

Blueberry-enriched diet ameliorates age-related declines in NMDA receptor-dependent LTP

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Abstract NMDA receptor-dependent long-term potentiation (LTP) in the hippocampus is widely accepted as a cellular substrate for memory formation. Age-related declines in the expression of both NMDAR-dependent LTP and NMDAR subunit proteins in the CA1 region of the hippocampus have been well characterized and likely underlie age-related memory impairment. In the current study, we examined NMDAR-dependent LTP in young Fischer 344 rats (4 months old) and aged rats (24 months old) given either a control diet or a diet supplemented with

blueberry extract for 6–8 weeks. NMDAR-dependent LTP was evoked by high-frequency stimulation (HFS) in the presence of nifedipine, to eliminate voltage-gated calcium channel LTP. Field excitatory postsynaptic potentials (fEPSPs) were increased by 57% 1 h after HFS in young animals, but this potentiation was reduced to 31% in aged animals. Supplementation of the diet with blueberry extract elevated LTP (63%) in aged animals to levels seen in young. The normalization of LTP may be due to the blueberry diet preventing a decline in synaptic strength, as measured by the slope of the fEPSP for a given fiber potential. The blueberry diet did not prevent age-related declines in NMDAR protein expression. However, phosphorylation of a key tyrosine residue on the NR2B subunit, important for increasing NMDAR function, was enhanced by the diet, suggesting that an increase in NMDAR function might overcome the loss in protein. This report provides evidence that dietary alterations later in life may prevent or postpone the cognitive declines associated with aging.

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Introduction

Loss of cognitive function in the elderly presents an overwhelming challenge to the families of those afflicted. With America's demographic shift toward

a more aged population, such mental declines will become an ever increasing challenge. In an effort to reduce the impact of age-related memory loss, intensive research efforts are underway to find ways to slow or reverse this cognitive impairment.

One aspect of cognitive function that appears to decline with age both in humans and in animal models is spatial learning (Barnes 1979, 1987; Rosenzweig and Barnes 2003). Several studies have focused on dietary alterations or supplementation to ameliorate the age-related loss of cognitive function in rat models (Stoll et al. 1993; Joseph et al. 1999; Eckles-Smith et al. 2000; Liu et al. 2002, 2003). In one such study, supplementation of the diet with extracts from blueberries, strawberries or spinach for eight weeks enhanced memory performance in the Morris water maze test (Joseph et al. 1999). While the beneficial behavioral effects of this diet in rats are clear, the cellular changes contributing to these effects have yet to be fully understood.

The major cellular mechanism thought to underlie spatial learning and memory is NMDAR-dependent long-term potentiation (LTP) in the hippocampus (Danysz et al. 1988; Morris 1989; Martin and Clark 2007). In the aged hippocampus voltage-gated calcium channel (VGCC)-dependent LTP is evoked by HFS, in addition to NMDAR-dependent LTP (Shankar et al. 1998). These two forms of LTP appear to oppose each other in learning and memory, blockade of NMDA receptors impairs (Danysz et al. 1988; Morris 1989), whereas blockade of VGCCs appears to enhance hippocampal dependent learning (Deyo et al. 1989; Meneses et al. 1997; Veng et al. 2003). In aged animals NMDAR-dependent LTP is reduced, while VGCC-dependent LTP is enhanced (Shankar et al. 1998). Because of the increase in VGCC LTP, the deficits in NMDAR-dependent LTP are only apparent when VGCCs are blocked or weak induction paradigms are used (Shankar et al. 1998; Clayton et al. 2002; Moore et al. 1993; Deupree et al. 1993; Eckles-Smith et al. 2000).

The age-related impairment of NMDAR-dependent LTP is proposed to be due to decreases in NMDAR function (Barnes et al. 1997) and NMDAR subunit expression (Eckles-Smith et al. 2000; Clayton and Browning 2001; Sonntag et al. 2000; Kuehl-Kovarik et al. 2000). Consistent with this, we found that spermine, which enhances NMDAR function, rescued NMDA-dependent LTP in aged animals (Clayton et al. 2002). In another study from our lab, the age-

related deficits in both NMDAR-dependent LTP and NR1 subunit expression were ameliorated by life-long caloric restriction (Eckles-Smith et al. 2000). Based on these previous studies, we chose to focus on age-related changes in NMDAR-dependent LTP. Therefore, in the current study, we wanted to investigate whether a diet enriched with blueberry extract could overcome the biochemical and electrophysiological sequela associated with aging. Consistent with previous reports, we found deficits in both NMDAR-dependent LTP and synaptic strength in hippocampal slices from aged animals. In slices from animals given the blueberry-enriched diet, both NMDAR-dependent LTP and synaptic strength were elevated to levels seen in young controls. While the deficits in NMDAR subunit expression were not rescued by the diet, phosphorylation of a key tyrosine residue in the NMDAR was enhanced.

