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COGNITIVE FUNCTION AND ITS NEURAL MECHANISMS IN NONHUMAN PRIMATE MODELS OF AGING, AlZHEIMER'S DISEASE, AND MENOPAUSE

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1. ABSTRACT

Nonhuman primates have been used as animal models in which to study cognitive changes associated with aging and age-related disease for decades. There are many advantages to using nonhuman primates for studies of aging including the capability to examine visual nonspatial cognitive processes and the ability to use operationally similar behavioral tasks to what is used with humans. Because some aspects of aging in humans do not develop naturally in nonhuman primates or do not follow the same course of natural development in monkeys, experimental models are necessary for some investigations. Research in our laboratory has identified similarities in the cognitive profiles of nonhuman primate models of aging, Alzheimer's Disease, and menopause with their human counterparts. In addition, through the use of a variety of different techniques we have used these nonhuman primate models to begin to determine the neural substrates of age-related cognitive dysfunction noted with advanced age and age-related disease. In this paper, we review our observations made in nonhuman primate models of aging, Alzheimer's Disease, and menopause and indicate areas for future research.

2. INTRODUCTION

Cognitive function gradually declines with advancing age and more precipitously deteriorates in Alzheimer's Disease (AD). Identification of the underlying neural mechanisms of these normal or neurodegenerative age-related cognitive changes is the subject of intense research in both humans and animal models. Nonhuman primates have been used as animal models in which to study cognitive changes associated with aging and age-related disease for decades. These animals are closest to humans in terms of phylogeny and in particular, development of many brain systems, especially cortex. The majority of research in nonhuman primate models of aging or AD have used macaque monkeys (rhesus and cynomolgus). Some aspects of aging in humans do not develop naturally in nonhuman primates or do not follow the same course of natural development in monkeys. Specifically, animals do not naturally develop AD with advanced age, and menopause in macaque monkeys occurs relatively late in the lifespan of the animal (1-3), thus significantly limiting the availability of sufficient numbers of naturally menopausal monkeys for study. Therefore, if one is interested in studying cognitive aspects of AD or of menopause in monkeys, it is necessary to use experimental models of these conditions to do so. Research in our laboratory has identified similarities in cognitive profiles between these animal models and their aged human counterparts and is focused on determining the neural substrates of these age-related cognitive dysfunctions. In this paper, we review these findings from our studies in nonhuman primate models of aging, AD, and menopause.

3. MODELS OF NORMAL AGING

Rhesus (*Macaca mulatta*) monkeys have been the most widely used Old World nonhuman primate for studies of normal aging. The estimated lifespan of a rhesus monkey is 35 to 40 years of age, with all major geriatric diseases increasing in incidence over the age of 20 years (4-6). Age-related cognitive impairments begin to occur around the age of 20 years in rhesus monkeys (7-8), with more significant impairments occurring after age 25 (7) Although it is commonly thought that aging in rhesus monkeys occurs at a rate of approximately three times that of humans, the ratio may not be uniform across every stage of life. Female rhesus monkeys are sexually mature at 2.5-3.5

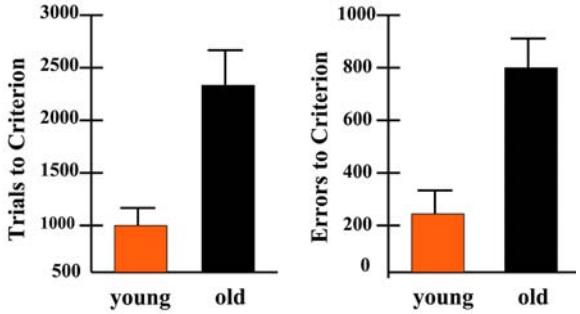

Figure 1. Acquisition of Delayed Response. Compared to young rhesus monkeys (n = 4) old rhesus monkeys (n = 8), were significantly impaired in their ability to learn a delayed response task as measured by both trials and errors to criterion. Error bars = SEM

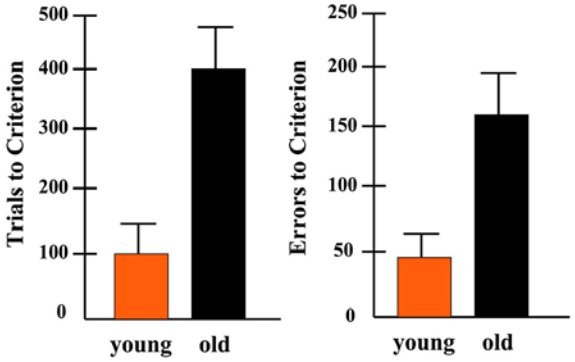

Figure 2. Acquisition of an Object Discrimination. Compared to young rhesus monkeys (n = 4), old rhesus monkeys (n = 8) were significantly impaired in their ability to learn a two choice object discrimination as measured by both trials and errors to criterion. (Data modified from (10)). Error bars = SEM

years of age (9-10) achieve adult stature around 8 years (11), and undergo menopause by about 27 years (1-2). These data suggest that the rate of aging in rhesus monkeys compared to humans may be 1:4 from birth to sexual maturity, 1:3 during young adulthood, and 1:2 before menopause. Although there is some debate over the specifics, in general, rhesus monkeys of 15-22 years of age are now considered to be of middle age, while those over 30 are considered to be of extreme old age. For the purposes of our discussion, old will be used to refer to monkeys that are older than 22 years of age.

3.1. Cognitive Studies

Normal aging in humans is accompanied by declines in a variety of functional domains, including aspects of learning, cognitive flexibility, memory, attention and motor skills (12-17). There are several caveats to these observations in humans, including that not all aspects of these functional abilities may be altered with advanced age, not all older individuals necessarily will demonstrate alterations in functional abilities, that cognitive performance can improve with repeated testing, and that not all functional abilities may be equally compromised by age within a given individual. Our research in old rhesus monkeys has demonstrated that functional aging in monkeys has many similarities with functional aging in humans, including the noted caveats.

Similar to elderly humans, old monkeys take longer to learn new information. This impairment in old monkeys has been observed in their ability to learn novel behavioral tasks. Acquisition of complex behavioral tasks is very difficult for old monkeys. One such task is the Delayed Response task (DR) in which the monkey must remember a previously cued spatial location through a delay period. Old monkeys are significantly impaired in learning DR compared to young monkeys (Figure 1). In a simpler task, behaviorally naïve old monkeys required four times as many trials to learn an object discrimination than young monkeys (Figure 2; 18). Analyses of backward learning curves revealed that many of the old monkeys performed near chance levels on this discrimination for a longer time than young monkeys, however performance was overlapping between the groups as they neared achievement of criterion (18). These old monkeys also demonstrated some difficulty learning a subsequent pattern discrimination, however they were comparable to young monkeys in learning additional visual discriminations and a spatial discrimination (18). These studies were the first to clearly demonstrate that acquisition of an object discrimination problem is impaired with advanced age in behaviorally naïve monkeys, but that continued exposure to similar behavioral paradigms diminishes this impairment. Findings from other laboratories support this notion of the influence of previous behavioral testing, in that learning discrimination problems is not impaired in old monkeys with prior exposure to complex behavioral paradigms or to discrimination problems (7, 19-23).

