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Behavioural Brain Research 160 (2005) 208–221
BEHAVIOURAL BRAIN RESEARCH
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Research report

Cognitive impairment in aged rhesus monkeys associated with monoamine receptors in the prefrontal cortex

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Received 26 September 2004; received in revised form 30 November 2004; accepted 4 December 2004
Available online 12 January 2005

Abstract

The "frontal aging hypothesis" has been proposed by many researchers suggesting that the earliest and most severe age-related changes in the cortex occur in the frontal lobes. Two of these changes include decreases in cognitive functions mediated by the prefrontal cortex (PFC) and significant decreases in norepinephrine (NE) and dopamine (DA). To investigate whether the changes in these neurotransmitter systems are directly related to the cognitive decline seen in aging we utilized the rhesus monkey as a model of normal human aging. Our goal was to determine if age-related changes in cognition is associated with changes in norepinephrine and dopamine receptor binding density in the PFC. Eight young monkeys between five and ten years of age (six males and two female) and eight aged monkeys between 25 and 32 years of age (five males and three females) were behaviorally characterized. Subsequently on-the-slide in vitro binding assays were used to quantify the α-1 adrenergic, α-2 adrenergic and DA1 receptors as well as the NE and DA uptake receptors. Aged animals as a group demonstrated significant cognitive impairments and aging produced a significant decrease in α-1 adrenergic and α-2 adrenergic receptor binding in the PFC but no significant change in binding for the DA1 receptor or the NE or DA uptake receptors. Further analysis revealed a significant relationship between monoamine receptor binding and cognitive performance on three tasks: delayed non-matching to sample, delayed recognition span test and the conceptual set-shifting task.

Keywords: Rhesus; Prefrontal cortex; Norepinephrine; Dopamine; Receptors; Cognition; Aging

1. Introduction

Biological changes that occur during the aging process have been extensively investigated in recent decades as the number of individuals over the age of 65 years in the population has grown. Of the many changes that have been described, impairments in cognitive function can significantly impair the quality of life. Mild, age-related cognitive changes have been identified in several cognitive domains but impairments in memory and executive function are among the earliest and most severely affected [1]. Although loss of cortical neurons is an important underlying factor in the cognitive impairments seen in major dementias like Alzheimer's disease, the majority of recent studies have failed to find any significant evidence of neuron loss in the process of normal aging (for review see [57] but for an exception see [68]). Hence it appears unlikely that a loss of cortical neurons will be able to account for the decline seen in cognition. This leaves the precise neurobiological basis of these age-related impairments in cognition yet to be determined.

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doi:10.1016/j.bbr.2004.12.003

One of the limitations of answering this question with human studies is the difficulty in obtaining optimally prepared tissue from cognitively characterized individuals. The rhesus monkey has proven to be a useful model that allows brain tissue from cognitively characterized subjects to be optimally prepared for a variety of analyses (see [52] for a review). This approach has identified age-related changes in memory [13,28,58,62,70,74] and executive function [35,36,47,51,61,69] quite similar to the patterns seen in humans. While examination of the brains has shown stability in neuron numbers [62], a variety of changes have also been reported including degenerative changes in forebrain myelin [53,54,55], changes in neurotransmitter systems of the medial temporal lobe [63] and changes in some synaptic properties [40] and action potential generation [18] in layer 3 neurons of the prefrontal cortex (PFC). While some of these variables appear to correlate with some aspects of age-related decline in recognition memory, others do not. Moreover, the profound age-related impairments in executive function in the normal aging rhesus monkey that we have recently described [47] are as yet, unexplained.

Executive function in humans and monkeys is largely attributed to the prefrontal cortex in general [23,42,43,44] and is profoundly dependent upon normal function of the monoamine systems of the frontal lobe [4,10,69]. Specifically, norepinephrine (NE) and dopamine (DA), two monoamine neurotransmitters known to play roles in the cognitive functions subserved by the prefrontal cortex, are reduced in aged monkeys, with the most significant reductions occurring in the prefrontal cortices [8,22,25]. Further, administration of α-2 adrenergic agonists, to aged monkeys improves their performance on the delayed response task [5,15], a classic test of frontal lobe function.

Despite numerous studies in non-human primates that have demonstrated age-related changes in NE and DA concentrations and the role of monoamine agonists and antagonists to modulate executive functions [3,6,9,14,17,69,75], few studies have investigated the effects of aging on the receptors of these two neurotransmitters or their relationship to cognitive decline. Accordingly, using a non-human primate model of normal human aging, the present study assessed the effects of aging on NE and DA receptor binding density in various regions of the PFC and the relationship between NE and DA receptor binding and performance on three cognitive tests. As previously published [28,47,49], aged monkeys are significantly impaired on cognitive tests (delayed nonmatching to sample, delayed recognition span test and the conceptual set shifting task) that rely in part on the PFC. Based on these findings, we wanted to determine if there was a relationship between age-related cognitive performance on these tests and changes in NE and DA receptor binding densities in the PFC.

2. Experimental procedures

2.1. Subjects

Sixteen rhesus monkeys (*Macaca mulatta*), both males and females, were used in this study. They were obtained from the colonies of the Yerkes National Primate Research Center. As shown in Table 1 eight subjects were young, (six males, two females) ranging in age from 5 to 10 with a mean of 7.6 and 8 were aged, (three males and five females) ranging in age from 25 to 32 years of age with a mean of 28.6. Three young monkeys (HM 034, 044, 059) were controls from a related study and were included as part of this study. They completed the behavioral tasks and their brains were processed

Table 1
This table shows the age and gender of each monkey and the behavioral tests completed by each monkey

Monkey	Age	Sex	DNMS (Acq, 2 and 10)	DRST (Spt, Obj)	Three-choice discrimination	CSST
Young						
AM055	10	M	Yes	Yes	No	No
AM057	10	M	Yes	Yes	No	No
AM078	6	F	Yes	Yes	No	No
AM094	5	M	Yes	Yes	Yes	Yes
AM095	7	F	Yes	Yes	Yes	Yes
HM034	9	M	Yes	No	Yes	Yes
HM044	7	M	Yes	No	Yes	Yes
HM059	7	M	Yes	No	Yes	Yes
Mean	7.6					
Aged						
AM024	29	F	Yes	No	Yes	Yes
AM048	30	M	Yes	No	Yes	Yes
AM051	32	F	No	Yes	No	No
AM061	31	F	Yes	No	No	No
AM063	25	F	Yes	Yes	Yes	Yes
AM068	25	M	Yes	Yes	Yes	Yes
AM073	30	M	No	No	No	No
AM098	27	F	Yes	Yes	Yes	Yes
Mean	28.6					

at the same time as the other animals in the control group. Their performance on all three tests and receptor binding densities fell within the range of the other control animals. All of the monkeys had known birth dates and complete health records were available. Before entering the study, monkeys received medical examinations that included serum chemistry, hematology, urine analysis and fecal analysis. In addition, explicit criteria were used to screen the health records and exclude monkeys with a history of splenectomy or thymectomy, exposure to radiation, cancer, organ transplantation, malnutrition, chronic illness, neurological diseases or chronic drug administration.

