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Spatial Memory Performance Associated with Measures of Immune Function in Elderly Female Rhesus Macaques

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Abstract

We recently reported that in aged female rhesus macaques, spatial learning and memory correlates with circadian sleep-wake measures and hippocampal muscarinic type 1 (M₁) receptor binding. To investigate if spatial memory also correlates with measures of immune function, we now assessed the magnitude of the adaptive immune response to vaccination in the same old female rhesus macaques. Cognitively characterized animals were classified as good spatial performers (GSP) or poor spatial performers (PSP) based on performance in the Spatial Foodport maze. The GSP group had higher frequency of CD8, but not CD4, interferon- γ (IFN- γ) producing cells following vaccination compared to the PSP group, suggesting a stronger CD8 T cell response in the GSP group. In addition, the number of CD-8 IFN- γ positive cells correlated with measures of sleep quality. Interestingly, the PSP group had a significantly higher antibody titer compared to the GSP group, and antibody titer negatively correlated with day-time activity. Thus, in aged female rhesus macaques, superior cognitive performance is correlated with a more robust CD8 T cell response but a reduced antibody response to vaccination.

Keywords

spatial learning and memory; immune senescence; circadian activity

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INTRODUCTION

The elderly show increased susceptibility to infection and respond less efficiently to vaccination (Chen, et al., 2009, Grubeck-Loebenstein, et al., 2009, McElhaney and Effros, 2009). This age-related dys-regulation in immune function is commonly referred to as “immune senescence” (Pawelec, et al., 2009, Weiskopf, et al., 2009) and is characterized by several hallmark changes, such as loss of naïve T cells, accumulation of terminally differentiated memory T cells, decreased frequency of B cells and increased production of inflammatory cytokines (Pawelec, et al., 2009, Weiskopf, et al., 2009). Studies have shown that these immuno-senescent changes are correlated with mortality as well as the development of several age-related morbidities such as Alzheimer's, osteoporosis and cancer (Larbi, et al., 2008, Pawelec, et al., 2006, Vasto, et al., 2009). For instance, the accumulation of memory T cells secreting high levels of TNF α in the bone marrow has been shown to result in increased osteoclast activity and in the development of osteoporosis (Ginaldi, et al., 2005, M. Neale Weitzmann, 2007, Weitzmann and Pacifici, 2005).

Immune deficient states such as HIV, chemotherapy, and immune senescence have also been hypothesized to have a negative impact on cognitive performance in humans (Brynskikh, et al., 2008, Kipnis, et al., 2008, Ron-Harel and Schwartz, 2009), but a specific link between immune function and spatial learning and memory has been made only in rodents. For example, young and old immune deficient mice have impaired spatial learning and memory (Brynskikh, et al., 2008, Kipnis, et al., 2004, Ron-Harel, et al., 2008, Ziv, et al., 2006). This impairment has been linked to the absence of T cells as reconstitution of the T cell repertoire recovers the ability to learn new spatial locations (Brynskikh, et al., 2008, Ron-Harel, et al., 2008, Ziv, et al., 2006). Interestingly, spatial learning and memory is heavily dependent on muscarinic receptor function in the hippocampus (Fredrickson, et al., 2008, Wisman, et al., 2008), which also declines with age (Walker, et al., 1988). Furthermore, muscarinic type 1 (M₁) receptor binding and affinity, but not type 2 (M₂), in the hippocampus but not PFC are correlated with spatial maze performance in elderly female rhesus macaques (Haley, et al., 2010). Although there is an apparent connection between the immune system and spatial learning and memory (Ron-Harel and Schwartz, 2009, Ron-Harel, et al., 2008), little is known about this connection.

Disruption of circadian activity, a prominent consequence of aging which negatively affect cognitive performance in humans and nonhuman primates (Haimov, et al., 2008, Haley, et al., 2009, Morin, et al., 1998). Interestingly, some immune functions such as stimulation of T cells cytokine secretion with mitogen produces explicit patterns depending on the time of day (Bonacho, et al., 2001, Haus and Smolensky, 1999). In addition, a longer sleep cycle is associated with an enhanced immune response to vaccination (Lange, et al., 2003, Spiegel, et al., 2002). Thus, a potential cause of a decreased immune response may be directly related to perturbed sleep cycles.