Reductions in cognitive flexibility, e.g. the ability to shift from one strategy of problem solving to another, occur with advanced age in humans (24) and in monkeys. Until recently, this functional construct conventionally has been tested in old monkeys through the use of the reversal discrimination task. In this task, the subject first learns a discrimination problem. Following this original learning, the reward contingencies of the stimuli are reversed so that now the previously unrewarded stimulus becomes the rewarded stimulus and vice versa. Thus, the subject must unlearn the original stimulus-reward association and learn a new one. Some studies of old monkeys have observed reversal learning impairments (19, 25), while others have not (22, 23). As before, prior behavioral testing experience may have influenced the outcomes in several of these investigations. We found that old monkeys, who were not subjected to prior behavioral testing, were impaired in learning visual discrimination reversals (18). Interestingly, the impairments in reversal learning by the old monkeys in our study were resolved with continued testing of reversal problems indicating that, like older humans, old monkeys can benefit from repeated practice. Further analyses indicated that the old monkeys were impaired at different stages of reversal learning depending on whether the animals were learning an object or a pattern discrimination (18). In learning object discrimination reversals, the old monkeys performed below chance for a significantly greater period of time, indicating that the animals were exhibiting

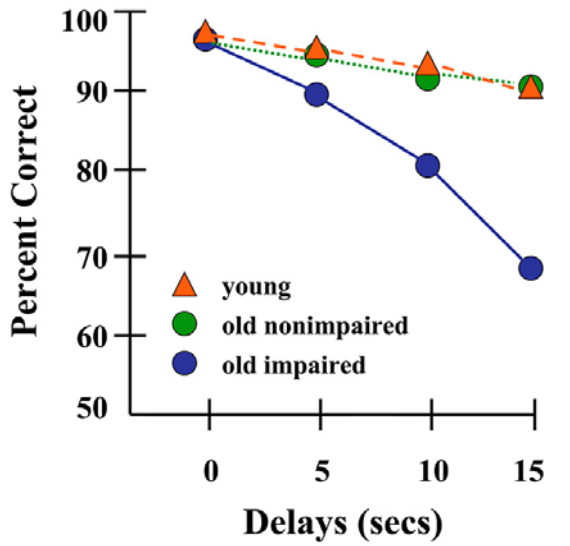

Figure 3. Memory in a Delayed Response Task. Some old rhesus monkeys (old nonimpaired; n=4) performed at comparable levels to young monkeys (n = 4) in a delayed response task, while other old monkeys of comparable age (old impaired; n=4) exhibited significantly greater difficulty with increasing delays.

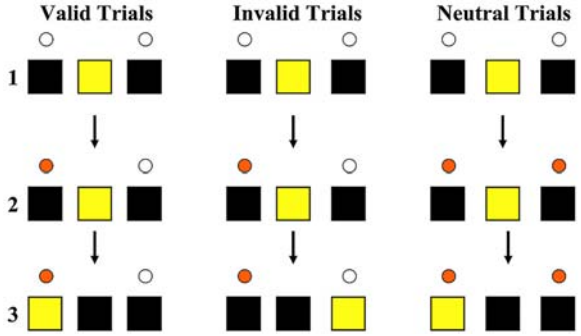

Figure 4. Visuospatial Attention Task. 1 = central square illuminated for 1-3 sec. 2 = peripheral cue illuminated for 200 msec while central square remains illuminated. 3 = target stimulus is illuminated. See text for details.

good retention of the original learning and demonstrating strong perseverative tendencies. In contrast, in learning pattern discrimination reversals, the old monkeys spent a significantly greater period of time performing at chance levels in their attempt to establish the new stimulus-response association. Only one other study of old monkeys has performed an analysis of reversal learning stages and, in agreement with our study, found that old monkeys spent significantly greater periods of time performing below chance than young monkeys during learning of the reversal of an object discrimination (23). Recently, age-related impairments in cognitive flexibility in monkeys was demonstrated in a monkey version of the Wisconsin Card Sorting Task in which old monkeys displayed a large number of perseverative errors (26) in parallel with observations made in elderly humans on this task (27).

Recent memory also is impaired with advanced age in monkeys. Numerous studies have demonstrated that older monkeys have significantly greater difficulty remembering spatial information as delays are increased in DR (7, 28-31). Parallel impairments in delayed memory for spatial location have been reported in aged humans on a similar delayed spatial recognition task in which the subjects must remember which room of a house had been illuminated (32). In our own laboratory, both young and old monkeys displayed changes in DR performance with increasing delays, however some of the old monkeys were more sensitive to the increased memory demands (Figure 3; 33). Despite the overwhelming data that support the notion that spatial recent memory is impaired with age in monkeys, there is evidence that spatial memory can improve with practice in old monkeys and that not all old monkeys have spatial memory deficits. For example, old monkeys showed considerable improvements in correct responding at long delays with repeated exposure to DR (30, 34). We made similar observations when we were determining the appropriate delays to use in DR to obtain an accuracy function that ranged from 100% - 65% correct. We found that old monkeys continued to improve their accuracy with repeated practice over 5 months so that the delays continually had to be lengthened to maintain a 65% accuracy level at the longest delay (Voytko, unpublished observations). In addition, as illustrated in Figure 3, we found that old monkeys are not homogenously affected by the increasing delays in DR indicating that individual variations in decline of cognitive abilities exist within populations of old monkeys as in older humans (35-38). Individual variability in cognitive performance across old monkeys also was evident in our studies of discrimination learning and reversal learning. In circumstances in which the old monkeys as a group were no longer significantly different from young monkeys, individual old monkeys demonstrated greatly impaired performance compared to the young monkeys or to the other old monkeys (18). Moreover, within an individual old monkey, not all functional abilities were affected equally (18).

