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RESEARCH ARTICLE

Increasing Progenitor Cell Proliferation in the Sub-Ventricular Zone: A Therapeutic Treatment for Progressive Multiple Sclerosis?

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Abstract: ***Introduction*:** The purpose of this study was to determine if pharmacological treatment could increase progenitor cell proliferation in the Sub-ventricular Zone of aged rats. Previous work had shown that increasing progenitor cell proliferation in this region correlated well (R2=0.78; *p*= 0.0007) with functional recovery in a damaged corpus callosum (white matter tract), suggesting that progenitor cell proliferation results in oligodendrocytes in this region.

***Methods*:** 10 month old male and female Sprague Dawley rats were fed the drugs for 30 days in cookie dough, then immunocytochemistry was performed on coronal brain sections, using Ki67 labeling to determine progenitor cell proliferation.

***Results*:** Female rats showed low endogenous (control) progenitor cell proliferation, significantly different from male rats (P<0.0001), at this age. Ascorbic Acid (20 mg/kg, daily for 30 days) increased progenitor cell proliferation overall, but maintained the innate gender difference in stem cell proliferation (P=0.001). Prozac (5 mg/kg, daily for 30 days) increased progenitor cell proliferation for females but decreased stem cell proliferation for males, again showing a gender difference (P<0.0001). Simvastatin (1 mg/kg for 30 days) also increased progenitor cell proliferation in females and decreased progenitor cell proliferation in males, leading to a significant gender difference.

***Discussion*:** The three drug combinations (fluoxetine, simvastatin, and ascorbic acid, patent # 9,254,281) led to ~ 4 fold increase in progenitor cell proliferation in females, while male progenitor cell proliferation was highest with 50 mg/kg ascorbic acid. However, the ascorbic acid increase in proliferation appears to be only on the sides of the ventricles, which is not the region that normally gives rise to oligodendrocytes.

***Conclusion*:** There are innate gender differences in progenitor cell proliferation at the Sub-Ventricular Zone at middle age in rats, possibly due to the loss of estrogen in females. We also see notable gender differences in progenitor cell proliferation in the Sub ventricular Zone in response to common drugs, such as fluoxetine, simvastatin and Vitamin C (ascorbic acid).

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1. INTRODUCTION

The Sub-ventricular zone of the lateral ventricles (the second brain neurogenesis niche) is important as it has been shown to give rise to oligodendrocytes after demyelination or injury from ischemic stroke in the white matter tracts, such as the corpus callosum [1-4]. In studying neurogenesis, numerous publications have pointed out the decrease in neurogenesis in aged animals, with a large portion of this work focusing on hippocampal neurogenesis [5-7]. However, it is interesting that progressive Multiple Sclerosis generally occurs around middle age or in the presence of great stress, both of which result in decreased neurogenesis in the Sub-ventricular zone [2, 8, 9]. In general, a good deal is known about adult brain neurogenesis, primarily through studies on rodents. Stress is known to inhibit stem/progenitor cell proliferation [10-13] in the dentate gyrus and the Sub-ventricular zone [14-16]. Previous studies show that sex hormones increase stem/progenitor cell proliferation, with estrogen [17-19], prolactin [20-22] and testosterone [23, 24]. We know that certain growth factors must be present for stem/progenitor cell proliferation to increase, including Brain-Derived Neurotrophic Factor (BDNF) [25-27], and that in general, stem/progenitor cell proliferation decreases in aged rats [6].

This study examines stem cell proliferation in the SVZ of the lateral ventricles focusing primarily on the anterior, middle and posterior regions in both genders of middle-aged

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rats, to determine if there are any regional differences (anterior, middle or posterior SVZ) in stem/progenitor cell proliferation. We also examined in particular whether this stem cell proliferation is increased or decreased by certain common individual drugs or drug combinations related to patent #9,254,281 (a combination of fluoxetine, simvastatin and ascorbic acid), given daily for a period of 30 days. All drugs were given orally, through voluntary eating, and prescription drugs were pharmacologically the same drugs that people would use; Prozac (brand name for fluoxetine), Zocor (generic for simvastatin). Ascorbic acid was purified ascorbic acid from Sigma Aldrich.