Previously we observed that animals with more robust sleep-wake cycles showed superior performance in the spatial maze test and enhanced M₁ receptor binding and affinity in the hippocampus (Haley, et al., 2009). The goal of this study was to evaluate whether superior spatial performance is also associated with a better immune response to vaccination, thereby establishing potential interactions between circadian activity, immune function, and cognition in aged primates.

METHODS

Animals

Old female rhesus macaques (*Macaca mulatta*; $n = 12$; age range 21–27 years) were cognitively tested in a previously described Spatial Foodport maze (Haley, et al., 2010, Haley, et al., 2009). The animals were cared for by the Division of Animal Resources at the Oregon National Primate Research Center (ONPRC) in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals. They were housed indoors under controlled conditions: 24°C temperature, 12L:12D photoperiod (lights on at 0700 h), regular meals at 0800 h and 1500 h (Purina High Protein Chow Diet, Purina Mills, Inc., St. Louis, MO) supplemented with fresh fruit, vegetables and candy treats; fresh drinking water was available *ad libitum*. All studies were approved by ONPRC IACUC. For the Spatial Foodport maze, briefly, non-tethered rhesus macaques searched a series of food ports, one of which was repeatedly baited with a preferred reward. The number of trials to criterion was recorded, and based on this parameter the animals were classified as GSP or PSP. GSP animals completed the task in less than 30 trials in either the first or second position ($n = 6$; 18.7 ± 4.8 trials $n = 4$ first location; 7 & 14 trials $n = 2$ for the second location), whereas PSP animals completed the task in more than 30 trials ($n = 6$; 81.8 ± 8.5 trials).

Circadian Activity

As previously reported (Haley, et al., 2009), animals were equipped with an Actiwatch activity recorder (MiniMitter-Philips-Respironics, Inc, Bend, OR) worn on a nylon collar (Primate Products, Inc., Immokalee, FL) during the testing period. Activity counts were averaged over 2 weeks of measurement. Data were analyzed with Actiware-Sleep version 3.4 (MiniMitter-Philips-Respironics, Inc) software for activity parameters and sleep quality (Urbanski, 2010).

Immune assays

After completion of a battery of cognitive tests, the animals were vaccinated twice with Modified Vaccinia Ankara (MVA), one-month apart. Blood samples were collected on days 7, 14 and 30 after each immunization. Peripheral blood mononuclear cells (PBMC) and plasma were isolated following centrifugation over a Histopaque gradient (Sigma, St Louis, MO). T cell responses to the vaccine were measured by intracellular cytokine staining (ICS). PBMC were incubated overnight with MVA at a multiplicity of infection of 1. Brefeldin A was then added for 6 additional hours after which PBMC were first stained with antibodies directed against surface molecules CD4 and CD8 (Biolegend, San Diego, CA). The cells were then fixed and permeabilized as per manufacturer's recommendation (Biolegend) followed by the addition of anti-IFN γ specific antibody (Biolegend). Stimulation with an irrelevant virus (simian varicella virus) was used as a negative control to ensure the specificity of the assay. Samples were acquired on the LSR II (BD instrumentation, San Diego, CA). Data was then analyzed using FlowJo (Tree Star, Ashland, OR).

Antibody titers were measured by end point ELISA using plates coated with MVA viral lysate. Three-fold dilutions of the plasma obtained on different days post vaccination were added in triplicates to the plates for 1 hr, followed by washing, staining with detection reagents (HRP-anti-IgG), and addition of chromogen substrate to allow for detection and quantitation of bound antibody molecules. Log-log transformation of the linear portion of the curve was then carried out with 0.1 OD units the cut-off to calculate end point titers. Each plate always contained a positive control sample, which was used to normalize the ELISA titers between assays. Plasma collected prior to vaccination served as the negative control to ensure assay specificity.

