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Sildenafil treatment of vascular dementia in aged rats

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ABSTRACT

Background: and purpose: In this study, we employed a multiple microinfarction (MMI) based vascular dementia (VaD) model in aged rats and tested the therapeutic effects of Sildenafil, a phosphodiesterase type 5 inhibitor, on cognitive decline, white matter damage, autophagy and inflammatory response associated with VaD.

Methods: Male, aged (16–18 months) Wistar rats were subjected to MMI (800 ± 100, 70–100 μm cholesterol crystals injected into the internal carotid artery) and treated with or without Sildenafil (2 mg/kg, i.p) starting at 24 h after MMI daily for 28 days. Four experimental groups were employed: Sham control, Sham + Sildenafil, MMI, and MMI + Sildenafil. A battery of cognitive tests were performed and rats were sacrificed at 28 days after MMI for immunohistochemical evaluation and PCR assay.

Results: Sildenafil treatment in aged MMI rats significantly improves short term memory evaluated by the novel object recognition test and improves spatial learning and memory in the Morris water maze test compared to aged control MMI rats. Sildenafil treatment of aged MMI rats significantly increases axon and myelin density in the corpus callosum and white matter bundles in the striatum, increases oligodendrocyte and oligodendrocyte progenitor cell number in the corpus callosum, cortex and striatum, and increases synaptic protein expression in the cortex and striatum compared to aged control MMI rats. In addition, Sildenafil treatment of MMI in aged rats significantly decreases Beclin1 expression and inflammatory factors Monocyte chemoattractant protein-1 and Interleukin-1β expression in brain. Sildenafil treatment in aged rats does not improve cognitive outcome compared to aged sham control rats.

Conclusions: Sildenafil treatment of MMI in aged rats significantly improves cognition and memory at 1 month after MMI. Sildenafil treatment increases axon and myelin density, increases Synaptophysin expression, decreases autophagic activity and exerts anti-inflammatory effects which in concert may contribute to cognitive improvement in aged rats subjected to MMI.

1. Introduction

Vascular dementia (VaD) is a complex neurodegenerative disease affecting cognition and memory, and may result from several mechanisms, one of which involves injury to blood vessels supplying deep white matter (WM) of the brain evoking silent, multifocal, brain microinfarcts, vascular dysfunction, decrease in cerebral blood flow (CBF) and induction of cerebral parenchymal cell damage (Laloux and Brucher, 1991; Rapp et al., 2008b; Steiner et al., 1980; Venkat et al., 2015, 2017; Wang et al., 2012, 2017; Yu et al., 2018). Extensive WM damage such as vacuolization, rarefaction, and demyelination in the periventricular region have been reported in VaD patients (Erkinjuntti et al., 1996; Tanabe et al., 1999). Such periventricular WM damage disrupts neuronal connections to the frontal lobe and may be central to VaD induced cognitive impairment (Sultzer et al., 1995). Cortical and sub cortical microinfarcts and periventricular demyelination have been routinely associated with VaD in post mortem studies of dementia cases of VaD or of mixed dementia subtype (Arvanitakis et al., 2011; Kovari et al., 2007). The multiple microinfarction (MMI) model in rodents closely mimics atheroembolization and represents global decline in CBF as a contributor to VaD (Laloux and Brucher, 1991; Rapp et al., 2008b; Steiner et al., 1980; Venkat et al., 2015, 2017; Wang et al., 2012, 2017; Yu et al., 2018). MMI in rodents induces cognitive impairment, periventricular WM damage, hippocampal damage, blood brain barrier (BBB) disruption, increased inflammatory responses, impaired water channel function and induces glymphatic dysfunction (Laloux and Brucher, 1991; Rapp et al., 2008b; Steiner et al., 1980; Venkat et al., 2015, 2017; Wang et al., 2012, 2017; Yu et al., 2018). VaD accounts for

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nearly 20% of all dementia patients, and in older adults it is the second leading form of dementia after Alzheimer's disease (Plassman et al., 2007). Yet, there is a lack of approved pharmacological treatments specifically for VaD creating a difficult situation for patients, caregivers, and healthcare providers.

Sildenafil is acyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 inhibitor and is marketed for the treatment of erectile dysfunction (Fink et al., 2002). Sildenafil increases cGMP and enhances nitric oxide-mediated vasodilation and increases blood flow (Fink et al., 2002). Sildenafil treatment (2 or 5 mg/kg per day) initiated at 2 or 24 h after ischemic stroke and administered orally for 7 consecutive days significantly promotes neurogenesis and improves neurological functional outcome in rats (Zhang et al., 2002). In neonatal rats with hypoxia-ischemia, 10 mg/kg Sildenafil citrate treatment significantly increases CBF, decreases apoptosis, decreases inflammatory responses such as reactive astrogliosis and macrophage/microglial activation, and improves motor locomotion (Charriaut-Marlangue et al., 2014). Sildenafil (15 mg/kg, i.p) administered daily for 5 weeks improves cognitive outcome in a transgenic mouse model of Alzheimer's disease without altering amyloid burden (Cuadrado-Tejedor et al., 2011). In a small group of 10 patients with mild to moderately severe stroke, Sildenafil (25 mg daily for 2 weeks) treatment initiated at 2–9 days after stroke onset was reported to be safe (Silver et al., 2009). The therapeutic effects of Sildenafil have not been investigated in VaD. In humans as well as rats and mice, cognitive outcome deteriorates with increasing age (Goldman et al., 1992; Park et al., 2007). Therefore, in a clinically relevant approach, we investigate the therapeutic effects of Sildenafil in improving cognitive outcome after MMI in aged rats. We also investigate the effects of Sildenafil treatment on WM remodeling and inflammatory response in aged rats subjected to MMI.

2. Material and methods

All experiments were conducted with institutional IACUC approval and in accordance with the standards and procedures of the American Council on Animal Care and Institutional Animal Care and Use Committee of Henry Ford Health System.

2.1. MMI model

The method for preparation of cholesterol crystals for the MMI model has been previously described (Rapp et al., 2008a, 2008b; Venkat et al., 2017; Wang et al., 2012). Briefly, freshly prepared crystals were filtered using a 100 μm cell strainer and filtrate passed through a 70 μm cell strainer to collect residual crystals of size 70–100 μm. The crystals were counted on a hemocytometer and diluted to yield a final concentration of 800 ± 100 crystals/300 μl saline. Male, aged (16–18 months) Wistar rats were anesthetized with 2% isoflurane in a chamber for pre-anesthetic, and spontaneously respired with 1.5% isoflurane in 2:1 N_2O:O_2 mixture using a facemask connected and regulated with a modified FLUOTEC 3 Vaporizer (Fraser Harlake, Orchard Park, NY). Rectal temperature was maintained at 37 °C throughout the surgical procedure using a feedback regulated water heating system. A 1 ml syringe connected to a PE-50 tube, with its tip tapered by heating near a flame was inserted from the external carotid artery (ECA) into the lumen of the internal carotid artery (ICA) (Venkat et al., 2017). The freshly prepared cholesterol crystals were slowly injected into the ICA over 1 min. The ECA was ligated after cholesterol injection while the ICA remained patent. Rats were moved back to their home cages to regain consciousness.

2.2. Blinding and randomization

Rats were randomly divided into control, and MMI groups. On day 1 after MMI, rats were randomized to treatment and non-treatment groups. Investigators blinded to the experimental groups performed behavioral tests and immunostaining measurements.

2.3. Experimental groups

Aged rats were randomized to: 1) sham control, 2) sham control + Sildenafil, 3) MMI, 4) MMI + Sildenafil. Sham control groups are included as reference to test the effects of the MMI model. Based on efficacy and toleration, the dose of Sildenafil citrate (Viagra; Pfizer, New York, NY, USA) used in patients with erectile dysfunction is 25–100 mg/day. The dosage of Sildenafil used in the present study is 2 mg/kg/day, which is equivalent to 27 mg/day in humans, using the BSA-based dose calculation (Reagan-Shaw et al., 2008). Administration of Sildenafil intravenously can induce hemodynamic changes (Al-Hesayen et al., 2006), while several studies have reported that intraperitoneal (i.p) administration of Sildenafil in rodents induces therapeutic effects without affecting blood pressure (Ali et al., 2011; Hassan and Ketat, 2005; Patil et al., 2006; Taskin et al., 2014). Therefore, i.p. administration was chosen. Sildenafil treatment (2 mg/kg, i.p.) was initiated at 24 h after MMI and administered daily for 28 days. Rats were sacrificed at 28 days after MMI for immunohistochemical analysis. There were no mortalities in the aged sham control group, 1 out of 6 rats died on day 14 in sham control + Sildenafil group, 2 out of 10 rats in MMI group died on days 1 and 4 after MMI, respectively, and 1 out of 8 rats died on day 1 after MMI in the Sildenafil treated aged MMI group. Therefore, the sample size for the groups is as follows: sham control: N = 10, sham control + Sildenafil: N = 5, MMI: N = 8, MMI + Sildenafil: N = 7.

