NEURAL REGENERATION RESEARCH
June 2016, Volume 11, Issue 6
www.nrronline.org

INVITED REVIEW

Discovery of nigral dopaminergic neurogenesis in adult mice

Brad E. Morrison*

Department of Biological Sciences, Boise State University, Boise, ID, USA; Biomolecular Ph.D. Program, Boise State University, Boise, ID, USA

Funding: *Work involving adult DA neurogenesis in the Morrison lab was supported by resources from Boise State University, The Biomolecular Research Center at Boise State University, and the Boise VA Medical Center, Boise, ID. Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408 and P20GM109095 provided undergraduate student internship support.*

How to cite this article: *Morrison BE (2016) Discovery of nigral dopaminergic neurogenesis in adult mice. Neural Regen Res 11(6):878-881.*

Abstract

Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra. As a result, intensive efforts have focused upon mechanisms that facilitate the death of mature dopaminergic neurons. Unfortunately, these efforts have been unsuccessful in providing an effective treatment to address neurodegeneration in this disease. Therefore, alternative theories of pathogenesis are being explored. Adult neurogenesis of dopaminergic neurons is an attractive concept that would provide a possible mechanism of neurodegeneration as well as offer an endogenous means to replenish affected neurons. To determine whether dopaminergic neurons experience neurogenesis in adult mice we developed a novel cell lineage tracing model that permitted detection of neurogenesis without many of the issues associated with popular techniques. Remarkably, we discovered that dopaminergic neurons are replenished in adult mice by Nestin+/Sox2- progenitor cells. What's more, the rate of neurogenesis is similar to the rate of dopaminergic neuron loss reported using a chronic, systemic inflammatory response mouse model. This observation may indicate that neuron loss in Parkinson's disease results from inhibition of neurogenesis.

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

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

doi: *10.4103/1673-5374.184449*

Accepted: *2016-05-17*

Key Words: *Parkinson's disease; adult neurogenesis; dopaminergic neurons; stem cells; nestin*

Introduction

Dopaminergic (DA) neurons located in the substantia nigral (SN) region of the midbrain serve as modulators for motor control. Progressive loss of these neurons is a hallmark of Parkinson's disease (PD), the second most common neurodegenerative disorder. The cellular and molecular mechanisms driving PD pathogenesis are not well understood. As a result, only symptomatic treatments exist and none address neurodegeneration in this disease. Current beliefs center around the notion that DA neurons in the SN are not replenished in adult mammals and that the observed loss associated with PD arises from death of mature neurons. Therefore, efforts have focused upon toxicants and/or trophic factor withdrawal in regard to mature neurons as principal mechanisms for neurodegeneration. However, determining whether DA neurons are naturally generated (neurogenesis) throughout our lifetime is fundamental to understanding demise of these cells as well as informing potential therapies. Adult neurogenesis has been described to occur for hippocampal neurons, olfactory bulb neurons (in rodents), and populations of striatal interneurons (Lois et al., 1996; Eriksson et al., 1998; Dayer et al., 2005; Ernst et al., 2014). These findings have spurred exploration of this process for DA neurons in the SN. Until recently, evidence for adult neurogenesis was lacking due to an inability to replicate the small number of studies favoring DA neurogenesis (Frielingsdorf et al., 2004). This is likely the result of technical limitations associated with popular methods for cell lineage tracing. To overcome these limitations, our group devised a novel lineage tracing approach that has produced compelling evidence for adult neurogenesis of DA neurons (Albright et al., 2016). This article will focus upon our recent discovery and the model employed.

Limitations of Current Neural Progenitor Cell Lineage Tracing Methods

The most prevalent method for mapping the progeny of neural precursor cells involves assessing the incorporation of a tagged nucleotide, such as bromodeoxyuridine (BrdU), into newly synthesized DNA. This procedure presents a number of potential complications. Firstly, BrdU and other nucleotide analogs are toxic, especially to dividing cells, as well as the organism as a whole. Secondly, this method presumes that cell division must occur for generation of new neurons. This notion will later be revisited. Additionally, incorporation of nucleotide analogs can occur through homeostatic DNA repair mechanisms leading to false-positive results. Moreover, these studies are modeled after work assessing adult neurogenesis of hippocampal neurons in the dentate gyrus where this process occurs at a rate roughly fifty times that reported for DA

neurons in our recent study (Albright et al., 2016). Another method for lineage tracing involves permanent labeling of neural progenitors and subsequent progeny using retrovirus. However, knowledge of the precise location of the progenitor population is required for successful labeling. To overcome these limitations, we formulated a model that utilizes a genetic approach coupled with a well-tolerated drug to activate progenitor labeling in adult mice. In addition, this model extended the monitoring time to six months post-labeling to ensure capture of this process which we predicted would be slow.