2. MATERIALS AND METHOD

2.1. Animals and Animal Husbandry

All animals were purchased as 10-month old Sprague Dawley outbred retired breeders, with equal number of male and female rats. Animals were weighed once a week, in order to keep the fixed dosage of medication constant for those animals receiving drugs or drug combinations. Animals were kept on a 12 hour light-dark-light cycle and were fed ad lib Harlan rat chow. Animals were given either vehicle (4 grams sugar cookie dough, Control groups) or drugs as outlined in Table **1** (encased in 4 grams sugar cookie dough), daily for a period of 30 days. This method of voluntary drug delivery alleviated daily stress associated with other drug delivery methods, such as oral gavage and intraperitoneal injections, which is important when trying to stimulate neurogenesis [28, 29]. The rats were euthanized on day 31 after IP injection with 100 mg/kg pentobarbital (Euthasol), through cardioperfusion with first Phosphate Buffered Saline (PBS), then PBS containing 4% Paraformaldehyde (PFA). The brain was dissected out and then prepared for immunocytochemistry. All animal studies in this paper were reviewed and approved by the IACUC.

2.2. Immunohistochemistry

The brain was blocked and then the anterior coronal piece was placed into PBS containing 4% PFA for a 24 hour post-fixation. The fixative was poured off the next day and replaced with 30% sucrose for at least 3 days, to prepare the brain for cryosectioning. We mounted our coronal section onto a cryostat post with TissueTek OCT, then froze the brain using the Peltier device on a model HM 550 cryostat. We cut the brain into 50 micron coronal sections and collected these into vials containing PBS. Brain sections were incubated in blocking solution (PBS with 0.3% Tween-20 and 3% goat serum) at room temperature for one hour on an orbital shaker. Primary antibody anti-Ki67 (AbCam, AB15580) was added to the blocking solution at a 1:1000 dilution, then incubated overnight at 5 $^{\circ}$C on an orbital shaker. The next day, the primary antibody solution was discarded and the sections were washed twice with PBS containing 0.3% Tween. The sections were then placed in a blocking solution containing a Vector biotinylated secondary antibody according to the manufacturer's instructions (from Vector ABC kit for Rabbit IgG with horseradish peroxidase) and incubated shaking at room temperature for one hour. This solution was removed from the sections and then washed twice with PBS containing 0.3% Tween. The ABC reagent was mixed and allowed to sit for at least 30 minutes; it was then placed onto the sections, incubating for one hour. Following this, the sections were washed twice with PBS containing 0.3% Tween. After removal of the solution, brain sections were incubated with the enzyme substrate (DAB with nickel enhancement) for about 10 minutes. At the end of this time, brain sections were rinsed with tap water and then resuspended in water. Sections were then mounted onto gel-subbed microscope slides, allowed to dry, then coverslipped using DPX as a permanent mountant.

2.3. Image Analysis

Digital photos were taken of the SVZ of the lateral ventricles in coronal sections of the brain slices using a total 40X magnification (oculars and objective). Multiple images of a single ventricle were montaged into one image using Adobe Photoshop. The montaged image was then opened with Image J (NIH) and the threshold adjusted until just the black Ki67 staining was highlighted in the white background. We enclosed the Ki67 staining with the free hand tool and then measured the area of particles within the enclosure in mm^2, after first setting our calibration scale for the image. For each ventricle, we first determined the location of the ventricle (anterior, middle or posterior), then indicated the average Ki67 staining area per brain slice for all of the replicate slices within that region for one particular rat.

Table 1. Drug dosages for male and female rats.

Group Name	Simvastatin (S)	Fluoxetine (F)	Ascorbic Acid (A)
Control	none	none	none
Simvastatin	1mg/kg/day	none	none
Fluoxetine	none	5mg/kg/day	none
Ascorbic Acid	none	none	20 mg/kg/day
FS	1 mg/kg/day	5mg/kg/day	none
FSA	1 mg/kg/day	5mg/kg/day	20 mg/kg/day

2.4. Graphs and Statistical Analysis

Data were graphed with Sigma Plot 11 Software and analyzed statistically with SAS, by the head of the Statistical Consulting Center (see Acknowledgements). Figures are shown with the Means ± SEM, with the number of animals in each group indicated.

3. RESULTS AND DISCUSSION

3.1. Effect of Drugs and Drug Combinations on Stem/Progenitor Cell Proliferation in Subventricular Zone Regions

Ki67 marks a protein that only appears during mitosis, so we are able to determine the number of stem/progenitor cells in the Subventricular Zone that were undergoing mitosis at the time of the animal's death: this is a measure of stem/progenitor cell proliferation [30]. We quantified the area of Ki67 staining throughout the Subventricular zone in these animals, using the NIH Image J program, as shown in Fig. (**1**). Panel A shows control female Ki67 staining in an anterior ventricle, and in panel C a mask from Image J that was used to measure the area of the particles is shown. Panel B shows Ki67 staining of an anterior ventricle from a female rat treated with Fluoxetine, Simvastatin and Ascorbic Acid (FSA) for thirty days, and the Image J mask used to measure the area of the particles is shown in panel D.