2.4. Cognitive tests

The time frame for cognitive tests is 21–28 days following MMI with Morris water maze (MWM) measurements conducted for 5 consecutive days on 21–25 days after MMI; and novel object recognition test (NOR) and odor test performed on days 26–28 after MMI, with no two tests being conducted on the same day.

a) The NOR test (Stuart et al., 2013) was used to assess short term memory based on animal bias to explore new objects. The test consists of a 5 min trial phase, and after a retention delay of 4 h, a 5 min test phase. During the trial phase, rats freely explore 2 identical objects placed equidistant from the walls of a large bin. During the test phase, one of the trial objects was randomly chosen and replaced with a novel object (different shape and color) and rats were freely allowed to explore for 5 min. Exploration was defined as sniffing, pawing, touching with snout or probing with whiskers within 1 cm of object, however sitting or climbing the object was not counted as exploration. Between trials, the bin and the objects were cleaned with water to prevent odor cues. The time spent exploring the novel and trial object was recorded and discrimination index calculated as exploration time on novel object/total time spent exploring either objects.
b) The odor test (Spinetta et al., 2008) with a retention delay of 24 h was employed to evaluate long term learning and memory based on an animal's preference for new smells. Twenty 2.5-cm round wooden beads (http://www.craftworks.com) were introduced into home cages of donor rats for one week prior to the test to serve as novel odors N1 and N2. The odor test spanning 3 days was performed in the home cages of animals and consisted of a habituation phase (day 1), trial phase (day 2) and test phase (day 3). During the 24 h habituation phase, 4 beads were placed in the home cages (familiar beads) and rats were allowed to freely explore the beads. One hour before the trial phase on day 2, all 4 beads were removed from the cage. The trial phase consisted of 3 1 min trials during which one of the 4 familiar beads were replaced with a novel odor bead (N1) with all beads placed in the middle of the testing cage. To maximize the sensitivity of the test, one novel (N1) and three

familiar-odor beads were used and the N1 bead was placed in a different position for each of the trial. An inter-trial interval of 1 min with no beads in cage was used to minimize olfactory adaptation. Following a retention delay of 24 h, during the 1 min test phase, four beads were used (N1, N2, and two familiar beads) since the four-choice procedure for assessing relative odor preference greatly increases sensitivity and reliability compared to two-choice procedures (Spinetta et al., 2008). Time spent exploring the 2 familiar beads, N1 bead and N2 bead were recorded and discrimination index calculated as time spent exploring N2/total exploration time.

c) The MWM test (Ohno et al., 2006) was used to evaluate spatial and visual learning and memory with averse motivation to assess hippocampal memory deficits. MWM was conducted on 5 consecutive days with 4 trials per animal per day. Data collection was automated using the HVS Image 2020 Plus Tracking System (US HVS Image, San Diego, CA.). For descriptive data collection, the pool was virtually subdivided into four equal quadrants (northeast (NE), north west, southeast and southwest). A platform was submerged in the NE quadrant and randomly moved within the quadrant for each of the 4 trials. At the start of each trial, the animal was placed randomly at one of four fixed starting points (north, south, east and west), facing the wall of the pool and allowed to swim for 90 s or until they found the platform. The time to reach the platform (latency) was recorded and the percentage of time traveled within the NE (platform) quadrant was calculated relative to the total amount of time spent swimming before reaching the platform.

2.5. Histological and immunohistochemical assessment

Rats were sacrificed and transcardially perfused with 0.9% saline. Brains were immediately removed and fixed in 4% paraformaldehyde. Brain coronal tissue sections were prepared and antibody against APC (oligodendrocyte (OL) marker, Genway, San Diego, CA, 1:20), NG2 (oligodendrocyte progenitor cell (OPC) marker, Chemicon (EMD Millipore), Billerica, MA, 1:400), Synaptophysin (synaptic protein, Abcam, Cambridge, MA, 1:400), Monocyte chemoattractant protein-1 (MCP-1, Abcam, Cambridge, MA, 1:100) and Beclin1 (Abcam, Cambridge, MA, 1:3000) were employed. Bielschowsky silver (axon marker) and Luxol fast blue (myelin marker) were used to stain axons and myelin. Control experiments consisted of similar procedures without addition of primary antibody.

2.6. Immunostaining quantification

Although cholesterol crystals were injected unilaterally into the right ICA, WM/axonal damage was observed in both the hemispheres, which is consistent with other studies (Wang et al., 2012, 2017). Therefore, for each slide, 8 fields of view of ipsi- and contralateral corpus callosum (CC), cortex and/or striatum were digitized under a 20 × or 40 × objective (Olympus BX40, Tokyo Japan) using a 3-CCD color video camera (Sony DXC-970MD) interfaced with MCID image analysis system (Imaging Research, St. Catharines, Ontario, Canada). For each field of view, positive cell numbers were counted, and to quantify density of positive immunolabeling, the positive stained area was measured using built-in densitometry function with a density threshold above that of the unstained set for all groups. Data were averaged to obtain a single value for each animal and presented as percentage positive area or number of positive cells/mm^2.

2.7. Real time polymerase chain reaction (PCR) assay

Total RNA was isolated with TRIzol (Invitrogen, Carlsbad, CA, USA), following standard protocol. Total RNA (2 μg) was used to make cDNA (complementary DNA) using the M-MLV (Invitrogen) standard protocol. A 2 μl aliquot of this cDNA was then used to run a quantitative polymerase chain reaction (PCR) using the SYBR Green real-time PCR method. Quantitative PCR was performed on a ViiA 7 PCR instrument (Applied Biosystems, Foster City, CA, USA) using 3-stage program parameters provided by the manufacturer, as follows; 2 min at 50 °C, 10 min at 95 °C, and then 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each sample was tested in triplicate, and analysis of relative gene expression was collected using the $2^{-\Delta\Delta CT}$ method.

Interleukin-1beta (IL-1β): Fwd: TGTCTGACCCATGTGAGCTG; Rev: CCCAAGGCCACAGGGATTTT

MCP-1: Fwd: TGTCACCAAGCTCAAGAGAGAG; Rev: CTGAAGTCCTTAGGGTTGATGC

Beclin1: Fwd: CAGTTTAACTCGGAGGAGCAGT; Rev: AACATCCCCTAAGGAGCAAGTC

GAPDH: Fwd: CGTATTCAGCATTCTATGCTCTCA; Rev: TTTGTAAGTATCTTGGTGCCTTTT

2.8. Statistical analysis

One-way Analysis of Variance (ANOVA) was used for the evaluation of functional outcome and histology. “Contrast/estimate” statement was used to test the group difference. If an overall treatment group effect was detected at $p < 0.05$, post-hoc analysis was performed and p-values were adjusted following Tukey multiple comparison testing, and are presented. Graphpad prism 7.00 was used to analyze the data. All data are presented as mean ± standard error (SE).

3. Results

3.1. Sildenafil treatment improves cognitive outcome in aged rats subjected to MMI

To test the effects of Sildenafil treatment on cognitive outcome, a battery of cognitive tests including NOR, odor test and MWM were performed and the results are presented in Fig. 1A–E. MMI in aged rats induces significant cognitive impairment affecting both poor short term and long term memory indicated by significantly decreased discrimination index in the NOR and odor test respectively ($p < 0.05$ vs. sham control), but MMI in aged rats does not significantly impair spatial learning and memory indicate by MWM compared to aged control rats ($p > 0.05$). Sildenafil treatment of MMI in aged rats significantly ($p < 0.05$ vs. aged MMI) improves short term memory indicated by higher discrimination index in NOR test, improves spatial learning and memory indicated by decreased latency and increased % time spent in the platform quadrant in the MWM test, but does not significantly improve long term memory in the odor test compared to aged control MMI rats. Sildenafil treatment of sham control rats does not significantly ($p > 0.05$) enhance memory indicated by NOR, odor and MWM tests compared to sham control rats. Fig. 1F shows that MMI in aged rats did not significantly alter body weight compared to sham control rats. Sildenafil treatment of aged control rats as well as aged MMI rats did not significantly alter body weight over 1 month compared to sham control and aged MMI rats, respectively.

