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Differential rescue of spatial memory deficits in aged rats by L-type voltage dependent calcium channel and ryanodine receptor antagonism

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Abstract

Age-associated memory impairments may result as a consequence of neuroinflammatory induction of intracellular calcium (Ca^{+2}) dysregulation. Altered L-type voltage dependent calcium channel (L-VDCC) and ryanodine receptor (RyR) activity may underlie age-associated learning and memory impairments. Various neuroinflammatory markers are associated with increased activity of both L-VDCCs and RyRs, and increased neuroinflammation are associated with normal aging. *In vitro*, pharmacological blockade of L-VDCCs and RyRs has been shown to be anti-inflammatory. Here, we examined whether pharmacological blockade of L-VDCCs or RyRs with the drugs nimodipine and dantrolene, respectively, could improve spatial memory and reduce age-associated increases in microglia activation. Dantrolene and nimodipine differentially attenuated age-associated spatial memory deficits but were not anti-inflammatory *in vivo*. Furthermore, RyR gene expression was inversely correlated with spatial memory, highlighting the central role of Ca^{+2} dysregulation in age-associated memory deficits.

1 Introduction

Calcium (Ca^{+2}) dysregulation via L-type voltage dependent Ca^{+2} channels (L-VDCCs) occurs during aging and may underlie age-associated memory impairments. During aging, L-VDCC activity and expression increases in the CA1 hippocampal subfield (Thibault and Landfield, 1996) which is related to increases in the slow afterhyperpolarization (sAHP) and reduced neuronal excitability (Disterhoft et al., 1996; Power et al., 2002). These electrophysiological changes are correlated with age-associated memory deficits (Thibault and Landfield, 1996), which can be improved with antagonism of L-VDCCs with nimodipine (Thompson et al., 1990; Moyer et al., 1992; Batuecas et al., 1998). Notably,

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blockade of the L-VDCC reduces the sAHP to a similar extent in both young and aged rats (Power et al., 2002) and improves memory in young and aged rats as well (Levy et al., 1991; Ingram et al., 1994). Furthermore, epidemiological evidence shows that L-VDCC antagonism is associated with reduced prevalence of Alzheimer's disease (AD; Anekonda and Quinn, 2011; Lopez-Arrieta and Birks, 2002; Forette et al., 2002) and AD patients given the L-VDCC antagonist nimodipine showed improvements over placebo-treated patients on several cognitive measures (Tollefson, 1990).

Similarly, increased ryanodine receptor (RyR) dysregulation also underlies age-associated Ca^{+2} dysregulation and increases in the sAHP. In young rats, *in vitro* RyR blockade reduces the sAHP to a lesser extent compared to L-VDCC antagonism (Borde et al., 2000). Aged neurons demonstrate prolonged increases in intracellular Ca^{+2} levels that are RyR-dependent (Clodfelter et al., 2002). Oxidative stress present in aged rats increases the sAHP by 50% and is dependent on RyRs but not other sources of Ca^{+2} , including inositol triphosphate receptors and L-VDCCs (Bodhinathan et al., 2010). Additionally, L-VDCCs and RyRs interact with each other: Ca^{+2} influx via L-VDCCs triggers Ca^{+2} -induced Ca^{+2} release via RyRs which in turn modulates L-VDCC activity (Chavis et al., 1996; Gant et al., 2014). Furthermore, a recent study on patients with late onset AD demonstrated a genetic interaction between L-VDCC and RyR mutations and amyloid deposition (Koran et al., 2014), demonstrating the importance of these two channels in the AD pathology.

Age-associated increases in L-VDCC and RyR activity may be due to changes in neuroinflammatory processes in aging, which may themselves contribute to the pathogenesis of AD (Akiyama et al., 2000; Cameron & Landreth, 2010). Production and release of pro-inflammatory cytokines and nitric oxide (NO) in the brain changes with age (Frank et al., 2006; Clarke et al., 2008), particularly in response to pro-inflammatory challenges (Hopp et al., 2014; Barrientos et al., 2009; Gayle et al., 1999). Both NO and cytokines are associated with increased RyR activity (Friedrich et al., 2014; Palmi and Meini, 2002; Kakizawa et al., 2013). Furthermore, the pro-inflammatory cytokine tumor necrosis factor α (TNF α) increases L-VDCC activity *in vitro* and this increase depends upon activation of the nuclear factor kappa B (NF κ B) pathway (Furukawa and Mattson, 1998). Additionally, *in vivo* treatment of aged rats with a TNF α inhibitor reduced L-VDCC activity, reduced age-associated increases in long-term depression (LTD), and improved spatial memory (Sama et al., 2012). *In vitro* studies have shown that L-VDCC and RyR antagonists are directly anti-inflammatory via their action on microglia and astrocytes (Hashioka et al., 2012; Li et al., 2009; Klegeris et al., 2007). The present *in vivo* study investigated whether an L-VDCC or RyR antagonist could reduce endogenous brain inflammation and improve spatial memory in aged Fisher 344 (F-344) rats.

