Effect of sertraline on central serotonin and hippocampal plasticity in pregnant and non-pregnant rats

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Highlights

- Pregnancy does not alter sertraline effects on serotonin in HPC or PFC
- Sertraline increases synaptophysin in DG and CA3 when non-pregnant
- Pregnancy increases synaptophysin in DG and CA3
- Hippocampal neurogenesis is decreased during late pregnancy
- Need to consider reproductive state on neuropharmacological effects of SSRIs

Abstract

One of the most frequently prescribed selective serotonin reuptake inhibitor medications (SSRIs) for peripartum mood and anxiety disorders is sertraline (Zoloft®). Sertraline can help alleviate mood and anxiety symptoms in many women but it is not known how sertraline, or SSRIs in general, affect the neurobiology of the brain particularly when pregnant. The aim of this study was to investigate how sertraline affects plasticity in the hippocampus, a brain area integral in depression and SSRI efficacy (particularly in males), during late pregnancy and whether these effects differ from the effects of sertraline in non-pregnant females. To do this pregnant and age-matched non-pregnant female Sprague-Dawley rats were used. For the last half of pregnancy (10 days), and at matched points in non-pregnant females, rats were given sertraline (2.5mg/kg/day or 10mg/kg/day) or vehicle (0mg/kg/day). Brains were used to investigate effects on the serotonergic system in the hippocampus and prefrontal cortex, and measures of neuroplasticity in the hippocampus. Results show that pregnant females have significantly higher serum levels of sertraline compared to non-pregnant females but that rates of serotonin turnover in the hippocampus and PFC are similar between pregnant and non-pregnant females. Sertraline increased synaptophysin density in the dentate gyrus and CA3 and was associated with a decrease in cell proliferation in dentate gyrus of non-pregnant, but not pregnant, females. During late pregnancy the hippocampus showed significant reductions in neurogenesis and increases in synaptophysin density. This research highlights the need to consider the unique effect of reproductive state on neuropharmacological effects of SSRIs.

Keywords: SSRI, perinatal depression, pregnancy, female, neurogenesis, maternal brain, peripartum, hippocampus

1. Introduction

Prescription rates for selective serotonin reuptake inhibitor medications (SSRIs) during pregnancy are between 4-10% in developed countries studied to date (Benard-Laribiere et al., 2018; Liang et al., 2019). SSRIs are prescribed to treat the growing number of women diagnosed with peripartum mood and anxiety disorders despite the fact that we know very little about how these medications affect the maternal brain (Drury et al., 2016; Gavin et al., 2005; Moses-Kolko et al., 2014; Pawluski et al., 2019a; Pawluski et al., 2019b; Pawluski et al., 2017). SSRIs and their metabolites cross the placenta (Angelotta and Wisner, 2017; Oberlander et al., 2009; Pohland et al., 1989) raising questions about the safety for the fetus of using these medications (Field, 2010; Gemmel et al., 2018a; Glover and Clinton, 2016; Homberg et al., 2010; Hutchison et al., 2018). Much research has focused on how these medications may affect the fetus and developing child but much less is known about how these medications affect the mother (Lonstein, 2018; Pawluski and Dickens, 2019; Pawluski et al., 2019b).

Recent work in peripartum women shows that SSRIs significantly increase serum brain derived neurotrophic factor (BDNF) levels suggesting a link between SSRIs and brain plasticity (Pawluski et al., 2019a). Direct effects of SSRIs on the brain plasticity in the peripartum female exists only in animal models, has only investigated effects during the *postpartum period*, and focuses on the effects of the SSRIs fluoxetine and escitalopram (Gemmel et al., 2018b; Gemmel et al., 2016c; Gobinath et al., 2018a; Gobinath et al., 2018b; Haim et al., 2016; Pawluski et al., 2012b; Workman et al., 2016). Serum SSRI levels are known to change markedly during gestation, particularly during the late stages of gestation when the activity of cytochrome P450 enzymes, key players in drug metabolism, is reduced (Kokras et al, 2019b). To date no research has investigated sertraline (Zoloft®), one of the most commonly prescribed SSRIs during pregnancy (Liang et al., 2019), on the brain when pregnant. A recent study of 6 pregnant women prescribed sertraline showed a decrease in the mean dose-corrected plasma concentration of sertraline during the last half of gestation with a return to early gestational levels at 12 weeks postpartum (Kokras et al., 2019b; Sit et al., 2008). How changes in sertraline metabolism affect the maternal brain during pregnancy remains to be determined.

During the postpartum period SSRIs have an effect on the maternal brain. Gemmel et al (2016) show that fluoxetine treatment decreases serotonin turnover in the hippocampus of the

dam and prevents the effect of gestational stress on serotonin turnover in the maternal prefrontal cortex (Gemmel et al., 2016c). Further research in this area has focused on postpartum SSRI effects on the hippocampus of the mother in part due to the role of the hippocampal neuroplasticity in mental illness and the actions of SSRIs in alleviating depressive-like and anxiety-like behaviors (Gemmel et al., 2018b; Haim et al., 2016; Workman et al., 2016). When administered perinatally from gestation day 10 to postpartum day 28 fluoxetine (Gemmel et al., 2018b), and the serotonin-norepinephrine reuptake inhibitor venlafaxine (Belovicova et al., 2017), increase the number of immature neurons in the hippocampus of the rat dam. The morphology of immature neurons in the granule cell layer (GCL) is also affected by SSRI treatment with 3 weeks of postpartum fluoxetine administration reducing the percent of cells in the intermediate stage and increasing the percent of cells in the postmitotic stage of development but with no effect on the overall number of immature neurons in the dam postpartum (Gemmel et al., 2016c; Gobinath et al., 2016; Pawluski et al., 2012a; Workman et al., 2016). Recent research has also reported that the discontinuation of sertraline treatment at birth had no effect on hippocampal neurogenesis in the rat dam 3 weeks after giving birth (Kott et al., 2018). Thus there are effects of SSRI treatment postpartum on the morphology of new neurons in the absence of overall differences in rates of neurogenesis. Whether these SSRI effects on hippocampal neurogenesis differ during pregnancy remain to be determined.

Apart from the effects of SSRIs on the hippocampus during the postpartum period, pregnancy and motherhood are times of marked plasticity in the female brain (Barba-Muller et al., 2019; Hoekzema et al., 2017; Kim et al., 2016; Kinsley and Lambert, 2006; Leuner et al., 2010; Levy et al., 2011; Pawluski et al., 2016). In the hippocampus of the female, motherhood and lactation are associated with a decrease in cell proliferation, neurogenesis and significant dendritic remodeling (Brus et al., 2014; Darnaudery et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007). Although less well studied, pregnancy is associated with a decrease in cell proliferation and dendritic remodeling in the hippocampus (Kinsley et al., 2006; Pawluski et al., 2010; Pawluski et al., 2015; Pawluski et al., 2012d; Pawluski et al., 2011). Even in humans recent research shows that hippocampal volume changes during pregnancy and this change lasts into the postpartum(Hoekzema et al., 2017). The heightened restructuring of the hippocampus in the female during pregnancy and postpartum suggests this brain area may be particularly

sensitive to, or play a significant role in, peripartum mood and anxiety disorders and subsequent SSRI treatment.

The primary aims of the current study were to determine how sertraline, one of the most commonly used SSRIs during pregnancy (Benard-Laribiere et al., 2018; Liang et al., 2019), 1) affects the serotonergic system of the hippocampus and prefrontal cortex (PFC) and 2) affects measures of neuroplasticity (cell proliferation, immature neurons, and synaptic protein levels) in the hippocampus when compared to age-matched non-pregnant females. Two doses of sertraline (2.5mg/kg/day and 10mg/kg/day) were administered via a biscuit and vehicle (0mg/kg/day) was administered as a control to pregnant females for the last half of pregnancy (10 days) and non-pregnant age-matched virgin females. Serum levels of sertraline and norsertraline were determined. It was expected that sertraline dose and reproductive state (pregnant vs non-pregnant) would significantly alter measures of hippocampal plasticity in the female brain and that these effects would be related to changes in central serotonergic measures. Increasing our understanding of how SSRIs affect the maternal brain during pregnancy will aid in developing safe and effective peripartum treatments for both mother and fetus.

2. Material and Methods

Animals. Eighteen pregnant (gestational day (GD) 10) (289-353g) and 18 non-pregnant (virgin) female (224-254g) Sprague-Dawley rats were obtained from Janvier Laboratories, France, and singly housed upon arrival at the animal facility. Animals were kept under standard laboratory conditions in a 12:12-h light/dark schedule with access to standard rat chow and tap water *ad libitum*. All experiments were approved by the Ethics Committee of the French Ministry of Research (authorization number APAFlS# 2015120114544585 v3). The animal facility is licensed by the French Ministry of Agriculture (agreement D35-238-19). All efforts were made to minimize animal stress and pain.

2.1. Pregnant and non-pregnant female rats were randomly assigned to 3 treatment groups: 0mg/kg (vehicle; n=6/group), 2.5mg/kg (n=6/group), and 10mg/kg (n=6/group) of sertraline for a total of 36 female rats in the study. On gestation day 14 (GD14) serum samples from tail nicks were taken in the morning (9-10am) and afternoon (2-3:30pm) in females to determine how serum sertraline and norsertraline levels may differ with sertraline dose during the day. Samples were pooled using three animals per sample per group and means are represented in Table 1. On

GD19, and at matched points in non-pregnant females, animals were deeply anesthetized with pentobarbital and decapitated. Trunk blood samples were taken and brains were extracted. In pregnant females the size of the litter, sex of the fetuses, anogenital distance and number of fetal anomalies were determined.

2.2. Sertraline administration. Sertraline (Fagron, Belgium) or vehicle was administered via a biscuit as previously described (Gemmel et al., 2018b; Pawluski et al., 2014). Females were fed 1/9th of a vanilla wafer biscuit (Crousti fondante, Delacre®, Belgium) once per day in the morning between 8-10am from GD10-19 and at matched points in non-pregnant females. Biscuit portions were injected with vehicle (66% propylene glycol) or sertraline (2.5mg/kg/day or 10mg/kg/day) dissolved in vehicle. After administration of the biscuit to the home cage, females were monitored to ensure that biscuits were eaten. Biscuits were consistently eaten by all females within 5 minutes.

2.3 Serum sertraline and norsertraline levels. To determine serum levels of sertraline and its active metabolite norsertraline blood samples were taken on ice, stored at 4°C overnight and centrifuged at 10,000 g for 10 minutes. Serum was collected and stored at −80°C until further use.

