Available online at www.sciencedirect.com
SCIENCE DIRECT®
Brain Research 990 (2003) 87–94
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Aging, estradiol and time of day differentially affect serotonin transporter binding in the central nervous system of female rats

Kristine Krajnak[a,*], Katherine L. Rosewell[b], Marilyn J. Duncan[c], Phyllis M. Wise[d]

[a] Department of Biology, West Virginia University, Morgantown, WV 26505, USA
[b] Department of Obstetrics and Gynecology, University of Kentucky Medical Center, Lexington, KY 40536, USA
[c] Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536, USA
[d] Division of Biological Sciences, University of California Davis, Davis, CA 95616-8536, USA

Accepted 30 July 2003

Abstract

Estrogen-related changes in serotonergic neuronal transmission, including changes in the number of serotonin transporter (SERT) binding sites, have been cited as a possible cause for changes in mood, memory and sleep that occur during the menopausal transition. However, both aging and estradiol regulate SERT binding sites in the brain. The goal of this experiment was to determine how aging and estrogen interact to regulate SERT levels in the forebrain of young and reproductively senescent female Sprague–Dawley rats using [^{3}H]paroxetine. The density of specific [^{3}H]paroxetine binding in various brain regions was compared in young (2–4 months) and reproductively senescent (10–12 months) female rats at three times of day. In most brain regions examined, estrogen and aging independently increased the number of [^{3}H]paroxetine binding sites. The only region that displayed a reduction in [^{3}H]paroxetine binding with age was the suprachiasmatic nucleus (SCN). Time of day influenced [^{3}H]paroxetine binding in the SCN and the paraventricular thalamus (PVT), two regions known to be involved in the regulation of circadian rhythms. Aging and/or estrogen also altered the pattern of binding in these regions. Thus, based on the results of this study, we conclude that aging and estrogen both act to regulate SERT binding sites in the forebrain of female rats, and that this regulation is region specific.

Keywords: Reproductive senescence; Hypothalamus; Paroxetine; Ovariectomy

1. Introduction

Serotonin (5-HT) is an ubiquitous neurotransmitter produced by cells in the dorsal and median raphe nuclei. Ascending projections from the raphe innervate forebrain regions including the frontal cortex, hypothalamus, hippocampus and amygdala [56]. Because of its wide distribution throughout the forebrain, it is not surprising that 5-HT is involved in regulating a number of processes, including but not limited to memory, mood, sleep/wake cycles, feeding and reproduction [10,22,35,37,43].

5-HT neuronal transmission is affected by the organizational activity of steroid hormones in the developing brain and the activational effects of steroids during adulthood. During development, estrogen acts on the CNS and results in the sexual differentiation of the serotonergic system, causing the density of 5-HT innervation to many forebrain regions to be different in females than in males [21,44]. During adulthood, 5-HT neuronal transmission is influenced by cyclic changes in estrogen and progesterone that occur during the reproductive cycle [31,32], or with ovariectomy and steroid hormone replacement [12]. For example, ovariectomy decreases tryptophan hydroxylase protein and mRNA levels in the raphe of macaques [7,42] and tryptophan hydroxylase protein concentrations in the hypothalamus of guinea pigs [29]. Estrogen replacement reverses these effects [42,29]. Estrogen also regulates the regional distribution of various 5-HT receptor subtypes throughout the brain [9,23,41,51]. Finally, estrogen modulates the distribution and density of SERT levels in many brain regions. For example, in rats [33] and macaques [30], SERT levels in the hypothalamus and other forebrain regions are

* Corresponding author. Current address: ECTB MS2027, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505, USA. Tel.: +1-304-285-6209/5964; fax: +1-304-285-6265.
E-mail address: mqk1@cdc.gov (K. Krajnak).

0006-8993/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0006-8993(03)03441-3

reduced after ovariectomy, and estradiol replacement prevents this.

Aging also alters the 5-HT system. In older animals, the density of 5-HT terminals is reduced in many brain regions and the morphology of these terminals is altered [15]. 5-HT turnover, metabolism and the expression of many 5-HT receptors are also different in young and aged animals [11,18–20]. Finally, a number of studies have demonstrated that SERT levels are altered in aged animals [20,34] and humans [54]. Thus, both aging and estrogen act to regulate 5-HT neuronal transmission in the CNS.

Depression and changes in sleep/wake patterns and memory that occur during the peri-menopausal period have been attributed to alterations in 5-HT neuronal transmission that occur in response to decreasing levels of steroid hormones [3,24]. In support of this hypothesis, there is evidence that some of these symptoms can be alleviated by estrogen replacement therapy [2]. Estrogen replacement therapy can also enhance the effectiveness of other treatments, such as anti-depressant treatment [47]. Although estrogen replacement therapy relieves or reduces certain symptoms associated with menopause, it does not completely reverse these changes in peri- and post-menopausal women [4,25]. Thus, changes in memory, sleep and mood could also be due to the effects of aging on the 5-HT system.

The goal of the present study was to determine how aging and estrogen interact to affect SERT levels in the CNS of young and reproductively senescent female rats. To do this we assessed the effects of aging on [^{3}H]paroxetine binding in a number of different brain regions. [^{3}H]Paroxetine has been used to quantify SERT levels in a number of different studies [26]. We specifically focused on analyzing [^{3}H]paroxetine binding in brain regions that show changes in SERT levels in response to changes in estrogen because we wanted to determine if the loss of estrogen mimicked the effects of aging, or if the effects of estrogen were independent of the effects of aging. 5-HT also modulates the expression of biological rhythms, including the sleep/wake cycle [36,59], and changes in sleep patterns are a hallmark of depression [46], so we also examined the effects of aging and estrogen on [^{3}H]paroxetine binding in two brain regions that modulate the expression of biological rhythms, the suprachiasmatic nucleus (SCN) and the paraventricular thalamus (PVT).