The stratification of this cohort into young and old was based on a survival study at the Yerkes National Primate Research Center from which these monkeys were obtained. In that study Tigges et al. [72], found that over half of the population was dead by about 16 years of age while almost no monkeys (<5%) lived beyond 30 years. Since monkeys reach sexual maturity by 5 years of age, this suggests a ratio of 1:3 between monkey and human years. Assuming that the relationship between monkeys and humans is constant across the life span, young monkeys between 5 and 10 likely correspond to humans between 15 and 30 years of age, while monkeys over 20 likely correspond to humans 60 years of age and older and monkeys 30 years of age and older correspond to humans over 90 years of age.

All monkeys were part of larger of study of normal aging (see [52] for a review) and underwent magnetic resonance imaging (MRI) to ensure that none of the monkeys had suffered a major cerebrovascular event, such as a stroke or head trauma and all had normal MRI findings. Throughout the course of the study monkeys were housed first at the Yerkes National Primate Research Center at Emory University and then at the Laboratory Animal Science Center of Boston University Medical Center. Both facilities are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care [33]. All care conformed to the standards of National Institutes of Health and the Institute of Laboratory Animal Resources Commission on Life Sciences' Guide for the Care and Use of Laboratory Animals and all procedures were approved by the Institutional Animal Care and Use Committees at both institutions.

2.2. Behavioral testing

Monkeys that completed behavioral testing on our battery of cognitive tasks are indicated in Table 1. The specific tasks that were used have been described in detail elsewhere [8,47,49]. In summary, the test battery begins with five tests in a Wisconsin General Test Apparatus (WGTA). The first is acquisition of the delayed non-match to sample task (DNMS-Acq) a test of rule learning. This is followed by DNMS with delays of 2 min (DNMS-2) and 10 min (DNMS-10) that assess recognition memory over delays. Subjects are then tested on two forms of the delayed recognition span task (DRST) that assess the depth of immediate memory capacity. The spatial form (DRST-Spt) requires the identification of each new stimulus in an increasing array of stimuli based solely on spatial location since all objects are identical, while the object form (DRST-Obj) requires the identification of each new stimulus based solely on its visual characteristics independent of spatial location. After completion of these tests in the WGTA, subjects are then tested on a simple three-choice visual discrimination task on a computer controlled touch screen apparatus that assesses simple associative learning. They are then tested in the same computer apparatus on the conceptual set shifting task (CSST), a monkey analog of Wisconsin Card Sorting Test that assesses executive functions including abstraction and set shifting. As a group, aged animals were significantly impaired in abstracting the non-matching principle (DNMS-Acq) and applying this principle across the delay conditions (DNMS-2 and -10). They evidence reduced memory capacity on the DRST task [28,49] and were also impaired on both abstraction and set shifting on the CSST as compared to young monkeys [47]. An examination of the error pattern made by the aged monkeys showed evidence of increased perseveration on the DRST and CSST as compared to young monkeys [47,49].

2.3. Tissue preparation for autoradiographic studies

Following completion of the battery of cognitive tasks [28,47,49] the monkeys were sedated with Ketamine and then deeply anesthetized with sodium pentobarbital and killed by exsanguination during transcardial perfusion with ice-cold Krebs buffer (pH 7.4). The brains were then blocked, in situ, in the coronal stereotactic plane, removed and quickly frozen in −70 °C isopentane. Time between the beginning of anoxia and freezing of the brain ranged from 10 to 15 min. Brains were stored at −80 °C until cut on a cryostat at −20 °C into interrupted series of 15 um thick sections. Each section was thaw mounted onto a poly-l-lysine coated slide and rapidly dried and stored at −20 °C until they were group-processed in binding assays with series from all the other subjects. This procedure eliminates between subject variance due to difference in processing for the assays described below.

2.4. Receptor binding assays

Standard published in vitro receptor assay techniques using well-characterized tritium labeled ligands were used to quantify the single concentration binding density of the following receptors: α-1 adrenergic, α-2 adrenergic, norepinephrine uptake transporter (NEU), dopamine-1 (DA1), and the dopamine uptake transporter (DAU) (see Table 2). Assays were run only with a single concentration of ligand with the ligand type and concentration and other incubation conditions based on published studies as summarized in

Table 2
Norepinephrine and dopamine receptor binding protocols

Receptor	H^3-ligand	Concentration (nM)	Blocker	Reference
α-1	Prazosin	0.5	Phenylephrine (100 UM/L)	Goldman-Rakic et al. (1990), Gross-Isseroff et al. (1990)
α-2	Clonidine	1.5	Norepinephrine (100 UM/L)	Rakic et al. (1988)
NEU	Nisoextine	3.0	Mazindol (1 UM/L)	Tejani-Butt et al. (1992)
DA1	SCH-23390	2.0	(+) Butaclamol 10 UM/L	Lidow et al. (1991), deKeyser et al. (1990)
DAU	WIN 35, 428	5.0	Cocaine (100 UM/L)	Coulter et al. (1995)

Table 2. The following general techniques were used for all the assays. The slide-mounted cryostat sections were removed from the freezer, rapidly thawed (10–20 s) on a warming plate (30–32 °C) and then transferred directly into the incubation solutions outlined in Table 2. Binding assays consisted of incubation in a solution containing a known concentration of radioactively labeled ligand known to bind to the receptor in question as well as appropriate ions to facilitate that binding. After a sufficient period of incubation to allow ligand binding to equilibrate, the sections were rinsed to remove unbound ligand and then rapidly dried. This assay constituted total binding of that particular ligand. Non-specific binding was assayed using an immediately adjacent section that was treated in the same way except that an excess amount of an appropriate unlabeled blocker was added to the incubation medium to competitively block specific receptor binding sites. After drying overnight at −80 °C with desiccant, the sections were loaded into an X-ray cassette along with tritium standards (Amersham Corp.) and apposed to tritium sensitive film. The cassettes were sealed and the film allowed to expose in the dark for several weeks at 4 °C before being developed.

2.5. Analysis of ligand binding

Quantification of binding densities was performed using Inquiry, a computer based, receptor autoradiographic image analysis system from Loats Associates (Westminster, MD). This system collects digitized images of film autoradiographs. Samples are then collected directly from these images using a mouse to lay down a sampling line within the region of interest on the digitized image. The average optical density of each pixel of the sampling line is determined. Based on the optical densities of the images of the tritium standards and their known activity as calibrated against brain paste, the program converts the optical densities of the tissue autoradiographic images to fmols of bound ligand per milligram of tissue for a given sampling region. Since tissue was only available to conduct single concentration assays, it was not possible to determine if differences in binding reflect changes in receptor numbers or changes in affinity.

Sections from all 16 animals were used in each assay but for technical reasons sections were run in two separate batches. To control for this, adjacent sections of tissue from one young and one aged monkey that were processed in the first batch were also run in the second batch to assess and control for any variability between batches. Analysis of the sections from subjects run in both batches demonstrated that observed binding densities were within 10% so data from both batches was pooled.

