NEURAL REGENERATION RESEARCH
November 2017, Volume 12, Issue 11
www.nrronline.org

RESEARCH ARTICLE

Nigral dopaminergic neuron replenishment in adult mice through VE-cadherin-expressing neural progenitor cells

Abir A. Rahman[1], Nathan K. Lai[2], Joshua E. Albright[2], Paige E. Urquhart[2], Abby R. Webb[2], Brad E. Morrison[1, 2, *]
1 Department of Biological Sciences, Boise State University, Boise, ID, USA; Biomolecular Ph.D. Program, Boise State University, Boise, ID, USA
2 Department of Biological Sciences, Boise State University, Boise, ID, USA

How to cite this article: *Rahman AA, Lai NK, Albright JE, Urquhart PE, Webb AR, Morrison BE (2017) Nigral dopaminergic neuron replenishment in adult mice through VE-cadherin-expressing neural progenitor cells. Neural Regen Res 12(11):1865-1869.*
Funding: *Student support was provided by the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants No. P20GM103408 and P20GM109095. Additional support was provided by The Biomolecular Research Center at Boise State University.*

Abstract
The function of dopaminergic neurons in the substantia nigra is of central importance to the coordination of movement by the brain's basal ganglia circuitry. This is evidenced by the loss of these neurons, resulting in the cardinal motor deficits associated with Parkinson's disease. In order to fully understand the physiology of these key neurons and develop potential therapies for their loss, it is essential to determine if and how dopaminergic neurons are replenished in the adult brain. Recent work has presented evidence for adult neurogenesis of these neurons by Nestin^{+}/Sox2^{-} neural progenitor cells. We sought to further validate this finding and explore a potential atypical origin for these progenitor cells. Since neural progenitor cells have a proximal association with the vasculature of the brain and subsets of endothelial cells are Nestin^{+}, we hypothesized that dopaminergic neural progenitors might share a common cell lineage. Therefore, we employed a *VE-cadherin promoter-driven CRE^{ERT2}:TH^{lox}/TH^{lox}* transgenic mouse line to ablate the *tyrosine hydroxylase* gene from endothelial cells in adult animals. After 26 weeks, but not 13 weeks, following the genetic blockade of *tyrosine hydroxylase* expression in VE-cadherin^{+} cells, we observed a significant reduction in *tyrosine hydroxylase*$^{+}$ neurons in the substantia nigra. The results from this genetic lineage tracing study suggest that dopaminergic neurons are replenished in adult mice by a VE-cadherin^{+} progenitor cell population potentially arising from an endothelial lineage.

Key Words: *nerve regeneration; neural progenitor cells; adult neurogenesis; endothelial cells; substantia nigra; dopaminergic neurons; Parkinson's disease; mouse model*

Correspondence to:
Brad E. Morrison, Ph.D., bradmorrison@boisestate.edu.

orcid:
0000-0002-6356-7149 (Brad E. Morrison)

doi: *10.4103/1673-5374.219050*

Accepted: *2017-09-23*

Introduction
The existence of adult neurogenesis for dopaminergic (DA) neurons is controversial. However, a major limitation of previous studies is their reliance upon nucleotide analog, such as bromodeoxyuridine (BrdU), incorporation to identify replicating neurons (Kay and Blum, 2000; Zhao et al., 2003; Frielingsdorf et al., 2004; Aponso et al., 2008). This approach presumes that DA neurons in adult animals are generated in a replication-dependent manner from neural progenitor cells (NPCs). Recent studies have demonstrated in multiple animal systems, including mice and non-human primates, that quiescent, nonreplicative neurogenesis occurs for some neural populations within the adult brain (Tandé et al., 2006; Nishimura et al., 2011; Barbosa et al., 2015; Fuentealba et al., 2015). Additionally, previous studies that have focused on DA adult neurogenesis employed relatively short labeling and post-labeling periods, thereby decreasing the ability to detect slow regeneration rates (Zhao et al., 2003; Frielingsdorf et al., 2004; Arias-Carrión et al., 2009). To avoid these potential pitfalls, we have established a transgenic mouse system that utilizes genetic cell lineage tracing observed over a 26 week period.