3.2. Sildenafil treatment increases axon and myelin density in WM bundles in aged rats subjected to MMI

The effect of Sildenafil treatment on WM/axonal remodeling was evaluated. Fig. 2 shows that MMI in aged rats significantly ($p < 0.01$) decreases axon and myelin density in WM bundles in the striatum but not in the CC ($p > 0.05$), compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly ($p < 0.05$) increases axon density (Bielschowsky silver) in WM bundles of striatum but not in the CC ($p = 0.23$), and significantly ($p < 0.05$) increases myelin density (Luxol fast blue) in CC and WM bundles of striatum compared to aged

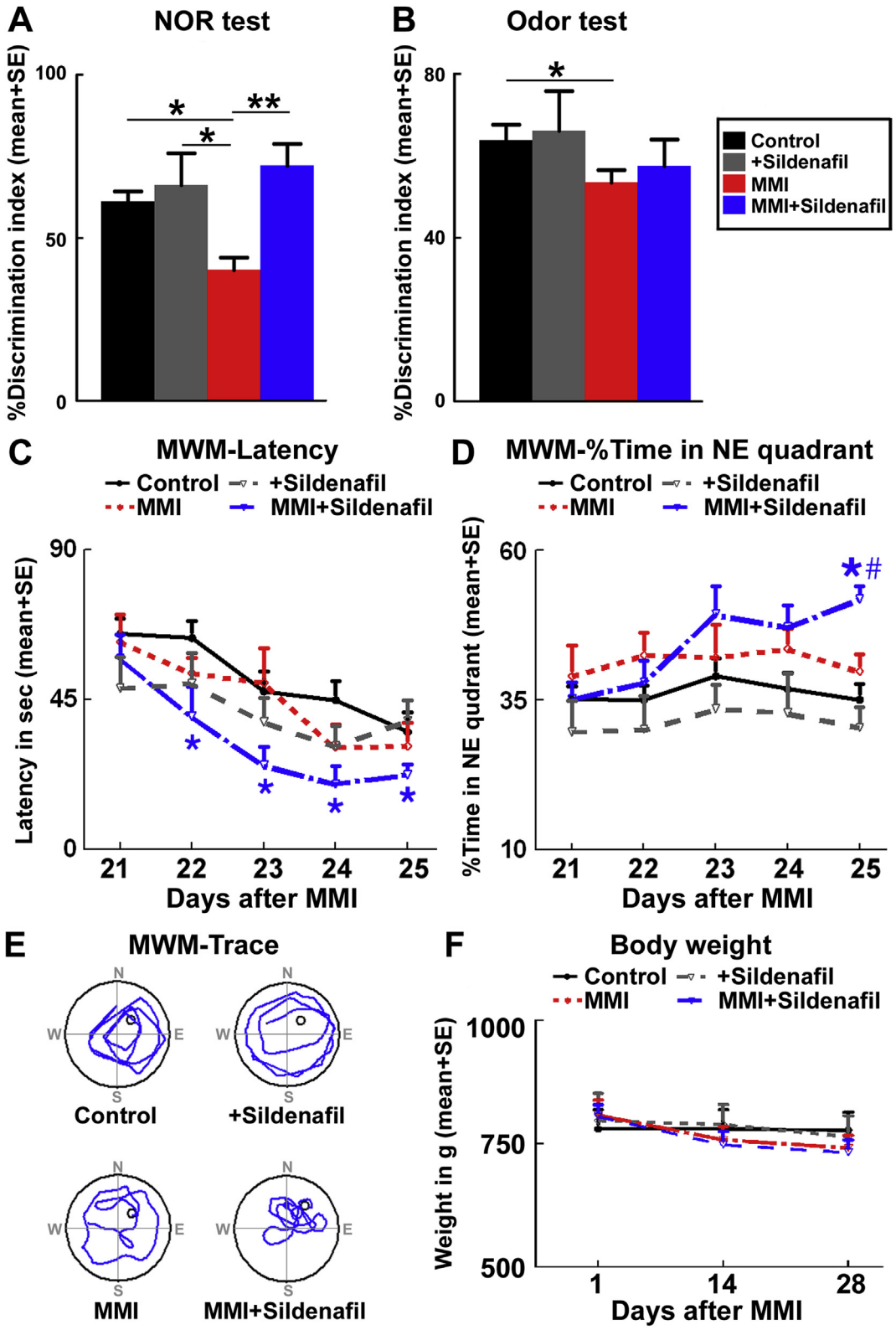

Fig. 1. Sildenafil treatment improves cognitive outcome in aged rats subjected to MMI. A battery of cognitive function tests were conducted including (A) novel object recognition (NOR) test to evaluate short term memory, (B) odor test to assess long term memory, and (C–E) Morris water maze (MWM) test to evaluate spatial learning and memory. Compared to sham control rats, aged rats subjected to MMI exhibit significant short term and long term memory deficits. MMI does not significantly alter latency or % time spent in the platform quadrant in the MWM test compared to control rats. In sham control rats, Sildenafil treatment does not significantly alter cognitive function. Sildenafil treatment of aged rats subjected to MMI significantly improves short term memory and improves spatial learning and memory but does not improve long term memory compared to age matched MMI rats, respectively, indicating that Sildenafil attenuates MMI induced cognitive impairment. (E) Representative trace of a trial on day 5 of MWM for each group. (F) There were no significant differences in body weight during the 1 month study period between the various experimental groups. Sample size: Control: N = 10, sham control + Sildenafil: N = 5, MMI: N = 8, MMI + Sildenafil: N = 7. In panels A–B *$p < 0.05$, **$p < 0.01$ between groups indicated by solid line, in panels C–D *$p < 0.05$ vs. MMI, #$p < 0.05$ vs. Control.

