Sex and regional differences in estradiol content in the prefrontal cortex, amygdala and hippocampus of adult male and female rats

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Abstract

In general, the behavioural and neural effects of estradiol administration to males and females differ. While much attention has been paid to the potential structural, cellular and sub-cellular mechanisms that may underlie such differences, as of yet there has been no examination of whether the differences observed may be related to differential uptake or storage of estradiol within the brain itself. We administered estradiol benzoate to gonadectomized male and female rats, and compared the concentration of estradiol in serum and brain tissue found in these rats to those of gonadectomized, oil-treated rats and intact rats of both sexes. Long-term gonadectomy (3 weeks) reduced estradiol concentration in the male and female hippocampus, but not in the male or female amygdala or in the female prefrontal cortex. Furthermore, exogenous treatment with estradiol increased estradiol content to levels above intact animals in the amygdala, prefrontal cortex and the male hippocampus. Levels of estradiol were undetectable in the prefrontal cortex of intact males, but were detectable in all other brain regions of intact rats. Here we demonstrate (1) that serum concentrations of estradiol are not necessarily reflective of brain tissue concentrations, (2) that within the brain, there are regional differences in the effects of gonadectomy and estradiol administration, and (3) that there is less evidence for local production of estradiol in males than females, particularly in the prefrontal cortex and perhaps the hippocampus. Thus there are regional differences in estradiol concentration in the prefrontal cortex, amygdala and hippocampus that are influenced by sex and hormone status.

Introduction

In various species, sex differences have been frequently reported on a number of learning and memory tasks (e.g. Gibbs and Johnson 2008), and any time a sex difference is observed this generally suggests that sex hormones, such as estradiol, play a role in mediating these differences. For example, males usually outperform females on hippocampus-dependent tasks (for reviews, see Galea *et al.* 1996; Luine 2008) and greater sex differences are observed in spatial learning when circulating gonadal hormone levels are high in adulthood (Galea *et al.* 1994; Galea *et al.* 1995). Generally, high levels of estradiol impair memory while low levels of estradiol facilitate spatial working and reference memory in adult female rats (Holmes *et al.* 2002). Some studies have found that estradiol can alter spatial performance in adult males (Luine and Rodriguez 1994; Moradpour *et al.* 2006 These findings suggest that hippocampus-dependent learning is modulated by estradiol in both adult males and females.

Adult levels of estradiol differentially affect the structure of the hippocampus between the sexes (Barker and Galea 2008; Galea et al. 2006; Lee et al. 2004; Leranth et al. 2003; McLaughlin et al. 2008). For example, repeated estradiol administration affects adult neurogenesis and cell death in the dentate gyrus of adult female, but not male, rats (Barker and Galea 2008; Spritzer and Galea 2007) and short-term estradiol increases apical dendritic spine density in the CA1 region of the hippocampus in female (Woolley and McEwen 1993) but not male rats (Leranth *et al.* 2003). Thus estradiol differentially affects hippocampus structure in adult female, but not male, rats.

Sex differences have also been reported in amygdala-dependent behaviours and structure, which are in part be mediated by estradiol levels in adulthood. Estradiol

disrupts conditioned fear performance in female, but not male, rodents (Toufexis *et al.* 2007; Gupta et al. 2001; Morgan and Pfaff 2001). There are also sex differences favoring males in size, morphology and responsiveness to sex steroids in various nuclei within the amygdala (Carrillo *et al.* 2007; Cooke *et al.* 2003; Hines *et al.* 1992; Mizukami *et al.* 1983; Vinader-Caerols *et al.* 1998). Thus activational effects of estradiol influence amygdala-based structure and function, and may do so differently in males versus females.

Males and females also differ prefrontal cortex-dependent cognition and morphology (Harness *et al.* 2008; Müller *et al.* 2007) that is in part dependent on levels of estradiol, (Alejandre-Gomez *et al.* 2007; Wide *et al.* 2004). In adult female rats, low levels of estradiol facilitate, and high levels of estradiol impair, performance on a non-spatial working memory task (Wide *et al.* 2004). In adult male rats, decreasing estradiol improves working memory on a delayed matching to sample task (Alejandre-Gomez *et al.* 2007. Further, estradiol increases dendritic spine density in the prefrontal cortex of both male and female rodents (Hajszan *et al.* 2007; Hao *et al.* 2006; Wallace *et al.* 2006). Thus estradiol influences prefrontal cortex function and morphology in both male and female rodents.

Collectively, these studies suggest that estradiol differentially affects the structure and function of the hippocampus, amygdala and prefrontal cortex in male and female rats. However, the amount of estrogen that can be bound in various brain regions - including the cortex, hippocampus, and amygdala - is very similar in male and female rats (Barley *et al.* 1977; Lieberburg *et al.* 1980; Ogren *et al.* 1976). Estrogen receptors (ER)-α and -β are found in the hippocampus and amygdala, and ERβ is found in the

prefrontal cortex (Shughrue *et al.* 1997) at similar levels between males and females in these regions (hippocampus ERα: Weiland *et al.* 1997; amygdala (ERα and ERβ: Kalita *et al.* 2005; Simerly *et al.* 1990; prefrontal cortex (ERα and ERβ: Kritzer 2002; Simerly *et al.* 1990). Thus sex differences in the influence of estradiol on the structure or function of these areas may be due to differences in estradiol content after administration in various brain regions. To date the concentration of estradiol in the hippocampus, prefrontal cortex or amygdala has yet to be compared in adult male and female rats. We sought to determine the concentration of estradiol in the amygdala, hippocampus, and prefrontal cortex of intact, gonadectomized, and estradiol-replaced rats of both sexes. We administered either oil or estradiol benzoate over 15 days to gonadectomized rats, a regimen that results in profound sex differences in neurogenesis and cell death in the hippocampus (Barker and Galea 2008). We also sought to determine whether the levels of estradiol in the brain reflect those in serum, or are actively regulated independently of serum concentrations. We expect that estradiol levels in the brain tissue of rats treated with estradiol should be higher than those in oil-treated rats, and that sex and regional differences may exist.

