Selective vulnerabilities of *N*-methyl-D-aspartate (NMDA) receptors during brain aging

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N-methyl-D-aspartate (NMDA) receptors are present in high density within the cerebral cortex and hippocampus and play an important role in learning and memory. NMDA receptors are negatively affected by aging, but these effects are not uniform in many different ways. This review discusses the selective age-related vulnerabilities of different binding sites of the NMDA receptor complex, different subunits that comprise the complex, and the expression and functions of the receptor within different brain regions. Spatial reference, passive avoidance, and working memory, as well as place field stability and expansion all involve NMDA receptors. Aged animals show deficiencies in these functions, as compared to young, and some studies have identified an association between age-associated changes in the expression of NMDA receptors and poor memory performance. A number of diet and drug interventions have shown potential for reversing or slowing the effects of aging on the NMDA receptor. On the other hand, there is mounting evidence that the NMDA receptors that remain within aged individuals are not always associated with good cognitive functioning. This may be due to a compensatory response of neurons to the decline in NMDA receptor expression or a change in the subunit composition of the remaining receptors. These studies suggest that developing treatments that are aimed at preventing or reversing the effects of aging on the NMDA receptor may aid in ameliorating the memory declines that are associated with aging. However, we need to be mindful of the possibility that there may also be negative consequences in aged individuals.

Keywords: aging, NMDA receptor, glutamate, binding, subunits, LTP, memory, learning

INTRODUCTION

Aging causes functional declines in many organs of the body, including the brain. One of the earliest cognitive dysfunctions that humans experience is a decline in learning and memory performance. This deterioration is already detectable in the fifth decade of life (Albert and Funkenstein, 1992). These declines in memory can range in severity from "benign senescent forgetfulness" (Kral, 1962), in which individuals have trouble accessing new and old information (Jolles, 1986), to the degenerative disorder, Alzheimer's disease (AD), which induces dementia and severe declines in cognitive functions (Terry and Katzman, 1983). A better understanding of the underlying causes of these memory declines during aging is necessary for the development of appropriate treatments or preventions for memory dysfunction as we grow older. These treatments may also be beneficial in delaying some of the symptoms of Alzheimer's Disease.

One subtype of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor, is expressed in high density in cortical and hippocampal regions and is very important in the initiation steps of learning and memory (Cotman et al., 1989; Morris and Davis, 1994). NMDA receptors are involved in the performance of many memory tasks, including those using spatial, reference, working, and passive avoidance memory, and in long-term potentiation (LTP), a cellular phenomenon that is believed to be involved in at least some types of memory (Mondadori et al., 1989; Morris and Davis, 1994; Lisman et al., 1998). The NMDA receptors appear to be more vulnerable to the aging process than other glutamate receptors and show declines in their binding densities, electrophysiological functions, and influence on other transmitter systems. The degree of decline in binding is not always the same across different brain regions. In addition, different binding sites of the NMDA receptor complex show differential decreases in binding density within the same regions. This might be explained in part by the findings that the subunits that comprise the NMDA receptor also show differential effects of aging on both mRNA and protein expression. There is evidence that suggests that these changes in NMDA receptor function should have an impact on learning and memory abilities and, in fact, several studies demonstrate an association between aging changes in the NMDA receptor and declining memory. However, there is also mounting evidence that, among older individuals, those that retain the highest levels of expression of NMDA receptors are the poorest learners. This suggests that there is a change in the role of the NMDA receptor with respect to plasticity between middle and old age. This review will present the normal features and functions of the NMDA receptor complex, discuss the changes that have been reported in the NMDA receptor and its related functions during aging, and suggest future directions for improving or preventing age-related changes in learning and memory processes by targeting the NMDA receptor complex. Some of this information has been reviewed previously (Muller et al., 1994; Magnusson, 1998b). This review represents an expansion of certain topics and an update on the progress achieved since

1998. The binding results, in particular, were more extensively discussed in a previous review (Magnusson, 1998b) and will only be summarized and updated here.

NMDA RECEPTOR COMPLEX

The NMDA receptor complex is a large protein assemblage, believed to be a tetramer (Dingledine et al., 1999). It has multiple binding sites for different ligands, including an NMDA binding site, a strychnine-insensitive glycine binding site, and a binding site within the channel for certain non-competitive antagonists. Each of these binding sites can bind several different compounds (Cotman et al., 1989; Corsi et al., 1996; Watkins and Jane, 2006). The NMDA binding site also binds L-glutamate and L-aspartate as endogenous agonists and D-2-amino-5-phosphonopentanoic acid (AP5), [(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (CPP), CGP39653, and CGS19755 as antagonists. The glycine binding site also binds serine and D-cycloserine, which act as agonists (Hood et al., 1989). Non-competitive antagonists, such as (+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohept-5,10-imine maleate (MK801), ketamine, phencyclidine (PCP) and 1-(1-thienyl-cyclohexyl)piperidine (TCP), can bind within the channel. There are also binding sites on the receptor complex for polyamines, protons, redox reagents, zinc and magnesium that can modulate the activity of the receptor (Cotman et al., 1989; Corsi et al., 1996). Some of these different binding sites and their interactions have been exploited to assess the effects of aging on the NMDA receptor complex.

SUBUNITS

The functional subunits of the NMDA receptor complex have been cloned for rats (Moriyoshi et al., 1991; Monyer et al., 1992; Ishii et al., 1993; Seeburg, 1993), mice (Ikeda et al., 1992; Kutsuwada et al., 1992; Meguro et al., 1992; Yamazaki et al., 1992) and humans (Karp et al., 1993; Le Bourdelles et al., 1994; Zimmer et al., 1995). The International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) has recently published guidelines for receptor and ion channel classification that aim to standardize the nomenclature used to refer to NMDA receptor subunits (Collingridge et al., 2009). We will use this new nomenclature throughout the paper and indicate the previous ones in parentheses here. There are three families of subunits identified for the NMDA receptor, including GluN1 subunit (previous designations: GLU_{N1}, NMDA-R1, NR1 and GluRζ1), four members of the GluN2 family, GluN2A, B, C, and D subunits (previous designations: GLU_{N2A-D}, NMDA-R2A-D, NR2A-D, and GluRε1-4); and two members of the GluN3 family, GluN3A or B subunits (previous designations: GLU_{N3A-B}, NMDA-R3A-B, NMDAR-L and chi-1) (Collingridge et al., 2009).

There is a 99% amino acid homology between the mouse, rat and human GluN1 subunits (Meguro et al., 1992; Nakanishi, 1992; Yamazaki et al., 1992; Karp et al., 1993; Le Bourdelles et al., 1994). The GluN1 subunit has the same distribution as NMDA-displaceable [³H]glutamate binding throughout the cortex and hippocampus (Moriyoshi et al., 1991; Meguro et al., 1992; Nakanishi, 1992). Based on studies in which *Xenopus* oocytes were injected with the mRNA for the GluN1 subunit alone, this subunit appears to be necessary and sufficient for the formation of functional channels and, in homomeric receptors, can respond to glutamate, glycine, and MK801 (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992; Ishii et al., 1993). Mutational analysis also suggests that the glycine site is associated with the GluN1 subunit (Kuryatov et al., 1994). The GluN1 subunit of the NMDA receptor has three transmembrane domains and one intramembrane loop domain. This configuration results in an extracellular N-terminal portion and an intracellular C-terminal portion (Zukin and Bennett, 1995).