2. Methods

2.1. Animals care and procedures

Female rats ($n=72$, Zivic Miller) were maintained on a 14:10 light/dark cycle (lights on at 04:00 h) with food and water available ad libitum. Vaginal cytology was examined daily to determine the reproductive status of all animals. Young females ($n=36$) were 3–4 months of age and these animals displayed consistent 4–5-day estrous cycles. Middle-aged animals ($n=36$) were 10–12 months of age and these animals no longer exhibited cycles in vaginal cytology. Instead, these reproductively senescent animals were in constant estrus, and vaginal smears from these animals only contained cornified epithelial cells. Numerous studies have demonstrated that the loss of reproductive cyclicity, and associated changes in circulating ovarian steroids in female rats, are associated with alterations in the synthesis, release and receptor expression for a number of different neurotransmitters including 5-HT [11,57]. Thus, we used this change in reproductive status as a biomarker for aging of the 5-HT system.

On day 0 of the study, all animals were ovariectomized under Metofane inhalant anesthesia. Animals were allowed to recover for 7 days and then half of the animals received a Silastic capsule containing estradiol (s.c., 30-mm capsule young, 40-mm capsule middle age, 180 μg/ml 17-B estradiol in sesame oil). The remaining animals received capsules containing oil (vehicle) replacement. Numerous studies have demonstrated that this estradiol treatment paradigm produces patterns in neuronal gene expression and hormone release that are similar to those seen in young animals on the day of proestrus (for review, see Refs. [57,58]), and that this replacement regime has significant effects on a number of neurochemical systems, including the 5-HT system [11]. On day 9 (2 days after estradiol replacement), animals were euthanized at 03:00, 12:00 or 20:00 h. Brains were collected and rapidly frozen on dry ice. All procedures and animal care protocols were approved by the West Virginia University and the University of Kentucky Animal Care Committees, and were in compliance with the NIH guidelines for the use of Laboratory Animals in Research.

2.2. [^{3}H]Paroxetine binding

Frozen brains were sectioned (12 μm) and thaw-mounted onto slides. Slides with sections were maintained at −80 °C until processed for [^{3}H]paroxetine binding. Coronal sections were collected starting at the level of the septum (bregma 1 mm [40]) and continuing caudally through the hypothalamus (to bregma −3.80 mm [40]).

Saturation analyses were performed on SCN tissue sections collected from additional groups of both young ($n=4$) and middle-aged ($n=4$) animals that were ovariectomized, treated with estradiol and euthanized at 12:00 h. Brain tissue was collected and processed as described above. Slides containing the SCN were chosen because of the high levels of SERT present in this region [20,26]. Slides containing the SCN of each animal were incubated in one the following concentrations of [^{3}H]paroxetine: 25, 50, 100, 200, 400, 800, 1000 or 2000 pM. Non-specific binding was assessed in the presence of 10 μM clomipramine.

[^{3}H]Paroxetine binding was performed in slides from the following regions: the septum, basolateral amygdala, hippocampus and ventromedial hypothalamus. Binding was

assessed in three slides containing consecutive sections from each region. Nissl-stained guide slides were used to choose sections from approximately the same anatomical location for each region analyzed. Examples of sections chosen are depicted in Fig. 1. Total binding was assessed in two slides/brain region/animal and non-specific binding was assessed in one slide/region/animal. [^{3}H]paroxetine (NEN Life Science Products, Boston, MA) binding was performed using a modification of a previously published procedure [20,26]. Briefly, slides were warmed to room temperature and then incubated in 50 mM Tris–HCl (pH 7.4) for 30 min at room temperature. Slides used for total binding were then incubated in [^{3}H]paroxetine (800 pM) diluted in 50 mM Tris–HCl buffer plus 120 mM NaCl and 5 mM KCl for 60 min at room temperature. After incubating with the [^{3}H]paroxetine, slides were washed twice (20 min each, at 37 °C) in incubation buffer, dipped in ice-cold water and allowed to dry. Slides and tritiated standards (Amersham) were apposed to Kodak [^{3}H]hyperfilm for 5 weeks and stored at room temperature. After the 5-week exposure period, all films were developed.

