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Differential effects of oxytocin on olfactory, hippocampal and hypothalamic neurogenesis in adult sheep.

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## Abstract

New neurons are continuously added in the dentate gyrus of the hippocampus, the olfactory bulb and the hypothalamus of mammalian brain. In sheep, while the control of adult neurogenesis by the social environment or the photoperiod has been the subject of several studies, its regulation by intrinsic factors, like hormones or neurotransmitters is less documented. We addressed this question by investigating the effects of central oxytocin administration on hippocampal, olfactory and hypothalamic neurogenesis. Endogenous markers, Ki67, Sox2 and DCX were used to assess cell proliferation, progenitor cells density and cell survival respectively in non-gestant ewes receiving a steroid treatment followed by intracerebroventricular injections of either oxytocin or saline. The results showed that oxytocin treatment significantly decreases the density of neuroblasts in the olfactory bulb, increases the density of neuroblasts in the ventromedian nucleus of the hypothalamus while no change is observed in both ventral and dorsal dentate gyrus. In addition, no change in the density of progenitor cells is found in the three neurogenic niches. These findings show for the first time that in females, oxytocin can regulate adult neurogenesis by acting on neuroblasts but not on progenitor cells and that this regulation is region specific.

Key words: cell proliferation; cell survival; dentate gyrus; doublecortin; olfactory bulb; social behavior; ventromedian hypothalamic nucleus

## Introduction

In most mammals, newborn neurons are continuously generated throughout life mainly in two structures of the brain, the dentate gyrus (DG) of the hippocampus and the olfactory bulb [OB; 9, 15, 45]. In the DG, stem cells reside in the subgranular zone and give rise to neuroblasts which become new granular neurons in the overlying granule cell layer [45]. The neural stem cells located in the wall of the lateral ventricles constituting the subventricular zone (SVZ) produce transient amplifying cells, which divide to produce neuroblasts that, in turn, migrate toward the OB along the rostral migratory stream. After reaching the OB, the new cells mature into granular interneurons for the majority of them [40]. A neurogenic activity is also reported within the hypothalamus of several mammalian species [28, 29, 43, 44]. Constitutive cell proliferation activity has been characterized in the arcuate and ventromedial nuclei and in the median eminence. Neural stem cells were detected among the cells lining the wall of the third ventricle and numerous immature neurons were found in the parenchyma of the ventromedial hypothalamus [VMH; 2, 30].

Extensive studies have shown that both extrinsic factors, like social environment or photoperiod, and intrinsic factors, like growth factors, neurotransmitters or physiological status and associated hormones, regulate each step of adult neurogenesis including proliferation and fate specification [for review: 32, 45, 51]. For instance, pregnancy and parturition **are associated with dramatic hormonal changes and specifically in progesterone and estradiol plasmatic levels**. In turn, steroid hormones, affect hippocampal, olfactory, and hypothalamic neurogenesis [for review: 3, 32, 51]. Some hormonal factors specifically regulate the activity of a neurogenic niche without affecting the other niches. For instance, in mice, prolactin increases cell proliferation in the SVZ although it does not affect

hippocampal neurogenesis [52] and high levels of corticosterone down-regulates hippocampal cell proliferation while no regulation of olfactory neurogenesis by corticosteroids has been reported in rats [7]. Regarding oxytocin (OT), while this hormone is capable of stimulating hippocampal neurogenesis in male rats [31], no evidence for its implication in the regulation of olfactory or hypothalamic neurogenesis has been so far reported. Interestingly, OT neurons in the hypothalamic paraventricular nuclei project to various brain regions including the OB, the CA3 hippocampal pyramidal cells and the mediobasal hypothalamus [MBH; 24, 39, 53] and OT receptors are found in the DG, **on the periglomerular and granular interneurons of the OB** and the VMH, the latest being included in the MBH [5, 54]. In sheep, parturition increases the expression of OT mRNA receptors in the VMH [5] and the OT release is enhanced in the OB at parturition and in the MBH during mating [25, 34].

Cell proliferation and neuronal survival are reduced in the early post-partum period in both the SVZ/OB and the DG of sheep [8, 11]. Since the whole oxytocinergic system is activated at this period [24] and can act on the olfactory, hippocampal and hypothalamic neurogenic niches, the present study was designed to assess whether OT alters adult neurogenesis in the three niches. To this end, cell proliferation and cell survival were measured in non-pregnant ewes receiving steroid treatment followed by intracerebroventricular injections of OT mimicking endocrine events occurring at parturition and enabling induction of maternal behavior [26, 27].