There are eight different splice variants of the mRNA for the GluN1 subunit that exist in the brain (Laurie and Seeburg, 1994; Zukin and Bennett, 1995). These are generated by alternative splicing of one N-terminal (Exon 5) and two C-terminal (Exons 21 and 22) cassettes in the mRNA (Anantharam et al., 1992; Durand et al., 1992; Nakanishi et al., 1992; Sugihara et al., 1992). The C2 cassette contains a translational stop codon and, in its absence, an additional sequence with the next stop codon, known as the C2′ cassette, becomes part of the mature mRNA (Durand et al., 1992). The N1 cassette is present on the extracellular side of the receptor and the C1, C2 and C2′ cassettes are present on the cytoplasmic side (Sugihara et al., 1992; Hollmann et al., 1993). We will use three subscripts to indicate the presence (1), absence (0) or either condition (X) of the N, C1, and C2 cassettes, in that order. Since mRNA probes have not been developed that span the N and C terminal cassettes, it has not been possible to examine each of the eight splice variants individually. Instead, studies have examined the N terminal cassette region separately from the C terminal cassettes, hence the X designation. The $GluN1_{0XX}$ (GluN1-a) splice variants lack the N1 insertion cassette, while the $GluN1_{1XX}$ (GluN1-b) splice variants contain the N1 insertion cassette (Laurie and Seeburg, 1994; Zukin and Bennett, 1995; Lynch and Guttmann, 2001). The designations for the C terminal splice variants are $GluN1_{X11}$ (GluN1-1:C1,C2 cassettes), $GluN1_{X01}$ (GluN1-2:C2 cassette), $GluN1_{X10}$ (GluN1-3: C1, C2′ cassette) and $GluN1_{X00}$ (GluN1-4: C2′ cassette) (Laurie and Seeburg, 1994; Zukin and Bennett, 1995; Lynch and Guttmann, 2001). The eight different splice variants are important to NMDA receptor function, as there is heterogeneity between variants with respect to agonist and antagonist affinity, zinc modulation, and regional and developmental expression patterns (Hollmann et al., 1993; Laurie and Seeburg, 1994; Zukin and Bennett, 1995).

There are at least four members of the GluN2 family of subunits that show high homology between species (Ikeda et al., 1992; Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992; Yamazaki et al., 1992; Ishii et al., 1993; Le Bourdelles et al., 1994). The GluN2A-D subunits each enhance the activity of the receptor when coupled with the GluN1 subunit (Ishii et al., 1993). The subtypes within this family of subunits confer different agonist/antagonist affinities to the GluN1/GluN2 heteromeric receptors (Kutsuwada et al., 1992; Yamazaki et al., 1992), as well as producing different gating behaviors, responses to Mg++, and I/V curves (Monyer et al., 1992; Ishii et al., 1993). They also differ from each other and the GluN1 subunit in distribution and developmental patterns of mRNA expression (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992, 1994; Ishii et al., 1993; Sheng et al., 1994). The different spatiotemporal expressions of these subunits suggest that multiple NMDA receptor populations

exist in the brain and that they differ both within and between brain regions. During the early stages of development, the GluN2B subunit is highly expressed throughout the brain but, at the onset of sexual maturity, the level of gene expression of the GluN2B subunit is down-regulated in many species and becomes restricted to the forebrain (Monyer et al., 1994; Wang et al., 1995; Wenzel et al., 1996; Laurie et al., 1997; Law et al., 2003). The visible decline in the expression of the GluN2B subunit post-development has been attributed to a programmed developmental switch from the GluN2B subunit to the GluN2A subunit in many brain regions (Liu et al., 2004).

Two subunits have been discovered in the GluN3 family, GluN3A and GluN3B subunits (Ciabarra et al., 1995; Sucher et al., 1995; Nishi et al., 2001; Chatterton et al., 2002). The GluN3 subunits have been localized to oligodendrocytes (Karadottir et al., 2005) and are found in excitatory glycine receptors that are unresponsive to glutamate (Chatterton et al., 2002). They can also be found in triheteromeric receptors with the GluN1 and GluN2 subunits on neurons (Yamakura et al., 2005). It is currently not known how aging affects these subunits or what role they play in cognitive aging, so they will not be discussed further.

NEURONAL PLASTICITY

The NMDA receptor appears to play an integral role in memory. Functional NMDA receptors have been shown, with antagonists and knockouts of the GluN1 subunit gene, to be necessary for successful performance of spatial reference memory tasks in the Morris water maze (Morris et al., 1986; Alessandri et al., 1989; Heale and Harley, 1990; Morris and Davis, 1994; Tsien et al., 1996). Overexpression of the GluN2B subunit gene enhances novel-object recognition, contextual and cued fear memory, fear extinction, spatial reference memory and social transmission of food preference, but not odor recognition memory (Tang et al., 1999; White and Youngentob, 2004; Cao et al., 2007). GluN2A subunit knockout mice demonstrate a role for this subunit in visual discrimination learning and reversal, eyeblink and spatial working memory, but not spatial reference memory (Kishimoto et al., 1997; Bannerman et al., 2008; Brigman et al., 2008). Correlations have been seen between NMDA-displaceable [³H]glutamate binding and the GluN1 and GluN2B subunit expressions in frontal cortical and hippocampal regions and spatial reference memory performance in the Morris water maze (Davis et al., 1993; Magnusson, 1998a, 2001; Magnusson et al., 2007; Zhao et al., 2009b). NMDA receptors also appear to be involved in some forms of passive avoidance learning (Mondadori et al., 1989), place cell stability (Kentros et al., 1998), expansion of place fields (Ekstrom et al., 2001), one-trial flavor-place association encoding (Bast et al., 2005) and in working memory functions (Li et al., 1997; Lisman et al., 1998). NMDA antagonists inhibit performance of spatial working memory tasks when a delay is induced between choices (Li et al., 1997) and NMDA application to the prefrontal cortex of macaques increases the retention time for short-term memory (Dudkin et al., 1996). These studies indicate that NMDA receptors are important for many different types of memory.

Some of the mechanisms for how NMDA receptors contribute to plasticity at the cellular level have been elucidated. Long-term potentiation (LTP) is a sustained increase in the efficiency of synaptic transmission that typically is induced by high-frequency stimulation (Collingridge and Bliss, 1987; Bashir et al., 1994). Antagonists of the NMDA receptor and knockouts of the GluN1 subunit gene block the induction of LTP in both the hippocampus (Harris et al., 1984; Morris et al., 1986; Bashir et al., 1991, 1994; Tsien et al., 1996) and neocortex (Artola and Singer, 1994) and, in some studies, this has been associated with declines in spatial memory performance (Morris et al., 1986; Morris, 1989; Morris and Davis, 1994; Tsien et al., 1996). Alterations of GluN2B subunit expression, including overexpression, show a role for NMDA receptors containing this subunit in LTP, long-term depression (LTD), and spatial memory (Kutsuwada et al., 1996; Tang et al., 1999; Cao et al., 2007; Zhou et al., 2007; von Engelhardt et al., 2008; Akashi et al., 2009). Antagonists for different GluN2 subunits suggest that distinct subpopulations of NMDA receptors characterized by different GluN2 subunits contribute to the induction mechanisms of both LTP and LTD (Massey et al., 2004; Fox et al., 2006; Bartlett et al., 2007; Li et al., 2007). Several pharmacological studies show that the GluN2B-containing NMDARs are required for LTP and/or LTD (Fox et al., 2006; Gardoni et al., 2006; Bartlett et al., 2007; Miwa et al., 2008; Hogsden and Dringenberg, 2009). Thus, one major way that NMDA receptors can contribute to behavioral plasticity is through induction of LTP and LTD.