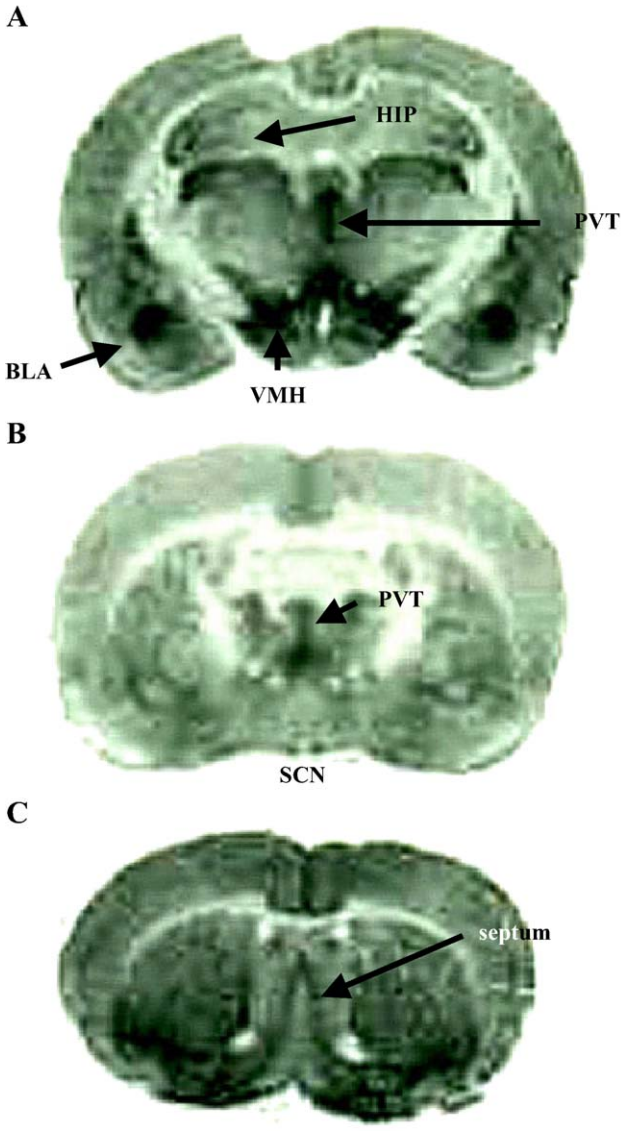

Fig. 1. Autoradiographs showing specific [^{3}H]paroxetine (free = 800 pM) binding in various brain regions. The following regions were analyzed: PVT, basolateral amygdala (BLA), hippocampus (HIP) and ventromedial hypothalamus (VMH) in A, the SCN and PVT in B, and the septum in C.

2.3. Analyses

Total and nonspecific [^{3}H]paroxetine binding was assessed from films using NIH Image. Films were placed on a light table that produced even and consistent illumination, and images from the film were captured using a Sony XC-77 camera with a CCTV lens to magnify the image. To control for slight differences in densities that may have occurred during film development, the aperture on the camera lens was adjusted so that the tritiated standards (range 2.7–126.4 Bq/mg tissue; Amersham) on each film gave equal density measurements from film to film. The density units for individual standards were used to make a standard curve and the density of labeling in samples was calculated using this curve. Both total and non-specific binding were measured by placing a defined box over the region of interest and taking a density measurement. Specific binding was calculated by subtracting non-specific binding from total binding. The K_d and B_{max} for specific [^{3}H]paroxetine binding in the SCN were calculated and the saturation curves were compared using Prism GraphPad (GraphPad Software). Specific binding in each region was analyzed using a 2 (age) × 2 (treatment) × 3 (time of day) ANOVA. Post-hoc analyses were made using a Tukey-HSD. All values are represented as means ± S.E.M. Significant differences were those with a probability of $p < 0.05$.

3. Results

The images in Fig. 1 demonstrate [^{3}H]paroxetine in the brain regions analyzed. The three-way ANOVAs analyzing the effects of time, estrogen and aging on specific [^{3}H]paroxetine binding in individual brain regions revealed that binding is differentially regulated by aging and/or estrogen in various brain regions. First, there was a main effect of estradiol treatment on specific [^{3}H]paroxetine binding in some of the brain regions examined (Table 1). Specific [^{3}H]paroxetine binding in the basolateral amygdala, ventromedial hypothalamus and hippocampus was significantly higher in estradiol-treated than in vehicle-treated animals, regardless of age. There was also a main effect of aging on specific [^{3}H]paroxetine binding in some regions, with aging significantly increasing binding in the basolateral amygdala, paraventricular thalamus and ventromedial hypothalamus (Table 2).

Table 1
The effects of ovariectomy and estradiol replacement on specific [^{3}H]paroxetine binding (Bq/mg tissue ± S.E.M.) in the brains of female rats

	Vehicle-treated	Estradiol-treated
Septum	139.58 ± 2.21	139.18 ± 2.34
Suprachiasmatic nucleus	9.54 ± 1.40	9.28 ± 1.24
Paraventricular thalamus	170.02 ± 2.11	164.70 ± 2.03
Basolateral amygdala	3.92 ± 0.16	4.49 ± 0.17*
Ventromedial hypothalamus	6.54 ± 0.40	7.96 ± 0.44*
Hippocampus	0.99 ± 0.11	1.48 ± 0.12*

Young and middle-aged female rats were ovariectomized and 7 days later, animals received estradiol replacement or oil (vehicle) replacement. Two days after vehicle or estradiol replacement, brains were collected and specific [^{3}H]paroxetine binding was assessed. These data show the main effects of estradiol-replacement on specific [^{3}H]paroxetine binding. Binding in the basolateral amygdala, ventromedial hypothalamus and hippocampus was significantly higher in animals receiving estradiol replacement than in vehicle-treated animals.

*$p < 0.05$.

In the SCN and PVT, not only was specific [^{3}H]paroxetine binding influenced by aging and estrogen, but it was also influenced by the time of day. There was a significant three-way interaction between aging, treatment and time of day on specific [^{3}H]paroxetine binding in the SCN of female rats [$F(2,61) = 3.54$, $p = 0.035$; see Fig. 2]. Further analyses of these interactions revealed that specific [^{3}H]paroxetine binding fluctuates over the day in all groups of animals. However, the pattern of binding is modulated by age and by estradiol. In young estradiol-treated animals, specific [^{3}H]paroxetine binding was highest during early morning (at 03:00 h) and lower at the other two time points ($p < 0.01$, compared to 12:00 and 20:00 h). In contrast, in young, vehicle-treated females, the highest levels of [^{3}H]paroxetine binding were seen later in the day at 12:00 h ($p < 0.01$ compared to 03:00 and 20:00 h). The highest levels of [^{3}H]paroxetine binding in both vehicle- and estradiol-treated middle-aged animals were detected at 20:00 h ($p < 0.01$ compared to 03:00 and 12:00 h). Thus, both aging and estrogen modulate daily fluctuations in specific [^{3}H]paroxetine binding in the SCN of female rats.