As shown in Fig. 1, samples were acquired from six specific regions of interest in the PFC on each of four sections from each case. These sections were matched for level and were always located in the anterior half of sulcus principalis. The six regions sampled in each section along with the cytoarchitectonic designation were: (a) dorsolateral convexity—area 9; (b) dorsal bank of sulcus principalis—area 46; (c) ventral bank of sulcus principalis—area 46; (d) ventrolateral convexity—area 12; (e) orbital frontal cortex—area 11, 14; and (f) medial frontal cortex—area 25. In each region, samples were taken in both the superficial layers (layers 1, 2 and 3) and deep layers (layers 5 and 6) for the DA1, α-1 adrenergic, and α-2 adrenergic receptors since there were clear laminar differences in binding. However, for the NEU and DAU assays, no laminar differences were observed and a pilot study confirmed the homogeneity of binding so the entire cortex was sampled from layer 1 to layer 6.

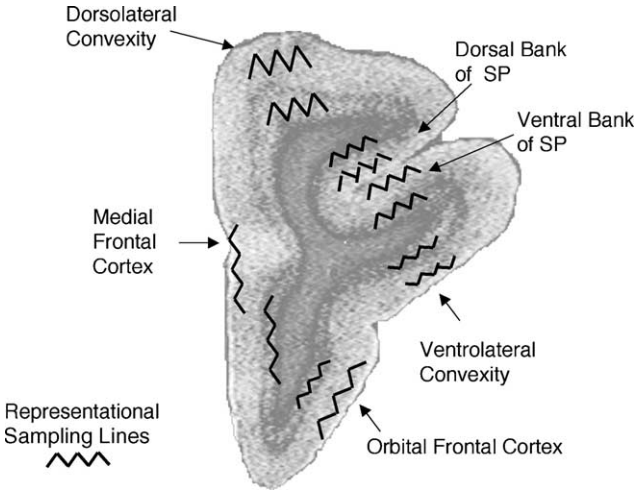

Fig. 1. This a representative coronal section of the prefrontal cortex showing the regions measured in the receptor binding experiments.

2.6. Data analysis

Since the non-specific assays for all ligands demonstrated no significant non-specific binding, measures of specific binding were taken from the total binding assays. For each receptor subtype the optical density data was converted to fmol/mg of protein based on tritium standards calibrated to brain paste (Amersham).

For α-1 adrenergic, α-2 adrenergic and DA1 receptors, in each of the six regions, the total fmol/mg of protein was averaged over all four sections and one number was assigned for each subject for each region (and lamina) measured. These data were analyzed with separate two way repeated measures analysis of variance with age as a between group variable and layer (superficial or deep) as a within subjects variable. These tests were followed, when appropriate, (i.e., the group by layer interaction was statistically significant) by between group comparisons using tests of simple main effects.

For the NEU and DAU, in each of the six regions, the total fmol/mg of protein across all layers was analyzed with separate two way repeated measures analysis of variance for each receptor, with age as a between group variable and region as a within subjects variable. These tests were followed, when appropriate (i.e., the group by region interaction was statistically significant) by between group comparisons using tests of simple main effects.

3. Results

3.1. α-1 Adrenergic receptors

The binding densities for the α-1 adrenergic receptor are illustrated in Fig. 2A. The detailed analysis indicated that there was a significant effect of layer in all regions with higher binding densities in the superficial compared to the deep layers. As indicated there was no affect of age on binding in the deep layers but there was a significant age-related reduction in binding density in the superficial layers of three out of the six regions examined with orbital, medial and dorsal bank of principalis not reaching significance.

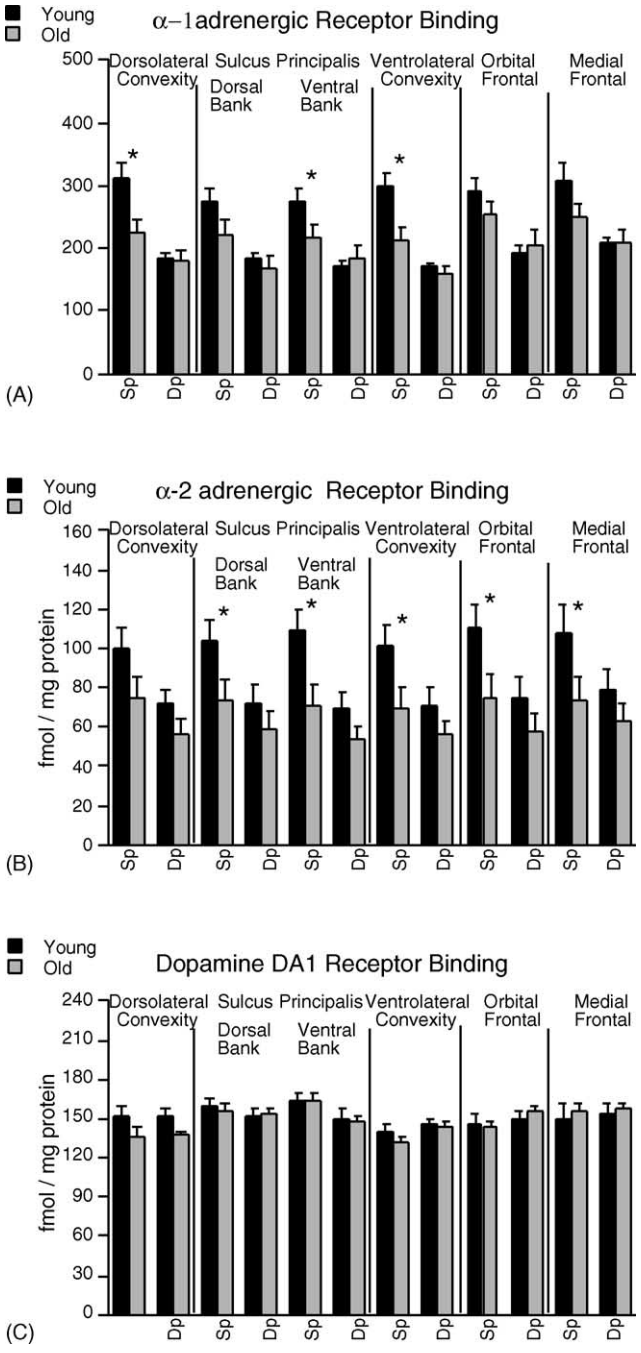

Fig. 2. These graphs depict the receptor binding densities for α-1 adrenergic (A), α-2 adrenergic (B) and DA1 (C) receptor binding. The areas of cortex measured are displayed across the top of the bars, the *x*-axis represents superficial and deep layers in each region and the *y*-axis represents a density measure in fmol/mg of protein. Asterisk indicates a significant group difference ($p \leq 0.05$).

3.1.1. Dorsolateral convexity

A two way repeated measures analysis of variance of binding density in the dorsolateral convexity revealed an overall effect of age group [$F(1,14) = 6.39$, $p = 0.02$], and of cortical layer [$F(1,14) = 115.9$, $p = 0.0001$] and a group by layer interaction [$F(1,14) = 13.46$, $p = 0.002$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the age groups in the superficial layers of the dorsolateral convexity [$F(1,28) = 15.45$, $p < 0.001$] (Fig. 2A), with the aged group showing a significant reduction in α-1 adrenergic binding density. There was no significant difference between the groups in the deep layers [$F(1,28) = 0.89$, $p < 0.36$].