Roles in cell survival and dendritic complexity have also been shown for the NMDA receptor. The GluN1 subunit appears to be important for the survival of new neurons in the dentate gyrus in young mice (Tashiro et al., 2006). The GluN2A and GluN2B subunits are both involved in development of dendritic arbor morphology (Ewald et al., 2008). The GluN2B subunit appears to allow temporally imprecise inputs to be considered coincident, whereas the GluN2A subunit requires more precise timing of inputs for plasticity to occur (Ewald et al., 2008). These studies all demonstrate an important role for NMDA receptors in memory processes and cellular plasticity and suggest that detrimental changes to the NMDA receptor during the aging process may explain, at least in part, the memory declines that people and animals experience during the aging process.

SELECTIVE EFFECTS OF AGING ON NMDA RECEPTORS AND SUBUNITS
EFFECTS OF AGING ON NMDA RECEPTOR BINDING
Selective vulnerability of NMDA receptors among glutamate receptors
The NMDA binding site is more affected by aging than the other ionotropic glutamate receptors in both C57Bl/6 and BALB/c mice (Magnusson and Cotman, 1993; Magnusson, 1995a). In multiple autoradiographic studies, there were more subregions and layers within the cerebral cortex and hippocampus that showed significant declines during aging in NMDA-displaceable [³H]glutamate binding than [³H]AMPA binding to its receptor (Magnusson and Cotman, 1993; Magnusson, 1995a, 1997a). In addition, the percent decrease in binding of [³H]AMPA in aged mice is always less than seen with [³H]glutamate binding to NMDA sites in these mice. Even in regions where there are significant changes in AMPA binding (frontal, parietal and entorhinal inner layers and caudate nucleus), the percent loss in NMDA-displaceable glutamate binding is greater (Magnusson and Cotman, 1993; Magnusson,

1995b, 1997a). These results, along with the lesser effect of aging on kainate and metabotropic glutamate binding sites (Magnusson and Cotman, 1993; Magnusson, 1997b), suggest that the NMDA receptor is more vulnerable to the aging process than other glutamate receptors in mice.

There is some evidence of this selective vulnerability in other animals as well. Dogs also show a greater decline in [³H]glutamate binding to NMDA sites in the cortex and hippocampus with increased age than [³H]AMPA binding (Magnusson et al., 2000). In monkeys, there was a greater loss in the proportion of projection neurons in the cortex exhibiting GluN1 subunit immunostaining than the AMPA receptor subunit, GluA2 (formerly GluR2), with the exception of region 46i (Hof et al., 2002). Fischer 344 rats exhibit a similar decrease in binding to both AMPA and NMDA receptors in the hippocampus, but less of an affect of aging on kainate receptors (Tamaru et al., 1991; Clark et al., 1992). However, Fischer 344 rats did show a greater age-related decline in protein expression of the NMDA receptor subunits GluN1 and GluN2B than the AMPA receptor subunits GluA1, GluA2, or GluA2/3 in the hippocampus (Clayton and Browning, 2001; Clayton et al., 2002b; Coultrap et al., 2008). Aged Wistar rats show a greater decline in MK801 binding to the NMDA receptor than AMPA binding in the forebrain (Ossowska et al., 2001). However, there were similar decreases during aging in the expression of the GluN2B and GluA2 subunit proteins and mRNAs, but no effect on the GluN1 or GluA1 subunit proteins in this same strain (Dyall et al., 2007). In Long-Evans rats, NMDA receptor binding is more decreased in certain regions than either AMPA or kainate binding (Nicolle et al., 1996; Nicolle and Baxter, 2003) and AMPA binding was significantly increased in the hippocampus in aged rats that were impaired in spatial learning as compared to young (Le Jeune et al., 1996; Andres et al., 2000). Fischer 344XBrown Norway (F344XBN) rats show significant declines during aging in both NMDA and AMPA receptor subunits in the hippocampus (Newton et al., 2007; Shi et al., 2007). These results suggest that other non-rodent species also show higher susceptibility of the NMDA receptor to aging than the AMPA receptors. However, there is variability in this relationship between different rat strains and between the effects of aging on AMPA binding and subunit expression.

Heterogeneous age-related changes in different binding sites on the NMDA receptor complex

The most consistent finding, with respect to binding studies investigating the effect of aging on the NMDA receptor complex, is that binding to the NMDA binding site by agonist (L-glutamate) and/or antagonists (CPP, CGS19755, and CGP39653) decreases with increasing age in the cerebral cortex and hippocampus, regions which are important to memory processing (Ammassari-Teule and Gozzo, 1982; Eichenbaum et al., 1990). This age-related decline in binding density to the NMDA binding site has been documented in Fischer 344 (Kito et al., 1990; Tamaru et al., 1991; Clark et al., 1992; Ingram et al., 1992; Ogawa et al., 1992; Davis et al., 1993; Castorina et al., 1994); but see (Bonhaus et al., 1990), Long-Evans (Pelleymounter et al., 1990), Wistar (Serra et al., 1994) and Sprague-Dawley (Fiore and Rampello, 1989) rats, C57Bl/6 and BALB/c mice (Magnusson and Cotman, 1993; Magnusson, 1995a,

1997a, 2000), dogs (Magnusson et al., 2000), and rhesus monkeys (Wenk et al., 1991). Therefore, we can conclude that the NMDA binding site is negatively affected by the aging process in multiple mammalian species.

The age-associated changes in the NMDA binding site do not appear to be homogeneous across brain regions, however. In most studies, the cerebral cortex of aged rats and mice show greater decreases in binding to the NMDA binding site than the hippocampus (Tamaru et al., 1991; Davis et al., 1993; Magnusson and Cotman, 1993; Castorina et al., 1994; Magnusson, 1995a, 2001). However, two studies involving rats showed that the change in binding in the hippocampus was equal to or slightly larger than the change in the cortex (Wenk et al., 1991; Serra et al., 1994). The decline in binding of glutamate to NMDA binding sites in aged dogs also shows some variability both within the cerebral cortex and between the cerebrum and hippocampus (Magnusson et al., 2000). The NMDA receptor binding sites in the intermediate hippocampus (located between the dorsal half and the ventral-most quarter; Moser and Moser, 1998) are more susceptible to aging than those in the dorsal hippocampus in mice (Magnusson et al., 2006). These findings may explain the differences seen in mouse studies examining the intermediate hippocampus (Magnusson and Cotman, 1993; Magnusson, 1995a, 1997a, 2000, 2001) and in rat studies examining the dorsal hippocampus (Kito et al., 1990; Nicolle et al., 1996). The protein concentration of the GluN1 subunit also shows decreases during aging in the ventral hippocampus, but not the dorsal in Sprague-Dawley rats (Liu et al., 2008). These studies suggest that the NMDA receptors in the cerebral cortex are more vulnerable to aging changes than those in the hippocampus.

There also appears to be a difference between the cerebrum and hippocampus with respect to the effects of aging on agonist versus competitive antagonist binding to the NMDA binding site. Studies in Long-Evans rats showed that there are significant declines in antagonist ([³H]CPP) binding in the hippocampus with increased age (Pelleymounter et al., 1990), but no significant change in agonist ([³H]glutamate) binding to NMDA sites in the same region (Nicolle et al., 1996). These studies support our findings in C57Bl/6 mice in which antagonist binding is more affected by aging than agonist binding in the hippocampus (Magnusson, 1995a). The percent declines during aging in [³H]glutamate and [³H]CPP binding in cerebral cortical regions of C57Bl/6 mice, however, are more equivalent to each other (Magnusson, 1995a). The percent decline during aging in the GluN2B subunit mRNA across different cortical and hippocampal subregions shows an association with changes in the binding of [³H]glutamate to NMDA receptors, while the decreases with age in the GluN1 subunit mRNA are more related to age-related declines in [³H]CPP binding in the same regions (Magnusson, 2000). These results show that the effects of aging on the NMDA binding site are not uniform throughout the brain.

