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Young and older good learners have higher levels of brain nicotinic receptor binding

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Abstract

Neuronal αβ heteromeric and α7 homomeric nicotinic acetylcholine receptors (nAChRs) were compared in 4- and 27-month rabbits selected for learning proficiency. Sixty 4- and 60 27-month rabbits received the α7 nAChR agonist (MEM-3389), galantamine, or vehicle during training in trace eyeblink classical conditioning. Brain tissue from the best and worst young and older learners was analyzed with radioligand binding. Vehicle-treated 4- and 27-month good learners had higher αβ heteromeric nAChR binding in hippocampus and temporal–parietal cortex than poor learners, and this result was replicated in both age groups of rabbits treated with galantamine. Results indicate that anatomically more numerous nAChRs or functional activation of a greater number of nAChRs may characterize animals demonstrating optimal learning. During normal aging the expression of high-affinity binding sites declines. Age-related changes in the expression of hippocampal αβ heteromeric nAChRs may account for some of the documented age-related impairment in learning. However, individual differences in αβ heteromeric nAChRs also exist early in life, as better learning in 4-month rabbits was associated with significantly higher binding.

Keywords: Radioligand binding; Hippocampus; Temporal–parietal cortex; Trace eyeblink classical conditioning; Age differences

1. Introduction

Individual differences in learning and memory have been reported for several mammalian species and a variety of behavioral paradigms (e.g., Matzel and Gandhi, 2000; Wehner et al., 2001). During normal aging this individual variability increases, with some older organisms showing preserved learning and memory and others showing impairment (Kempermann and Gage, 2002; Lee et al., 2005; Olton et al., 1991; Van der Zee et al., 1997). Associated with preserved learning and memory in normal older organisms has been electrophysiological functioning comparable to young adult levels (McEchron et al., 2001; Schoenbaum et al., 2006). For example, excitability of CA1 neurons studied 24 h after training in hippocampus-dependent trace eyeblink conditioning differentiated good and poor learning in aged rabbits. Comparisons were made between young rabbits and aged rabbits that reached a behavioral criterion of 60% conditioned responses (CRs) called "learning-intact," rabbits trained for 30 days that never demonstrated more than 30% CRs per session, called "failed to learn," and naïve aging rabbits (McEchron et al., 2001). In general, aged rabbits required significantly more training trials to reach learning criterion than did young rabbits. However, hippocampal CA1 neurons from aged learning intact animals had significantly reduced post-burst afterhyperpolarizations and reduced spike frequency adaptation comparable to young rabbits. Hippocampal CA1 neurons from control groups of naïve and aging rabbits that failed to learn had significantly elevated post-burst afterhyperpolarizations and increased spike frequency adaptation. The data suggest that postsynaptic excitability of CA1 neurons is correlated with learning the hippocampus-dependent trace eyeblink conditioning task in both young and aged rabbits.

Among the mechanisms that support hippocampal electrophysiological function in adult organisms are neurotransmitter and receptor systems (Zhang et al., 2007).

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Extensive evidence indicates that nicotinic acetylcholine receptors (nAChRs) act as neuromodulators in communicative processes in the brain (Lindstrom, 1996) and that nAChRs are involved in cognitive and memory functions (Changeux et al., 1998; Dani and Bertrand, 2007). nAChRs in the central nervous system are composed of five subunits arranged around a ligand-gated excitatory ion channel. The most abundant nAChR subtypes are those that participate in high-affinity agonist binding associated with α4 and β2 subunits, and those sensitive to blockade by α-bungarotoxin and containing α7 subunits. In normal aging, it is the high-affinity agonist binding nAChR subunits that show the greatest deficits in human (e.g., Giacobini, 1992) and rodent brain (Araujo et al., 1990; Zhang et al., 1990). The loss in these sites is coincident with changes of expression of subunit proteins. Data in humans, rabbits, and rodents show consistency in the age-related loss of expression of αβ heteromeric nAChRs (Birtsch et al., 1997; Gahring et al., 2005; Li et al., in press). The pattern of age-related changes in neurons containing α7 homomeric nAChRs demonstrates age-related loss in a few brain sites but stability in most regions (Court et al., 1997; Falk et al., 2003; Gahring et al., 2005; Nordberg and Winblad, 1986).