Fig. (**2**), Black bars represent the mean Ki67 staining area in the anterior SVZ, white bars represent the mean Ki67 staining area in the middle SVZ, and gray bars represent the mean Ki67 staining area in the posterior SVZ, with the error bars representing SEM. Data from male rats are shown at the left and data from female rats are shown at the right, as labeled, with the number of animals in each group (Ns) indicated in the figure legend. We can see from this figure, in the Control Panel A, that mean endogenous female stem/progenitor cell proliferation in the SVZ is quite low at this age, showing a significant difference from the male rats ($F=13.494$, $DF=1$, $P<0.001$, eta-square = 0.55): this is likely due to these female rats having estrogen loss at this age while the male rats are still producing testosterone. A daily dose of 20 mg/kg ascorbic acid (Panel B; vitamin C) increased stem cell proliferation (see y axis change) approximately 2 fold, but maintained the innate gender difference seen in control animals ($F=14.40$, $DF=1$, $P=0.001$, eta-square= 0.42). There is not sufficient evidence to suggest that there is a significant mean difference between any of the regions ($P = 0.08$). A daily dose of 1 mg/kg simvastatin (Panel C) did significantly increase stem/progenitor cell proliferation in females in a two way ANOVA ($F=6.207$, $DF=1$, $P=0.02$, eta-square = 0.19) compared to males. Fluoxetine (Panel D) given daily at a 5 mg/kg dose also increased stem/progenitor cell proliferation in females, and caused a profound decrease in males (2 way ANOVA, $F=19.011$, $DF=1$, $P<0.0001$, eta square = 0.57), again

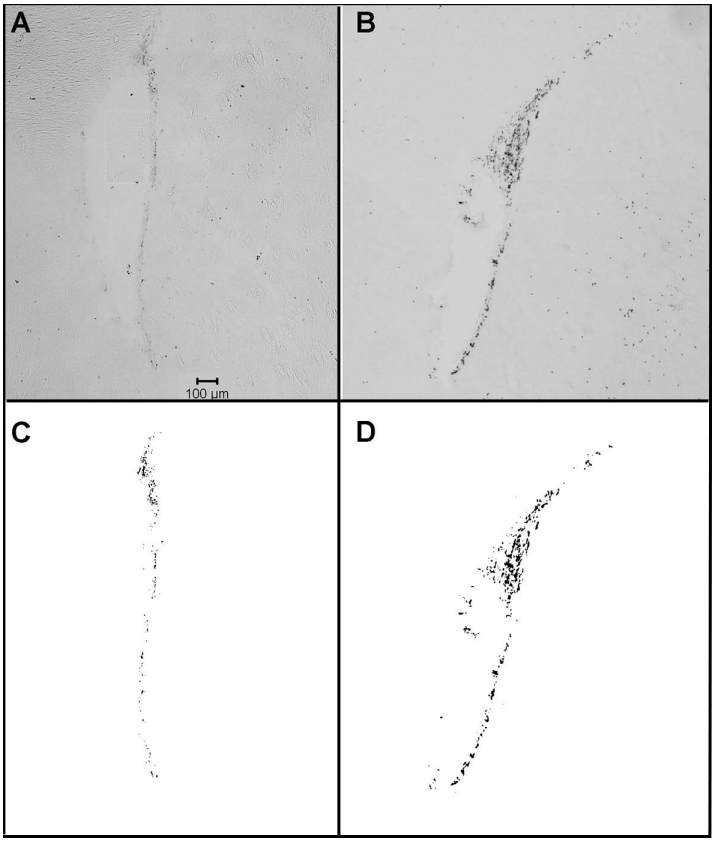

Fig. (1). Representative images of Ki67 staining in anterior ventricles of 11 month old female rats. Panel (**A**) shows the normal Ki67 staining (no drugs) and the respective mask used to measure the area of Ki67 staining in Image J from panel (**A**) is shown in panel (**C**). Panel (**B**) showing Ki67 staining in the anterior ventricle of an 11 month old female rat in response to a 30 day drug treatment to FSA (Fluoxetine, Ascorbic acid and simvastatin), with the respective mask used to determine Ki67 staining area shown in panel (**D**). Scale bar in panel (**A**) shows 100 micrometers. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