2.3.1 Serum sample preparation. Serum samples frozen at -80°C were thawed at room temperature, vortexed and centrifuged during 5 min at 13400 rpm. To 50 µL of each serum sample was added 100 µL of 5% orthophosphoric acid, 50 µL of formic acid 0.1% and 50 µL of internal standard solution (50 ng/mL) in a 96-well polypropylene plate. An Oasis µElution MCX 96 well plate was used to clean up the samples for the analysis. The strong cation-exchanger polymeric sorbent was first conditioned by 300 µL methanol followed by 300 µL water. The entire sample was then transferred in the corresponding well of the extraction plate and drawn through the sorbent under vacuum. The plate was washed with 200 µL of 2% formic acid followed by 200 µL methanol. The extracts were eluted with 2 × 50 µL of methanol/ammonium hydroxide (95:5, v/v). After elution, 400 µL of 2% formic acid was added to each well. A calibration curve in serum was made with standard concentrations of 5, 20, 50, 200 and 500 ng/ml.

2.3.2 LC-chip-MS/MS analysis

2.3.2.1 LC-chip conditions. Chromatographic separation was achieved on a 1200 series LC-chip system consisting of a nanoflow pump, a capillary pump, a wellplate sampler and a LC-chip/MS

interface. Chromatographic separation was performed on a Ultra High Capacity chip including a 500 nL trapping column and a 150 mm x 75 µm analytical column, both packed with a Zorbax 80SB 5 µm C_{18} phase (Agilent Technologies). The mobile phase was composed of H_2O/FA (100:0.1, v/v) (A) and ACN/H_2O/FA (90:10:0.1, v/v/v) (B). The analysis was performed using a gradient starting at 21% B that linearly ramped up to 76% B in 7 min at a flow rate of 400 nL/min. Column was then rinsed with 90% B for 2 min. All the experiments were carried out with a 5 µL sample injection volume.

2.3.2.2 MS/MS detection. MS detection was performed using a 6340 Ion Trap equipped with a nanoESI source operating in positive mode (Agilent Technologies, Waldbronn, Germany). Skimmer, capillary exit, octopoles and lenses voltages were optimized by means of the Smart Ramp tool included in TrapControl software in order to maximize precursor ion intensity for both analytes and internal standard. Capillary voltage was set on 1800 V and the endplate offset on 500 V. Nitrogen was used as drying gas at a flow rate of 4 L/min and the source temperature was set at 325°C. Quantification was obtained using the MRM (multiple reaction monitoring) mode of the transitions at m/z 306.1→274.9 for sertraline, m/z 292.1→274.9 for norsertraline, and m/z 298.1→280.9 for norsertraline $^{13}C6$ (internal standard). ChemStation (Agilent Technologies) was used for instrument control. MS detection parameters were set by TrapControl (Bruker Daltonik GmbH, Bremen, Germany). Raw MS data were processed using Data Analysis software (Bruker Daltonik GmbH).

2.4 Brain collection. On gestation day 19 and at matched points in non-pregnant female animals were deeply anesthetized with pentobarbital and rapidly decapitated. Brains were rapidly dissected with the right hemisphere flash-frozen for high-performance liquid chromatography with electrochemical detection (HPLC-ED) techniques and the left hemisphere immersion fixed in 4% paraformaldehyde for 48h, and then placed in saturated 30% sucrose solution for a week. We are aware that pentobarbital can have an effect on brain serotonin levels particularly 60 minutes post pentobarbital injection (Kuschinsky et al., 1973). In our study all animals were treated in the same fashion and were decapitated within 5 minutes of injection. We are confident that any group differences that exist are primarily a result of sertraline treatment or reproductive state and not pentobarbital. Immersion fixed brains were stored at -80°C until coronally sliced in

40 micron sections by cryostat (Leica Biosystems, Wetzlar, Germany) in series of 12. Tissue sections were transferred to a glycol based tissue antifreeze solution and stored at -15°C.

2.5. HPLC-ED analysis. Monoamine levels in the hippocampus and PFC were measured using high performance liquid chromatography with electrochemical detection (HPLC-ED). Dissected brain tissue was weighed, homogenized and deproteinized in 0.1N perchloric acid solution (Applichem, Darmstadt, Germany) containing 7.9mM Na2S2O5 and 1.3mM Na2EDTA (Riedel-de Haën AG, Seelze, Germany), centrifuged at 20,000g for 45min at 4°C. The supernatant was stored at -80°C until analysis. The analysis was performed using a GBC LC1150 HPLC pump (GBC Scientific Equipment, Braeside, Victoria, Australia) coupled with a BAS-LC-4C (Bioanalytical Systems Inc., West Lafayette, IN) electrochemical detector, as previously described (Kokras et al., 2009; Kyratsas et al., 2013; Novais et al., 2013), with the working electrode of the electrochemical detector set up at þ800mV. Reverse phase ion pairing chromatography was used to assay serotonin (5HT) and its metabolite 5-hydroxyindoleacetic (5HIAA). The mobile phase consisted of a 50mM phosphate buffer regulated at pH 3.0, containing 5-octylsulfate sodium salt (ion pairing reagent) at a concentration of 300mg/L and Na2EDTA at a concentration of 20mg/L (Riedelde Haën AG); acetonitrile (Merck, Darmstadt, Germany) was added at a 8–15% concentration. The reference standards were prepared in 0.1N perchloric acid containing 7.9mM Na2S2O5 and 1.3mM Na2EDTA. The column used was an Aquasil C18 HPLC Column, 1502.1mm, 5mm Particle Size (Thermo Electron Corporation, UK). Samples were quantified by comparison of the area under the curve against known external reference standards using a PC compatible HPLC software package (Chromatography Station for Windows ver.17 Data Apex Ltd). In addition, the 5HT turnover rate was calculated separately for each chromatograph as the ratio of 5HIAA/5HT. Turnover rates estimate the serotonergic activity better than individual neurotransmitter and metabolite tissue levels as they reflect 5HT release and/or metabolic activity (Bessinis et al., 2013; Dalla et al., 2008; Kokras et al., 2009; Mikail et al., 2012). Prior to analysis outliers 2.5 standard deviations above the mean were removed. This occurred two times for 5HT levels in the hippocampus; once in non-pregnant vehicle group and once in 2.5 mg/kg/day pregnant group.

2.6. Immunohistochemistry. Immersion fixed brain halves were coronally sliced in 40 micron sections by cryostat (Leica Biosystems, Wetzlar, Germany) in a series of 12. Brain tissue was used for immunohistochemical analysis of cell proliferation (anti-Ki67) and immature neurons

(anti-doublecortin (DCX)) in the dentate gyrus (DG) of the hippocampus and analysis of synaptophysin density in CA3, and DG as previously described (Gemmel et al., 2016c). For all immunohistochemical protocols, tissue sections were rinsed between steps with TBS and TBS plus 0.01% Triton X-100 (TBST). Tissue was incubated in 0.6% H_2O_2 for 30 minutes at room temperature, blocked in 5% donkey serum in TBST for 30 minutes at room temperature, and then incubated at 4°C in primary rabbit anti-Ki67 (1:200 Abcam ab1666, USA), primary goat anti-doublecortin (1:300, Santa Cruz Biotechnology, USA), mouse anti-synaptophysin (1:500, Sigma s5768, France) in 2% donkey serum and TBS overnight. Sections were incubated for 2h at room temperature in appropriate secondary antibody (1:400 donkey anti-rabbit, DAKO EO432; donkey anti-goat 1:500 705-065-003 Jackson ImmunoResearch Laboratories;1:200 horse anti-mouse DAKO EO433). Further processing of the tissue was completed using the avidin-biotin complex (ABC Elite kit; 1:1000; Vector laboratories, France) and DAB (3,3-diaminobenzidine; Vector laboratories, France). Sections were mounted on Superfrost Plus slides (Fischer Scientific, Europe), dried, dehydrated, and cover-slipped with Permount (Fisher Scientific, Europe).

2.6.1 Quantification. The number of Ki67- and DCX-immunoreactive (-ir) cells were counted under a 40x objective, as described (Rayen et al., 2015; Rayen et al., 2011) in the granule cell layer (GCL) and subgranular zone (SGZ) of the dorsal and ventral hippocampus on every 6th section throughout the left hemisphere of the brain. The dorsal region was considered up to -4.92 and the ventral region was considered after this. Cells were considered Ki67-ir or DCX-ir if they were darkly stained, had oval or circular cell bodies, and in the area of interest (GCL/SGZ). Total estimates of cell numbers were calculated by multiplying the counts by 12. The number of hippocampal sections counted throughout the hippocampus were recorded to ensure that a similar number of sections were analyzed in each female.

DCX-ir cell morphology was quantified and characterized based in dorsal and ventral hippocampal regions. A total of fifty DCX-ir cells were randomly assessed, twenty-five cells per dorsal or ventral region and categorized as either proliferative (no process or short process), intermediate (medium process with no branching) or post-mitotic neurons (neurite branching, see full description in (Dechartres et al., 2019; Plumpe et al., 2006; Workman et al., 2016). DCX-ir cells for morphology characterization were selected from a minimum of 3 dorsal and 3 ventral

sections. The percentage of these cell types per animal was calculated as a function of the total cells investigated per region.

For optical densities of synaptophysin-ir, four sections of the dorsal hippocampus between stereotaxic coordinates -2.64 mm to -4.92 mm bregma were analyzed as previously described (Gemmel et al., 2016b; Rayen et al., 2015). Photomicrographs were taken for two areas within the CA3 and the DG. Immunoreactivity for all sections was examined under a 40x objective using an Olympus AX70 TRF and a DP71 digital camera with Cell^F software (Olympus). ImageJ64 software (Wayne Rasband, NIH, Bethesda MD, USA) was used for quantification of optical densities. The relative optical density was defined as the difference in optical density (grey level), after calibration, between the area of interest and the background, which was an equivalent area adjacent to the area of interest with minimal staining. All quantification was carried out by a researcher blind to the conditions.

2.7 Statistical Analysis. Data were analyzed using the Statistica 13 software (Dell Inc.). Analysis of variance tests (ANOVAs) were conducted on serum levels of sertraline and norsertraline with reproductive state (pregnant/non-pregnant) and treatment dose (2.5mg/kg, 10mg/kg) dose as factors. ANOVAs were conducted on neurotransmitter levels (5HT, 5HIAA, 5HIAA/5HT) and synaptophysin-ir density with reproductive state (pregnant/non-pregnant) and treatment dose (0mg/kg, 2.5mg/kg, 10mg/kg) as factors. For measures of Ki67-ir and DCX-ir cells, repeated measures ANOVAs were conducted for cell counts in the dorsal and ventral regions of the hippocampus with condition (pregnant/non-pregnant) and treatment dose (0mg/kg, 2.5mg/kg, 10mg/kg) as factors. DCX-ir cell types (stages of cell maturity) were analyzed using repeated measures ANOVAs with cell type (proliferative, intermediate, postmitotic) as the within-subjects factor. Significant interaction effects were analyzed in more detail by a Bonferroni test. Pearson correlations were analyzed between serum drug levels, measures of 5HT in the hippocampus and PFC, and measures of plasticity in the hippocampus. Significance was set at $p<0.05$.

