 0.21	1.90 ± 0.09	0.18	1.69 ± 0.13
Second spike duration (ms)	2.68 ± 0.28	2.54 ± 0.18	0.67	2.09 ± 0.17

Data are given as mean ± SEM, statistical comparsions by t-test or Mann-Whitney U test

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Table 2: Cortical synaptic response properties

	aIP derived	OP derived	p-value	Unlabeled
Amplitude (pA)	1.70 ± 0.44	2.08 ± 0.62	0.77	1.50 ± 0.33
Duration (ms)	143.9 ± 14.48	99.30 ± 10.47	0.03	112.81 ± 11.76
Rise time (ms)	5.73 ± 0.42	5.87 ± 0.95	0.83	4.47 ± 0.29
Decay time (ms)	66.20 ± 6.98	44.63 ± 5.32	0.03	55.86 ± 5.90

Data are given as mean ± SEM, statistical comparsions by Mann-Whitney test.

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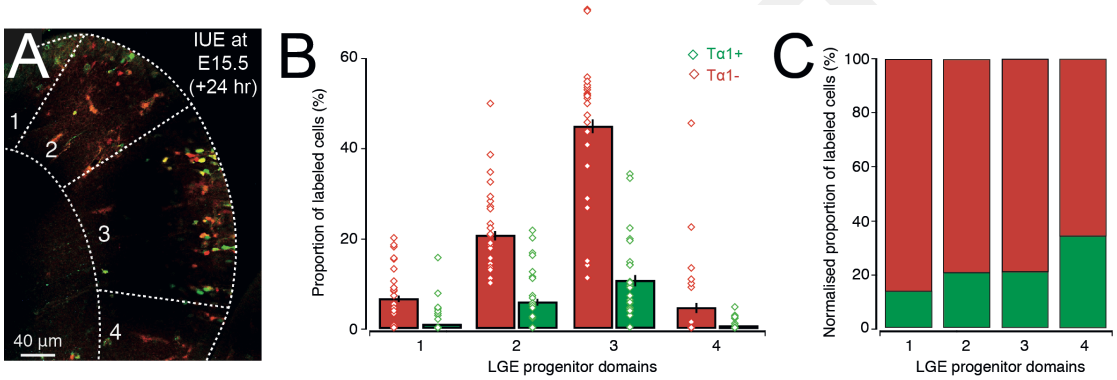
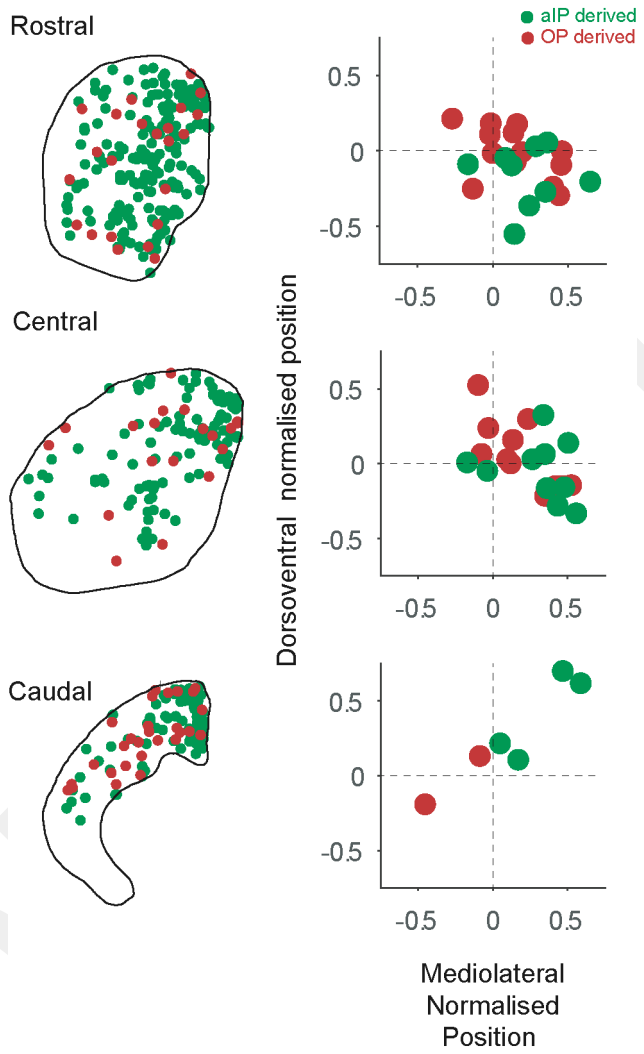
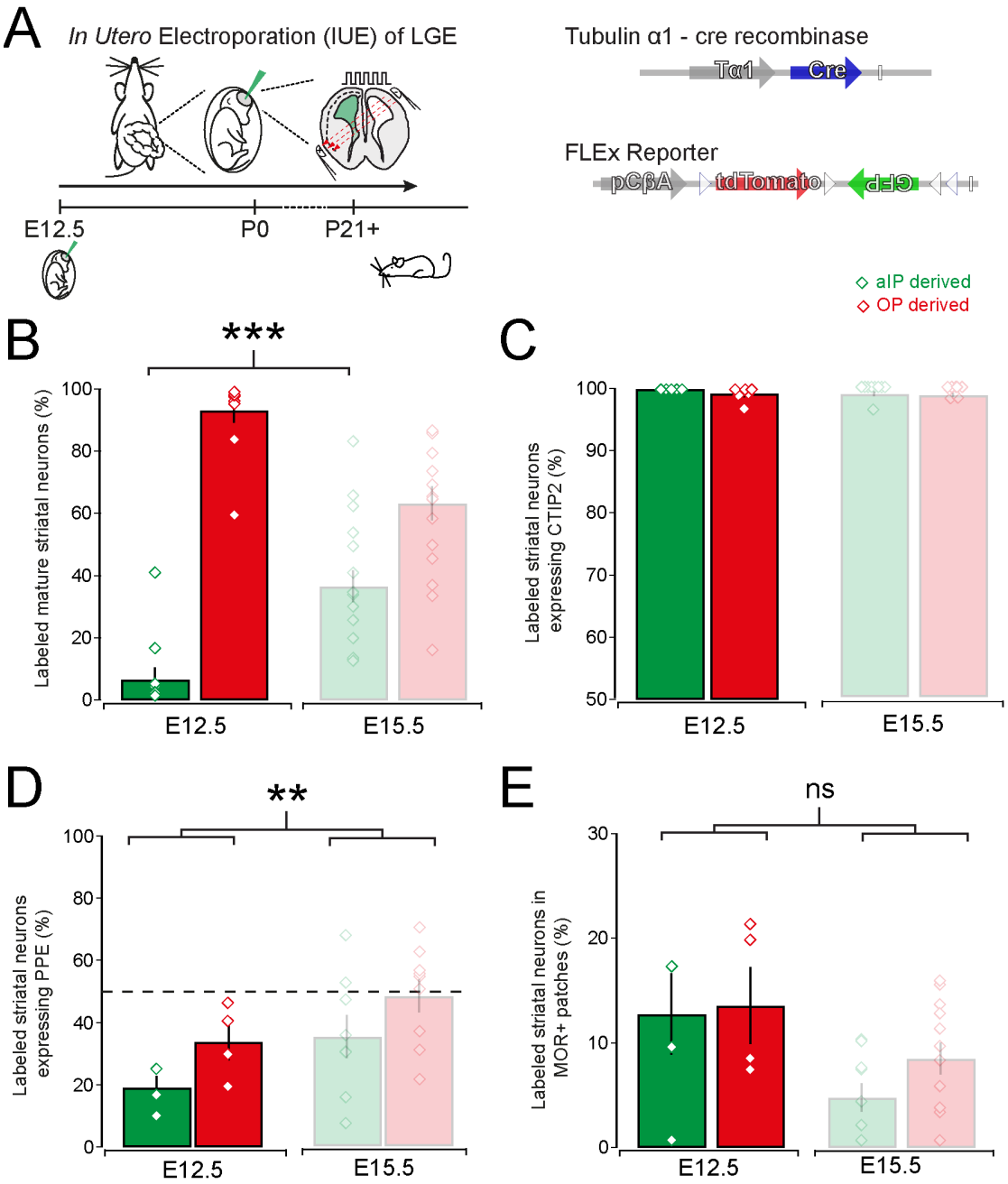
Supplementary Figures