Materials and methods

Animals Five young adult (4 months old) and eight aged (22 months old) Fischer 344 rats were obtained from the National Institutes of Aging breeding colony at Harlan Laboratories. Animals were housed two to three per cage in a climate-controlled environment with a 12-h light/dark cycle with water and rat chow available ad libitum. Aged animals were randomly divided into two groups of four animals: one received a control diet, while the other group received a diet enriched with blueberry extract for 6–8 weeks (Joseph et al. 1999). Aged animals were 23–24 months old at the time of experiment.

Tissue preparation Following euthanasia, the brain was removed to ice-cold oxygenated artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2.5 mM CaCl₂, 10 mM dextrose, 1 mM KH₂PO₄, 25.7 mM NaHCO₃). Both hippocampi were removed, and the CA1 region was isolated as previously described (Clayton et al. 2002). Transverse CA1 mini-slices were made by chopping the CA1 (400 μm) on a McIlwain tissue chopper. Slices were recovered for 90 min at 32°C in a perfusion chamber with aCSF (oxygenated with 95% O₂/5% CO₂) flowing at 3 ml/min.

After recovery some slices were prepared for Western blot by sonication in 1% sodium dodecyl sulfate

(SDS), 1 mM ethylenediamineteraacetic acid, 10 mM Tris (pH 8). Samples were heated to 95°C for 5 min, and then frozen at –80°C. The remaining slices were kept in the perfusion chamber for electrophysiology.

Extracellular field recordings One or two slices from each animal were used for electrophysiological studies. Field excitatory post-synaptic potentials (fEPSPs) were obtained by stimulating the Schaffer collateral pathway using a bipolar tungsten stimulating electrode. The recording electrode, a silver chloride recording electrode inside a finely drawn glass capillary containing aCSF, was placed in the dendritic layer of the CA1. Stimuli were given at a rate of 0.033 Hz. Both the initial slope and the peak amplitude of the resulting fEPSP were measured.

An input/output curve was obtained for each slice by increasing the stimulus intensity and measuring the resulting fiber potential and slope of the fEPSP. To compare input/output curves from different groups the fiber potential was measured and plotted against the initial slope of the corresponding fEPSP. Before the LTP experiment began, the applied stimulus was adjusted to produce an fEPSP that was 30–50% of the maximum amplitude. Baseline responses before potentiation had average initial slopes of -160 ± 22 , -128 ± 11 , and -147 ± 12 mV/ μ s in young, aged control and aged blueberry diet animals, respectively. Baseline responses in aged control animals were slightly (but not significantly) lower than the other groups. Once a stable baseline was recorded for at least 20 min, 25 μ M nifedipine, the voltage-gated calcium channel (VGCC) blocker, was perfused for 30 min. As previously described (Shankar et al. 1998), aged animals exhibit robust potentiation via VGCCs and this obscures NMDA-dependent LTP in these animals. LTP was then induced by four 1-s trains of 100-Hz stimulation separated by 30 s. Following this high frequency stimulation (HFS), nifedipine perfusion was then terminated. Responses were continuously recorded at 0.033 Hz for at least 1 h after HFS. LTP was measured as the percent change in the initial slope of the fEPSP at 1 h post HFS by comparing the fEPSP responses at 55–65 min with those spanning the 10 min prior to HFS, using one-way ANOVA with Tukey's post-hoc.

Semi-quantitative Western blotting Briefly, protein concentrations were determined by BCA assay