Muscarinic receptor binding

Animals participating in the Spatial Foodport maze were euthanized within 1 week of completing their final cognitive battery at ONPRC by trained pathologists according to Veterinarian approved procedures. The animals were euthanized with ketamine (15–25 mg/kg i.m.) followed by pentobarbital sodium (25–30 mg/kg i.v.), a procedure consistent with the recommendations of the American Veterinary Medical Association's Panel on Euthanasia. Their brains were flushed with 1 liter of 0.9% (w/v) saline via a cardiac catheter, and the brain was removed from the skull. The prefrontal cortex (PFC) and hippocampus were dissected from the right side of the brain by the same individual to ensure consistency. The PFC and hippocampus tissue blocks were kept cold on ice, and subsequently frozen in liquid nitrogen, and stored at -80°C .

The tissues were homogenized in 1 ml of ice-cold buffer solution (140 mM NaCl, 0.2 mM KCl, 10 mM MgCl_2 , 0.5 mM EDTA, 50 mM Tris, pH=7.5), centrifuged at $16,000\times g$ for 25 minutes, washed in ice-cold buffer, and re-centrifuged. This was repeated for a total of 4 times and the pellet was stored at -80°C until assayed. Pellets were re-suspended in ice cold buffer (10 mM NaH_2PO_4 , 10 mM Na_2HPO_4 , 1 mM MgCl_2 , pH=7.5) and the protein concentration of each pellet was measured using the BCA protein assay (Pierce Pharmaceuticals, Rockford, IL) protein assay kit. Specific binding and non-specific binding were measured in duplicate. Eight different increasing concentrations of the M_1 receptor ligand [^3H]pirenzepine (312.5 pM to 80 nM) was used. Non-specific binding was assessed using 2.5 μM of atropine. Once radioligand was added to the tissue samples, they were incubated for 1 hour at room temperature. The incubation was terminated by adding 5 ml of ice-cold binding buffer. Tissue-bound and free radioligand tissues were separated using Whatman filter paper and a Brandell cell harvester. Subsequently, the filters were placed in vials and after adding 5 ml of scintillation fluid counted in a beta scintillation counter (LS 6000SC Beta counter, Beckman, Fullerton CA, USA).

Statistical Analyses

Statistical analyses were performed using Prism Software (Graphpad, La Jolla, CA). Repeated Measures ANOVAs were used to assess CD8 IFN- γ positive cells, CD4 IFN- γ positive cells, and antibody titers during the vaccine priming and booster periods. Pearson's product correlations were used to assess potential correlations between measures of circadian activity, immune function, M_1 receptor function, and cognitive performance.

RESULTS

Spatial maze performance and CD4 and CD8 T cell response to MVA

There was no significant difference in the percentage of IFN- γ -producing MVA-specific CD4 T cells between GSP and PSP at any of the time points measured ($P = 0.4$; Figure 1A). However, the GSP group had a significantly greater percentage of CD8 IFN- γ positive cells compared to the PSP group, notably at Day 7 and 14 after booster vaccination [$F(5, 49) = 72.8$, $P = 0.001$ Figure 1B].

Circadian activity, hippocampal M_1 receptor binding, and CD4 and CD8 T cell response to MVA

The percentage of CD8 IFN- γ positive cells at Day 7 after the booster vaccination correlated positively with the Light:Dark activity ratio ($r = 0.66$; $r^2 = 0.43$; $P = 0.03$; Table 1) and negatively with the number of wake bouts ($r = -0.76$; $r^2 = 0.58$; $P = 0.006$). The percentage of CD8 IFN- γ positive cells at Day 14 after the booster vaccination correlated positively with Day-time Activity Counts ($r = 0.79$; $r^2 = 0.50$, $P = 0.02$), Light:Dark activity ratio ($r =$

0.89; $r^2 = 0.79$, $P = 0.0006$; Figure 2A), and sleep latency ($r = 0.71$; $r^2 = 0.52$, $P = 0.01$) but negatively with the number of wake bouts ($r = -0.82$; $r^2 = 0.69$, $P = 0.003$; Figure 2B).

