Published in final edited form as:

Behav Brain Res. 2012 June 15; 232(1): 1–6. doi:10.1016/j.bbr.2012.03.032.

Association of microtubule associated protein-2, synaptophysin, and apolipoprotein E mRNA and protein levels with cognition and anxiety levels in aged female Rhesus macaques

Gwendolen E. Haley^{1,2}, Dominique H. Eghlidi^{1,2}, Steven G. Kohama², Henryk F. Urbanski^{1,2,3}, and Jacob Raber^{1,2,4,*}

¹Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR 97239

²Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR 97006

³Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR 97239

⁴Department of Neurology, Oregon Health and Science University, Portland, OR 97239

Abstract

The dendritic protein microtubule associated protein 2 (MAP-2), the presynaptic marker synaptophysin (SYN), and apolipoprotein E (apoE), a protein which plays a role in lipid transport and metabolism and affects synaptic activity show changes with age. We analyzed post-mortem tissue from aged female Rhesus macaques cognitively tested in a spatial maze and classified as good spatial performers (GSP) or poor spatial performers (PSP) and behaviorally tested in a playroom and classified as bold or reserved animals. *MAP2*, *SYN*, and *APOE* mRNA and protein levels in the prefrontal cortex (PFC), hippocampus, and amygdala, were assessed using qRT-PCR and western blot. In the amygdala, bold monkeys had higher levels of *MAP2* and *SYN* mRNA than reserved monkeys. *MAP2* mRNA correlated positively with amygdala size on the right, left, and combined left and right sides, while *SYN* mRNA levels correlated positively with the size of the right amygdala. In the hippocampus, SYN and apoE protein levels were higher in GSP than PSP animals. Thus, in aged nonhuman primates, classification of measures of anxiety is associated with differences in selected mRNA, but not protein, levels. In contrast, classification of cognitive performance is associated with differences in selected protein, but not mRNA, levels.

Keywords

MAP-2; SYN; APOE; cognitive function

© 2012 Elsevier B.V. All rights reserved.

*Corresponding Author, Jacob Raber, Ph.D., Department of Behavioral Neuroscience, Oregon Health and Science University, 3181 SW Sam Jackson Pkwy L470, Portland, OR 97239; raberj@ohsu.edu, Ph: (503) 494-1524; Fax: (503) 494-6877.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no conflict of interest to declare.

1. Introduction

Age-related decline in cognitive function is observed in humans, nonhuman primates, and rodents [1–3]. Neuron numbers is preserved in the hippocampus [4] and parahippocampal region [5] of aged rats with impairments in spatial learning and the dentate gyrus might be most vulnerable to aging [6]. However, neurobiological changes in the prefrontal cortex (PFC) and hippocampus, brain regions necessary for cognitive function, and the amygdala, which plays a role in the regulation of anxiety [7–9] and can affect hippocampus-dependent cognitive performance [10–13], might play a role in this loss of function [11]. The dendritic protein microtubule associated protein 2 (MAP-2), the presynaptic marker synaptophysin (SYN), and apolipoprotein E (APOE), a protein which plays role in lipid transport and metabolism and also affects synaptic activity, have integral roles in cognition [14–18]. MAP-2 is a dendritic protein involved in the assembly of the microtubules in the dendrites and in dendritic plasticity [19]. SYN is a presynaptic vesicle protein located in axons and responsible for formation of synapses [20]. SYN levels positively correlate with cognitive function in rodents [17, 21]. However, in the hippocampus of aged memory-deficient rats SYN and the other synaptic proteins synaptotagmin and synaptosomal associated protein-25 were preserved [22, 23] while metabotropic glutamate receptor-mediated phosphoinositide turnover was blunted [24]. In humans, SYN levels in the hippocampus do not show age-related changes but SYN mRNA levels in the parahippocampal gyrus did show age-related decline [25]. In the nonhuman primate, MAP-2 and apoE protein levels increase with age, while SYN decreases with age [26]. Although these proteins show age-related changes and SYN correlates with cognitive performance in rodents [20, 21], it is less clear if these changes are associated with cognitive ability in aged nonhuman primates. As these three proteins show pronounced age-related changes, we focused on them in the current study.

Recently, we classified cognitive performance in nonhuman primates in a spatial maze as good spatial performers (GSP) or poor spatial performers (PSP) and behavioral performance of these animals in the same maze as bold or reserved [10, 11]. In this study, we performed quantitative real-time PCR (qRT-PCR) and western blot analyses using post-mortem tissue from these animals to determine whether *MAP2*, *SYN*, and *APOE* mRNA and protein levels in the PFC, hippocampus, and amygdala are associated with the cognitive or anxiety classification.