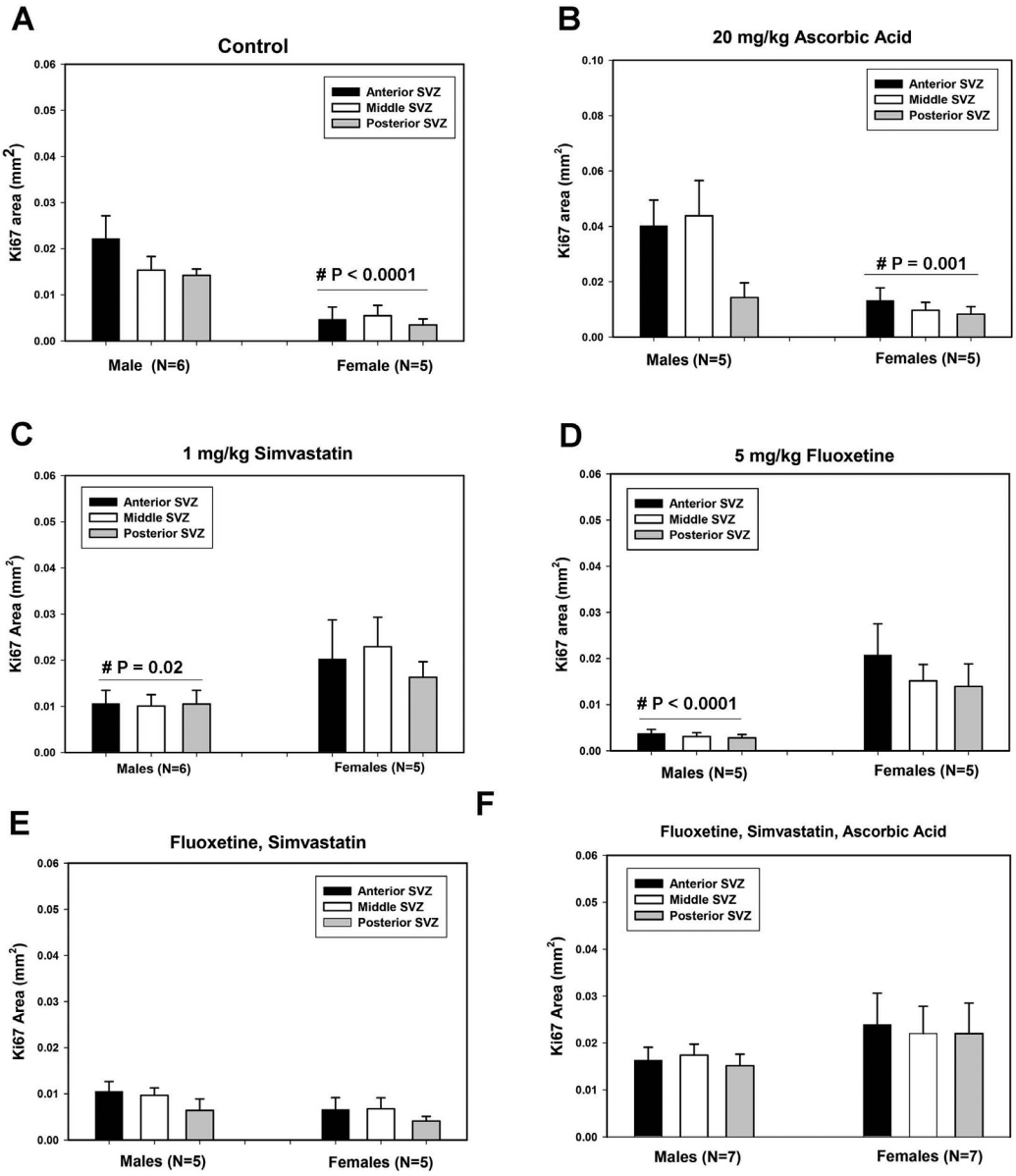

Fig. (2). Each bar represents the mean area of Ki67 staining from the anterior (black), middle (white) and posterior (gray) Subventricular zone (first independent variable in 2-Way ANOVA) The bars are also separated by gender (second independent variable in 2-way ANOVA). The mean for the treatment group is indicated by each bar and the error bar represents the standard error for the group. The statistical test was two way ANOVA using SAS. If gender showed a statistical difference, then a bar was placed over the entire male or female group with the P value above the bar. Panel **A**: Control Treatment Groups. Panel **B**: 20 mg/kg Ascorbic Acid Treatment Groups. Panel **C**: 1 mg/kg Simvastatin Treatment Groups. Panel **D**: 5 mg/kg Fluoxetine Treatment Groups. Panel **E**. 5 mg/kg Fluoxetine, 1 mg/kg Simvastatin Treatment Groups. Panel **F**: 5 mg/kg Fluoxetine, 1 mg/kg Simvastatin, 20 mg/kg Ascorbic Acid Treatment Groups. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