3.1.2. Sulcus principalis (dorsal bank)

A two way repeated measures analysis of variance of binding density in the dorsal bank of sulcus principalis revealed an effect of layer [$F(1,14) = 80.34$, $p < 0.0001$] but no overall effect of age group [$F(1,14) = 2.54$, $p < 0.133$]. There was a trend towards significance for the group by layer interaction [$F(1,14) = 3.71$, $p = 0.07$]. Follow-up comparisons with independent samples one-way analysis of variance did not show significant (p's > 0.05) differences

3.1.3. Sulcus principalis (ventral bank)

A two way repeated measures analysis of variance of binding density in the ventral bank of principalis revealed an overall effect of layer [$F(1,14) = 141.75$, $p < 0.0001$] and a group by layer interaction [$F(1,14) = 8.25$, $p = 0.01$]. However, there was no effect of age group [$F(1,14) = 2.04$, $p = 0.17$] in this region. Follow-up analyses with between group comparisons using a test of simple main effects were conducted on the data for the superficial and deep layers of the Sulcus principalis (ventral bank). This analysis revealed a significant difference between the groups in the superficial layers of the Sulcus principalis (ventral bank) [$F(1,28) = 5.09$, $p < 0.05$] (Fig. 2A) with the aged group showing a significant reduction in α-1 adrenergic binding density. Like the dorsal convexity, there was no significant difference between the groups in the deep layers [$F(1,28) = 0.37$, $p = 0.55$].

3.1.4. Ventrolateral convexity

A two way repeated measures analysis of variance of binding density in the ventrolateral convexity revealed an overall effect of age group [$F(1,14) = 5.05$, $p = 0.04$] and an overall effect of layer [$F(1,14) = 124.73$, $p < 0.0001$] as well as a significant group by layer interaction [$F(1,14) = 14.00$, $p = 0.002$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the groups in the superficial layers of the ventrolateral convexity [$F(1,28) = 13.03$, $p < 0.01$] (Fig. 2A) with the aged group showing a significant reduction in α-1 adrenergic binding density. Again, there was no significant difference between the groups in the deep layers [$F(1,28) = 0.56$, $p = 0.46$].

3.1.5. Orbitofrontal cortex

A two way repeated measures analysis of variance of binding density in the orbitofrontal cortex revealed no significant effect of age group [$F(1,14) = 0.04$, $p = 0.85$] and while there was an overall effect of layer [$F(1,14) = 81.92$, $p < 0.0001$]

there was no group by layer interaction [$F(1,14) = 0.007$, $p = 0.93$].

3.1.6. Medial frontal cortex

A two way repeated measures analysis of variance of binding density in the medial frontal cortex revealed no significant effect of age group [$F(1,14) = 0.03$, $p = 0.85$] and although there was an overall effect of layer [$F(1,14) = 120.81$, $p < 0.0001$] there was no significant group by layer interaction [$F(1,14) = 0.37$, $p = 0.55$].

3.2. α-2 Adrenergic receptors

The binding densities for the α-2 adrenergic receptor are illustrated in Fig. 2B. Similar to the α-1 adrenergic receptor findings, there was a significant effect of layer in all regions. Moreover there was a significant age-related reduction in binding density in the superficial layers of five of the six regions examined with only the dorsolateral convexity failing to reach significance.

3.2.1. Dorsolateral convexity

A two way repeated measures analysis of variance of binding density in the dorsolateral convexity cortex revealed an effect of layer [$F(1,14) = 18.37$, $p < 0.0008$] but there was no overall effect of age group [$F(1,14) = 2.58$, $p < 0.13$] or a group by layer interaction [$F(1,14) = 0.87$, $p = 0.37$].

3.2.2. Sulcus principalis (dorsal bank)

A two way repeated measures analysis of variance of binding density in the dorsal bank of sulcus principalis revealed an overall effect of layer [$F(1,14) = 82.26$, $p < 0.0001$] and a group by layer interaction [$F(1,14) = 12.38$, $p < 0.003$] but there was no effect of group [$F(1,14) = 2.34$, $p = 0.15$]. Follow-up analyses with between group comparisons using a test of simple main effects conducted on the data for the superficial and deep layers revealed a significant difference between the groups in the superficial layers [$F(1,28) = 4.45$, $p < 0.05$] with the aged group showing a significant reduction in α-2 adrenergic binding density as shown in Fig. 2B. There was no significant difference between the groups in the deep layers [$F(1,28) = 1.03$, $p = 0.32$].

3.2.3. Sulcus principalis (ventral bank)

A two way repeated measures analysis of variance of binding density in the ventral bank of sulcus principalis revealed an overall effect of layer [$F(1,14) = 87.81$, $p < 0.0001$] and of age group [$F(1,14) = 4.22$, $p = 0.05$] as well as a significant group by layer interaction [$F(1,14) = 12.47$, $p = 0.003$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the groups in the superficial layers of the cortex [$F(1,28) = 7.88$, $p < 0.01$] with the aged group showing a significant reduction in α-2 adrenergic binding density as shown in Fig. 2B. There was no significant difference between the groups in the deep layers [$F(1,28) = 2.10$, $p = 0.17$].

3.2.4. Ventrolateral convexity

A two way repeated measures analysis of variance of binding density in the ventrolateral convexity revealed no effect of age but there was a significant effect of layer [$F(1,14) = 40.51$, $p < 0.0001$] as well as group by layer interaction [$F(1,14) = 6.02$, $p = 0.02$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the groups in the superficial layers of the ventrolateral convexity [$F(1,28) = 5.12$, $p < 0.05$]. As shown in Fig. 2B, there was a significant reduction in α-2 adrenergic binding density in this region for the age group relative to the young. There was no significant difference between the groups in the deep layers [$F(1,28) = 1.52$, $p = 0.24$].

3.2.5. Orbitofrontal cortex

Separate two way repeated measures analysis of variance of binding density in the orbitofrontal cortex revealed no significant effect of age group [$F(1,14) = 2.48$, $p = 0.13$] but there was an overall effect of layer [$F(1,14) = 88.21$, $p < 0.0001$] and a group by layer interaction [$F(1,14) = 12.71$, $p = 0.003$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the groups in the superficial layers of the orbitofrontal cortex [$F(1,28) = 4.58$, $p < 0.05$]. As shown in Fig. 2B, there was a significant reduction in α-2 adrenergic binding density for the aged subjects relative to the young. There was no significant difference between the groups in the deep layers [$F(1,28) = 0.92$, $p < 0.31$].

3.2.6. Medial frontal cortex

Separate two way repeated measures analysis of variance of binding density in the medial frontal cortex revealed no significant effect of age group [$F(1,14) = 2.04$, $p = 0.17$] but there was a significant overall effect of layer [$F(1,14) = 26.64$, $p < 0.0001$] and a group by layer interaction [$F(1,14) = 6.15$, $p = 0.02$]. Follow-up analyses with between group comparisons using a test of simple main effects revealed a significant difference between the groups in the superficial layers of the medial frontal cortex [$F(1,28) = 3.62$, $p < 0.05$] as shown in Fig. 2B with the aged group showing a significant reduction in α-2 adrenergic binding density. There was no significant difference between the groups in the deep layers [$F(1,28) = 0.80$, $p < 0.30$].