There was an inverse correlation between CD8 IFN- γ and M₁ binding affinity in the hippocampus ($r = 0.65$, $r^2 = 0.41$, $P = 0.04$; Figure 2C). No significant correlation was observed between CD8 IFN- γ and M₁ binding number ($P = 0.2$), nor between CD8 IFN- γ and M₁ binding number or affinity in the PFC ($P > 0.7$). Similarly, no significant correlation was found between M₂ receptor binding number or affinity in the PFC or hippocampus ($P > 0.4$).

Spatial maze performance, circadian activity, and antibody titer

At day 14 and 30 after booster vaccination, the antibody titer was significantly higher in the PSP than the GSP group [$F(2, 29) = 7.6$, $P = 0.01$; Figure 3]. At Day 14 after the booster vaccination, the antibody titer negatively correlated with Day-time Activity Counts ($r = -0.68$; $r^2 = 0.47$, $P = 0.02$; Table 1) and Night-time Activity Counts ($r = -0.77$; $r^2 = 0.59$, $P = 0.009$; Figure 2D).

DISCUSSION

Data from several rodent studies suggest that a compromised immune system decreases cognitive function (Kipnis, et al., 2008, Ron-Harel, et al., 2008). However, little is known about a potential correlation between the age-related decline in immune function and cognitive performance in the elderly. Here, we report strong correlations between measures of the immune response to vaccination and performance in a Spatial Foodport maze in aged female rhesus macaques. Monkeys with superior cognitive performance generated a more robust CD8 IFN- γ response after vaccination. Interestingly, the frequency of CD8 IFN- γ positive cells also correlated with the inverse of the K_d of the M₁ receptor in the hippocampus.

Moreover, we found a strong correlation between sleep quality and the frequency of CD8 IFN- γ positive T cells. These observations are in agreement with studies that showed a connection between immune function and circadian activity. For instance, disruption of circadian rhythms has a negative effect on the immune response to a foreign antigen (Haus and Smolensky, 1999). Superior performers also showed better sleep quality indices, including an increased Light:Dark activity ratio and fewer nocturnal wake bouts (Haley, et al., 2009). Together, these results suggest that animals with a more robust circadian rhythm generate a better T cell response to vaccination.

The M₁ receptor has a crucial role in CD8 cell differentiation (Zimring, et al., 2005) and T cell differentiation (Vezys, et al., 2007). Furthermore, the M₁ receptor appears to be involved in antibody conversion and other cytokine production (Fujii, et al., 2007). We observed a significant correlation between the affinity of the M₁ receptor in the hippocampus, but not the PFC, and the number of responding IFN- γ positive CD8 T cells. The fact that animals with a lower M₁ affinity have a lower functioning immune function suggests that M₁ elsewhere, including within the immune system itself (Eglen, 2006, Fujii, et al., 2007, Kawashima and Fujii, 2004), could be dysfunctional.

The relationship between T cell function and cognitive performance has been previously described in rodent studies where hippocampus-dependent learning is impaired in T cell deficient mice (Brynskikh, et al., 2008, Kipnis, et al., 2008, Ron-Harel and Schwartz, 2009, Ziv, et al., 2006). It has been suggested that T cells modulate neuronal plasticity, especially hippocampal neurogenesis, the process of which immature neurons migrate into the granule layer of the dentate gyrus (Kuhn, et al., 1996) and develop into functional neurons (van

Praag, et al., 2002). In support of this hypothesis, mice deficient in T cells have decreased neurogenesis and transfer of T cells into these mice rescues neurogenesis as well as performance in a water maze (Ziv, et al., 2006). Neurogenesis occurs readily in a healthy hippocampus (Eriksson, et al., 1998, Kuhn, et al., 1996), is most pronounced in adulthood (Cardon, et al., 2010), and is decreased in elderly (Galvan and Jin, 2007, Kempermann, 2002). It is therefore possible that the loss of T cell function that occurs with age contributes to diminished neurogenesis and cognitive decline in the elderly.

