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Dopaminergic Innervation of the Subventricular Zone in the Murine Brain

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University Scholar Thesis

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I. ABSTRACT

The subventricular zone (SVZ) is one of two areas in the brain that, in a healthy mouse, continually generate neurons throughout adulthood. While it was previously thought that only the A9 neurons of the substantia nigra sent dopaminergic afferents to the SVZ, recent studies suggest that the A10 neurons of the ventral tegmental area may innervate this area. This project has aimed to discover which, if either, model is correct.

Examination of the Aphakia (AK) mouse was used to determine the role of distinct midbrain regions in SVZ regulation. Additionally, intraperitoneal injections of the chemical MPTP were used to deduce the role of A9 and A10 dopamine signaling in the SVZ. Anterograde and retrograde neuronal tracing were used to identify the specific midbrain region that sends dopaminergic afferents to the SVZ. Our results, while preliminary, suggest that the currently accepted notion of the A9 dopaminergic innervation may need to be replaced by a model in which the A10 VTA neurons, or a combination of A9 and A10 neurons, innervate the SVZ.

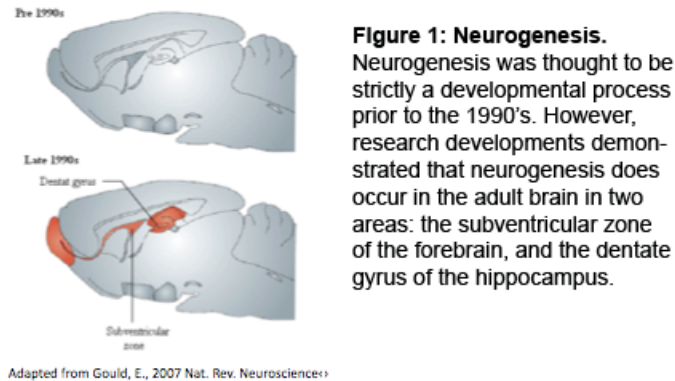
Elucidation of the regulatory mechanisms of the SVZ impacts fields including learning, aging, and Parkinson's disease, and hence has profound implications for biomedical science.

II. INTRODUCTION

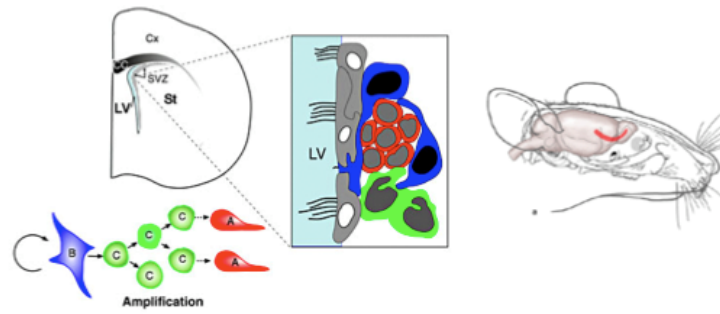
i. Neurogenesis and the Subventricular Zone

Before the 1990's, the idea of neurogenesis, or the generation of new neurons, in the adult mammalian brain was considered far-fetched. Advances in neurobiology soon demonstrated that two areas of the adult mammalian brain do maintain the ability to

generate neural stem cells throughout adulthood (**Fig. 1**). The two largest areas of adult neurogenesis are the subventricular zone (SVZ) of the forebrain and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Lois & Alvarez-Buylla, 1994; Gould & Cameron, 1996; Kempermann et al., 1997).



The SVZ is a neurogenic region about three to five cell layers thick that lines the lateral walls of the lateral ventricles. The major cell types of the SVZ are ependymal cells, astrocytes (Type B cells, or stem cells), transit amplifying progenitor cells (TAP, or Type C cells), and neuroblasts (Type A cells) (**Fig. 2**). Ependymal cells form the barrier between the cerebral spinal fluid in the ventricle and the SVZ. Type B cells in the SVZ give rise to type C cells, which subsequently differentiate into type A cells (Doetsch et al., 1999). These type A cells (neuroblasts) travel in tight chains along a specific route known as the rostral migratory stream (RMS), eventually incorporating into the olfactory bulb as interneurons (Lois & Alvarez-Buylla, 1994; Belluzi et al., 2003).



Jackson, and Alvarez-Buylla, 2008, Cells Tissues Organs; Lenington et al., 2003, Rep. Bio. and Endo.

Figure 2: The subventricular zone. The SVZ of the murine brain is a tightly regulated niche. Ependymal cells line the ventricle, and astrocytes (type B cells, blue) wrap around clusters of neuroblasts (type A cells, red). Transit amplifying progenitor cells (type C cells, green) are also present. Type B cells, which are considered the stem cells of the SVZ, give rise to type C cells, which subsequently give rise to type A cells. The type A cells travel in chains along the rostral migratory stream to incorporate into the olfactory bulb.