3.3. DA1 receptors

The binding densities for the DA1 receptor are illustrated in Fig. 2C. While there was an overall effect of layer in 4 of the 6 regions (no effect in the dorsolateral convexity and medial frontal regions), there was no overall effect of age or any age by layer interactions in any of the regions. Hence there was no evidence of an overall effect of age on binding density in any

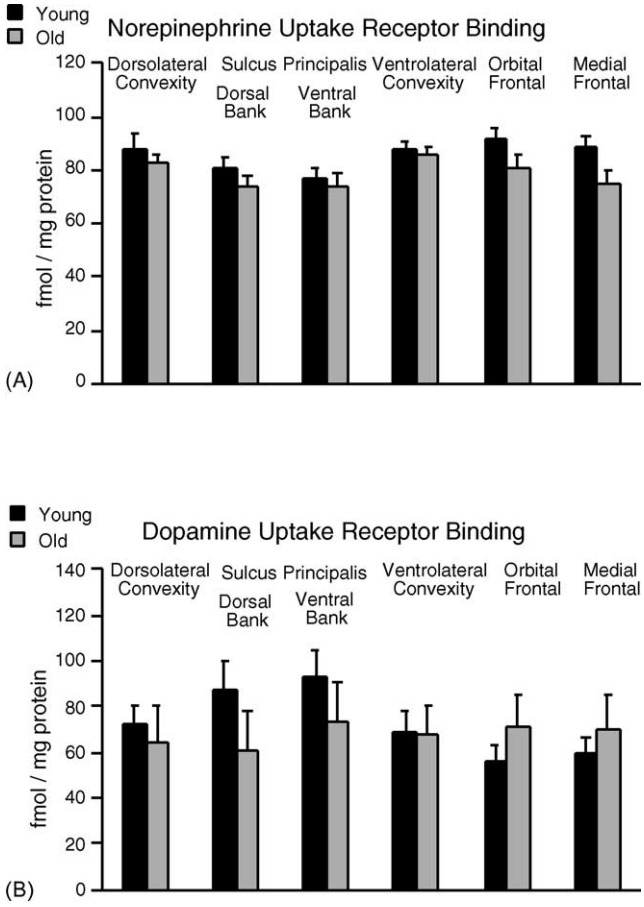

Fig. 3. These graphs depict the receptor binding densities for NEU (A) and DAU (B) receptor binding. The areas of cortex measured are displayed across the top of the bars, and the *y*-axis represents a density measure in fmol/mg of protein.

of the regions or layers examined so the detailed ANOVAs are not presented. These results indicate that in normal aging monkeys, despite evidence of loss of DA innervation, DA1 receptor binding density is stable across age.

3.4. Norepinephrine uptake (NEU) transporter

The results of receptor binding assays for the norepinephrine transporter are illustrated in Fig. 3A. Separate two-way repeated measures analysis of variance for binding density across all the regions examined revealed no overall effect of group [$F(1,14) = 1.61$, $p = 0.225$], region [$F(1,70) = 8.50$, $p = 0.0001$] or group by region interaction [$F(5,70) = 1.91$, $p = 0.10$] These results suggest that the NEU transporter is stable across age.

3.5. Dopamine uptake (DAU) receptors

The results of binding assays for the dopamine transporter are illustrated in Fig. 3B. A two-way repeated measures analysis of variance of binding density for all the regions examined revealed no effect of age group [$F(1,12) = 0.07$, $p = 0.791$] but there was a significant overall effect of region [$F(5,60) = 4.01$, $p = 0.003$] and a group by region interaction [$F(5,60) = 5.20$, $p = 0.0005$]. Follow-up analyses with tests of simple main effect revealed no significant difference between the age groups for any of the 6 regions examined.

3.6. Principle component analysis of relationship of binding changes to behavior

There were six to twelve regional density values for each of the five receptors examined and multiple behavioral outcome measures in the present study. In order to reduce the numbers of variables in this data set to a manageable and sensible number, we used a principle component analysis that is a factor analysis procedure. This allowed us to reduce the number of observed variables to a set of variables or principle components that best describes or accounts for the variance in all of the original variables. The first principle component accounts for the maximum possible variance that exists in all the variables. Then, the second principle component is essentially identical to the first except that it accounts for the variation remaining in the data after the variation attributed to the first principle component is removed from the analysis. Additional components are added until as many components have been computed as there are variables. To the extent that numbers of variables assess the same underlying processes, they will "load" onto the same principle component. As a result of this analysis, principle, multiple variables that load heavily on a single principle component can be "collapsed" or averaged together, resulting in a small number of derived variables.

3.7. Principle component analysis 1 (behavior)

For the DNMS and DRST the outcome variables included total errors, percent correct and total span. Given the small number of outcome variable for these tests a PCA appeared to be unnecessary. Hence, the Principle Component Analysis (PCA) was first applied to the error and perseverative error measures for each condition from the CSST and age to reduce the number of these variables in this data set. This analysis revealed that total errors in each condition loaded heaviest on a single factor, and perseverative errors as a percent of total shift trials loaded on a separate factor. On the basis of these loadings, the error measures (total errors for the red, triangle, blue and star conditions) were collapsed (averaged) together and perseverative errors as a percent of total shift trials was used as a second CSST measure (Table 3).

3.8. Principle component analysis 2 (receptor binding)

In PCA 2, standardized z-scores for the receptor binding values from each receptor subtype were used to determine which of these variables loaded together. For the α-1 adrenergic, α-2 adrenergic and DA 1 receptor subtypes, the PCA was computed on the correlational matrix of 13 variables; age, receptor binding in the superficial and deep layers

Table 3
This table shows the results for the principle component analyses to reduce the number of variable from the CSST and receptor binding experiments

PCA 1 reduced variables	PCA 2 reduced variables
Total errors (CSST)	Total α-1 adrenergic binding
Total PE as a % of Shift trials (CSST)	Total α-2 adrenergic binding
	Total NEU binding
	Total DA1 binding
	Total DAU Binding

of the medial frontal cortex, orbitofrontal cortex, dorsolateral prefrontal cortex, ventrolateral cortex, and the dorsal and ventral banks of sulcus principalis. For the NEU and DAU receptor subtypes, the PCA was computed on the correlational matrix of seven variables; age, receptor binding in the medial frontal, orbitofrontal, dorsolateral, ventrolateral and banks of the sulcus principalis. For all receptor subtypes, binding in all regions and all layers loaded heaviest on a single factor for each receptor. On the basis of these loadings, the values for all regions were grouped together to generate a single density value for each receptor (Table 3).

3.9. Relationship between binding and behavioral variables

Based on these findings total CSST errors, total CSST perseverative errors as a percent of shift trials, total DNMS errors, percent correct on DNMS delays, DRST span and the collapsed receptor binding values for each receptor, were used in five separate Pearson's product moment correlations to determine if there was a correlational relationship between any of these variables. The results are summarized in Table 4 and present in detail below according for each receptor.