Neurons are believed to arise from ectodermal tissue during embryonic development, while endothelial cells likely arise from the mesoderm. However, a strong connection exists among vascular physiology, NPCs, and Parkinson's disease (PD). NPCs in the adult brain have been reported to reside and differentiate in close association with capillaries, suggesting an intimate relationship with endothelial cells (Siegenthaler and Pleasure, 2010). One unexplored possibility, supported by mounting evidence, is that specialized endothelial cells give rise to NPCs. For example, a number of factors associated with endothelial physiology (e.g., VEGF and endothelin) have robustly influenced outcomes in pre-clinical models of neurodegenerative disease (Wang et al., 2007; Kirby et al., 2015). Remarkably, similar to DA neurons, subsets of endothelial cells have been demonstrated to produce and respond to dopamine (Basu et al., 2001; Sorriento et al., 2012). In addition, when co-cultured with endothelial cells, it has been reported that mouse NPCs differentiate into endothelial cells (~6% rate) capable of forming capillary networks, which blurs the lines between these cell types (Wurmser et al., 2004).

NPCs express a number of endothelial cell markers and

share common niches within the brain. For instance, Pramel7 was recently identified as a marker and mediator for a pre-implantation embryonic stem cell pluripotency ground state that has limited replicative self-renewal capacity (Graf et al., 2017). *In situ* RNA hybridization data on adult mouse brains available from the Allen Institute for Brain Science indicates that Pramel7 is expressed in the meninges, most likely in endothelial cells (Lein et al., 2007). Moreover, the greatest concentration of positive signal arises from the meninges immediately ventral to the substantia nigra (SN). This is particularly interesting given that Bifari et al. (2016) recently reported that quiescent NPCs generated during embryogenesis migrate from the meninges to differentiate into cortical neurons without replication in adult mice. Previous work has also shown that DA neurons in the SN express IL-13RA1, a histological marker for endothelial cells (Morrison et al., 2012). Additionally, the discovery of a $Nestin^{+}/Sox^{2-}$ DA NPC population in adult mice reported by Albright et al. (2016) may indicate an atypical origin for these cells since Sox2 is broadly reported as a canonical marker for NPCs. $Nestin^{+}/Sox^{2-}$ cells ($NeuN^{-}$; non-neural) were also described by Hendrickson et al. (2011) and reside in a satellite position directly neighboring mature neurons in the adult rat brain. This suggests that these cells may represent previously uncharacterized NPCs (Hendrickson et al., 2011). In addition, $Nestin^{+}$ endothelial cells are found throughout the body (Suzuki et al., 2010). Whether there is a connection between $Nestin^{+}/Sox^{2-}$ DA NPCs and $Nestin^{+}$ endothelial cells, Nestin+ endothelial precursor cells, or $Nestin^{+}$ mesenchymal stem cells (Pacini and Petrini, 2014) that are known to regulate endothelial progenitor cell differentiation and which might directly transdifferentiate into endothelial cells (Xie et al., 2015), could be fundamental toward understanding DA neurogenesis. We therefore seek to determine if adult DA NPCs are derived from an endothelial cell lineage.

Materials and Methods

Experimental animals

Procedures and husbandry for studied animals were performed under Boise State University and Boise Veterans Affairs Medical Center Institutional Animal Care and Use Committee guidelines (Approval number: 006-AC15-018) and in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). The TH^{lox} mouse line (Jackson et al., 2012) used in this study was a kind gift from Dr. Martin Darvas and Dr. Richard Palmiter (The University of Washington). The *VE-cadherin-CRE*^{ERT2} mice were generously provided by Dr. Luisa Iruela-Arispe (The University of California, Los Angeles, USA). During the course of this study, all mice were of a C57Bl/6 lineage that were provided food *ad libitum* and housed with 12-hour day/night cycles. Tamoxifen treatment was administered to activate *TH* gene excision in 3-month-old mice (male average weight = 25.2 ± 2.3 g; female average weight = 20.1± 1.7 g) *via* standard rodent chow infused with 400 mg tamoxifen citrate (Envigo, Huntingdon, Cambridgeshire, U.K.; TD.130860) per kg of chow, which was provided as the only food source for 6 weeks. Following treatment, mice were returned to a standard chow diet. Male and female mice were utilized in all groups at approximately equal ratios. We did not observe any sex-linked variation in DA neurogenesis (data not shown).