A Novel Lineage Tracing Model and Discovery of Adult DA Neurogenesis

The model utilized in our study assessed adult neurogenesis of DA neurons by removing a DA neuron marker gene, *tyrosine hydroxylase (Th)*, from neural progenitor cells in adult animals (**Figure 1**). Either *nestin* or *Sox2* promoters were employed to drive the expression of a CRE recombinase (CRE^{ERT2}) in neural progenitors (Battiste et al., 2007; Arnold et al., 2011). These promoters are known to be expressed by neural precursor populations and not expressed in mature neurons making them good candidates for this study. At 3 months of age, mice were fed tamoxifen-laden chow for 6 weeks to activate CRE activity. These mice possessed a *Th* gene containing *loxP* sites flanking exon 1 (Jackson et al., 2012) thereby allowing for excision and silencing of the gene following CRE activation. Six months following tamoxifen treatment, DA neurons in the SN were labeled by immunohistochemistry using an anti-Tyrosine Hydroxylase antibody and quantified. A reduction in DA neurons (TH^+ cells) in the SN *versus* control mice would signify that DA neurogenesis had occurred in these adult mice since the loss of *Th* in progenitors would have affected the mature DA neuron population. Surprisingly, this is precisely what was observed for mice possessing *nestin*-driven CRE^{ERT2} expression following tamoxifen treatment (**Figure 1B**). However, it was interesting to find that *Sox2*-driven CRE expression had no effect. This is an unexpected result given that Sox2 has been reported to be a ubiquitous neural progenitor marker.

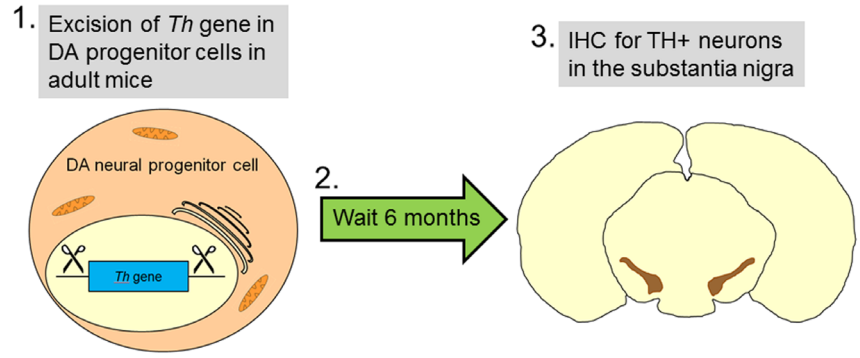
Prospective on Adult Nigral DA Neurogenesis

Our study uncovered similar rates for DA neurogenesis to those reported for DA neuron loss in an inflammatory response model of PD (**Figure 2A**) which poses a provocative correlation. Heightened inflammatory response has been closely linked to PD pathology and epidemiology. For example, PD patient brain material exhibits activated microglia and elevated inflammatory factor expression. In addition, non-steroidal anti-inflammatory drug use, particularly ibuprofen, has been associated with protection against the development of PD. Taken together, these results may indicate that inflammatory response facilitates loss of DA neurons through inhibition of adult neurogenesis. Future investigations could directly address this by combining the DA progenitor tracing model and the inflammatory response model of DA neuron loss to determine if heightened inflammation results in added loss of TH^+ cells in the SN.

Sox2 is one of four factors identified by Dr. Yamanaka's group whose overexpression can transform somatic fibroblasts into pluripotent stem cells (iPSC's) (Takahashi and Yamanaka, 2006). Consequently, Sox2 might be an indication of a less differentiated cell in comparison with Nestin which is only expressed following differentiation of iPSC's toward specific lineages. An interesting possibility is that $Nestin^+/SOX2^-$ DA neural progenitor cells represent a more mature and potentially post-mitotic population of cells (**Figure 2B**). This would be in line with recent work by Fuentbealba et al. (2015) suggesting that neural stem cells that replenish olfactory bulb (OB) neurons in adult mice are generated embryonically and remain quiescent until activated to differentiate. Their study provides evidence supporting the notion that some adult OB neural progenitors have very limited or possibly no replicative capability. Whether OB and DA neural progenitors in the SN share this feature will require further work but will be fundamental to understanding adult DA neurogenesis. Interestingly, $Nestin^+/SOX2^-$ cells have been reported to be present in abundance within the adult mouse brain and that these cells reside in a satellite position adjacent to mature neurons (Hendrickson et al., 2011). The reported $Nestin^+/SOX2^-$ cells do not express neuronal (NeuN, βIII-tubulin, doublecortin), astrocyte (GFAP), or oligodendrocyte (GST-ϖ, NG2) markers suggesting that these cells could be novel neural progenitor cells and therefore might serve as a potential source of DA neurons.