Materials and methods

All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines regarding appropriate treatment of animals and were approved by the University of British Columbia. Every effort was made to minimize the number of animals used per group and to minimize the suffering of animals used throughout all experimental procedures.

Experimental Subjects

Twenty-four male and twenty-seven female adult (80-90 days old) Sprague-Dawley rats (Charles River Canada, Québec, Canada) were kept on a 12-hour light-dark cycle (lights on at 0700), housed in same-sex groups of 2-3 rats in opaque polyurethane bins (48 x 27 x 20 cm) with aspen chip bedding and Purina rat chow and tap water ad libitum.

Procedure

Eleven male (n = 5-6 per group) and twelve female rats (n = 6 per group) were gonadectomized under isoflurane anaesthesia one week after their arrival in the laboratory. Briefly, all rats were anaesthetized with 1-chloro-2, 2,2-trifluoroethyl difluoromethyl ether (isoflurane) using an initial flow rate of 4% and a maintenance flow rate of 2% during surgery. All females were bilaterally ovariectomized through bilateral flank incisions, and all males were bilaterally castrated through an incision in the scrotal sac. Beginning 7 days after surgery (Day 1), rats received subcutaneous injections of estradiol benzoate (EB; dissolved in 0.1 mL sesame oil) or sesame oil (vehicle, 0.1 mL) each day, between 1000 h and 1230 h, for 15 consecutive days (Fig. 1). This regimen was used as it has been previously shown to result in sex differences in hippocampal neurogenesis and cell death (Barker and Galea 2008). A dose of 33 μg/kg EB was used to match previous studies investigating the effects of estradiol on hippocampal neurogenesis and spine density (e.g. Barker and Galea 2008; Leranth et al. 2003; Rössler et al. 2006; Spritzer and Galea 2007; Tanapat et al. 1999; Woolley and McEwen 1993).

Beginning 6 days after surgery (Day -1), up to 200 μL of blood was collected from gonadectomized rats via a tail nick every 2-3 days (Fig. 1), one hour after injection (Woolley and McEwen 1993).

The remaining 6 males and 8 females were left intact and untreated, except for daily vaginal lavage of the females each day for 21 days to ensure that females were all in proestrus (and therefore maximal normal serum estradiol concentration) at the time of tissue collection. On Day 16, all rats (gonadectomized and intact) were heavily anaesthetized with isoflurane and rapidly decapitated. Trunk blood was collected from all rats, and the brain removed and dissected within 10 minutes. The hippocampus, amygdala, and prefrontal cortex were dissected out and stored at -80°C until processing.

Hormone assays

Following each blood collection, whole blood was allowed to clot at 4°C for 24 hours, then spun in a rotating centrifuge (8000 rpm for 8 minutes). The serum was collected and stored at -20°C until processing. The estradiol concentration of each sample was measured in duplicate using a commercial radioimmunoassay kit (MP Biomedicals). Briefly, 25 μL of each serum sample was added to each of two borosilicate glass tubes, along with a known amount of radioactive estradiol and anti-estradiol antibody. After incubation at 37°C for 90 minutes, a precipitant solution was added to remove the antibody (and bound estradiol) from solution. Tubes were spun in a rotating centrifuge (4°C, 1000 *g* for 20 minutes), the liquid portion discarded and the radioactivity of the remaining pellets measured using a gamma counter. A standard curve created by adding known amounts of estradiol to tubes was used to calculate the amount

of estradiol present in each experimental sample. The detection limit of this assay was 7.2 pg/mL, and the intra- and inter-assay coefficients of variation were 5.9% and 11.5%, respectively. Cross-reactivity of this kit with estrone is 20%, and below 1.6% with other steroid hormones.

Frozen brain tissue was weighed to obtain the wet weight of each brain region of interest. Tissue was then homogenized with 400 μL of phosphate-buffered saline (ph 7.4) using a Teflon manual homogenizer, and 25 μL of this homogenate (each sample in duplicate) was added to borosilicate glass test tubes. Two millilitres of diethyl ether was added to each tube to extract out steroid hormones, and the tubes were centrifuged (4°C, 1000 *g* for 5 minutes), then snap-frozen in a slurry of dry ice and 70% methanol. The ether fraction was decanted, and dried down under nitrogen at 37°C. The resulting crystalline hormones were re-dissolved in 25 μL of stripped (estradiol-free) human serum and analyzed using the radioimmunoassay kit as described above. Estradiol concentration in brain tissue was expressed in pg/g wet weight of tissue assayed.

To verify that the method used to measure estradiol was appropriate, additional samples of brain tissue were homogenized as described above. From each of these samples, 25 μL of the homogenate was treated and re-dissolved as described above. In separate tubes, 2.5 μL of 1000 pg/mL estradiol standard from the radioimmunoassay kit was added to an additional 22.5 μL of the homogenate from each sample. The concentration of estradiol measured in the original samples was used to calculate expected estradiol concentrations in the samples to which standard had been added. These expected values were compared to those obtained by direct measurement by

radioimmunoassay. Estradiol recovery was determined by comparing spiked samples with unspiked samples from the same homogenate (n = 35 pairs).

Data analyses

Concentrations of estradiol obtained from tail blood were analyzed using repeated-measures ANOVA, with sex (male, female) and treatment (oil, 15d estradiol) as between-subjects factors, and day (0 through +15) as the within-subjects factor. Estradiol concentrations in trunk blood and brain tissue on day 16 were analyzed using ANOVA, with sex and treatment (oil, 15d estradiol, intact) as between-subjects factors. Post-hoc tests utilized the Newman-Keuls procedure.