Tissue processing and immunohistochemistry

Mice were anesthetized by isoflurane (Piramal, Bethlehem, PA, USA) inhalation and transcardially perfused with a 50 mL phosphate buffer (PB; pH 7.2) containing heparin sodium salt (20 units/mL) followed by 50 mL of 4% paraformaldehyde (PFA) in PB solution. Mouse brains were collected and placed in 4% PFA/PB overnight at 4°C. The following day, the brains were placed in 30% sucrose/PB at 4°C until they sunk (~72 hours). Tissue was then rapidly frozen in optimal cutting temperature (OCT) compound and stored at –80°C until immunohistochemistry (IHC) was performed.

For IHC, OCT-embedded brains were equilibrated to a cryostat (Leica CM1950) at –20°C overnight. Sections were then cut at a thickness of 35 μm, placed into 12 well plates containing PB, and processed by free-floating IHC. Endogenous horseradish peroxidase (HRP) activity was quenched with a 3% H_2O_2/10% methanol/PB solution incubated for 15 minutes at room temperature. Sections were then blocked and permeabilized with 0.5% bovine serum albumin (BSA)/0.25% Triton X100/PB. Rabbit anti-tyrosine hydroxylase (TH) antibody (Millipore; AB152) was used at 1:2,000 in 0.5% BSA/PB, 4°C overnight. A biotinylated goat anti-rabbit secondary antibody (JacksonImmuno Research, West Grove, PA, USA; BA-1000) solution (1:500) was then added and incubated at room temperature for 1 hour. Next, streptavidin-HRP was added according to manufacturer instructions (Vector Labs, Burlingame, CA, USA; PK-6200). Wash steps were performed using PB. TH labeling was visualized using 50 mg/mL 3,3′-diaminobenzidine tetrahydrochloride hydrate (DAB; VWR cat # AAJ62216-09) in PB. Sections were dried overnight, placed on slides, and then coverslips were mounted with Vectamount (Vector Labs; H-5000).

Quantification of DA neurons in the SN

DAB-labeled DA neurons were visualized by bright-field microscopy (OMAX microscope, Gyeonggi-do, Korea). For counting purposes, the left hemisphere was marked by piercing with a 20-gauge needle prior to sectioning the SN. Each tissue section containing SN from the right brain hemisphere was quantified following IHC. The SN was represented in an average of 38.9 sections/hemisphere across all samples. DA neuron counts included the substantia nigra pars compacta and excluded TH^{+} neurons located in the adjoining ventral tegmental area.

Isolation of endothelial cells and assessment of TH excision

Primary mouse endothelial cells were obtained by magnet-

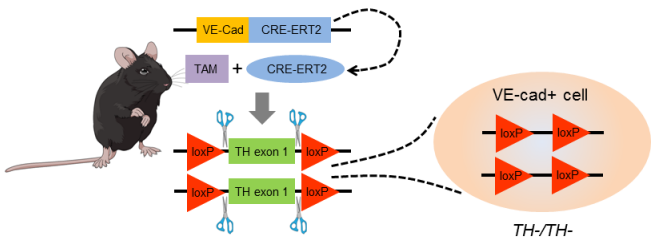

Figure 1 Inducible transgenic mouse model for adult dopaminergic neurogenesis assessment.
Three-month-old mice expressing CRE-ERT2 under the control of a VE-cadherin promoter were given tamoxifen (TAM)-laden chow, resulting in excision and silencing of the loxP-containing tyrosine hydroxylase (TH) gene (homozygous) in VE-cadherin-positive cells.