Table 2
Specific [^{3}H]paroxetine binding (average Bq/mg tissue ± S.E.M.) in the brains of young (2–4 months) and middle-aged (10–12 months) female rats

	Young	Middle-age
Septum	141.37 ± 2.26	137.39 ± 2.29
Suprachiasmatic nucleus	9.11 ± 1.32	9.72 ± 1.21
Paraventricular thalamus	160.51 ± 1.95	175.77 ± 2.19*
Basolateral amygdala	3.82 ± 0.17	4.59 ± 0.16*
Ventromedial hypothalamus	6.62 ± 0.42	7.89 ± 0.43*
Hippocampus	1.24 ± 0.11	1.20 ± 0.12

Specific [^{3}H]paroxetine binding was assessed in various brain regions in young and middle-aged females that had been ovariectomized and treated with estradiol or oil. These data show the main effects of age on specific [^{3}H]paroxetine binding. Binding was significantly increased in the paraventricular thalamus, basolateral amygdala and ventromedial hypothalamus and hippocampus of middle-aged animals.

*$p < 0.05$.

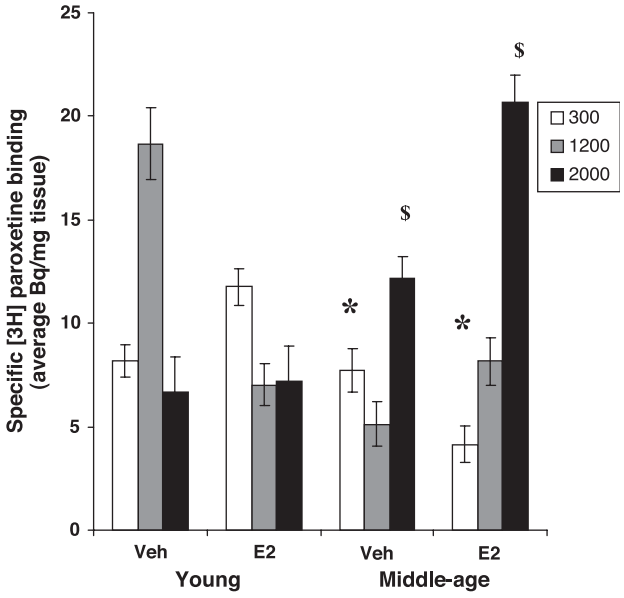

Fig. 2. The density of specific [^{3}H]paroxetine binding in the SCN of young and middle-age vehicle- and estradiol-treated females at three times of day. The ANOVA revealed a three-way interaction between the variable [$F(2,61) = 3.54$, $p = 0.035$]. Analyses of this interaction revealed that the density of specific [^{3}H]paroxetine binding exhibited significant fluctuations over the day in all groups examined. However, the pattern of these fluctuations was different in each group, indicating that age and estradiol interact to regulate paroxetine binding in the SCN. There was also a significant two-way interaction between age and time of day, with binding being significantly higher in the middle-age animals than the young animals at 20:00 h ($^{\$}p < 0.05$) and significantly lower than binding in the young animals at 03:00 h (*$p < 0.05$).

There was also a significant two-way interaction between aging and time of day [$F(2,61) = 7.944$, $p = 0.0009$] on the level of paroxetine binding sites in the SCN. Specific [^{3}H]paroxetine binding in the SCN of young animals was higher than binding in the SCN of middle-aged animals at 03:00 h [$F(1,22) = 4.74$, $p = 0.04$] and binding in the SCN of middle-aged animals was higher than binding in young animals at 20:00 h [$F(1,20) = 4.71$, $p = 0.042$]. Based on these results, we conclude that the number of specific [^{3}H]paroxetine binding sites changes over the day in the

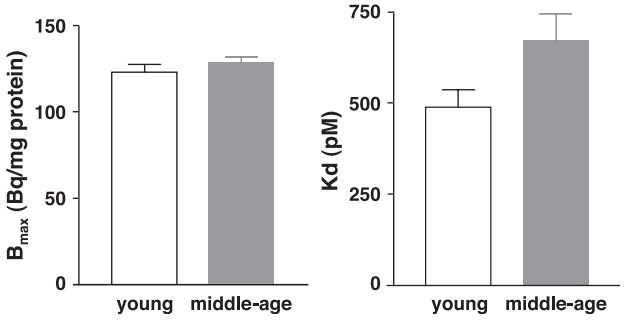

Fig. 3. These graphs show the mean (± S.E.M.) B_{max} and K_d for specific [^{3}H]paroxetine binding in the SCN of young and middle-age females that were ovariectomized and treated with estradiol. Neither the B_{max} nor the K_d were significantly different between the two groups.

SCN in all of the experimental groups, and that aging does not simply increase or decrease the paroxetine binding sites, but instead alters the daily pattern in binding in the SCN.

The B_{max} for specific [^{3}H]paroxetine binding in the SCN of female rats was not altered by age (means ± S.E.M.; 123.8 ± 4.77 and 125.9 ± 3.64 Bq/mg tissue for young and middle-aged animals, respectively; see Fig. 3). The K_d for binding in the SCN was also not significantly affected by age (629 ± 73.44 vs. 509 ± 48.53 pM, respectively). Thus, aging and estrogen do not affect the B_{max} or K_d for specific [^{3}H]paroxetine binding in the SCN at 12:00 h. However, because there are daily fluctuations in binding, the B_{max} or K_d could be affected by aging or estrogen at different time points during the day.

Specific [^{3}H]paroxetine binding also fluctuated over the day in the PVT. The three-way ANOVA revealed that there was a significant interaction between time of day and condition [$F(2,59) = 3.89$, $p = 0.03$; see Fig. 4], with specific [^{3}H]paroxetine binding being higher in the estradiol-treated animals than in the ovariectomized animals at 20:00 h [$F(1,24) = 7.19$, $p = 0.013$]. There were no differences between ovariectomized animals and estradiol-treated animals at the other time points. Because the PVT may play a role in modulating various circadian rhythms, we performed specific post-hoc analyses to determine if [^{3}H]paroxetine binding fluctuated over the day in the PVT, and how aging and estrogen regulated these fluctuations. Specific [^{3}H]paroxetine binding in the PVT of young animals varied over the day, with the estradiol-treated animals exhibiting high levels at 03:00 and 20:00 h and low levels at 12:00 h ($p < 0.05$), while the vehicle-treated animals showed high levels at 12,000 and low levels at 03:00 and 20:00 h ($p < 0.05$). Specific [^{3}H]paroxetine binding in the PVT of middle-aged estradiol-treated animals also fluctuated throughout the day with the peak occurring at 20:00 h ($p < 0.05$); in contrast, specific [^{3}H]paroxetine binding in the middle-aged vehicle-treated animals did not exhibit daily variations. Based on these results, we conclude that estrogen modulates the daily pattern of specific [^{3}H]paroxetine binding in the PVT. Although estradiol does restore daily fluctuations in binding in the middle-aged animals, this pattern is different from that seen in young animals.