(Pierce). Samples were diluted to equal protein concentrations in sample loading buffer (2.3% SDS, 67.5 mM Tris, 10% glycerol, 5% β -mercaptoethanol, 0.017% bromophenol blue). To allow comparison of samples run on different gels, each gel contained a five-point standard curve with 1.5–12 μ g of whole hippocampal homogenate in sample loading buffer. The standards used on all gels in the study were from the same batch. Samples and standards in loading buffer were boiled for 5 min then loaded onto 7.5% polyacrylamide gels. Gels were run at 200 V until the bromophenol blue exited the gel. Proteins were transferred to polyscreen PVDF (New England Nuclear) using the Genie transfer apparatus (Idea Scientific). Blots were blocked with 3% BSA in Tris-buffered saline (140 mM NaCl, 20 mM Tris, pH 7.6) with 0.1% Tween 20 (TTBS) for 1 h, then probed with primary antibody overnight at 4°C. Antibodies to NR1 (BD Pharmingen), NR2B (Snell et al. 1996), p-Y1472 NR2B (Phosphosolutions) and GluR2/3 (Chemicon) were diluted 1:3,000 in 1% BSA. Blots were probed with HRP-conjugated goat anti-mouse or goat anti-rabbit (Bio-Rad) antibodies and detected using Super Signal west dura ECL reagent (Pierce). Digital images of the blots were captured using a Chemiimager 4400 (Alpha Innotech). Briefly, each blot contained samples from young, aged control and aged blueberry diet in duplicate as well as a standard curve of hippocampal homogenate. An integrated density value (IDV) for samples and standards was determined as the sum of the intensity of the pixels constituting each band. A standard curve was generated by plotting the IDV of each standard by the micrograms of total protein loaded in that lane. The IDV of each sample was then compared with the standard curve to determine the immunoreactivity of that sample. Only bands that had an IDV within the standard curve were used. The immunoreactivity was divided by the amount of sample protein (in micrograms) loaded in each lane and reported in the figures.

Statistics One-way ANOVAs with Tukey's post-hoc were performed with GraphPad Prism (GraphPad). Because two slices were used from several animals in the LTP study, a nested ANOVA was performed with rat as a random factor nested in condition. This test confirmed that the variance was due to condition and not a clustering effect of rat as a subgroup within the condition group.

Results

NMDAR-dependent LTP in the CA1 of aged rats

We examined the effects of a blueberry-enriched diet on NMDAR-dependent LTP in area CA1 of the hippocampus in aged animals. Previously, we reported that the LTP of population spikes induced by primed burst stimulation was absent in aged animals and that the deficit was relieved by a life-long calorically restricted diet (Eckles-Smith et al. 2000). Furthermore, we have shown that addition of certain berry extracts to the diet can slow the loss of function in memory tasks in aged rats (Joseph et al. 1999). In the current study, the decline of LTP in aged animals was examined by supplementation of the diet with blueberry extract later in life to determine the effectiveness of the diet for reducing the age-related decline in synaptic plasticity.

Field excitatory post-synaptic potentials (fEPSPs) were recorded from CA1 mini-slices of young (4 months old) and aged (24 months old) Fisher 344 rats. NMDAR-dependent LTP was induced by application of four trains of high-frequency stimulation (HFS) in the presence of nifedipine. Because VGCC-dependent LTP is reportedly elevated in aged animals, slices were treated with nifedipine before and during LTP induction to insure that only NMDAR-dependent LTP was induced (Shankar et al. 1998). Nifedipine had no effect on the slope or amplitude of the fEPSP.

The initial slope of the fEPSP was measured before and after induction of LTP (Fig. 1). The degree of LTP was reported as the percent increase in fEPSP slope at 1 h after LTP induction relative to the fEPSP slope just before induction. NMDAR-dependent LTP was reduced in aged animals (30.8±4% potentiation; Fig. 1) compared with young (57.2±7%). LTP in aged animals receiving the blueberry enriched diet for 6–8 weeks was comparable with that seen in young animals (63.3±9.5%). Potentiation was equivalent in young and aged animals given the blueberry diet, and both were elevated relative to aged control animals as determined by one-way ANOVA with Tukey's post-hoc [$F(2,19)=5.59$, $p<0.05$].