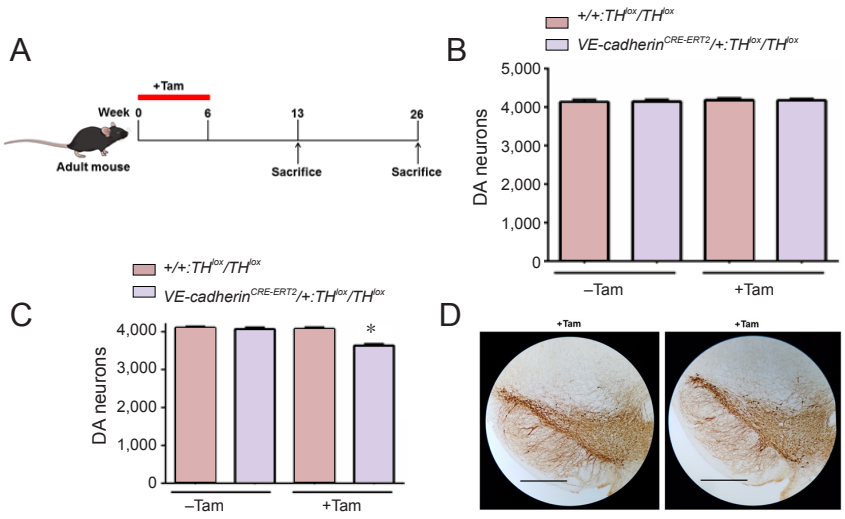

Figure 2 Adult nigral dopaminergic (DA) neurogenesis by VE-cadherin^{+} progenitor cells.
Tamoxifen (Tam) citrate-laden chow (400 mg/kg food pellet) was fed ad libitum to 3-month-old transgenic mice (A). Brains were harvested, immunohistochemistry was performed for tyrosine hydroxylase (TH) immunoreactivity, and dopaminergic (DA) neurons quantified at 13 and 26 weeks following tamoxifen administration. No difference was observed among groups at 13 weeks following tamoxifen treatment (B). However, at 26 weeks post-treatment, *VE-cadherin*$^{CRE\text{-}ERT2}$*:TH*^{lox}*/TH*^{lox} mice showed significantly reduced DA neurons in the substantia nigra compared with control groups (C). Every tissue section in the right hemisphere containing substantia nigra was counted. Statistical analysis was performed using multiple-way analysis of variance in conjunction with post hoc Tukey's test (6 mice/group; error bars = SEM; $*P < 0.05$, *vs.* all other groups). Nigral DA neurons were observed by immunohistochemistry using a tyrosine hydroxylase (TH) antibody (3′-diaminobenzidine staining). Representative images are shown for mice at 26 weeks post tamoxifen treatment (scale bars: 500 μm) (D).

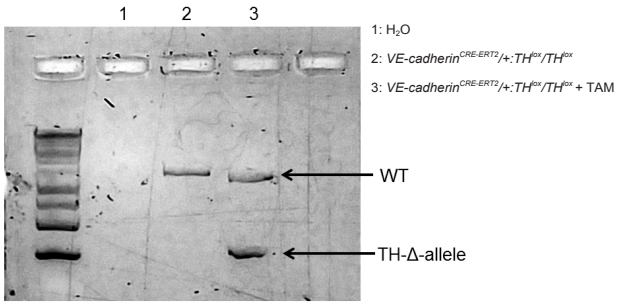

Figure 3 Endothelial tyrosine hydroxylase (TH) gene excision from *VE-cadherin*$^{CRE\text{-}ERT2}$*:TH*^{lox}*/TH*^{lox} transgenic mice treated with tamoxifen.
Endothelial cells were isolated by magnetic cell sorting (CD31-conjugated beads) from livers harvested from untreated or tamoxifen (TAM)-treated (400 mg/kg chow, 6 weeks) mice. DNA was then extracted and PCR was performed to verify excision of TH gene in endothelial cells. WT: Wild type.