3.10. α-1 Adrenergic and behavior

The principal component analysis revealed a significant linear relationship between α-1 adrenergic receptor binding in the PFC and perseverative errors as a percent of shift trials ($r = -0.725$, $p < 0.05$) (Table 4 and Fig. 4A) and errors to criterion on DNMS ($r = -0.820$, $p < 0.01$) (Table 4 and Fig. 4B).

Table 4
This table shows the results from the Pearson's product moment correlational analysis using the reduced variables from Table 3

Receptor	CSST % PE (of shift trials)	CSST errors	DNMS errors	DRST object
α-1 Adrenergic	0.05	N.S.	0.01	N.S.
α-2 Adrenergic	0.05	0.05	N.S.	N.S.
DA 1	N.S.	N.S.	N.S.	0.05
DAU	N.S.	N.S.	0.05	N.S.
NEU	N.S.	N.S.	N.S.	N.S.

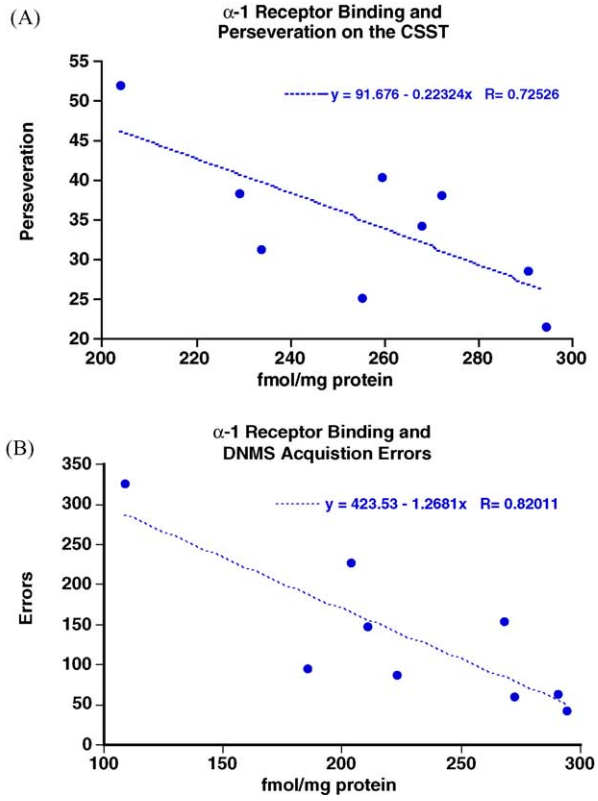

Fig. 4. These graphs depict the relationship between α-1 adrenergic receptor binding and perseveration on the CSST (A) and α-1 adrenergic receptor binding and DNMS acquisition errors (B).

3.11. α-2 Adrenergic and behavior

The Principal Component analysis revealed a significant linear relationship between α-2 adrenergic receptor binding in the PFC and total errors on the CSST ($r = -0.655$, $p < 0.05$) and (Table 4 and Fig. 5A), and perseverative errors as a percent of shift trials ($r = -0.675$, $p < 0.05$) (Table 4 and Fig. 5B).

3.12. NEU and behavior

The principal component analysis revealed no linear relationship between NEU receptor binding in the PFC and any of the behavioral measures in this analysis.

3.13. DA1 and behavior

Since there was a trend towards a significant age-related difference for the DA1 receptor and based on evidence in the literature of an age-related changes in this receptor it was included in this analysis. The principal component analysis revealed a significant linear relationship between DA1 receptor binding in the PFC and total span on DRST object ($r = 0.698$, $p < 0.05$) (Table 4 and Fig. 6A).

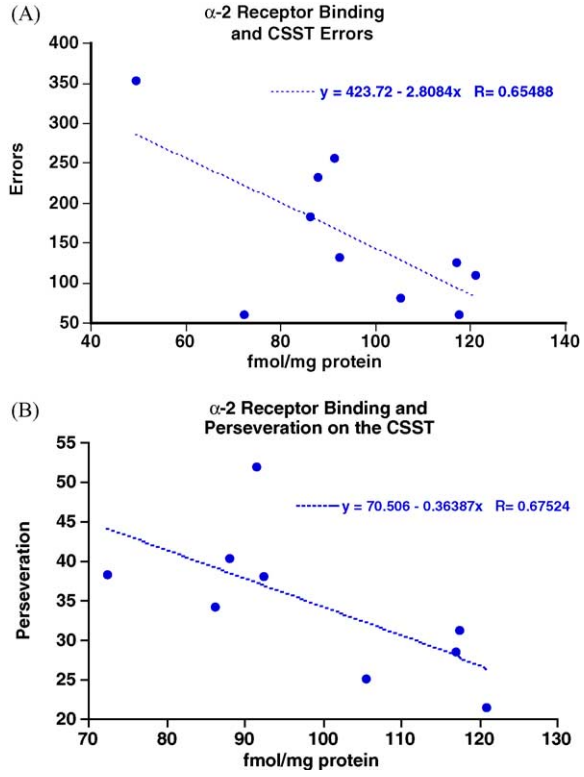

Fig. 5. These graphs depict the relationship between α-2 adrenergic receptor binding and CSST errors (A) and α-2 adrenergic receptor binding and perseveration on the CSST (B).

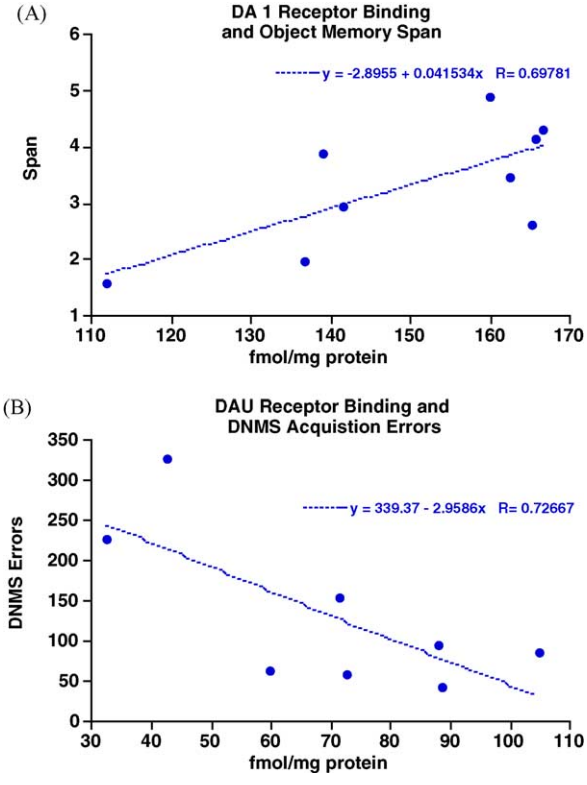

Fig. 6. These graphs depict the relationship between DA1 receptor binding and object memory span on the DRST (A) and DAU receptor binding and DNMS acquistion errors (B).

3.14. DAU and behavior

The principal component analysis revealed a significant linear relationship between DAU receptor binding in the PFC and errors to criterion on DNMS ($r = -0.727$, $p < 0.05$) (Table 4 and Fig. 6B).