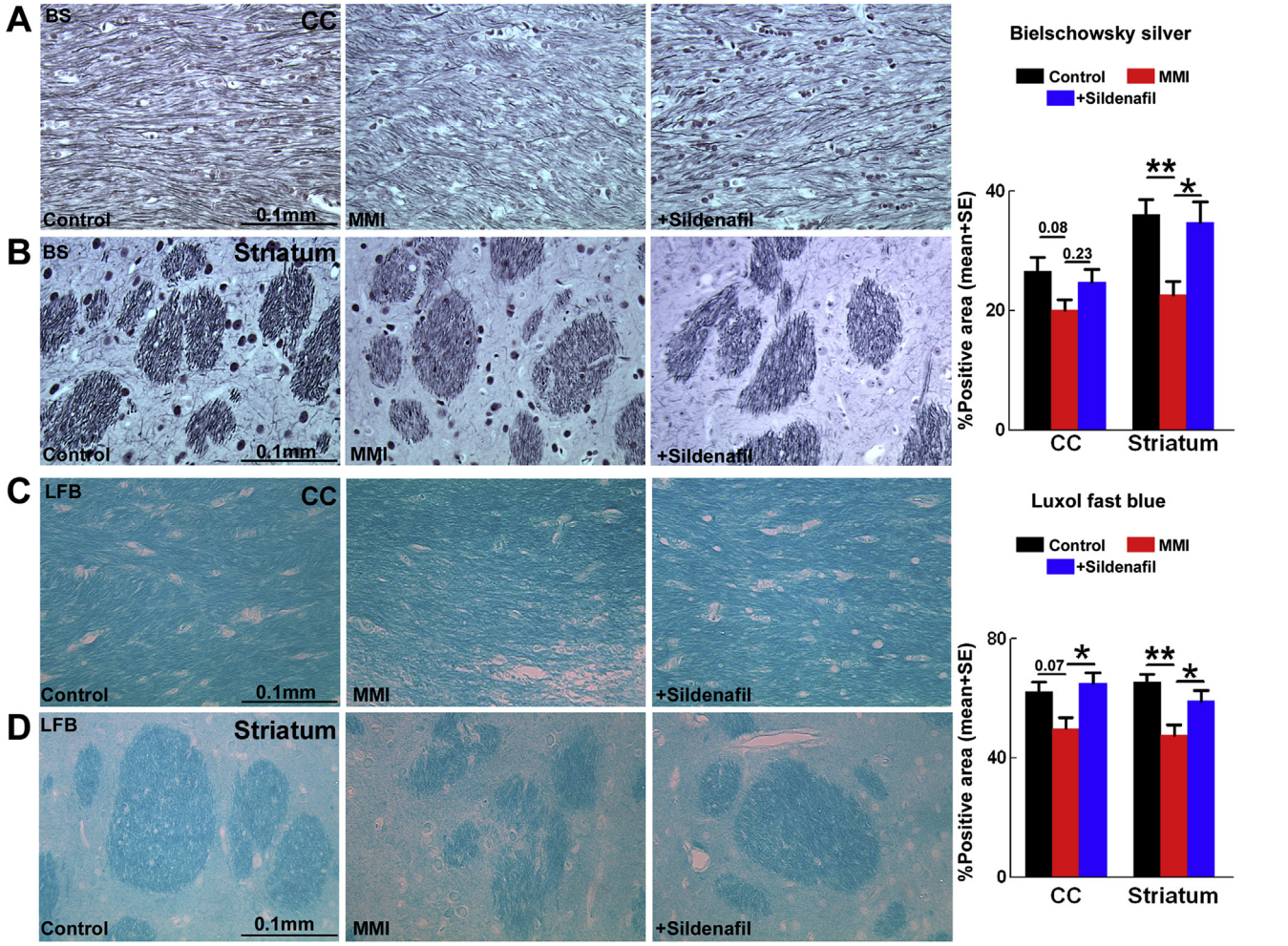

Fig. 2. Sildenafil treatment significantly promotes axon and myelin density in aged rats subjected to MMI. Representative images and quantitative data for density of positive immunolabeling with (A–B) Bielschowsky silver and (C–D) Luxol fast blue in WM tracts of corpus callosum (CC) and WM bundles in striatum to measure axon and myelin density, respectively. MMI in aged rats significantly decreases axon density and myelin density in striatal WM bundles but not WM tracts of CC compared to aged control rats. Sildenafil treatment of MMI in aged rats significantly increases axon density in striatal WM bundles but not WM tracts of CC, and significantly increases myelin density in CC and striatal WM bundles compared to aged control MMI rats. Sample size: Control: N = 10, MMI: N = 8, MMI + Sildenafil: N = 7; *$p < 0.05$ and **$p < 0.01$ between groups indicated by solid line.

MMI control rats. OPCs generate OLs which are required for re-myelination and repair after injury to the brain. Therefore, APC and NG2 staining were used to evaluate OL and OPC number in brain, respectively. Fig. 3 shows that MMI in aged rats significantly decreases the number of OLs in the CC ($p < 0.0001$) and cortex ($p < 0.05$) but not in the striatum ($p = 0.38$) compared to sham control rats. MMI also significantly decreases the number of OPCs in the CC ($p < 0.0001$) and striatum ($p < 0.05$) but not in the cortex ($p = 0.17$) compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly increases OL number in the CC ($p < 0.05$), cortex ($p < 0.01$) and striatum ($p < 0.001$) as well as significantly increases OPC number in cortex ($p < 0.01$) and striatum ($p < 0.01$) but not in the CC ($p = 0.27$) compared to aged MMI control rats (see Fig. 3).

3.3. Sildenafil treatment increases synaptophysin expression in aged rats subjected to MMI

To assess whether Sildenafil treatment improves synaptic protein expression in brain of aged rats subjected to MMI, we measured the expression of Synaptophysin, the major integral membrane glycoprotein of neuronal synaptic vesicles that is present in nearly all synapses. Fig. 4 shows that MMI significantly decreases Synaptophysin protein expression in the striatum ($p < 0.05$) but not in the cortex ($p = 0.1$) compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly increases Synaptophysin protein expression in the cortex and striatum compared to aged MMI rats ($p < 0.05$).

3.4. Sildenafil treatment of MMI significantly decreases inflammatory responses in aged rats

To evaluate the anti-inflammatory effects of Sildenafil treatment in aged rats subjected to MMI, immunostaining and PCR were used to test inflammatory factor expression in brain. Fig. 5A and B shows that MMI significantly increases MCP-1 expression in the CC ($p < 0.01$) but not in the cortex ($p = 0.45$) and striatum ($p = 0.99$) compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly decreases numbers of MCP-1 positive cells in the CC ($p < 0.001$) and striatum ($p < 0.05$) but not in cortex ($p = 0.91$) compared to aged sham rats, as well as significantly decreases MCP-1 expression in striatum ($p < 0.05$) compared to aged control MMI rats.

Fig. 6A shows that MMI in aged rats increases inflammatory factor gene expression such as IL-1β and MCP-1 in brain tissue compared to sham control rats ($p < 0.05$). Sildenafil treatment of MMI in aged rats significantly ($p < 0.05$) decreases brain tissue inflammatory factors IL-1β and MCP-1 gene expression compared to aged MMI rats.

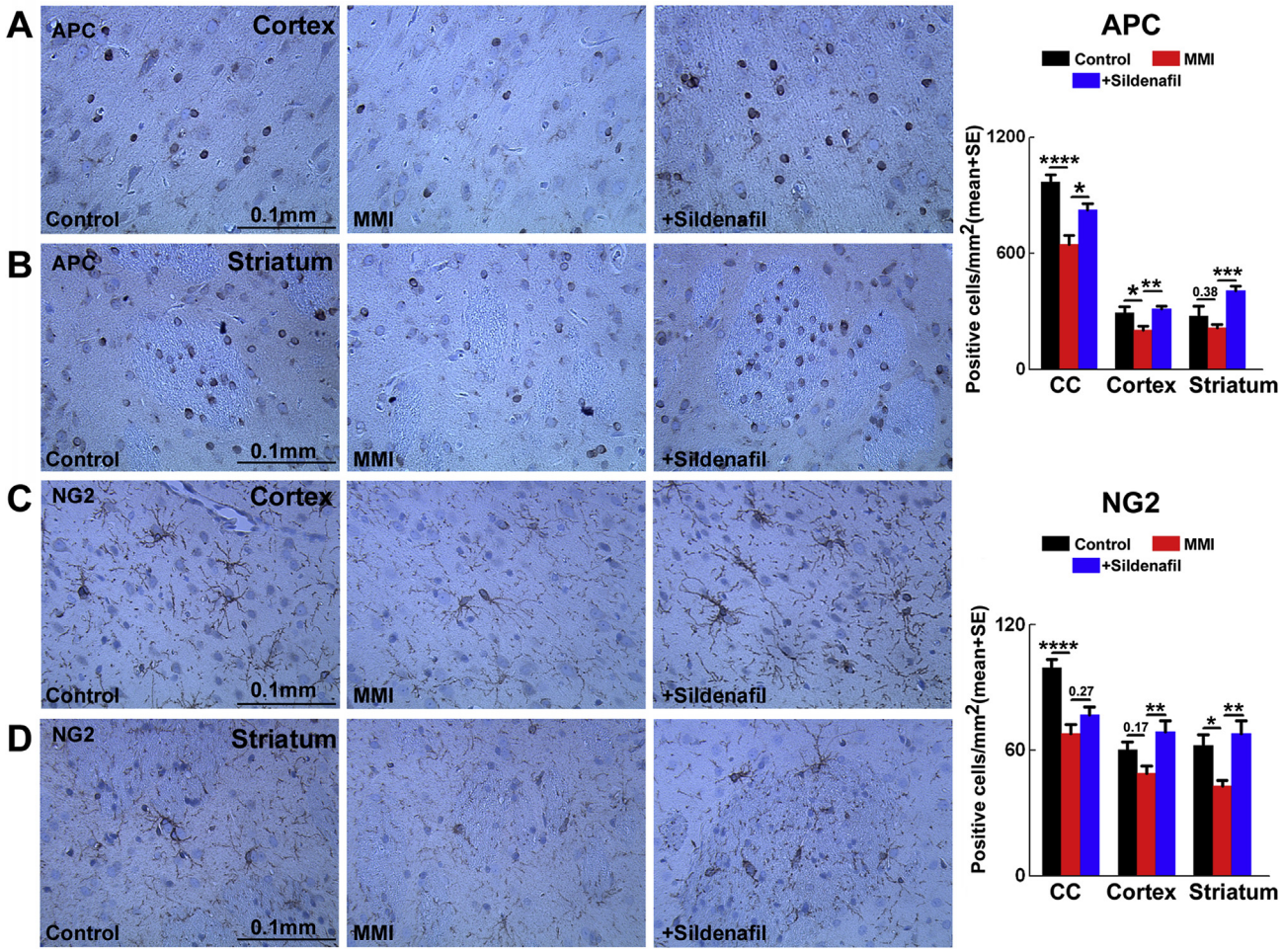

Fig. 3. Sildenafil treatment significantly promotes oligodendrogenesis in aged rats subjected to MMI. Representative images and quantitative data for number of positive cells immunolabeled with (A–B) APC to identify oligodendrocytes (OL) and (C–D) NG2 to identify oligodendrocyte progenitor cells (OPC). MMI in aged rats significantly decreases number of OL in the CC and cortex but not in the striatum as well as significantly decreases OPC number in the CC and striatum but not cortex, compared to aged control rats. Sildenafil treatment of MMI in aged rats significantly increases numbers of OLs in the CC, cortex and striatum as well as increases numbers of OPCs in the cortex and striatum but not in the CC, compared to aged MMI control rats. Sample size: Control: N = 10, MMI: N = 8, MMI + Sildenafil: N = 7; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 between groups indicated by solid line.