3. Results

3.1 Serum levels of sertraline and norsertraline were increased in pregnant females. When comparing serum levels at sacrifice of the low (2.5mg/kg/day) and high (10mg/kg/day) doses of

sertraline and its metabolite, norsertraline, there were significantly higher levels of sertraline ($F(1, 19)=50.096$, $p=.000001$: Figure 1A) and norsertraline ($F(1, 19)=24.969$, $p=.00008$: Figure 1B) in late pregnant females compared to age-matched non-pregnant female rats. There was also a significant dose effect on serum sertraline levels ($F(1, 19)=5.6440$, $p=.02819$) with rats receiving the high dose (10mg/kg/day) having higher serum levels at sacrifice. This effect of reproductive state on serum levels of sertraline and norsertraline was also evident from the pooled serum samples taken from tail nicks on GD14 (Table 1) where pregnant females had higher mean levels of sertraline and norsertraline particularly at the high sertraline dose (10mg/kg) compared to non-pregnant females (Table 1). Serum norsertraline levels at perfusion on GD19 did not show significant dose effects ($F(1, 19)=4.1187$, $p=.057$). There were no other significant main effects or significant interactions ($p> 0.158$).

3.2 5HT levels in the hippocampus and PFC were affected by sertraline and pregnancy. In the hippocampus, sertraline treatment at both doses significantly decreased 5HIAA levels (p's <0.003:main effect of dose $F(2, 30)=17.320$, $p=.00001$: Figure 2B) and sertraline treatment at 10mg/kg dose significantly decreased 5HIAA/5HT ratios compared to vehicle ($p=0.009$: main effect $F(2, 30)=5.0962$, $p=.01244$; Figure 2C). There was a significant main effect of reproductive state on 5HIAA ($F(1, 30)=5.3195$, $p=.02818$) with pregnant females having less 5HIAA. The reproductive state x dose interaction effect for 5HT levels was not significant ($F(2, 28)=3.2233$, $p=.05498$: Figure 2A).

In the PFC, sertraline at the high dose significantly increased 5HT (high vs low $p=0.009$; main effect of dose $F(2, 30)=5.7225$, $p=.00785$; Figure 2D), significantly decreased 5HIAA (high vs vehicle $p=0.0005$; main effect of dose $F(2, 30)=9.1731$, $p=.00078$; Figure 2E) and lowered 5HIAA/5HT ratios (high vs low and vehicle p's < 0.005: main effect of dose $F(2, 30)=10.308$, $p=.00039$; Figure 2F). There was also a significant main effect of reproductive state on 5HT ($F(1, 30)=4.6989$, $p=.03825$) and 5HIAA/5HT($F(1, 30)=7.4165$, $p=.01067$) with pregnant females having more 5HT and a smaller 5HIAA/5HT ratio in the PFC (Figure 2D, F).

There were no other significant main effects or interaction effects on 5HT measures in the hippocampus or PFC (p's > 0.06).

Serum levels of sertraline and norsertraline were negatively correlated with 5HIAA levels and the ratio of 5HIAA/5HT in both the hippocampus and the PFC of pregnant and non-pregnant females (see Table 2 for list of correlations and p values).

3.3 Measures of hippocampal neuroplasticity are affected by sertraline and pregnancy.

3.3.1 *Synaptophysin-ir is affected by sertraline in non-pregnant rats and by pregnancy.* Sertraline at low and high doses increased synaptophysin-ir in the DG of non-pregnant rats but not pregnant rats ($p < 0.01$: DG interaction effect $F(2, 30)=3.3386$, $p=.04908$; Figure 3B). In the CA3 region, the highest dose of sertraline increased synaptophysin-ir in non-pregnant females ($p<0.03$: CA3 interaction effect $F(2, 30)=3.3525$, $p=.04852$; Figure 3C) but not in pregnant females. Pregnancy increased synaptophysin-ir density compared to vehicle non-pregnant females in both the DG and CA3 regions (p's <0.03; Figures 3B and 3C). There were also significant main effects of reproductive state on synaptophysin-ir density in the DG ($F(2, 30)=12.162$, $p=.00014$) and CA3 ($F(1, 30)=7.0857$, $p=.01236$). There were main effects of dose in the DG ($F(2, 30)=12.162$, $p=.00014$) and CA3 ($F(2, 30)=4.9952$, $p=.01341$) with higher doses of sertraline resulting in greater density of synaptophysin-ir cells. There were no other significant main effects or interaction effects on synaptophysin density.

3.3.2 *Hippocampal cell proliferation and neurogenesis is reduced in late pregnancy.* There were no significant differences in the number of sections counted per brain ($p > 0.2$) thus estimates of total cell counts were used. Overall, pregnant females had significantly fewer Ki67-ir cells (main effect $F(1, 26)=26.568$, $p=.00002$; Figure 4A) and DCX-ir cells (main effect $F(1, 30)=13.279$, $p=.00101$; Figure 4B) in the GCL/SGZ of the hippocampus. There were significantly more Ki67-ir cells and DCX-ir cells in the dorsal than in the ventral hippocampus (Ki67-ir; $F(1, 26)=69.412$, $p=.000001$: DCX-ir; $F(1, 30)=14.243$, $p=.00071$). There were no significant main effects of sertraline treatment or other significant interactions ($p > 0.4$).

When investigating DCX-ir cell type (proliferative, intermediate, postmitotic) in the dorsal hippocampus there was a significant cell type x reproductive state effect ($F(2, 60)=4.0793$, $p=.02182$: Figure 5A). Further post hoc tests revealed that pregnant females had significantly more intermediate cells than proliferative cells, while non-pregnant females had significantly more intermediate cells than postmitotic cells and proliferative (p's <0.01; Figure 5A). Non-pregnant females also had more intermediate cells than postmitotic cells in pregnant females

while pregnant females had more intermediate cells than postmitotic cells in non-pregnant females (p's <0.01: significant cell type x reproductive state effect (F(2, 60)=4.0793, p=.02182; Figure 5A). In the dorsal region of the hippocampus the cell type x reproductive state x dose interaction was not significant (F(4, 60)=2.4267, p=.05759). In both the dorsal and ventral regions of the hippocampus there were significant main effects of cell type (dorsal - F(2, 60)=15.415, p=.000001; ventral - F(2, 60)=24.204, p=.000001; Figure 5B) with significantly more intermediate cells than proliferative or postmitotic cells (p's < 0.00008).

3.3.3 *Sertraline levels are generally negatively correlated with hippocampal measures.* There were significant correlations between serum sertraline levels and synaptophysin-ir density in the DG (r=0.4622, p=0.026; Figure 6A), number of DCX-ir (r=-0.6438, p=0.001; Figure 6B), and number of Ki67-ir cells (r=-0.5791, p=0.006; Figure 6C). Serum norsertraline levels were also significantly negatively correlated with number of Ki67-ir cells (r=-0.5886, p=0.005). Further analysis of correlations per condition (pregnant/non-pregnant) revealed that in non-pregnant females only there were significant negative correlations between serum sertraline or norsertraline levels and number of Ki67-ir cells in the GCL/SGZ (sertraline - r=-0.6290, p=0.038: norsertraline - r=-0.6136, p=0.045; Figure 6C). There were no other significant main effects or interaction effects.

3.4 Weight increased during pregnancy. As expected pregnant females had a greater change in body weight gain during the study than non-pregnant females (F(1, 30)=188.47, p=.000001: Table 3). There was no effect of sertraline treatment on change in weight from the beginning to the end of the study (p=0.45).

3.5 Fetal characteristics were not affected by sertraline treatment. There was no significant effect of sertraline on number of fetuses (p=0.9), the number of female (p=.23) or male (p=0.06) fetuses, number of obvious fetal abnormalities (p=0.521) or anogenital distance per litter of male (p=0.400) or female (p=0.579) fetuses (Table 3).

4. Discussion

Up to 10% of women are prescribed SSRIs while pregnant with sertraline being one of the most popular SSRIs prescribed to date (Andrade et al., 2008; Benard-Laribiere et al., 2018; Gemmel et

al., 2018a; Liang et al., 2019). Virtually nothing is known about how sertraline or SSRIs affect the female brain during pregnancy. In fact treatment and dosing strategies of antidepressant medications are often based on data from healthy male volunteers and little consideration has been given to the impact of sex/gender or the complex physiology of pregnancy and their unique effects on mental illness (Feghali et al., 2015; Kokras et al., 2019a; Pawluski and Dickens, 2019). Findings from this study show that sertraline has a significant impact on the female brain with differences in serum medication levels, the serotonergic system in the hippocampus and PFC, and measures of neuroplasticity in the hippocampus. Importantly, pregnancy is a significant factor contributing to the effects of sertraline on the female brain.

The main function of sertraline, as with all SSRIs, is as a serotonin reuptake inhibitor acting on the serotonin transporter to increase serotonin levels in the synaptic cleft. Sertraline and its metabolite norsertraline are evident in the bloodstream and previous studies in pregnant women report a decrease in the mean dose-corrected plasma concentration of sertraline during the last half of gestation with a return to early gestational levels at 12 weeks postpartum (Freeman et al., 2008; Kokras et al., 2019b; Sit et al., 2008). Reduced levels of sertraline and increased levels of its main metabolite are attributed mainly to pregnancy-related changes in the activity of the cytochrome P450 family of enzymes. In our study, sertraline and norsertraline levels decreased from GD14 to GD19 in non-pregnant rats and the metabolic ratio (norsertraline/sertraline) slightly increased from 1.2 to 1.6 on average between GD14 and GD19. This suggests that sertraline treatment did not reach steady state pharmacokinetics during the 10 days of treatment as evidenced by the differences between GD14 and GD19. Interestingly in pregnant rats, the metabolic ratio was on average 0.9-1.0 on GD14 and GD19 and for both doses (2.5mg/kg and 10mg/kg). This clearly demonstrates that the activity of CYP P450 was reduced in pregnant rats. Moreover, blood levels of sertraline and its metabolite were higher in pregnant rats in comparison to non-pregnant rats, and surprisingly sertraline and norsertraline levels increased from GD14 to GD19 in females treated with the low dose, whereas they decreased from GD14 to GD19 in females treated with the high dose. This further strengthens the hypothesis that steady state pharmacokinetics were not achieved in the timeframe of the study. Future research is needed to determine the precise change of sertraline/norsertraline levels during pregnancy in humans and rodent models.

From our study it becomes clear the pregnancy affects the metabolism of sertraline. The steroid hormone profile of pregnancy can indeed affect the activity of the CYP P450 enzymes and it differs between humans and rodents (Pawluski et al., 2009). For example, near the end of gestation in the rat estradiol begins to increase whereas estradiol increases more remarkably throughout pregnancy in humans. Changes in estrogens can both increase and decrease CYP450 isozymes (for example decrease 1A2 and induce 2D6) (Jeong, 2010; Kokras et al., 2011; Kokras et al., 2019b) and because sertraline is metabolized by many CYP isozymes, it could be that the responsible isozymes for sertraline metabolism are differentially affected during the rat and the human pregnancy.