Synaptic strength in the CA1 region of aged rats

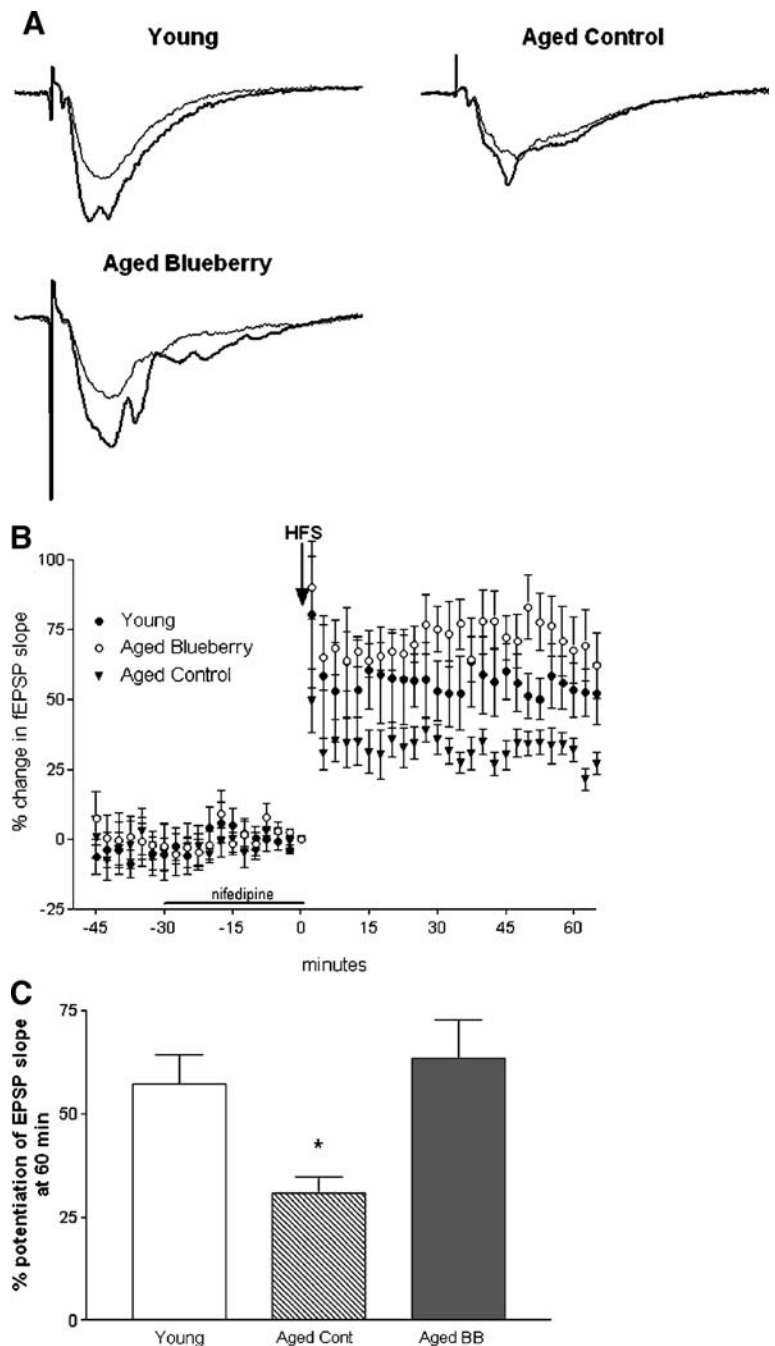
It has been reported previously that there is a reduction in the fEPSP size relative to the fiber potential in aged animals, and this synaptic weakening may contribute to

cognitive impairments (Barnes et al. 1992, 1997; Hsu et al. 2002; Deupree et al. 1991; Potier et al. 2000). To that end, we examined the input/output curve (I/O curve) by comparing the relationship between the fiber potential and the initial slope of the corresponding fEPSP. Figure 2a shows amplitude of the fiber potential relative to the stimulus intensity. The fiber potential generated for a given stimulus was slightly elevated in young animals relative to aged [$F(2,17)=37.2$, $p<0.001$; 0.134±0.007, 0.073±0.004, and 0.086±0.004 in young, aged controls, and aged blueberry diet, respectively]. There was, however, no difference between slices from aged animals given the control diet and those given the blueberry diet. As previously reported, there was a rightward shift of the input/output function in aged animals compared with young as measured by the slope of the I/O lines (2,702±163 in young vs 1,223±73 in aged; Fig. 2). This reduction in synaptic strength was ameliorated in the aged animals given the blueberry enriched diet (2,988±156), suggesting that this diet may prevent or delay this important synaptic change. The slope of the I/O curve was comparable in young and aged animals on the blueberry diet, and both were significantly elevated from aged controls [$F(2,17)=39.5$, $p<0.001$]. The loss of synaptic strength is postulated to represent a loss of functional synapses in the aged animal (Barnes et al. 1997; Rosenzweig and Barnes 2003). Thus, the age-related loss of synaptic strength and the amelioration of this loss by the blueberry diet may provide a mechanism for the decline of LTP with age and the subsequent normalization by the blueberry diet.

Age-related changes in NMDAR expression and phosphorylation

In addition to loss of synaptic strength, age-related decreases in expression of the NR1 and NR2B subunits of the NMDA receptor may also contribute to the LTP deficits (Eckles-Smith et al. 2000; Clayton and Browning 2001; Gemma et al. 2004). Therefore, we examined the expression of NMDA receptor subunit proteins from the CA1 mini-slices of young, aged, and aged animals given the blueberry diet. The expression of both NR1 and NR2B subunits was reduced in the aged animals compared with the young adults by 25% [Fig. 3, NR1: $F(2,9)=5.17$, $p<0.05$, NR2B: $F(2,9)=7.7$, $p<0.05$]. However, the blueberry-enriched diet failed to rescue the deficits in NR1 or NR2B expression ($p>0.05$).