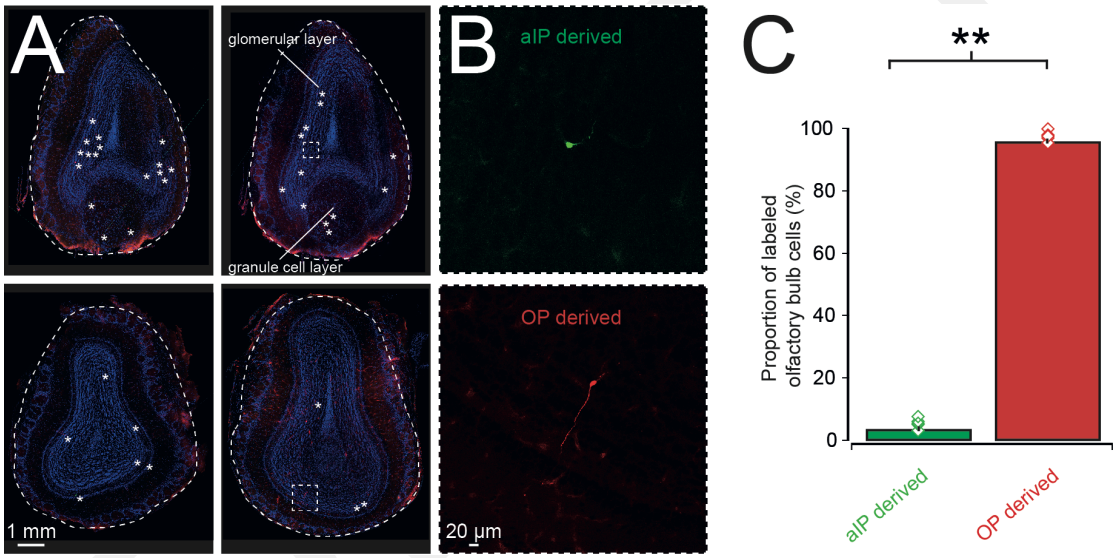
Supplementary Figure 1: IUE labeled cells can be found in all domains of the LGE. (**A**) Example coronal section of an embryonic mouse brain 24 hours after IUE at E15.5 with Tα1-cre and FLEx plasmids. Note that GFP$^+$ and TdTomato$^+$ labeled cells can be seen in all progenitor domains of the LGE. (**B**) Average barplot of the proportion of Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ labeled cells in the different progenitor domains of the LGE. Note that our labeling strategy predominantly targets LGE3 (all labeled neurons: LGE1: 7.6%, LGE2: 32.0%, LGE3: 59.0% and LGE4: 1.3%) and that each progenitor domain of the LGE contains both Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ progenitors. (**C**) The relative proportion of Tα1$^+$/GFP$^+$ and Tα1$^-$/TdTomato$^+$ labeled cells appears relatively constant between the different domains of the LGE.



Supplementary Figure 2: Labeled aIP and OP derived striatal neurons in rostral, central and caudal striatum. Left column, positions of aIP and OP derived neurons in rostral, central and caudal sections of a single animal. Right column, normalised positions of aIP and OP derived neurons in each plane (rostral n = 13, central n = 11, caudal n = 4 mice).



Supplementary Figure 3: Labeled aIP and OP derived striatal neurons after IUE during early stages of neurogenesis. (**A**) Animals that underwent IUE at E12.5 with Tα1-cre and FLEx reporter plasmids were left to mature untill young adulthood (P21+) (**B**) Significantly more aIP derived neurons are generated using IUE at E15.5 than at E12.5 (E12.5: 6.6 ± 4.0% and E15.5: 36.7 ± 5.5%, Mann-Whitney test, p=0.0002, n = 10 and 16 brains). (**C**) IUE at both E12.5 and E15.5 generate mostly striatal CTIP$^+$ neurons. (**D**) There is no significant difference in the production of PPE$^+$/D2 SPNs by aIP or OP progenitors at E12.5 or E15.5. However, grouping all labeled neurons together at E12.5 a smaller proportion of PPE$^+$/D2 SPNs is labeled at this early stage of neurogenesis (E12.5 vs. E15.5, Mann-Whitney test; p = 0.006; n = 4 and 9 brains). (**E**) There is a trend towards more labeled neurons in the patch compartments after IUE at E12.5 (E12.5: 12.4 ± 2.4% vs. E15.5: 6.9 ± 1.3% in MOR+ patches; Mann-Whitney test, p = 0.170, n = 4 and 12).



Supplementary Figure 4: IUE of LGE neural progenitors also labels a small number of olfactory bulb cells. (**A**) Example images of 4 different olfactory bulbs with labeled neurons indicated by asterisks. (**B**) High magnification image of an aIP derived cell (top) and an OP derived cell (bottom) in the olfactory bulb. (**C**) The majority of labeled cells in the olfactory bulb are OP derived (aIP derived: 3.9 ± 1.6% and OP derived: 96.1 ± 1.6%, p=0.0079, Mann-Whitney test, n = 5 brains).