When we investigated the 5HT turnover levels in the brain of pregnant and non-pregnant female rats, as a function of sertraline dose, a higher dose of sertraline (10mg/kg/day) decreased 5HT turnover in the hippocampus and PFC of pregnant and non-pregnant females. 5HT turnover was significantly reduced in the PFC of pregnant females in comparison to non-pregnant female rats. This suggests that even though serum drug levels are significantly higher in pregnant females, the effects on 5HT in the brain is not as markedly affected and these effects are brain region specific (PFC differs from hippocampus). Interestingly, when looking at behavioral outcomes, recent work in male rats shows that sertraline treatment affects forced swim test performance associated with changes in 5HT levels in the PFC (Mikail et al., 2012). Furthermore, when tested on the same paradigm, immobility levels of female rats were more responsive to lower sertraline doses than males. This suggests that the effects of sertraline in mental health are partially brain region and sex/gender-dependent (Dalla et al., 2010; Kokras et al., 2015). Whether similar effects are evident in females across reproductive stages is the focus of further research by our group.

We also found that serum levels of sertraline were associated with a decrease in cell proliferation and the number of immature neurons, as well as an increase in synaptophysin density in the DG of female rat, particularly in non-pregnant females. The negative association between serum sertraline levels and the measures of hippocampal neurogenesis (cell proliferation and immature neurons) are particularly pronounced in non-pregnant females. This association in non-pregnant females is in contrast with a growing body of literature showing that serotonin and SSRIs can act to increase hippocampal neurogenesis in male rats (Kraus et al., 2017; Malberg et al., 2000; Santarelli et al., 2003). However, others have also reported minimal effects of SSRIs

on hippocampal neurogenesis in the female. It has been shown that while 14 days of the SSRI fluoxetine can increase hippocampal cell proliferation in adult male rats there are no effects in adult female rats (Hodes et al., 2009). We have also reported that 14 days of fluoxetine treatment has minimal effects on hippocampal neurogenesis in virgin females with only serum fluoxetine levels of 50ng/ml, administered with a minipump, increasing cell proliferation while higher or lower serum levels did not have the same effect (Pawluski et al., 2014). In the present study we did not find a main effect of sertraline dose on measures of neurogenesis in the hippocampus of pregnant or non-pregnant females. It may be that this 10 day treatment with sertraline was not long enough to show alterations in hippocampal neurogenesis. However, previous research shows that the SSRI fluoxetine significantly increases hippocampal cell proliferation after 7 days of treatment to male rats (Micheli et al., 2018). In human cell culture derived from hippocampus, 3-10 days of treatment with sertraline was enough to increase neurogenesis (Anacker et al., 2011). Thus it is likely that SSRI duration, type and dose of treatment, sex/gender and reproductive state alter the effects of antidepressant medications on neuroplasticity in the hippocampus.

In the present study sertraline increased synaptophysin density in the DG and CA3 of non-pregnant females with a high dose showing promise in increasing synaptophysin density in the DG of pregnant females. Synaptophysin is a membrane glycoprotein found in presynaptic vesicles of neurons. An increase in synaptophysin density after sertraline treatment may be indicative of increased synaptic plasticity and function. Thus while there are no effects of 10 days of sertraline treatment on hippocampal cell proliferation or neurogenesis there is an effect on synaptic structure which may translate into behavioral outcomes. This increase of synaptophysin density in the DG after sertraline treatment to non-pregnant females is in contrast with our previous work using fluoxetine. Previously we reported that fluoxetine treatment for 14 days significantly decreased synaptophysin density in the DG of non-pregnant females (Pawluski et al., 2014). Although not previously investigated during pregnancy, fluoxetine treatment for 3 weeks postpartum has minimal effects on synaptophysin density in the DG of rat dams (Gemmel et al., 2016a). However, this SSRI treatment does prevent the increase in synaptophysin density in the cingulate cortex after gestational stress in the mother (Gemmel et al., 2016a). This suggests that the actions of SSRIs on the brain depend on the type of SSRI, reproductive status as well as brain region investigated.

Given the marked physiological changes during pregnancy it is not surprising that pregnancy and motherhood have significant effects on the female brain (for review see (Barba-Muller et al., 2019; Leuner et al., 2010; Pawluski et al., 2016). During late pregnancy and the early postpartum period there is a marked decrease in hippocampal neurogenesis in the maternal brain (Darnaudery et al., 2007; Leuner et al., 2007; Pawluski et al., 2015; Pawluski and Galea, 2007) as well as changes in dendritic morphology and spine densities of pyramidal neurons (Kinsley et al., 2006; Pawluski et al., 2012d). We expand this work and show that in addition to significantly lower rates of cell proliferation in the hippocampus and fewer immature hippocampal neurons pregnant females have significantly elevated levels of synaptophysin in the CA3 and DG compared to non-pregnant females. Thus, even though there are lower rates of neurogenesis during late pregnancy it may be that synaptic communication is strengthened. We also found that while pregnant a female rat shows differences in the morphological stages of immature neurons compared to non-pregnant females, particularly in the dorsal hippocampus. Pregnant females have more intermediate immature neurons than proliferative cells and non-pregnant females have more intermediated immature neurons compared to both proliferative and postmitotic cells. This suggests there may be a higher turnover of immature neurons or more new cell death in the hippocampus of late-pregnant females. Taken together the hippocampus during pregnancy shows significant remodeling, and perhaps fine-tuning, of mature neurons via changes in synapse formation and communication.

4.1 Limitations and future directions

This is a first study demonstrating the complexities of SSRI neuropharmacology in the hippocampus during pregnancy. This work has generated more questions than answers and there are a number of directions for future research. For example, future research should aim to expand these findings by 1) investigating changes in other brain areas such as the PFC which is known to play a role in peripartum mental illness and maternal behaviors (Gemmel et al., 2016c; Haim et al., 2016); 2) using a longer treatment regimen of the SSRI as previous research has shown that 4 weeks of postpartum fluoxetine treatment can alter neurogenesis in the maternal hippocampus (Gemmel et al., 2018b); 3) using a model of maternal depression or repeated stress to understand the actions of SSRI in this more clinically relevant context (Gemmel et al., 2016c; Pawluski et al., 2012c; Pawluski et al., 2012d); and 4) investigating the role of SSRI treatment on

behavioral outcomes related to mood and anxiety measures as well as maternal care-giving patterns (Pawluski et al., 2019b; Pawluski et al., 2011).

Although we have much to learn about how and when SSRIs are effective in treating peripartum mental illness, when looking specifically at sertraline only one study investigated how sertraline treatment during gestation affects maternal behaviors in the postpartum period (Kott et al., 2018). This work showed that with the cessation of sertraline treatment there were minimal effects of the SSRI on maternal affective-like behaviors or hippocampal neurogenesis in the dam during the postpartum. However the delay between gestational sertraline treatment and testing may have contributed to the lack of findings. In adult virgin rats sertraline can diminish depressive-like behaviors (Kokras et al., 2015; Mikail et al., 2012) and this effect is associated with changes in 5HT in the PFC. Thus, a key first step for further research in pregnant females is to understand how sertraline may act to counteract stress effects in the hippocampus as well as the PFC.

4.2 Conclusions

In the present study we report that the effects of sertraline treat on the hippocampus of pregnant and non-pregnant females differs as a function of reproductive state as well as dose. We found that although serum drug levels were elevated in late pregnant females the rates of serotonin turnover in the hippocampus and PFC were similar in pregnant and non-pregnant females. However, we found that sertraline increased synaptophysin density and was associated with decreased cell proliferation rates in the hippocampus of non-pregnant females only. Pregnancy alone resulted in significant reductions in hippocampal neurogenesis and increased synaptophysin density, regardless of sertraline treatment. In conclusion, this work highlights the need to consider how reproductive state affects the central actions of antidepressant medication treatment for peripartum mood and anxiety disorders.

Figure legends

Figure 1. Mean (+SEM) serum levels of (A) sertraline and (B) norsertraline in age-matched non-pregnant and pregnant females treated with 2.5mg/kg/day and 10mg/kg/day of sertraline. (*n* = 5–6/group). '∗' denotes significant main effect of reproductive state p<0.05. 'a' denotes 2.5mg/kg differs from 10mg/kg dose p <0.05.

Figure 2. Mean (+SEM) levels of A) 5HT, B) 5HIAA and C) 5HIAA/5HT in the hippocampus and D) 5HT, E) 5HIAA and F) 5HIAA/5HT in the prefrontal cortex (PFC) (B). (*n* = 5–6/group). (ug/g wet tissue). In the hippocampus B)sertraline treatment at both doses significantly decreased 5HIAA levels (p's <0.003) and C) sertraline treatment at 10mg/kg dose significantly decreased 5HIAA/5HT ratio compared to vehicle (p=0.009). In the PFC D) sertraline at the high doses significantly increased 5HT (high vs low p=0.009), E) significantly decreased 5HIAA (high vs vehicle p=0.0005) and F) significantly lowered 5HIAA/5HT ratios (high vs low and vehicle p's < 0.005). * denotes significance p <0.05. [a] denotes main effect of 2.5 and 10 mg/kg dose p < 0.05. [b] denotes main effect of 10mg dose p <0.05.

Figure 3. A) Photomicrograph of synaptophysin-ir in the dentate gyrus (DG) and CA3 of the hippocampus. Mean (+SEM) synaptophysin-ir density (OD) in the B) DG and C) CA3 region of the hippocampus. (n=6/group). 'a' denotes non-pregnant vehicle significantly different from all other groups (p's < 0.01);'b' denotes non-pregnant vehicle significnatly difference from 10mg/kg non-pregnant group (p's < 0.03);* denotes significance p<0.05.

Figure 4. Photomicrograph of A) Ki67-ir cells and B) DCX-ir cells in the GCL/SGZ of the dentate gyrus of non-pregnant and pregnant females under 40x objective (scale bar=20um). Mean (+SEM) total number of C) Ki67-ir cells and D) DCX-ir cells in the GCL/SGZ. (n=5-6/group). * denotes significance p<0.05. DCX=doublecortin

Figure 5. A) Photomicrograph of morphological subtypes of DCX-ir cells (scale bar=20um). Mean (+SEM) percentage of proliferative (Prol), intermediate (Inter) and postmitotic (Post) DCX-ir cells in the GCL/SGZ of the B) dorsal and C) ventral hippocampus. C) Main effect of

cell morphology with intermediate cells significantly differing from other cell types (p's <0.00008). '*' denotes significance. DCX=doublecortin

Figure 6. Correlations between serum sertraline levels (ng/ml) in non-pregnant and pregnant females administered sertraline and A) synaptophysin-ir density in the dentate gyrus, B) number of Ki67-ir cells and C) number of DCX-ir cells in the GCL/SGZ. * denotes significance in non-pregnant females only. DCX=doublecortin. Hatched lines note regression separately in non-pregnant and pregnant females.