Several attentional processes decline with age in humans (13, 39-41) and others appear to be relatively unaltered (42-43). Interestingly, attentional processes have not been well investigated in old monkeys. Other than a study by Davis (44, pp. 164-169), which demonstrated that old monkeys are more distracted during initial registration of information, the extent to which aging alters attention in monkeys is unknown. Because attentional processing of information controls allocation of processing resources to incoming stimuli, the integrity of attention processes is an important issue in old monkeys as deficits in attention could produce behavioral impairments that are unrelated to the specific cognitive demands of the tasks. To begin to address this issue, we investigated visuospatial attention abilities in old monkeys using a peripherally-cued task that was adapted from one developed by Posner (45-46). In this task, an advanced peripheral cue identifies the probable location of a target appearing in one of two spatial locations (Figure 4). The cue is referred to being "valid" when it appears in the same location as the target, being "invalid" when it appears in the opposite location as the target, or being "neutral" when it provides no information about the spatial location of the target. Decreases in reaction time

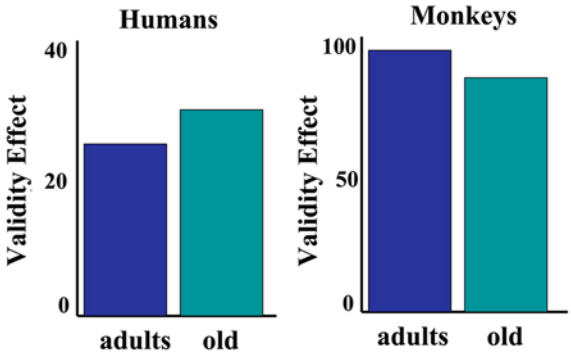

Figure 5. Comparison of Human and Monkey Performance on the Visuospatial Attention Task. Validity effect refers to the difference in response time at the appearance of the target stimulus on invalid trials compared to valid trials. Although the absolute response times differ between monkeys and humans, there is no difference in the validity effect with age in either monkeys or humans. (Human data modified from (40). Monkey data modified from (43)).

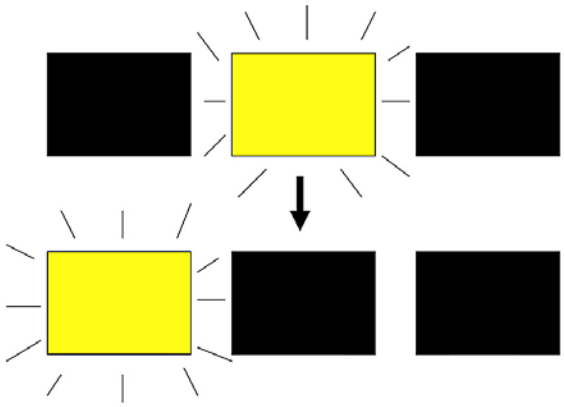

Figure 6. Simple Reaction Time Task. See text for details.

(benefits) occur when an advanced cue correctly directs a subject's attention to the target location (valid trials), while increases in reaction time (costs) occur when the cue incorrectly directs attention to a location because of the need to "shift" attention to the true target location (invalid trials) (45). The probability of valid:neutral:invalid trials was 70:15:15 and the dependent measure was the time to release hold on a center screen at the appearance of the target. This task was chosen for our assessments of attention in old monkeys for two major reasons. First, the old monkeys in our colony had significant difficulty on DR in which a peripheral cue identifies the spatial location that is to be remembered (33). An impairment in shifting or orienting attention to a spatial location could impair DR performance even if mnemonic ability were intact. Thus, it was important to determine if shifting of attention to a peripherally-cued location was impaired in these old monkeys. Second, the efficiency with which attention is shifted to a peripherally-cued spatial location is unaltered with normal aging in humans (42-43, 47-49**).** Because one of our aims in studying old monkeys is to identify the extent to which functional aging is similar in monkeys and humans, we were interested in determining if shifting of attention to a peripherally-cued location was unaltered with normal aging in monkeys.

We found that old monkeys demonstrated equivalent proficiency as young adult monkeys at shifting attention to a peripherally-cued spatial location as measured by the difference in reaction time on invalid and valid trials (validity effect) (Figure 5; 50). Both young adult and old monkeys had faster reaction times following valid cues and slower reaction times following invalid cues indicating that the monkeys were using information provided by the advanced cues. In general, the costs, benefits, or combined costs plus benefits (validity effect), associated with attention to the cues were equivalent in young adult and old monkeys in three separate experiments. Furthermore, facilitation of processing at the cued location was not affected differentially by age, was most apparent at a stimulus onset asynchrony of 200 msec, and was eliminated at longer asynchrony durations (50). Our findings in old monkeys are in direct correspondence with reports of intact orienting of attention in aged humans when examined with similar peripherally-cued tasks (42-43, 47-48). In those studies, young adult and aged subjects were faster in responding to targets following valid cues than following invalid cues and age did not alter the combined costs plus benefits (validity effect) associated with attention to a peripheral cue (Figure 5). Moreover, the time course of facilitation of processing the peripheral cues in young adult and aged humans were analogous to that of our study of young adult and old monkeys. Thus, orienting of attention to peripheral cues is preserved with age in humans and monkeys. The extent to which other aspects of attention may be altered with age in monkeys remains to be determined.

Although some studies have measured response speed during cognitive testing of old monkeys, few studies have determined if basic reaction time capabilities are altered with age in monkeys. Using a simple reaction time task, we found that old monkeys were comparable to young monkeys in their motoric ability (50). The task was similar in many respects to the attention task that we used except that it did not have advanced cueing (Figure 6). In this task, the monkeys had to release hold of a centrally-illuminated screen and respond to a peripherally-illuminated side screen ("target"). The target always appeared in the same spatial location and the duration of target appearance was decreased incrementally until the time at which the monkey could no longer make a response while the target was available. Fastest reaction time was defined as the shortest time in which the target could appear and a response to it made. The fastest reaction time achieved by old monkeys was comparable to that achieved by young monkeys. Moreover, the number of trials to achieve the fastest reaction time was equivalent in young and old monkeys. There were no mnemonic or unpredictable elements in the simple reaction time task used in our experiment (50) compared to other studies of reaction/response time in old monkeys (7, 25, 51), and our findings parallel reports that simple reaction time is not increased with age in people when task parameters are kept stable (52-54).

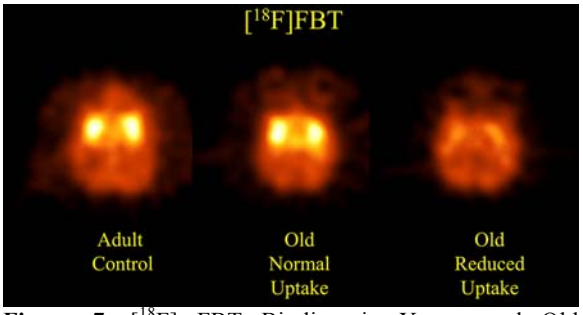

Figure 7. [^{18}F] FBT Binding in Young and Old Monkeys. Striatal binding of [^{18}F] FBT in a young adult monkey and in two comparably aged older monkeys. There is individual variability in striatal binding of [^{18}F] FBT in old monkeys (Data modified from (76)).