The effects of aging on the glycine binding site of the NMDA receptor appear to be more variable than the changes in the NMDA binding site. There is evidence for age-related increases (130%) in maximal binding (B_{max}) of [³H]glycine in NMRI mice (Saransaari and Oja, 1993), a 27–49% decrease in [³H]glycine binding in Fischer 344 rats (Kito et al., 1990; Tamaru et al., 1991), and no change

in C57BL/6 mice (Magnusson, 1995a) and Sprague-Dawley rats (Bresink et al., 1995). All of these studies were performed with protocols that measured strychnine-insensitive glycine binding (Ogita et al., 1989; McDonald et al., 1990), so should not include inhibitory glycine receptors. These studies indicate that highly variable changes occur in the glycine binding site of the NMDA receptor across ages in different rodents.

Autoradiographic examination of glutamate-stimulated [^{3}H]MK801 and [^{3}H]TCP binding within the channel in different ages of C57BL/6 mice showed that there are decreases in binding with increasing age (Magnusson, 1995a). This decrease is significant in less brain regions than the age-related changes in [^{3}H]glutamate binding to the NMDA binding site in the same mice (Magnusson, 1995a). Age-related declines in [^{3}H]MK801 binding in dogs, however, are similar to the decreases in [^{3}H]glutamate binding to the NMDA site (Magnusson et al., 2000). A decrease in the B_{max} for [^{3}H]MK801 and [^{3}H]TCP binding sites has been seen with increased age in the cortex and hippocampus of other strains of mice and rats (Tamaru et al., 1991; Cohen and Muller, 1992; Kitamura et al., 1992; Serra et al., 1994; Scheuer et al., 1995) and in human frontal cortex (Piggott et al., 1992). Sprague-Dawley rats show a non-significant increase in both B_{max} and K_D during aging (Bresink et al., 1995) and F344XBN male rats show no influence of aging on [^{3}H]MK801 binding in the neocortex (Palmer, 2000). In the majority of mammals studied, therefore, it appears that there is a decrease in the number of channel binding sites, although the percentage change may be less than for the NMDA binding site in some species.

The effects of aging on the channel binding sites also appear to be brain region dependent. There appears to be more change with age in the channel binding site in the cerebral cortex (up to a 24% decline in binding) than in the hippocampus (up to a 14% decline), similar to the results with other binding sites on the NMDA receptor complex in C57BL/6 mice (Magnusson, 1995a). No changes in [^{3}H]MK801 binding were detectable across ages in the hippocampus, entorhinal cortex or cerebellum of humans (Perry et al., 1993), but declines were reported in human frontal cortex (Piggott et al., 1992). This appears to fit with the higher susceptibility of this receptor within the cortex to aging changes, as compared to the hippocampus, in C57Bl/6 mice (Magnusson, 1995a).

NMDA receptor binding changes: Interpretations

This variability of the effects of aging on different binding sites of the NMDA receptor, along with the data about the subunits (see below) suggests that, although there may be reduced numbers of receptors in aged animals, there are other changes occurring in the remaining receptors as well. The differences in susceptibility of the NMDA receptors within different brain regions could be due to the differing populations of receptors, with respect to subunit composition, within different regions. It also could be due to a "rate of living" effect, with some regions sustaining more oxidative damage than others. The fact that frontal and parietal cortices and striatum show aging effects in multiple glutamate receptors supports this possibility. In addition, the redox site on NMDA receptors in the hippocampus of aged rats are in a more oxidized state than in young (Bodhinathan et al., 2007), suggesting that the

receptors are in a more oxidizing environment in old individuals. The amount of oxidation and susceptibility of the receptors to this environment may vary between regions. Heterogeneity in the subunit makeup of receptors between different brain regions may also contribute to the differences seen in the effects of aging on the agonist versus competitive antagonist binding to the NMDA binding site. It is interesting, though, that the more susceptible antagonist binding was related to the GluN1 subunit mRNA, which does not change as significantly as the GluN2B subunit, in either mRNA or protein expression, during aging (see below). This may be because the amount of mRNA expression does not directly reflect the amount of protein produced. It is also possible that there are alterations during aging in the tertiary structure of the NMDA binding site that influence the binding of antagonist molecules more than glutamate. This decline in binding of competitive antagonists during aging could be important to the design of treatments aimed at blocking NMDA receptor-related neurotoxicity for conditions such as stroke. Competitive antagonists may become less effective with increasing age. Whatever the cause, it seems likely that these binding site changes alter the functions associated with the NMDA receptor. In addition, given the importance of this receptor to learning and memory processes, it also seems probable that the binding site changes are, at least in part, responsible for some of the age-related declines in memory.

DIFFERENTIAL SUSCEPTIBILITY OF DIFFERENT NMDA RECEPTOR SUBUNITS TO AGING

GluN1 subunit expression

The GluN1 subunit has been observed to be vulnerable to the effects of aging. In mice, there are decreases in mRNA expression between 3 and 30 months of age within the frontal and occipital cortices and the dentate gyrus of the hippocampus (Magnusson, 2000). Protein levels of the GluN1 subunit also decline with increasing age in mice within the hippocampus and cerebral cortex (Magnusson et al., 2002). These declines in the expression of the GluN1 subunit are less than those seen for the GluN2B subunit (Magnusson, 2000; Magnusson et al., 2002) and are not always consistent between studies involving aged C57BL/6 mice (Ontl et al., 2004; Magnusson et al., 2005; Das and Magnusson, 2008; Zhao et al., 2009b). Fischer 344 and F344XBN rats also show declines in protein expression of the GluN1 subunit in the hippocampus during aging (Eckles-Smith et al., 2000; Mesches et al., 2004; Newton et al., 2007; Shi et al., 2007; Coultrap et al., 2008). Wistar and Long-Evans rats, however, show no age-related change in the expression of the GluN1 subunit in hippocampus (Adams et al., 2001b; Dyall et al., 2007). A decrease in the protein expression of the GluN1 subunit protein is also observed in the distal dendrites of the dentate granule cells in aged macaque monkeys, as compared to young adults (Gazzaley et al., 1996a). There is also a loss of GluN1 protein in corticocortically projecting neurons in both macaque and patas monkeys (Hof et al., 2002). The mRNA expression for the GluN1 subunit is also decreased in the hypothalamus of middle-aged female Sprague-Dawley rats and C57BL/6 mice, which may play a role in reproductive senescence (Zuo et al., 1996; Adjan et al., 2008). These studies indicate that the GluN1 subunit of the NMDA receptor can be susceptible to the effects of

aging. However, this susceptibility appears to be very variable both within and between certain species and strains. This variability may indicate that the changes observed in the expression of the GluN1 subunit are not due to a programmed decline, but rather the result of environmental factors and experiences during aging.