2 Methods

2.1 Subjects

The subjects were male F-344 (National Institutes of Aging, Taconic) rats, 3 (young) or 22 (aged) months old, individually housed with *ad libitum* access to food and water and maintained on a reverse 12/12 light/dark cycle with lights off at 8AM. Rats received daily subcutaneous drug injections at a volume of 1 ml/kg per day with vehicle (polyethylene

glycol 300, Thermo Fisher Scientific, Waltham, MA, USA), dantrolene sodium salt (5 mg/kg/day, Sigma), or nimodipine (5 mg/kg/day, Sigma) for 4 weeks. The six treatment groups were: young + vehicle (n=6), young + nimodipine (n=5), young + dantrolene (n=6), old + vehicle (n=11), old + nimodipine (n=10), old + dantrolene (n=12). Body weights were monitored daily and aged rats were given saline injections and supplemental food as needed due to health concerns unrelated to the study. This research was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and The Ohio State University Institutional Animal Care and Use Committee.

2.2 Behavioral testing

Rats were handled daily until behavioral testing, which took place on the third week after drug administration was begun. Drugs were administered immediately after testing was completed each day. Spatial learning was assessed in the Morris water maze (MWM), using a 170 cm diameter pool with gray walls surrounded by multiple visual distal and proximal cues. The water was maintained at room temperature (RT; 21 °C–22 °C). During the hidden platform portion of the task, a transparent, circular escape platform was present in a consistent location and submerged 2.5 cm below the water surface. The rats were tracked using overhead cameras and Noldus Ethovision 3.1 tracking and analysis stem (Noldus, Lessburg, VA, USA). Each rat performed 6 trials per day separated by a 60 minute inter-trial interval. The rat was released into the water at 1 of 6 randomized locations which were varied such that rats could not take the same path to the hidden platform more than once per day. After the rat located the hidden platform or swam for a maximum of 60 seconds, it was placed on the platform for 30 seconds. At the end of the fourth day, rats were tested in a 60 second probe trial, during which the platform was removed. During the probe trial, we measured how much time rats spent in the location of the platform to test their memory for the location. At the conclusion of the probe trial, rats were tested in a visual platform test in order to control for any group- or drug-related differences in visual acuity. The platform was moved to a different quadrant and raised 2 cm above the surface of the water. All rats included in the data analysis were able to locate the visible platform.

2.3 Tissue collection

All of the rats were deeply anesthetized using isoflurane prior to sacrifice. The histology cohort underwent transcardiac perfusion with cold saline containing 1 U/mL heparin followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were post-fixed overnight in fixative and then stored at 4 °C in phosphate-buffered saline (PBS), pH 7.4. The biochemistry cohort were rapidly decapitated and their hippocampi dissected on ice and separated such that the right or left side were randomly chosen for either protein or gene expression analysis. The hippocampi were stored at –80 °C until RNA or protein extraction.

2.4 Histological procedures

Free-floating coronal sections (40 µm thickness) were generated using a vibratome and stained using standard avidin/biotin peroxidase labeling methods as previously described (Bardou et al., 2013). The mouse monoclonal antibody against OX-6 (final dilution 1:200; BD Pharmingen, San Diego, CA, USA) was used to label major histocompatibility complex