While adult neurogenesis is the focus of this study, knowledge of embryonic neurogenic processes is helpful. The embryonic mammalian brain begins development as ectodermal tissue arising from the neural tube. The pseudostratified neuroepithelial cells that line the ventricles early in embryonic development give rise to radial glia (RG) cells (Kriegstein & Alvarez-Buylla, 2009). During cortical neurogenesis, these RG cells have the option of symmetrically dividing to self-renew, or of asymmetrically dividing to produce daughter cells (Haubensak et al., 2004; Noctor et al., 2004, 2007). Some of these daughter cells populate the SVZ and are capable of dividing to generate more neurons (Miyata et al., 2004; Noctor et al., 2004).

Given the importance of RG cells in embryonic neurogenesis, it is fitting that recent studies have shown that SVZ type B cells retain a number of RG-like characteristics. RG cells have long basal processes that contact the brain surface during embryonic development. Similarly, SVZ B cells have short processes that extend through the ependymal layer to contact the ventricle, with end feet characteristic of RG cells. The B cells can also have radial or tangential projections to contact blood vessels, reminiscent

of the RG processes (Kriegstein & Alvarez-Buylla, 2009). This coupling of type B cells to blood vessels is part of a vascular niche that signals to and supports the SVZ (Tavazoie et al., 2008). It is common, and possibly essential, for proliferating SVZ cells to be in close contact with blood vessels. Additionally, while the SVZ is often discussed as a uniform neurogenic niche, there is evidence that subregions exist, producing different types of olfactory interneurons (Merckle et al., 2006).

ii. Dopamine

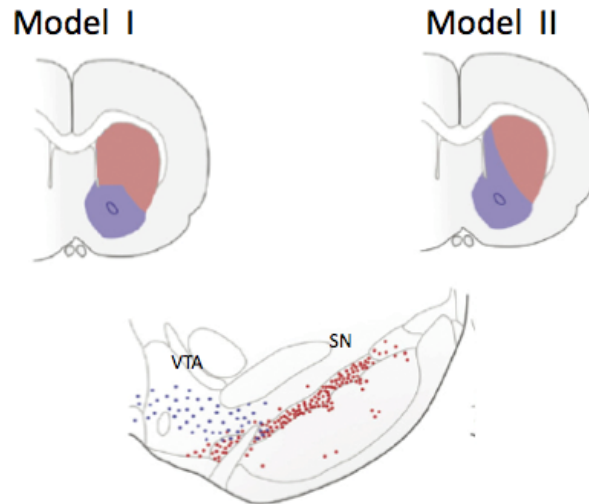
While the major cell types of the SVZ are known, the mechanisms by which the cells are regulated and maintained as a neurogenic niche are not fully understood. Dopamine, a neurotransmitter produced in the midbrain, has been suggested to regulate the SVZ (Van Kampen, 2004; Höglinger, 2004; Baker, 2004). Although dopaminergic neurons comprise fewer than 1% of total brain neurons, they play a role in regulation of major brain functions such as motor behavior, motivation and working memory (Chinta and Andersen, 2005). Support for an additional role for dopamine as a regulatory molecule in the SVZ is based on the localization of dopaminergic projections along and within the SVZ (Bjorklund and Dunnet, 2007). Additionally, dopamine signaling initiates neurogenesis in embryonic development (Ohtani et al., 2003), suggesting a similar role may occur in adulthood.

While *in vivo* studies are best when dealing with physiology, *in vitro* studies have shown that dopamine promotes SVZ cell proliferation by increasing local EGF production in the area, activating EGF receptors to produce the effect (O'Keeffe et al.,

2009). In the same set of studies, it was shown that depleting dopamine *in vitro* results in reduced neurogenic activity in the SVZ.

ii. Substantia Nigra and Ventral Tegmental Area

The largest population of dopaminergic neurons in the brain reside in the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA) of the ventral midbrain (Hwang et al., 2003). The SNpc contributes to the control of voluntary motor movement, and dopaminergic neurons in this region have been designated “A9”. The VTA contains “A10” dopaminergic neurons, and it is associated with emotion-based behaviors (Chinta and Andersen, 2005). While it was previously thought that only the A9 neurons of the SNpc innervated the striatum along the wall of the lateral ventricle, recent studies suggest that the A10 neurons of the VTA may innervate this area (**Fig. 3**; Bjorklund and Dunnett, 2007). In considering the roles of the SNpc and VTA (movement and emotion, respectively), it is logical to think that the supposed relationship between the A9 movement-associated neurons of the SNpc and the olfaction-supporting SVZ might be incomplete. Olfaction is widely known to play an important role in the limbic system of the brain, encouraging studies into the ability of the A10 emotion-based neurons of the VTA to impact the SVZ and, ultimately, olfaction.