## 2. Material and methods

### 2.1. Animals and ethics

Experiments were conducted on 16 non-gestant Ile de France ewes (4-5 years old) from the IPAO, INRA (Animal physiology experimental facility, doi:10.15454/1.5573896321728955E12). Animals were permanently housed indoors, with free access to water and were fed with lucerne, maize, straw and a supplement of vitamins and minerals. Animal care and experimental treatments complied with the guidelines of the French Ministry of Agriculture for animal experimentation and European regulations on animal experimentation (86/609/EEC). They were performed in accordance with the local animal regulation (authorization N°. 006352 of the French Ministry of Agriculture in accordance with EEC directive) and with the ethical committee Val de Loire (agreement N° 2015062415046597). Ewes were sacrificed by a licensed butcher in an official slaughterhouse (authorization No. E37-175-2 agreement UEPAO). All efforts were made to reduce the number of animals (8 animals per group).

## 2.2. Neurosurgery

OT was stereotaxically injected by means of an injection needle inserted into a guide cannulae and connected to battery-driven syringe pumps (GrasebyMedical, 166 Watford, UL) tied on the animal's backs. For stereotaxic surgery, animals were anaesthetized with a mixture of Nesdonal (Hippocampe, Bressuieres, France) and atropine (20mg; CDM Lavoisier, Paris, France), followed by 3% isoflurane gaseous anesthesia in oxygen (CSP Center Specialty Pharmaceutical, Cornon, France). Full sterile procedures were used throughout surgery. The animal's head was fixed in a stereotaxic frame adapted to sheep and modified for X-ray radiographic control. Stereotaxic coordinates were calculated using lateral and dorsoventral X-rays of the skull after infusion of a radio-opaque agent (Iopamiron 300; Shering, Germany) in the lateral ventricle. The middle of the third ventricle was used to calculate the lateral

coordinates. Guide-cannulae were placed into the lateral ventricle with a six-degree angle of incidence in order to avoid the venous sinus. According to the atlas of Richard (1967), the stereotaxic coordinates of the guide-cannulae tips (stainless steel tubes, external diameter 1.2 mm, internal diameter 0.8 mm, length 18 mm, Thiebaud Biomedical Instruments, Thonon-les-Bains, France) were as follows: rostrocaudal plane, 30mm; mediolateral plane,  $1.9 \pm 0.4$  mm from the middle of the third ventricle; depth,  $18.1 \pm 1$  mm from the cortex surface (Fig. 1A). A pilot study allowed us to confirm that these coordinates were appropriate to avoid tissue damage to the SVZ and the hippocampus. Stainless steel guide-tubes were fixed to the bone with acrylic cement closed with obturators. The entire assembly was then protected with a Teflon ring anchored to the skull with stainless steel screws and acrylic cement. Following surgery, animals received daily intramuscular injections of a solution containing an antibiotic (5 days, 5 ml of amoxicillin, Centravet, Plancoët, France), an anti-inflammatory (5 ml diurizone for 3 days, 2 ml finadyne on the first day, Centravet, Plancoët, France) and an analgesic (morphine (dose) for the first day, CDM Lavoisier).

### 2.3. Treatment

Two weeks after surgery, ewes were housed in 2×1 m individual pens and were daily accustomed to human presence and handling for a week (Fig. 1B). After receiving vaginal sponges impregnated with a progestagen for 14 days, ewes received two daily subcutaneous injections of estradiol benzoate (200µg /day). This steroid treatment was used because progesterone suppresses the basal release of OT sensitizing the brain to a surge of OT following estrogen injections and estradiol increases OT synthesis in the brain as well as the number of OT receptors [4, 12]. The day following the steroid treatment and for two days, ewes were unilaterally

infused with artificial cerebrospinal fluid (aCSF; pH 7.4; Control group, N= 8) or bilaterally with OT (OT group, N= 8; 25µg/per side/day) by inserting perfusion needles (Cooper Needles; UK) into the guide cannulae positioned into the lateral ventricle. One milliliter of solution was infused **over a period of 60 minutes** in one side for the CSF or both sides for OT. The OT dose was adapted from Keverne and Kendrick's study [27] showing that central administration of OT in non-pregnant ewes induces the all repertoire of maternal behavior normally observed at parturition. **We chose two daily OT i.c.v. injections as it was reported that repeated OT injections enhanced the number of adult-generated neurons [31].**

