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Telencephalic neural precursor cells show transient competence to interpret the dopaminergic niche of the embryonic midbrain

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ABSTRACT

Neural Precursor Cells (NPCs) generate complex stereotypic arrays of neuronal subtypes in the brain. This process involves the integration of patterning cues that progressively restrict the fate of specific NPCs. Yet the capacity of NPCs to interpret foreign microenvironments during development remains poorly defined. The aim of this work was to test the competence of mouse telencephalic NPCs to respond to the dopaminergic niche of the mesencephalon. Telencephalic NPCs isolated from midgestation mouse embryos (E10.5) and transplanted to age-matched mesencephalic explants efficiently differentiated into neurons but were largely unable to produce midbrain dopaminergic (mDA) neurons. Instead, E10.5 telencephalic NPCs behaved as restricted gabaergic progenitors that maintained ectopic expression of *Foxg1* and *Pax6*. In contrast, E8.5 telencephalic NPCs were able to differentiate into $Lmx1a^{+}/Foxa2^{+}/TH^{+}$ neurons in the dopaminergic niche of the mesencephalic explants. In addition, these early telencephalic NPCs showed region-dependent expression of *Nkx6.1*, *Nkx2.2* and site-specific differentiation into gabaergic neurons within the mesencephalic tissue. Significant dopaminergic differentiation of E8.5 telencephalic NPCs was not observed after transplantation to E12.5 mesencephalic explants, suggesting that inductive signals in the dopaminergic niche rapidly decay after midgestation. Moreover, we employed transplantation of embryonic stem cells-derived precursors to demonstrate that extinction of inductive signals within the telencephalon lags behind the commitment of residing NPCs. Our data indicate that the plasticity to interpret multiple instructive niches is an early and ephemeral feature of the telencephalic neural lineage.

Introduction

Neural Precursor Cells (NPCs) give rise to a wide diversity of neuronal and glial subtypes during embryogenesis. This process occurs in stereotyped spatiotemporal patterns. Thus, according to the position in the embryo, NPCs limit their choices to certain neuron types and, expectedly, greater restrictions are imposed on NPCs as development proceeds. It is known that combinations of intrinsic and extrinsic factors define the fate of NPCs (Anderson, 2001; Jessell, 2000). The differentiation potential of NPCs is an intrinsic property that dynamically changes as cells develop. Expression of specific markers, mainly transcription factors, is an indication that the NPCs are being specified according to the embryonic region in which they reside; however, this is not evidence of commitment. In order to determine NPC plasticity and commitment, it is necessary to assess NPC behavior in a context different to that found in their natural niche. Commonly, these evaluations are performed by transplanting NPCs or embryo fragments into a region capable of promoting specific differentiation (Anderson, 2001; Baizabal et al., 2003). Conversely, the instructive capacity of a niche is evaluated by transplanting competent cells to the region of interest at different developmental stages.

NPCs emerge during induction of the neural plate around day 7 of embryonic development (E7) in mice (Wood and Episkopou, 1999). The most anterior part of the neural plate is then specified to become the telencephalon around E8.5. This event is marked by the presence of the forkhead transcription factor Foxg1 (previously known as Bf1) in the prospective telencephalic region (Shimamura and Rubenstein, 1997; Tao and Lai, 1992b). Genetic studies have implicated *Foxg1* as a critical determinant of ventral telencephalic fate (Dou et al., 1999; Martynoga et al., 2005; Shimamura and Rubenstein, 1997). After closure of the neural tube (NT), the telencephalon becomes regionalized in dorsal and ventral domains; the former giving rise to the cerebral cortex, while the latter produces the medial, lateral and caudal ganglionic eminences. Most NPCs in the dorsal telencephalon differentiate into pyramidal glutamatergic neurons, whereas ventral telencephalic NPCs generate several subtypes of gabaergic interneurons (Hebert and Fishell, 2008). Cortical inhibitory interneurons are also generated within the ganglionic eminences, a process that involves migration from ventral to dorsal telencephalic regions (Nery et al., 2002; Wichterle et al., 2001). Despite this well-defined pattern of differentiation, it is not known when telencephalic NPCs become committed to particular fates and how this event is coordinated with the signals that control telencephalic specification.