Adapted from Bjorklund and Dunnett, 2007, Trends Neuroscience

Figure 3: Models for midbrain innervation of the SVZ. The current dogma regarding midbrain innervation of the SVZ follows Model I, in which the substantia nigra (SN) projects to the SVZ. However, Model II, in which the ventral tegmental area (VTA) innervates the SVZ has been suggested. The possibility of dopaminergic innervation of the SVZ by the VTA has been further supported by data from our studies.

iii. AK Mouse

The aphakia (AK) mouse lacks the transcription factor Pitx-3. This homozygous deficiency results in the selective embryonic degeneration of most of the A9 SNpc neurons, while sparing the A10 neurons of the VTA (Hwang, 2003; Nunes, 2003). Another side effect of this mutation is blindness. AK mice are commonly used as a model for Parkinson's disease; While there are obvious problems with the model (such as lacking the Parkinsonian tremors and a number of other human symptoms), the AK was appropriate for this study due to its degenerated SNpc. AK mice were compared to wild type C57Bl/6 mice (henceforth referred to as C57 mice) and were used as indicators of the role of A9 neurons in neurogenesis.

iv. MPTP

The chemical MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is thought to selectively degenerate A9 neurons (Przedborski et al., 2001; Hoglinger et al., 2004; Jackson-Lewis and Przedborski, 2007). MPTP treatment is known to decrease neurogenesis. This chemical is popular because it is the only known dopaminergic neurotoxin capable of mirroring the clinical picture of Parkinson's disease in both humans and monkeys (Jackson-Lewis and Przedborski, 2007). MPTP is typically administered via a series of intraperitoneal injections. The AK mouse served as a control to test the assumption that MPTP targets A9 neurons, as injecting MPTP into the A9 neuron-free AK mice should not have caused any change. We hypothesized that MPTP also targets A10 neurons.

III. METHODOLOGY

BrdU Immunohistochemistry

2-3 month old C57 and AK mice were injected with 300mg BrdU/kg 2 hr prior to perfusion. Mice were perfused transcardially with 0.9% saline followed by 3% paraformaldehyde in PBS. BrdU immunostaining of 50 μ m sections (A/P coordinates 0.5-1.4mm, relative to bregma) were conducted by the following procedure: 2 N HCl was used to dissociate the DNA followed by neutralization (sodium borate) then blocking and permeabilization (10% horse serum in PBS/0.1% Triton X-100). The floating brain sections were incubated overnight (12 hrs) at 4°C using Rat BrdU primary antibody diluted in 10% horse serum in PBS/0.1% Triton X-100. Sections were washed three times for five minutes in PBS and incubated for 2 hours at room temperature with donkey secondary antibodies (Alexa Fluor, 1:500). Sections were again washed three times for

five minutes in PBS, mounted and dried overnight. Epifluorescence imaging of the lateral wall of the lateral ventricle was performed and BrdU+ cells along the entire lateral wall of the lateral ventricle, from the dorsolateral aspect to the level of the anterior commissure, were counted using Openlab 3.1.5 imaging software (Improvision, Lexington, MA) in 18 anterior forebrain sections (50µm), from coordinates 0.5-1.4 anterior, relative to bregma and statistical analyses were performed using Students' t-test.

Subventricular Zone and Midbrain Immunohistochemistry

Free floating brain sections were blocked and permeabilized for 1 hour at room temperature in a solution of 10% horse serum in PBS/0.1% Triton X-100. Sections were incubated overnight (12 hours) with primary antibodies (diluted in 10% horse serum in PBS/0.1% Triton X-100) against: (SVZ) Tyrosine hydroxylase (1:500); Ki67 (1:1000); Caspase 3 (0.6 µl/ml) and (Midbrain) Tyrosine hydroxylase (1:500); Girk2 (1:500); and Calbindin (1:500). Sections were then washed three times for five minutes in PBS and incubated for 2 hours at room temperature with the appropriate fluorescent secondary antibodies (Alexa Fluor, 1:500). Sections were again washed three times for five minutes in PBS, mounted and dried overnight. Cell nuclei were labeled with DAPI and slides were mounted using aquapolymount. Slides were imaged using a Leica TCS SP2 confocal microscope and processed using Adobe Photoshop CS2.

For analysis of Caspase 3, cells along the entire lateral wall of the lateral ventricle, from the dorsolateral aspect to the level of the anterior commissure, were counted using Openlab 3.1.5 imaging software (Improvision, Lexington, MA) in 18

anterior forebrain sections (50 μ m), from coordinates 0.5-1.4 anterior relative to bregma, and statistical analyses were performed using Students' t-test.