Fig. 1 a–c Age-related deficit in NMDAR-dependent LTP in the CA1 is rescued by blueberry-enriched diet. **a** Sample traces showing EPSPs before and after HFS from young, aged control diet, and aged blueberry diet. The *thin line* depicts control EPSPs and the *thicker line* represents responses 60 min after HFS. **b** Time course of NMDAR-dependent LTP as measured by the initial slope of the EPSP in young (*open circles*; $n=8$), aged control diet (*closed triangles*; $n=7$) and aged blueberry diet (*closed squares*; $n=7$). Baseline responses were obtained for 15 min with drugs and for 30 min in the presence of nifedipine. Then LTP was induced by application of four trains of 100-Hz stimulation for 1 s per train, each train was 30 s apart. Nifedipine perfusion was terminated after the last train. **c** Potentiation of the slope of the EPSP at 60 min after HFS. LTP in aged control diet is significantly lower than in young or aged blueberry diet as determined by one-way ANOVA with Tukey's post-hoc analysis ($*p<0.05$)



Expression of the AMPAR subunits GluR2/3 was not significantly altered by aging [$F(2,9)=0.66$, $p>0.05$].

NMDAR function is strongly regulated by phosphorylation (Wang and Salter 1994; Yu et al. 1997; Lu et al. 1999; Chen and Leonard 1996). Indeed, Norris et al. (1998a, b) suggested that reduced synaptic strength in aged animals could be due to a

shift in the balance of protein kinase and phosphatase activities. Therefore, we investigated whether the blueberry diet could have modified NMDAR function via effects on phosphorylation. We examined the level of phosphorylation of NR2B on tyrosine 1472, a site thought to be important in regulation of receptor function (Nakazawa et al. 2001). Phosphorylation of