A heterogeneous effect of aging has also been observed on the expression of the individual GluN1 splice variants in rodents. Analysis of the C-terminal splice variants shows that there are declines seen in the mRNA expression of the GluN1$_{X11}$ (1-1) and GluN1$_{X10}$ (1-3) splice variants in some regions of the frontal cortex and hippocampus during aging in C57BL/6 mice, regardless of whether or not there are significant changes in the mRNA for all GluN1 subunits (Magnusson et al., 2005; Das and Magnusson, 2008). The mRNA for the GluN1$_{X11}$ (1-1) splice variants is decreased in the lateral frontal cortex, including the lateral orbital and insular cortices, and in the dentate gyrus, both in the presence or absence of significant changes in the unprocessed mRNA for the whole GluN1 subunit (Magnusson et al., 2005; Das and Magnusson, 2008). Age-related changes in the GluN1$_{X10}$ (1-3) splice variant mRNA also can be observed in the CA3 and dentate gyrus regions without any significant decrease in the total pool of GluN1 subunit mRNAs (Magnusson et al., 2005). However, in the medial and lateral prefrontal cortex there either has to be an overall change in the whole subunit mRNA expression or a behavioral experience (see below) to detect significant changes in the GluN1$_{X10}$ (1-3) splice variants (Magnusson et al., 2005; Das and Magnusson, 2008). No changes have been observed between different adult ages in the mRNA expression of the GluN1$_{X01}$ (1-2) or GluN1$_{X00}$ (1-4) splice variants (Magnusson et al., 2005). A reduction in the GluN1$_{1XX}$ (1-b) splice variant mRNA has only been seen in the insular cortex and only between young and middle-aged mice (Das and Magnusson, 2008). Collectively these data indicate that there is differential susceptibility of different GluN1 splice variants to the aging process. In addition the splice variants show heterogeneous effects of aging between different brain regions, similar to the receptor as a whole.

Experience has differential effects on the GluN1 subunit during aging. Experience in a set of memory tasks, involving tests of reference, working and associative memory in the Morris water maze, lead to an increase in the mRNA expression of the GluN1$_{0XX}$ (1-a) splice variants in old mice only, but exacerbated the age-related decrease in GluN1$_{X10}$ (1-3) splice variant mRNA densities (Das and Magnusson, 2008). Following stimulation of LTP, the activity dependent increase in the surface expression of GluN1 subunit protein that is observed in the young rats, is absent in the old rat hippocampus. This is associated with a decline in protein expression of the C2 cassettes of the GluN1 subunit (Clayton et al., 2002a). These results suggest that behavioral experience can enhance expression of the mRNA for the GluN1$_{0XX}$ (1-a) splice variants, but it is unclear whether this translates to enhanced surface expression of the protein. This also provides support for experience influencing some aspects of GluN1 subunit expression during aging.

GluN2A subunit expression

There is little or no effect of aging on the mRNA expression of the GluN2A subunit in the cortex or hippocampus of C57Bl/6 mice (Magnusson, 2000, 2001; Magnusson et al., 2006). The expression of the GluN2A subunit mRNA, however, does show a positive correlation with NMDA-displaceable [³H]-glutamate binding levels in cortical regions and the CA3 region of the hippocampus both across ages and in old mice (Magnusson, 2001). There is a decline in the protein expression of the GluN2A subunit within the hippocampus, but only between middle-aged and old C57BL/6 mice (Magnusson et al., 2002). A lack of change during aging in the GluN2A subunit is seen in the hippocampus of Fischer 344 rats (Clayton and Browning, 2001; Clayton et al., 2002b; Mesches et al., 2004) and in the striatum of Wistar rats (Martinez-Villayandre et al., 2006). F344XBN rats show similar age-related declines in protein expression of the GluN2A and GluN2B subunits in the hippocampus as a whole, but the GluN2A subunit is not affected by aging within the dentate gyrus alone (Newton et al., 2007). Female, Sprague-Dawley rats show a significant, but decreased decline in expression of the GluN2A subunit during aging, as compared with GluN1 and GluN2B subunits (Adams et al., 2001a). Males of the same strain show no significant decreases during aging in the GluN2A subunit within the hippocampus, but a decline does occur in perirhinal, entorhinal and postrhinal cortices (Delibas et al., 2005; Liu et al., 2008). These results suggest that, in most rodent strains examined, the GluN2A subunit of the NMDA receptor is less susceptible to the effects of the aging process than the GluN1 and GluN2B subunits. This can alter the function of the remaining receptors (see "NMDA Receptor Subunit Changes: Interpretations" below).

GluN2B subunit expression

The greatest age-related declines in NMDA receptor subunit expression in C57BL/6 mice are in the GluN2B subunit. In aged C57BL/6 mice, the mRNA expression of the GluN2B subunit declines significantly with age throughout the cerebral cortex and in some subregions of the hippocampus (Magnusson, 2000, 2001; Ontl et al., 2004; Magnusson et al., 2006). These changes in GluN2B subunit mRNA expression correlate positively with age-related changes in binding of [³H]glutamate to NMDA receptors in both cortical and hippocampal regions (Magnusson, 2000, 2001). In the cerebral cortex and hippocampus, there are also significant declines in the protein expression of GluN2B subunit (Magnusson et al., 2002, 2007; Ontl et al., 2004; Zhao et al., 2009b). The declines in the mRNA expression of the GluN2B subunit in the frontal cortex show a pattern that suggests that the aging change may be a continuation of the programmed developmental decline of the GluN2B subunit (Ontl et al., 2004). In the frontal cortex, the protein levels of the GluN2B subunit showed a greater decline with aging in the synaptic membrane fraction than in the whole homogenate (Zhao et al., 2009b). This suggests that, in addition to the declines already discussed in mRNA expression of the GluN2B subunit, there may be an additional effect of aging on GluN2B subunit localization within the synaptic membranes of the frontal cortex. In the hippocampus, a similar age-related decline was observed in both fractions (Zhao et al., 2009b), suggesting that the decline in expression is primarily due to changes in the transcript. Monkeys and rats also show similar declines during aging in the expression of the GluN2B subunit (Sonntag et al., 2000; Clayton and Browning, 2001; Clayton et al., 2002b; Bai et al., 2004; Dyall et al., 2007; Shi et al., 2007; Coultrap et al., 2008). Together, these data suggest that

expression of the GluN2B subunit is significantly decreased across species in both the cerebral cortex and in regions of the hippocampus in aged animals.

NMDA receptor subunit changes: Interpretations

Given the necessity of having the GluN1 subunit present in order to have a functioning NMDA receptor (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992; Ishii et al., 1993), it seems likely that the declines seen in the GluN1 subunit may account for the changes observed in B_{max} in binding studies (Tamaru et al., 1991; Cohen and Muller, 1992; Kitamura et al., 1992; Serra et al., 1994; Scheuer et al., 1995). Because of the evidence that the glycine binding site is located on the GluN1 subunit (Kuryatov et al., 1994), it is also possible that the variability seen in glycine binding to the NMDA receptor during aging may be explained by the variable effects of aging on the GluN1 subunit. The variability in the effects of aging and influence of behavioral experience and interventions (see below) also suggest that we may be better able to correct the effects of aging on the GluN1 subunit than the GluN2B subunit.

The enhancement of the splice variants with the N terminal cassette spliced out and the loss of the C1 cassette mRNA and C2 cassette protein seem to be the most prominent changes during aging in the GluN1 subunit. Splicing out of the N1 insertion cassette has been shown to reduce affinity for agonist (Durand et al., 1993), decrease the current amplitude in oocytes expressing NR1 subunits (Hollmann et al., 1993; Zheng et al., 1994), and enhance stimulation of the NMDA receptor by polyamines, proton inhibition and zinc modulation (Durand et al., 1992, 1993; Hollmann et al., 1993; Zheng et al., 1994). The C-terminal is essential for NMDA-receptor dependent gene expression because of the role it has on the downstream signaling and receptor inactivation (Bradley et al., 2006). Loss of the C1 cassette would lead to loss of phosphorylation sites, two for protein kinase C (PKC) and one for protein kinase A (PKA) activity (Tingley et al., 1993, 1997), and loss of the interaction of the NMDA receptor with two proteins, yatiao and neurofilament L, which could alter clustering of receptors (Ehlers et al., 1998; Lin et al., 1998). These age-associated changes in the GluN1 splice variants thus could lead to an alteration in the composition of the receptors remaining in aged individuals, which could alter NMDA receptor function.