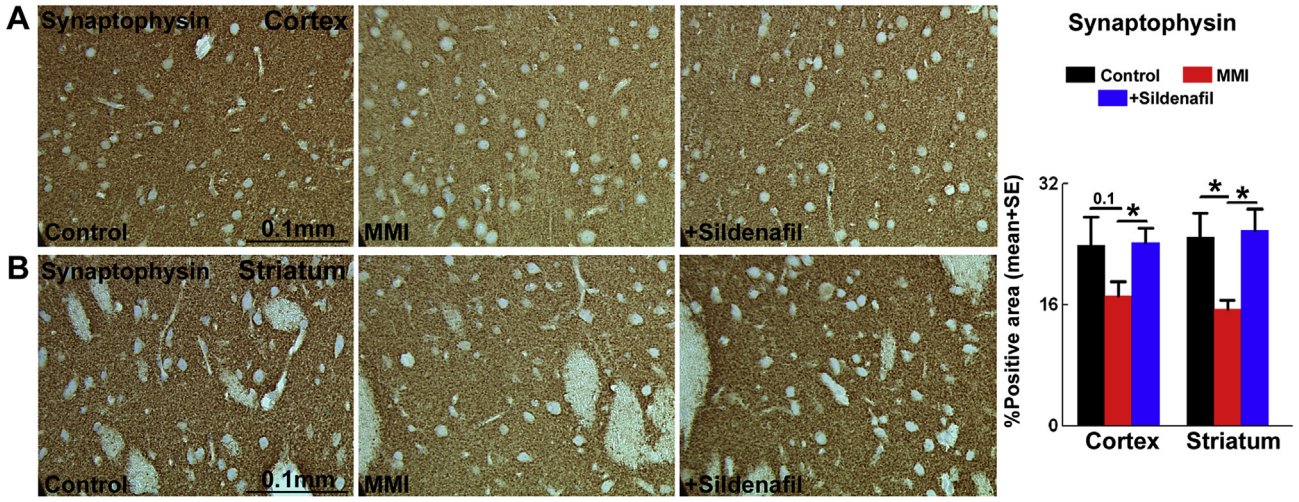

Fig. 4. Sildenafil treatment significantly increases Synaptophysin expression in aged rats subjected to MMI. Representative images and quantitative data for density of positive immunolabeling of Synaptophysin immunostaining in (A) cortex and (B) striatum. MMI in aged rats significantly decreases Synaptophysin expression in striatum but not cortex compared to aged sham control rats, while Sildenafil treatment of MMI in aged rats significantly increases Synaptophysin expression in the cortex as well as striatum compared to aged MMI control rats. Sample size: Control: N = 10, MMI: N = 8, MMI + Sildenafil: N = 7; *p < 0.05 between groups indicated by solid line.

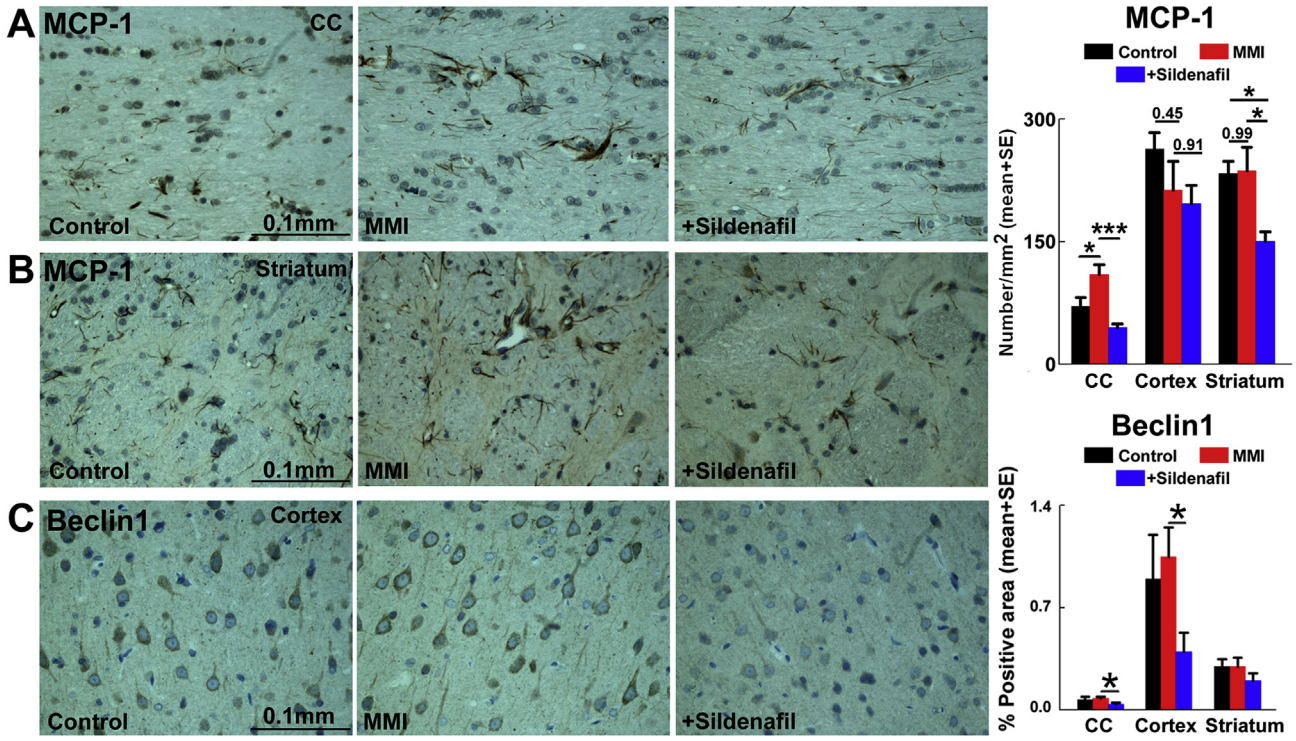

Fig. 5. Sildenafil treatment significantly decreases inflammatory factor and Becin-1 expression in brain of aged rats subjected to MMI. (A–B) Immunostaining and quantification data for Monocyte chemoattractant protein 1 (MCP-1) positive cells in the CC, cortex and striatum. MMI in aged rats significantly increases MCP-1 positive cells in the CC but not in the cortex and striatum compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly decreases inflammatory factor MCP-1 in the CC and striatum but not in the cortex compared to aged sham rats, as well as significantly decreases MCP-1 expression in the striatum compared to aged control MMI rats. (C) Immunostaining and quantification data for density of positive immunolabeling of Beclin1 in CC, cortex and striatum. MMI in aged rats does not significantly alter Beclin1 expression in CC, cortex or striatum, compared to sham control rats. Sildenafil treatment of MMI in aged rats significantly decreases Beclin1 expression in CC and cortex but not striatum compared to aged control MMI rats. Sample size: Control: N = 10, MMI: N = 8, MMI + Sildenafil: N = 7; *p < 0.05, ***p < 0.001 between groups indicated by solid line.

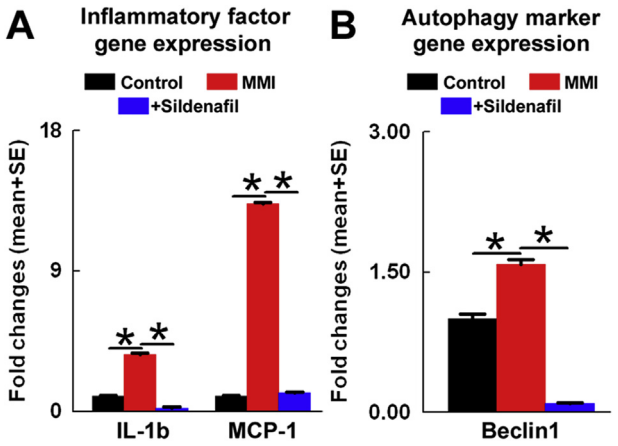

Fig. 6. Sildenafil treatment significantly decreases Beclin-1 and inflammatory factors gene expression in brain of aged rats subjected to MMI. Real time PCR data indicating that MMI significantly increases gene expression of (A) inflammatory markers IL-1β and MCP-1 and (B) autophagy marker Beclin1 in brain of aged rats compared to aged sham control rats. Sildenafil treatment of MMI in aged rats significantly decreases brain tissue IL-1β, MCP-1 and Beclin1 gene expression compared to aged MMI rats. N = 3/group; *p < 0.05.