In contrast to the T cell responses, the antibody titer was significantly higher in the PSP than in the GSP group. Different mechanisms might regulate T cell senescence and B cell senescence. Studies have suggested that aging results in a shift from Th1 to Th2 T cell response (Jing, et al., 2007, Walsh and Falsey, 2004). The GSP group has a better preserved CD8 T cell response, which is mediated mostly by a Th1 T cells (IFN- γ production), whereas the responses to MVA vaccination in the PSP group are mediated by a Th2 T cells and associated with antibody production. These observations suggest that the GSP group might have a delayed onset or reduced severity of immune senescence.

Immune dysfunction can cause cognitive decline, observed in immune compromised states such as HIV and during chemotherapy (Ances and Ellis, 2007, Ferguson and Ahles, 2003, Schifitto, et al., 2009). However, with aging, the potential mechanistic relationship between cognition and immune function is unclear. As circadian disruption also causes cognitive decline (Gruber, et al., 2010, Harrison and Horne, 2000, Harrison and Horne, 2000, Harrison, et al., 2000), it is possible that during aging circadian rhythm changes contribute to alterations in the immune system and that both contribute to cognitive decline. Rodent studies indicate that M₁ receptor function might alter circadian activity (Kafka, et al., 1986, Liu, et al., 1996, Tanaka, et al., 2002), suggesting that hippocampal M₁ receptor function might mediate these effects.

Increased efforts are warranted for understanding the mechanisms underlying the effects of immune measures, and more specifically, T cell senescence, on age-related cognitive decline. They could provide new strategies for interventions and prevention of age-related changes in cognition. The immune system could serve as a window into the cognitive performance and might provide an opportunity to identify individuals at increased risk, especially for those whose sleep cycles are not regularly monitored.

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Figure 1.

Percentage of CD4 (A) and CD8 (B) of IFN- γ positive cells following primary (Days a) as well as booster vaccination (Days b) in good spatial performers (GSP; black bars) and poor spatial performers (PSP; white bars). ** $P < 0.01$.

Figure 2.

Representative correlations between immune measures, circadian activity, and muscarinic receptor binding affinity. Correlation between the percentage of CD8 IFN- γ 14 days following booster vaccination (14b) and (A) Average Light:Dark (L:D) activity ratio ($r = 0.89$), (B) Average number of wake bouts ($r = -0.82$), (C) and $1/M_1$ binding affinity (K_d ; $r = 0.66$). (D) Correlation between Antibody Titer (Day 14b) and average night-time activity ($r = 0.77$).

Figure 3. Antibody titers on days 7, 14, and 30 following booster vaccination in good spatial performers (GSP; black) and poor spatial performers (PSP; white). * $P < 0.05$

Table 1

Correlations between immune measures and circadian activity

	r value
CD8 IFN- γ day 7b - Light:Dark activity ratio	0.66 [*]
CD8 IFN- γ day 7b - Number of wake bouts	-0.76 ^{**}
CD8 IFN- γ day 14b - Day-time activity	0.79 [*]
CD8 IFN- γ day 14b - Light:Dark activity ratio	0.89 ^{**}
CD8 IFN- γ day 14b - Sleep Latency	0.71 [*]
CD8 IFN- γ day 14b - Number of wake bouts	-0.82 ^{**}
CD8 IFN- γ day 14b - 1/hippocampus M ₁ K _d	0.66 [*]
Antibody titer day 14b - Day-time activity	-0.68 [*]
Antibody titer day 14b - Night-time activity	-0.77 ^{**}

Significant correlations between immune parameters and circadian activity, as well as M₁ binding.^{*}
 $P < 0.05$ ^{**}
 $P < 0.01$