Abstract

Macaques (Macaca spp.) are useful models to evaluate effects of ovarian sex steroids and selective estrogen receptor modulators (SERMs) on mood and cognitive function due to similarities to women in their reproductive and central nervous systems. The results of nonhuman primate studies support the hypothesis that estrogen mediates specific aspects of attention and memory, yet much work is needed to understand which cognitive processes are affected, whether natural versus surgical menopause effects are different, and the interaction of age and ovarian senescence on cognitive function. This knowledge is necessary to determine whether to support the cognitive function of women in the menopausal phase of life and, if so, to determine efficacious therapeutic interventions. Mood disorders are prevalent in women and are associated with reproductive function in women and macaques. Exogenous steroid therapies, including oral contraceptives and postmenopausal hormone replacement therapies, have behavioral effects in women and appear to affect the behavior and underlying neural substrates of monkeys. Additional research is necessary to confirm and extend these observations. Ovarian steroids have multiple effects on serotonin synthesis, reuptake, and degradation, on neural activity that drives serotonin release, and on receptor activation in primates. This system modulates cognitive function and mood and is the target of a broad class of antidepressant therapies. Understanding the effects of ovarian steroids on the neural serotonergic system is necessary to understand depression in women. These studies are best carried out in primate models, which are more similar to humans in neural serotonergic function than other animal models.

Key Words: cognition; depression; monkeys; mood; SERMs; serotonin; sex hormones; social stress

Introduction

Data from human studies are mixed as to whether mood or cognitive processes are affected by endogenous or exogenous sex hormones, probably for several reasons. First, in determining natural variation in mood and cognitive processes associated with fluctuating ovarian steroids, it is experimentally challenging to characterize women’s menstrual cycles well enough or long enough to establish a database with the power to address the question. Added to this difficulty is the time-consuming task of administering multiple tests to evaluate multiple cognitive and emotional domains. When experimental demands are excessive, human subjects are noncompliant with experimental protocols, and the quality of the data base suffers. The effects of exogenous sex steroids or selective estrogen receptor modulators (SERMs) are also difficult to ascertain due to the time demands of assessing multiple cognitive domains. Furthermore, the assessments may be required at specific times in hormone replacement regimens that vary over the month in steroid content (e.g., estrogen only, progestin only, or estrogen plus progestin).

A second complication is that exogenous hormone treatments vary in their formulation and route of delivery. Different formulations may have different effects, and route of delivery may also influence the effects of the hormone regimen on cognitive processes due to differences in metabolism. Finally, a large proportion of women are noncompliant with exogenous sex hormone or SERM regimens because of concern about effects on health (e.g., breast cancer risk) or because of the way the drugs make them feel. Thus, clinical studies may be composed of self-selected subject populations. It is reasonable to presume that these subjects may have fewer side effects than those who are noncompliant with the treatment regimen. Animal models may ameliorate these difficulties.

This article reviews the nonhuman primate literature addressing the question of the effects of sex hormones on cognitive processes and mood. Because most of the research has focused on estrogens, the role of estrogens is predominant in this review. However, where data are available, the effects of other endogenous and exogenous sex steroids are considered.
Estrogen, SERMs, and Cognition

Public Health Issue

It is important for several reasons to understand whether menstrual cyclicity has an impact on cognitive processes. This knowledge will promote our understanding of basic sex differences in cognition and emotion. Small fluctuations in cognitive performance may become critical, particularly in cognitively demanding situations such as space flight or combat. Knowledge of the impact of endogenous ovarian steroid fluctuations on cognitive processes will help our understanding of the effects of exogenous hormone replacement therapies. Although the human lifespan has increased over the last century, the timing of ovarian senescence has remained relatively constant (Hawkes 2003). Whereas 100 yr ago few women lived a long time beyond menopause, now women may live one third or more of their lives after ovarian function cessation. To deal with the health of our aging population effectively, it is important to determine both the effects of the cessation of fluctuating ovarian steroids on cognition and whether replacement therapies protect against cognitive decline and neurodegeneration.

Results of experimental studies suggest several mechanisms by which exogenous estrogen replacement could be neuroprotective and have beneficial effects on cognition (Leranth et al. 2002; McEwen and Alves 1999). The results of human studies that address this hypothesis are mixed. In spite of conflicting results, some literature reviews conclude that estrogen helps maintain some aspects of verbal and spatial memory in women, and that cognitive changes due to estrogen deprivation are reversible (Resnick et al. 1997; Sherwin 2003). In contrast, other reviews point out that the estrogen-cognition link is unconvincing due to problems of experimental design mentioned above (Barrett-Connor 1998; Yaffe et al. 1998). A recent large, double-blind prospective clinical trial of a commonly prescribed hormone replacement therapy (HRT1), conjugated equine estrogen plus medroxyprogesterone acetate, resulted in no cognitive benefit, cognitive detriment to a small number of subjects, and an increase in dementia (Rapp et al. 2003; Shumaker et al. 2003). Progestins are a necessary component of HRT formulations to protect against uterine cancer. Little is known about differences in the effects of estrogen versus estrogen plus progestin formulations on cognition.