3.5. Sildenafil treatment of MMI significantly decreases Beclin1 expression in aged rats

To evaluate the effects of Sildenafil treatment on autophagy in aged rats subjected to MMI, immunostaining and PCR was used to test Beclin1 expression in brain. Beclin1 is an indicator of autophagic activity, and upregulation of Beclin-1 promotes autophagy (Kang et al., 2011). Fig. 5C shows that there were no significant differences in Beclin1 positive immunostaining between aged control and aged MMI rats. However, Sildenafil treatment of MMI in aged rats significantly ($p < 0.05$) decreases Beclin1 expression in CC and cortex compared to aged control MMI rats. There were no changes in striatal Beclin1 expression between aged control, MMI and Sildenafil treated groups ($p > 0.05$). Fig. 6B shows that MMI significantly ($p < 0.05$) increases Beclin1 gene expression compared to sham control rats and treatment of MMI in aged rats with Sildenafil significantly ($p < 0.05$) decreases Beclin1 gene expression compared to aged MMI control rats.

4. Discussion

In this study, we show for the first time that Sildenafil treatment of MMI in aged rats improves cognitive outcome which may be mediated at least in part by enhanced axon and myelin density, increased synaptic protein expression and anti-inflammatory effects.

Sildenafil has been shown to readily cross the blood brain barrier (Gomez-Vallejo et al., 2016) and has been previously investigated as a cognitive enhancer (Prickaerts et al., 2004; Singh and Parle, 2003). Sildenafil treatment enhances learning and memory in normal mice evaluated using elevated plus maze (Singh and Parle, 2003), and improves memory performance in novel object recognition task and passive avoidance test outcomes in rodents (Prickaerts et al., 2004). Cognitive impairment is a hallmark of VaD and VaD patients exhibit slowed thinking, forgetfulness, depression and anxiety, disorientation, and loss of executive functions like problem solving, working memory, thinking, reasoning, judgment, planning and execution of tasks, with

performance declining with increasing task complexity (Venkat et al., 2015). Thus, improvement of cognition and memory is a primary outcome measure for VaD treatments. No single measure of disability describes all dimensions of recovery for rodents subjected to a VaD model. Therefore, multiple cognitive tests were conducted. Our data indicate that aged-MMI rats have significant cognitive deficits compared to aged control rats in NOR and odor test. However, MMI induced cognitive deficits were not detected using MWM and there were no significant differences in % time in the platform quadrant and latency between control and MMI groups. This may, in part, be attributed to aging induced decline in spatial learning and memory deficits, that are evident when MWM results between young adult (3–4 months) sham control rats and aged sham control (16–18 months) rats are compared (Supplementary Data). Supplementary Fig. 1 shows that aged control rats (16–18 months) exhibit significantly increased latency to reach the submerged platform as well as reduced % time spent in the platform quadrant, indicating spatial learning and memory deficits. Cognitive testing also reveals that Sildenafil treatment of MMI in aged rats significantly improves short term memory indicted by NOR and spatial learning and memory indicated by MWM but not long term memory indicated by odor test compared to aged control rats.

Cerebral WM is highly susceptible to hypoxia, and in VaD, WM damage arises primarily from demyelination that is attributed to hypoxia induced OL damage (Ihara et al., 2010). WM in VaD exhibit structural damage characterized by WM rarefaction with loss of axonal and myelin density, vacuolization, edema, and mild gliosis (McAleese et al., 2016). Myelin enables rapid conduction and coordination of impulse transmission between distant cortical regions which are vital for optimal cognition and learning (Fields, 2008). Failure of remyelination and WM disease progression occur due to impaired survival, proliferation, migration, recruitment, and differentiation of OPCs (Maki et al., 2013). Our data indicate that Sildenafil treatment of MMI in aged rats significantly increases axon and myelin density as well as numbers of OPCs and OLs in the periventricular WM in the CC and striatal WM bundles. While the effects of synaptic dysfunction in VaD are not completely understood, Synaptophysin is the most abundant integral synaptic vesicle protein and synaptic plasticity and is crucial for memory storage (Clare et al., 2010; Okano et al., 2000). Our data indicate that Sildenafil treatment of MMI in aged rats significantly increases Synaptophysin expression compared to aged MMI rats which may contribute to improved cognition and memory.

Inflammatory responses mediated by glial activation can aggravate OL damage and demyelination in the MMI model of VaD, as well as in other neurodegenerative diseases (Acosta et al., 2017; Solito and Sastre, 2012; Wang et al., 2012, 2017). In post-mortem samples from patients with Alzheimer's disease or VaD, a significant increase in IL-1β expression was observed in frontal cortex, parietal cortex, temporal cortex, hypothalamus, thalamus, and hippocampus regions of brain tissue compared to aged-matched control samples (Cacabelos et al., 1994). Excess of microglia-derived IL-1β inhibits OPC maturation and induces hypomyelination and periventricular WM damage in infectious disease and sepsis (Xie et al., 2016). In addition, MCP-1 is a key chemokine that mediates inflammation in Alzheimer's disease, and elevated MCP-1 expression in brain, plasma and cerebrospinal fluid has been reported in patients with Alzheimer's disease (Pola et al., 2004). Elevated plasma MCP-1 levels have been associated with increased severity and faster cognitive decline in patients with mild cognitive impairment and Alzheimer's disease (Lee et al., 2018). Compared to wild type mice, MCP-1 −/− mice exhibit less severe demyelination and OL loss in the cortex when subjected to an experimental model of multiple sclerosis (Janssen et al., 2016). Therefore, inflammatory factors such as MCP-1 and IL-1β, may contribute to demyelination in rodents. Here, we tested whether Sildenafil exerts anti-inflammatory effects such as decreasing inflammatory factor expression in brain of aged MMI rats. Our data indicate that Sildenafil treatment of MMI in aged rats significantly decreases brain tissue inflammatory factor IL-1β and MCP-1 expression compared to aged MMI control rats.

Autophagy is an intracellular degradation process that recycles nonfunctional proteins and organelles to provide nutrients and promote cell survival under metabolic distress (Mizushima and Komatsu, 2011). The activation of autophagy in response to ischemia may extend neuronal survival and provide neuroprotection; however, excessive autophagy may be detrimental and enhance brain injury by excessive cytoplasmic degradation and/or inducing apoptosis or necrosis (Chen et al., 2014; Marino et al., 2014). Beclin1 is a central positive regulator in the early stage of autophagy, and is one of the core elements to regulate autophagy, apoptosis and inflammatory reaction (Jaeger and Wyss-Coray, 2010; Kang et al., 2011). In neurodegenerative diseases, decrease in Beclin1 expression decreases neuronal autophagy (Pickford et al., 2008). Conversion of LC3-II, autophagosome formation, and expression levels of Beclin1 were significantly increased in the ipsilateral thalamus at 7 and 14 days after stroke induced by distal middle cerebral artery occlusion model while, Beclin1 knockdown inhibited activation of autophagy and prevented secondary neurodegenerative damage in the ipsilateral thalamus (Xing et al., 2012). In rats subjected to a modified Pulsinelli four-vessel occlusion (4-VO) model of VaD, Beclin1 and Cathepsin B protein expression were significantly increased compared to sham control rats (Liu et al., 2014). Similarly, our data indicate that MMI in aged rats significantly increases Beclin1 gene expression compared to sham control rats. Administration of the autophagy inhibitor Wortmannin to rats 1 h prior to 4-VO, significantly reduced VaD induced hippocampal injury by suppressing autophagic activity and exhibited neuroprotective effects (Liu et al., 2014). Inhibition of autophagy also correlated with decreasing inflammatory factors such as MCP-1 and TNFα in cultured adipocytes of obese WOKW rats as compared to LEW.1W control rats (Kosacka et al., 2018). In our study, we show that Sildenafil treatment not only significantly decreases inflammatory factor MCP-1 and IL-1β, but also decreases brain tissue Beclin1 gene expression and improves cognitive outcome compared to aged MMI control rats. Therefore, modulation of autophagy related protein Beclin-1 expression may play a role in the onset, development and treatment of VaD.

4.1. Limitations

This is a proof-of-concept study in which we show that Sildenafil improves cognitive outcome in aged rats, which may, in part (directly or indirectly), be attributed to improved axon and myelin density, and reduction of inflammatory factors. The interactions between cognitive outcome, WM/axonal damage, and inflammatory responses warrant further investigation. Causal mechanistic studies were not performed and future studies investigating the molecular mechanisms of Sildenafil treatment induced cognitive improvement and anti-inflammatory effects are warranted. Investigations of sex differences in VaD and other dementias are warranted. Hypertension and diabetes are high risk factors for VaD and a majority of VaD patients present with co-morbidities. Thus, investigation of therapeutic effects of Sildenafil in treating VaD with comorbidities such as diabetes and/or hypertension is warranted.