3.2. Neurobiological Studies

The aging primate brain undergoes structural and chemical changes that likely are responsible for the functional alterations noted in aging populations. Many of the age-related brain changes that occur in humans also occur in monkeys (6, 8, 55, 56). In both instances, these brain changes are not global but tend to be region-specific and sometimes variable. It is this regional specificity and variability that may play an important role in the individual variations in behavior that have been noted with aging in people and monkeys. As a first approach to address this issue, our laboratory has examined age-related changes that occur in the cholinergic system in monkeys because this neural system has been implicated in a number of cognitive functions that we have assessed in monkeys, including memory and attention (57-60).

We have used different approaches to determine the integrity of the cholinergic system in old monkeys and we have begun to determine its relationship to age-related changes in cognitive function. One approach was to examine the cholinergic neurons themselves located in the nucleus basalis of Meynert (NBM) in the basal forebrain, which is the major source of cholinergic input to the telencephalon (61-62). Numbers of magnocellular Nissl-stained neurons in the NBM or numbers of cholinergic neurons labeled with antibodies to p75 nerve growth factor receptor were not altered with age in monkeys (63). However, the size of these neurons was larger overall in the old monkeys compared to young monkeys, especially in the posterior subdivisions of the NBM. A subset of these monkeys had been extensively tested on a variety of cognitive and motor tasks shortly before death (7, 64), thus presenting an unique opportunity to assess the interrelationships among age, behavior, and morphometric measures in the NBM. Partial correlation analyses revealed that increased age was associated with declines in performance on several behavioral tasks, independent of cholinergic cell number. More importantly, independent of age, decreased numbers of cholinergic neurons in the intermediate regions of the NBM were associated with poorer spatial recent memory on a DR task and difficulty learning concurrent object discriminations (63). These relationships are consistent with the innervation of lateral prefrontal and lateral temporal cortices by the intermediate regions of the NBM and the involvement of these brain regions in these behaviors.

Another means by which the integrity of neural systems in aged individuals has been determined, without the need for autopsy material, is through *in vivo* functional imaging studies using Positron Emission Tomography (PET). Our laboratory was one of the first to use PET methodology to assess the effects of age on the integrity of the cholinergic system in monkeys. For our PET studies, we used [^{18}F]fluorobenzyltrozamicol ([^{18}F] FBT) as the cholinergic radiotracer because it has a high affinity and selectivity for the vesicular acetylcholine transporter (VAChT) (65-68) and binding of this radiotracer is correlated with ChAT activity (69). Thus, this ligand provides information about the presynaptic functional activity of the cholinergic system. In our initial study, we demonstrated that [^{18}F] FBT has an appropriate binding profile for use in PET studies of the VAChT in primates (70). In this study, the highest accumulations of [^{18}F] FBT that could be visualized with the 6 mm anatomical resolution of the PET scanner were found in the basal ganglia, which is consistent with the labeling of cholinergic interneurons in this region, the distribution of [^{3}H]hemicholinium-3 binding in rhesus monkey and human brain (71), and the *in vivo* binding of other radioactive vesamicol analogs (67, 72-75). To determine if functional activity of the cholinergic system was altered with age, we performed PET studies using [^{18}F] FBT in male rhesus monkeys that ranged in age from 10-37 years (76). Binding of [^{18}F] FBT was reduced with advancing age. However, we also found individual differences in the binding of [^{18}F] FBT in older monkeys, in that binding in some old monkeys was comparable to that observed in young monkeys (Figure 7). Indeed, only 17% of the variance in [^{18}F] FBT binding was associated with age suggesting that although presynaptic cholinergic function may decrease with aging, there may be differential susceptibility of the cholinergic system to the aging process in different individuals. Further analysis of the data revealed that the individual differences in the binding of [^{18}F] FBT among the older monkeys were not related to chronological age. These individual differences in cholinergic activity of aged rhesus monkeys, that are beginning to be revealed by PET, serve to emphasize the individual variation that occurs in the neurobiology of the brain of old monkeys. This neurobiological variability may underlie some of the variations in cognition that we and others have noted in aged rhesus monkey populations.

4. MODELS OF ALZHEIMER'S DISEASE

Alzheimer's Disease (AD) is an age-related neurodegenerative disease that is characterized by severe alterations in cognitive and noncognitive behavioral functions, and by significant neuropathological and biochemical changes in the brain. Because animals do not naturally develop AD, experimental animal models have been created to investigate specific components of the disease. Of particular interest has been the determination of how specific structural or chemical changes in the brain contribute to the behavioral symptoms noted in patients with

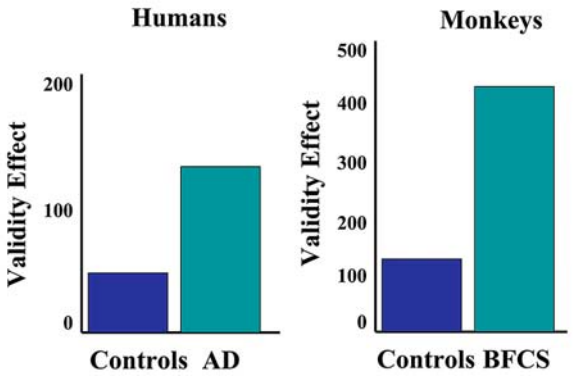

Figure 8. Performance on the Visuospatial Attention Task in patients with Alzheimer's Disease and in Monkeys with Basal Forebrain Lesions. Although the absolute response times differ between monkeys and humans, there is a greater validity effect in patients with Alzheimer's Disease (AD) and in monkeys with basal forebrain lesions (BFCS) than in their respective controls. In both cases, the greater validity effect reflects a greater response time on invalid trials compared to valid trials. (Human data modified from (77). Monkey data modified from (52)).

AD. While pathological alterations occur in many different systems of the AD brain, the changes that occur in the basal forebrain cholinergic system (BFCS) and their relationship to altered cognitive function have been of considerable interest. The focus on the BFCS in AD was precipitated by an hypothesis put forth by Bartus and colleagues in the early 1980's which stated that dysfunction of cholinergic neurons in the BFCS may be partially responsible for the learning and memory deficits of aging and AD (77). Many different lines of evidence were used to formulate this hypothesis including early pharmacological, neurochemical and neuropathological studies performed in humans and monkeys that highlighted the similarity in the nature of cognitive impairments in old monkeys, aged humans, and AD patients, and that also indicated that the integrity of the BFCS was associated with cognitive function (78).