II (MHC-II) on activated microglia. Briefly, endogenous peroxidase activity was quenched and nonspecific binding was blocked with 5% normal goat serum (NGS) in PBS. Sections were then incubated overnight at 4 °C in primary antibody diluted in the same blocking solution. The next day, sections were rinsed and then incubated for 1.5 hours at RT in biotinylated secondary antibody from the appropriate species (final dilution 1:200, Vector, Burlingame, CA, USA). Sections were then rinsed and incubated for 1 hour at RT with avidin-biotinylated horseradish peroxidase (Vectastain, ABC kit, Vector). After another rinse, sections were incubated with 0.05% 3, 3'-diaminobenzidine tetrahydrochloride (Vector) as a chromogen. The reaction was stopped by rinsing the sections with PBS. No staining was detected in the absence of primary or secondary antibodies. Sections were mounted on superfrost slides, air-dried, dehydrated with a series of ethanol and xylene rinses, and coverslipped with cyto seal (Allan Scientific, Kalamazoo, MI, USA) mounting medium. Images of the hippocampi were captured with light microscopy, stitched together, and analyzed with a Nikon 90i system with a DS-5M-L1 digital camera using Elements 3.1 Software (Nikon Instruments, Melville, NY, USA). Subfields of interest were determined as previously reported (Rosi et al., 2005). To detect positive staining, an intensity threshold was set to detect staining in the regions of interest and was quantified using intensity threshold densitometry; data is expressed as % area (of the region of interest).

2.5 Gene expression analysis

Total RNA was isolated from hippocampi with phenol-chloroform extraction using PureZol (Bio-Rad, Hercules, CA) followed by clean-up using NucleoSpin RNA II kits (Machery-Nagel, Düren, Germany) according to manufacturers' instructions. RNA quantity was measured using a Synergy Plate reader equipped with a Take-3 plate (Bio-Tek, Winooski, VT, USA) and 1 µg from each sample was used to generate cDNA using the iScript reverse transcription Supermix kit (Bio-Rad) using a C1000 Thermal Cycler (Bio-Rad). Selected RNA samples were run without reverse polymerase as a control to ensure no contamination from genomic DNA. Primer were designed using the PrimerQuest software (Integrated DNA Technologies, Coralville, IA, USA; Table 1). Gene expression was quantified using SsoAdvanced Universal SYBR green (Bio-Rad) quantitative polymerase chain reaction (qPCR) on a CFX96 Real-Time PCR detection system (Bio-Rad). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene. Data were analyzed using the comparative threshold cycle method with results expressed as fold change versus young + vehicle rats.

2.6 Magnetic bead immunoassays

Hippocampal levels of TNFα, interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, interferon-γ (INFγ), and granulocyte-macrophage-stimulating factor (GM-CSF) were quantified simultaneously with a magnetic bead-based immunoassay (Bio-Rad; BioPlex Pro Rat Cytokine Th1/Th2, 171-K1002M) according to the manufacturer's instructions. Briefly, protein homogenates generated above were diluted to 2 µg/µl with lysis buffer and then combined at a 1:1 ratio with BioPlex Sample Diluent for a final protein concentration of 1 µg/µl. Samples, standards, and blanks were incubated on a shaker with antibody-conjugated magnetic beads for 1 hr at RT, followed by incubation with a detection antibody for 30 minutes at RT, and finally incubated for 15 minutes with streptavidin-

phycoerythrin reporter. Plates were then read with a BioPlex MAGPIX Multiplex reader (Bio-Rad) and results were analyzed using BioPlex Manager MP software. Results are reported as pg/ml.

2.7 Statistical analyses

Analyses of variance (ANOVA) were performed followed by Fisher's protected least significant difference for post hoc comparisons (SigmaPlot 12.5, Systat, San Jose, CA, USA). Graphs display the mean plus standard error of the mean (SEM). A $p < 0.05$ was considered statistically significant.