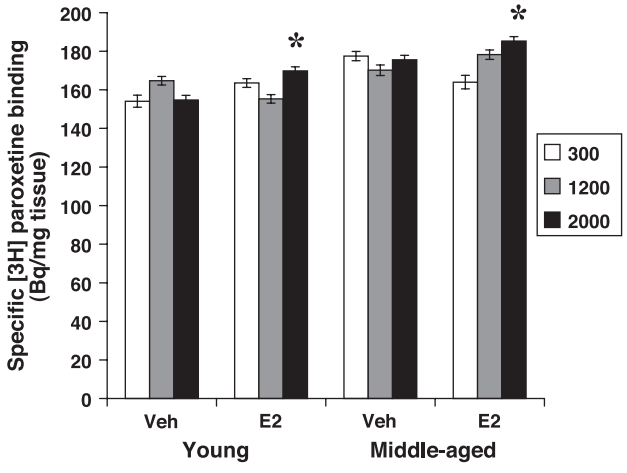

Fig. 4. Specific [^{3}H]paroxetine binding in the PVT of young and middle-age female rats that were ovariectomized and treated with estradiol (E2) or oil (veh). The analyses of paroxetine binding in the PVT revealed that there was a significant interaction between time of day and condition [$F(2,59) = 3.89$, $p = 0.03$], with binding being higher in estradiol-treated animals than vehicle-treated animals at 20:00 h (*greater than estradiol-treated animals of the same age, $p < 0.05$). Planned comparisons also revealed that there were daily fluctuations in paroxetine binding in the PVT of all animals except the middle-aged vehicle-treated animals. Age and estradiol affected the patterns of these fluctuations and the time of peak binding.

4. Discussion

It has been hypothesized that changes in mood, memory and sleep/wake patterns that occur during the peri-menopausal period may be associated with changes in the regulation of SERT levels or binding [7,24,33]. However, to date, most studies have only examined the effects of ovarian steroids in young animals, and have not taken into account the effects of age on SERT levels in females. The goal of this study was to determine how aging and changes in circulating estradiol levels interact to affect SERT levels, as measured by [^{3}H]paroxetine, in the forebrain of female rats. We found that in most, but not all, brain regions we analyzed, aging and estrogen affected specific [^{3}H]paroxetine binding, and that these variables acted independently.

Specific [^{3}H]paroxetine binding was significantly increased by estradiol replacement in the basolateral amygdala, ventromedial hypothalamus and hippocampus of both young and middle-aged female rats. These findings are consistent with the results of other studies showing that estrogen increases specific [^{3}H]paroxetine binding and SERT mRNA in the brains of young female rats [33] and SERT levels in the hypothalamus of macaques [30,48]. Since SERT mRNA levels appear to be regulated by estrogen, and because previous work has demonstrated that estrogen does not alter the affinity of the SERT for paroxetine in the CNS of female rats [33], these estrogen-induced changes are most likely the result of changes in the number of binding sites in these specific regions.

In contrast to previous reports, where estrogen increased specific [^{3}H]paroxetine binding in the septum of female rats [33], we did not see any difference in specific [^{3}H]paroxetine binding in the septum of ovariectomized and estradiol-treated rats. The differences between our results and those of Ref. [33] may be due to the length of time animals were ovariectomized and exposed to estradiol before tissue was collected. In Ref. [33], animals were immediately injected with estradiol after being ovariectomized and they were euthanized the next day. However, in this study, animals were ovariectomized for one week before receiving estradiol replacement and tissue was collected 2 days after replacement. Since animals in the current study were ovariecto-

mized longer than in McQueen's study, it is possible that they needed an extended exposure to estradiol replacement to see an increase in specific [^{3}H]paroxetine binding in the septum.

Aging also altered specific [^{3}H]paroxetine binding in a number of brain regions. In most regions examined, specific [^{3}H]paroxetine binding was higher in middle-aged than in young animals. These findings are consistent with data collected in male hamsters that demonstrate that aging increases [^{3}H]paroxetine binding in specific brain regions [20], and with data collected in male rats indicating that aging results in an increase in SERT mRNA in the raphe [34]. Although there is evidence demonstrating that reductions in 5-HT release are associated with a decrease in the number of [^{3}H]paroxetine binding sites [28], suggesting that SERT levels may be influenced by the amount of 5-HT being released, it is unlikely that the increase in [^{3}H]paroxetine binding in aged animals and humans is due to an increase in 5-HT release in the CNS. In fact, a number of studies have demonstrated that aging reduces 5-HT turnover [49] and causes degeneration of the 5-HT system [55]. It is possible that the age-related increase in the number of [^{3}H]paroxetine binding sites in various brain regions reduces the effects of 5-HT by lowering the concentrations of 5-HT in the synapse. However, it is also possible that this age-related increase in the number of SERT binding sites may serve as a mechanism to rapidly remove 5-HT from the synapse so that it can be metabolized and the tryptophan used to synthesize more 5-HT. To fully understand how aging affects 5-HT release and uptake, both these variable must be measured in specific brain regions over the same time course so that the relationship between these two variables can be accurately described.