MPTP Treated Mice

3-5 month old male C57Bl/6 and AK mice (n=4-5 for all conditions) were given 4 injections of 18mg/kg 1-methyl-1,4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP-HCl) or 0.9% saline, each 2 hours apart. Immediately following MPTP or saline administration, mice were given 8 injections of 50 mg/kg 5-Bromo-2-deoxyuridine (BrdU), each 6 hours apart. Mice were sacrificed at 3 days following the final injection of MPTP-HCl. Mice were anesthetized using 2.5% Avertin and transcardially perfused with 0.9% saline followed by 3% PFA. Brains were removed and fixed in 3% PFA overnight at 4°C. The subventricular zones of these brains were sectioned into 25 μ m sections and the ventral midbrains were sectioned into 40 μ m sections. 2 N HCl was used to dissociate the DNA, followed by neutralization (sodium borate) then blocking and permeabilization (10% horse serum in PBS/0.1% Triton X-100). The floating brain sections were incubated overnight (12 hrs) at 4°C using the following primary antibodies diluted in 10% horse serum in PBS/0.1% Triton X-100: Rat BrdU (1:100), Sheep tyrosine hydroxylase (1:500); and goat doublecortin (DCX) (1:250). Sections were then washed three times for five minutes in PBS and incubated for 2 hours at room temperature with the appropriate fluorescent secondary antibodies (Alexa Fluor, 1:500). Sections were again washed three times for five minutes in PBS, mounted and dried overnight. Cell nuclei were labeled with DAPI and slides were mounted using aquapolymount. Every other section between brain coordinates -0.1mm to 1.34mm relative to bregma was

imaged using a Leica axiscope epifluorescence microscope. All BrdU+ cells in these sections were counted in Adobe Photoshop CS2 and all statistical analyses were performed using Students' t-test.

Neuronal Tracing

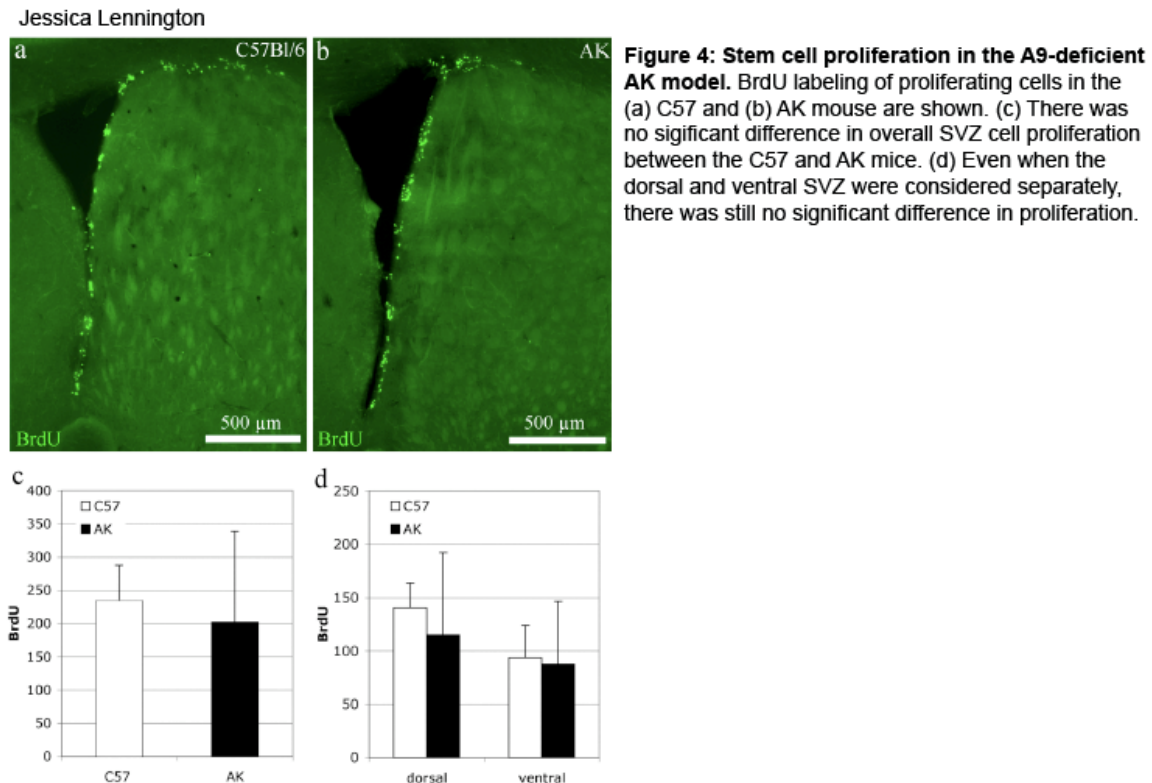
3-5 month old male C57Bl/6 and AK mice ($n \geq 3$) were anesthetized using isofluorane in 2% oxygen. They were then injected with a 2mg/mL solution of Wheat Germ Agglutinin conjugated to FITC (WGA-FITC) into the following coordinates relative to bregma: lateral ventricle (bregma: 0.0mm, lateral: 0.75mm, ventral: 2.25mm) and ventral midbrain (bregma: -3.08mm, lateral: 0.5mm, ventral: 4.7mm). Mice were allowed to survive for 5-7 days and were then anesthetized using 2.5% Avertin and transcardially perfused with 0.9% saline followed by 3% paraformaldehyde (PFA). Brains were removed and fixed in 3% PFA overnight at 4°C. The subventricular zone and ventral midbrain of these brains were sectioned into 40 μ m section using a Leica vibratome and then processed for immunohistochemistry.