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In the mesencephalon (i.e. the embryonic midbrain), the region surrounding the ventral midline is the niche where NPCs become specified into midbrain dopaminergic (mDA) neurons. This event is molecularly defined as the beginning of *Lmx1a* expression (E9), which actively represses alternative ventral fates (Andersson et al., 2006). The Lmx1a$^+$ dopaminergic domain forms the medial region of the floor plate (FP) (Kittappa et al., 2007; Ono et al., 2007), the organizer that patterns the ventral NT through the production of the morphogen sonic hedgehog (Shh; Lupo et al., 2006). Interestingly, it was demonstrated that *Foxa2* expression, a known characteristic of FP, is a requirement for the proper dopaminergic differentiation in the ventral mesencephalon (Ferri et al., 2007). Foxa2 positively regulates Lmx1a in the ventral midline and, subsequently, both transcription factors cooperatively determine mDA fate through activation of common downstream target genes like *Ngn2* and *Nurr1* (Lin et al., 2009; Nakatani et al., 2009). In addition, Lmx1a activates *Msx1/2* expression (Andersson et al., 2006) whereas Foxa2 positively regulates *En1* in immature mDA neurons and tyrosine hydroxylase (TH) in post-mitotic mDA neurons (Ferri et al., 2007). Generation of TH$^+$ mDA neurons initiates around E10.5 and peaks by E12/13 (Bayer et al., 1995). At present, it is unknown whether NPCs residing outside of the embryonic midbrain have the potential to interpret the mesencephalic niche of dopaminergic neurogenesis at some point in development.

In a recent work, we used mesencephalic explant cultures to test the developmental potential of NPCs derived from Embryonic Stem Cells (ESCs) (Baizabal and Covarrubias, 2009). We found that only those ESC-derived NPCs generated within the context of the mesencephalic niche give rise to significant numbers of mDA neurons. In contrast, ESC-derived NPCs generated in a culture dish and then transplanted to mesencephalic explants, do not produce large numbers of mDA neurons. These data suggested that NPCs are able to interpret patterning cues of neuronal specification only during early stages of maturation within the neural lineage. To test this hypothesis, in this study we isolated telencephalic NPCs at different stages of development and then transplanted these cells to mesencephalic explants. We present evidence that telencephalic NPCs have the capacity to differentiate into mDA-like neurons upon incorporation into the dopaminergic niche of the mesencephalon. Ectopic differentiation of telencephalic NPCs was restricted to a short developmental window at stages prior to the onset of neurogenesis in the embryonic forebrain (i.e. between E8.5 and E10.5). Furthermore, E8.5 telencephalic NPCs acquired the correct positional identity within mesencephalic domains of gabaergic neurogenesis. Our data also suggest that the instructive mesencephalic niche swiftly decays beyond midgestation. Finally, we show that commitment of telencephalic NPCs is apparently not synchronized to the extinction of telencephalic gabaergic cues.

Materials and methods

Collagen explant culture

Mesencephalic explants were obtained from either E10.5 or E12.5 CD-1 mice embryos and cultivated in a collagen matrix as previously described (Baizabal and Covarrubias, 2009). Briefly, mesencephalons were isolated by two coronal cuts: one along the mesencephalic–diencephalic border and other at the level of the rhombic lip. Subsequently, mesencephalic explants were cut along the dorsal midline, transferred to 35 mm Petri dishes, and placed in the "open book" configuration with the ventricular surface facing upwards. Media was removed as far as possible and approximately 40–60 µl of collagen mixture was added over each explant. The collagen mixture contained: rat collagen (100 µl; gift from Dr. Alfredo Varela-Echavarría), 1.5 M NaCl (10 µl), 7.5% $NaHCO_3$ (10 µl) and explant media (300 µl). Explant media (hereafter referred as Optimix) was prepared by mixing Optimem with Glutamax (72% v/v; Gibco), DMEM-F12 (25% v/v; Gibco), glucose 2 M (2% v/v; Sigma) and GPS 1X (glutamax/penicillin/streptomycin; Gibco). Explants were incubated for 1 h at 37 °C in 5% CO_2–95% atmospheric air incubators to allow collagen polymerization before proceeding to cell transplantation (see below). Ventral telencephalic explants were embedded in collagen as described above for mesencephalic explants.