3 Results

3.1 Spatial learning and memory in aged rats

We tested rats' spatial learning and memory in the MWM (Figure 1) after three weeks of treatment with vehicle, dantrolene, or nimodipine ($n=5-12/\text{group}$). Only rats that located the platform during visible trials were included in these analyses; there was a significant main effect of age on path length taken to locate the visible platform ($p < 0.001$, data not shown) similar to previous reports (Bizon et al., 2009; Lindner and Gribkoff, 1991). Aged rats had significantly longer latencies (data not shown) to locate the hidden platform ($F(1, 172)=200.37$, $p < 0.001$), although this is likely due to the significantly slower swim speed of the aged rats ($F(1, 1289)=784.132$, $p < 0.001$; Figure 1a); indeed, latency across ages is a poor measure of MWM performance (Guidi & Foster, 2012). Due to this difference in swim speed, we also examined the path length that rats took to locate the hidden platform (Figure 1b), with longer path lengths indicative of a spatial learning deficit. A 3-way ANOVA revealed a significant learning deficits due to age ($F(1, 172)=18.185$, $p < 0.001$) and improvement due to testing day ($F(3, 172)=17.186$, $p < 0.001$). There was also a significant interaction between age and drug ($F(2, 172)=4.42$, $p=0.013$). A 2-way repeated measures (RM-) ANOVA revealed significant effects of group ($F(5, 129)=2.533$, $p=0.043$), day ($F(3, 129)=31.692$, $p < 0.001$), and an interaction between day and group ($F(15, 129)=4.409$; $p < 0.001$). Vehicle-treated aged rats took significantly longer paths to locate the hidden platform on days 2, 3, and 4 ($p < 0.01$); additionally, vehicle-treated aged rats showed no improvement in performance between days 1 and 4 ($p > 0.05$), although there was an improvement between days 2 and 3 ($p=0.039$) and days 2 and 4 ($p < 0.001$). Dantrolene treatment improved performance in aged rats with respect to path length, revealing a partial recovery (no difference vs. young + dantrolene rats) on days 1 through 4 ($p > 0.150$) and a trend towards improvement compared to aged vehicle-treated rats on day 4 ($p=0.062$). Furthermore, aged rats treated with dantrolene had significantly improved performance between days 1 and 4 ($p=0.007$), days 2 and 3 ($p=0.006$), and days 2 and 4 ($p < 0.001$). In contrast, nimodipine-treated aged rats were significantly impaired compared to young nimodipine-treated rats on days 3 and 4 ($p=0.043$ and $p=0.005$, respectively), showed no improvement over vehicle-treated aged rats on any day ($p > 0.3$), and demonstrated no significant improvement between any days ($p > 0.05$). All of the young rats, regardless of treatment group, improved between days 1 and 4 ($p < 0.001$), days 1 and 3 (nimodipine and vehicle $p < 0.05$, dantrolene $p=0.09$) and days 1 and 2 (vehicle $p < 0.05$, dantrolene and nimodipine $p < 0.09$). Dantrolene- and nimodipine-treated young rats took significantly

longer paths than vehicle-treated young rats on day 2 ($p<0.05$) and dantrolene-treated young rats took longer paths on day 3 ($p<0.05$), with no difference between any of the young rats on day 4 ($p>0.43$).

For the probe trial (Figure 1c–d), we measured the amount of time rats spent in the exact location as well as a 25% annulus where the platform was previously located, with more time in the location indicative of better learning of the platform location. Aged rats were impaired, spending less time in the exact platform location ($F(1, 43)=19.956$, $p<0.001$) and in the 25% annulus ($F(1,43)=15.651$, $p<0.0001$). Post-hoc analyses revealed that aged rats treated with either vehicle or dantrolene spent significantly less time at the exact platform location than their young controls ($p<0.005$). Nimodipine treatment increased the amount of time that aged rats spent at the platform location, with nimodipine-treated aged rats spending a similar amount of time near the platform location compared to their young controls. Overall, these data indicate that dantrolene treatment improved learning of the MWM task in aged rats, while nimodipine treatment slightly improved recall during the probe.

3.2 Age-associated microglia activation in the hippocampus

MHC-II immunoreactive microglia present throughout the CA3 hippocampal subfield of aged rats, with few labeled microglia present in the hippocampus of young rats (Figure 2). Two-way ANOVAs revealed a significant main effect of age in the CA3 and dentate gyrus (DG) ($F(1, 83)=31.047$, $p<0.001$; $F(1, 83)=5.458$, $p=0.022$, respectively). In the CA1, there was a significant interaction between age and drug treatment ($F(2,83)=3.225$, $p=0.045$). Overall, blockade of RyRs and L-VDCCs did not significantly reduce age-associated microglia activation in the hippocampus.

3.3 Inflammatory gene and protein expression in aging

Neither aging nor drug treatment significantly altered gene expression (see Table 1) of any of the inflammatory markers tested ($p>0.05$). Protein analyses showed no change in the M1 markers TNF α , IL-1 α , IL-1 β , IL-2, IL-6, IL-12p70, INF γ , or GM-CSF or the M2 markers IL-4, IL-5, IL-10, and IL-13 (Boche et al., 2013). For the M2 cytokine IL-5, there was a main effect of drug treatment group ($F(2, 21)=5.902$, $p<0.01$). Post-hoc analyses revealed nimodipine-treated rats had overall higher expression of IL-5 compared to dantrolene-treated rats ($p<0.01$).