The only two regions that showed a significant interaction between estradiol and age on specific [^{3}H]paroxetine binding were the SCN and the PVT. These regions were also the only two regions where specific [^{3}H]paroxetine binding was influenced by the time of day. Although we only examined specific [^{3}H]paroxetine binding at three different time points during the day, our results suggest that there may be a rhythm in specific [^{3}H]paroxetine binding in the SCN and that the pattern of this rhythm is altered by aging and estrogen. In young animals, ovariectomy appears to delay the peak in specific [^{3}H]paroxetine binding in the SCN. Ovariectomy and aging also have been shown to regulate the rhythm in 5-HT turnover in the SCN of female rats, where ovariectomy and aging attenuate the rhythm in 5-HT turnover [11,12]. 5-HT release into the SCN is highly correlated with activity, with 5-HT release being highest during the early evening bout of activity in nocturnal rodents [6,17]. A number of studies have demonstrated that ovarian hormones can regulate the time of activity onset and level of activity in female rodents, with activity onset occurring earlier when estrogen levels are high, and later when estrogen levels are lower [1,5,52]. Thus, it is possible that steroid hormones regulate activity/rest cycles and that changes in activity/rest cycles affect 5-HT activity (turnover and SERT levels) in the SCN.

Aging also alters the expression of activity rhythms [53] and results in changes in the rhythmic pattern of the sleep/wake cycles in humans and rats [14,27]. Post-menopausal women often sleep less, wake earlier and go through fewer bouts of slow-wave sleep than younger women [2,3]. Age-related changes in both endocrine and sleep/wake rhythms have been correlated with the declining levels of estrogen during menopause [3,24]. However, based on the results of this study, it does not appear as if estrogen replacement restores the pattern of specific [^{3}H]paroxetine binding in the SCN of older animals. Estrogen replacement also does not restore the rhythm in 5-HT turnover in the SCN of aged female rats [12]. However, estrogen replacement does appear to alleviate some of the disturbances in sleep patterns seen in post-menopausal women [2]. Thus, age-related changes in the expression of biological rhythms and sleep/wake patterns are not solely due to changes in circulating estradiol levels, or to changes in 5-HT neural transmission in the SCN.

The pattern of specific [^{3}H]paroxetine binding in the PVT is also affected by estrogen and age. In young animals treated with estradiol, specific [^{3}H]paroxetine binding was high at 03:00 and 20:00 h and low at 12:00 h. In contrast, in young ovariectomized vehicle-treated animals, this peak was altered, with specific [^{3}H]paroxetine binding being highest at 12:00 h and lower at 03:00 and 20:00 h. Based on these results, we conclude that estrogen regulates the pattern of specific [^{3}H]paroxetine binding in the PVT of young animals.

The ability of estrogen to regulate specific [^{3}H]paroxetine binding in the PVT appears to be altered in reproductively senescent animals. Estradiol-treatment restored daily fluctuations in paroxetine binding in middle-aged animals. However, this pattern of binding was different from that seen young animals, indicating that changes seen in paroxetine binding in the PVT of reproductively senescent female rats are regulated by both changes in estradiol and aging.

The PVT receives dense serotonergic input from the raphe [16,45] and input from the circadian system (i.e., the SCN [50]). This region of the brain appears to play a role in the regulation of activity, sleep/wake [38,39] and corticosteroid rhythms [8] in rodents. It is also a site of action for many antispychotic agents [13]. Thus, estrogen- and age-related changes in serotonergic activity and specific [^{3}H]paroxetine binding in this region may affect the expression the expression of certain biological rhythms and mood. In fact, because changes in biological rhythms and mood are often coupled in individuals, we hypothesize that the PVT may serve as one of the central regions integrating mechanisms that regulate these processes.

In conclusion, the regulation of specific [^{3}H]paroxetine binding in many forebrain regions of females appears to be independently regulated by aging and estrogen. Thus, certain age-related changes in mood, cognition and/or biolog-

ical rhythms in post-menopausal women may not be due to a reduction in estrogen, but instead may be solely due to the effects of age. In contrast, age- and estrogen-related changes in the rhythmic release of corticosteriods and sleep may be related to changes in serotonergic activity in the PVT and future studies will be designed to determine how these factors interact to influence inputs and outputs of this region.

Acknowledgements

This work was funded by an American Federation of Aging grant to KK and AGO2224 to PMW.