Value of a Primate Model

Old World monkey and ape species are valuable models in which to study complex cognitive processes. More simple animals have one or a few potential responses to specific environmental stimuli, which are often immutable. Flexible cognitive strategies, which rely on the use of some form of representation, allow individuals to choose from a number of responses. Flexibility in perceiving and understanding the environment, as well as in choice of behavioral responses, requires more complex neural circuitry.

The cognitive adaptations of primates are driven by two problems that must be solved: foraging for food and interacting with conspecifics. Foraging theorists have pointed out the special difficulties that many primates face in maintaining an adequate diet given their dependence on certain food types that are patchily distributed in space and time (Milton 1993). Social cognition theorists have pointed out that most primates live in complex social groups involving various types of competition, display complex forms of emotional expression and communication, and form a variety of long-term social relationships based on individual experiences (Cheney et al. 1987). These two sets of adaptive problems present special difficulties that have resulted in the mammalian order of primates and are characterized by the special adaptation of increased brain: body size, with an expanded neocortex that allows for extensive learning, flexibility, and mental representation; and a common dependence on visual sensory information.

Old World monkeys and apes have another unique similarity to humans that makes them ideal for the study of sex steroid effects on cognitive processes—the menstrual cycle (Knobil and Hotchkiss 1988). These animals have menstrual cycles that are similar to those of women both in length and in sex steroid and gonadotropin fluctuations across the menstrual cycle. In the estrus cycle of small rodent species, specific behavior patterns such as sexual interactions are closely synchronized with the 3- to 4-day estrus cycle. Thus, female rats mate only during a few specific hours on 1 day of their estrus cycle. In primates, the cycle is much longer and, consistent with their adaptations for flexibility, the relation between time of cycle and specific behavior patterns is loosened. However, although that relation is looser than in rodents, sex hormone fluctuations still influence the behavior of human and nonhuman primates (Michael 1980). Thus, similarities in the cognitive processes, neural adaptations, and reproductive systems of Old World monkeys and apes make them ideal models for the study of sex hormone influences on cognition.

Monkey Studies of the Relation Between Sex Hormones and Cognitive Performance

Endogenous Sex Hormones and Cyclic Variation in Cognitive Performance

If ovarian sex steroids modulate cognitive functioning, then fluctuations should occur in cognitive performance coincident with endogenous fluctuations of estradiol and progesterone. Lacreuse and colleagues (2001) examined fluctuations in spatial recognition memory across the menstrual cycle in female rhesus monkeys (Macaca mulatta). They tested four young (5- to 7-yr) females daily during one entire menstrual cycle on three cognitive tasks (matching-to-sample no delay, matching-to-sample 30-sec delay, and spatial-delayed recognition span test) using a computerized touch screen on which the monkeys had been previously
trained. Matching-to-sample is a visual recognition memory task that is dependent on the medial temporal lobes (Blaizot et al. 2000; Mishkin 1990).

In the 30-sec delay form, monkeys see an image, the screen is blank for 30 sec, and then two images appear. The monkey is rewarded for identifying the image that matches the first sample image presented. The procedure for the matching-to-sample with no delay is identical except that the two test images appear immediately after the sample image. The spatial-delayed recognition span test is a memory test that depends on hippocampal function (Beason-Held et al. 1999). The monkey must identify, trial by trial, the new location of a stimulus among an increasing number of serially presented identical stimuli. Initially a disk is presented in a certain location. Then two disks appear, one in the location of the original sample and one in a new location. The monkey is rewarded for touching the disk in the new location. Each time the monkey makes a correct response the number of test disks is increased, up to a maximum of eight at any one time. Blood samples were collected throughout the cycle for estradiol and progesterone assay.

There were no significant differences in matching-to-sample task performance; however, spatial-delayed recognition span test performance was better during the follicular and luteal phases than the periovulatory phase, when estradiol levels are high. Thus, spatial memory performance appears sensitive to estradiol variations, and better performance is associated with low estradiol levels. This finding is consistent with the widely held belief that male mammals have better spatial ability than females (Jones et al. 2003). If the difference in spatial ability is mediated in part by estrogen concentrations, then we might expect spatial memory to peak when estradiol is low, as we observed in this study.

Effects of Menopause and Estrogen Replacement on Cognitive Function

In macaques, menopause generally occurs in the third decade of life (Gilardi et al. 1997). Roberts and colleagues (1997) investigated the effects of natural menopause on performance on a delayed response task in 13 young (3- to 11-yr) adult and 12 aged (> 20-yr) rhesus monkeys (Figure 1). The delayed response task depends on the prefrontal cortex and memory-related structures in the medial temporal lobe. Briefly, a food reward was hidden in one of two locations before a delay, which varied up to 60 sec. Performance in the aged group varied substantially. In general, aged monkeys took longer to learn the task at longer delays and performed less well in general at longer delays than young monkeys. Among the aged animals, four were premenopausal and eight were peri- or postmenopausal (based on urinary estrone conjugates and pregnanediol-3-glucuronide levels, as well as frequency and regularity of menses), and these two groups did not differ by age. Delayed response learning was impaired in the peri/postmenopausal group compared with the premenopausal-aged and young monkeys. Urinary estrogen metabolites were correlated with performance, providing additional evidence that natural menopause is associated with memory impairment in rhesus monkeys.