3.4 Expression of Ca²⁺ channels and plasticity markers in aging

Gene expression analysis was performed on the three RyR isoforms as well as the $\alpha 1C$ and $\alpha 1D$ subunit of the L-VDCC. The $\alpha 1D$ subunit has been correlated with age-related increases in membrane density and electrophysiological activity (Chen et al., 2000). No significant changes in L-VDCC subunit or RyR isoform were detected as compared to young control rats. While these markers were not significantly altered by age or drug, they had significant correlations with MWM performance as measured by the Pearson test that differed between the two age groups when pooled across all treatment groups (Figure 3). In aged rats, RyR1, RyR2, and RyR3 expression were negatively correlated with the amount of time spent in the vicinity (25% of total pool area) of the platform location during the probe trial ($R^2=0.2667$, 0.3327 , and 0.5749 , respectively; $p<0.05$); this relationship was not

evident in young rats ($p=0.03322$, 0.8973 and $p=0.4999$, respectively). There was no significant change in the expression of doublecortin, post-synaptic density 95, and synapsin 1 ($p>0.05$).

4 Discussion

To the best of our knowledge, this is the first study to examine the ability of the RyR antagonist dantrolene to attenuate age-associated spatial memory deficits. This is particularly relevant in the context of our data herein demonstrating that poor water maze performance correlated with increased RyR expression in aged rats (Figure 3). Increased RyR activity with aging drives increases in the sAHP (Kumar and Foster, 2004; Bodhinathan et al., 2010), and age-associated increases in the sAHP correlates with MWM deficits (Tombaugh et al., 2005). RyR expression is also altered in patients with AD and mild cognitive impairment (Bruno et al., 2012) and Ca^{+2} handling is altered in non-neuronal tissues in AD patients and family members (Etcheberrigaray et al., 1998). Similarly, the 3xTg-AD transgenic mouse model of AD has alterations in Ca^{+2} handling that is related to RyR dysregulation; this dysfunction is normalized by blockade of RyRs with dantrolene (Chakroborty et al., 2012). Furthermore, deletion of RyR3 in the AD APP/PS1 transgenic mouse model improved neuronal function in older mice (Liu et al., 2014). From a behavioral perspective, dantrolene has been shown to improve memory in transgenic AD mouse models expressing mutations in amyloid precursor protein or tau (Oulés et al., 2012; Peng et al., 2012) and in the current study, four weeks of dantrolene treatment produced a minor reduction in path length on day four of testing in aged rats and an improvement in learning between testing days. The treatment period (4 weeks) in the current study may have been too short to improve memory; the studies with transgenic AD mice dose animals for 3–8 months (Oulés et al., 2012; Peng et al., 2012). Additionally, blockade of RyRs with dantrolene may interfere with memory in young rats, as we observed on days 2 and 3 of the MWM. This is consistent with studies demonstrating that dantrolene can disrupt memory acquisition and retention in young chicks and mice (Edwards and Rickard, 2006; Ohnuki and Nomura, 1996). Furthermore, RyR knockdown in AD APP/PS1 transgenic mice reduces spine density in young mice but improves spine density in aged mice (Liu et al., 2014). This discrepancy between young and aged animals may be due to age-associated changes in RyR expression and activity that lead to a lengthened sAHP which interferes with N-methyl-D-aspartate receptor (NMDAR)-dependent long term potentiation (LTP; Kumar and Foster, 2004). In aged rats, reducing RyR function would be beneficial to memory performance by reducing the sAHP, while in young rats this would blunt RyR-mediated amplification of NMDAR signaling (Lei et al., 1992). Overall, these data indicate that RyR-dependent alterations in Ca^{+2} handling is a major factor in aging and AD and may be a relevant target for treatment or prevention of these neurodegenerative diseases, but may disrupt memory processes outside of aging.