Our data may also indicate that DA neural progenitor cells only express Nestin transiently in adult mice. As mentioned previously, our calculated rate of DA neurogenesis from our prior study correlates well with DA neuron loss reported using an inflammatory mouse model of PD (**Figure 2A**) (Frank-Cannon et al., 2008; Morrison et al., 2012). The rate of DA neurogenesis shown in **Figure 2A** is calculated by dividing TH^+ neurons missing by the tamoxifen treatment duration (42 days). Future studies are needed to confirm this prospect but the finding of DA neurogenesis as possibly a function of tamoxifen duration suggests that *Th* is not being excised from a homogenous pool of stem cells but rather from a transient $Nestin^+$ population. One could speculate that the removal of *Th* from the entire DA progenitor pool would only require a few days of tamoxifen treatment and thus would not be correlated with the prolonged tamoxifen treatment duration of 42 days. This may indicate that the progenitor cells only express Nestin transiently in our system. Therefore, the prospect exists for a non-replicative SOX2-negative progenitor pool that transiently expresses Nestin (**Figure 2B**). This possibility is supported by studies involving incorporation of nucleotide analogs (BrdU) or isotopic nucleotides (from cold war atomic weapon testing) that have been unsuccessful in detecting adult DA neurogenesis suggesting that cell division might not be involved in this process (Frielingsdorf et al., 2004; Spalding et al., 2005). Whether progenitors transiently express Nestin could be tested by extending the post-treatment (tamoxifen)

A



B

	DA neurons in SN	
Genotype	**No Tam**	**Tam**
+/+; $Th^{lox/lox}$	4176 ± 31	4198 ± 32
nestin-CRE^{ERT2}/+; $Th^{lox/lox}$	4185 ± 39	3603 ± 143*
Sox2-CRE^{ERT2}/+; $Th^{lox/lox}$	4123 ± 53	4210 ± 78

Figure 1 Genetic model used to discover nigral dopaminergic neurogenesis in adult mice.
(A) Tamoxifen (Tam) treatment (42 days) in adult mice activates CRE activity in *nestin* or *Sox2*-expressing dopaminergic (DA) progenitor cells resulting in tyrosine hydroxylase (*Th*) gene silencing. Six months later, TH^+ neurons in the substantia nigra (SN) were labeled by immunohistochemistry (IHC) using a TH antibody and counted. (B) The table displays counts of DA neurons in the SN for control and tamoxifen treated mice from Albright et al. (single hemisphere average; standard error of the mean). A significant reduction for *nestin-CRE*^{ERT2} mice was observed (*; multivariate analysis of variance (ANOVA) followed by *post hoc* Tukey's test; $n = 6$ mice/group; P-value < 0.05 for all groups compared to *nestin-CRE*^{ERT2} with Tam).

A

Study	Model	Rate (neurons/day)
Albright et al.	Nigral DA neurogenesis	13.9 ± 1.2
Frank-Cannon et al.	Chronic, systemic LPS	14.3 ± 2.5
Morrison et al.	Chronic, systemic LPS	12.3 ± 0.75

B

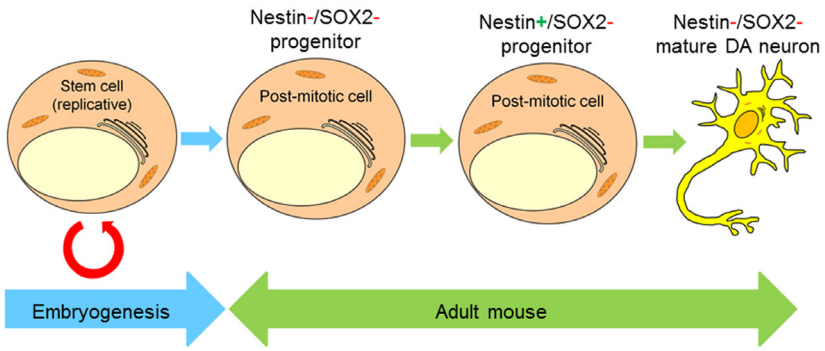


Figure 2 Proposed model of adult neurogenesis for nigral dopaminergic neurons.
(A) The rate of dopaminergic (DA) neurogenesis in our model and the rates of DA neuron loss reported in two other studies utilizing a systemic inflammatory mouse model of Parkinson's disease are shown. The rates were calculated as DA neuron loss/days of treatment. Days of treatment were 42 and 180 for tamoxifen (neurogenesis model) and lipopolysaccharide (LPS) (inflammatory model), respectively (average rate per brain hemisphere; standard error of the mean; approximation used for Frank-Cannon et al.'s study). (B) Our data is consistent with the presented model. DA neural stem cells replicate during embryogenesis and then enter a quiescent state as post-mitotic neural progenitors. In adulthood, post-mitotic progenitor cells differentiate into DA neurons in the substantia nigra (SN) through a transient $Nestin^+/SOX2^-$ intermediate.