An age-related decline in the GluN2B subunit within the synapse, with little or no change in the GluN2A (Magnusson, 2000; Magnusson et al., 2002) and GluN1 subunits could lead to a synaptic population of NMDA receptors that have decreased agonist affinity, faster kinetics, and reduced LTP associated with binding of calcium calmodulin kinase II (Kutsuwada et al., 1992; Monyer et al., 1992; Yamazaki et al., 1992; Ishii et al., 1993; Barria and Malinow, 2005) than in the young adult. These changes in receptor response may have an influence on the binding studies involving MK801 and TCP, because these compounds are dependent on the channel opening to bind and often the studies use glutamate to promote channel opening (Foster and Wong, 1987; Ransom and Stec, 1988; Reynolds et al., 1992). To our knowledge, no study has examined the relationship between GluN2B and MK801 or TCP binding changes during aging. If some of the decline in synaptic membrane expression were due to a shift to more extra-synaptic localization of GluN2B subunits in the frontal cortex of aged animals, there could also be an

increase in LTD (Massey et al., 2004) and/or activation of a CREB shut-off pathway that interferes with induction of brain-derived neurotrophic factor (BDNF) and leads to a loss of mitochondrial membrane potential and cell death (Hardingham et al., 2002). Thus, the aging changes observed in synaptic membrane expression of the GluN2B subunit could be associated with changes in subunit composition and function of the synaptically-active NMDA receptors and/or a change in the interaction of NMDA receptors with other proteins involved in synaptic plasticity.

Further knowledge about alterations in the subunits of the NMDA receptor during the aging process and the functional consequence may be the key to preventing the aging changes that occur in this receptor. Electrophysiological examination of changes in the subunit ratios and splice variants would enhance understanding of the consequences of the aging alterations. The ability to target single subunits or splice variants may aid our ability to enhance NMDA receptor functions without inducing neurotoxicity.

CHANGES IN ELECTROPHYSIOLOGICAL RESPONSES INVOLVING NMDA RECEPTORS

Some changes during aging in NMDA receptor expression are reflected in changes in the electrophysiological responses, but interpretations can be complicated by changes in other systems. Intracellular responses show a reduced sensitivity to applied NMDA in slices of the frontoparietal cortex from old Fischer 344 rats, as compared to young (Baskys et al., 1990). There is also no evidence of LTP in this same region in old rats when using a protocol that produces LTP in the young, (Baskys et al., 1990). NMDA responses from *Xenopus* oocytes injected with mRNA isolated from aged frontal cortex show decreased maximal NMDA currents and increased sensitivity to magnesium, as compared to mRNA from young mice (Kuehl-Kovarik et al., 2000). There was also a decrease in the low affinity and an increase in the high affinity ifenprodil components in oocytes injected with aged mRNA (Kuehl-Kovarik et al., 2000), which is consistent with the findings of decreased GluN2B subunit mRNA in the frontal cortex of aged mice (Magnusson, 2000, 2001). Thus, there are changes in the function of the NMDA receptor in the frontoparietal cortex with age that are consistent with the changes in NMDA receptor expression.

The data from the hippocampus are more complicated. NMDA responses in both the dentate gyrus (Yang et al., 2008) and in the CA1 region of the hippocampus show decreased excitatory postsynaptic potentials for a given presynaptic fiber volley in aged Fischer 344 rats (Barnes et al., 1997). However, NMDA-induced inhibition of the extracellular field potential shows no age-related difference in dose in the CA1 regions of Sprague-Dawley rats (Billard et al., 1997). The ratio of NMDA/AMPA responses also remains constant across age in the CA1 region of the hippocampus in Fischer 344 rats (Barnes et al., 1997), but decreases in the dentate gyrus (Yang et al., 2008). Certain aspects of LTP in the hippocampus are altered as rats age; the enhancement of LTP reaches maximum at a slower rate and the decay rate is faster in old rats, as compared to young (Barnes, 1979; Barnes and McNaughton, 1985). There is evidence that NMDA-dependent LTP induction is normal in the CA1 region of old rats when post-synaptic cells are depolarized by intracellular injection of current (Barnes et al., 1996) or when high-frequency tetanization is used (Deupree et al.,

1991; Moore et al., 1993; Billard et al., 1997; Shankar et al., 1998). However, a decline in both the magnitude of short-term potentiation (STP) and LTP in the CA1 region are seen with increasing age with peri-threshold stimulation protocols (Deupree et al., 1991; Moore et al., 1993; Barnes et al., 1997). These results suggest that, although the aged hippocampus is still capable of producing NMDA-dependent LTP under maximal stimulation conditions, it is not as good as the young hippocampus at lower stimulation rates, which are likely to be more physiological. This could be due to changes in the NMDA receptor itself and/or due to alterations in other steps of the signaling pathways that are that are up- or down-stream from the NMDA receptors. In addition, the electrophysiological responses that are attributable to NMDA receptors in young animals can be more influenced by other signaling systems in the elderly. The maintenance of LTP in aged animals is characterized by a decrease in NMDA receptor-induced LTP and a compensatory increase in calcium channel-dependent LTP (Norris et al., 1998; Shankar et al., 1998). NMDA receptor-dependent LTD and reversal of LTP by low frequency stimulation are increased in the CA1 region of aged Fischer 344 rats (Norris et al., 1996). The interpretation of these changes in NMDA receptor functions are complicated by alterations in the regulation of calcium and metabotropic glutamate receptor activity in the same region (Norris et al., 1998; Kumar and Foster, 2004, 2007). There are, therefore, some electrophysiological differences in NMDA receptor responses that are evident between the dentate gyrus and CA1 regions of the hippocampus in aged rodents. However, other alterations in NMDA receptor function during aging may be influenced by changes in other systems.

RELATIONSHIPS OF NMDA RECEPTOR CHANGES TO COGNITIVE AGING

Aged humans show declines in performance (30–80% decreases from young performance) in problems involving spatial memory (Evans et al., 1984; Moore et al., 1984; Sharps and Gollin, 1987; Kirasic and Bernicki, 1990; Cherry and Park, 1993). The role of NMDA receptors in spatial learning and memory in the young, behaving animal has been predominantly studied by Morris and his associates with the use of the Morris water maze and a reference spatial memory task (Morris et al., 1986; Morris, 1989; Morris and Davis, 1994; Bannerman et al., 1995). This task (Gage et al., 1984; Rapp et al., 1987; Gallagher et al., 1993; Magnusson, 1997a) and others, including the Barnes circular platform maze and Olton radial arm maze (Barnes et al., 1980), also have been used extensively in aging research to demonstrate and characterize the age-related declines that occur in spatial memory performance in rodents and the relationship between changes in NMDA receptor binding during aging to memory dysfunctions. Several studies show that lower binding of agonist or antagonist to the NMDA binding site in the frontal cortex and/or hippocampus of aged animals is associated with poorer performance in spatial memory tasks utilizing the water maze (Pelleymounter et al., 1990; Davis et al., 1993; Magnusson, 1998a, 2001). There is also evidence for a role for NMDA receptors in passive avoidance retention memory (Mondadori and Weiskrantz, 1993) and there is a significant correlation between [³H]MK801 B_{max} and passive avoidance latency during aging; high binding was associated with better retention (Scheuer et al., 1995).