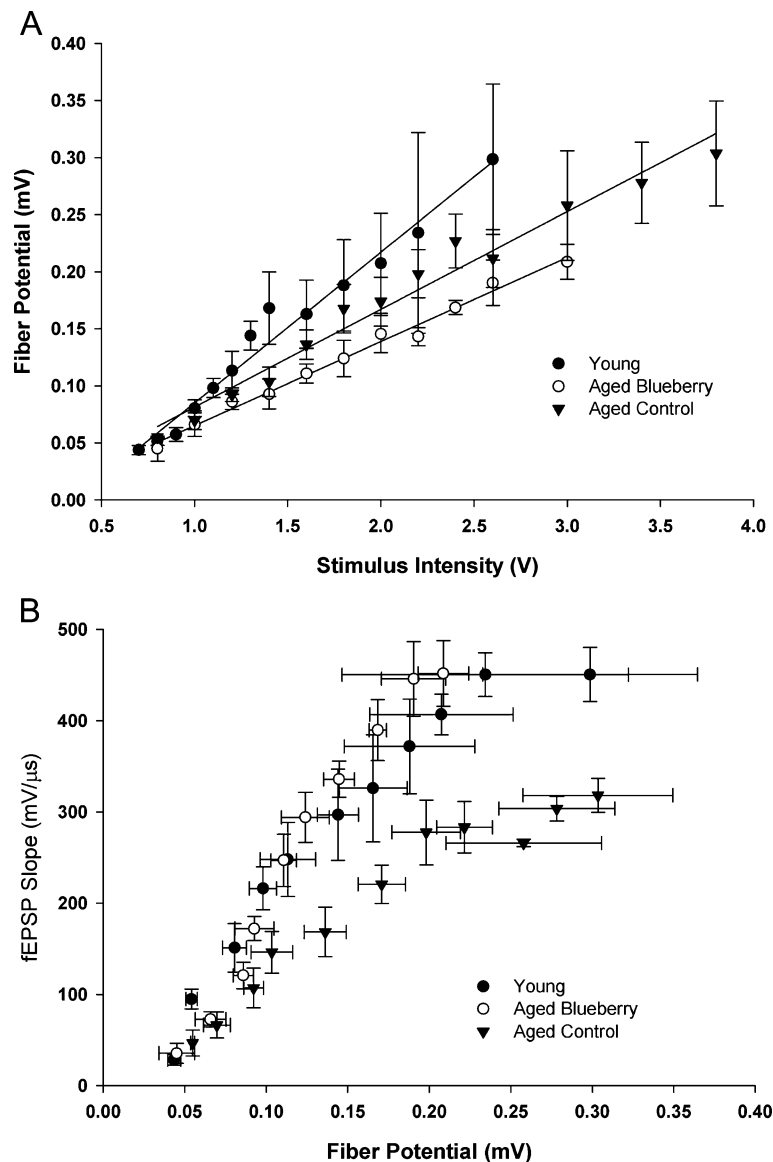


Fig. 2 a, b Age-related decrease in input/output curve is rescued by blueberry-enriched diet. **a** Stimulus intensity vs fiber potential in CA1 from young (closed circles; $n=8$), aged control diet (closed triangles; $n=6$) and aged blueberry diet animals (open circles; $n=6$). The fiber potential from a given stimulus intensity was reduced in aged animals on control or blueberry diet compared with young (the slope of the regression line was 0.134 ± 0.007 , 0.073 ± 0.004 , and 0.086 ± 0.004 in young, aged controls, and aged blueberry diet, respectively, $p < 0.001$ young vs aged control and aged blueberry). **b** I/O curve showing initial slope of the EPSP as a function of the

fiber potential evoked in young (closed circles; $n=8$), aged control diet (closed triangles; $n=6$) and aged blueberry diet (open circles; $n=6$). A linear regression was performed on each group. The resulting slopes of the linear portion of the I/O curves were $2,702 \pm 63$, $1,223 \pm 73$ and $2,988 \pm 156$ for young, aged control and aged blueberry, respectively. The resulting I/O curve slopes from the young and aged blueberry diet animals were significantly different from the aged control diet by one-way ANOVA with Tukey's post hoc ($p < 0.001$ young vs aged control and aged blueberry vs aged control)

this site was elevated, though not significantly, in aged animals compared with young. In aged animals given the blueberry enriched diet, however, there was a significant [$F(2,9)=4.31$, $p < 0.05$] 90% increase in

Y1472 phosphorylation compared with young animals (Fig. 4). In addition to the effects on synaptic strength, the blueberry diet could also contribute to the rescue of LTP in aged animals via an increase in