Table 1.

	Sertraline (ng/ml) Morning	Sertraline (ng/ml) Afternoon	Norsertraline (ng/ml) Morning	Norsertraline (ng/ml) Afternoon
Non-pregnant				
2.5mg/kg	10.30±0.36	12.49±4.57	14.00 ±3.94	13.74 ±3.13
10mg/kg	11.46 ± 0.19	16.42 ± 0.44	16.31 ± 1.01	20.52 ± 1.62
Pregnant				
2.5mg/kg	10.8 ± 6.60	14.79 ± 2.06	11.33 ± 3.15	13.52 ± 3.07
10mg/kg	31.18 ± 17.16	36.70 ± 26.50	34.59 ± 9.62	35.28 ± 12.87

Serum levels of sertraline and norsertraline during the morning (9-10am) and afternoon (2-3:30pm) in pregnant and non-pregnant females. Samples were pooled (3 females per group pooled) for analysis to provide an indication of any change in drug levels.

Table 2.

	5HIAA (ug/g)	5HT (ug/g)	5HIAA/5HT (ug/g)
Hippocampus			
All females			
Sertraline (ng/ml)	r = -.6241, p=.000*	r = -.0347, p=.843	r = -.3631, p= .032*
Norsertraline (ng/ml)	r = -.6892, p=.000*	r = -.1153, p=.509	r = -.4204, p=.012*
Non-pregnant			
Sertraline (ng/ml)	r = -.7852, p=.000*	r = -.2164, p=.389	r = -.5439, p= .020
Norsertraline (ng/ml)	r = -.8087, p=.000*	r = -.1784, p=.479	r = -.6182, p=.006*
Pregnant			
Sertraline (ng/ml)	r = -.6745, p=.003*	r = -.0349, p=.894	r = -.2957, p=.249
Norsertraline (ng/ml)	r = -.6598, p=.004*	r = -.1303, p=.618	r = -.2672, p=.300
PFC			
All females			
Sertraline (ng/ml)	r = -.6025, p=.000*	r = .1144, p=.513	r = -.4080, p= .015
Norsertraline (ng/ml)	r = -.6622, p=.000*	r = .1260, p=.471	r = -.4548, p=.006*
Non-pregnant			
Sertraline (ng/ml)	r = -.4584, p= .056	r = .4994, p=.035	r = -.5464, p=.019
Norsertraline (ng/ml)	r = -.5971, p=.009*	r = .7099, p=.001*	r = -.6917, p=.001*
Pregnant			
Sertraline (ng/ml)	r = -.7265, p=.001*	r = -.1369, p=.600	r = -.3122, p=.222
Norsertraline (ng/ml)	r = -.7200, p=.001*	r = -.2237, p=.388	r = -.2331, p=.368

Correlations between serum sertraline or norsertraline levels (ng/ml) and serotonin measures in the hippocampus and PFC of pregnant and non-pregnant female rats. * denotes significant correlation.

Table 3.

	Non-pregnant			Pregnant		
	0mg/kg	2.5mg/kg	10mg/kg	0mg/kg	2.5mg/kg	10mg/kg
Weight change (%)	16.7 ± 1.1	14.7 ± 1.9	16.1 ± 0.8	37.0 ± 1.3	37.1 ± 1.7	33.9 ± 3.1
Fetal Characteristics						
No. of males	-	-	-	6.8±0.9	7.3±0.7	4.3±1.0
No. of females	-	-	-	6.3±1.0	5.5±0.9	8.0±1.1
Anogenital distance males (mm)	-	-	-	4.1±0.03	4.1±0.1	4.3±0.2
Anogenital distance females (mm)	-	-	-	2.2±0.08	2.3±0.1	2.2±0.1
Fetal anomalies	-	-	-	0.0±0.0	0.3±0.2	0.7±0.7

Mean (±SEM) weight change (%) of non-pregnant and pregnant females and fetal characteristics in pregnant females. There were no significant effects.

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Fig 1

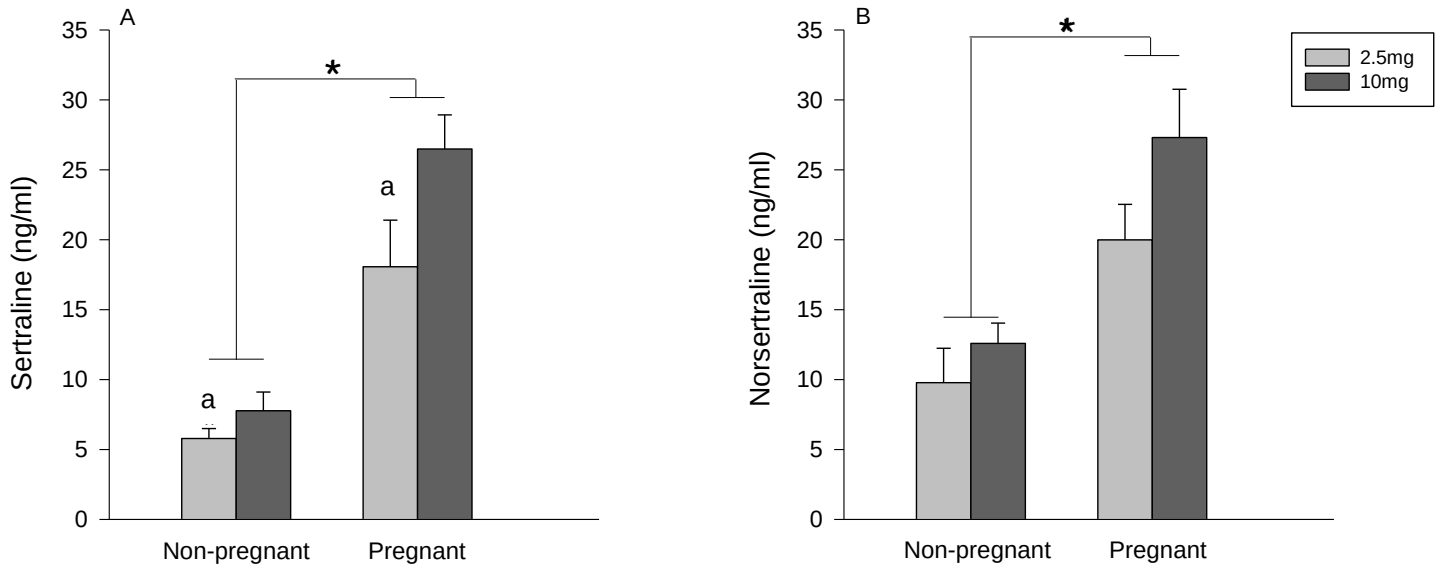

A
Sertraline (ng/ml)
35
30
25
20
15
10
5
0
*
a
a
Non-pregnant
Pregnant
B
Norsertraline (ng/ml)
35
30
25
20
15
10
5
0
*
2.5mg
10mg
Non-pregnant
Pregnant

Fig 2

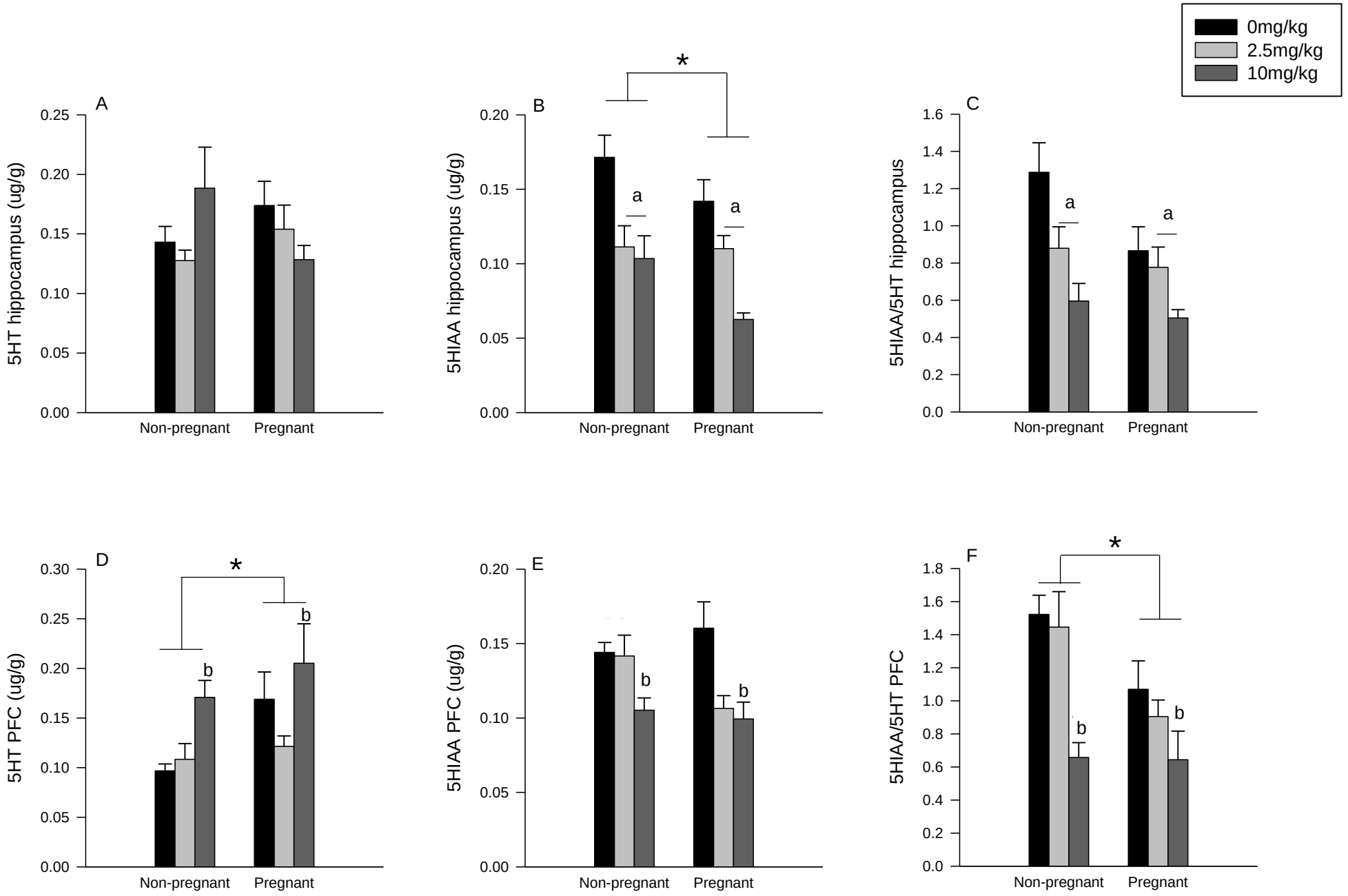
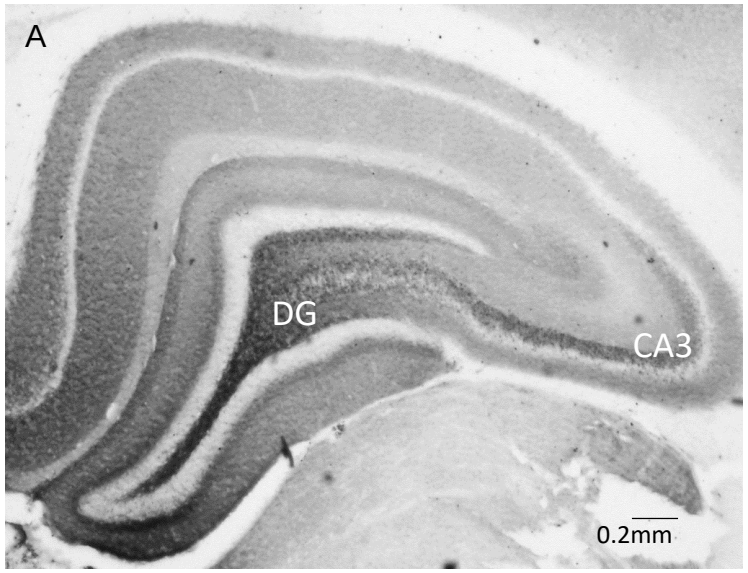
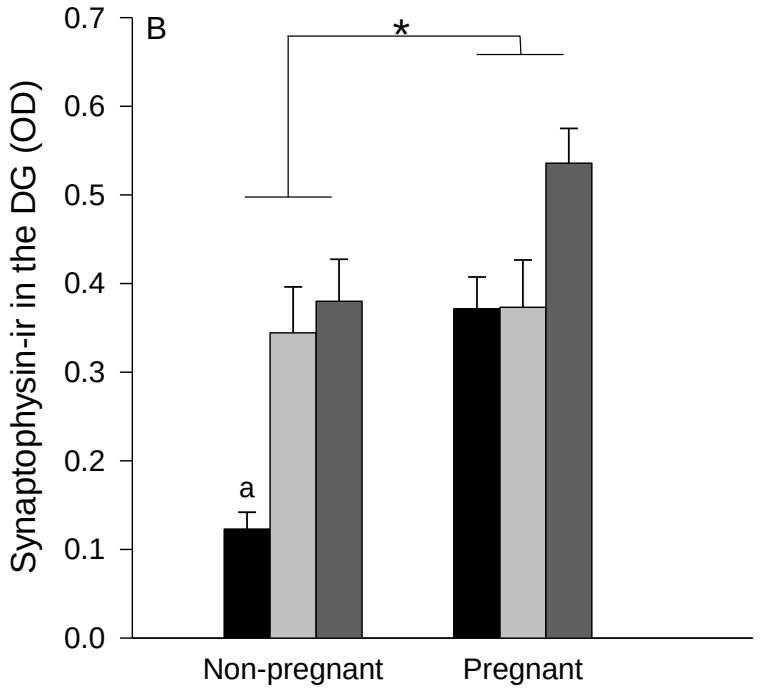