2. Materials and Methods

2.1. Spatial maze cognitive classification

The Spatial Food Port Maze consisted of a bank of 10 ports in a testing room, each port containing a one way, opaque swinging door. Freely moving old female rhesus macaques (*Macaca mulatta*; $n = 16$; age range 21–27 years; reproductively inactive) were placed in the room and were allowed to search the ports. The animals were acclimated to daily removal from their home cage, transport to the testing room in transfer boxes, and human handlers. The animals were not food restricted and received preferred candy or nuts as positive reinforcement in all phases of testing. There were four phases of testing: acclimation, training, initial, and shift phase. Animals were tested five days per week, with at least four trials per day, and each trial was 2 min long. Each phase of testing was completed once criteria were achieved and the monkey was immediately moved to the next phase of testing until four trials per day were completed. In the acclimation phase of testing, monkeys were placed in the room with all ten ports baited, with the port doors propped open so that the food rewards were prominently visible. The criterion for the acclimation phase was retrieval of the treats from more than eight ports per trial in two consecutive trials. During the training phase, all ten ports were baited but with the rewards hidden behind opaque doors. The criterion for the training phase was retrieval of rewards from at least eight ports in two

consecutive trials. For the initial phase of testing, only one designated port was baited. Monkeys were pre-assigned randomly to having either the third or seventh port baited. The criterion for the initial phase was retrieving the reward from the baited port within the first two ports searched for three consecutive trials. Once the initial phase criterion was reached, a different pre-assigned port was baited for the shift phase. Monkeys who were initially trained to retrieve the reward from port three were then trained to retrieve the reward from port six, while monkeys initially trained to retrieve the reward from port seven were trained to retrieve the reward from port four. As before, the criterion for the shift phase was to locate the reward within the first two searches in three consecutive trials. The sequence of ports searched on each trial was recorded as well as the number of trials to reach each criterion. The cohort of cognitively characterized animals was separated into two groups, GSP and PSP [19], based on performance in this maze [27]. GSP was defined as animals that completed the task in less than 30 trials in either the first or second position, while PSP was defined as animals that completed the task in more than 30 trials.

2.2. Playroom behavioral classification

Monkeys were introduced to a playroom (2.44×3.45 m) one at a time for a total of 2 minutes per trial. There were 6 blue food boxes equally spaced around the room and baited with preferred food rewards. Each monkey was repeatedly placed in the room until they were trained to a criterion level. In order to start the cognitive task, each monkey had to enter and exit the room and reliably search all the boxes in the room. The average number of trials for all monkeys was 67 ± 8.0 . The average number of trials to criterion for the high anxiety monkeys was 79.5 ± 8.4 trials, whereas the average number of trials to criterion for the low anxiety was 50 ± 7.6 trials. The room consisted of a human entry door, one-way observation window, monkey entry door, and a series of 10 ports [27]. Three of the 10 ports were baited with preferred rewards. Movement and location of the animal within the room were recorded with Ethovision video tracking (Noldus Information Technologies, Leesburg, VA).

For data analysis, the room was divided into 5 areas (Figure 1), the animal door (A), the ports (B), the middle of the room (C), corners of the room (D), and the perimeter of the room (E). Videos were analyzed using Button Box (Behavior Research Solutions LLC, Hudson, WI) to measure the duration of time spent in each area. The time spent in the corner and perimeter was collapsed (protected areas) as were the time spent in the middle of the room, the jump door, and the ports (unprotected areas). In the corner of the room, the animals are protected by 2 walls and have their back to the walls, while examining the rest of the room (Figure 1, areas D and E). In the unprotected areas of the room, the monkeys are more exposed to the rest of the room (Figure 1, areas A, B, and C). Specifically at the jump door, the monkeys have their back to the rest of the room, a posture that would not or hardly be present in the high anxiety monkeys. If a monkey did not show preference for one area of the room, the monkey was considered low anxiety. Based on their exploratory behavior in the playroom, 7 out of 16 monkeys were classified as low anxiety monkeys and 9 out of 16 monkeys were classified as high anxiety monkeys [11].

2.3. Voxel Based Morphology (VBM)

Within 2 weeks of completing the testing, an MRI was performed on each animal. Using a voxel based morphology (VBM) method, the size of the amygdala and hippocampus was assessed for each monkey, as described [11]. Monkeys were fasted the night before and the morning of the MRI experiments for the anesthesia. They were sedated with ketamine (1 mg/kg body weight) and transported from their home cages to a Siemens whole-body 3-T trio MRI system (Erlanger, Germany) located on the ONPRC campus. Animals were immediately intubated and respiration, free-breathing, with 0.9–1.1% isoflurane.