IV. RESULTS

AK Mice Do Not Have Reduced SVZ Proliferation

Because AK mice have a severely reduced SNpc neuronal population, we hypothesized that they would have decreased SVZ proliferation as compared to the control C57 mice. The proposed cause of decreased neurogenesis was the depletion of midbrain-based dopamine in the striatum of the AK mice. However, we found that the AK mice did not have significantly less proliferation than the C57 mice (**Fig. 4**). There

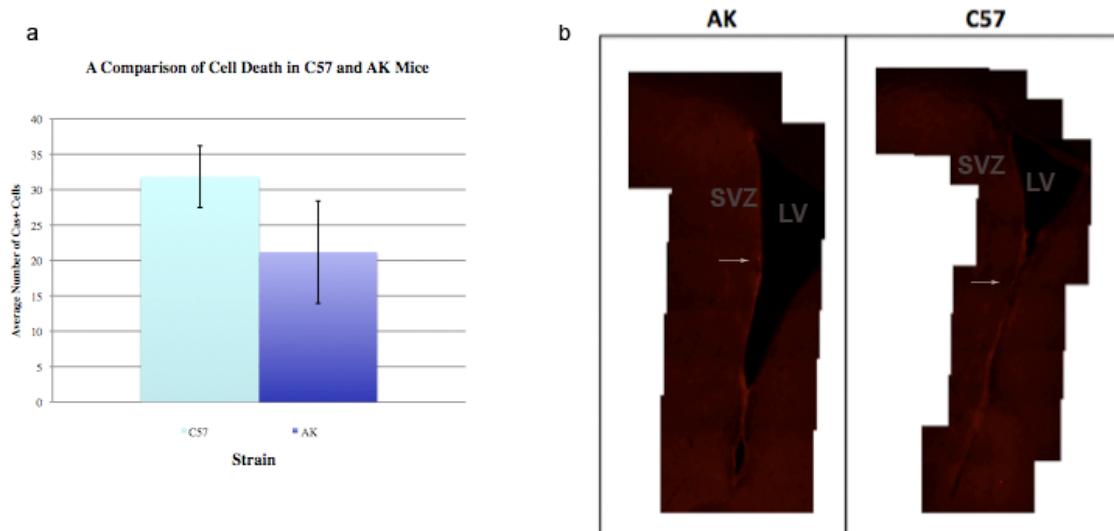
was robust labeling of BrdU in C57 (4a) and AK (4b) mice. When the BrdU cell counts were statistically analyzed (4c), there was no significant difference in SVZ cell proliferation between C57 and AK mice. In considering the dorsal and ventral SVZ separately (4d), there was still no significant difference between the two strains.



AK Mice Have Similar Levels of Cell Death in the SVZ Compared to C57 Mice

In order to further characterize the AK mouse, a cell death study was carried out (**Fig. 5**). Caspase 3 labels apoptotic cells, and it was used to visualize levels of cell death in C57 and AK mice. It was found that there is no significant difference in apoptotic cell death between the C57 and AK mice (5a).

Figure 5: Cell Death in the AK and C57 Mice. In a comparison of C57 and AK mice, (a) there was no significant difference in SVZ cell death as measured by caspase 3. Images of caspase 3 staining in the SVZ are shown in (b), with arrows pointing to caspase 3-positive cells. (LV: lateral ventricle)



MPTP Decreases Cell Proliferation in the SVZ

The chemical MPTP has been reported to deplete A9 SNpc neurons, and to decrease neurogenesis as a result. Given our results, showing that the AK mouse has normal levels of neurogenesis despite lacking most SNpc cells, we hypothesized that MPTP might decrease neurogenesis by depleting more than just A9 SNpc neurons. Other studies have explored this issue: MPTP was found to be directly cytotoxic to SVZ cells (He et al., 2006), particularly neuroblasts. An older study found that VTA A10 neurons were 42% depleted following administration of MPTP (German et al., 1996). While issues with the experimental methods of the German study could be the source of A10 depletion, there is also a possibility that MPTP is targeting a population of cells that cannot be identified by the A9/A10 designation.