Voytko (2002) assessed the effects of ovariectomy and percutaneous estradiol replacement on reaction time and visuospatial attention in 18 young adult (6- to 11-yr) cynomolgus monkeys (Macaca fascicularis). The monkeys were trained, tested, ovariectomized, and retested. Then they were assigned to either the estradiol or placebo treatment group and tested again at 1 wk and 4 mo of treatment. In addition, after 4 mo of treatment, the effects of scopolamine were examined. The monkeys were tested for the last time after 14 mo of treatment. Reaction time was unaffected by ovariectomy or estradiol. Ovariectomy decreased attention and estradiol increased attention after 14 mo of treatment relative to placebo controls. Scopolamine, a cholinergic antagonist, disrupts cognitive performance. The deleterious effects of scopolamine were more apparent in the estradiol-treated group than the placebo group. These results suggest that the loss of estradiol deleteriously affects attention in monkeys, and that one mechanism of this effect is through the cholinergic system. Voytko (2000) had also assessed the effects of ovariectomy and percutaneous estradiol replacement on learning and memory in 13 young adult (6- to 10-yr) cynomolgus monkeys. Learning and memory performance were comparable to baseline values at 2, 12, and 24 mo after ovariectomy. The ability to learn, remember, and perform reversals of object discriminations (a test of cognitive flexibility) and accuracy on a spatial delayed response task did not differ between treated monkeys and placebo controls after 5 or 16 mo of treatment. However, ovariectomy appeared to increase sensitivity to the disruptive effects of scopolamine.

Figure 1 Delayed response performance in young monkeys (n = 3), aged premenopausal monkeys (PRE; n = 4), and peri/postmenopausal monkeys (PERI/POST; n = 8). Mean percentage correct (± s.e.) across delayed response retention intervals of 5 to 60 sec. Adapted from Roberts JA, Gilardi KV, Lasley B, Rapp PR. 1997. Reproductive senescence predicts cognitive decline in aged female monkeys. Neuroreport 27:2047-2051.
Estrogen effects on cognition may be age dependent. Lacreuse and colleagues (2000) compared the performance of six aged rhesus monkeys that had been ovarioctomized early in life with that of eight aged intact monkeys (19- to 27-yr), and five young adult intact monkeys (4- to 7-yr) on tests of visual recognition memory, object and spatial memory, and executive function (keeping cognitive processes focused on the task until completion). Of the seven tasks, ovarioctomized-aged females were slightly impaired on only one, the longest delay in a delayed nonmatch-to-sample task, relative to intact-aged females. However, the ovarioctomized-aged females had greater memory spans on a delayed recognition span test than intact-aged females, suggesting that long-term ovarioctomy may protect against aging-associated spatial memory deficits. Unfortunately, the quality of ovarian function among old and young intacts was not characterized in this experiment, therefore conclusions are difficult, and more studies are needed to confirm this observation.

In a subsequent study in five of the same aged animals that had been ovarioctomized early in life, Lacreuse and colleagues (2002) demonstrated that oral ethinyl estradiol improved spatial working memory (as measured by the spatial-delayed recognition span test but not the delayed response test). In contrast, another commonly prescribed menopausal replacement therapy, raloxifene, had no effect on performance of age-sensitive cognitive tasks such as the delayed recognition span test, delayed response, and delayed nonmatch-to-sample. Thus, beneficial effects of estrogen on cognition may be more apparent in older than in younger subjects.

Estrogen effects on cognition may depend on the pattern of estrogen exposure. Rapp and colleagues (2003) examined the effects of estradiol on cognitive function in 16 aged nonpostmenopausal monkeys that had been ovarioctomized for approximately 7 mo. The monkeys were treated with monthly injections of estradiol, which provided high (≈300 pg/mL), transient (above baseline for approximately 3 days) exposure to estradiol. This treatment reversed age-related impairment of spatial working memory and improved performance on a delayed nonmatching-to-sample recognition memory task. The estrogen administration method used in this monkey cognition experiment is not widely used either experimentally or in hormone replacement regimens; however, it does more closely mimic endogenous estradiol production.