#### 2.4. Brain perfusion and immunohistochemistry

Four hours after the end of the second intracerebroventricular infusion, ewes were deeply anesthetized with thiopental and decapitated by a licensed butcher. **Ewes were rapidly killed after the second injection since one of our aim was to investigate the effects of OT injection on cell proliferation. In the case of a longer time interval between injection and sacrifice, a causal link would have been difficult to establish.**

The heads of the ewes were immediately perfused via the carotid arteries with 2 L of warm 1% sodium nitrite in phosphate buffer saline, followed by 4 L of ice-cold 4% paraformaldehyde solution in 0.1M phosphate buffer (pH 7.4). The brain was dissected out, cut into several blocks (MOB, dHipp, vHipp, SVZ and VMH) and post-fixed in the same fixative for 48 h. The tissues were then stored in 20% sucrose for at least 2 days until sectioned. Frontal sections of the SVZ, the MOB, the dorsal and ventral hippocampus (dHipp and vHipp) and the VMH were cut on a cryostat at a thickness of 30µm. Sections were stored at 4 °C in TBS Azide buffer until their use



for immunostaining. Examination of frontal sections at the level of the lateral ventricle confirmed that all the cannulae were localized within the lateral ventricle.

To reveal the putative effect of OT administration on cell proliferation in the 3 neurogenic niches, Ki-67, an endogenous marker of cell division **expressed for the entire duration of the mitotic process** [23], was used in the SVZ and the dHipp. In the VMH, Sox2, used as a marker for neural stem /progenitor cells [41], was used in 6 animals out of 8, since this area was irreversibly damaged in 2 animals during dissection [16, 17, 44].

To reveal Ki67-positive cells a peroxidase single-immunolabeling was used. Sections were mounted on a glass slide and dried at 37°C. Then the sections were warmed up to 90°C in citrate buffer 0.01M using a microwave. After cooling 1 h to room temperature and three rinses in TBS-Triton 0.3% (TBST), sections were pre-incubated for 10 min in TBST-0.1%-sodium azide-BSA 1% (TBSTA-BSA 1%), then incubated overnight at room temperature in primary mouse anti-Ki67 (1:250; BD Pharmingen, USA). The next day, sections were rinsed four times in TBST, and incubated at room temperature for 2.5 h with a sheep anti mouse peroxidase antibody (1:300; Jackson, USA). Finally, after two rinses in TBS and two rinses in Tris-HCl, sections were reacted for peroxidase detection in DAB-Ni-H<sub>2</sub>O<sub>2</sub> that allows a permanent labeling of Ki67 positive cells appropriate for counting.

In the VMH, previous experiments showed that, Sox2 fluorescent immunolabeling was a relevant indicator for the neurogenic activity [1]. After 30mn in 0.1% sodium borohydride in TBS at room temperature followed by three rinses in TBS, the sections were incubated for 1 h in TBSTA-BSA 1% at room temperature. They were then incubated for 48 h in goat anti-Sox2 (1:300; R and D Systems, USA). They were rinsed four times for 5 min in TBST before incubation (for 3h at 4°C) in

CY3-donkey anti goat (1:1000; Jackson, USA). After three rinses in TBST, cells nuclei were counterstained in a Hoechst solution diluted at 1:5000 in TBS, followed by three rinses of TBS. The immunostained sections were then mounted on slides, dried and immersed 10 min in 0.1% Sudan Black B (Merck, Germany) diluted in 70% ethanol. Finally, slides were washed two times in ethanol (5mn) and one time in TBS (5 min). They were then dried and coverslipped with Fluoromount-G (Southern Biotech, Birmingham, AL).

Doublecortin (DCX) is expressed in migrating neuroblasts [6, 20, 47] and is a reliable and specific marker that reflects levels of adult neurogenesis [6, 10]. **Young proliferating neuroblasts could also express DCX together with either Ki67 or Sox2 indicating cell division of neuroblasts [6]. This marker was used to reveal the effect of OT administration on olfactory, hippocampal and hypothalamic neurogenesis.**

For DCX immunolabeling, peroxidase technique was used for the OB, the dHipp, the vHipp. After two rinses in TBST and two rinses in Tris-HCl, sections were reacted in DAB-Ni-H<sub>2</sub>O<sub>2</sub> allowing a permanent labeling of DCX positive cells appropriate for counting. The slides were then rinsed, dried, dehydrated through graded alcohol, cleared in toluene and cover-slipped with Depex®.