4.1. Cognitive Studies

To understand how altered BFCS function contributes to the cognitive impairments observed in AD, we conducted a large systematic study of monkeys with BFCS lesions (57). At the time, the majority of studies of this issue had been performed in rodents or in monkeys with either restricted BFCS lesions or who were tested only for visual memory abilities (reviewed in 79). In our study, monkeys were preoperatively characterized on a series of behavioral tasks that tapped into different cognitive domains and modalities, including delayed nonmatching-to-sample (DNMS), object discriminations, delayed response (DR), and visuospatial cued attention. In a two-stage surgery, magnetic resonance imaging was used to place ibotenic acid injections throughout the basal forebrain to model the degeneration in this region in patients with AD. Neuropathological and neurochemical analyses conducted at the conclusion of the study, almost one year following the neurotoxic injections, demonstrated damage throughout the BFCS, with greatest cell loss in the NBM which projects to cortex (61), and significant reductions in choline acetyltransferase activity throughout the cortex (57). Following the neurotoxic injections, monkeys were postoperatively assessed on the same series of behavioral tasks, on more demanding versions of the behavioral tasks, and on novel tasks. Although spatial memory in DR and visual recognition memory in DNMS were sensitive to increasing memory demands with increasing delays or increasing stimuli, memory was not disproportionately disrupted in the monkeys with lesions compared to control monkeys (57). Similarly, learning and retention of object discriminations with simultaneous or concurrent presentations also was not impaired following BFCS lesions. In addition, spatial and object discrimination reversal problems were learned comparably by control monkeys and by those with BFCS lesions (57).

In contrast to the findings on learning and memory, the BFCS lesions in monkeys disrupted orienting of visuospatial attention in a peripherally-cued attention task that had been shown to be sensitive to attentional impairments in patients with AD (80-82) and that we used in our studies of old monkeys (see Figure 4; 50). Similar to AD patients, monkeys with BFCS lesions were generally slower in responding than control monkeys, particularly when targets appeared in unexpected locations in invalid trials (57). The large difference in reaction time between invalid and valid trials resulted in a greater validity effect in both AD patients and in monkeys with BFCS lesions (Figure 8). These observations indicated that monkeys with BFCS lesions, like patients with AD, have an impairment in attention that may involve difficulty in shifting attention to a particular spatial location. The impairments in the visuospatial attention task were not related to motor difficulties as time to touch the target was equivalent in control and lesion monkeys (57).

4.2. Neurobiological Studies

We performed pharmacological studies to determine the extent to which the cholinergic system is compromised following neurotoxic injections in the BFCS of monkeys. We determined if lesions of the BFCS in monkeys increased sensitivity to anticholinergic drugs by evaluating responses to injections of scopolamine in the DNMS task that assesses visual recognition memory (57). Reactions to scopolamine were assessed by determining the "most effective dose" of scopolamine for each monkey that produced a particular memory function on the task and by determining the magnitude of response to several doses of scopolamine. These two measures of responses to scopolamine indicated that monkeys with BFCS lesions were more sensitive than control monkeys. First, having equated performance between lesion and control monkeys on DNMS, monkeys with BFCS lesions required half the dose of scopolamine of control monkeys to produce similar levels of impairment. Second, the magnitude of impairment in response to a 0.04 mg/kg dose of scopolamine was greater in the monkeys with lesions. The increased sensitivity to scopolamine on DNMS in monkeys with BFCS lesions mirrored the enhanced sensitivity of AD patients to scopolamine challenges (83-85).

Although we were not the first to examine cognitive function in a monkey model of AD with BFCS lesions, our study was seminal in several respects. First, by virtue of the large number of neurotoxic injections made throughout the BFCS, and in particular the NBM, damage produced by our lesions was greater than that produced in other monkey studies. Second, the breadth of types of learning and memory and modalities tested in our study was greater than what had been assessed in previous monkey studies. For example, previous studies of monkeys with ibotenic acid lesions of the BFCS only examined performance on the DNMS task (86-87) or on visual or spatial discriminations (88-90).Third, the assessment of attention function in monkeys with BFCS lesions was completely novel and the similarity in the visuospatial attention deficit in monkeys with BFCS lesions and in patients with AD was the first demonstration of parallel cognitive impairments using the same behavioral task in a monkey model of AD.

5. MODELS OF MENOPAUSE

Cessation of ovarian estrogen production with menopause occurs around the age of 50 years in women and has been associated with changes in somatic physiological functions**.** Cognitive processes, including memory and attention, also may be altered with menopause in women and improved following ovarian hormone therapy, although these issues are still controversial (91-95). The cognitive changes noted by postmenopausal women may be related not only to the loss of estrogen, but also to other factors including advancing age.

Until recently, the rodent has been the most ubiquitous animal model used to study the effects of ovariectomy (OVX) or estrogen replacement therapy (ERT) on cognitive or neurobiological function. The use of the female nonhuman primate as a model for menopause is in its infancy. This fact is surprising given that nonhuman female primates, unlike rodents, share many reproductive and endocrine features in common with women. In particular, female macaque monkeys have 28 day menstrual cycles and patterns of ovarian hormone fluctuations that closely resemble those of women (96-97), they undergo a similar menopause (1, 2, 98), and they have physiological responses to surgical menopause and ERT that are similar to women (99-101). Rodents, on the other hand, have a four-day estrous cycle and cessation of ovarian function in rodents does not closely resemble primate menopause (102). Moreover, the use of nonhuman primates provides the opportunity to investigate the effects of ovarian hormones on cognitive domains in different modalities, aside from primarily spatial memory that has been the focus of rodent studies.

5.1. Cognitive Studies

The fact that cognitive changes noted by postmenopausal women may be related to the loss of estrogen or to other factors that also influence cognitive abilities, including advanced age, has made it difficult to determine the independent or interactive contributions of these factors to the cognitive function of women. To begin to address this issue in a nonhuman primate model of menopause, we assessed the cognitive function of young adult monkeys following OVX and ERT in a series of experiments (103-104). In this manner, the effects of estrogen alone on cognitive abilities could be examined. A series of behavioral tasks were used to assess cognitive function in monkeys preoperatively, following OVX, and following ERT. The behavioral tasks tapped into different functional domains and included DR (spatial memory), visual discriminations (associative learning and retention), discrimination reversals (cognitive flexibility), visuospatial cued reaction time (visuospatial attention), and simple reaction time (motor skills). Performance on DR, visual discriminations and reversals, and simple reaction time were not affected by 24 months of OVX or by 14-16 months of ERT (103-104). Our findings of an absence of an effect of OVX or ERT on aspects of spatial and visual memory in young monkeys was recently corroborated in a study that examined surgically menopausal young monkeys treated with placebo or ERT over 8 months (105). In contrast to our findings in memory, visuospatial attention function in our peripherally-cued attention task (see Figure 4) was disrupted as early as one week following OVX in monkeys, with significantly increasing impairments obvious by two months postoperatively (104). These visuospatial attention impairments were reversed with ERT, with improvements noted within one month of treatment initiation and being sustained through the 14 months of treatment observation (104). These findings from our studies in young adult monkeys indicated that loss of ovarian hormones, without the influence of other confounding factors, could alter visuospatial attention function and that treatment with estrogen could reverse these impairments. These observations in monkeys suggest that loss of estrogen alone may be contributing to the attentional impairments noted by postmenopausal women. Various forms of attention may be altered in postmenopausal women (106-112) and it will be important for future studies in monkey models of menopause to determine the extent to which loss and replacement of estrogen affects diverse types of attention function.