0mg/kg
2.5mg/kg
10mg/kg
A
5HT hippocampus (ug/g)
0.25
0.20
0.15
0.10
0.05
0.00
Non-pregnant
Pregnant
B
5HIAA hippocampus (ug/g)
0.20
0.15
0.10
0.05
0.00
*
a
a
Non-pregnant
Pregnant
C
5HIAA/5HT hippocampus
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
a
Non-pregnant
Pregnant
D
5HT PFC (ug/g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
b
b
Non-pregnant
Pregnant
E
5HIAA PFC (ug/g)
0.20
0.15
0.10
0.05
0.00
b
b
Non-pregnant
Pregnant
F
5HIAA/5HT PFC
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
b
b
Non-pregnant
Pregnant

Fig 3


A
DG
CA3
0.2mm


B
Synaptophysin-ir in the DG (OD)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
a
Non-pregnant
Pregnant

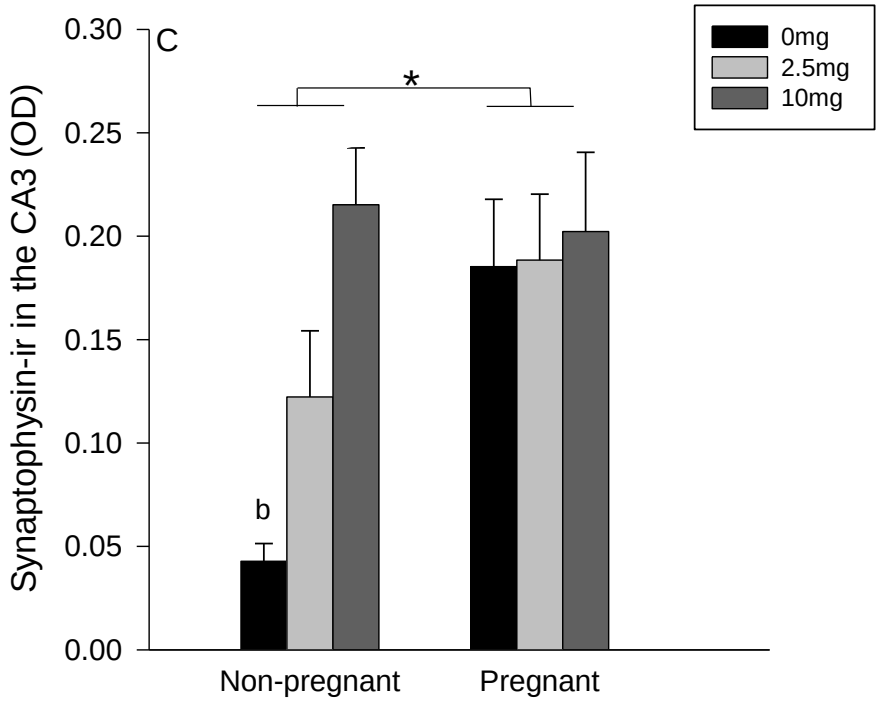
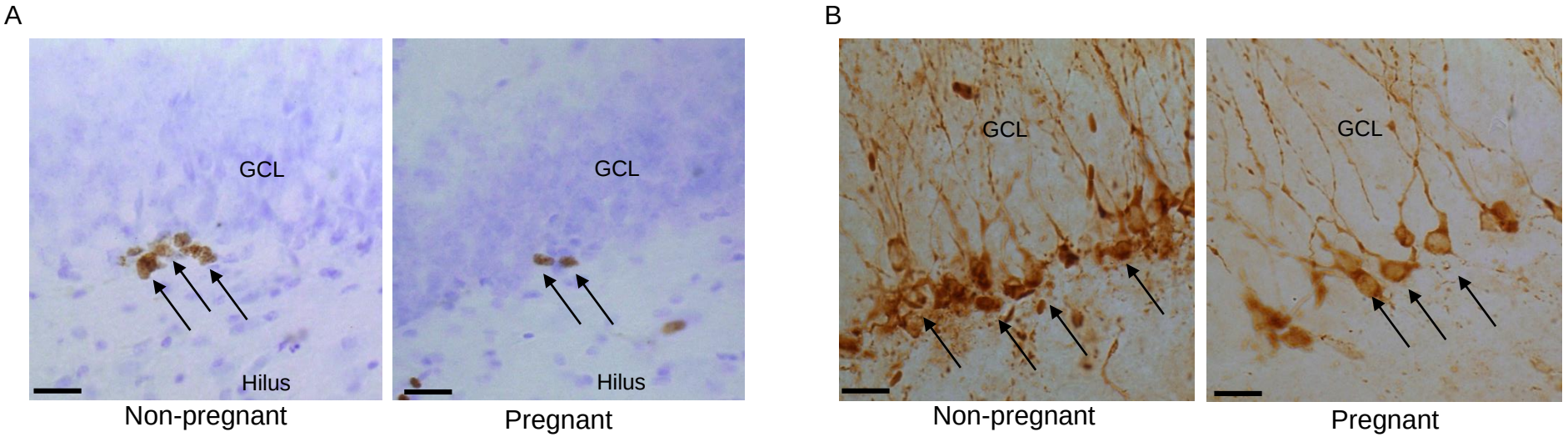

C
Synaptophysin-ir in the CA3 (OD)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
b
Non-pregnant
Pregnant
0mg
2.5mg
10mg

Fig 4


A
GCL
Hilus
Non-pregnant
GCL
Hilus
Pregnant
B
GCL
Non-pregnant
GCL
Pregnant

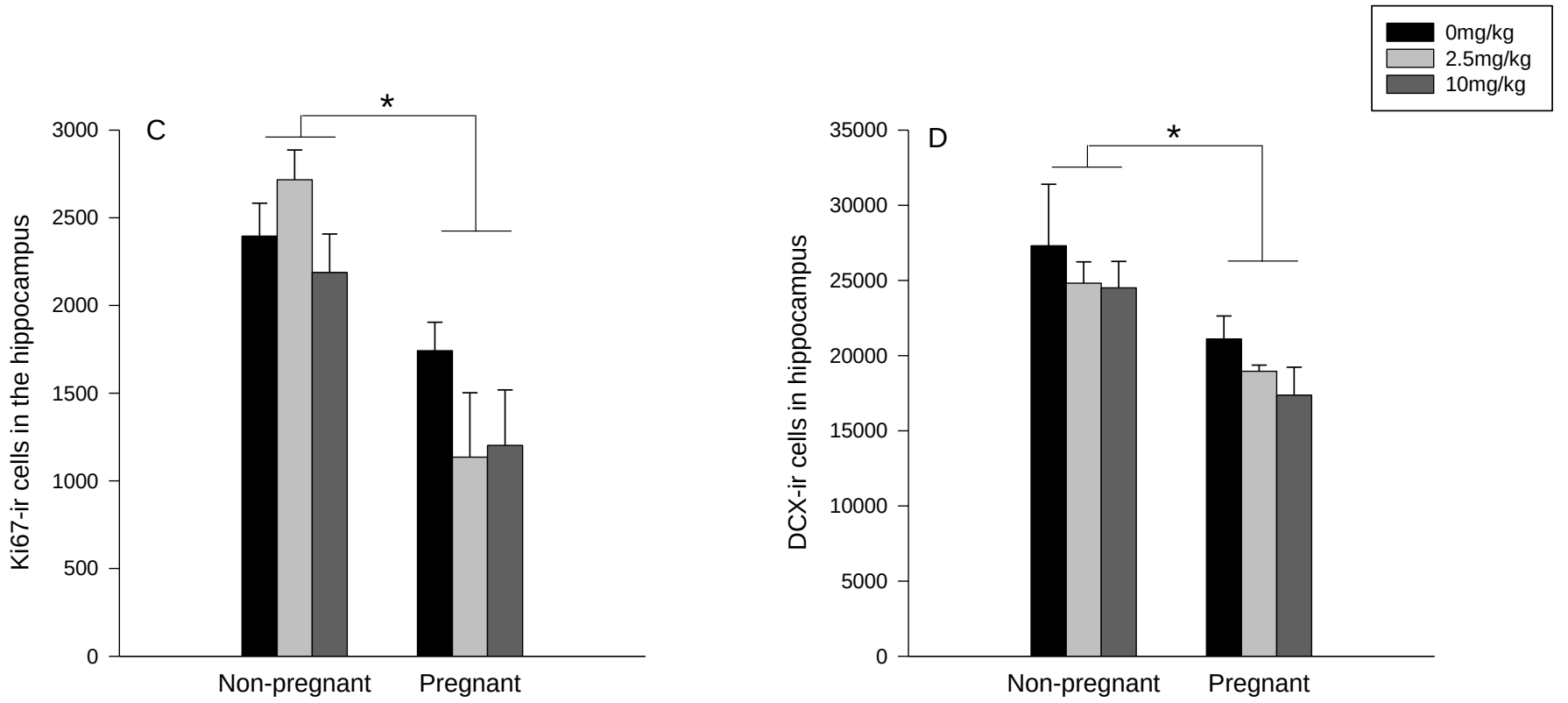

0mg/kg
2.5mg/kg
10mg/kg
C
*
Ki67-ir cells in the hippocampus
3000
2500
2000
1500
1000
500
0
Non-pregnant
Pregnant
D
*
DCX-ir cells in hippocampus
35000
30000
25000
20000
15000
10000
5000
0
Non-pregnant
Pregnant