Cell transplantation

This procedure was essentially as previously described (Baizabal and Covarrubias, 2009). Telencephalic NPCs were isolated from a transgenic mouse line (gift from Andras Nagy) that constitutively produces Green Fluorescent Protein (GFP). The ventral regions of 8–12 telencephalons were dissected from E10.5 mouse embryos and treated for 10 min at 37 °C with 0.1% trypsin (Gibco) diluted in Versene (EDTA 0.02%; Gibco). Trypsin was then inactivated with Optimix containing 10% Fetal Bovine Serum (FBS; Gibco). The tissues were mechanically dissociated with a serological pipette, and centrifuged at 3000 rpm for 5 min. The resulting pellet was resuspended in 5–20 µl of Optimix. Early NPCs were isolated from E8.5 prospective telencephalons by directly suctioning the anterior-most region of the neuroepithelium. Cell dissociation was carried out by treating tissue fragments for 7 min at 37 °C with 0.025% trypsin diluted in Versene. Trypsin was then inactivated and the cell suspension concentrated as described above. In some cases, trypsin dissociation was not carried out in order to evaluate the rosette-forming capacity of transplanted telencephalic cells. For transplantation, a flame-stretched Pasteur pipette coupled to a hose with a mouthpiece was used to deposit donor cells over the ventricular surface of collagen-embedded explants (see above). Transplanted cells were allowed to attach for 3 h at 37 °C before adding Optimix to completely cover the tissues. To achieve a higher efficiency of transplanted cells around the mesencephalic ventral midline, a modification of the described protocol was used. Briefly, mesencephalic explants were laid over Millicell culture plate inserts (Millipore) and donor cells were deposited over the ventral midline. Culture plate inserts were transferred to 35 mm Petri dishes containing 2 ml of Optimix and explants were incubated overnight to allow attachment of donor cells. The next day, mesencephalic explants were transferred to clean 35 mm Petri dishes and cultivated in collagen as described above. Collagen explants were maintained at 37 °C in humidified 5% CO_2–95% atmospheric air incubators for 7 days. Half of the media was replaced every 2 days.

Embryonic stem cells culture and differentiation

The R1B5 line of mouse ESCs (gift from Andras Nagy) which constitutively produces GFP was propagated and differentiated into Embryoid Body (EB) precursors as previously described (Baizabal and Covarrubias, 2009). Day-4EB cells were dissociated for 15 min at 37 °C with 0.25% trypsin diluted in Versene. Trypsin was then inactivated as described above and EB precursors were mechanically dissociated with a serological pipette. The cell suspension was centrifuged at 3000 rpm for 5 min and concentrated in 40–80 µl of Optimix for transplantation to collagen explants of the ventral telencephalon.

Tissue processing and immunofluorescence

Collagen explants were fixed and processed for cryo-sectioning as previously described (Baizabal and Covarrubias, 2009). Primary antibodies were used to detect Nestin (1:500; mouse), β-III Tubulin (1:100; mouse), NeuN (1:400; mouse), GAD65/67 (1:500; rabbit) and TH (1:500; rabbit), all from Chemicon; β-III Tubulin (1:2000; rabbit; Covance), Lmx1a (1:1000; rabbit; gift from M. German), En1, Nkx6.1, Nkx2.2, Msx1/2, Pax6 (supernatants 1:2; mouse), all from Developmental Studies Hybridoma Bank; Foxg1/Bf1 (1:100; rabbit; Abcam),