The observations of an absence of an effect of OVX or ERT on spatial memory in young monkeys are in contrast to numerous studies in young adult rodents in which spatial abilities have been shown repeatedly to be affected by OVX and ERT (113-116). Several points need to be made regarding this apparent species difference. First, spatial memory typically is assessed in rodents as they physically navigate through mazes, while spatial memory in monkeys typically is assessed in operant chambers in which the animals are stationary. Given that cells in the hippocampus of monkeys fire differentially on the basis of whether an animal is stationary or moving during testing of spatial memory (e.g., 117-120), these differences in task parameters between monkeys and rodents may be critically important. Second, a variety of different behavioral paradigms have been used to assess spatial memory in rodents, many of which do not have parallels with those used to assess spatial memory in monkeys. However, the use of similar paradigms may lead to similar observations. Specifically, our findings that OVX and ERT in young

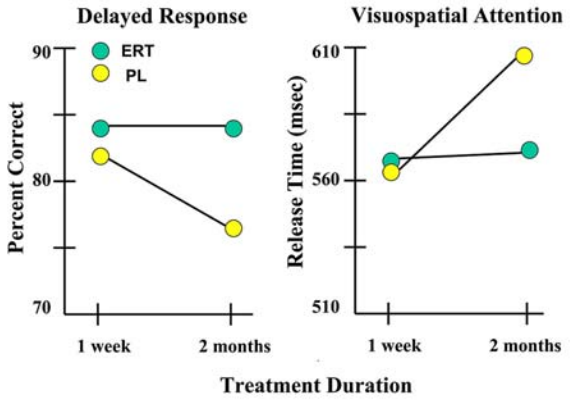

Figure 9. Performance of Middle-Aged Ovariectomized Monkeys on a Delayed Response and Visuospatial Attention Task. For both tasks, monkeys treated with estrogen (ERT; n = 3) maintained their performance levels during two months of treatment, while monkeys treated with placebo (PL; n = 3) demonstrated worse performance. Data are collapsed across delays or trial types.

adult monkeys did not affect spatial memory in DR are in agreement with the equivalent performance of OVX and ERT rats in the conceptually comparable spatial memory task of delayed nonmatching-to-position (121). Third, it appears that either acquisition or memory of spatial information may be more sensitive in rodents to manipulation of estrogen depending upon the specific task. Indeed, while acquisition of a delayed nonmatching-to-position task in rodents was impaired by OVX, memory performance in the task was not (121). We only tested spatial memory performance in DR in young OVX monkeys and do not know if acquisition of the task may be more sensitive to OVX or ERT.

Our studies were the first in a nonhuman primate model of menopause to determine the effects of ovarian hormones alone on cognitive processes. However, age may interact with the loss of ovarian hormones to influence cognitive function in the middle-aged postmenopausal woman, particularly learning and memory which were found to be unaffected by estrogen states in the young adult monkeys of our studies. Indeed, a small group of naturally peri/postmenopausal monkeys exhibited impairments in acquisition and performance of DR compared to similarly aged premenopausal monkeys or to young adult control monkeys (122). However, there was not an interaction between group and delay in this study indicating that the DR performance impairment was not related to deficits in memory per se, but may be related to deficits in attention or other cognitive domains. Importantly, the premenopausal older monkeys also were impaired relative to young controls indicating that the age-related deficit in DR was not entirely related to endocrine status.

Because of limited resources of very old monkeys and the fact that natural menopause occurs relatively late in the lifespan of female monkeys (>27 years) (1-3), it is extremely difficult to amass a sufficient number of naturally menopausal monkeys to conduct large-scale well-designed studies. A more direct and controlled approach to addressing the interaction of advanced age and loss of estrogen in monkeys will be the use of the OVX model. In one recent study, middle-aged (mean of 22 yrs) female monkeys were ovariectomized and after waiting 30 weeks, treated with estrogen and behaviorally characterized (123). Placebo monkeys generally were impaired relative to ERT monkeys in the DNMS test of visual recognition memory and in DR (123). However, in both instances, the placebo monkeys were not disproportionately different in their performance at the longer delays relative to ERT monkeys. These observations again suggest that other cognitive domains may be more sensitive than spatial memory to ovarian hormones, even in older females.

We addressed the issue of whether age interacts with estrogen loss to affect cognitive processes beyond memory function in a preliminary study of six middle-aged (22 years) female rhesus monkeys (124). Monkeys were trained on both the DR and our peripherally-cued visuospatial attention task. Following OVX and ERT, the monkeys were reassessed on both tasks at one week and two months following treatment initiation. In both spatial memory and visuospatial attention, placebo monkeys demonstrated decreased performance across the two months, while ERT monkeys maintained their performance (Figure 9). These observations in middle-aged surgically menopausal monkeys differ from those we made in younger surgically menopausal monkeys in which only visuospatial attention function was affected by OVX or ERT (103-104). Collectively, these data suggest that OVX and ERT may affect multiple cognitive domains with advancing age, including attention function.

The duration of estrogen deficiency may be an important factor in determining the cognitive domains that respond to ERT when it is initiated. Many postmenopausal women do not begin ERT immediately and therefore are in an estrogen deficient state for many years before initiating ERT. How well cognitive functions respond to this delayed ERT is unknown and has not been well studied in rodent models of menopause. To begin to address this issue, a small group of monkeys (19-27 years), who had been ovariectomized 8-18 years (mean of 13 years) previously, were behaviorally characterized prior to and following ERT (125). In comparison to intact monkeys, long-term OVX impaired visual recognition memory in DNMS, but had no effect on either visual or spatial discriminations or their reversals. Treatment with estrogen had no effect on performance in DR or DNMS, however, ERT did improve spatial memory abilities in a delayed recognition span task (126). In concert, studies in older female rhesus monkeys demonstrate that ERT given immediately (124) or within six months (123) following OVX can improve performance in DR and DNMS, but that ERT given many years after OVX in older monkeys has no influence on performance in these memory tasks (126).