References

[1] H.E. Albers, Gonadal hormones organize and modulate the circadian system of the rat, Am. Physiol. Soc. (1981) 62–66.
[2] I.A. Antonijevic, G.K. Stalla, A. Steiger, Modulation of sleep electroencephalogram by estrogen replacement in postmenopausal women, Am. J. Obstet. Gynecol. 182 (2000) 277–282.
[3] I.A. Antonijevic, H. Murck, R.M. Frieboes, M. Uhr, A. Steiger, On the role of menopause for sleep-endocrine alterations associated with major depression, Psychoneuroendocrinology 28 (2003) 401–418.
[4] N.E. Avis, S. Crawford, R. Stellato, C. Longcope, Longitudinal study of hormone levels and depression among women transitioning through menopause, Climacteric 4 (2001) 243–249.
[5] J.F. Axelson, A.A. Gerall, H.E. Albers, Effect of progesterone on the estrous activity cycle of the rat, Psychol. Behav. 26 (1981) 631–635.
[6] S. Barassin, S. Raison, M. Saboureau, C. Bienvenu, M. Maitre, A. Malan, P. Pevet, Circadian tryptophan hydroxylase levels and serotonin release in the suprachiasmatic nucleus of the rat, Eur. J. Neurosci. 15 (2002) 833–840.
[7] C.L. Bethea, C. Gundlah, S.J. Mirkes, Ovarian steroid action in the serotonin neural system of macaques, Novartis Found. Symp. 230 (2000) 112–130.
[8] S. Bhatnagar, M.F. Dallman, The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner, Brain Res. 851 (1999) 66–75.
[9] A. Bigeon, C.T. Fischette, T.C. Rainbow, B.S. McEwen, Serotonin receptor modulation by estrogen in discrete brain nuclei, Neuroendocrinology 35 (1982) 287–291.
[10] P.G. Clifton, Meal patterning in rodents: psychopharmacological and neuroanatomical studies, Neurosci. Biobehav. Rev. 24 (2000) 213–222.
[11] I.R. Cohen, P.M. Wise, Age-related changes in the diurnal rhythm of serotonin turnover in microdissected brain areas of estradiol-treated ovariectomized rats, Endocrinology 122 (1988) 2626–2633.
[12] I.R. Cohen, P.M. Wise, Effects of estradiol on the diurnal rhythm of serotonin activity in microdissected brain areas of ovariectomized rats, Endocrinology 122 (1988) 2619–2625.
[13] B.M. Cohen, W. Wan, M.P. Froimowitz, D.J. Ennulat, S. Cherkerzian, H. Konieczna, Activation of midline thalamic nuclei by anti-psychotic drugs, Psychopharmacology 135 (1998) 37–43.
[14] C.A. Czeisler, J.F. Duffy, T.L. Shanahan, E.N. Brown, J.F. Mitchell, D.W. Rimmer, J.M. Ronda, E.J. Silva, J.S. Allan, J.S. Emens, D.-J. Dijk, R.E. Kronauer, Stability, precision, and near-24-hour period of the human circadian pacemaker, Science 284 (1999) 2177–2181.
[15] M.S. Davidoff, I.S. Lolova, Age-related changes in serotonin-immunoreactivity in the telencephalon and diencephalon of rats, J. Hirnforsch. 32 (1991) 745–753.
[16] E.B. DeSouza, B.L. Kuyatt, Autoradiographic localization of ^{3}H-paroxetine-labeled serotonin reuptake sites in rat brain, Synapse 1 (1987) 488–496.
[17] T.E. Dudley, L.A. DiNardo, J.D. Glass, Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus, J. Neurosci. 18 (1998) 5045–5052.
[18] M.J. Duncan, J.G. Hensler, Aging alters in a region-specific manner serotonin transporter sites and 5-HT$_{1A}$ receptor-G protein interactions in hamster brain, Neuropharmacology 43 (2002) 36–44.
[19] M.J. Duncan, J. Short, D.L. Wheeler, A comparison of the effects of aging of 5-HT$_{7}$ and 5-HT$_{1A}$ receptors in discrete regions of the circadian timing system in hamsters, Brain Res. 829 (1999) 39–45.
[20] M.J. Duncan, C.J. Crafton, D.L. Wheeler, Aging regulates 5-HT$_{1B}$ receptors and serotonin reuptake sites in the SCN, Brain Res. 856 (2000) 213–219.
[21] M.I. Gonzalaz, M.L. Leret, Extrahypothalamic serotonergic modification after masculinization induced by neonatal gonadal hormones, Pharmacol. Biochem. Behav. 41 (1992) 329–332.
[22] A.C. Gore, E. Terasawa, Neural circuits regulating pulsatile luteinizing hormone release in the female guinea pig: opiod, adrenergic and serotonergic interactions, J. Neuroendocrinol. 13 (2001) 239–248.
[23] C. Gundlah, T.M. Pecins, W.E. Schutzer, C.L. Bethea, Ovarian steroid effects on serotonin 1A, 2A and 2C receptor mRNA in Macaque hypothalamus, Mol. Brain Res. 63 (1999) 325–339.
[24] C.B. Hammond, Menopause and hormone replacement therapy: an overview, Obstet. Gynecol. 87 (1996) 2s–15s.
[25] L.D. Hollander, E.W. Freeman, M.D. Sammel, J.A. Berlin, J.A. Grisso, M. Battistini, Sleep quality, estradiol levels, and behavioral factors in late reproductive age women, Obstet. Gynecol. 98 (2001) 391–397.
[26] P.D. Hrdina, B. Foy, A. Hepner, R.J. Summers, Antidepressant binding sites in brain: autoradiographic comparison of [^{3}H]paroxetine and [^{3}H]imipramine localization and relationship to serotonin transporter, J. Pharmacol. Exp. Ther. 252 (1990) 410–418.
[27] H. Li, E. Satinoff, Changes in circadian rhythms of body temperature and sleep in old rats, Am. J. Physiol. 269 (1995) R208–R214.
[28] K. Linnet, K. Koed, O. Wiborg, N. Gregersen, Serotonin depletion decreases serotonin transporter mRNA levels in rat brain, Brain Res. 697 (1995) 251–253.
[29] N.Z. Lu, T.A. Shlaes, C. Gundlah, S.E. Dziennis, R.E. Lyle, C.L. Bethea, Ovarian steroid action on tryptophan hydroxylase protein and serotonin compared to localization of ovarian steroid receptors in midbrain of guinea pigs, Endocrine 11 (1999) 257–267.
[30] N.Z. Lu, A.J. Eshleman, A. Janowsky, C.L. Bethea, Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution and function in female macaques, Mol. Psychiatry 8 (2003) 353–360.
[31] S. Maswood, G. Stewart, L. Uphouse, Gender and estrous cycle effects of the 5HT1a agonist, 8-OH-DPAT, on hypothalamic serotonin, Pharmacol. Biochem. Behav. 51 (1995) 807–813.
[32] S. Maswood, W. Truitt, M. Hotema, M. Caldarola-Patuszka, L. Uphouse, Estrous cycle modulation of extracellular serotonin in mediobasal hypothalamus: role of the serotonin transporter and terminal autoreceptors, Brain Res. 831 (1999) 146–154.
[33] J.K. McQueen, H. Wilson, G. Fink, Estradiol-17B increases serotonin transporter (SERT) mRNA levels and the density of SERT-binding sites in female rat brain, Mol. Brain Res. 45 (1997) 13–23.
[34] B. Meister, H. Johnson, B. Ulfhake, Increased expression of serotonin transporter messenger RNA in raphe neurons of the aged rat, Mol. Brain Res. 33 (1995) 87–96.
[35] A. Meneses, 5-HT system and cognition, Neurosci. Biobehav. Rev. 23 (1999) 1111–1125.
[36] J.D. Miller, L.P. Morin, W.J. Schwartz, R.Y. Moore, New insights into the mammalian circadian clock, Sleep 19 (1996) 641–667.
[37] J.A. Moguilevsky, W. Wuttke, Changes in the control of gonadotropin secretion by neurotransmitters during sexual development in rats, Exp. Clin. Endocrinol. Diabetes 109 (2001) 188–195.
[38] C.M. Novak, A.A. Nunez, Daily rhythms in Fos activity in the rat