Physiological parameters, including blood oxygen levels, carbon dioxide, blood pressure, heart rate, temperature, and respiration rate were monitored continuously throughout the experiment using an MRI-compatible Precess monitor (Invivo, Orlando, FL). The animal's head was placed inside a quadrature transmit/8-channel receive human extremity RF coil (Invivo) and fitted with pads to eliminate head movement. After acquisition of a series of "localizer images", 20 contiguous T₂*-weighted multi-shot echo planar images were obtained over the course of 7 minutes. (TR = 1800 ms, TE = 30 ms, 4 segments, 2 mm slices, flip angle 90°, 24 slices, 20 volumes). Voxel based morphology (VBM) was assessed for each amygdala, right and left side, and hippocampus, right and left side, and PFC. MRI analyses revealed that reserved monkeys had smaller amygdala volumes than bold monkeys and the total time spent in safer corners was negatively correlated with the right amygdala and total amygdala size [11]. In contrast to the amygdala, the volumes of the right or left hippocampus and right or left PFC were not different in reserved and bold monkeys.

2.4. qRT-PCR

Post-mortem tissues from the PFC, hippocampus, and amygdala from the right hemisphere of the animals previously cognitively and behaviorally tested [7, 8, 18] were used for the mRNA and protein studies. There were 16 monkeys able to complete anxiety testing but only 12 monkeys able to complete the spatial maze. Therefore the *n* for the neurochemical data is 16 for the amygdala and 12 for the PFC and hippocampus. The animals were euthanized and brains were flushed with 1 liter saline at room temperature and removed. The perfusion rate was 500 ml/minute. Subsequently, coronal slabs were made through the prefrontal cortex, and the dorsal and ventral banks adjacent to the principal sulcus of cortical area 46 were collected for the analysis. The hippocampus was dissected free of the surrounding temporal cortex and a coronal piece was removed through the main body of the hippocampus. The main body of the hippocampus, which spans approximately the middle-third of the structure was used in this analysis.

A coronal cut just rostral to the hippocampus was made and the amygdala was dissected free from the surrounding cortex and frozen. The entire amygdala was collected and used in this analysis. All tissues were immediately frozen in liquid nitrogen and subsequently stored at -80°C until use. qRT-PCR and western blot analyses were performed for MAP-2, SYN, and apoE. Quantitative real-time PCR data were obtained using a 7900HT Fast Real-Time PCR thermal cycler and sequence detection system software (version 2.2.4; Applied Biosystems (AB), Foster City, CA). Complementary DNA (cDNA) synthesis using random hexamers was followed by reverse transcription using Superscript III reverse transcriptase (1 U/μg of RNA (Invitrogen Life Technologies, Carlsbad, CA USA). Table 1 shows the custom TaqMan qPCR assays (AB) used for quantification of mRNA levels were based on NCBI rhesus macaque reference sequences. In addition to *APOE*, *MAP2*, *SYN*, levels of three housekeeping genes (*ALG9*, *GAPDH*, *RPL13A*) that are expressed in the rhesus hippocampus and amygdala [28], were also analyzed. As reported in our earlier studies, these three genes are good candidates for reference genes as they showed high expression stability in the rhesus macaque brain [29, 30]. Consistent with these data, the levels of the combination of these three genes did not differ between the groups analyzed by cognitive performance ($p = 0.5208$) or anxiety level ($p = 0.5433$).

For each mRNA, pooled cDNA was used to create standard curves (1, 0.25, 0.0625, 0.015625 ng), and the experimental samples were subsequently diluted to fall within the linear part of the curve. The PCR mixtures contained 5 μl of TaqMan Universal PCR Master Mix, 0.3 μl of each specific forward and reverse primer (300 nM final concentration), 0.25 μl of a specific probe (250 nM final concentration), and 2 μl of cDNA. The reaction sequence included 2 min at 50°C, 10 min at 95°C, and 50 cycles of 15 sec at 95°C and 1 min at 60°C. Automatic baseline and threshold levels were determined using AB sequence

detection system software. The final expression values were calculated as ratios relative to the average expression of the three reference genes, *GAPDH*, *ALG9*, and *RPL13A*. Individual cDNAs were examined together in the same 384-well optical plate. In the negative controls, cDNA templates were omitted from the reaction mixture. Initially, whole brain cDNA samples were used as positive controls and amplified during 40 cycles to produce amplicons of a predicted size, using the ABI 7900. The genes for the probes are shown in Table 1.