Changes in specific subunits of the NMDA receptor have also shown relationships with memory declines during aging. Age-related decreases in the protein expression of the GluN1 subunit within crude synaptosomes of the frontal cortex of C57BL/6 mice show a relationship to the declines in performance in a spatial reference memory task across age groups (Magnusson et al., 2007). Lower expression of the GluN1 subunit within the synaptic membrane of the hippocampus of middle-aged mice is also associated with poorer performance in the same task, though this relationship is reversed in the old mice (see "Not Your Young Adult's NMDA Receptor" below) (Zhao et al., 2009b). Lower protein expression of the GluN1 subunit was also found in the CA3 region of aged Long-Evans rats that performed the worst in a spatial reference memory task (Adams et al., 2001b). High mRNA expression of $GluN1_{0XX}$ (1-a) splice variants in the ventral orbital cortex of old C57BL/6 mice is associated with good spatial working memory performance and showed a trend for a similar relationship to spatial reference memory (Das and Magnusson, 2008). Interestingly, this splice form was negatively associated with performance in the cued association task (Das and Magnusson, 2008). The reason for this is unclear. This finding represents another example of the variability among the GluN1 splice forms, since other splice variants that were affected by aging, $GluN1_{X11}$ (1-1) and $GluN1_{X10}$ (1-3), did not show a relationship with the learning declines (Das and Magnusson, 2008).

Although there is less of an affect of aging on the GluN2A subunit than the other subunits, the expression of the GluN2A subunit in aged mice does appear to influence spatial learning. A high ratio of mRNA for GluN2A subunits to either GluN1 or GluN2B subunits in parietal cortex and/or hippocampus within aged mice is associated with good performance in a spatial reference memory task (Magnusson, 2001). This suggests that enhancing the expression of the NR2A subunit in aged individuals may be beneficial in light of the declines seen in the other subunits with age.

In aging, the decline seen in expression of the GluN2B subunit in both frontal cortex and hippocampus is significantly correlated with impaired memory function in old or middle-aged mice and rats (Clayton and Browning, 2001; Magnusson et al., 2007; Zhao et al., 2009b). A study by Clayton et al. (2002b) investigated whether or not the deficit of the GluN2B subunit seen in aged rats was sufficient to account for the spatial memory deficit that had been previously observed (Clayton and Browning, 2001). They found that GluN2B subunit antisense treatment in the hippocampus diminished NMDA-dependent LTP and spatial learning (Clayton et al., 2002b). Significant reductions in GluN2B subunit expression have also been observed in the susceptible regions of brains with AD (Sze et al., 2001; Hynd et al., 2004). These studies suggest that age-related reductions in the levels of the GluN2B subunit are likely to contribute to memory impairment in aged individuals.

The relationships observed between NMDA receptor expression and cognitive declines during aging suggest that aging interventions aimed at retaining or improving NMDA receptor function could be beneficial to relieving some age-related memory dysfunctions.

INTERVENTIONS THAT ENHANCE NMDA RECEPTOR EXPRESSION

Many different interventions have been used successfully *in vivo* to attenuate the effect of aging on the NMDA receptor complex, as shown by improvements in receptor binding and/or subunit

expression. Acetyl-L-carnitine (ALCAR), a compound that demonstrates multiple anti-aging effects in the brain when administered systemically to an aged animal (see review, Castorina et al., 1994), improves antagonist binding to the NMDA binding site, slightly in the hippocampus and frontal cortex and significantly in the striatum (Castorina et al., 1994). ALCAR also decreases the age-related difference in NMDA-displaceable [³H]glutamate binding, slightly in the hippocampus (Fiore and Rampello, 1989) and significantly in the anterior cortex (Davis et al., 1993). In the latter study, there was a slight improvement in spatial memory performance associated with the aged animals that received ALCAR treatment (Davis et al., 1993). Other cognitive enhancing and free radical scavenging drugs, such as memantine (Bresink et al., 1995), phosphatidylserine (Cohen and Muller, 1992), piracetam (Cohen and Muller, 1993), pyrintol (Hartmann et al., 1993), vitamin E (Martinez-Villayandre et al., 2006) and alpha-lipoic acid (Stoll et al., 1993), all produce significant increases in the binding of [³H]MK801 in aged rodents. Bifemalane hydrochloride, a drug used for cerebrovascular diseases, increases CPP binding in most cortical, hippocampal and thalamic regions studied in aged rats (Ogawa et al., 1992). D-cycloserine, a partial glycine agonist, improves the NMDA-stimulated norepinephrine release in older treated rats over non-treated, age-matched controls (Pittaluga et al., 1993).

Caloric restriction is an aging intervention that has been shown to improve memory performance (Witte et al., 2009), especially in spatial tasks (Algeri et al., 1991; Pitsikas and Algeri, 1992; Magnusson, 2001). Restricting the dietary intake of calories to 60% of *ad-libitum* fed animals, beginning from 3 months of age, resulted in the maintenance of slighter higher levels of [³H]glutamate binding to the NMDA site in older C57Bl/6 mice (Magnusson, 1997a, 2001) and this effect is correlated with improved spatial memory performance in the water maze (Magnusson, 1998a, 2001). Caloric restriction also results in an increase in mRNA expression of the GluN1 subunit in frontal cortical regions of middle-aged and old mice, as compared to the *ad-libitum* fed animals, (Magnusson, 2001). Fischer 344 rats also show a positive effect of caloric restriction on GluN1 subunit protein expression in the aged hippocampus (Eckles-Smith et al., 2000). The F344XBN rats exhibit a reduction in the age-related differences seen in the GluN1 and GluN2A subunits in the hippocampus, but this appears to be due to a reduction in the expression of the subunits in the calorically-restricted young rats, as compared to young rats fed *ad-libitum* (Newton et al., 2007; Shi et al., 2007). There was no reversal of the age-related declines in GluN2B subunit mRNA expression with these calorie restriction protocols (Magnusson, 2001; Newton et al., 2007). In the dentate gyrus, the aged animals on the restricted diets actually show the greatest decreases in GluN2B subunit mRNA densities (Magnusson, 2001; Newton et al., 2007). However, a recent study by Fontan-Lozano et al. (2007) employed the long-term intermittent fasting diet (L-IFD), a variant of caloric restriction. The L-IFD resulted in an increase in protein expression of the GluN2B subunit in both the hippocampus and perirhinal cortex (Fontan-Lozano et al., 2007). There was also a significant increase in GluN1 in the perirhinal cortex, but no effect on GluN2A subunit expression in any region examined (Fontan-Lozano et al., 2007). The enhanced memory in aged L-IFD mice appeared to be due to the enhanced expression of the GluN2B subunit in the hippocampus, as demonstrated by region-specific antagonism (Fontan-Lozano et al., 2007).

Other dietary interventions, such as dietary supplementation, also show promise for ameliorating the cognitive declines associated with aging. Aging is associated with oxidative stress and declines in omega-3 polyunsaturated fatty acids (*n*-3 PUFA) in the brain (Butterfield et al., 1999; Ulmann et al., 2001). This deficit in *n*-3 PUFA is associated with memory impairment (Greiner et al., 1999; Ulmann et al., 2001). A study by Calon et al. (2005) showed that in a mouse model of AD with *n*-3 PUFA deficiency, the expression levels of GluN1, GluN2A and GluN2B subunits are significantly decreased compared to non-transgenic mice. This change can be partially rescued by dietary supplementation with docosahexaenoic acid (DHA), a key component of *n*-3 PUFA (Calon et al., 2005). Likewise, in aged rats, dietary supplementation with *n*-3 PUFA reversed deficits in the expression of the GluN2B subunit (Dyall et al., 2007). Recently, Chytrova et al. (2009) showed that both the actions of exercise and dietary supplementation of DHA in young adult rats enhances spatial memory and increases expression of the GluN2B subunit.