Summary: Estrogen and Cognition in Primates

Age, ovarian condition, hormone replacement regimen, and task vary over this set of experiments. Among young adult females, spatial memory may peak when estradiol is low. Memory appears to be better in age-matched premenopausal versus peri/postmenopausal aged females. Ovarioctomy reduces and estradiol restores attention, but estradiol does not affect learning and memory in young adults. Among aged females, preliminary evidence indicates that those without ovaries for many years may have better spatial memory than intacts, and estrogen replacement therapy in such animals improves spatial memory. Thus, age and estrogen may interact to influence some cognitive processes. Some aspects of cognitive function may be responsive to estrogen replacement even after long-term ovarian hormone deprivation. Much evidence from rodent and some data from monkey studies support the hypothesis that the positive effects of estrogen on spatial memory are mediated in the hippocampus (Lacreuse et al. 2002; McEwen and Alves 1999). The ability to respond to estrogen after long-term deprivation suggests that the aged primate hippocampus may retain plasticity similar to that observed in aged rats (Adams et al. 2001; Miranda et al. 1999). However, the length of time of hormone deprivation before onset of estrogen replacement may determine how neurons respond to estrogen replacement therapy (Adams et al. 2001; Silva et al. 2003).

Some evidence suggests that the estrogen effect appears to be specific to spatial (vs. nonspatial) working (vs. reference) memory in rodents and primates (Lacreuse et al. 2002). However, delayed response is considered a test of spatial working memory, yet no effect of estrogen on this task was observed in aged intact versus ovarioctomized females (Lacreuse et al. 2002) or young adult ovarioctomized females (Voytko 2000). In contrast, Roberts and colleagues (1997) observed better delayed response performance in premenopausal versus peri/postmenopausal monkeys. This effect may be specific to natural menopause. Despite the difficulty of such studies due to low availability, more research is needed in naturally menopausal monkeys.

Finally, recent findings suggest that chronic versus episodic estrogen replacement may have different effects on cognition. Taken together, the results of nonhuman primate studies support the hypothesis that estrogen mediates specific aspects of attention and memory. Nonetheless, much work is needed to understand exactly which cognitive processes are affected, whether natural versus surgical menopause effects are different, the interaction of age and estrogen deprivation on cognitive function, and to determine the best way to support the cognitive function of women in the menopausal phase of life.

Stress-related Mood Disorders

Public Health Issue

Depression is prevalent (approximately 10% of the population experience a clinically significant depression) and increasing in frequency. The risk of depression in women increases shortly after puberty and remains elevated through menopause. The lifetime rate of major depression is two to three times higher in women than men. Women are prone to depression during times of reproductive hormone change such as puberty, the postpartum period, the premenstrual phase of the menstrual cycle, and the perimenopause, and during oral contraceptive use. Thus, female susceptibility to
mood disorder appears to be influenced by reproductive system function, but we have little understanding of the nature of the relation between mood and ovarian function (Burt and Stein 2002; Parry and Newton 2001).

Value of the Primate Model

Darwin (1905) wrote, “pain or suffering of any kind, if long continued, causes depression and lessens the power of action, yet is well adapted to make a creature guard itself against any great or sudden evil.” In recent years, attempts have been made to understand the evolution of anxiety, low mood states, and anhedonia in an effort to distinguish evolutionary explanations from proximate studies, and to integrate proximate mechanisms with evolutionary explanations (Nesse 1999). From an evolutionary perspective, low mood and anhedonia have been hypothesized to reduce interaction with the physical and social environment, to allow the individual to be sensitive to environment quality and adjust resource investments accordingly, and to disengage from goal achievement efforts when success seems unlikely (Sloman et al. 2003). Price suggests that depressive behavior is associated with low social rank because this mood state allows individuals to accept defeat in ritual agonistic encounters associated with social status (Price 1967; Price et al. 1994). From this perspective, anhedonia and low mood may serve the best interests of subordinates because it reduces exploration, engagement with the environment, and the pursuit of resources that may elicit attacks from more dominant group members who are competing for the same resources (e.g., food or mates). However, the hypothesis that anxiety, low mood states, and depression may have evolved in response to selective pressures remains controversial, as does the potential function of these emotional states that might have been the focus of selection (McLoughlin 2002; Watson and Andrews 2002).

From an evolutionary perspective, monkeys are an ideal model in which to study low mood states for the following reasons: (1) Similarities between human and nonhuman primates are likely due to homology; (2) as in human society, status in monkey society is a key social organizing mechanism; (3) monkeys have evolved the ability to engage in complex cognitive processes, a range of emotional expression, and dependence on social relationships; and (4) monkeys rely on close attachment bonds, not only between mother and offspring but also between group mates. Loss of the maternal attachment bond immediately results in protest and despair, which are accompanied by physiological changes (e.g., high heart rate and cortisol, disrupted sleep) similar to those seen in human depression (Dettling et al. 2002; Levine and Mody 2003; Reite et al. 1981; Sanchez et al. 2001; Schino et al. 2001). Loss of an attachment bond between adults also results in behavioral depression, although it has been little studied (Rasmussen and Reite 1982). Thus, like humans, monkeys are capable of a depressive response to adverse social environmental conditions in which goals cannot be achieved.