ic-activated cell sorting (MACS) as previously described (Shi et al., 1999). Briefly, 6-month-old mice treated with or without tamoxifen citrate chow (400 mg/kg) for 6 weeks were sacrificed, one liver lobe harvested, and placed in DMEM on ice. Livers were then minced using sterile razor blades. Each diced liver sample was then transferred to a tube containing 100 mg type I collagenase (Rockland Immunochemical, Limerick, PA, USA; MB-118-0100) in 25 mL of HBSS (+calcium, +magnesium, +1% BSA). Tubes were incubated with occasional mixing in a 37°C water bath for 60 minutes. Samples were then filtered through a 70 μm sterile cell strainer and centrifuged at 300 × *g* for 5 minutes at 4°C. Supernatants were discarded and pellets washed once with 0.1% BSA/PBS and centrifuged at 300 × *g* for 5 minutes at 4°C. Supernatants were aspirated and pellets resuspended in 0.5% BSA/PBS with 2 mM ethylenediaminetetraacetic acid (EDTA). MACS was performed according to the manufacturer's (MiltenyiBiotec, Auburn, CA, USA) protocol using positive selection with CD31 microbeads and MS columns. Following endothelial cell isolation, DNA was extracted using a mouse tissue DNA extraction kit (Biopioneer, San Diego, CA, USA; MAQ-1). PCR was performed using the following primers: TAG GGA GAT GCC AAA GGC TA; CAG GAC CCA ACA GAA GCA TT. Thermocycling was done using the following parameters: annealing temperature = 62°C, 30 seconds; extension time = 30 seconds; cycles = 35. PCR products were labeled with SYBR safe and resolved on a 1.5% agarose gel.

Statistical analysis

DA neuron counts were analyzed for significance ($P < 0.05$) among groups using a multiple-way analysis of variance (ANOVA) in conjunction with a *post hoc* Tukey's test using GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA). Means are shown and the standard error of the mean is represented by error bars.

Results

Nigral dopaminergic NPCs express VE-cadherin

To test whether DA neurons arise from an endothelial cell lineage in adult animals, we generated the transgenic mouse shown in **Figure 1** where, in effect, the *TH* gene is being utilized as a genetic cell lineage tracing marker. A Vascular Endothelial Cadherin promoter was used to drive expression of a tamoxifen-activatable CRE recombinase (*VE-cadherin CRE^{ERT2}*) in endothelial cells (Monvoisin et al., 2006). Upon activation of CRE activity by tamoxifen treatment in adult mice (three months of age), the *TH* gene was silenced by excision in VE-cadherin positive cells. Twenty-six weeks after initiation of a 6-week tamoxifen treatment, the mice were assessed for loss of nigral DA (TH$^+$) neurons (**Figure 2A**). Interestingly, *VE-cadherin$^{CRE-ERT2}$* mice receiving tamoxifen displayed a reduction in DA neurons within the SN (**Figure 2C**). To verify whether this loss resulted from TH excision in DA progenitors or from existing mature DA neurons, we assessed DA neurons in a cohort of *VE-cadherin$^{CRE-ERT2}$:TH^{lox}/TH^{lox}* mice 13 weeks after tamoxifen administration (**Figure 2B**). We observed no loss in nigral DA neurons in this group, indicating that TH excision occurred in cells other than in mature DA neurons. In addition, we confirmed successful TH excision from endothelial cells isolated from tamoxifen treated *VE-cadherin$^{CRE-ERT2}$:TH^{lox}/TH^{lox}* mice (**Figure 3**).

Discussion

Determining whether DA neurons undergo adult neurogenesis is very important to understand fundamental brain physiology as well as develop potential therapies to combat their loss. Our current study has presented evidence in favor of dopaminergic neuron regeneration in adult mice through VE-cadherin-expressing NPCs. This finding builds upon previous work demonstrating that Nestin$^+$/Sox^{2-} NPCs in the adult mouse brain regenerate nigral DA neurons in a slow, progressive manner (Albright et al., 2016). These results are also consistent with the possibility of an endothelial cell-derived DA NPC population in adult mice.