2.5. Western blots

Western blot analyses were performed as previously described [26]. Briefly, primary antibodies against MAP-2 (1:1000, Cell Signaling, raised in rabbit), SYN (1 ug/ml, Sigma, raised in mouse), and APOE (1:4000, Calbiochem, raised in goat) were used in 3% bovine serum albumin (BSA) and incubated overnight at 4°C. Secondary antibodies (1:1000 anti-rabbit, anti-mouse, or anti-goat, respectively, (Santa Cruz (Santa Cruz, CA))) were incubated for 2 hours at room temperature. Bands were visualized with the SuperSignal Pico detection system (Pierce Technologies) and analyzed using Flurochem Q software. All membranes were also hybridized with antibodies against β -actin (1:2000, raised in mouse, Santa Cruz) as a loading control. Data are presented as the relative protein levels compared to that of β -actin. The levels of β -actin did not differ between the groups analyzed by cognitive performance ($p = 0.7686$) or anxiety level ($p = 0.7166$).

2.6. Statistical analysis

All data analyses were performed using GraphPad Prism (San Diego, CA) software. Based on cognitive performance and anxiety level, group differences were assessed using Student's t-tests. Pearson correlations were used to assess potential correlations. Significance was considered at $P < 0.05$.

3. Results

3.1. qRT-PCR

In the amygdala, bold monkeys had higher levels of *MAP2* ($t = 2.2$; $P < 0.05$) and *SYN* ($t = 2.5$; $P < 0.05$) mRNA than reserved monkeys (Figure 2A). In contrast to *MAP2* and *SYN*, bold and reserved monkeys had similar *APOE* mRNA levels in the amygdala (Figure 2A). *MAP2* mRNA was positively correlated with MAP2 protein ($r = 0.5829$, $p = 0.0298$, 2-tailed). In nonhuman primate brain, MAP2 immunoreactivity has been successfully used to determine infarct volumes at early time points in an ischemic stroke model [31]. Therefore, we explored whether MAP2 was correlated with amygdala volumes in this study. Significant positive correlations were observed between *MAP2* mRNA levels and amygdala size. *MAP2* mRNA correlated with the size of the amygdala on the right side ($r = 0.54$; $P = 0.04$, Figure 2B), left side ($r = 0.55$; $P = 0.04$, Figure 2C), and combined left and right sides ($r = 0.56$; $P = 0.03$, Figure 2D). *SYN* mRNA levels correlated positively with the size of the right amygdala ($r = 0.55$; $P = 0.04$, Figure 2E). *MAP2*, *SYN*, or *APOE* mRNA levels did not correlate with the size of the PFC or hippocampus. In contrast to *MAP2* mRNA, *SYN* mRNA was not correlated with SYN protein ($p = 0.8940$). There was a trend towards a positive correlation between *APOE* mRNA with apoE protein level ($r = 0.1195$, $p = 0.0684$, 2-tailed). When classified by cognitive performance or anxiety level, there was no group difference in *MAP2*, *SYN*, or *APOE* mRNA levels in the PFC or hippocampus (Table 2).

3.2. Western blots

In the hippocampus, SYN ($t = 3.1$; $P < 0.01$) and apoE ($t = 2.4$; $P < 0.05$) levels were higher in GSP than PSP animals (Figure 2F). In contrast, there was no difference in hippocampal

MAP-2 protein levels in the GSP and PSP groups. In contrast, in the amygdala or PFC, there was no difference in MAP-2, SYN, or apoE protein levels between the GSP and the PSP groups (Table 3).

There was a trend towards higher SYN levels in the amygdala of reserved than bold monkeys ($t = 1.8$; $P = 0.08$). No differences in MAP-2 or apoE protein levels were observed between the bold and reserved monkeys in the amygdala (Table 2). In the PFC and hippocampus, there were no correlations between MAP-2, SYN, or apoE protein levels and size of the brain region. In the PFC and the hippocampus, no group differences were observed in MAP-2, SYN, or apoE levels between the bold and reserved monkeys (Table 2). Finally, there were no correlations between protein expression levels and amygdala size, cognitive performance or measures of anxiety.

4. Discussion

In aged animals, classification of cognitive performance is associated with differences in selected protein, but not mRNA, levels, while classification of measures of anxiety is associated with differences in selected mRNA, but not protein, levels. In the amygdala, bold monkeys had higher levels of *MAP2* and *SYN* mRNA than reserved monkeys. *MAP2* mRNA correlated positively with the size of the amygdala on the right side, left side, and combined left and right sides, while *SYN* mRNA levels correlated positively with the size of the right amygdala. There was anatomical specificity to these effects, as *MAP2*, *SYN*, or *APOE* mRNA levels did not correlate with the size of the PFC or hippocampus. In the hippocampus, SYN and APOE protein levels were higher in GSP than PSP animals. The distinct group differences in mRNA versus protein levels are remarkable, as *MAP2* mRNA was positively correlated with MAP2 protein and there was a trend towards a positive correlation between *APOE* mRNA and apoE protein level.