We focused on the most abundant nAChR subtypes, those that participate in high-affinity agonist binding associated with α4 and β2 subunits and those containing α7 subunits. We used radioligand binding of tissue from hippocampus and temporal–parietal cortex to determine whether higher binding levels accompanied superior learning. Trace eyeblink classical conditioning was used to assess learning. This is a relatively difficult paradigm for rabbits in which a conditioned stimulus (CS) such as a tone is presented and then turned off before the unconditioned stimulus (US; corneal airpuff) is turned on. A learned or CR occurs when the rabbit produces an eyeblink before the onset of the US. We used trace procedures with sufficiently long trace intervals (400–500 ms) to insure that the task would be hippocampus-dependent for rabbits (Moyer et al., 1990; Rose et al., 2007).

One question of interest was whether cognition-enhancing drugs would improve learning in the difficult trace procedure in young and older rabbits. We used two drugs with different mechanisms of action as cognition enhancers. Galantamine is among the most effective cognition enhancers tested in the eyeblink classical conditioning model. A dose of 3.0 mg/kg galantamine ameliorates learning impairment in older rabbits in both the trace (Simon et al., 2004; Weible et al., 2004) and delay eyeblink classical conditioning paradigms (Woodruff-Pak et al., 2001, 2003). Galantamine has mechanisms of action that include both mild acetylcholinesterase inhibition and allosteric potentiating effects at nAChRs (Popa et al., 2006). AR-R-17779 (now called MEM-3389) is an agonist shown to be selective to α7 homomeric nAChRs in frog oocytes (Papke et al., 2004). We anticipated that MEM-3389 would have efficacy in this model as a partial α7 agonist did improve eyeblink conditioning in 25-month rabbits (Woodruff-Pak et al., 1994). A dose–response study using the 750 ms delay eyeblink classical conditioning paradigm demonstrated that 1.0 mg/kg MEM-3389 significantly improved learning over vehicle (Li et al., in press).

A second question of interest was whether individual differences in nAChR binding levels would be associated with variation in learning rate in young and older organisms. Hippocampal and temporal–parietal cortical tissue from the best ("good learners;" $n = 36$) and worst ("poor learners;" $n = 36$) vehicle- and drug-treated performers was analyzed with radioligand binding ([^{3}H]Epibatidine, [^{3}H]Methyllycaconitine), and results from good and poor learners were compared.

A third question was whether cognition-enhancing drugs would increase nAChR binding levels in good learners above the levels in vehicle-treated good learners. A related issue was whether there would be differences in nAChR binding levels between drug-treated poor learners and vehicle-treated poor learners. We addressed these questions by comparing receptor binding in hippocampus and temporal–parietal cortex of young and older vehicle-treated good and poor learning rabbits to binding at these sites in young and older good and poor learning rabbits treated with MEM-3389 or galantamine.

2. Methods

2.1. Study population

A total of 120 female New Zealand white specific pathogen free (SPF) rabbits were tested. Sixty rabbits were retired breeders of a mean age of 27.4 months (S.D. = 2.5) and a mean weight of 4.1 kg (S.D. = 0.4) and 60 rabbits were young adults of a mean age of 4.0 months (S.D. = 0.0) and a mean weight of 2.8 kg (S.D. = 0.3). All rabbits were purchased from Covance (Denver, PA). They were individually housed in stainless steel cages in temperature and humidity-controlled rooms in an Association for Assessment and Accreditation of Laboratory Animal Care International-(AAALAC-) approved animal facility. They had ad lib access to food and water. The light/dark cycle was 12/12-h. The Institutional Animal Care and Use Committee (IACUC) at Temple University approved research procedures used in this study. This research was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

2.2. Behavioral testing

Over the course of 7 days prior to training, the rabbits were gradually familiarized and adapted to Plexiglas restrainers for 30 min per day. Familiarization training took place in rabbits' individual cages during the first 5 days. At the end of each familiarization session rabbits were rewarded with a treat formulated for rabbits (Kaytee Yogurt Dips). The last 2 days of familiarization took place outside the individual cages, and rabbits were fully restrained. On the seventh day, a local oph-