The data presented here might also explain the mounting evidence linking DA neuron and endothelial physiology. In addition to the ability to produce and respond to dopamine, endothelial cells exhibit acute sensitivity to inflammatory response and oxidative stress (Pober and Sessa, 2007). Interestingly, oxidative stress has been shown to cause mesenchymal transdifferentiation of endothelial cells *in vivo*, providing further support for the plasticity of endothelial cells (Montorfano et al., 2014). If DA neurons share a close lineage with endothelial cells, our findings offer an explanation for the unique sensitivity of DA neurons toward oxidative and inflammatory response-based systemic insults. For example, peripheral administration of the potent oxidizing agent paraquat has been reported to cause the specific loss of nigrostriatal DA neurons (McCormack et al., 2002). In addition, systemic (intraperitoneal) administration of bacterial lipopolysaccharide twice weekly for 6 months results in chronic inflammatory response and in a selective, slow, and progressive loss of DA neurons in the SN (Qin et al., 2007; Frank-Cannon et al., 2008). Furthermore, the neurotoxic effect of MPTP on DA neurons can be completely halted with anti-inflammatory therapy (Aubin et al., 1998; Nomura et al., 2011). Similarly, NSAID use, particularly ibuprofen, has been correlated with a reduced incidence for PD suggesting the presence of an inflammatory instigator in human disease that targets these neurons (Chen et al., 2003; Gao et al., 2011). Therefore, a growing body of data indicates that DA neurons experience heightened sensitivity, compared with other neural populations, to insults that could, in part, be explained by physiology shared with endothelial cells.

Recent work by multiple groups in diverse systems has revealed the existence of replication-independent adult neurogenesis (Nishimura et al., 2011; Barbosa et al., 2015; Fuentealba et al., 2015; Bifari et al., 2016). These studies have shown that quiescent NPCs, produced and expanded during embryogenesis, directly differentiate into neurons as needed in the adult animals. If a similar mechanism is responsible for the generation of DA neurons in adults, it would provide an explanation for an inability to detect DA neurogenesis using assays that rely upon nucleotide analog incorporation (e.g., BrdU) (Frielingsdorf et al., 2004). Additionally, previous studies employing a nucleotide labeling strategy have utilized a relatively short post-labeling period before brain harvesting, ranging from 8 hours to 6 weeks (Kay and Blum, 2000; Zhao et al., 2003; Frielingsdorf et al., 2004; Aponso et al., 2008). This approach could dramatically limit the ability to detect DA neurogenesis if, as our results suggest, this process occurs at a very slow rate and is, using our method, undetectable at 13 weeks post-labeling. If a replicative population of DA NPCs exists, then it will be essential to allow enough time for differentiation into a TH$^+$ neuron to occur before assessment. Therefore, further investigations using alternative strategies, like the one demonstrated here, are warranted to identify the precise source of DA NPCs and characterize this regenerative process so that it can be harnessed for therapeutic benefit.

Acknowledgments: *The authors extend sincere thanks to Dr. Richard Palmiter and Dr. Martin Darvas (The University of Washington) for gifting the TH^{lox} mouse line as well Dr. Luisa Iruela-Arispe (The University of California, Los Angeles, USA) for providing the VE-cadherin-CRE^{ERT2} transgenic mice used in this study.*

Author contributions: *AAR and BEM designed and performed critical analysis of this study. AAR, NKL, JEA, PEU, ARW, and BEM performed experiments and analyzed experimental data. BEM wrote the manuscript. All listed authors approved this manuscript in its final form.*

Conflicts of interest: *None declared.*

Research ethics: *Procedures and husbandry for studied animals were performed under Boise State University and Boise Veterans Affairs Medical Center Institutional Animal Care and Use Committee guidelines (Approval # 006-AC15-018) and in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).*

Data sharing statement: *Datasets analyzed during the current study are available from the corresponding author on reasonable request.*

Plagiarism check: *Checked twice by iThenticate.*

Peer review: *Externally peer reviewed.*

References

Albright JE, Stojkovska I, Rahman AA, Brown CJ, Morrison BE (2016) Nestin-positive/SOX2-negative cells mediate adult neurogenesis of nigral dopaminergic neurons in mice. Neurosci Lett 615:50-54.