Monkeys are also an ideal model in which to study female-specific depression because they have menstrual cycles like women and experience similar life cycle changes in reproductive function associated with puberty, parturition, and menopause. Interestingly, in baboons, abnormal, abusive, and stress-related behaviors have been observed to increase immediately during the postpartum period; and in vervets, aggression, avoidance of social overtures, and retreats from threat have been observed to increase during the late luteal phase. These changes suggest that mood changes may be associated with changes in reproductive function (Brent et al. 2002; Rapkin et al. 1995). Thus, a monkey model of adult depression in females would provide a valuable tool in which to investigate the role of the reproductive system in depression in females.

Monkey Studies on Stress, Mood, and Sex Hormones

Social Stress-associated Depression in Adult Female Monkeys

We have been developing a monkey model of adult female depression over the last 15 yr with the long-term goal of understanding how reproductive function modulates mood states in female primates. Among adult female monkeys in small (4-6) social groups in the laboratory, social subordination is stressful. Compared with dominant females, subordinates are aggressed more, spend more time fearfully scanning the social environment, spend less time receiving the active affiliative behavior of being groomed, have more variable and higher heart rate responses to novelty, and are hypercortisolemic (Shively 1998b). In addition, socially subordinate females have poor ovarian function, characterized by low luteal phase progesterone concentrations, and a greater portion of impaired or anovulatory cycles than dominants (Adams et al. 1985; Shively et al. 1997b).

Prolactin secretion is inhibited by dopamine via D2 receptors, and haloperidol administration, a D2 antagonist, results in increased prolactin secretion. Prolactin responses to haloperidol are positively associated with being groomed, negatively associated with being aggressed, and lower in subordinates than dominants, observations consistent with the hypothesis that subordinate females have decreased D2 receptor function (Shively et al. 1997a). This hypothesis was supported by evaluation of striatal D2 receptor availability in dominant and subordinate females using positron emission tomography. Subordinate females had lower striatal D2 receptor availability than dominants (Grant et al. 1998). The striatal dopamine system mediates reward or hedonic value of stimuli. The anhedonia characteristic of depression is moderated by this system. These observations suggest that social subordination is stressful and may alter brain dopaminergic function in primates. The neurophysiological characteristics of subordinates may contribute to susceptibility to a depressive response to stress.
We studied depressive behavior in these small social groups of females. Based on previous research, we define depressive behavior as a slumped or collapsed body posture accompanied by a lack of responsiveness to environmental stimuli (Figure 2). Subordinate females exhibited more of this depressed posture than dominants, particularly if they had a history of being subordinate in previous social groups (Shively et al. 1997b). Like humans, depressed monkeys have higher heart rates, impaired hypothalamic-pituitary-adrenal (HPA1) function, and poor ovarian function compared with their nondepressed counterparts (Shively et al. 2002).

**Behavioral Neurobiology of Exogenous Sex Steroid Regimens**

Female cynomolgus monkeys have been used to study the effects of commonly used exogenous hormone regimens on mood, behavior, and related neurobiology. A significant proportion of women experience negative changes in mood and irritability with oral contraceptive (OC) use, and the mechanisms of this effect are not known (Kahn and Halbreich 2001; Oinonen and Mazmanian 2002). For this reason, we evaluated the effects of 2 yr of exposure to a commonly used triphasic OC (ethinyl estradiol and levonorgestrel) on behavior, HPA, and central nervous system function in 75 adult female cynomolgus monkeys housed in small social groups. This evaluation was part of a larger experiment, which is discussed further below. OC use increased contact aggression received as well as time spent in locomotion and in sitting close to another animal, and it decreased time spent fearfully scanning. OC use decreased heart rate and increased activity levels, basal cortisol, and the cortisol response to ACTH compared with controls. OC-treated females also had a decreased prolactin response to fenfluramine, a partial serotonin agonist, which suggests decreased serotonergic activity. Increased aggression is associated with decreased central serotonergic activity in a number of clinical and experimental studies. These results suggest that this triphasic OC disrupts social behavior, HPA axis regulation, and the underlying central nervous system function (Henderson and Shively 2004).

After 2 yr of OC or placebo control, OC treatment ended and these monkeys were ovariectomized. After 1 yr, we re-evaluated the monkeys. Relative to the animals that had never been exposed to OCs, a history of OC exposure increased contact aggression received and reduced the prolactin response to fenfluramine. The findings suggested persistent changes in behavior and reductions in serotonergic activity for at least 1 yr after cessation of OC treatment in these ovariectomized females. Furthermore, a history of OC exposure increased cardiovascular and HPA responses to stress in socially dominant but not socially subordinate females, which suggests complex interactions between social stress and exogenous steroid administration (Shively 1998a). These observations may be relevant to the central nervous system health of the several million women who have taken oral contraceptives.