5. Conclusions

Sildenafil treatment initiated at 1 day after MMI and administered daily for 28 days significantly improves cognition and memory in aged rats subjected to MMI. Sildenafil treatment promotes axonal/WM remodeling, increases Synaptophysin expression and decreases inflammatory factor expression and Beclin-1 expression in brain which in concert may contribute to cognitive improvement in aged MMI rats.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2018.12.015.

References

Acosta, C., Anderson, H.D., Anderson, C.M., 2017. Astrocyte dysfunction in Alzheimer disease. J. Neurosci. Res. 95, 2430–2447. https://doi.org/10.1002/jnr.24075.

Al-Hesayen, A., Floras, J.S., Parker, J.D., 2006. The effects of intravenous sildenafil on hemodynamics and cardiac sympathetic activity in chronic human heart failure. Eur. J. Heart Fail. 8, 864–868. https://doi.org/10.1016/j.ejheart.2006.03.007.

Ali, B.H., Abdelrahman, A.M., Al-Salam, S., Sudhadevi, M., AlMahruqi, A.S., Al-Husseni, I.S., Beegam, S., Dhanasekaran, S., Nemmar, A., Al-Moundhri, M., 2011. The effect of sildenafil on cisplatin nephrotoxicity in rats. Basic Clin. Pharmacol. Toxicol. 109, 300–308. https://doi.org/10.1111/j.1742-7843.2011.00724.x.

Arvanitakis, Z., Leurgans, S.E., Barnes, L.L., Bennett, D.A., Schneider, J.A., 2011. Microinfarct pathology, dementia, and cognitive systems. Stroke 42, 722–727. https://doi.org/10.1161/STROKEAHA.110.595082.

Cacabelos, R., Alvarez, X.A., Fernandez-Novoa, L., Franco, A., Mangues, R., Pellicer, A., Nishimura, T., 1994. Brain interleukin-1 beta in Alzheimer's disease and vascular dementia. Methods Find. Exp. Clin. Pharmacol. 16, 141–151.

Charriaut-Marlangue, C., Nguyen, T., Bonnin, P., Duy, A.P., Leger, P.L., Csaba, Z., Pansiot, J., Bourgeois, T., Renolleau, S., Baud, O., 2014. Sildenafil mediates blood-flow redistribution and neuroprotection after neonatal hypoxia-ischemia. Stroke 45, 850–856. https://doi.org/10.1161/strokeaha.113.003606.

Chen, W., Sun, Y., Liu, K., Sun, X., 2014. Autophagy: a double-edged sword for neuronal survival after cerebral ischemia. Neural Regen Res 9, 1210–1216. https://doi.org/10.4103/1673-5374.135329.

Clare, R., King, V.G., Wirenfeldt, M., Vinters, H.V., 2010. Synapse loss in dementias. J. Neurosci. Res. 88, 2083–2090. https://doi.org/10.1002/jnr.22392.

Cuadrado-Tejedor, M., Hervias, I., Ricobaraza, A., Puerta, E., Pérez-Roldán, J.M., García-Barroso, C., Franco, R., Aguirre, N., García-Osta, A., 2011. Sildenafil restores cognitive function without affecting β-amyloid burden in a mouse model of Alzheimer's disease. Br. J. Pharmacol. 164, 2029–2041. https://doi.org/10.1111/j.1476-5381.2011.01517.x.

Erkinjuntti, T., Benavente, O., Eliasziw, M., Munoz, D.G., Sulkava, R., Haltia, M., Hachinski, V., 1996. Diffuse vacuolization (spongiosis) and arteriolosclerosis in the frontal white matter occurs in vascular dementia. Arch. Neurol. 53, 325–332. https://doi.org/10.1001/archneur.1996.00550040053014.

Fields, R.D., 2008. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 31, 361–370. https://doi.org/10.1016/j.tins.2008.04.001.

Fink, H.A., Mac Donald, R., Rutks, I.R., Nelson, D.B., Wilt, T.J., 2002. Sildenafil for male erectile dysfunction: a systematic review and meta-analysis. Arch. Intern. Med. 162, 1349–1360. https://doi.org/10.1001/archinte.162.12.1349.

Goldman, H., Berman, R.F., Gershon, S., Murphy, S., Morehead, M., Altman, H.J., 1992. Cerebrovascular permeability and cognition in the aging rat. Neurobiol. Aging 13, 57–62. https://doi.org/10.1016/0197-4580(92)90009-M.

Gomez-Vallejo, V., Ugarte, A., Garcia-Barroso, C., Cuadrado-Tejedor, M., Szczupak, B., Dopeso-Reyes, I.G., Lanciego, J.L., Garcia-Osta, A., Llop, J., Oyarzabal, J., Franco, R., 2016. Pharmacokinetic investigation of sildenafil using positron emission tomography and determination of its effect on cerebrospinal fluid cGMP levels. J. Neurochem. 136, 403–415. https://doi.org/10.1111/jnc.13454.

Hassan, M.A., Ketat, A.F., 2005. Sildenafil citrate increases myocardial cGMP content in rat heart, decreases its hypertrophic response to isoproterenol and decreases myocardial leak of creatine kinase and troponin T. BMC Pharmacol. 5, 10. https://doi.org/10.1186/1471-2210-5-10.

Ihara, M., Polvikoski, T.M., Hall, R., Slade, J.Y., Perry, R.H., Oakley, A.E., Englund, E., O'Brien, J.T., Ince, P.G., Kalaria, R.N., 2010. Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies. Acta Neuropathol. 119, 579–589. https://doi.org/10.1007/s00401-009-0635-8.

Jaeger, P.A., Wyss-Coray, T., 2010. Beclin 1 complex in autophagy and Alzheimer disease. Arch. Neurol. 67, 1181–1184. https://doi.org/10.1001/archneurol.2010.258.

Janssen, K., Rickert, M., Clarner, T., Beyer, C., Kipp, M., 2016. Absence of CCL2 and CCL3 ameliorates central nervous system grey matter but not white matter demyelination in the presence of an intact blood-brain barrier. Mol. Neurobiol. 53, 1551–1564. https://doi.org/10.1007/s12035-015-9113-6.

Kang, R., Zeh, H.J., Lotze, M.T., Tang, D., 2011. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ. 18, 571–580. https://doi.org/10.1038/cdd.2010.191.

Kosacka, J., Nowicki, M., Paeschke, S., Baum, P., Bluher, M., Kloting, N., 2018. Up-regulated autophagy: as a protective factor in adipose tissue of WOKW rats with metabolic syndrome. Diabetol. Metab. Syndrome 10, 13. https://doi.org/10.1186/s13098-018-0317-6.

Kovari, E., Gold, G., Herrmann, F.R., Canuto, A., Hof, P.R., Bouras, C., Giannakopoulos, P., 2007. Cortical microinfarcts and demyelination affect cognition in cases at high risk for dementia. Neurology 68, 927–931. https://doi.org/10.1212/01.wnl.0000257094.10655.9a.

Laloux, P., Brucher, J.M., 1991. Lacunar infarctions due to cholesterol emboli. Stroke 22, 1440–1444.

Lee, W.-J., Liao, Y.-C., Wang, Y.-F., Lin, I.F., Wang, S.-J., Fuh, J.-L., 2018. Plasma MCP-1 and cognitive decline in patients with Alzheimer's disease and mild cognitive impairment: a two-year follow-up study. Sci. Rep. 8, 1280. https://doi.org/10.1038/s41598-018-19807-y.

Liu, B., Tang, J., Zhang, J., Li, S., Yuan, M., Wang, R., 2014. Autophagy activation aggravates neuronal injury in the hippocampus of vascular dementia rats. Neural Regen Res 9, 1288–1296. https://doi.org/10.4103/1673-5374.137576.

Maki, T., Liang, A.C., Miyamoto, N., Lo, E.H., Arai, K., 2013. Mechanisms of oligodendrocyte regeneration from ventricular-subventricular zone-derived progenitor cells in white matter diseases. Front. Cell. Neurosci. 7, 275. https://doi.org/10.3389/fncel.2013.00275.

Marino, G., Niso-Santano, M., Baehrecke, E.H., Kroemer, G., 2014. Self-consumption: the interplay of autophagy and apoptosis. Nat. Rev. Mol. Cell Biol. 15, 81–94. https://doi.org/10.1038/nrm3735.

McAleese, K.E., Alafuzoff, I., Charidimou, A., De Reuck, J., Grinberg, L.T., Hainsworth, A.H., Hortobagyi, T., Ince, P., Jellinger, K., Gao, J., Kalaria, R.N., Kovacs, G.G., Kovari, E., Love, S., Popovic, M., Skrobot, O., Taipa, R., Thal, D.R., Werring, D., Wharton, S.B., Attems, J., 2016. Post-mortem assessment in vascular dementia: advances and aspirations. BMC Med. 14, 129. https://doi.org/10.1186/s12916-016-0676-5.