Before carrying out the MPTP study, we first created a comparison of SVZ cell proliferation in C57 and AK mice (6a). No significant difference was found. When MPTP was applied to the C57 and AK mice, there was a significant decrease in cell proliferation in the C57. The AK showed a downward trend in proliferation, though it was not significant. There is a possibility, however, that increasing the number of experiments for the AK would produce a statistically significant shift in proliferation. On the other hand, it is possible that dopamine does not directly impact the SVZ. In that case, the difference could be due to MPTP causing more dopaminergic neurons to die in the C57, releasing dangerous factors and negatively impacting proliferation. The AK, having fewer dopaminergic neurons, would experience less SVZ cytotoxicity in this case.

Jessica Lennington

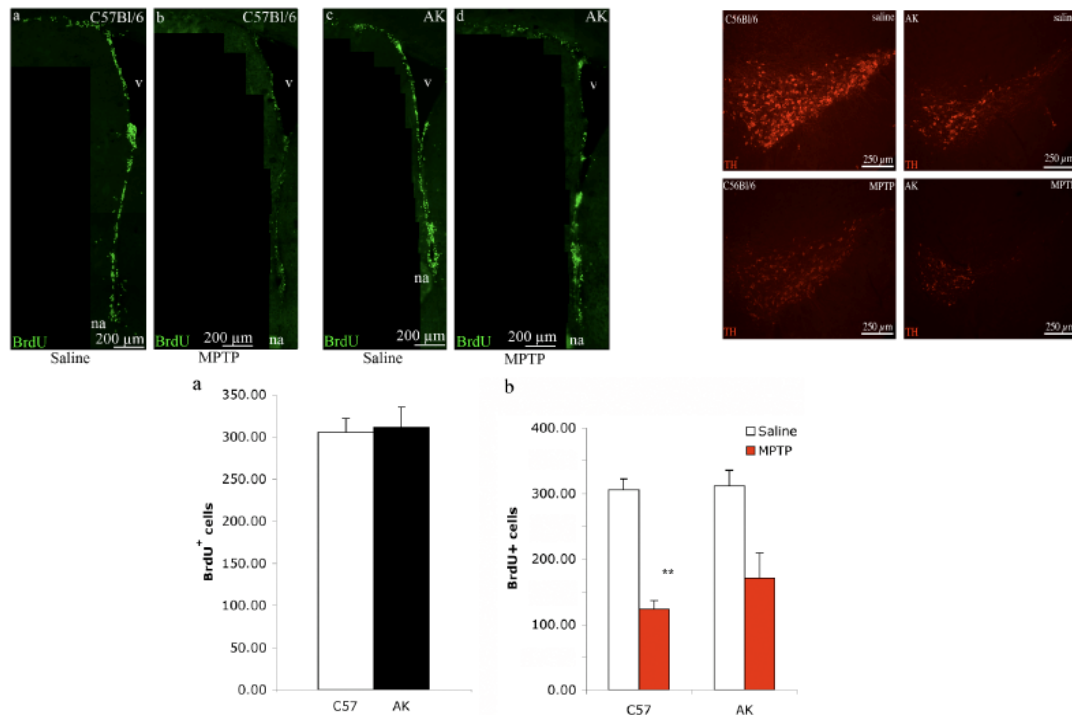


Figure 6: Effects of MPTP on cell proliferation. Images of the effects of MPTP on neurogenesis (upper left) and midbrain dopaminergic neurons (upper right) in C57 and AK mice. Using BrdU cell counts, (a) there was no significant difference in SVZ cell proliferation between the C57 and AK mice. (b) 3 days after the MPTP injections, C57 mice showed a statistically significant decrease in cell proliferation. The AK mice did show a downward trend in proliferation, although it was not significant.

VTA A10 Neurons Send Dopaminergic Projections to the SVZ in the AK Mouse

Based on work by Sara Pope demonstrating that the SVZ in AK mice still receives dopaminergic projections to the SVZ, and that the majority of remaining SNpc/VTA neurons display VTA markers, we hypothesized that the VTA is responsible for dopaminergic innervation of the SVZ in AK mice.

To test this hypothesis, we first did anterograde neuronal tracing (**Fig. 7**) via injections of WGA-HRP into the midbrain of C57 mice. This was meant to confirm that the midbrain does send dopaminergic projections to the SVZ. We expected tracing to be present in all parts of the SVZ, as C57 mice have both the SNpc and VTA intact. The procedure involved treating brain sections from injected mice with DAB and hydrogen peroxide. The HRP converts DAB into a brown precipitate wherever tracing has occurred. Cell bodies were visualized using nissl stain. The results showed that the midbrain does track to the SVZ in C57 mice (Fig. 7, a-c), as expected.

With the confirmation that the midbrain provides dopaminergic innervation of the SVZ in C57 mice, we wanted to find out if the A10 dopaminergic neurons of the VTA innervate the SVZ of AK mice. While pinpointing the character of the midbrain cells that were injected in the C57 would have proved extremely difficult (due to the proximity of the SNpc and the VTA, and the diffusion of the tracer after injection), we were able to assume that the injections into the AK brain were hitting mostly VTA neurons, due to the degenerated SNpc in AK mice. Instead of using WGA-HRP, as in the C57 mice, the AK mice were injected with WGA-FITC. FITC is a fluorescent molecule.