We recognize that the animals were not behaviorally naïve. Therefore, we cannot exclude that prior behavioral and cognitive testing might have contributed to the brain region-dependent differences in mRNA and protein levels. For instance, the animals were tested for spatial learning and memory in a spatial maze [27] and spatial learning and memory is heavily reliant on the hippocampus [32–35] and less so on the amygdala and the PFC. Thus, it is possible that there might be differences in protein expression in the hippocampus of animals classified by spatial learning and memory. Likewise, the animals were tested for anxiety levels in the play room [11] and the amygdala plays an important role in the regulation of anxiety [36–38].

SYN levels show age-dependent changes in the hippocampus and cortex of nonhuman primates [26] and mice, and in mice these changes are associated with cognitive performance [2, 17, 21, 30]. Here, we demonstrate that SYN protein levels are higher in the hippocampus, but not the PFC, of GSP than PSP animals. The higher SYN levels could be due to higher levels of SYN per presynaptic terminal or a higher number of synaptic connections in the cognitively enhanced GSP animals. Either way, this could contribute to the better cognitive performance of GSP than PSP animals.

In the amygdala, *SYN* mRNA levels were higher in the bold monkeys, but SYN protein levels were slightly, although not significantly, lower in the bold monkeys compared to the reserved monkeys. Rodent studies have demonstrated changes in levels of selected protein levels due to chronic stress [40, 41]. It is conceivable that repeated exposure to the playroom might have differential effects on synaptic plasticity depending on the temperament of the animal.

ApoE levels increase with age and are also associated with cognitive performance [19, 27]. Increased apoE levels might be the result of a challenge or injury or reflect enhanced neuronal function, recovery or repair. ApoE colocalizes with β -amyloid plaques in the nonhuman primate [26] and might also play an important role in the development and clearance of β -amyloid plaques [42–46]. We observed higher levels of apoE in the hippocampus of GSP than PSP animals, supporting the latter two possibilities. In the amygdala, there was not a significant difference in *APOE* mRNA or protein levels between the bold and reserved monkeys. As apoE has been shown to play a role in the regulation of anxiety in mice and probable AD patients [47, 48], in the aged nonhuman primate apoE might be more important in the regulation of cognition than anxiety.

In conclusion, we describe here that selected mRNA and protein levels are associated with measures of anxiety and cognitive performance. The mRNA and protein levels are not following the same patterns in the different areas of the brain. Increased efforts are warranted to investigate the reasons underlying these different patterns as they could reveal therapeutic targets for age-related cognitive decline.

Highlights

1. Bold monkeys have higher amygdala levels of *MAP2* mRNA than reserved monkeys
2. Bold monkeys have higher amygdala levels of *SYN* mRNA than reserved monkeys
3. *MAP2* mRNA correlates positively with amygdala size.
4. *SYN* mRNA levels correlates positively with right amygdala size.
5. Hippocampal *SYN* and apoE levels higher in good than poor performers

Acknowledgments

This work was supported by NIH Grants AG-029612, AG-023477 (Dr. Henryk Urbanski) and RR-000163 (Dr. Joseph Robertson), the Medical Research Foundation of Oregon (Dr. Gwendolen Haley), and an OHSU Development Account (Dr. Jacob Raber).

References

1. Albert, MS.; Moss, MB. Cognitive profiles of normal human aging. In: Peters, A.; Morrison, JH., editors. *Cerebral cortex*. New York: Kluwer/Plenum; 1999. p. 1-20.
2. Benice TS, Rizk A, Kohama S, Pfankuch T, Raber J. Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity. *Neuroscience*. 2006; 137:413–423. [PubMed: 16330151]
3. Moss, MB.; Killiany, RJ.; Herndon, JG. Age-related cognitive decline in the rhesus monkey. In: Peters, A.; Morrison, JH., editors. *Cerebral cortex*. New York: Kluwer/Plenum; 1999. p. 21-48.
4. Rapp PR, Gallagher M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci*. 1996; 93:9926–9930. [PubMed: 8790433]
5. Rapp PR, Deroche PS, Mao Y, Burwell RD. Neuron number in the parahippocampal region is preserved in aged rats with spatial learning deficits. *Cereb Cortex*. 2002; 12:1171–1179. [PubMed: 12379605]
6. Small SA, Chawla MK, Buonocore M, Rapp PR, Barnes CA. Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc Natl Acad Sci USA*. 2004; 101:7181–7186. [PubMed: 15118105]