In the VMH, previous experiments showed that fluorescent immunolabeling of DCX+ cells provided a detailed distribution pattern of immature and developing neurons in sheep [6, 20, 48]. Before the antigen retrieval with a microwave method, sections were mounted on a glass slide and dried at 37°C. Then the sections were warmed up to 90°C in citrate buffer 0.01M using a microwave. After cooling to room temperature and three rinses in 0.3% TBST, sections were pre-incubated for 10 min in TBST- 1% horse serum, and then incubated in primary goat anti-DCX (1:300, Santa Cruz Biotechnology, California, USA) in TBST- 0.1% sodium azide- 1% horse

serum overnight at room temperature. The next day, sections were rinsed three times for 10 min in TBST, and incubated at room temperature for 3 h with a HRP-donkey anti-goat (1:300; Jackson, USA) for peroxidase labeling or with CY3-donkey anti-goat for immunofluorescence (1:1000; Jackson, USA). After three washes in TBST slides were immersed for 2 min in a Hoechst solution (1:5000; Molecular Probes/Invitrogen, Carlsbad, CA) for nuclear staining and rinsed in TBS. The slides were dried, immersed 10 min in 0.1% Sudan Black B (Merck, Germany) diluted in 70% ethanol and washed twice in ethanol (5 min) and once in TBS (5 min). They were then dried and coverslipped with Fluoromount-G (Southern Biotech, Birmingham, AL).

## 2.5. Quantification

The number of Ki67, Sox2 and DCX positive cells was assessed by counting the immunostained sections using a photonic microscope (Axioscop, Zeiss, Germany) with a motorized stage on a magnification of x20 and a cell-count analysis software (Mercator, Explora Nova, La Rochelle, France), according to a predetermined grey level and size criteria. Sections per animal were selected through different levels along the rostrocaudal axis of the structures: 3 sections for the OB, the SVZ, the vHipp and the dHipp and 4 sections every 500  $\mu\text{m}$  for the VMH. Each area of interest was drawn with a x2.5 magnification. In the OB, granular layer was drawn and randomly covered by probes (300  $\mu\text{m}$  X 200  $\mu\text{m}$ ) separated from each other by 250  $\mu\text{m}$ . Both in the dHipp and vHipp, 6 rectangles (300  $\mu\text{m}$  X 50  $\mu\text{m}$ ) were randomly distributed along the dentate gyrus. The density of immunostained cells was then calculated. In the VMH, counting was performed within 2 rectangles (800  $\mu\text{m}$  X 1400  $\mu\text{m}$ ) placed next to the fornix, one on the right side of the brain, one on the left side.

Because Ki67 and Sox2 can also be expressed by some young proliferating neuroblasts [6], it is possible that counting of the population of proliferative cells in the three neurogenic niches involves some immature neurons.

## 2.6. Statistical analysis

Due to lack of normality, data were analyzed with non-parametric Mann-Whitney tests. Statistical analyses were performed using the statistical package Statistica 10 and the level of statistical significance was set at  $p \leq 0.05$ . All data are represented as median and interquartile ranges.

## 3. Results

### 3.1 Proliferation

Representative cell labelings for Ki67 in the SVZ and in the dHipp are shown in Fig. 2A. Following immunolabeling quantification, Ki67<sup>+</sup>cell density in the SVZ did not significantly differ between OT and control groups ( $Z=1.26$ ,  $p=0.2$ ; Fig. 2B). Similarly, no significant difference was observed in the dHipp for Ki67<sup>+</sup>cell density between OT and control group ( $Z=0.3$ ,  $p=0.7$ ; Fig. 2B.) and in the vHipp (9.7 [5.2-21.4] vs 10.2[8.9-15.2],  $Z=0.2$ ,  $p=0.8$ , OT vs control groups, respectively).

The figure 2A illustrates the Sox2 immunopositive cells detected in the VMH. No change was observed in Sox2<sup>+</sup> cell density between OT and control groups (Fig. 2B;  $Z=0$ ,  $P=1$ ).

### 3.2 Cell survival.

DCX<sup>+</sup> cells in the OB and the dHipp from control and OT animals are shown in figures 3A and 3C. Quantification analysis reveals that in the granular layer of the

OB, OT infusion significantly decreased DCX<sup>+</sup> cell density ( $Z=2.2$ ,  $p=0.02$ ; Fig. 3B). In contrast, no significant difference was observed, between OT and control groups, in the DCX<sup>+</sup> cell density, in the dHipp ( $Z=0.3$ ,  $p=0.7$ ; Fig. 3D) or in the vHipp (429 [406-476] vs 430[377-552],  $Z=0.05$ ,  $p=0.9$ , OT vs control groups, respectively).