Estrogen replacement therapy in peri- and postmenopausal women is accompanied by reports of improved mood and well-being, whereas combination HRTs that include a progestin component often appear to cause dysphoria and irritability (Genazzani et al. 2002). In the experiment discussed above, after ovariectomy, half of the monkeys were untreated, and the other half were treated with conjugated equine estrogens (CEE), a common estrogen replacement therapy. We evaluated the effects of CEE on behavioral and neurobiological endpoints after 1 yr of treatment. Like OCs, current CEE treatment also increased cardiovascular and HPA responses to stress in socially dominant but not socially subordinate females. CEE also appeared to increase indices of dopaminergic, serotonergic, and noradrenergic activity as reflected in neuroendocrine challenge tests (Shively 1998a). An opposing progestin in combination with an estrogen is necessary to reduce the risk of reproductive system cancers in women, but recent reports suggest potentially deleterious health effects of combination HRT (Rapp et al. 2003; Shumaker et al. 2003; Writing Group for the WHI 2002).

Soy phytoestrogens (SPE) are SERMs with no agonist effects in mammary or uterus, which have been considered as a potential alternative to traditional HRT because no opposing progestin is necessary. Tryptophan hydroxylase (TPH) is the rate limiting enzyme for serotonin production, and nearly all serotonin in the brain is produced in the dorsal raphe. We evaluated the effects of social status, CEE, and SPE on dorsal raphe TPH and serotonin reuptake transporter (SERT) levels in adult ovariectomized females housed in small social groups of four to six animals. The study comprised a three-group, parallel arm design, with a 36-mo treatment period. At the end of the study, the monkeys were sacrificed and the dorsal raphe frozen for Western blot.

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**Figure 2** (Left) A normal, alert monkey. (Right) A depressed monkey.
analysis. Whereas CEE and SPE increased SERT, social status had no effect. Like CEE, SPE increased TPH levels. Social subordinates had markedly lower TPH protein levels than dominants regardless of hormone replacement (Figure 3). Thus, subordinate females that were untreated had the lowest TPH levels of all (Shively et al. 2003). Consequently, although CEE and SPE appear to promote serotonin synthesis, social subordinate status is associated with reduced serotonin production in ovariectomized females.

Summary: Sex Steroids and Stress-related Mood Disorders in Primates

The hypothesis that depression may be an adaptive response to adverse environmental conditions, which is shared by many species, is currently being debated. Depression is prevalent in women and often associated with changes in reproductive function. Female monkey models of social stress-induced depression are promising because the neural systems that regulate mood and their reproductive systems are similar to those of humans. Exogenous steroid therapies including OCs and postmenopausal HRTs appear to affect the behavior and underlying neural substrates of monkeys. Additional research is necessary to confirm and extend these observations. No interactive effects of exogenous sex steroids or SERMs and social stress were observed. However, the effects of cessation of ovarian function plus the stress of social subordination appeared to have additive adverse effects on the neural serotonin system which may increase vulnerability to a depressive reaction to stress. The effects of sex steroids on the neural serotonin system are discussed in detail below.

Sex Steroid Effects in the Neural Serotonin System

Public Health Issue

The impact of women’s mental health on society as a whole cannot be overestimated. When women who have children are stressed, depressed, anxious, phobic or psychotic, and/or self-medicating with drugs of abuse, the repercussions are both dire and multigenerational. As described above, women have affective pathologies, frequently including depression and anxiety, which are associated with reproductive function such as premenstrual syndrome, premenstrual dysphoric disorder, postpartum depression and psychosis, peri- and postmenopausal negative affect, as well as memory and cognition problems after menopause. Of note, the selective serotonin reuptake inhibitors (SSRIs) are used to treat many of the symptoms associated with these disorders and a host of others including obsessive compulsive disorder, phobias, violent aggression, and Alzheimer’s disease. Thus, it is not entirely surprising that the serotonergic neurons of the dorsal and medial raphe nuclei, located in the pontine midbrain, project to almost every area of the forebrain and regulate diverse neural processes from higher order functions in the prefrontal cortex such as integrative cognition and memory, to limbic system control of arousal and mood, to diencephalic functions such as pituitary hormone secretion, satiety, and sexual behavior or libido. The more caudal serotonin neurons project to the spinal cord and interact with numerous autonomic and sensory systems. All of these neural functions, subserved by serotonin, are sensitive to the presence or absence of the ovarian hormones, estrogen and progesterone. A better understanding of the mechanisms by which estrogen, progesterone, stress, and genetics affect serotonin neural function is necessary to determine which aspects of the system go awry in the different pathologies and, ultimately, to identify effective and tolerable therapeutic interventions. Moreover, any new treatment strategies may require testing in primates before approval for human use. It is also important to recognize that currently there are no cures for mental illness. Pharmacotherapies treat symptoms and are needed for life; however, they have unwanted side effects and are not tolerated by a large number of individuals.