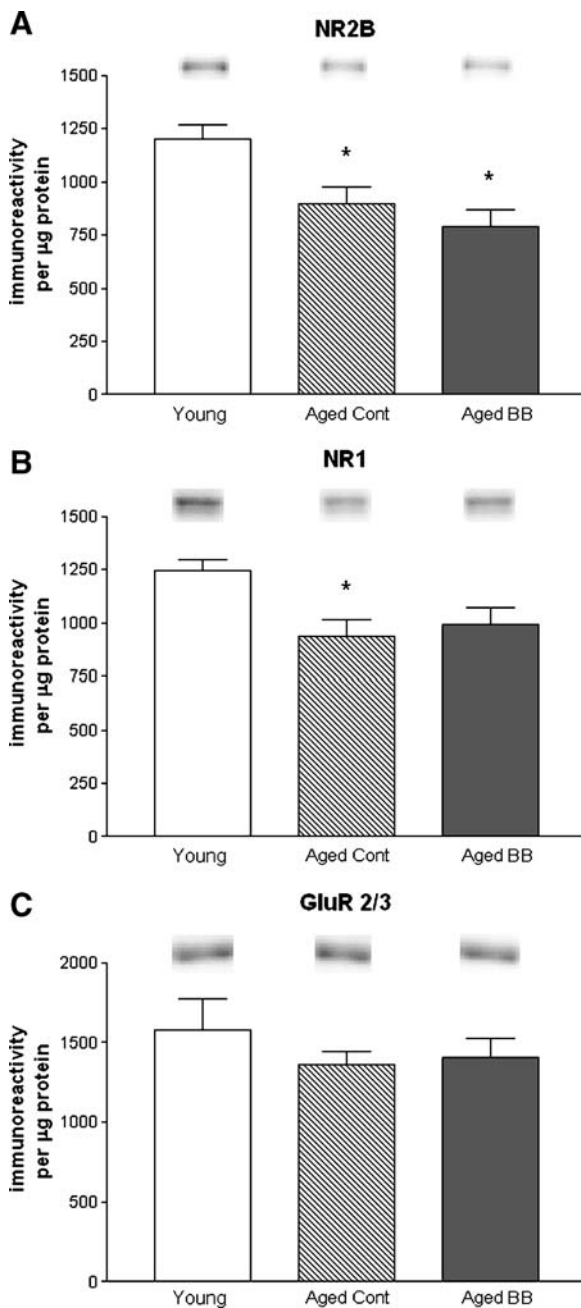


Fig. 3 a–c Age-related decreases in NMDAR subunit expression is not rescued by blueberry-enriched diet as determined by Western blot. **a** NR2B subunit expression in young, aged control and aged blueberry was 1,199 \pm 67, 895 \pm 82 and 790 \pm 80 immunoreactivity units per μg protein, respectively ($n=4$). Expression in aged animals was reduced by 25% compared with young (one-way ANOVA with Tukey's post-hoc, $*p<0.05$ young vs aged control and young vs aged blueberry). **b** NR1 subunit expression in young, aged control and aged blueberry was 1,243 \pm 52, 936 \pm 78 and 992 \pm 83, respectively ($n=4$). Expression in aged animals was reduced by 25% compared with young (one-way ANOVA with Tukey's post-hoc, $*p<0.05$ young vs AC). **c** Expression of GluR2/3 AMPAR subunits in young, aged control and aged blueberry was 1,577 \pm 196, 1,360 \pm 84 and 1,405 \pm 120, respectively ($n=4$). Expression was not reduced in aged animals compared with young (one-way ANOVA $p>0.05$)

tyrosine phosphorylation of the NMDA receptor, which enhances function.

Discussion

In the current report, we show that LTP induced by HFS is reduced in aged animals when VGCCs are

blocked, providing further evidence that NMDAR-dependent LTP is reduced with aging. More importantly, the LTP deficit can be prevented by addition of blueberry extract to the diet for 6–8 weeks. This diet or other diets such as spinach or spirulina, which are also enriched in bioflavonoids, have previously been shown to enhance the performance of aged rats in the Morris water maze (Joseph et al. 1999), enhance performance on a classical conditioning task (Cartford et al. 2002), reduce ischemic brain damage (Wang et al. 2005) and enhance the survival of transplanted neuronal tissue (Willis et al. 2005). It is well established that these diets can reduce markers of oxidative stress and inflammation (Gemma et al. 2002; Joseph et al. 1998). The reduction in oxidative

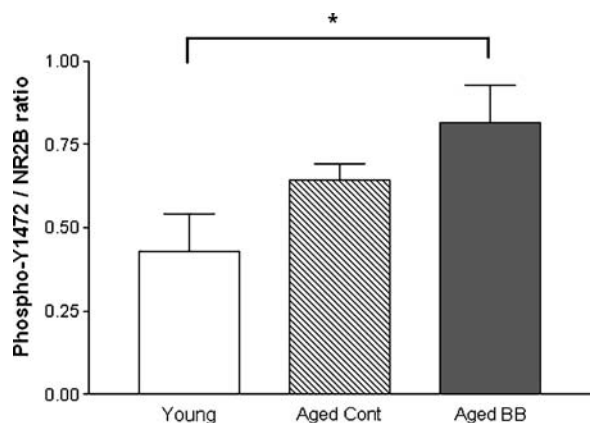


Fig. 4 Age-related increase in the phosphorylation of tyrosine 1472 on the NR2B subunit. The ratio of phospho-Tyr1472 immunoreactivity to NR2B immunoreactivity was 0.43 \pm 0.11, 0.64 \pm 0.04, and 0.82 \pm 0.11 in young, aged control and aged blueberry, respectively ($n=4$; one-way ANOVA with Tukey's post-hoc, $*p<0.05$ young vs aged blueberry)

damage to proteins, membranes and DNA is likely to be the mechanisms by which these diets can lead to functional improvement in several domains. For example, in the cerebellum a functional improvement in the β -adrenergic receptor is linked with the improvement in classical conditioning, and this is also associated with decreased oxidative damage markers and pro-inflammatory (Cartford et al. 2002; Gemma et al. 2002). Thus, the mechanism underlying the improvement in LTP may be related to increased function of receptor signaling involved in NMDAR-dependent LTP.