Fig 5

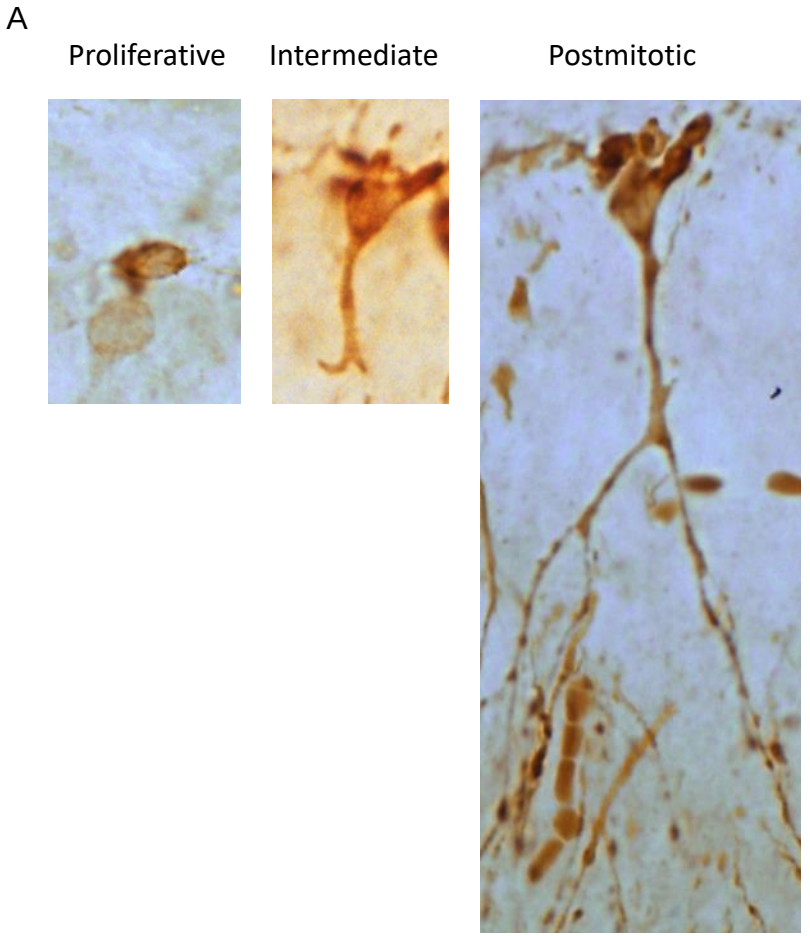
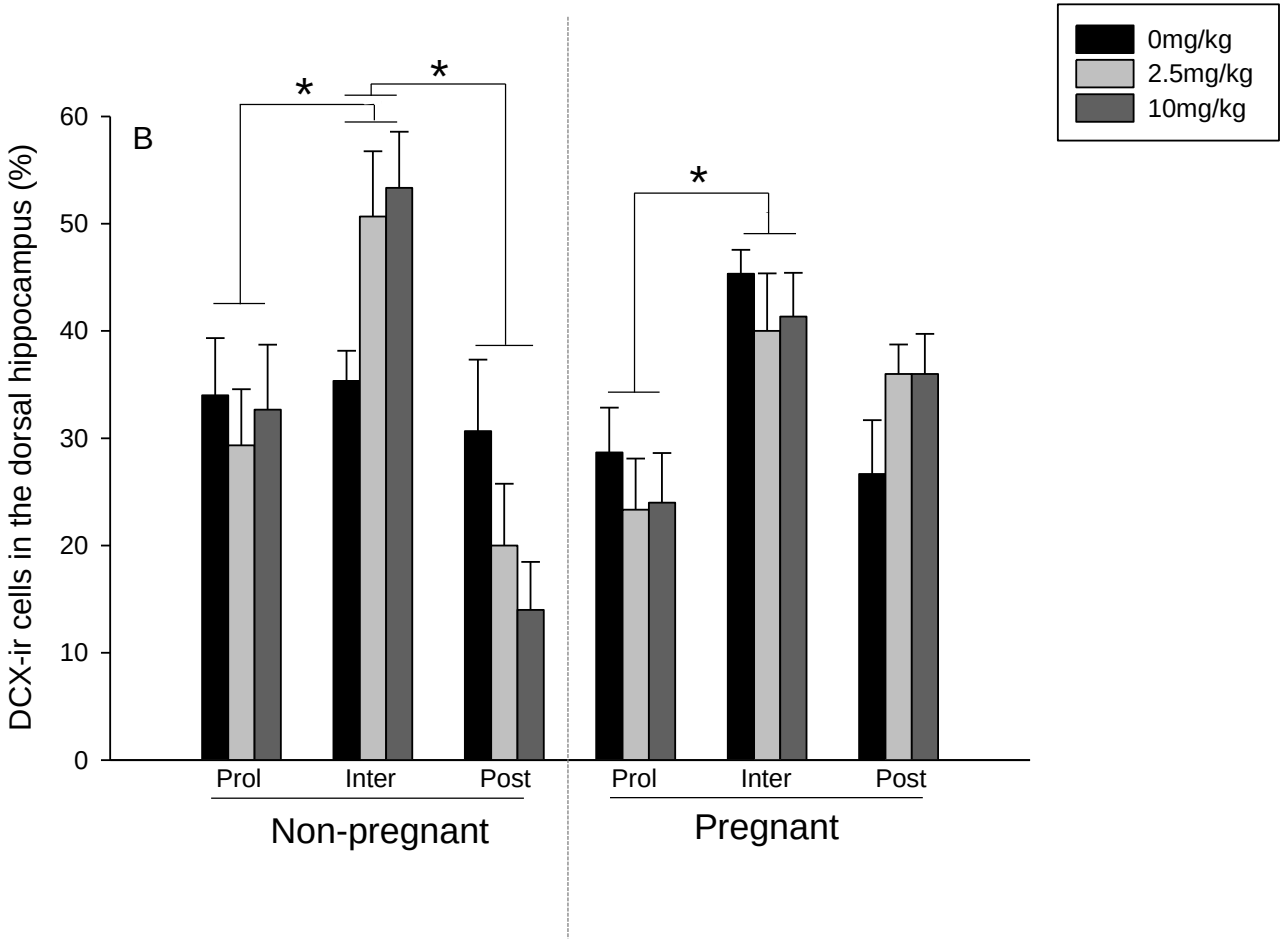
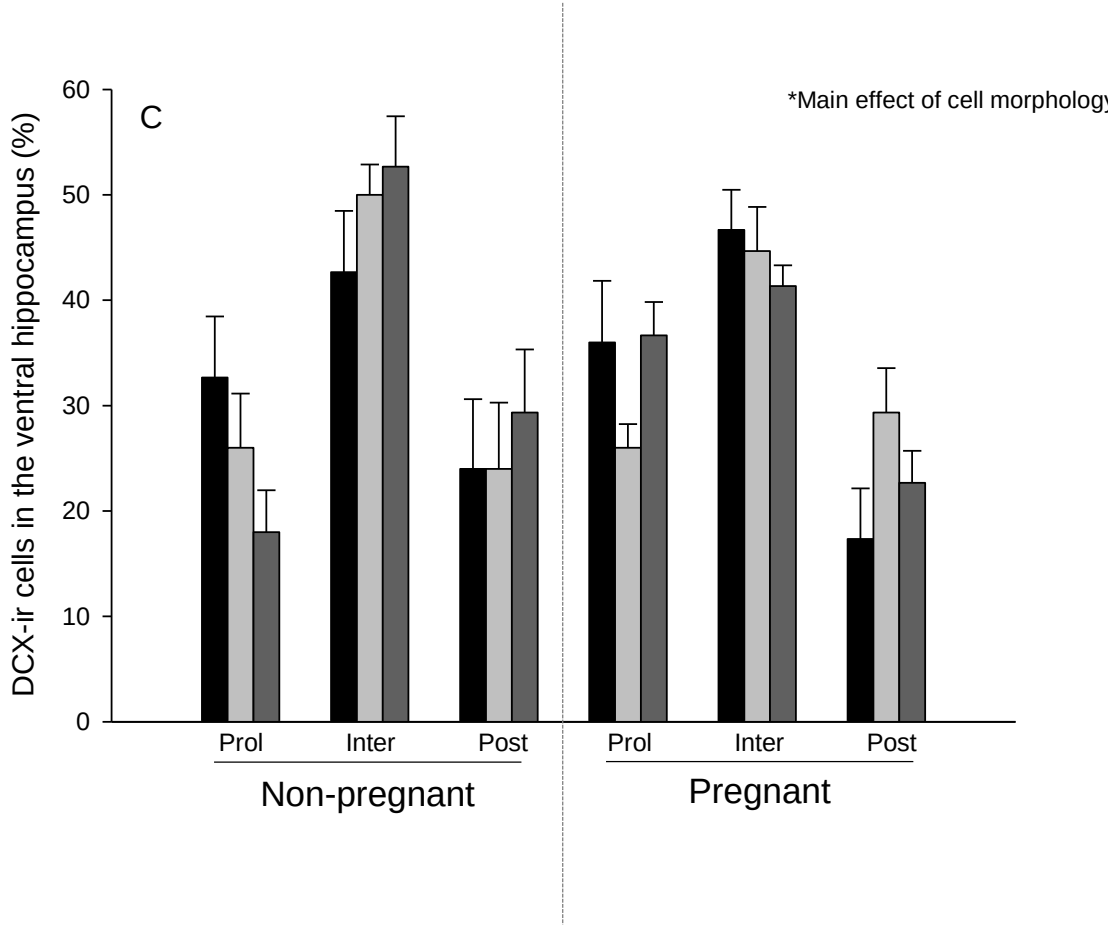
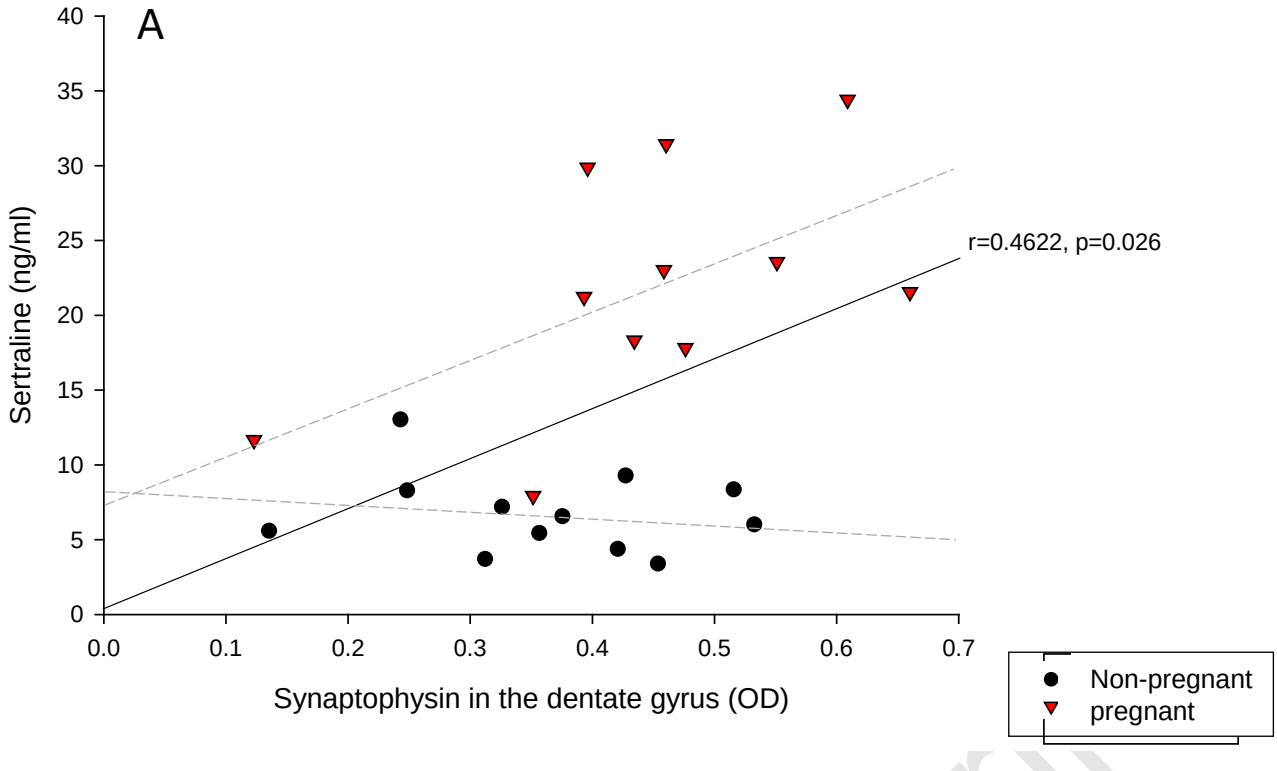
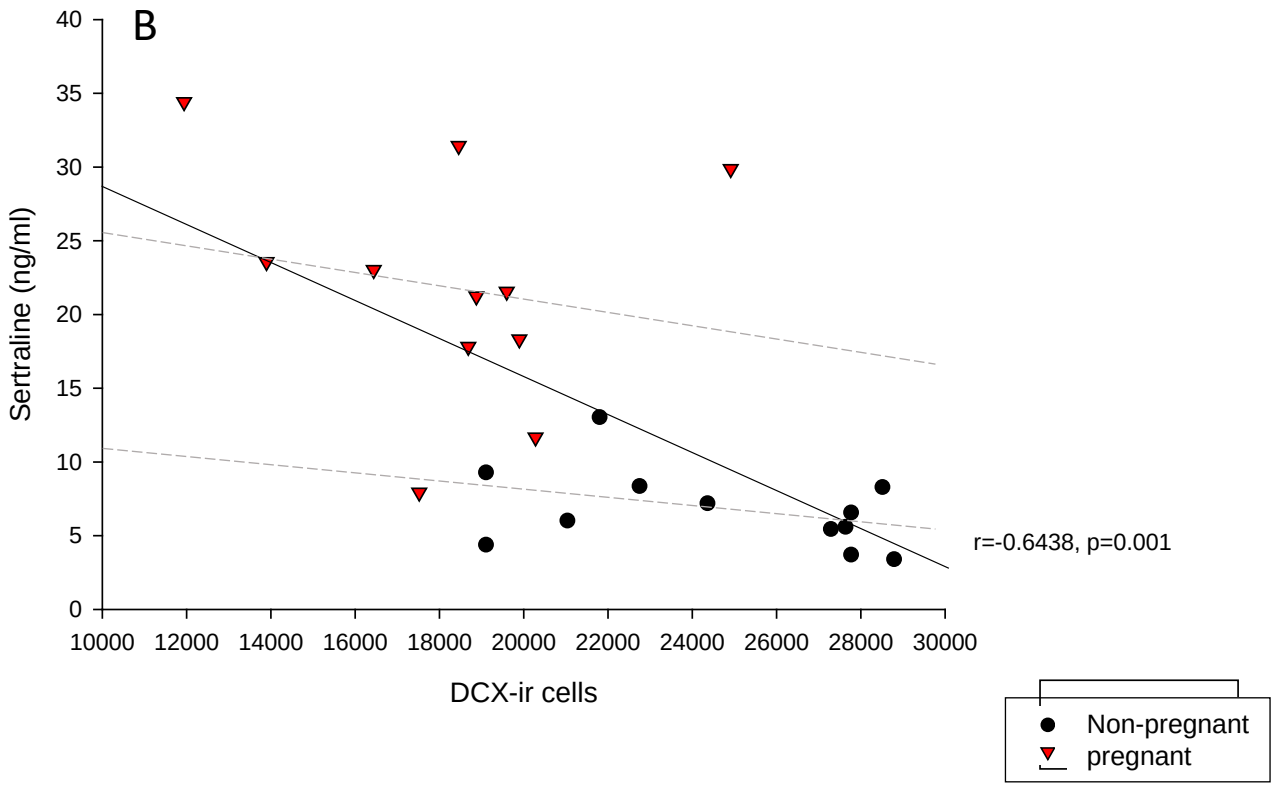