Results

Four blood samples were missing and these values were replaced with the mean value of the group in which these animals were assigned, for the day in question (missing values were from one each: oil-treated male and EB-treated female on day +1, oil-treated male on day 7, and EB-treated male on day 15). The overall ANOVA of serum levels during days 0-15 revealed a significant main effect of group ($F(1,19) = 182$; $p < 0.0001$) and of day ($F(6,114) = 8.47$; $p < 0.0001$), but not of sex ($F(1,19) = 0.45$; $p = 0.51$). There was also a significant group x day interaction effect ($F(6,114) = 9.22$; $p < 0.0001$). Post-hoc tests revealed that rats treated with estradiol for 15 days had higher serum levels of estradiol than oil-treated rats of the same sex from day 1 onwards (all comparisons $p < 0.009$; Fig. 2A).

An ANOVA of the concentration of estradiol in serum from estradiol-injected, oil-injected, and intact rats at the time of perfusion revealed a significant main effect of

group ($F(2,31) = 57.0$, $p < 0.0001$) but no significant main effect of sex ($F(1,31) = 0.05$, $p = 0.82$) or sex x group interaction effect ($F(2, 31) = 2.18$, $p = 0.13$). Post-hoc tests revealed that rats of either sex treated with estradiol for 15 days had higher serum levels of estradiol than rats treated with oil (both comparisons $p < 0.0001$). The concentration of estradiol in the serum of female rats treated with estradiol for 15 days was also higher than that of intact females in proestrus ($p < 0.0002$; Fig. 2B). In male rats, serum estradiol levels of rats treated with estradiol for 15 days were higher than those in intact males ($p < 0.0002$).

To verify the estradiol assay with brain tissue, the concentrations of estradiol measured in brain tissue to which estradiol had been added were highly correlated with those calculated for the same samples that were not spiked with known estradiol ($r = 0.93$, $p < 0.0001$, Fig. 3). Recovery of estradiol was 104 ± 7.5% in brain tissue. The experimental brain tissue was therefore also processed using ether extraction, resuspension, and radioimmunoassay.

Separate ANOVAs on the concentration of estradiol in the amygdala, hippocampus, or prefrontal cortex revealed a significant main effect of group in the amygdala ($F(2,31) = 11.4$, $p = 0.0002$; Fig. 4A), hippocampus ($F(2,31) = 16.6$; $p = 0.00001$; Fig. 4B), and prefrontal cortex ($F(2,31) = 11.3$, $p = 0.0002$; Fig. 4C). There were no other significant main or interaction effects for any region (all p`s > 0.19;). In the amygdala and prefrontal cortex, estradiol treatment increased the concentration of estradiol above that of both oil-treated and intact animals (both $p < 0.0004$; oil vs. intact $p = 0.85$). Furthermore, gonadectomy did not significantly reduce the amount of estradiol in the amygdala or prefrontal cortex compared to intact rats ($p < 0.69$, $p < 0.88$,

respectively). In contrast gonadectomy decreased the amount of estradiol in the hippocampus ($p < 0.002$). Furthermore, the estradiol treatment resulted in estradiol concentrations above both oil-treated and intact animals $p < 0.0001$, $p < 0.02$, respectively). *A priori* we were interested in sex differences in differential uptake of estradiol, and *a priori* tests revealed that estradiol treatment raised the estradiol concentration in the hippocampus above that found in intact animals in males ($p < 0.001$), but not females ($p = 0.45$).

Discussion

The effects of gonadectomy on estradiol concentration differed with the specific tissue examined. As expected, in serum, gonadectomized rats had lower concentrations of estradiol than their intact counterparts. Long-term gonadectomy (3 weeks) reduced estradiol concentration in the male and female hippocampus (Fig. 4B), but not in the male or female amygdala or in the female prefrontal cortex (Figs. 4B and 4C). This suggests greater local production of estradiol in the male and female amygdala and female prefrontal cortex, but perhaps not in the hippocampus or prefrontal cortex of the adult male rat. Furthermore, exogenous treatment with estradiol increased estradiol content to levels above intact animals in the amygdala, prefrontal cortex and the male hippocampus. These results further suggest that the female rat hippocampus may respond differently to exogenous estradiol than males, and that the female prefrontal cortex differs from that of males in endogenous estradiol content. In addition, our results indicate that changes in serum estradiol concentration alone do not necessarily reflect changes in estradiol concentration in brain tissue. Taken together, our results point to evidence that different

brain regions differ in the uptake, storage, or metabolism of estradiol arriving from the periphery.

In intact rats, we did not find evidence for a sex difference in estradiol content in the prefrontal cortex, hippocampus, or amygdala. This is consistent with findings in perinatal rats showing that although there sex differences at birth in estradiol content in the cortex, favoring males, these sex differences were no longer evident 32 h after birth (Amateau *et al.* 2004). In contrast to our results from the adult hippocampus, in rat pups there are higher levels of estradiol in the female hippocampus compared to the male hippocampus 32 h after birth (Amateau *et al.* 2004). Despite differences in serum levels of estradiol seen in the present study, and in neural and behavioural responses to exogenous estradiol administration (e.g. Barker and Galea 2008; Gibbs and Johnson 2008), intact rats of both sexes are remarkably similar in the amount of estradiol present in various brain regions.