Aponso PM, Faull RL, Connor B (2008) Increased progenitor cell proliferation and astrogenesis in the partial progressive 6-hydroxydopamine model of Parkinson's disease. Neuroscience 151:1142-1153.

Arias-Carrión O, Yamada E, Freundlieb N, Djufri M, Maurer L, Hermanns G, Ipach B, Chiu WH, Steiner C, Oertel WH, Höglinger GU (2009) Neurogenesis in substantianigra of parkinsonian brains? J Neural Transm Suppl:279-285.

Aubin N, Curet O, Deffois A, Carter C (1998) Aspirin and salicylate protect against MPTP-induced dopamine depletion in mice. J Neurochem 71:1635-1642.

Barbosa JS, Sanchez-Gonzalez R, Di Giaimo R, Baumgart EV, Theis FJ, G?tz M, Ninkovic J (2015) Neurodevelopment. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. Science 348:789-793.

Basu S, Nagy JA, Pal S, Vasile E, Eckelhoefer IA, Bliss VS, Manseau EJ, Dasgupta PS, Dvorak HF, Mukhopadhyay D (2001) The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. Nat Med 7:569-574.

Bifari F, Decimo I, Pino A, Llorens-Bobadilla E, Zhao S, Lange C, Panuccio G, Boeckx B, Thienpont B, Vinckier S, Wyns S, Bouche A, Lambrechts D, Giugliano M, Dewerchin M, Martin-Villalba A, Carmeliet P (2016) Neurogenic radial glia-like cells in meninges migrate and differentiate into functionally integrated neurons in the neonatal cortex. Cell Stem Cell 20:360-373.

Chen H, Zhang SM, Hernán MA, Schwarzschild MA, Willett WC, Colditz GA, Speizer FE, Ascherio A (2003) Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. Arch Neurol 60:1059-1064.

Frank-Cannon TC, Tran T, Ruhn KA, Martinez TN, Hong J, Marvin M, Hartley M, Treviño I, O'Brien DE, Casey B, Goldberg MS, Tansey MG (2008) Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. J Neurosci 28:10825-10834.

Frielingsdorf H, Schwarz K, Brundin P, Mohapel P (2004) No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 101:10177-10182.

Fuentealba LC, Rompani SB, Parraguez JI, Obernier K, Romero R, Cepko CL, Alvarez-Buylla A (2015) Embryonic origin of postnatal neural stem cells. Cell 161:1644-1655.

Gao X, Chen H, Schwarzschild MA, Ascherio A (2011) Use of ibuprofen and risk of Parkinson disease. Neurology 76:863-869.

Graf U, Casanova EA, Wyck S, Dalcher D, Gatti M, Vollenweider E, Okoniewski MJ, Weber FA, Patel SS, Schmid MW, Li J, Sharif J, Wanner GA, Koseki H, Wong J, Pelczar P, Penengo L, Santoro R, Cinelli P (2017) Pramel7 mediates ground-state pluripotency through proteasomal-epigenetic combined pathways. Nat Cell Biol 19:763-773.

Hendrickson ML, Rao AJ, Demerdash ONA, Kalil RE (2011) Expression of nestin by neural cells in the adult rat and human brain. PLoS One 6:e18535.

Jackson CR, Ruan G-X, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM, McMahon DG (2012) Retinal dopamine mediates multiple dimensions of light-adapted vision. J Neurosci Off J Soc Neurosci 32:9359-9368.

Kay JN, Blum M (2000) Differential response of ventral midbrain and striatal progenitor cells to lesions of the nigrostriatal dopaminergic projection. Dev Neurosci 22:56-67.