Value of the Primate Model

The nonhuman primate not only has a menstrual cycle like that of women, along with higher cortical function and complex social interactions, but also has a serotonin system that is identical to that of humans (and different in various aspects from that of rodents) (Alves et al. 1997, 1998). For this reason, new treatment strategies that alter neural serotonergic activity should be tested in primates before approval for human use. We have shown that serotonin

Figure 3 Social status and effects of selective serotonin reuptake inhibitors on raphe tryptophan hydroxylase (TPH). Optical density (OD) of TPH in dominant (Dom) and subordinate (Sub) ovariectomized females that were untreated controls or treated with conjugated equine estrogens (CEE) or soy phytoestrogens (SPE). Adapted from Shively CA, Mirkes SJ, Lu NZ, Henderson JA, Bethea CL. 2003. Soy and social stress affect serotonin neurotransmission in primates. Pharmacogenomics J 32:114-121.
neurons in nonhuman primates contain the beta isoform of the estrogen receptor and progestin receptors (Bethea 1993, 1994; Gundlah et al. 2000, 2001b) (Figure 4). The presence of a receptor defines a cell as a target for the cognate hormone. Thus, serotonin neurons are targets for ovarian steroids that, in turn, modify gene expression. Any change in serotonergic neural function could be manifested by a change in any of the projection target systems, and in this manner, serotonin neurons integrate steroid hormone information and transduce their action in the brain.

**Monkey Studies on Sex Hormones and Serotonin**

Serotonin transmission can be envisioned as the sum of several processes including (but not limited to) serotonin synthesis, reuptake, degradation, and neural activity, which drive release and receptor activation. In turn, serotonin synthesis is governed by TPH, the rate limiting enzyme in the conversion of tryptophan to 5-hydroxytryptophan. Serotonin reuptake is accomplished by the SERT, and serotonin neural activity is inhibited by the 5HT1A autoreceptor. Serotonin is degraded by monoamine oxidases (MAO1), and neural activity is inhibited by the 5HT1A autoreceptor. Serotonin is degraded by monoamine oxidases (MAO1), and when there is less 5HT1A autoreceptor available, there will be a change in any of the projection target systems, and in this manner, serotonin neurons integrate steroid hormone information and transduce their action in the brain.

**Figure 4** Serotonin neurons in macaques contain estrogen receptor beta (ERβ) and progestin receptor (PR). These gene transcription factors define the neuron as a target for the cognate hormones, estrogen and progesterone. (Top left) ERβ mRNA expression in the dorsal raphe is shown with in situ hybridization. (Bottom left) ERβ protein is shown in the nuclei of dorsal raphe neurons with immunocytochemistry. (Top right) Dorsal raphe neuron is shown that has been double labeled for serotonin (cytoplasm) and for nuclear progestin receptors (nucleus). (Bottom right) Histogram reflects the increase in the number of PR-positive neurons in the dorsal raphe of monkeys treated with estrogen or estrogen plus progesterone.

To determine whether ovarian steroids alter the expression of pivotal genes and proteins in serotonin neurons, we have used spayed rhesus macaques as a model for surgical menopause, treating them for 28 days with either estrogen alone or estrogen supplemented with progesterone in a manner similar to hormone replacement regimens, although mostly using Silastic implants instead of oral dosing. The majority of the serotonin neurons that project to forebrain areas involved in the regulation of mood, arousal (stress sensitivity), and cognition are located in the midbrain region of the primate brain. We have examined the regulation of TPH, SERT, 5HT1A autoreceptor, MAO-A, and MAO-B genes and proteins within these serotonin neurons and several projection fields with in situ hybridization, Western blotting, receptor autoradiography, and immunocytochemistry.

In spayed monkeys, the TPH mRNA signal is barely detectable, but upon estrogen treatment, there is a marked induction of TPH mRNA. TPH is still easily detectable when progesterone is added to the estrogen regimen (Pecins-Thompson et al. 1996). The TPH mRNA detected in these studies is TPH-1. Another form, called TPH-2, has recently been described (Walther et al. 2003), and studies are currently under way to examine the regulation of TPH-2 by sex hormones. TPH protein mass on Western blots also increases with estrogen and estrogen plus progestin treatment in a manner similar to the mRNA (Bethea et al. 2000). When there is more TPH in the cell, there is likely more synthesis of serotonin. However, TPH requires phosphorylation for activation, and a coregulator protein called 14-3-3 also plays a role. We know little about these factors.

There was robust expression of 5HT1A mRNA in the spayed monkey raphe. After continuous treatment with estrogen or estrogen plus progesterone using Silastic capsule implants for 1 mo, there was a significant decrease in the autoradiographic signal for 5HT1A mRNA (Pecins-Thompson and Bethea 1998). A decrease in 5HT1A mRNA was not observed with 1 mo of once daily oral dosing of estrogen (Bethea et al. 2002), suggesting that responses to continuous versus intermittent dosing may be different. Nonetheless, to determine whether the decrease in 5HT1A mRNA was manifested at the protein level, we used a radiolabeled 5HT1A agonist, [3H]8-OH-DPAT, to label the receptor, and then autoradiography to detect the radiolabeled receptor. Animals treated with continuous estrogen or estrogen plus progesterone for 1 mo had significantly less 5HT1A receptor binding than did the spayed animals. Moreover, hormone-treated animals also had less of the G protein that is utilized by the 5-hydroxytryptamine 1A (5HT1A) receptor (Lu and Bethea 2002). The 5HT1A autoreceptor controls neural firing of serotonin neurons, so when there is less 5HT1A autoreceptor available, there will
be more neural firing. The addition of a 5HT1A antagonist to a SSRI may shorten the onset of efficacy in some patients. Buspar (busiperone) is a 5HT1A antagonist that decreases anxiety in a small percentage of patients.