duration until recovery of TH^+ cells is observed in the SN. No recovery, regardless of post-treatment duration, would be expected if the initial source progenitor population in adults expresses Nestin. The consequence for lack of replicating progenitors in adults would be significant. Factors that negatively impact these progenitors during development through adulthood could irreversibly deplete this pool leading to a gradual loss of DA neuron replenishment.

Prevalent genetic models of PD in mice present many pitfalls. Results from our study suggest that nigral DA neurogenesis is a slow process that could further limit the usefulness of these models. The rate of TH^+ cell loss in the SN can be calculated as a function of tamoxifen treatment duration (42 days) or the duration following tamoxifen treatment (6 months) yielding 13.9 ± 1.2 or 3.2 ± 0.3 neurons/day (per hemisphere), respectively. Future studies incorporating multiple time points and treatment schemes will be needed to determine the appropriate rate. However, with that information withstanding, both rates for DA neurogenesis are sufficiently low to partially explain the difficulty in generating nigral DA neuron loss in genetic murine models of PD. Genetic models have utilized pan overexpression (*e.g.*, SNCA (A53T), SNCA (wild-type)) or knockout (*e.g.*, Parkin, PINK1) of PD-causing genes. Yet, loss of nigral DA neurons has not been observed. This could be explained by the relatively short lifespan of mice (~2 years) in concert with a partial blockade of adult neurogenesis. Supporting this notion are reports of decreased neurogenesis in the subventricular and subgranular zones in SNCA overexpressing mouse models of PD although this remains controversial (reviewed in Le Grand et al., 2015). Additionally, in humans, expression of PD-causing mutations can take several decades, and over 10 years for juvenile forms, to induce clinical pathology. Therefore, observing DA neuron loss using existing genetic mouse models may be problematic for this reason and require additional considerations before their use.

Prospects of Adult DA Neurogenesis Modeling and Therapy for PD

Discovery of DA neurogenesis in adult mammals provides an opportunity to harness this naturally occurring process for therapeutic benefit. This will require locating and characterizing the DA progenitor cells responsible. Characterization of this process *in vivo* will potentially allow for modeling in cell culture systems that could expedite new therapeutic invention strategies. Such a system might facilitate drug screening efforts to modulate this process. In addition, furthering our understanding of adult DA neurogenesis will better inform stem cell replacement therapy for PD. Previous clinical trials have utilized fetal tissue with little manipulation or standardization prior to transplantation into PD patients (Freed et al., 2001; Olanow et al., 2003). This was believed to underlie the outcome variability from these studies. Understanding DA progenitor expression markers will permit differentiation in cell culture toward the appropriate phenotype and serve as a consistent source for transplantation therapy. Theoretically, this source material could be provided by the patient themselves using iPSC methods. Finally, the discovery of adult neurogenesis for DA neurons adds to the limited number of neural populations reported to experience this phenomenon and enhances our collective foundational knowledge of brain biology.

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.

Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, Seandel M, Geijsen N, Hochedlinger K (2011) Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell 9:317-329.

Battiste J, Helms AW, Kim EJ, Savage TK, Lagace DC, Mandyam CD, Eisch AJ, Miyoshi G, Johnson JE (2007) Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. Dev Camb Engl 134:285-293.

Dayer AG, Cleaver KM, Abouantoun T, Cameron HA (2005) New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. J Cell Biol 168:415-427.

Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313-1317.

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisén J (2014) Neurogenesis in the striatum of the adult human brain. Cell 156:1072-1083.

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 Off J Soc Neurosci 28:10825-10834.

Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 344:710-719.

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.

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 GX, 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.

Le Grand JN, Gonzalez-Cano L, Pavlou MA, Schwamborn JC (2015) Neural stem cells in Parkinson's disease: a role for neurogenesis defects in onset and progression. Cell Mol Life Sci CMLS 72:773-797.

Lois C, García-Verdugo JM, Alvarez-Buylla A (1996) Chain migration of neuronal precursors. Science 271:978-981.

Morrison BE, Marcondes MCG, Nomura DK, Sanchez-Alavez M, Sanchez-Gonzalez A, Saar I, Kim KS, 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 Baltim Md 189:5498-5502.

Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB (2003) A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol 54:403-414.

Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisén J (2005) Retrospective birth dating of cells in humans. Cell 122:133-143.

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.