Other dietary interventions have also shown promise. Dietary supplementation with blueberry extract in aged rats rescues NMDA receptor-dependent LTP in the hippocampus, but did not prevent aged related declines in the protein expression of the GluN1 or GluN2B subunits (Coultrap et al., 2008). However, an increase in phosphorylation of a key tyrosine residue on the GluN2B subunit in aged animals is associated with improved LTP in these animals (Coultrap et al., 2008). Dietary supplementation with ginsenoside, the effective ingredient in ginseng, improves memory in aged C57BL/6 mice and enhances the expression of phosphorylated GluN1 subunits back to the levels seen in young adults (Zhao et al., 2009a).

Drug related interventions have shown promise for reversing age-related cognitive deficits. Nicotine reversed the effects of aging on the GluN2B subunit in the hippocampus, but showed no influence on expression of the GluN2A subunit in aged Sprague-Dawley rats (Delibas et al., 2005). Likewise, sulindac, a non-sterioidal anti-inflammatory drug (NSAID), attenuated age-related deficits in memory by decreasing inflammation, while increasing expression levels of the NMDA receptor subunits, GluN1 and GluN2B (Mesches et al., 2004).

Hormone treatments have also been used successfully as aging interventions for the NMDA receptor complex. Insulin-like growth factor 1 (IGF-1) alleviates the decline in both GluN2A and GluN2B subunits seen in older F344XBN rats (Sonntag et al., 2000). Growth hormone enhances the mRNA expressions of the GluN1 and GluN2A subunits in the hippocampus (Le Greves et al., 2002). Estradiol can increase the protein expression of the GluN1 subunit, but not the mRNA, in the hippocampus of female Sprague-Dawley rats (Gazzaley et al., 1996b).

Many of these interventions have selective effects on certain subunits, which further supports the idea that there are different mechanisms of aging acting on the NMDA receptor subunits. These interventions do offer some hope for correcting the effects of aging on the NMDA receptor complex. However, a more in depth examination of how these improvements in the NMDA

receptor relate to improvements in receptor function and memory processing is needed before deciding which of these represent the optimal treatment.

"NOT YOUR YOUNG ADULT'S NMDA RECEPTOR"

Although there is evidence that declines in NMDA receptor binding densities and subunit expression are associated with the declines in memory during aging, there is also mounting evidence that the NMDA receptors that remain in aged animals are less associated with good learning and memory than the receptors of young animals. Middle-aged mice showed that good spatial memory was associated with higher expressions of the NMDA receptor subunits, GluN1 and GluN2B, in the synaptic membrane of the hippocampus (Zhao et al., 2009b). This would be expected given the importance of hippocampal NMDA receptors in acquisition of spatial memory in younger animals (Morris, 1989; Heale and Harley, 1990; Steele and Morris, 1999). In contrast, the highest levels of expression of both GluN1 and GluN2B subunits in the hippocampal synaptic membrane were found in the aged mice with the poorest spatial memory (Zhao et al., 2009b). The negative relationship with memory in the old mice was not simply due to the subunit expression falling below a certain threshold (Zhao et al., 2009b). This suggests that a change occurred in the functioning of NMDA receptors in the synaptic membrane between 11 and 26 months of age in C57BL/6 mice. This change could be due to compensatory changes, induced by the decline in NMDA receptor expression, that did not occur until later ages, and/or aging changes that affect other molecules that interact with NMDA receptors.

There is other evidence of a change in the role of NMDA receptors in aged animals. High densities of NMDA receptor binding within old rats in subregions of the hippocampus have been shown to be associated with poor long-term memory retention in the water maze (Topic et al., 2007) and a non-spatial complex maze task (Ingram et al., 1992) and in the striatum are related to poor set shifting (Nicolle and Baxter, 2003) and poor spatial learning (Nicolle et al., 1996). Aged rats that were unimpaired in a spatial memory task showed greater age-related declines in MK801 binding in the cortex and hippocampus than those that were impaired (Le Jeune et al., 1996). NMDA receptor antagonists, including mementine, a therapeutic used for AD, improve memory (Norris and Foster, 1999; Danysz and Parsons, 2003; Pieta Dias et al., 2007; Beracochea et al., 2008) and increase neurogenesis (Nacher et al., 2003) in aged individuals. Cholinesterase inhibitors used to treat AD are also believed to act by inhibiting NMDA receptors (Chen et al., 2008). Some evidence suggests that the blockade of the NMDA receptor may be protecting against excitotoxicity and oxidative damage (Pieta Dias et al., 2007). There is an increased responsiveness in aged animals to NMDA receptor-dependent low frequency stimulations leading to LTD or paired pulse stimulation that is inversely correlated with memory ability, as compared to young (Norris et al., 1996; Huang and Kandel, 2006). Non-NMDA receptor-dependent LTP and LTD are more associated with good memory in aged rats than NMDA receptor-dependent (Lee et al., 2005; Boric et al., 2008). It has been suggested that the decline in NMDA receptor contribution to plasticity in aged animals may be beneficial by reducing the overwriting of old information (Yang et al., 2008). All of these studies suggest that the NMDA receptors present in aged animals

function differently from those in young. Potentially this could be due to changes in subunit composition or the environment in which the receptor functions. It remains to be seen whether this could or should be reversed or prevented.

PERSPECTIVE

Evidence from multiple studies show that the NMDA receptor complex is vulnerable to the negative effects of the aging process. Aging seems to affect everything from the number and functionality of NMDA receptors to the expression of its subunits and their composition within various brain regions. Future studies should focus on determining the underlying cause of the alterations. It seems likely that the heterogeneous changes in the subunits play a role in the differential effect of aging on the different binding sites, but why are certain subunits more affected by aging than others? What causes certain splice variants of the GluN1 subunit to be altered by aging and behavioral experience, while others show no influence of the aging process? Why is the GluN2A subunit relatively spared by aging in most strains of rodents? Is there an alteration in trafficking of the GluN2B subunit to the synaptic membrane? What role does environment and experience (environmental enrichment, stress and social interaction) during aging have on receptor expression?

Is it the composition of the NMDA receptor in old animals or the aged brain environment that determines how the receptor will function? If different populations of receptors, based on subunit composition, are involved, it behooves us to focus more on subdivisions of the cerebral cortex and hippocampus in order to better understand the regional and cellular changes that are occurring. A comparison of electrophysiological responses *in vitro*, obtained by artificially altering subunit ratios to mimic aging changes, with responses *in situ* from different brain regions in different ages of animals could be informative about the functional consequences of subunit changes versus changes in the environment of the aged brain.

Changes in the NMDA receptor complex are associated with age-related cognitive decline, especially in terms of learning and memory. This loss of cognitive function appears to be partially reversible through dietary and drug interventions. Yet, none of these interventions seem to be capable of completely reversing the effects of aging by restoring individuals to a young profile. The differences within and between species suggest that the aging process is not an exact or programmed deterioration and further investigation into these discrepancies may shed light on causes and interventions that could be applicable across species. Information for designing optimal interventions might also be gained by a greater focus on the similarities and differences between the sexes. Evidence suggests that interventions that enhance the expression of the NMDA receptor should be beneficial for preventing or reversing the memory declines that occur as we grow older. However, the negative associations between NMDA receptors and cognitive ability that have been seen among old individuals suggest that we also need to assess whether enhancement of NMDA receptors may make the elderly more susceptible to neurotoxicity or other negative side effects.

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