DCX<sup>+</sup> cells in the VMH, from control and OT animals are shown in figure 4A. OT infusion induced a significant increase in DCX<sup>+</sup> cell density ( $Z=2.4$ ,  $p=0.01$ , Fig. 4B).

#### 4. Discussion

The present study shows for the first time in steroid-primed females that acute central administration of OT regulates adult neurogenesis in a region specific manner. OT treatment results in a decreased density of neuroblasts (DCX<sup>+</sup> cells) in the OB, an increased density of neuroblasts in the VMH while no change is found in both vHipp and dHipp. In addition, no change in cell proliferation (Ki67<sup>+</sup> and Sox2<sup>+</sup> cells) is observed in the three neurogenic niches.

Previous studies in sheep reported that, at parturition, an increased release of OT occurs within the granular layer of the OB [34]. In addition, during the peripartum period, a down-regulation of the number of neuroblasts (DCX<sup>+</sup> cells) has been reported within the granular layer of the OB [11]. Thus, the present study suggests that OT can be responsible for this reduction in new olfactory neurons occurring at parturition. A considerable body of evidence reveals that OT is involved in various olfactory guided behaviors [22, 38]. In the context of mother-young interactions, OT regulates preference of rat pup for maternal odors [49] and OT administration within the OB induces maternal behavior in ovariectomized rats primed with estrogen [57]. In ewes, i.c.v. OT administration induces olfactory preference for amniotic fluid odors

[33, 35]. Thus, since olfactory newborn neurons are involved in lamb odor recognition [13, 14], it is possible that OT drives changes in odor preference by acting on olfactory neurogenesis. Interestingly, interactions with the lamb at parturition down-regulate olfactory neurogenesis in sheep mothers favoring maturation of neuroblasts [11] and disruption of this neurogenesis prevents olfactory memory of the lamb [14]. Therefore, OT by decreasing the density of neuroblasts could also participate in the formation of this olfactory memory. The effect of OT could be direct through the activation of specific receptors expressed by olfactory neuroblasts (DCX<sup>+</sup> cells) or by more mature adult-generated neurons. Alternatively, an indirect effect of OT through an intermediate neuronal population can be also considered. In rodents, neural progenitors in the DG do not express OT receptors and OT stimulates neurogenesis in the DG via oxytocin receptor expressed in CA3 neurons [39]. The fact that OT can activate autoreceptors on OT neurons of the paraventricular nucleus of the hypothalamus [18] that provides afferent input to the OB [19, 36] could be a mechanism by which OT regulates olfactory neurogenesis.

Intracerebroventricular administration of OT has no influence on cell proliferation (Ki67<sup>+</sup> cells) in the SVZ. Similarly, a lack of effect on cell proliferation in the SVZ of rats was reported after OT administration [31]. This lack of OT effect could be due to the absence of OT receptors in the SVZ, although this hypothesis requires further analysis. Alternatively, OT may modulate cell proliferation indirectly by acting on OT-responsive neurons within regions providing afferents to the SVZ, as it was reported in the control of the SVZ neural stem cells by hypothalamic proopiomelanocortin neurons [50]. According to this hypothesis, the amount of OT administered in the lateral ventricles would not have been sufficient to activate the OT-responsive neurons.

No change in either cell proliferation (**Ki67<sup>+</sup> cells**) or in neuroblasts (**DCX<sup>+</sup> cells**) production in the DG is found after i.c.v. OT administration. In rodents, contradictory results were reported: an enhancement of cell proliferation in the vHipp of male rat, but not the dHipp, following peripheral or central OT administration [31] whereas no change in progenitor cell proliferation and differentiation into immature neurons induced by conditional deletion of OT receptors in male mice [39]. However, beside sex differences between rodent and sheep studies, OT receptor distribution varies between species [19, 54]. For instance, OT binding sites are lacking in the hippocampus, amygdala, entorhinal cortex, and olfactory bulb of human brains [42]. In sheep, very low levels of OT receptor mRNA expression has been found in the DG and steroid treatment did not contribute to increase expression [5]. Consequently, regulation of hippocampal neurogenesis would not involve OT due to a **paucity of OT receptors** in the DG which is consistent with the inability of OT to stimulate neurogenesis in the dHipp of rats which contains low levels of OT receptors [37]. It is possible that other neuromodulatory factors contributed to the regulation of hippocampal neurogenesis, such as glucocorticoids, which are potent inhibitors of neurogenesis in rodents [7, 46, 55, 56]. **Alternatively, doses and timing of OT injections would not be appropriate. Considering the low level of OT receptors in the DG, the OT treatment would not be long enough to induce changes in hippocampal neurogenesis.**