Evidence for local production of estradiol in the amygdala and prefrontal cortex but limited evidence in the hippocampus of adult female rats

Although serum estradiol was higher in intact rats than in gonadectomized rats of the same sex, this difference was not necessarily reflected in brain tissue. In the male and female amygdala and female prefrontal cortex, the concentration of estradiol was similar in gonadectomized and intact rats, perhaps suggesting local production of estradiol in these two regions of the brain (Baulieu 1998; Tsutsui *et al.* 2000). Interestingly, only females demonstrated a reliable supply of estradiol to the prefrontal cortex in the absence of gonadal production, as estradiol levels were undetectable in the prefrontal cortex of

gonadectomized or intact males. These findings suggest the absence of local production of estradiol in the male prefrontal cortex. However, it should be noted that the apparent absence of estradiol may be due to the detection limits of the assay we used, and that there could be estradiol present at a very low concentration in the male PFC and amygdala. To our knowledge, this is the first demonstration of estradiol content in the amygdala and prefrontal cortex of adult rats of both sexes, and the first evidence of local steroid production in the adult amygdala and female prefrontal cortex.

In the hippocampus, estradiol concentration was significantly lower in long-term gonadectomized rats than their intact counterparts, suggesting that the hippocampus did not locally synthesize *substantial* quantities of estradiol under these conditions in either sex. Male rats had no detectable estradiol in the hippocampus after gonadectomy, suggesting limited local production of estradiol, and estradiol was detected in only a single female rat. Thus it is unlikely that three weeks after gonadectomy adult rats of either sex produce substantial amounts of estradiol in the hippocampus itself under these conditions. In contrast to our findings, local endogenous production of estradiol has been reported in embryonic, perinatal and postnatal tissue of intact rats (Amateau *et al.* 2004; Prange-Kiel *et al.* 2008; Rune *et al.* 2006). However, the sex of the embryos used in these studies was not determined, so the possibility of sex differences in local estradiol production rates *in vitro* could not be assessed. Consistent with our results, Hojo *et al.* (2004) found evidence for estradiol in the hippocampus of intact adult male rats, and McCarthy's group showed that *in vivo* inhibition of aromatase in perinatal male rat pups does not affect estradiol content in the hippocampus (Amateau *et al.* 2004). Although the failure to detect estradiol in the hippocampus of adult male rats may have been due to the

detection limits of the assay, it is clear that the level of estradiol in the hippocampus is lower in gonadectomized compared to intact rats. This. suggests that local production of estradiol is limited in the hippocampus in long-term gonadectomized rats. Hippocampal slices from intact adult male rats produce estradiol after incubation with NMDA (Hojo *et al.* 2004) and hippocampal slices of intact postnatal rats produce estradiol after application of gonadotropin-releasing hormone (GnRH) (Prange-Kiel *et al.* 2008) suggesting that under different stimulating circumstances local production of estradiol may be seen in the intact adult male and female hippocampus. It is therefore possible that long-term gonadectomy alters the environment of the hippocampus, limiting or eliminating local production of estradiol.

In the present study, we used adult rats whereas prior studies used *in vitro* systems, embryonic, perinatal and/or postnatal tissue, underscoring the possible differences between these different developmental stages and using a dissociated system. Overall, the concentrations we detected in all brain regions are considerably lower than those reported overall in embryonic brain tissue (e.g. less than 25% of the lowest mean reported by Pei *et al.* 2006) but are on the same order of magnitude as those reported in young postnatal female rats (Bixo *et al.* 1986) and female human frontal cortex (Bixo *et al.* 1995). Taken together, these studies suggest that estradiol content is much lower in adult versus embryonic brain tissue.

With the exception of the present study, there have been no studies examining the potential local production of estradiol after long-term gonadectomy in the adult male and female hippocampus, suggesting that the capacity for local estradiol production in the brain decreases with time after gonadectomy. Certainly there are a number of other

changes that occur after long-term gonadectomy that could potentially alter the brain's ability to locally produce estradiol or respond to estradiol: for example, in adult females 3 or 4 wks after ovariectomy estradiol no longer upregulates cell proliferation in the dentate gyrus of the hippocampus (Tanapat et al. 2005) and 3 months after ovariectomy there are decreases in estrogen receptor (ER) β density in the brain (Rose'Meyer et al. 2003). We also cannot exclude the possibility that the hippocampus was producing estradiol and metabolizing it (for example, to catechol estrogens or estrone) or clearing it away at a higher rate than the other areas examined.

Differential metabolism or sequestering of estradiol by different regions of the brain

Systemic administration of estradiol to both males and females increased the estradiol concentration in the amygdala and prefrontal cortex to levels above those found in intact animals of the same sex, suggesting either sequestration or a relatively slow breakdown of estradiol within these brain regions. However, there was a sex difference in estradiol content in the hippocampus. Estradiol administration to gonadectomized male rats increased hippocampal estradiol levels above those found in intact males. In females, however, estradiol administration did not significantly increase hippocampal estradiol above that found in intact females. This may suggest the rapid use of estradiol, or the presence of a mechanism to restrict estradiol concentration within the female hippocampus, such as blood flow changes, metabolism differences, or lack of sequestering in female rats.

Estradiol access to the hippocampus may be controlled by modulation of blood flow to particular brain regions, as estradiol administration rapidly increases blood flow to most brain regions, most notably to the frontal cortex and hippocampus (Goldman *et al.* 1976). This effect on blood flow is greater in females than in males, suggesting a potential for differential delivery of peripheral estradiol. Although increased blood flow to these regions may result in increased availability of estradiol, this could be counteracted by a concurrent increase in blood flow *from* these regions, rapidly clearing estradiol or its metabolites and maintaining normal, physiological levels of estradiol despite supra-physiological peripheral levels.