4. Discussion

4.1. Receptor binding summary

The principal findings of this study were: (1) a significant decrease in α-1 adrenergic receptor binding density in the superficial layers of the dorsolateral convexity, ventrolateral convexity and sulcus principalis of aged monkeys; (2) a significant decrease in α-2 adrenergic receptor binding density in the superficial layers of the ventrolateral convexity, sulcus principalis, orbital frontal cortex and medial frontal cortex of aged monkeys; (3) no significant age-related decrease in the binding density of the dopamine DA1 receptor subtype in the PFC and (4) no significant decrease in the binding density of uptake receptors for either NE or DA in the PFC of aged monkeys. The stability of the uptake sites suggests that in normal aging both the NE and DA innervation are relatively preserved even though the levels of transmitter present may be reduced. In addition, the stability of the DA1 receptor suggests that dopamine neurotransmission for this subtype is relatively preserved. In contrast, the significant and widespread age-related reduction in the density of the α-1 adrenergic and α-2 adrenergic receptor subtypes suggests that noradrenergic neurotransmission is likely to be significantly altered despite the apparent preservation of innervation.

4.2. Behavioral correlation summary

The principle findings of the correlational analysis were: (1) a significant relationship between age-related reductions in α-1 adrenergic receptor binding and elevated perseverative responses on the CSST and with impaired acquisition (total errors to criterion) on DNMS; (2) a significant relationship between age-related reductions in α-2 adrenergic receptor binding and elevated total errors and perseverative responses on the CSST; (3) a significant relationship between DA1 receptor binding and memory span on the DRST object; (4) a significant relationship between DAU receptor binding and total errors on DNMS acquisition and (5) no relationship between NEU receptor binding and performance on the CSST, DNMS or DRST.

4.3. Age-Related changes in NE receptors

Age-related reductions in NE receptor binding were found in the superficial layers of the dorsal and ventral lateral convexities, both banks of sulcus principalis and in the orbital and medial frontal cortices. These areas approximately corre-

spond to Walker's areas 8, 9, 10, 11, 12, 14, 25 and 46 in rhesus monkey, which are areas of the PFC that contain high concentrations of NE fibers and receptors [24,37,38]. These findings are supported by several studies demonstrating marked decreases in α-2 adrenergic receptors in the PFC of aged humans, and decreased α-1 adrenergic and α-2 adrenergic receptors in the non-human primate [7,8,11,14,16]. Findings in rodents are quite variable though there does seem to be an overall tendency towards a decrease in all NE receptors with advanced age [27,30].

4.4. Age-related changes in DA receptors

The majority of studies quantifying receptor densities have found an age-related decrease in DA1 receptor density in the PFC [6,21,50,70]. In the present study, only post-synaptic DA1 receptor binding in the cortex was measured and was found not to be significantly affected by age. However, there was a trend towards significance ($p \leq 0.08$) in the dorsolateral prefrontal cortex. A power analysis revealed that to reach a statistical level of significance for the deep layers of the dorsolateral convexity approximately one more animal per group would be needed and five more animals per group would be needed for the superficial layers of the dorsolateral convexity. In addition, variability in binding density within the aged group may in part be responsible for falling just short of a statistical level of significance in this study. Interestingly, though there was not an overall group effect of age for this receptor, there was a significant correlation between DA 1 density and age-related impairment on the DRST object span. Finally, since the DA1 ligand used in this study only binds to the post-synaptic receptor, future studies with ligands that bind to the pre-synaptic receptor are necessary, as this group of receptors may account for the age-related changes in DA receptor binding widely reported in the literature.

4.5. Stability of DA and NE uptake sites

In the present study, there was no significant change in receptor binding density for either NE or DA uptake receptors in the PFC of aged monkeys. The stability of the uptake sites suggests that in normal aging both NE and DA innervation to the PFC are relatively preserved. However, another study in this same animal model demonstrated a significant loss of neurons in three DA brainstem nuclei, the substantia nigra pars compacta (SNpc), the paranigral (VTApn), and the parabrachial pigmentosus (VTApbp) nuclei of the ventral tegmental area, that project to the PFC [67]. Similar findings have been reported for age-related decreases in the density of projections from the locus coeruleus to the PFC [30–32,46]. However, there is also a marked increase in the degree of terminal arborizations and an increase in the electrical excitability of axon terminals of LC neurons in the PFC [31,32,66]. While a decrease in innervation would typically alter the uptake site, the changes in the terminal branching of the LC neurons may account for the stability in the NE uptake receptor binding in this study. Whether a similar process accounts for stability of DA uptake sites is unknown.

4.6. Possible mechanisms of age-related changes in NE receptors

Though age-related changes in the NE system have been demonstrated in a variety of different species, the precise mechanisms that underlie these changes are not understood. It is well known that NE receptors are located both presynaptically on nerve terminals and postsynaptically on somata and dendrites and act to modulate neurotransmission and neuronal activity in the PFC [8,17,24,64,65,71,77]. Given the marked decrease in NE levels and the increased turnover rate that occurs with age [11] one might expect compensatory upregulation of at least some of the NE receptor subtypes [73]. Since up-regulation was not observed for either the α-1 adrenergic and α-2 adrenergic receptors, it seems likely that age-related decreases in binding densities result from processes independent of those responsible for reductions in neurotransmitter levels.

One possibility is that changes in neuronal function and/or synaptic transmission may account for age-related changes in NE receptors. For example, there is an increase in the electrical excitability of LC neurons in the PFC with aging that would likely lead to increased release of NE and could subsequently cause a down-regulation of NE receptors as observed in this study [31,32,66].

An alternative explanation for the decrease in NE receptors in the PFC with aging may be related to specific age-related changes in layer 1 of the PFC. These changes include decreased thickness, significant degeneration of apical dendrites and loss of dendritic branches [56,62]. In conjunction with a loss of dendrites, there is a substantial loss of dendritic spines and synapses in layer 1 of the PFC [56,62]. These findings are of particular relevance to this study in that the decrease in receptor binding density was localized to the superficial layers of the cortex, including layer 1. Therefore, any degenerative changes in layer 1 could, very likely, underlie these decreases in receptor binding density.

4.7. Relationship of monoamine receptors and cognition

The use of a principle component analysis allowed for a comparison of several cognitive and receptor binding variables to determine the strength of the relationship between performance on DNMS, DRST and CSST and NE and DA receptor binding in the prefrontal cortex. This analysis revealed a relationship between α-1 adrenergic and α-2 adrenergic receptor binding and performance on the CSST and DNMS acquisition and between DA1 receptor binding and performance on the DRST object task.

The CSST is a test of abstraction and set shifting, which are cognitive functions thought to be mediated primarily by the PFC. In addition, the acquisition phase of the DNMS task, requires the monkey to abstract and apply the concept

of "choosing the novel object". The profile of performance observed in the aged monkeys on the CSST and DNMS-Acq resembles that of monkeys with lesions of the PFC (Walker's areas 46 and 9 and small portions of areas 8 and 10), which results in a high incidence of perseverative errors and an inability to shift set once established [48]. Similarly, these subjects required over 1200 trials to acquire the basic principle of the DNMS task which is three to four times as many trials as required by normal young monkeys [48]. Taken together, performance on the CSST and DNMS-Acq by monkeys with damage to the PFC suggests that, at least in part, these tests rely on the functional integrity of the PFC. It is not surprising therefore, that age-related impairments on these tasks are thought to be the result of frontal lobe dysfunction [19,26,29,34,39,45,59,60,76].