7. Canteras NS, Resstel LB, Bertoglio LJ, Carobrez Ade P, Guimaraes FS. Neuroanatomy of anxiety. *Curr Top Behav Neurosci*. 2010; 2:77–96. [PubMed: 21309107]
8. Kalin NH, Shelton SE, Davidson RJ. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. *J Neurosci*. 2004; 24:5506–5515. [PubMed: 15201323]
9. Vinkers CH, Bijlsma EY, Houtepen LC, Westphal KG, Veening JG, Groenink L, Olivier B. Medial amygdala lesions differentially influence stress responsivity and sensorimotor gating in rats. *Physiol Behav*. 2010; 99:395–401. [PubMed: 20006965]
10. Haley GE, Kroenke C, Schwarz D, Kohama S, Urbanski HF, Raber J. Hippocampal M1 Receptor Function Associated with Spatial Learning and Memory in Aged Female Rhesus Macaques. *AGE*. 2010; 33:309–320. [PubMed: 20890730]
11. Haley GE, McGuire A, Berteau-Pavy D, Weiss A, Patel R, Messaoudi I, Urbanski HF, Raber J. Measures of anxiety, amygdala volumes, and hippocampal scopolamine phMRI response in elderly female rhesus macaques. *Neuropharmacology*. 2012; 62:385–390. [PubMed: 21867720]
14. Shackman AJ, Sarinopoulos I, Maxwell JS, Pizzagalli DA, Lavric A, Davidson RJ. Anxiety selectively disrupts visuospatial working memory. *Emotion*. 2006; 6:40–61. [PubMed: 16637749]
15. Siegel JA, Haley GE, Raber J. Apolipoprotein E isoform-dependent effects on anxiety and cognition in female TR mice. *Neurobiol Aging*. 2012; 33:345–358. [PubMed: 20400205]
16. Levy R, Goldman-Rakic P. Association of storage and processing functions in the dorsolateral prefrontal cortex of the non-human primate. *J Neurosci*. 1999; 19:5149–5158. [PubMed: 10366648]
17. Dawson GR, Seabrook GR, Zheng H, Smith DW, Graham S, O'Dowd G, Bowery BJ, Boyce S, Trumbauer ME, Chen HY, Van der Ploeg LH, Sirinathsinghi DJ. Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. *Neuroscience*. 1999; 90:1–13. [PubMed: 10188929]
18. Harada A, Teng J, Takei Y, Oguchi K, Hirokawa N. MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. *J Cell Biol*. 2002; 158:541–549. [PubMed: 12163474]
19. Kadish I, Thibault O, Blalock EM, Chen KC, Gant JC, Porter NM, Landfield PW. Hippocampal and cognitive aging across the lifespan: a bioenergetic shift precedes and increased cholesterol trafficking parallels memory impairment. *J Neurosci*. 2009; 29:1805–1816. [PubMed: 19211887]
20. Tarsa L, Goda Y. Synaptophysin regulates activity-dependent synapse formation in cultured hippocampal neurons. *Proc Natl Acad Sci U S A*. 2002; 99:1012–1016. [PubMed: 11792847]
21. Mulder M, Jansen PJ, Janssen BJ, van de Berg WD, van der Boom H, Havekes LM, de Kloet RE, Ramaekers FC, Blokland A. Low-density lipoprotein receptor-knockout mice display impaired spatial memory associated with a decreased synaptic density in the hippocampus. *Neurobiol Dis*. 2004; 16:212–219. [PubMed: 15207278]
22. Nicolle M, Gallagher M, McKinney M. No loss of synaptic proteins in the hippocampus of aged, behaviorally impaired rat. *Neurobiol Aging*. 1999; 20:343–348. [PubMed: 10588583]
23. Barnes CA. Do synaptic markers provide a window on synaptic effectiveness in the aged hippocampus? *Neurobiol Aging*. 1999; 20:349–351. [PubMed: 10588584]
24. Nicolle MM, Colombo P, Gallagher M, McKinney M. Metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in spatial learning-impaired aged rats. *J Neurosci*. 1999; 19:9604–9610. [PubMed: 10531462]
25. Eastwood SL, Weickert CS, Webster MJ, Herman MM, Kleinman JE, Harrison PJ. Synaptophysin protein and mRNA expression in the human hippocampal formation from birth to old age. *Hippocampus*. 2006; 16:645–654. [PubMed: 16807900]
26. Haley GE, Kohama SG, Urbanski HF, Raber J. Age-related decreases in SYN levels associated with increases in MAP-2, apoE, and GFAP levels in the rhesus macaque prefrontal cortex and hippocampus. *AGE*. 2010; 32:283–296. [PubMed: 20640549]
27. Haley GE, Landauer N, Renner L, Weiss A, Hooper K, Urbanski HF, Kohama SG, Neuringer M, Raber J. Circadian activity associated with spatial learning and memory in aging rhesus monkeys. *Exp Neurol*. 2009; 217:55–62. [PubMed: 19416674]
28. Noriega NC, Eghlidi DH, Garyfallou VT, Kohama SG, Kryger SG, Urbanski HF. Influence of 17beta-estradiol and progesterone on GABAergic gene expression in the arcuate nucleus,