Mizushima, N., Komatsu, M., 2011. Autophagy: renovation of cells and tissues. Cell 147, 728–741. https://doi.org/10.1016/j.cell.2011.10.026.

Ohno, M., Sametsky, E.A., Silva, A.J., Disterhoft, J.F., 2006. Differential effects of alphaCaMKII mutation on hippocampal learning and changes in intrinsic neuronal excitability. Eur. J. Neurosci. 23, 2235–2240.

Okano, H., Hirano, T., Balaban, E., 2000. Learning and memory. Proc. Natl. Acad. Sci. U. S. A. 97, 12403–12404. https://doi.org/10.1073/pnas.210381897.

Park, L., Anrather, J., Girouard, H., Zhou, P., Iadecola, C., 2007. Nox2-derived reactive oxygen species mediate neurovascular dysregulation in the aging mouse brain. J. Cerebr. Blood Flow Metabol. 27, 1908–1918. https://doi.org/10.1038/sj.jcbfm.9600491.

Patil, C.S., Singh, V.P., Kulkarni, S.K., 2006. Modulatory effect of sildenafil in diabetes and electroconvulsive shock-induced cognitive dysfunction in rats. Pharmacol. Rep. 58, 373–380.

Pickford, F., Masliah, E., Britschgi, M., Lucin, K., Narasimhan, R., Jaeger, P.A., Small, S., Spencer, B., Rockenstein, E., Levine, B., Wyss-Coray, T., 2008. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice. J. Clin. Invest. 118, 2190–2199. https://doi.org/10.1172/JCI33585.

Plassman, B.L., Langa, K.M., Fisher, G.G., Heeringa, S.G., Weir, D.R., Ofstedal, M.B., Burke, J.R., Hurd, M.D., Potter, G.G., Rodgers, W.L., Steffens, D.C., Willis, R.J., Wallace, R.B., 2007. Prevalence of dementia in the United States: the aging, demographics, and memory study. Neuroepidemiology 29, 125–132. https://doi.org/10.1159/000109998.

Pola, R., Flex, A., Gaetani, E., Proia, A.S., Papaleo, P., Di Giorgio, A., Straface, G., Pecorini, G., Serricchio, M., Pola, P., 2004. Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism and risk of Alzheimer's disease in Italians. Exp. Gerontol. 39, 1249–1252. https://doi.org/10.1016/j.exger.2004.05.001.

Prickaerts, J., Sik, A., van Staveren, W.C., Koopmans, G., Steinbusch, H.W., van der Staay, F.J., de Vente, J., Blokland, A., 2004. Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. Neurochem. Int. 45, 915–928. https://doi.org/10.1016/j.neuint.2004.03.022.

Rapp, J.H., Hollenbeck, K., Pan, X.M., 2008a. An experimental model of lacunar infarction: embolization of microthrombi. J. Vasc. Surg. 48, 196–200. https://doi.org/10.1016/j.jvs.2008.01.038.

Rapp, J.H., Pan, X.M., Neumann, M., Hong, M., Hollenbeck, K., Liu, J., 2008b. Microemboli composed of cholesterol crystals disrupt the blood-brain barrier and reduce cognition. Stroke 39, 2354–2361. https://doi.org/10.1161/strokeaha.107.496737.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. Faseb. J. 22, 659–661. https://doi.org/10.1096/fj.07-9574LSF.

Silver, B., McCarthy, S., Lu, M., Mitsias, P., Russman, A.N., Katramados, A., Morris, D.C., Lewandowski, C.A., Chopp, M., 2009. Sildenafil treatment of subacute ischemic stroke: a safety study at 25-mg daily for 2 weeks. J. Stroke Cerebrovasc. Dis. 18, 381–383. https://doi.org/10.1016/j.jstrokecerebrovasdis.2009.01.007.

Singh, N., Parle, M., 2003. Sildenafil improves acquisition and retention of memory in mice. Indian J. Physiol. Pharmacol. 47, 318–324.

Solito, E., Sastre, M., 2012. Microglia function in Alzheimer's disease. Front. Pharmacol. 3, 14. https://doi.org/10.3389/fphar.2012.00014.

Spinetta, M.J., Woodlee, M.T., Feinberg, L.M., Stroud, C., Schallert, K., Cormack, L.K., Schallert, T., 2008. Alcohol-induced retrograde memory impairment in rats: prevention by caffeine. Psychopharmacology (Berlin) 201, 361–371. https://doi.org/10.1007/s00213-008-1294-5.

Steiner, T.J., Rail, D.L., Rose, F.C., 1980. Cholesterol crystal embolization in rat brain: a

model for atheroembolic cerebral infarction. Stroke 11, 184–189.
Stuart, S.A., Robertson, J.D., Marrion, N.V., Robinson, E.S., 2013. Chronic pravastatin but not atorvastatin treatment impairs cognitive function in two rodent models of learning and memory. PLoS One 8, e75467. https://doi.org/10.1371/journal.pone.0075467.
Sultzer, D.L., Mahler, M.E., Cummings, J.L., Van Gorp, W.G., Hinkin, C.H., Brown, C., 1995. Cortical abnormalities associated with subcortical lesions in vascular dementia. Clinical and position emission tomographic findings. Arch. Neurol. 52, 773–780. https://doi.org/10.1001/archneur.1995.00540320049012.
Tanabe, J.L., Ezekiel, F., Jagust, W.J., Reed, B.R., Norman, D., Schuff, N., Weiner, M.W., Chui, H., Fein, G., 1999. Magnetization transfer ratio of white matter hyperintensities in subcortical ischemic vascular dementia. AJNR Am J Neuroradiol 20, 839–844.
Taskin, M.I., Yay, A., Adali, E., Balcioglu, E., Inceboz, U., 2014. Protective effects of sildenafil citrate administration on cisplatin-induced ovarian damage in rats. Fertil. Steril. 102, e146. https://doi.org/10.1016/j.fertnstert.2014.07.500.
Venkat, P., Chopp, M., Chen, J., 2015. Models and mechanisms of vascular dementia. Exp. Neurol. 272, 97–108. https://doi.org/10.1016/j.expneurol.2015.05.006.
Venkat, P., Chopp, M., Zacharek, A., Cui, C., Zhang, L., Li, Q., Lu, M., Zhang, T., Liu, A., Chen, J., 2017. White matter damage and glymphatic dysfunction in a model of vascular dementia in rats with no prior vascular pathologies. Neurobiol. Aging 50, 96–106. https://doi.org/10.1016/j.neurobiolaging.2016.11.002.
Wang, M., Ding, F., Deng, S., Guo, X., Wang, W., Iliff, J.J., Nedergaard, M., 2017. Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. J. Neurosci. 37, 2870–2877. https://doi.org/10.1523/jneurosci.2112-16.2017.
Wang, M., Iliff, J.J., Liao, Y., Chen, M.J., Shinseki, M.S., Venkataraman, A., Cheung, J., Wang, W., Nedergaard, M., 2012. Cognitive deficits and delayed neuronal loss in a mouse model of multiple microinfarcts. J. Neurosci. 32, 17948–17960. https://doi.org/10.1523/jneurosci.1860-12.2012.
Xie, D., Shen, F., He, S., Chen, M., Han, Q., Fang, M., Zeng, H., Chen, C., Deng, Y., 2016. IL-1beta induces hypomyelination in the periventricular white matter through inhibition of oligodendrocyte progenitor cell maturation via FYN/MEK/ERK signaling pathway in septic neonatal rats. Glia 64, 583–602. https://doi.org/10.1002/glia.22950.
Xing, S., Zhang, Y., Li, J., Zhang, J., Li, Y., Dang, C., Li, C., Fan, Y., Yu, J., Pei, Z., Zeng, J., 2012. Beclin 1 knockdown inhibits autophagic activation and prevents the secondary neurodegenerative damage in the ipsilateral thalamus following focal cerebral infarction. Autophagy 8, 63–76. https://doi.org/10.4161/auto.8.1.18217.
Yu, P., Venkat, P., Chopp, M., Zacharek, A., Shen, Y., Ning, R., Liang, L., Li, W., Zhang, L., Landschoot-Ward, J., Jiang, R., Chen, J., 2018. Role of microRNA-126 in vascular cognitive impairment in mice. J. Cerebr. Blood Flow Metabol. https://doi.org/10.1177/0271678x18800593. 0271678X18800593.
Zhang, R., Wang, Y., Zhang, L., Zhang, Z., Tsang, W., Lu, M., Zhang, L., Chopp, M., 2002. Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats. Stroke 33, 2675–2680. https://doi.org/10.1161/01.STR.0000034399.95249.59.