Cognitive functions subserved by the PFC include a variety of abilities such as abstraction, cognitive flexibility and set shifting that are typically referred to as executive function. These abilities are thought to be modulated in part by norepinephrine, which appears to be essential for reducing the effects of interference, directing attention and integrating cognitive processes [5,15,20]. In fact, aged monkeys, naturally depleted of catecholamines such as NE, are vulnerable to interference from irrelevant stimuli. Further, evidence has shown that deficits demonstrated by aged monkeys on frontal lobe tasks are reversed with the administration of postsynaptic α-2 adrenergic agonists [5,11,25]. Also, it has been demonstrated that the α-2 adrenergic agonists have the greatest effects on improving performance for stimulus trials with distractors, which supports the notion that NE action at the α-2 adrenergic receptors is involved in attention, control of interfering information and inhibition of irrelevant stimuli [5,15]. So while age-related reduction in NE levels has been identified as a contributor to age-related cognitive impairments, the correlation reported here between age-related reduction of α-2 adrenergic receptors and cognitive performance on tasks requiring the normal function of NE in the PFC suggests that these receptor changes may also contribute. Whether changes in NE levels and α-2 adrenergic receptors are part of a unitary age-related change or reflect separate processes remains to be determined.

Whereas evidence suggests that activation of α-2 adrenergic receptors facilitates cognitive processes of the PFC, norepinephrine actions at the α-1 adrenergic receptor appear to have a detrimental effect on cognition. In young monkeys, α-1 adrenergic agonists impair performance on spatial working memory tasks, though the administration of α-1 adrenergic antagonists does not further impair the performance of aged monkeys [9,10,15]. This suggests that the α-1 and α-2 adrenergic receptors may work together to maintain an "optimal" level of adrenergic tone for different behavioral situations.

The extensive evidence of the opposing roles of α-1 adrenergic and α-2 adrenergic receptors in the cognitive functions of the prefrontal cortex [9,10,12,41] supports the present findings of a relationship between impairments in cognition and decreased α-2 adrenergic receptor binding density but not the observed decreases in α-1 adrenergic receptor binding density. Administration of α-2 adrenergic agonists improves working memory in young and aged monkeys while similar administration of α-1 adrenergic agonists impairs performance [9,69]. Therefore, it is not surprising that a significant decrease in α-2 adrenergic receptors is related to impaired performance on the CSST and DNMS tasks. However, if activation of the α-1 adrenergic receptor impairs cognitive performance on tasks of working memory it would seem logical that a decrease in this receptor would not be related to impaired cognitive abilities. While it is possible that the correlation between the decrease in α-1 adrenergic receptors and cognitive performance does not reflect a true functional relationship there are two possible explanations that may account for these findings. First, in the present study, though there was a decrease in both α-1 and α-2 adrenergic receptors, the decrease in α-2 receptors was between 5 and 30% greater in the dorsolateral and sulcus principalis regions than for the α-1 receptor. Based on findings that these receptors are co-localized in the superficial layers of the PFC and are thought to be preferentially located on dendritic spines [24], a greater decrease in α-2 receptors may allow an increased activation of remaining α-1 receptors at any given level of NE release. This increased activation of the α-1 receptor would produce impaired cognitive function in the same way as systemic administration of α-1 agonists that cause high levels of α-1stimulation and PFC dysfunction [9,12].

However, while it is thought that the α-1 and α-2 receptors are likely co-localized on similar parts of the neuron, little is known about the precise location of the α-1 receptor (i.e., whether it is on the dendritic shaft or spine). A great deal is known though about the location of the α-2 receptor. The α-2 receptor is located most prominently on the dendritic spines post-synpatically but it is also located pre-synaptically on the axon [2,10]. Pre-synapatically, it functions as an autoreceptor and inhibits NE release [2]. This function as an autoreceptor provides a second possible explanation of the correlation between α-1 receptor binding density and cognitive impairment. If the reduction of the α-2 receptor occurs at its pre-synaptic sites as well as at postsynaptic sites then a decrease in this receptor could alter its ability to inhibit the release of norepinephrine and therefore result in increased norepinephrine levels in the synapse. This increase in NE may be activating the α-1 receptors and as a result causes impaired cognitive function. This notion is supported by findings in the aged rhesus monkey that high levels of α-1 receptor activation results in cognitive impairment [9]. While these two scenarios provide possible explanations for the relationship between decreased α-1 receptor binding density and cognitive impairment reported in this study, further investigation of the localization and the functional interaction of α-1 and α-2 receptors in the PFC is still needed.

While NE in the PFC may play a role in enhancing cortical functioning by reducing the effects of interference, dopamine appears to support neuronal processing necessary

for the temporal integration of behaviors [17,65]. The role of dopamine in this type of cognitive function is supported by the findings in this study that DA1 receptor binding densities were related to performance on the DRST with lower densities associated with reduced performance. The DRST, is a test of immediate memory capacity in which the monkey must identify, trial-by-trial, a new stimulus among an increasing array of serially presented familiar stimuli, requires temporal integration of behavior for successful completion of this task. Though there was no significant age-related decrease in DA1 receptor or DAU binding in this study, there was a modest decrease in binding in the dorsolateral convexity that while not statistically significant may have been sufficient to contribute to cognitive impairment on this task. Also, similar types of cognitive impairments on frontal lobe tasks are observed in aged animals naturally depleted of DA in the PFC and in young monkeys following the administration of DA1 antagonists, whereas the impaired performance demonstrated by the aged monkeys is significantly improved with the administration of DA1 agonists [6,17,54,65].

5. Conclusions

In this study, we have demonstrated that α-1 adrenergic, and α-2 adrenergic receptor binding density is significantly decreased in the PFC of aged rhesus monkeys. Also, there was a relationship between α-1 adrenergic, α-2 adrenergic and DA1 receptor binding and performance on tests of abstraction, set shifting, concept formation and working memory capacity. The importance of NE and DA for performance on tests of frontal lobe functions is supported by a wealth of evidence in the literature for the role of these two neurotransmitters in the cognitive functions of the PFC. Nevertheless, it is unlikely that decreased receptors alone account for the impaired performance by the aged monkeys. Instead, these changes in receptor density are likely one part of a cascade of neurobiological changes that occur in the brain that contribute to age-related cognitive impairment. Further investigation is needed to examine the relationship between the changes in the various neurobiological variables demonstrated in this animal model and how these changes relate to cognitive performance.

Acknowledgments

This research was supported by NIH grants R37-AG17609, PO1-AG00001 and RR00165. The authors wish to thank Sarah Hix. Mark Goh, and Reece Edwards for their valuable assistance with this project. The authors also wish to thank Ben Wallace at the Eunice Kennedy Shriver Center for the Glyph software we used to develop the Conceptual Set Shifting Task and Dr. Howard Cabral of the Boston University School of Public Health for his statistical expertise.

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