- amygdala and hippocampus of the rhesus macaque. *Brain Res.* 2010; 1307:28–42. [PubMed: 19833106]
29. Noriega NC, Kohama SG, Urbanski HF. Microarray analysis of relative gene expression stability for selection of internal reference genes in the rhesus macaque brain. *BMC Mol Biol.* 2010; 11:47. [PubMed: 20565976]
 30. Eghlidi DH, Haley GE, Noriega NC, Kohama SG, Urbanski HF. Influence of age and 17 β -estradiol on kisspeptin, neurokinin B, and prodynorphin gene expression in the arcuate-median eminence of female rhesus macaques. *Endocrinology.* 2010; 151:3783–3794. [PubMed: 20519367]
 31. Kharlamov A, LaVerde GC, Nemoto EM, Jungreis CA, Yushmanov VE, Jones SC, Boada FE. MAP2 immunostaining in thick sections for early ischemic stroke infarct volume in non human primate brain. *J Neurosci Methods.* 2009; 182:205–210. [PubMed: 19540877]
 32. Cho YH, Friedman E, Silva AJ. Ibotenate lesion of the hippocampus impair spatial learning but not contextual fear conditioning. *Behav Brain Res.* 1999; 98:77–87. [PubMed: 10210524]
 33. Banta Lavenex P, Lavenex P. Spatial memory and the monkey hippocampus: not all space is created equal. *Hippocampus.* 2009; 19:8–19. [PubMed: 18727046]
 34. Lavenex PB, Amaral DG, Lavenex P. Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. *J Neurosci.* 2006; 26:4546–4558. [PubMed: 16641234]
 35. Raber J. Androgens, apoE, and Alzheimer's disease. *Sci Aging Knowledge Environ.* 2004:re2. [PubMed: 15028864]
 36. Irle E, Lange C, Sachsse U, Weniger G. Further evidence that post-traumatic stress disorder but not dissociative disorders are related to amygdala and hippocampal size reduction in trauma-exposed individuals. *Acta Psychiatr Scand.* 2009; 119:330–331. discussion 331. [PubMed: 19207126]
 37. Weniger G, Lange C, Sachsse U, Irle E. Amygdala and hippocampal volumes and cognition in adult survivors of childhood abuse with dissociative disorders. *Acta Psychiatr Scand.* 2008; 118:281–290. [PubMed: 18759808]
 38. Weniger G, Lange C, Sachsse U, Irle E. Reduced amygdala and hippocampus size in trauma-exposed women with borderline personality disorder and without posttraumatic stress disorder. *J Psychiatry Neurosci.* 2009; 34:383–388. [PubMed: 19721849]
 39. Hai J, Su SH, Lin Q, Zhang L, Wan JF, Li H, Chen YY, Lu Y. Cognitive impairment and changes of neuronal plasticity in rats of chronic cerebral hypoperfusion associated with cerebral arteriovenous malformations. *Acta Neurol. Belg.* 2010; 110:180–185. [PubMed: 20873448]
 40. Carvalho-Netto EF, Myers B, Jones K, Solomon MB, Herman JP. Sex differences in synaptic plasticity in stress-responsive brain regions following chronic variable stress. *Physiol Behav.* 2011; 104:242–247. [PubMed: 21315096]
 41. Gilabert-Juan J, Castillo-Gomez E, Perez-Rando M, Molto MD, Nacher J. Chronic stress induces changes in the structure of interneurons and in the expression of molecules related to neuronal structural plasticity and inhibitory neurotransmission in the amygdala of adult mice. *Exp. Neurol.* 2011; 232:33–40. [PubMed: 21819983]
 42. Burns MP, Noble WJ, Olm V, Gaynor K, Casey E, LaFrancois J, Wang L, Duff K. Co-localization of cholesterol, apolipoprotein E and fibrillar A β in amyloid plaques. *Brain. Res. Mol. Brain Res.* 2003; 110:119–125. [PubMed: 12573540]
 43. Cork LC, Masters C, Beyreuther K, Price DL. Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits. *Am J Pathol.* 1990; 137:1383–1392. [PubMed: 1701963]
 44. Nakamura S, Kiatipattanasakul W, Nakayama H, Ono F, Sakakibara I, Yoshikawa Y, Goto N, Doi K. Immunohistochemical characteristics of the constituents of senile plaques and amyloid angiopathy in aged cynomolgus monkeys. *J Med Primatol.* 1996; 25:294–300. [PubMed: 8906609]
 45. Sani S, Traul D, Klink A, Niaraki N, Gonzalo-Ruiz A, Wu CK, Geula C. Distribution, progression and chemical composition of cortical amyloid-beta deposits in aged rhesus monkeys: similarities to the human. *Acta Neuropathologica.* 2003; 105:145–156.
 46. Uno H, Alsum PB, Dong S, Richardson R, Zimbric ML, Thieme CS, Houser WD. Cerebral amyloid angiopathy and plaques, and visceral amyloidosis in aged macaques. *Neurobiol Aging.* 1996; 17:275–281. [PubMed: 8744409]