MAO-A and MAO-B are the main catabolic or degradative enzymes for serotonin and catecholamines. Inhibitors of MAO were among the first pharmacotherapies successfully used for the treatment of depression. The ability of MAO inhibitors to relieve depression suggests that MAO plays a functionally significant role in serotonin metabolism. MAO-A prefers serotonin and MAO-B usually degrades catecholamines, but it can also degrade serotonin. It appears that cells contain the highest concentrations of the enzyme that degrades the transmitter that impinges on the cell, not the transmitter the cell makes. For example, serotonin neurons receive a major noradrenergic afferent projection from the locus coeruleus, and so they contain high levels of MAO-B but low levels of MAO-A (Saura et al. 1996). Nonetheless, we found that MAO-A, within serotonin neurons and within hypothalamic target neurons, was significantly decreased by estrogen or estrogen plus progesterone. MAO-B within serotonin neurons was not affected by the sex hormones, but MAO-B in specific hypothalamic areas was decreased by estrogen and estrogen plus progesterone (Gundlah et al. 2001a). We are currently examining MAO-A and MAO-B protein expression on Western blots. Any decrease in MAO-A or MAO-B would likely result in less degradation and, therefore, more available serotonin.

SERT mRNA is an extremely abundant message in the dorsal raphe of spayed macaques. In the estrogen and estrogen plus progesterone treated groups, the SERT mRNA signal in the dorsal raphe decreases markedly (Pecins-Thompson et al. 1998). However, SERT protein, as detected with [3H]citalopram-binding immunofluorescent histochemistry and Western blotting, does not follow from the mRNA expression. Rather, with 1 mo of estrogen or estrogen plus progesterone treatment, citalopram binding increases in specific serotonin projection areas of the forebrain, but the binding does not change in the dorsal raphe (Lu et al. 2003). However, after prolonged (2-yr) treatment of macaques with conjugated equine estrogens, SERT protein mass increased in the dorsal raphe also (Shively et al. 2003). Whether the difference in apparent SERT concentrations in the dorsal raphe is due to the length of treatment or to a difference between natural estrogen and CEE is unknown. There appears to be a consistent correlation between serotonin and the transporter, such that when there is more serotonin present, there is more serotonin transporter present. Likewise, lower amounts of SERT are detected in depressed patients than nondepressed control subjects (Malison et al. 1998). We do not completely understand the relation between serotonin, SERT protein, and SERT mRNA, nor do we fully understand the functional consequences of the increase in SERT binding in animals treated with sex hormones. Nonetheless, this protein is the target of the entire class of SSRI antidepressants, therefore we believe it is important to seek a better grasp of its regulation.

Summary, Prospects, and Need for Additional Research

Monkey studies of sex hormones, mood, and cognition have been helpful in expanding our understanding of the role of sex hormones in cognition and in mood disorders in primates. From these studies, it is clear that complex cognitive processes may be affected by sex hormones; however, our understanding of these effects in primates is based on remarkably few experiments with relatively small sample sizes. Although complex cognitive processes are necessarily studied in higher primates, the techniques to evaluate monkey cognitive processes are expensive and time consuming. Age may well interact with hormonal status to affect cognitive processes, yet aged primates are rare and difficult to incorporate into demanding cognitive assessment paradigms due to fragile health. Our understanding of menopause effects on mood and cognition is based almost entirely on a surgically menopausal model. This model differs from natural menopause in that the monkeys are usually young relative to the time of natural menopause. Surgical menopause is sudden, whereas natural menopause involves a slow decline in ovarian function. The effects of sudden versus slow declines in ovarian steroids are not known. Improved cognitive assessment methods, which reduce experimental subject contact time, social isolation, and diet and water restriction, are needed to expand the available studies and to include naturally menopausal female monkeys in the studies. Finally, there is a critical need for a national mechanism to support older monkeys between experimental protocols.

Social stress-induced depression in adult female monkeys is greatly understudied. However, this is an attractive model in which to study the relation between reproductive function and depression because depression is associated with changes in ovarian function. This monkey model of depression is also promising for investigations of comorbidities such as coronary heart disease and depression. Finally, the model allows the combination of experimental rigor with state-of-the-art disease and depression. Finally, the model allows the combination of experimental rigor with state-of-the-art imaging techniques, which may reveal neural substrates of social stress-induced depression in subjects that are naïve to pharmacotherapies. Refinement of the induction of depression using experimentally manipulated social stressors are needed. The identification and study of subpopulations of individuals at risk would be helpful in the identification of epigenetic factors that promote social stress-induced depression.

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