Another possible explanation for our results is differential metabolism of estradiol in different brain regions. For example, estradiol within the hippocampus may be efficiently converted to a variety of metabolites (e.g. by oxidation or conjugation; Martucci and Fishman 1993; Raftogianis et al. 2000, as well as conversion to estrone Akinola *et al.* 1996; Miettinen *et al.* 1996; Wu *et al.* 1993) that would not necessarily be detectable, but could be related to behavioural or neural effects of estradiol administration (Barha et al. *in press;* Zhu and Conney 1998). In the amygdala and prefrontal cortex, on the other hand, the metabolism of estradiol may not proceed as rapidly or as efficiently, allowing estradiol arriving from the periphery to accumulate in these regions. Similar effects have been reported from the intake of dietary phytoestrogens, after which various phytoestrogens (daidzein, genistein, and equol) are increased in the frontal cortex and amygdala, but not in the hippocampus, of intact male rats (Lund *et al.* 2001). There is evidence for sex differences in the metabolism of estrogens within the brain; for example, the enzyme CYP1B1 (which metabolizes

estradiol to catecholestrogens) is widely distributed in the female rhesus monkey brain (including the frontal cortex, hippocampus, and amygdala), but in males is primarily restricted to the hippocampus (Scallet *et al.* 2005). Thus it is possible that metabolism of estrogens within the brain also differs in males and females.

The differences in estradiol concentrations between brain regions may be due to specific sequestration and storage of estradiol in particular regions. For example, the hypothalamus accumulates more estradiol from the periphery than does the cortex in female rats (Feder *et al.* 1974), and in male and female guinea pigs (Eaton et al. 1975; Sholl and Goy 1981). Within the amygdala of both male and female rats, a substantial population of cells selectively take up peripheral estradiol into their nuclei, whereas in the hippocampus a much smaller subset of cells take up peripheral estradiol (Parvizi et al. 1985; Pfaff and Keiner 1973; Stumpf and Sar 1971). This sequestration may in part be due to differences in the number of ERs present in the different brain regions, as the presence of more ERs would be expected to increase the amount of estradiol that could be bound and sequestered within a cell. Overall levels of ERβ and its mRNA are similar in the hippocampus and amygdala in both sexes (Kalita *et al.* 2005), but the density of cells containing ERα mRNA is higher in the amygdala than in the hippocampus, in both male and female rats (Simerly *et al.* 1990). The amygdala therefore has the potential to hold more estradiol than the hippocampus, and we found estradiol levels in the amygdala approximately twice those in the hippocampus.

Estradiol concentrations do not clearly explain sex differences in the behavioural or neural effects of estradiol treatment

Perhaps surprisingly, the present study did not show dramatic sex differences in estradiol concentration in any brain area examined, despite the fact that estradiol can have disparate effects in males versus females, particularly in the hippocampus and amygdala. The lack of dramatic differences in estradiol concentration in the hippocampus of adult rats was somewhat surprising, given the sex differences in the effects of estradiol on the hippocampus (Barker and Galea 2008; Leranth et al. 2003). Although the absolute tissue concentration of estradiol was similar in males and females, the relationship between serum and tissue concentrations of estradiol differed between the sexes. For example, intact males had lower serum estradiol concentrations than intact females, but the estradiol concentration in the hippocampus of intact rats was similar in both sexes. Furthermore, exogenous estradiol raised serum estradiol concentrations to similar levels in both sexes, but raised the concentration of estradiol in the hippocampus above that of intact rats only in males. In addition, although serum estradiol levels in gonadectomized rats were similar, ovariectomized females had detectable levels of estradiol in all three brain regions examined, whereas none of the oil-treated males had detectable levels of estradiol in the hippocampus or prefrontal cortex. Such results could potentially be explained by sex differences in ER levels, however, overall levels of estrogen binding and ERα and ERβ levels in the cortex, hippocampus, and amygdala are very similar in male and female rats (Barley *et al.* 1977; Lieberburg *et al.* 1980; Ogren *et al.* 1976; Weiland *et al.* 1997; Kritzer 2002; Simerly *et al.* 1990). However, it remains possible that subtle sex differences in localization of ERs within particular brain regions or their inputs determine the extent to which estradiol can affect those regions and the behaviours they mediate, in males and females(Isgor and Watson 2005; Mazzucco et al. 2006).

Conclusions

We have demonstrated that intact male and female rats, though they differ in serum concentration of estradiol, have similar estradiol content in the amygdala and hippocampus. Gonadectomy substantially reduces the serum concentration of estradiol in both sexes, but does not reduce estradiol in the female amygdala or prefrontal cortex, consistent with reports that regions of the adult brain are capable of local synthesis of steroid hormones (Baulieu 1998; Naftolin 1994; Tsutsui *et al.* 2000). However, long-term gonadectomy in males reduces estradiol content of the hippocampus to undetectable levels, and both intact and gonadectomized males had no detectable levels of estradiol in the prefrontal cortex, providing little evidence for local production of estradiol in the male hippocampus or prefrontal cortex. It is also possible that levels of estradiol were present in low amounts that were beyond the detection limit of our assay and future studies should examine this possibility. However this does not negate the fact that levels of estradiol were lower in the male and female hippocampus and the male amygdala after approximately one month after gonadectomy, suggesting limited local production of estradiol under these conditions. Administration of estradiol to gonadectomized rats increases estradiol concentration in the amygdala and prefrontal cortex to roughly the same levels in both sexes. Thus in the current study we provide evidence for sex differences in the concentration of estradiol in the amygdala, PFC and hippocampus under different hormonal conditions.