NMDAR-dependent LTP in the CA1 region of the hippocampus is thought to be the cellular mechanism responsible for spatial memory formation (Barnes 1987). In aged animals, two types of LTP are induced by high-frequency stimulation: NMDAR-dependent LTP and VGCC-dependent LTP (Shankar et al. 1998). NMDAR-dependent LTP is thought to contribute to memory formation, while VGCC-dependent LTP is thought to be detrimental to memory formation (Shankar et al. 1998; Norris et al. 1998b). There is an age-related decline in NMDAR-dependent LTP coincident with an increase in VGCC-dependent LTP (Shankar et al. 1998). Consistent with these changes in the mechanisms of LTP induction, age-related alterations in the expression of NMDARs and VGCCs in the CA1 have been identified. Expression of the NR1 and NR2B subunits of the NMDA receptor decline with age leading to a reduction in NMDAR function, while expression of the VGCC α_{1D} subunits increase with age in area CA1 (Eckles-Smith et al. 2000; Clayton and Browning 2001; Clayton et al. 2002; Barnes et al. 1997; Potier et al. 2000; Veng et al. 2003; Veng and Browning 2002). In fact, antisense knockdown of NR2B expression in young animals reduced LTP in a manner similar to aging (Clayton et al. 2002). We have previously reported that lifelong caloric restriction prevented the age-related deficits in NMDAR-dependent LTP expression as well as the reduction in NMDAR expression (Eckles-Smith et al. 2000). In the current study, administration of a blueberry enriched diet later in life prevented the deficit in NMDAR-dependent LTP, but did not prevent the age-related loss of NMDA receptors. One possibility is that the function of the existing NMDARs is somehow enhanced following the blueberry diet. Consistent with this, we report an increase in the phosphorylation of tyrosine 1472 on

the NR2B subunit. Tyrosine phosphorylation of NMDARs has previously been reported to increase receptor function (Wang and Salter 1994; Chen and Leonard 1996; Yu et al. 1997; Lu et al. 1999). Phosphorylation of Y1472 in particular has been implicated in LTP (Nakazawa et al. 2001). Thus, the increase in tyrosine phosphorylation of NR2B associated with the blueberry diet may result in an increase in NMDAR function. Such an increase in function could provide a mechanism to compensate for the loss of NMDA receptors.

In addition to the age-related decline in LTP, we and others also found age-related deficiencies in the I/O curve of the fEPSP (Barnes et al. 1992, 1997; Hsu et al. 2002; Deupree et al. 1991; Potier et al. 2000). The changes underlying the loss of synaptic strength are a matter of some debate. Similar changes in the dentate gyrus are likely due to a decrease in the number of synapses; however, in the CA1 there are no significant age-related losses of principal cells nor are there reductions in the number of synapses (Rapp and Gallagher 1996; Rasmussen et al. 1996; Geinisman et al. 1995, 2004). It has been proposed that the reduction in synaptic strength is due to a decrease in functional synapses in older rats (Barnes et al. 1992; Geinisman 1999). This decrease is not likely due to presynaptic changes as synaptic proteins involved in neurotransmitter release, such as synaptophysin, synaptotagmin, SNAP-25 and synapsin, are not altered (Nicolle et al. 1999). Likewise, studies from several laboratories have concluded that there is no change in paired pulse facilitation ratios with age in the CA1 (Deupree et al. 1993; Landfield and Lynch 1977; Landfield et al. 1978). Thus, the mechanism for the age-related decrease in the fEPSP is likely postsynaptic. We report here that expression of the AMPAR subunits GluR1 and 2 is not altered in aged animals, confirming findings from previous studies (Eckles-Smith et al. 2000; Clayton and Browning 2001; Nicolle et al. 1999). Sensitivity of synaptic responses to AMPA is reportedly reduced in aged animals (Barnes et al. 1992). One study found that the age-related reduction in synaptic strength was due to a shift in the balance of protein kinase and phosphatase activities (Norris et al. 1998a). In this study, blockade of serine/threonine phosphatase activity increased synaptic strength in aged animals. The function of AMPA receptors is thought to be enhanced by phosphorylation (Boehm and Malinow 2005). It is

possible that the functional expression of AMPARs in the synaptic membrane or the conductance of existing AMPARs is reduced with aging, resulting in a weakened AMPA current (Geinisman 1999). Further studies will be needed to determine if this is the case. In the current study, we found that the age-related decrease in synaptic strength is prevented by the blueberry enriched diet, although the mechanism for this decline remains to be elucidated.

While lifelong caloric restriction may prevent or delay the onset of the decline in LTP by preventing the loss of NMDARs, supplementation of the diet with blueberry extract later in life may provide a means to offset the decline in NMDAR expression by enhancing cellular mechanisms which compensate for the loss. This diet has previously been shown to reverse age-related declines in cognitive and motor behavior (Joseph et al. 1999). Here we show that age-related deficits in NMDAR-dependent LTP, a cellular substrate for learning and memory, are also prevented by the blueberry enriched diet. The normalization of LTP was not due to reversal of NMDAR subunit loss, but may in fact be due to preventing the loss of synaptic strength or by producing an increase in NMDAR function resulting from increases in tyrosine phosphorylation of the NR2B subunit.

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