Kirby ED, Kuwahara AA, Messer RL, Wyss-Coray T (2015) Adult hippocampal neural stem and progenitor cells regulate the neurogenic niche by secreting VEGF. Proc Natl Acad Sci U S A 112:4128-4133.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445:168-176.

McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, Di Monte DA (2002) Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 10:119-127.

Montorfano I, Becerra A, Cerro R, Echeverría C, Sáez E, Morales MG, Fernández R, Cabello-Verrugio C, Simon F (2014) Oxidative stress mediates the conversion of endothelial cells into myofibroblasts via a TGF-β1 and TGF-β2-dependent pathway. Lab Investig J Tech Methods Pathol 94:1068-1082.

Monvoisin A, Alva JA, Hofmann JJ, Zovein AC, Lane TF, Iruela-Arispe ML (2006) VE-cadherin-CreERT2 transgenic mouse: a model for inducible recombination in the endothelium. Dev Dyn Off Publ Am Assoc Anat 235:3413-3422.

Morrison BE, Marcondes MCG, Nomura DK, Sanchez-Alavez M, Sanchez-Gonzalez A, Saar I, Kim K-S, Bartfai T, Maher P, Sugama S, Conti B (2012) Cutting edge: IL-13Rα1 expression in dopaminergic neurons contributes to their oxidative stress-mediated loss following chronic peripheral treatment with lipopolysaccharide. J Immunol 189:5498–5502.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011) Guide for the Care and Use of Laboratory Animals, 8th ed. Washington (DC): National Academies Press (US). Available at: http://www.ncbi.nlm.nih.gov/books/NBK54050/.

Nishimura K, Inoue T, Yoshimoto K, Taniguchi T, Kitamura Y, Agata K (2011) Regeneration of dopaminergic neurons after 6-hydroxydopamine-induced lesion in planarian brain. J Neurochem 119:1217-1231.

Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MCG, Ward AM, Hahn YK, Lichtman AH, Conti B, Cravatt BF (2011) Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science 334:809-813.

Pacini S, Petrini I (2014) Are MSCs angiogenic cells? New insights on human nestin-positive bone marrow-derived multipotent cells. Front Cell Dev Biol 2:20.

Pober JS, Sessa WC (2007) Evolving functions of endothelial cells in inflammation. Nat Rev Immunol 7:803-815.

Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong J-S, Knapp DJ, Crews FT (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55:453-462.

Shi GP, Villadangos JA, Dranoff G, Small C, Gu L, Haley KJ, Riese R, Ploegh HL, Chapman HA (1999) Cathepsin S required for normal MHC class II peptide loading and germinal center development. Immunity 10:197-206.

Siegenthaler JA, Pleasure SJ (2010) There's no place like home for a neural stem cell. Cell Stem Cell 7:141-143.

Sorriento D, Santulli G, Del Giudice C, Anastasio A, Trimarco B, Iaccarino G (2012) Endothelial cells are able to synthesize and release catecholamines both in vitro and in vivo. Hypertension 60:129–136.

Suzuki S, Namiki J, Shibata S, Mastuzaki Y, Okano H (2010) The neural stem/progenitor cell marker nestin is expressed in proliferative endothelial cells, but not in mature vasculature. J Histochem Cytochem Off J Histochem Soc 58:721-730.

Tandé D, Höglinger G, Debeir T, Freundlieb N, Hirsch EC, François C (2006) New striatal dopamine neurons in MPTP-treated macaques result from a phenotypic shift and not neurogenesis. Brain J Neurol 129:1194-1200.

Wang Y, Jin K, Greenberg DA (2007) Neurogenesis associated with endothelin-induced cortical infarction in the mouse. Brain Res 1167:118-122.

Wurmser AE, Nakashima K, Summers RG, Toni N, D'Amour KA, Lie DC, Gage FH (2004) Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature 430:350-356.

Xie L, Zeng X, Hu J, Chen Q (2015) Characterization of nestin, a selective marker for bone marrow derived mesenchymal stem cells. Stem Cells Int 2015:762098.

Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 100:7925-7930.

Copyedited by Li CH, Song LP, Zhao M