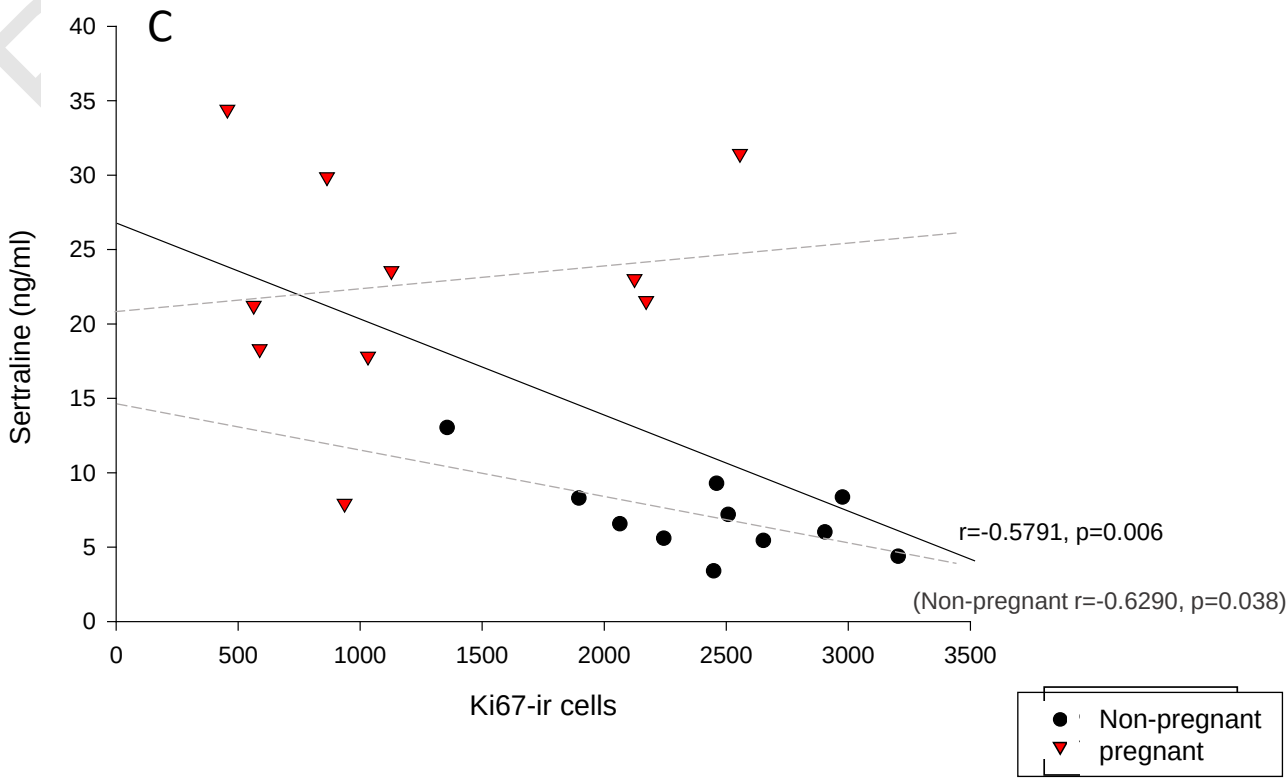
Fig 5


B
0mg/kg
2.5mg/kg
10mg/kg
DCX-ir cells in the dorsal hippocampus (%)
0
10
20
30
40
50
60
*
*
*
Prol
Inter
Post
Prol
Inter
Post
Non-pregnant
Pregnant


C
*Main effect of cell morphology
DCX-ir cells in the ventral hippocampus (%)
0
10
20
30
40
50
60
Prol
Inter
Post
Prol
Inter
Post
Non-pregnant
Pregnant


A
Sertraline (ng/ml)
Synaptophysin in the dentate gyrus (OD)
r=0.4622, p=0.026
Non-pregnant
pregnant


B
Sertraline (ng/ml)
DCX-ir cells
r=-0.6438, p=0.001
Non-pregnant
pregnant


C
Sertraline (ng/ml)
Ki67-ir cells
r=-0.5791, p=0.006
(Non-pregnant r=-0.6290, p=0.038)
Non-pregnant
pregnant