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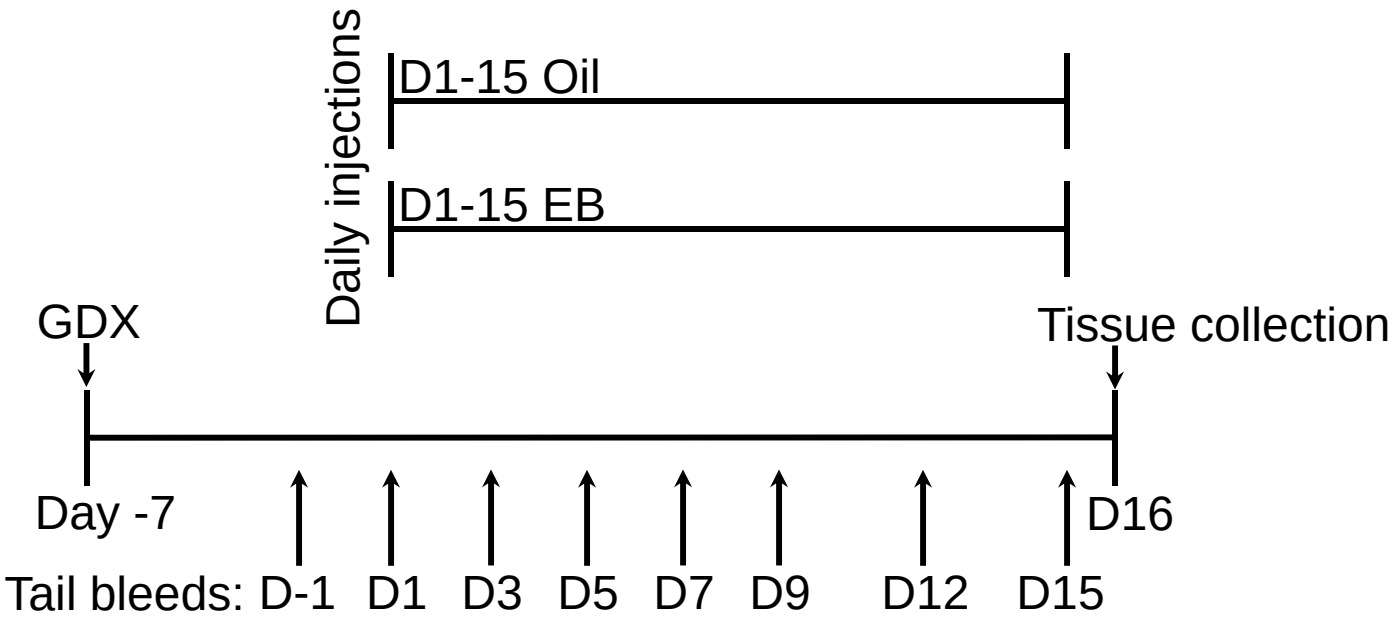
Figure 1: Experimental treatment timeline. All male and female rats treated with oil or estradiol benzoate were gonadectomized on day -7, and allowed to recover for 6 days. On day -1, prior to the start of injections, blood was collected from the tail. Daily injections were given for 5 days (estradiol benzoate) or 15 days (estradiol benzoate or oil). Trunk blood and brain tissue was collected from all rats on day 16, including intact males and females.

Figure 2: Estradiol concentrations in the serum of gonadectomized rats over time. (A) Blood was collected via tail nick one hour after estradiol or oil injection. Both female (○) and male rats (□) treated with oil for 15 days have low but detectable serum concentrations of estradiol. Female (●) and male rats (■) treated with estradiol benzoate for 15 days show an initial increase in serum estradiol concentration, and subsequently maintain high concentrations. Estradiol-treated rats had significantly higher serum estradiol concentrations at all time points examined from day 1 through day 15. (B) On the day of perfusion, 24 hours after the final round of injections, serum estradiol remained higher in the estradiol-treated (females: FEB, males: MEB) than the oil-treated gonadectomized rats (females: FO, males: MO), and was also higher than in intact rats of the same sex (females: FI, males: MI). Data is shown as group mean ± SEM (standard error of the mean). * significantly different groups ($p < 0.05$).

Figure 3: The extraction and assay methods used were adequate for comparing relative concentrations of estradiol in rat brain samples. Brain tissue from adult rats was homogenized and extracted with ether, with or without the addition of a known amount

of estradiol to the sample measured. The measured concentration of estradiol in samples to which estradiol had been added was plotted against the expected value. There was a high correlation between the measured and expected values ($r^2 = 0.86$, $p < 0.05$).