showing a gender difference in response to an individual drug. The combination of Simvastatin and Fluoxetine (Panel E) had a rather surprising effect, in that the increases seen with the individual drugs in female rats were not additive: we actually saw a decrease in the stem/progenitor cell proliferation area to a mean (.00886 mm^2) very similar to control (0.00637 mm^2) female rats. In males, we saw slightly lower stem/progenitor cell proliferation in the combination drugs (mean Ki67 area =0.00886 mm^2) compared to that with statin alone (mean Ki67 area = 0.0104 mm^2), but again not additive. In this drug combination of fluoxetine and simvastatin, no statistical differences between the genders (2 way ANOVA, F=3.117, DF=1, P=0.086) and between regions in the SVZ (2 way ANOVA, F=1.384, DF=2, P=0.264) were seen. In Panel F, we gave the rats daily doses of our three drugs combination (1 mg/kg Simvastatin, 20 mg/kg Ascorbic Acid, and 5 mg/kg Fluoxetine) for 30 days. Female stem/progenitor cell proliferation was increased compared to female control approximately 4 fold (see Fig. **3B**) with this drug combination, but it was not significantly different from the male stem/progenitor cell proliferation within this treatment group (2 way AN0VA, F=2.466, DF=1, P=0.125), and we saw no regional differences in stem/progenitor cell proliferation (2-way ANOVA, F=0.0369, DF=2, P=0.964).

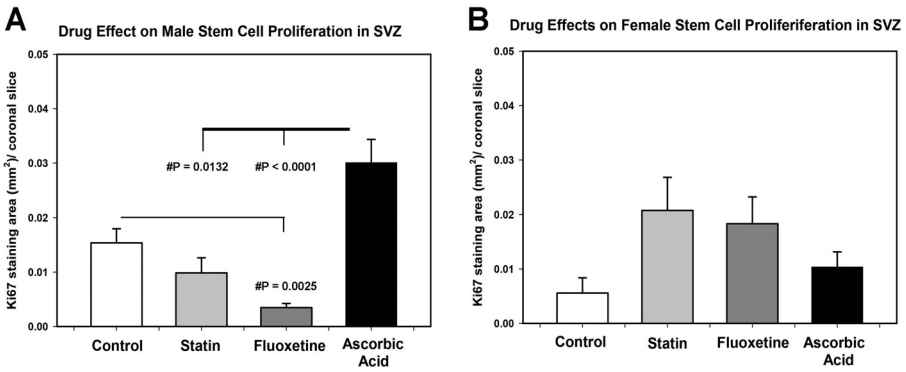

Fig. (3). Individual drug effects on stem cell proliferation in SVZ of male (Panel **A**) or female (Panel **B**) rats tested in one way ANOVA. In this analysis, we used the average Ki67 staining area in a coronal 50 micron brain slice for the entire SVZ for a given rat in a group, with the error bar representing SEM for the group. The treatment groups were 1(white bar): control (no drugs; male N=6; female N=5), 2(light grey bar): 1 mg/kg simvastatin (male N=5; female N=5), 3 (dark grey bar): 5 mg/kg fluoxetine (male N=5; female N=5); and 4 (black bar): 20 mg/kg ascorbic acid (male N=5, female N=5), which the animals received daily for a period of 30 days. Statistical Analysis was by one-way ANOVA using SAS. Ki67 Staining for Male Fluoxetine group ($p<0.0001$) and Male Simvastatin group was significantly decreased for the Male Fluoxetine group ($P<0.001$), the simvastatin group ($P<0.0132$) compared to the Male Ascorbic Acid Group. The Fluoxetine group Ki67 staining was significantly decreased compared to male control ($P=0.0025$). There were no significant differences in the one-way ANOVA for the female group ($P=0.09$). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Effect of Individual Drugs on Stem/Progenitor Cell Proliferation in the Subventricular Zone in Male and Female Rats