We initially hypothesized that blockade of L-VDCCs in aged rats would reduce age-associated increases in the sAHP (Disterhoft et al., 1996) thus reducing interference with NMDAR-dependent LTP (Kumar and Foster, 2004) and improving NMDAR-dependent spatial reference memory (Woodside et al., 2004), thus improving MWM performance in aged rats. Indeed, acute nimodipine has been shown to enhance recent short-term memory in

aged rats trained on the radial arm maze (Lever and Walker, 1992). Here, rats were tested in the probe trial 60 minutes following the last training trial, which is soon enough after training to represent a short-term memory. Nimodipine-treated aged rats were no different from young rats or from aged vehicle rats, which may represent a partial recovery. In contrast, we did not observe improvement between testing days in nimodipine-treated aged rats. Previous studies with chronic nimodipine treatment in aged rats observed improved acquisition of the MWM (Riekkinen et al., 1997), presumably via enhancement of NMDAR-dependent learning. That study treated aged animals for 3.5 months rather than 4 weeks, and their MWM paradigm used only 2 training trials per day, whereas our study used 6 trials per day; longer treatment periods may be required to facilitate memory improvement. Previous studies in young rats have shown that L-VDCC antagonism with a different drug, verapamil, can interfere with long-term memory retention while NMDAR antagonism interferes with more rapid acquisition (Woodside et al., 2004; Borroni et al., 2000). L-VDCC and NMDAR forms of LTP are differentially affected by age, which may account for the differences we observed in the studies herein (for review, Foster et al., 2012). We did observe a memory deficit in nimodipine-treated young rats on day 2, but we did not test long-term retention of the task with nimodipine treatment. These data demonstrate, similar to the data with dantrolene, that there made by differential age effects of these treatments as well as effects on different types of memory that are relevant for development of therapies.

Treatment with dantrolene or nimodipine did not reduce the number of activated microglia present in the hippocampus of aged rats. *In vitro*, modulation of RyRs or blockade of L-VDCCs can reduce microglia activation directly, preventing neurotoxicity (Klegeris et al., 2007; Hashioka et al., 2012). However, this does not appear to be the case *in vivo* with aged activated microglia in the rat hippocampus. While microglia contain functional RyRs *in vivo* (Klegeris et al., 2007), there is some debate as to whether they express functional L-VDCCs, despite their responsiveness to L-VDCC antagonists (Hashioka et al., 2012). L-VDCC mediated blockade of microglia neurotoxicity might be due to direct modulation of the superoxide production pathway (Li et al., 2009), rather than Ca^{+2} -mediated induction of this pathway (Colton et al., 1994). Regardless, L-VDCC or RyR blockade is anti-inflammatory in young microglia *in vitro* (Klegeris et al., 2007; Hashioka et al., 2012), but not aged microglia *in vivo*. Here, we did not observe any age- or treatment-related changes in expression of pro- or anti-inflammatory cytokine mRNA or protein, which is in line with several other studies have shown no change in basal brain expression with aging (Hopp et al., 2014; Bardou et al., 2013; Tripathy et al., 2010; Henry et al., 2009; Gayle et al., 1999). Rather, aged microglia appear to be primed towards a pro-inflammatory cytokine response following inflammatory insult (Barrientos et al., 2009; Henry et al., 2009), although some studies have shown a basal increase in pro-inflammatory cytokine and a decrease in anti-inflammatory cytokine expression with aging (Clarke et al., 2008; Frank et al., 2006; Maher et al., 2005). Aged microglia may be defective in receiving or processing “off” signals: acute neuroinflammation in aged rats resolves more slowly than in young rats (Hopp et al., 2014; Henry et al., 2009). Overall, these findings align with other data from our lab demonstrating that interventional treatments during aging do not alleviate microglia activation to the same extent as identical treatments in young rats with experimentally induced neuroinflammation (Brothers et al., 2013; Brothers et al., 2010; Hauss-Wegrzyniak et al., 1999). In the study

herein, the lack of anti-inflammatory action of the two drugs tested may be due to alterations in expression levels of L-VDCCs or RyRs on microglia specifically, or age-related differences in post-translational modifications of these channels that are cell-type specific.

Previously, we found that MWM deficits in aging are correlated with markers of glutamate dysregulation and can be improved by increasing glutamate uptake (Brothers et al., 2013). In the present study, a pivotal finding was that performance of aged rats in the MWM was correlated with RyR expression levels, with higher levels associated with poor performance in the probe trial; this correlation was not evident in young rats (Figure 3). Interestingly, a substantial portion of NMDAR-initiated intracellular Ca^{+2} increase is via release from RyR-gated intracellular Ca^{+2} stores (Lei et al., 1992), suggesting that these two mechanisms may be linked. Overall, these findings utilizing selective pharmacological tools suggest that age-associated memory deficits are potentially related to two different yet interrelated modes of Ca^{+2} dysregulation.