Figure 4: Estradiol concentration in brain tissue. (A) Rats treated with estradiol benzoate for 15d (females: FEB, males: MEB) had higher concentrations of estradiol in the amygdala than gonadectomized oil-treated rats (females: FO, males: MO). However, intact rats of both sexes (females in proestrus: FI, intact males: MI) were similar to oil-treated rats. (B) In the hippocampus, oil-treated gonadectomized rats had lower concentrations of estradiol than either estradiol-treated or intact rats. Estradiol treatment raised levels to above those found in intact rats in male rats only. (C) In the prefrontal cortex, rats treated with estradiol benzoate for 15d had higher concentrations of estradiol than gonadectomized oil-treated rats. However, intact rats of both sexes were similar to oil-treated rats, further male rats had undetectable levels of estradiol in both intact and oil-treated controls. Numbers immediately adjacent to the x-axis indicate the percentage of detectable samples. Bars represent group means + SEM (standard error of the mean). * significantly different groups ($p < 0.05$).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Day -7
D16
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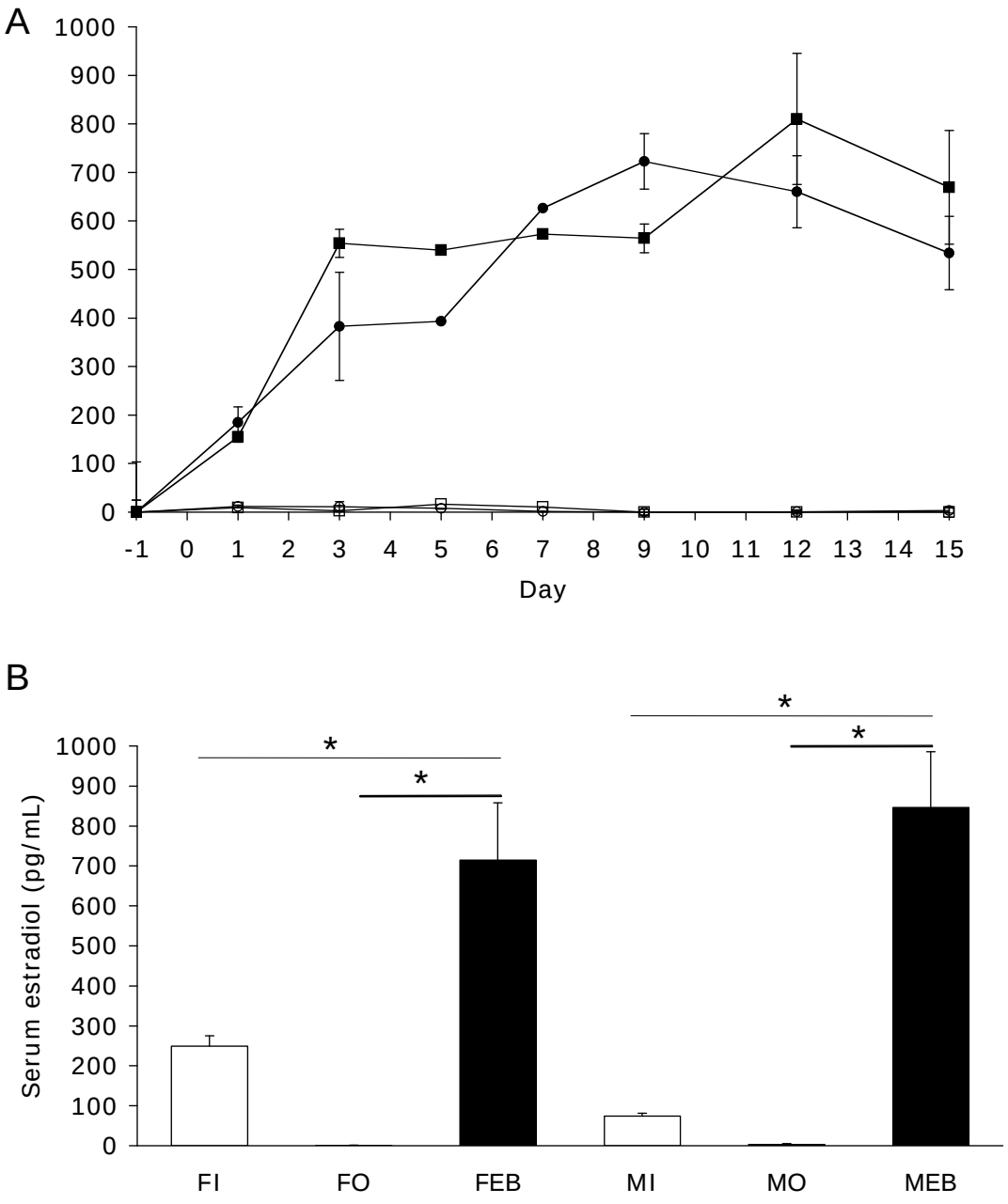
A
1000
900
800
700
600
500
400
300
200
100
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Day
B
Serum estradiol (pg/mL)
1000
900
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*
*
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FI
FO
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MO
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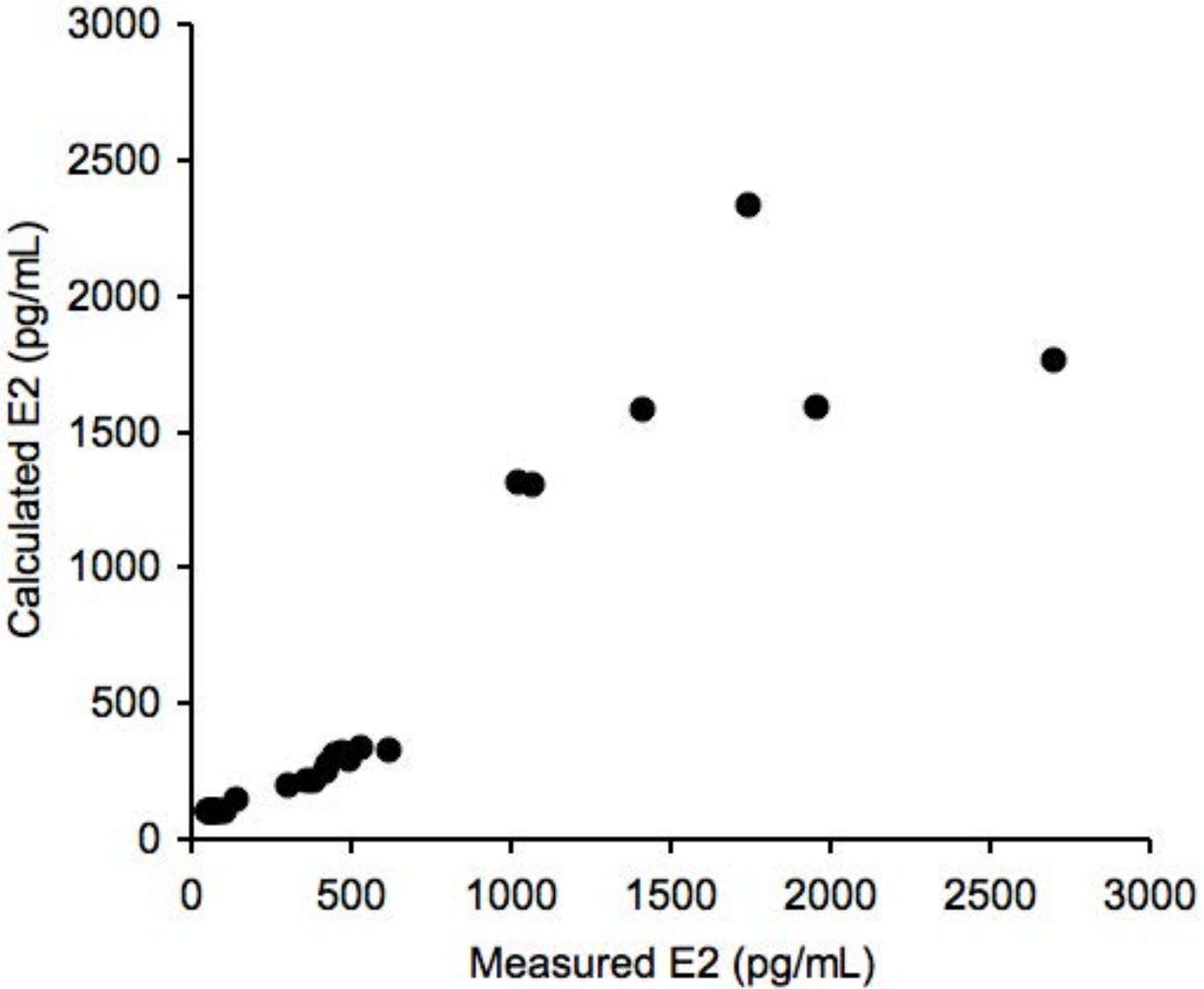
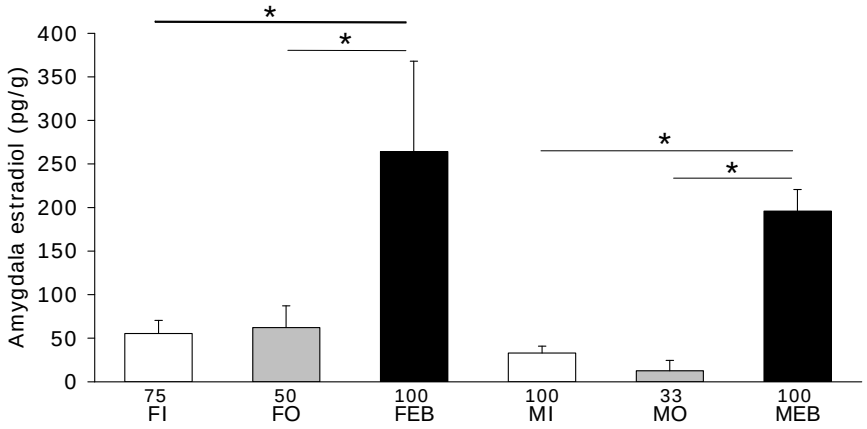
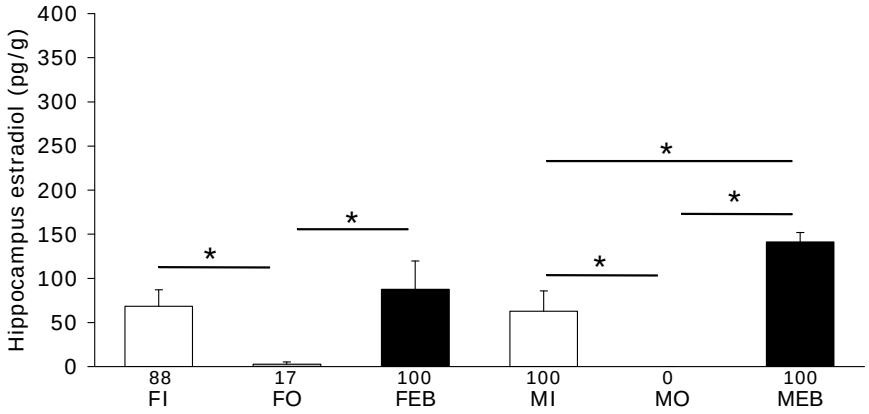