ventrolateral preoptic area and midline thalamic nuclei, Am. J. Physiol. 275 (1998) R1620–R1626.
[39] C.M. Novak, L. Smale, A.A. Nunez, Rhythms in Fos expression in brain areas related to the sleep–wake cycle in the diurnal *Arvicanthus niloticus*, Am. J. Physiol. 278 (2000) R1267–R1274.
[40] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, NY, 1997.
[41] M. Pecins-Thompson, C.L. Bethea, Ovarian steroid regulation of serotonin-1A autoreceptor messenger RNA expression in the dorsal raphe of *Rhesus macaques*, Neuroscience 89 (1999) 267–277.
[42] M. Pecins-Thompson, N.A. Brown, S.G. Kohama, C.L. Bethea, Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in *Rhesus macaques*, J. Neurosci. 16 (1996) 7021–7029.
[43] C.M. Portas, B. Bjorvatn, R. Ursin, Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies, Prog. Neurobiol. 60 (2000) 13–35.
[44] A.G. Reznikov, N.D. Nosenko, Early postnatal changes in sexual dimorphism of catecholamine and indolamine content in the brain of prenatally stressed rats, Neuroscience 70 (1996) 547–551.
[45] P.E. Sawchenko, L.W. Swanson, H.W. Steinbusch, A.A. Verhofstad, The distribution and cells of origin of serotonergic inputs to the paraventricular and supraoptic nuclei of the rat., Brain Res. 277 (1983) 355–360.
[46] J. Shaffery, R. Hoffman, R. Armitage, The neurobiology of depression: perspectives from animal and human sleep studies, Neuroscientist 9 (2003) 82–98.
[47] B.B. Sherwin, Hormones, mood, and cognitive functioning in postmenopausal women, Obstet. Gynecol. 87 (1996) 20s–26s.
[48] C.A. Shively, S.J. Mirles, N.Z. Lu, J.A. Henderson, C.L. Bethea, Soy and social stress affect serotonin neurotransmission in primates, Pharmacogenomics J. 3 (2003) 114–121.
[49] J.W. Simpkins, Regional changes in monoamine metabolism in the aging constant estrous rat, Neurobiol. Aging 5 (1984) 309–313.
[50] P.J. Sollars, G.E. Pickard, Vasoactive intestinal peptide efferent projections of the suprachiasmatic nucleus in anterior hypothalamic transplants: correlation with functional restoration of circadian behavior, Exp. Neurol. 136 (1995) 1–11.
[51] B.E. Summer, G. Fink, Estrogen increases the density of 5-hydroxytryptamine(2A) receptors in cerebral cortex and nucleus accumbens in the female rat, J. Steroid Biochem. Mol. Biol. 54 (1995) 15–20.
[52] J.S. Takahashi, M. Menaker, Interaction of estradiol and progesterone: effects in circadian locomotor rhythm of female golden hamsters, Am. J. Physiol. 239 (1980) 497–504.
[53] F.W. Turek, P.Z.Y. Penev, O. van Reeth, P. Zee, Effects of age on the circadian system, Neurosci. Biobehav. Rev. 19 (1995) 53–58.
[54] C.H. van Dyck, R.T. Malison, J.P. Seibyl, M. Laruelle, H. Klumpp, S.S. Zoghbi, R.M. Baldwin, R.B. Innis, Age-related decline in central serotonin transporter availability with [^{123}I]β-CIT SPECT, Neurobiol. Aging 21 (2000) 497–501.
[55] M.G.P.A. van Luijtelaar, H.W.M. Steinbusch, J.A.D.M. Tonnaer, Similarities between aberrant serotonergic fibers in the aged and 5,7-DHT de-innervated young adult rat brain, Exp. Brain Res. 78 (1989) 81–89.
[56] D. Verge, A. Calas, Serotonergic neurons and serotonin receptors: gains from cytochemical approaches, J. Chem. Neuroanat. 18 (2000) 41–56.
[57] P.M. Wise, M.L. Kashon, K.M. Krajnak, K.L. Rosewell, A. Cai, K. Scarbrough, J.P. Harney, T.M. McShane, J.M. Lloyd, N.G. Weildand, Aging of the female reproductive system: a window into brain aging, Rec. Prog. Horm. Res. (1997) 279–305.
[58] P.M. Wise, M.J. Smith, D.B. Dubal, M.E. Wilson, K.M. Krajnak, K.L. Rosewell, Neuroendocrine influences and repercussions of the menopause, Endocr. Rev. 20 (1999) 243–248.
[59] M.E. Yacoubi, S. Bouali, D. Popa, L. Naudon, I. Leroux-Nicollet, M. Hamon, J. Costentin, J. Adrien, J.-M. Vaugeois, Behavioral, neurochemical and electrophysiological characterization of a genetic mouse model of depression, PNAS 100 (2003) 6227–6232.