In this analysis, the Stem/Progenitor cell proliferation, measured by Ki67 staining area in mm^2, was averaged across the entire Subventricular Zone for each rat in a treatment group. We then compared changes in the stem/progenitor cell proliferation with each individual drug treatment to control to determine if there were significant increases or decreases in stem/progenitor cell proliferation for a given gender, male or female. In Fig. **3**, Panel A shows the results from the male rats and Panel B shows the results from the female rats. White bars indicate control treatment groups (male N=6, female N=5), light gray bars indicate simvastatin treatment groups (male N=5, female N=5), dark gray bars indicate fluoxetine treatment groups (male N=5, female N=5) and black bars indicate ascorbic acid treatment groups (male N=5, female N=5). In a 1-way ANOVA, we saw a significant difference in Ki67 area (indicating stem/progenitor cell proliferation) between the treatment groups ($F=14.937$, $DF=3$, $P<0.0001$, Eta-square = 070). In post hoc analysis with the Tukey's multiple comparison method, treatment with 20 mg/kg Ascorbic acid (Vitamin C) increased stem/progenitor cell proliferation significantly in male rats over treatment with 1 mg/kg simvastatin (simvastatin comparison, difference between means = 1.316585, p = 0.0132, simultaneous 95% confidence limits (0.247465, 2.385706) and 5 mg/kg fluoxetine (fluoxetine comparison, difference between means = 2.2493, adjusted P value < 0.0001, simultaneous 95% confidence limits (1.180261, and 3.3118503). Treatment with 5 mg/kg fluoxetine significantly decreased stem/progenitor cell proliferation in SVZ in males compared to control (difference between means = 1.549221, p = 0.0025, simultaneous 95% confidence level (0.525616, 2.572826). While all three drugs increased stem/progenitor cell proliferation in female rats (panel B), none of these was statistically significant ($F=2.587$, $DF=3$, $P=0.09$) from control, although the power of the performed test (0.331) was less than the desired power of 0.800, so the negative result should be interpreted cautiously.

3.3. Effect of Drug Combinations on Stem/Progenitor Cell Proliferation in the Subventricular Zone of Male and Female Rats

The stem/progenitor cell proliferation, measured by Ki67 staining area in mm^2, was averaged across the entire Subventricular Zone for each rat in a treatment group. We then compared drug combination treatments (1. 5 mg/kg Fluoxetine and 1 mg/kg Simvastatin; male N=7, female N=6; **2**. 5 mg/kg Fluoxetine, 1 mg/kg Simvastatin, 20 mg/kg Ascorbic Acid; male N=7, female N=7) against a control group (male N=6, female N=5), and looked for differences in stem/progenitor cell proliferation with one way ANOVA, shown in Fig. **4**. Control groups are shown in white bars, the Fluoxetine and Simvastatin drug combination groups are shown with diagonal striped bars, and the Fluoxetine, Simvastatin and Ascorbic Acid combination groups are shown with diagonally hatched bars. In Panel A, although the individual drugs had each significantly altered stem cell proliferation in the males, the drug combinations did not ($F=3.116$, $DF=2$, $P=0.070$), although the negative result should be interpreted cautiously since the power of the performed test (0.364) was below the desired power of 0.800. In this panel, the combination of Fluoxetine and Statin showed the only decrease in stem/progenitor cell proliferation compared to either control or the three drug combination (fluoxetine, simvastatin, ascorbic acid). In Panel B, the Fluoxetine, Simvastatin and Ascorbic acid drug combination significantly increased stem/progenitor cell proliferation compared to either control or the fluoxetine and simvastatin combination ($F(2,17)=4.180$, $DF=2$, $p = 0.004$, eta square = 0.36), showing approximately a 3.8 fold increase in proliferation. The individual fluoxetine and simvastatin effects are not additive in the female rats in the drug combination. It is possible that oxidation of some of the components in the fluoxetine and simvastatin combination abolishes its normal