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Highlights

- Ryanodine receptor expression is negatively correlated with memory in aged rats
- Antagonism of ryanodine receptors improves spatial memory acquisition in aged rats
- Blocking L-type voltage dependent Ca^{+2} channels improves recent memory in aged rats

Figure 1.

A. Aged rats swam significantly slower than young rats, regardless of drug treatment. B. Aged rats treated with vehicle or nimodipine took significantly longer paths to locate the hidden platform compared to young treatment-matched controls, and the aged rats treated with vehicle or nimodipine did not significantly improve between days 1 and 4. Aged rats treated with dantrolene significantly improved between days 1 and 4, and there was no significant difference between young and old rats treated with dantrolene on day 4, indicating a partial recovery. * indicates significant ($p < 0.05$) difference from treatment-matched young rats. C–D. During the probe trial, young rats spent significantly more time in the exact location of the previous placement of the platform and an annulus that was 25% total pool diameter compared to aged rats. The dashed line in panel D indicates chance performance. ¥ indicates significant ($p < 0.05$) main effect of age.

Figure 2.

A. Very little MHC-II immunoreactivity was observed in the hippocampus of young rats, whereas aged rats had increased MHC-II immunoreactivity. B. There was a significant interaction ($p=0.045$) between age and drug for MHC-II expression in CA1, but post-hoc analyses revealed no significant differences. C–D. Aged rats have more MHC-II staining in the CA3 and DG compared to young rats. ¥ indicates significant ($p<0.05$) main effect of age.

Figure 3.

Performance in the MWM negatively correlates with RyR expression in aged but not young rats. Decreased expression of all three RyR isoforms in aged rats correlates with improved MWM performance as measured by an increased amount of time spent in an area equivalent to 25% of the total pool area surrounding the platform. In young rats, RyR expression does not correlate with MWM performance. Gene expression data is expressed as fold change compared to young vehicle treated rats.

Table 1

Primer sequences

Target	Accession #	Forward	Reverse
GAPDH	NM_017008	TGACTCTACCCACGGCAAGTTCAA	ACGACATACTCAGCACCAGCATCA
RyR1	AF112256	TGCGCTCCAACCAGGATCTCATT	TCACCTCGAAGTACCACTTGCCATAC
RyR2	AF112257	GGACTTGAAGGAACTGACGGAGGAAA	CACTGAGACCAGCATTTGGGTGTG
RyR3	AF130881	TGGCCTCCTGGCTGTAGTTGTTTA	ACCTGCTCTTACGCCCACATACAT
L-VDCC $\alpha 1C$	NM_012517	GGCTATGAGTTGCCCTGGGTGTATTT	CGAGCTTTGGCTTTCTCCCTCTCTTT
L-VDCC $\alpha 1D$	NM_017298	TGCATGACATTGGGCCAGAAATCC	AGTTTCCAAGCAGGGCACCATTTC
Interleukin (IL)- 1β	NM_031512	ACCTGCTAGTGTGTGATGTTCCCA	AGGTGGAGAGCTTTCAGCTCACAT
TNF α	X66539.1	CTGGCCAATGGCATGGATCTCAA	AGCCTTGTCCCTTGAAGAGAACCT
Transforming growth factor (TGF) β	NM_021578	TGATACGCCTGAGTGGCTGTCTTT	TTTGCTGTCACAAGAGCAGTGAGC
Inducible nitric oxide synthase (iNOS)	NM_012611	AGTTTCCAAGCAGGGCACCATTTC	TGGGTGTCAGAGTCTTGTGCCTTT
Arginase	NM_017134	AGCTACCTGCTGGGAAGGAAGAAA	CTTCTCTGTAAGATAGGCCTCCCACAAC
CD200 ligand	NM_031518	CCTGAACGTGTTTCCCTGGTCTACTT	GTCAAATCCCTCACAGGCTTCCTTCT
CD200 receptor	NM_023953	GCGGCTGAGTCAAGTTGTCCTGATA	TGAAATAGAAGGGCAGCAGAGCAGAG
Doublecortin	NM_053379	TTGTGCCCTGGCTGACACTGAATA	CACGTACAGATCACGTTGCCCTAACT
Post synaptic density 95	NM_019621	AGCGACGAGAGTGGTCAAGGTTAAAG	CCAAGGATGATGATGGGACGAGCATAG
Synapsin 1	NM_019133	TCTCCAGCACACCTTGCTCCTAATC	CTGGTTGGTGGATAGTAGGTGGCATT