Thus to date, only six full studies have been performed in female monkeys to examine estrogen's effects on cognitive processes; two in young adults and four in older animals. Five of these studies were intervention studies, i.e. ERT was provided at some point

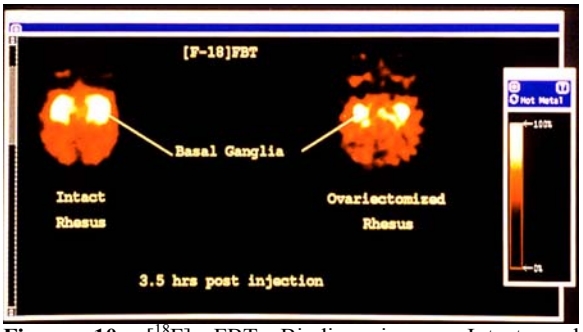

Figure 10. [^{18}F] FBT Binding in an Intact and Ovariectomized Monkey. Striatal [^{18}F] FBT binding is reduced in the ovariectomized monkey compared to the intact monkey. The intact monkey was scanned during the follicular phase of her menstrual cycle.

following OVX, with the duration since OVX before ERT varying from 2 months (103-104) to 18 years (126). The ERT regimens employed were different across studies and included silastic implants that delivered continuous ERT (103-104), daily oral administration (126), and cyclical regimens of injections every three weeks (123). Despite these differences, these studies collectively suggest that attention function may be more sensitive to OVX and ERT than other cognitive domains in young adult monkeys, but that multiple cognitive domains are sensitive to ovarian hormone manipulations in older female monkeys that more closely mimic the middle-age postmenopausal woman. There are many avenues for future studies in nonhuman primate models of menopause, including the need to further determine the types of memory and attention function that may be sensitive to ovarian hormones, identify the information processing parameters that are hormone sensitive, and investigate the ability of ERT to not only treat already manifested cognitive deficits but also to prevent cognitive decline from occurring.

5.2. Neurobiological Studies

Our understanding of the neurobiological mechanisms by which estrogen may affect cognitive function in nonhuman primate models of menopause also is in its beginning stages, however several different approaches are being used to add to our knowledge. One neural mechanism through which estrogen may affect cognition is through the cholinergic system and the cholinergic neurons located in the BFCS. To examine how estrogen may use the cholinergic system to influence cognition in primates, we injected the muscarinic antagonist scopolamine into young adult monkeys that were intact, OVX, or OVX treated with ERT or placebo. Spatial memory in DR and visuospatial attention in the visuospatial cueing task were examined following injections with scopolamine. Scopolamine impaired visuospatial attention function in monkeys, in a manner dependent on the presence or absence of estrogen (104). Monkeys with ERT responded to scopolamine in a manner similar to intact monkeys, while monkeys with placebo were relatively resistant to disruption by scopolamine in a manner similar to male rats. In contrast to visuospatial attention, accuracy in DR was decreased comparably by scopolamine in intact and ovariectomized monkeys (103) and in ERT and placebo monkeys (104). These observations indicate that disruption of spatial memory in DR by scopolamine does not depend upon estrogen. These observations in OVX monkeys are in contrast to what has been reported with scopolamine in OVX rodents, where disruption of spatial memory by scopolamine was prevented by ERT (115, 127-129).

Aside from cholinergic receptors, other cholinergic mechanisms in primates also respond to ovarian hormones. Cholinergic fiber innervation pattern of area 46 in dorsolateral prefrontal cortex is disrupted following 3 months OVX in monkeys and reversed with 28 days of ERT (130). We found an identical disruption of cholinergic fibers in area 46 of dorsolateral prefrontal of monkeys that had been OVX for two years and this decrease was prevented with two years of ERT (131). Despite the differences in type, route, and dose of ERT between these studies, and that one study modeled intervention and the other prevention, the results compare favorably and suggest that changes in cholinergic fiber innervation to prefrontal cortex may persist for multiple years following ovariectomy and that these changes can be reversed or prevented with ERT. Although studies in rodents have suggested that the effects of ERT might diminish as treatment lengthens (132), the findings from our study indicate that long-term ERT is as viable as short-term ERT, at least for cholinergic fibers in monkey prefrontal cortex. Unlike the dorsolateral prefrontal cortex, cholinergic markers in other regions of primate brain may not respond to ERT. No difference was found in choline acetyltransferase activity, acetylcholinesterase activity, or cholinergic fiber density in ventrolateral prefrontal cortex between monkeys that received ERT for two years and OVX controls (131, 133). In addition, two years of OVX in monkeys did not alter cholinergic fiber input to PG-type cortex (area 7a) of the inferior parietal lobule (131) or alter choline acetyltransferase or acetylcholinesterase activity in parietal area PE/PEa (133). The cholinergic neurons in the BFCS that project to cortex also do not seem to be affected by two years of OVX or ERT in monkeys, either in terms of cell number or size (131). The absence of ERT effects in these studies may be related to true regional differences in response to OVX and ERT or to the timeframe in which the studies were conducted, e.g. changes in these regions may have occurred with shorter durations of OVX or ERT.

In addition to the behavioral and postmortem studies being conducted in surgically menopausal monkeys, functional imaging approaches also are being used to identify how estrogen modulates the primate cholinergic system. Although functional imaging studies have been performed in postmenopausal women (e.g., 134-136), until recently, these studies have not measured the response to ovarian hormone therapy in specific neurochemical systems, such as the cholinergic system. For our imaging studies, we used PET and the cholinergic radiotracer, [^{18}F]-Fluorobenzyltrozamicol ([^{18}F] FBT), that we had used in our previous aging studies (76). A large reduction in striatal binding of [^{18}F] FBT was observed in a monkey who had been ovariectomized for three years compared to an intact monkey (Figure 10). Treatment with estrogen

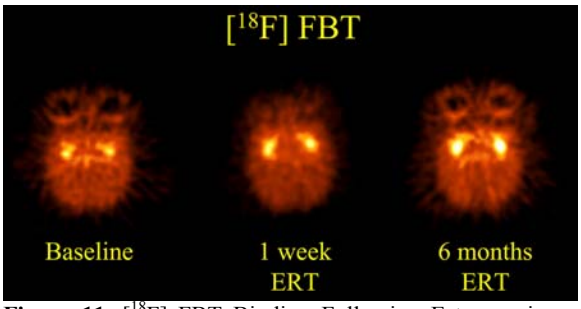

Figure 11. [18F] FBT Binding Following Estrogen in an Ovariectomized Monkey. Striatal [18F] FBT binding was greatly increased by as little as one week of estrogen treatment and this increase was maintained for the six months of treatment. Baseline represents [18F] FBT binding in the monkey when she was in an ovariectomized state before receiving estrogen.