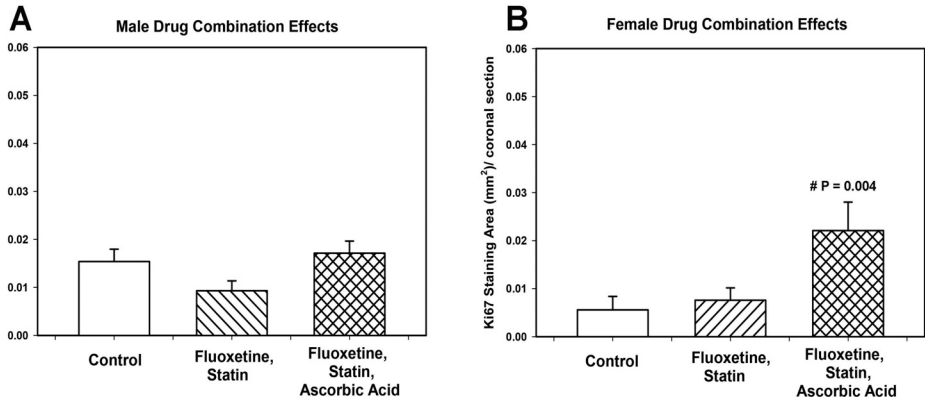

Fig. (4). Drug combination effects on stem cell proliferation in male (Panel **A**) or Female (Panel **B**) rats tested in one way ANOVA. In this analysis, we used the average Ki67 staining area in a coronal 50 micron brain slice for the entire SVZ for a given rat in a group, with the error bar representing SEM for the group. The treatment groups were 1(white bar): control, no drugs (male N=6, female N=5); 2. (Diagonal striped bar): 5 mg/kg fluoxetine, 1 mg/kg simvastatin drug combo treatment group (male N=7; female N=6); and 3 (cross-hatched bar): 5 mg/kg fluoxetine, 1 mg/kg simvastatin and 20 mg/kg ascorbic acid drug combination treatment group (male N=7; female N=7). Statistical analysis was by one way ANOVA using SAS. P value for Panel **A** (males) was 0.070. The P value for Panel **B** (females) was 0.004, showing a significant difference between groups. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

effect, as the addition of an antioxidant, ascorbic acid, in the three drug combination increases the stem cell proliferation greatly.

3.4. Functional Recovery of White Matter Injury in Ischemic Stroke Correlated with Increased Progenitor Cell Proliferation in the Sub-Ventricular Zone

In the previous work in our lab, we had induced ischemic stroke in female middle aged rats with an endothelin injection targeting the forelimb motor cortex, then assaying for functional recovery with the Montoya Staircase [2, 28]. During these experiments, it became evident from the functional assay with Montoya Staircase that we have damaged the white matter tract in the Corpus Callosum, since approximately 25% of the animals showed a bilateral deficit in grasping after stroke induction. While we could not find a good correlation between progenitor cell proliferation and total functional recovery (R squared values of 0.008895 for thirty day treatment and 0.01024 for sixty day treatment), we did see a very good correlation between progenitor cell proliferation and ipsilateral recovery (R squared = 0.7827; p=0.0007). While only 25% of the animals in this study showed damage to the corpus callosum and bilateral deficits in forepaw function, those with ipsilateral damage showed full recovery of the ipsilateral functional deficit by the end of 30 days of treatments.

3.5. Innate Sex Differences in Stem Cell Proliferation in the Subventricular Zone of 11-month Rats

Rats of this age are generally considered retired breeders. Although we sometimes find a female rat of this age with an elevated estrogen, the vast majority of them have very low levels of sex hormones [31], corresponding to the post-menopause stage in humans. Male rats, however, still have elevated levels of testosterone at this age, which accounts for their innate high stem/progenitor cell proliferation level compared to the females [24]. Stem/progenitor cell proliferation is a reflection of the number of growth factors that are available in that particular niche [32-34] and a number of researchers have hypothesized that this loss of growth factors in the aging brain may increase the susceptibility of neurodegenerative diseases, such as Alzheimer's disease [35, 36].

3.6. Drug Effects on Subventricular Zone Neurogenesis

The method of drug delivery (voluntary oral delivery) was important in this study, as it removed the daily stress of either a daily injection of drugs or oral gavage from this study. By minimizing stress, it enabled us to examine the sole effect of drug(s) on neurogenesis. The human equivalent dosages of drugs given to rats would correspond to approximately a 40 mg dose of fluoxetine, a 8 mg dose of simvastatin and a 160 mg dose of ascorbic acid (vitamin C), which are all within a normal therapeutic range [37].