3000
2500
2000
1500
1000
500
0
Calculated E2 (pg/mL)
0
500
1000
1500
2000
2500
3000
Measured E2 (pg/mL)

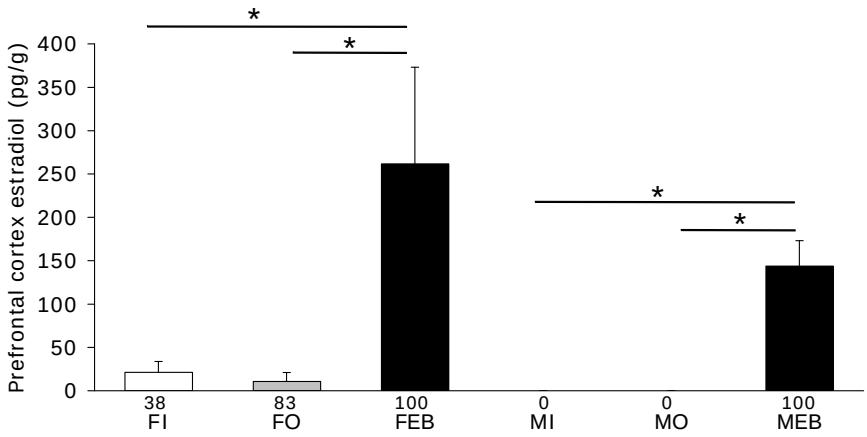
A


Amygdala estradiol (pg/g)
400
350
300
250
200
150
100
50
0
*
*
*
*
75
FI
50
FO
100
FEB
100
MI
33
MO
100
MEB

B


Hippocampus estradiol (pg/g)
400
350
300
250
200
150
100
50
0
*
*
*
*
88
FI
17
FO
100
FEB
100
MI
0
MO
100
MEB

C


Prefrontal cortex estradiol (pg/g)
400
350
300
250
200
150
100
50
0
*
*
*
*
38
FI
83
FO
100
FEB
0
MI
0
MO
100
MEB