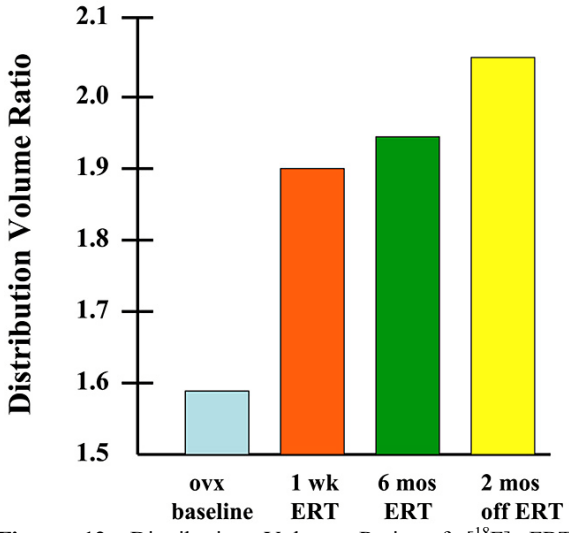

Figure 12. Distribution Volume Ratio of [18F] FBT Binding in an Ovariectomized Monkey. The ratio of binding of [18F] FBT in the striatum compared to the cerebellum (control region) in an ovariectomized monkey before receiving estrogen (ovx baseline) and following estrogen replacement therapy (ert) for one week (1 wk) and six months (6 mos), and two months after ert was stopped. Compared to baseline, the [18F] FBT binding ratio was increased following treatment with estrogen and this increase was sustained for at least two months despite having stopped estrogen therapy.

increased [18F] FBT binding by 17% over pretreatment binding levels within one week of initiating ERT, with continued elevation of [18F] FBT binding during six months of treatment (Figure 11). Interestingly, levels of [18F] FBT binding remained elevated for at least two months once ERT was stopped (Figure 12). These preliminary PET data in monkeys demonstrate that ERT modulates the *in vivo* functional activity of cholinergic terminals in the primate brain and that the cholinergic system remains responsive to ERT despite not being exposed to normal levels of estrogen for several years. Although our studies focused on the basal ganglia because of resolution limitations of our PET scanner, the changes that were observed may reflect a more generalized effect of estrogen on the cholinergic system in the primate brain. Indeed, a recent single photon emission tomography study in a small group of postmenopausal women found that the duration of ERT correlated positively with the binding of a radiotracer for the vesicular acetylcholine transporter in multiple cortical areas (137).

6. PERSPECTIVE

Our cognitive studies have demonstrated that there are several functional parallels between nonhuman primate models of aging or age-related disease and their human counterparts. For example, learning and memory abilities are compromised in both old monkeys and humans, while visuospatial attention measured by the peripherally-cued attention task is preserved with advanced age in both monkeys and humans. Similarly, lesions of the BFCS in young monkeys impaired visuospatial attention function in a manner that mirrored the visuospatial impairment on a similar task in patients with AD. Although it is tempting to try to draw inferences across our studies of monkey models of aging, one has to be cautious in doing so for two major reasons. First, the cognitive changes noted in old monkeys, who are undergoing age-related changes in numerous neural systems, may not be replicated in more selective experimental monkey models of aging which are primarily performed in healthy young monkeys. Thus, the cognitive profile of young monkeys with BFCS lesions or young surgically menopausal monkeys may not mimic the profile of old monkeys. Indeed, we are finding that even within surgically menopausal monkey models, that more cognitive domains are affected by ovariectomy and ERT in middle-aged monkeys than in young adults. Second, historically, the studies of cognition in old monkeys have involved mixed sex populations in which the reproductive status of female monkeys in those studies was not determined. This point again makes it difficult to draw conclusions across most behavioral studies of old monkeys and those involving surgically menopausal monkeys whether they are young or old. Noting these caveats, the peripherally-cued visuospatial attention task has been a component of the test batteries that we have given to all of our monkey models of aging. Although impairments on the task have been found in several of our studies, the task is not a nonspecific assay in that performance on the task is not disrupted in all monkey models of aging, as noted by the absence of impairment on the task with normal aging in monkeys (50). Rather, the peripherally-cued visuospatial attention task is dependent upon the cholinergic system, as shown in our BFCS studies (57) and pharmacological studies in other laboratories (138-139), and it is sensitive to factors that modulate the cholinergic system, such as OVX and ERT (e.g., 130, 131, 140). Our PET studies in monkeys have shown that there is a great deal of variability in the susceptibility of the cholinergic system to change with age in monkeys and perhaps that is the reason that we did not find visuospatial attention impairments in our study of old monkeys. In

addition, as noted above, our behavioral studies of old monkeys have included both males and females, again making it difficult to draw conclusions across these studies and those involving female surgically menopausal monkeys.

Nonhuman primate models have contributed substantially to our understanding of cognitive and neural dysfunction in normal aging, AD, and more recently menopause. There are many advantages to using nonhuman primates in studies of aging over other animal species. For example, because primates are largely visually-oriented, use of nonhuman primate models affords the ability to examine visual nonspatial and spatial cognitive processes, therefore providing opportunities to examine additional critical cognitive functions that are relevant for the human conditions we are trying to model. Another advantage of nonhuman primate models is that behavioral apparati and paradigms used to test cognitive function in humans can easily be modified for testing of nonhuman primate models and vice versa (141). For example, the computerized visuospatial attention task that we have used in several of our aging studies (50, 57, 104) was adapted from one originally developed for use in the clinics (45). The ability to use operationally similar behavioral tasks in both humans and monkeys allows for greater extrapolation between these animal models to humans. In addition, although rodents are commonly used for studies of aging and age-related disease, there are critical neural and endocrine differences between rodents and primates that likely contribute to the differences in behavioral observations made on more than one occasion between primate and rodent models of aging (e.g. in models of AD and menopause). Collectively, these factors highlight the continued importance of using nonhuman primates to investigate aspects of human aging and age-related disease.

There remain many avenues of further research to more completely determine the extent to which the cognitive profile of the monkey models of aging that have been described mimic the human condition. In particular, besides determining the similarities in performance, equally important is the endeavor to determine if similar cognitive strategies are being used by nonhuman primates and humans to perform the same behavioral task. In terms of the neural basis of cognitive function, neurobiological studies of nonhuman primate models of aging are revealing the alterations that occur in specific primate neural systems with advanced age and age-related disease and are demonstrating that not all brain regions or neurobiological parameters are equally affected in normal aging or menopause. A great deal of further investigation is required at all levels of neural circuitry and with a variety of neurobiological approaches, including molecular and functional imaging, to unveil the full complement of structural and neurochemical changes that occur in nonhuman primate models of advanced age, age-related disease and menopause. One future avenue of fruitful research is the noninvasive continuous monitoring with functional imaging technology of neural systems in nonhuman primate models of aging throughout the course of their behavioral investigations. By this means, the dynamic relationship between neural and cognitive parameters can be determined in the living subject. With an understanding of the neural basis of the changes in cognitive function in nonhuman primate models of age and age-related disease, novel therapeutic approaches can be designed to prevent or treat cognitive dysfunction and significantly improve the quality of life of the elderly.

7. ACKNOWLEDGEMENTS

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