In this study, we see some clear gender based differences in response to the different drugs tested in the stem/progenitor cell proliferation in the Subventricular Zone. Fluoxetine, in particular, stimulates stem/progenitor cell proliferation in female rats after estrogen loss but significantly decreases stem cell proliferation in males. There has been previous work showing that fluoxetine decreases stem cell proliferation in the Subventricular zone of lateral ventricles in 8 month old male rats, but it was unclear in that study whether the author saw the effect of the fluoxetine itself or the effect of the drug delivery method (intraperitoneal injection), which was stressful [38]. In an earlier study [39], using subcutaneous fluoxetine pellets to deliver the drugs in mice for different lengths of time, the authors found that chronic delivery for either 6 or 9 weeks decreased stem cell proliferation at the Subventricular zone in adult (8 week old) male mice, while it increased stem cell proliferation in the Subgranular zone.

Administration of low dosage statin (1 mg/kg simvastatin) stimulates stem/progenitor cell proliferation in the Subventricular Zone in females but decreases it in males, showing a statistical gender difference in 2 way ANOVA. Simvastatin has been shown to increase stem/progenitor cell proliferation in other studies [40, 41], in which the age of

the animals was much younger. Interestingly, in a study in male mice [42] in which lesions were induced in the corpus callosum, treatment with simvastatin (ip injections for at least a two week period of time) inhibited central nervous system remyelination [43] by blocking SVZ proliferation and differentiation, which supports the data we show, where stem/progenitor cell proliferation is ultimately decreased with simvastatin in male rats.

Ascorbic Acid (20 mg/kg) increased stem/progenitor cell proliferation in both males and females, but males had significantly greater stem/progenitor cell proliferation than females (2-way ANOVA). Vitamin C (ascorbic acid) has specific transporters across the brain [44, 45] and is an important antioxidant [46-48]. It is interesting that while we see increased stem cell proliferation with this drug, we appear to be missing an increased stem cell proliferation across the top of the ventricle, where oligodendrocyte precursor markers are found [49].

The highest stem cell/progenitor cell proliferation was seen with a combination of statin, fluoxetine and ascorbic acid in the female rats (Fig. **4**). The statin, we believe, is working through stimulation of endothelial nitric oxide synthase to produce BDNF [27], and it also stimulates a pathway that produces plasmin, which is capable of cleaving any released pro-BDNF to BDNF [50, 51]. The fluoxetine in this combination is working on microglial cells in an injury model [28], changing an M1 inflammatory type microglial cell to an M2 neuroprotective microglial cell [52]. Even though this study used normal animals, they were also aged animals, and studies have shown that microglia become increasingly inflammatory with age [53-55]. One obvious limitation in this study is that stem cells in the SVZ of rats may not behave in the same way as stem cells in the SVZ of humans, but there is some evidence of gender differences in response to fluoxetine in humans [56].

CURRENT & FUTURE DEVELOPMENTS

This research offers a therapeutic means of increasing progenitor cell proliferation for both male and female middle-aged rats, although the therapeutic means showed a gender difference. We would like to next test these drug combinations after a focal demyelination of the corpus callosum under the forelimb motor cortex with lysolecithin, to determine if increasing progenitor cell proliferation with these drugs does result in more remyelination and functional recovery.

CONCLUSION

There are innate gender differences in progenitor cell proliferation at the sub-ventricular zone at middle age in rats, possibly due to loss of estrogen in females. We also see notable gender differences in progenitor cell proliferation in the subventricular zone in response to common drugs, such as fluoxetine, simvastatin, and vitamin C (ascorbic acid).

LIST OF ABBREVIATIONS

Anova	=	Analysis of Variance
Bdnf	=	Brain Derived Neurotrophic Factor
Fsa	=	Fluoxetine, Simvastatin and Ascorbic Acid Combination
Sem	=	Standard Error of the Mean
Svz	=	Sub-Ventricular Zone

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Prior to initiation of this study, animal protocols were approved by the Wright State University IACUC.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal experiments were performed in compliance with the US National Research Council's "Guide for the Care and Use of Laboratory Animals".

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Our data is not stored in any external repository but is available to any reviewers upon request.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHORS CONTRIBUTIONS

TS performed the majority of the animal work and analysis of Ki67 staining; he also contributed in writing the manuscript. NS performed the next highest amount of work on this project, in both animal work and analysis. DN performed some essential correlation of recovery of function with white matter injury in an ischemic stroke model in middle aged rats, showing that increased neurogenesis was correlated with increased white matter functional recovery. AA performed some additional analysis needed for increasing Ns in each group, along with AJ. AC performed all statistics for data analysis, prepared the figures, and wrote the majority of the manuscript. We thank Mike Bottomley of the Statistical Consulting Center for help with all of the statistical analysis.

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