The results showed tracking to all areas of the SVZ (7 d-f). Tyrosine hydroxylase (TH) and Ki67 staining were used to show dopaminergic and proliferating neurons,

respectively. We found that the AK mice had midbrain dopaminergic neurons that projected to the SVZ, lying in close proximity to proliferating cells (7g). Since AK mice lack a SNpc, it was likely that these midbrain neurons were A10 VTA neurons. While helping to characterize the AK mouse, this data also suggested that C57 mice could also have at least partial VTA innervation of the SVZ.

Sara Pope

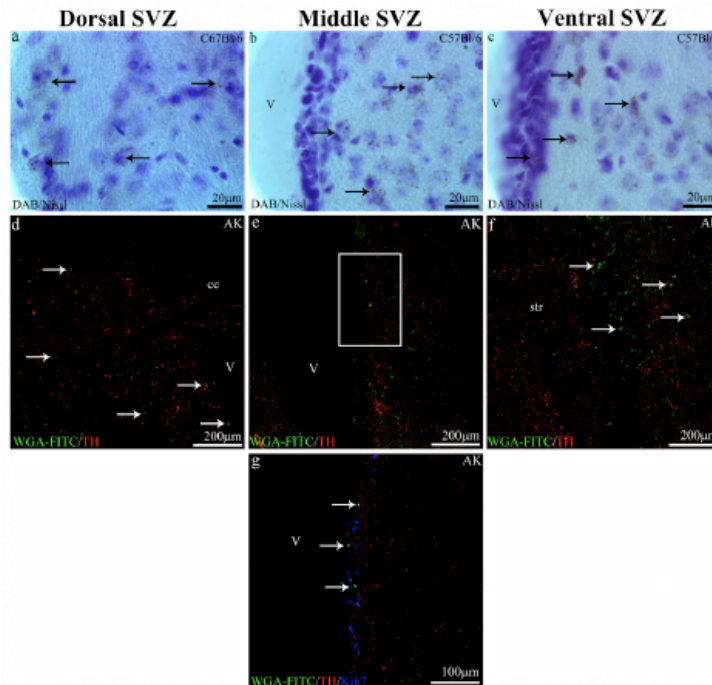


Figure 7: Anterograde tracing. Anterograde tracing was carried out with injections of WGA-HRP into the midbrain of C57 mice (a-c) and injections of WGA-FITC into the midbrains of the AK mice (d-g). Tracking was present in all areas of the SVZ in both mouse strains. In (g), the boxed area of (e) is magnified to show the close association of WGA-FITC with TH+ (dopaminergic) and Ki67+ (proliferation) cells.

A Small Nucleus of Dopaminergic Neurons of VTA Origin Innervate the SVZ

With data suggesting a role for the VTA in the SVZ of AK mice, we hypothesized that might happen in the C57 mouse. Since anterograde injections could not be specific enough to guarantee SNpc versus VTA injections, we decided to use retrograde tracing from the lateral ventricle to find the source of SVZ innervation. Previous studies have shown injecting factors into the ventricle as an acceptable way to drive those factors

across the ventricular wall and into the SVZ (Doetsch et al., 2002; Bauer and Patterson, 2006; Ferron et al., 2007).

After performing retrograde injections of WGA-FITC into the lateral ventricles of C57 mice and waiting 5-7 days, the mice were perfused and the brains processed. The A9 and A10 neurons of the SNpc and VTA, respectively, were labeled with the following set of markers: All dopaminergic midbrain neurons were visualized with TH (Fig. 8a); A10 VTA neurons were stained with Calbindin (8b); and SNpc A9 neurons were stained with Girk2. The WGA-FITC that was injected into the lateral ventricles tracked to an area at the juncture of the A9 and A10 regions of the midbrain (8c). No tracking was seen in other areas of the VTA or SNpc. When neurons in this region were analyzed further using immunohistochemistry, TH and calbindin positivity indicated that they were A10 neurons (8d). These results indicate that the VTA's role in dopaminergic innervation of the SVZ in AK mice is not an anomaly resulting from a lack of SNpc; rather, it seems that the VTA plays a role in dopaminergic innervation in the wild type C57 mouse as well.