47. Raber J, Akana SF, Bhatnagar S, Dallman MF, Wong D, Mucke L. Hypothalamic-pituitary-adrenal dysfunction in Apoe(−/−) mice: possible role in behavioral and metabolic alterations. *J Neurosci*. 2000; 20:2064–2071. [PubMed: 10684907]
48. Robertson J, Curley J, Kaye J, Quinn J, Pfankuch T, Raber J. apoE isoforms and measures of anxiety in probable AD patients and Apoe−/− mice. *Neurobiol Aging*. 2005; 26:637–643. [PubMed: 15708438]

Figure 1. Division of the playroom into distinct areas for assessing anxiety levels in nonhuman primates. The jump door (A), the ports (B), the middle of the room (C), the corners (D), and the perimeter of the room (E).

Figure 2.

A. Relative mRNA levels (normalized to GAPDH, RPL13A, and ALG9) of microtubule associated protein 2 (*MAP2*), synaptophysin (*SYN*), and apolipoprotein E (*APOE*) in the amygdala of bold and reserved monkeys ($n = 16$). B. Positive correlation between the right amygdala size and the relative *MAP2* mRNA expression ($r = 0.54$; $P = 0.04$; $n = 16$). C. Positive correlation between the left amygdala size and the relative *MAP2* mRNA expression ($r = 0.55$; $P = 0.04$; $n = 16$). D. Positive correlation between the total amygdala size and the relative *MAP2* mRNA expression ($r = 0.55$; $P = 0.03$; $n = 16$). E. Positive correlation between the right amygdala size and the relative *SYN* mRNA expression ($r = 0.54$; $P = 0.04$; $n = 16$). F. Relative protein levels (normalized to β -actin) of MAP-2, SYN, and apoE in the hippocampus of good spatial performers (GSP) and poor spatial performers (PSP; $n = 12$). Representative blots for the proteins, MAP-2, SYN, and apoE are next to the graph. Lane 1 is a representative blot for the GSP and Lane 2 is a representative blot for the PSP. * $P < 0.05$

Table 1

Genes used for qRT-PCR

Target Genes	GenBank Accession ID.	RefSeq	Applied Biosystems Assay ID.
<i>MAP2</i>	NC_007869	XM_001109205.1	Rh02836496_m1
<i>SYN</i>	XM_001106095	NM_001194329.1	Rh00913691_m1
<i>APOE</i>	U52030	XM_001104482.2	Rh02788292_m1

Table 2

Gene expression levels in the prefrontal cortex (PFC) of good spatial performers (GSP) and poor spatial performers (PSP).

PFC	GSP	PSP
<i>MAP2</i>	1.42 ± 0.08	1.43 ± 0.40
<i>SYN</i>	3.01 ± 0.09	2.87 ± 0.10
<i>APOE</i>	1.12 ± 0.06	1.26 ± 0.07
HPC		
<i>MAP2</i>	1.30 ± 0.11	1.49 ± 0.09
<i>SYN</i>	2.10 ± 0.17	1.84 ± 0.18
<i>APOE</i>	1.45 ± 0.07	1.43 ± 0.06

n = 12 for the PFC and hippocampus

Table 3

Protein levels in the prefrontal cortex (PFC) of good spatial performers (GSP) and poor spatial performers (PSP) and in the amygdala of bold and reserved monkeys.

PFC	GSP	PSP
MAP-2	1.9 ± 0.7	1.1 ± 0.4
SYN	3.5 ± 0.7	2.2 ± 0.6
ApoE	0.9 ± 0.1	1.2 ± 0.3
Amygdala	Bold	Reserved
MAP-2	1.1 ± 0.2	0.9 ± 0.2
SYN	9.4 ± 2.3	5.0 ± 1.0
ApoE	12.1 ± 3.3	7.6 ± 1.9

n = 12 for the PFC

n = 16 for the amygdala