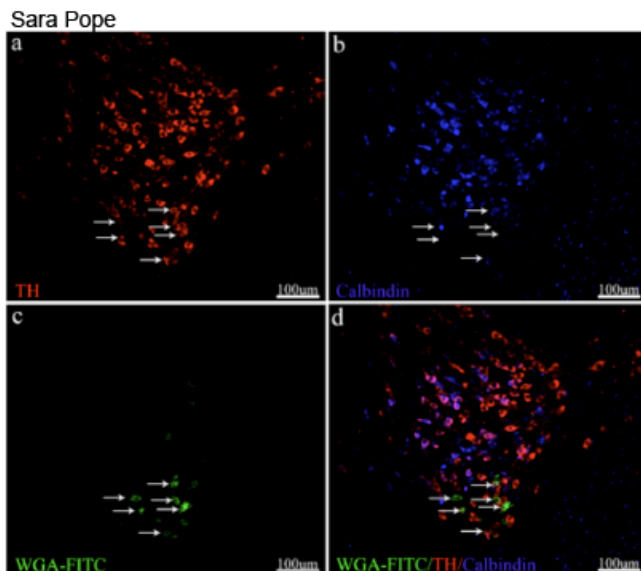


Figure 8: Retrograde tracing. Injections of WGA-FITC were made into the lateral ventricle of C57 mice. In (a), TH immunohistochemistry is used to show all midbrain dopaminergic neurons of SNpc or VTA origin. (b) Calbindin is used to visualize A10 neurons of the VTA. (c) WGA-FITC tracked from the dopaminergic neurons of the SVZ to the "elbow region" of the midbrain, where the SNpc and VTA merge. (d) Arrows show triple-labeled cells. These cells indicate that some dopaminergic fibers in the SVZ of C57 mice track back to a population of Th+/Calbindin+ (VTA) neurons in the midbrain.

V. DISCUSSION

The data suggest that the paradigm of dopaminergic innervation in the SVZ may need to shift. We hypothesized that AK mice would have decreased neurogenesis due to a depletion of dopamine in the SVZ, based on the commonly accepted idea that the SVZ receives dopaminergic afferents from the SNpc. However, our data showed no significant difference in neurogenesis between AK and C57 mice. This suggests that innervation from the SNpc is not necessary to support regular levels of neurogenesis in the SVZ.

To explain why the AK mice had similar levels of neurogenesis to the C57 mice, our hypothesis regarding the VTA's role in innervating the SVZ becomes relevant. Two scenarios seem plausible based on the data: Either the VTA is completely responsible for the dopaminergic innervation of the SVZ, or the VTA is partially responsible, sharing its role with the SNpc. In the latter case, the data would be explained by an upregulation of dopamine from the VTA due to the absent SNpc in AK mice, salvaging the neurogenesis to wild type levels.

When MPTP injections were given to C57 and AK mice, we expected to have a decrease in cell proliferation in the C57 and no change in the AK. Technically, statistical analysis of our results supported our expectations. However, a closer look at the data trends reveals another possibility. The AK mice did show a discernible, yet not statistically significant, decrease in cell proliferation following MPTP treatment. Repeating this experiment for the AK mice could plausibly bring the cell counts to the threshold for statistical significance.

Based on the opinion that AK mice do show considerable decreases in neurogenesis in response to MPTP, it seems that MPTP may not target SNpc neurons

alone. The impact of MPTP on AK mice suggests that the injections impact the A10 VTA neurons as well as the SNpc population, and thus further implicates the A10 neuronal population as a source of dopamine signaling in the SVZ.

The next step toward pinpointing the source of dopaminergic innervation of the SVZ was to conduct neuronal tracing experiments. The anterograde experiments demonstrated that, despite lacking most neurons of the SNpc, AK mice maintain dopaminergic afferents in the SVZ. This suggests that, at the least, the VTA supplies some dopamine to the SVZ. In the anterograde tracing study (**Fig. 7**), we show the dorsal, middle and ventral SVZ as separate images. The B cells, or stem cells, in the SVZ niche are often thought of as a homogenous proliferative group, but evidence exists that these cells are diverse and regionally specified within the SVZ. B cells from different regions of the SVZ are already heterogeneous by the time they reach the rostral migratory stream, and they form different types of interneurons upon reaching the olfactory bulb (Kriegstein & Alvarez-Buylla, 2009). From this information, the question emerges of whether the dopaminergic afferents in the SVZ follow any sort of spatial pattern corresponding to the subgroups of B cells with different fates.

Since the anterograde tracing data in the AK mice demonstrated that the VTA may innervate the SVZ, we conducted retrograde tracing studies in C57 mice to see if we would find the same result in the wild type model. Injections of WGA-FITC into the lateral ventricles of C57 mice resulted in tracing to a nucleus of cells into the border of the SNpc and the VTA. These cells triple labeled for WGA-FITC, TH and calbindin, signifying that the tracer had tracked to dopaminergic neurons in the VTA. This indicates that, in the wild type C57 mouse, some A10 neurons of the VTA are responsible for

dopaminergic innervation of the SVZ. While the data is intriguing, more trials should be carried out to solidify the significance.

Achieving a better understanding of the SVZ and its regulation has implications in biomedical science, from the bench to the clinic. Studies like these will help researchers better understand the brain's ability to regenerate neurons and will ultimately contribute to the knowledge base that drives science forward.

VI. ACKNOWLEDGEMENTS

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