Central OT administration induces an increase in the number of neuroblasts (**DCX<sup>+</sup> cells**) in the VMH. It remains unclear whether the effects of OT are mediated via the activation of OT receptors on neuroblasts and it would be interested to examine whether hypothalamic neuroblasts express OT receptors. Nevertheless, the VMH of female sheep contains high levels of OT receptors and a steroid treatment

greatly enhances OT receptor expression [4]. Increased OT release within the MBH, which comprises the VMH, is reported in ewes during mating and OT infusion by retrodialysis in the MBH decreases sexual receptivity [25]. The up-regulation of neurogenesis induced by OT release during mating could provide a mechanism by which OT inhibits sexual receptivity. **The mechanism could involve an effect of OT on VMH neuroblasts by modulating their dendrite length reaching the lateral fiber complex, those projections being involved in the control of female sexual behavior [21]. In addition, VMH neuroblasts could migrate towards rostral parts of the hypothalamus, including the preoptic area involved in sexual behavior [1].**

In conclusion, these findings constitute a first evidence for OT regulation of adult neurogenesis in female. This regulation could be involved in the major role played by OT in socio-sexual interactions [22].

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## Figure captions

### **Figure 1:** Timetable and protocol design.

(A). Photograph of a sagittal view of the sheep brain stained with cresyl violet showing the site of infusion. Scale bar: 10 mm. dHipp: dorsal hippocampus; LV: lateral ventricle; OB: olfactory bulb; SVZ: subventricular zone.

(B). Approximately one month after cannulation in the lateral ventricle, ewes received a steroid treatment (progestagen for 14 days + one oestradiol benzoate injection per day for 2 days) followed by one i.c.v. bilateral injection of OT or unilateral injection of aCSF per day for 2 days. Ewes were sacrificed 4 h after the last i.c.v. injection and their brain were perfused for immunohistological analysis.

### **Figure 2:** Effects of OT i.c.v. infusion on cell proliferation in the SVZ, the dHipp and the VMH.

(A). Ki67 immunostainings of the SVZ, the dHipp, (scale bar: 15 $\mu$ m) and double immunostaining Sox2 with DCX (red) and Hoechst (blue) in the VMH (scale bar: 50 $\mu$ m).

(B). Results showing the number of Ki67+ cells per mm<sup>2</sup> in the SVZ, the dHipp and of the number of Sox2 + cells in the VMH are presented as median and interquartiles. No significant difference was observed between OT and control groups in the three structures.

### **Figure 3:** Effects of OT i.c.v. infusion on neurogenesis in the OB and the dHipp.



(A). Frontal section of the OB stained with Cresyl Violet (scale bar: 1mm) and immunostaining of DCX+ cells in the control and OT groups (scale bar: 50µm). VOB: ventricular olfactory bulb, Gl: glomerular cell layer, Gr: granular cell layer.

(B). Results are presented as median and interquartiles of the number of DCX+ cells per mm<sup>2</sup> in the OB. OT infusion induces a significant decrease in the number of DCX+ cells per mm<sup>2</sup>.

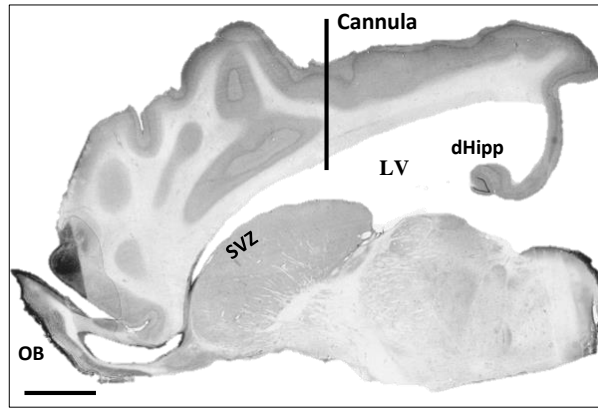
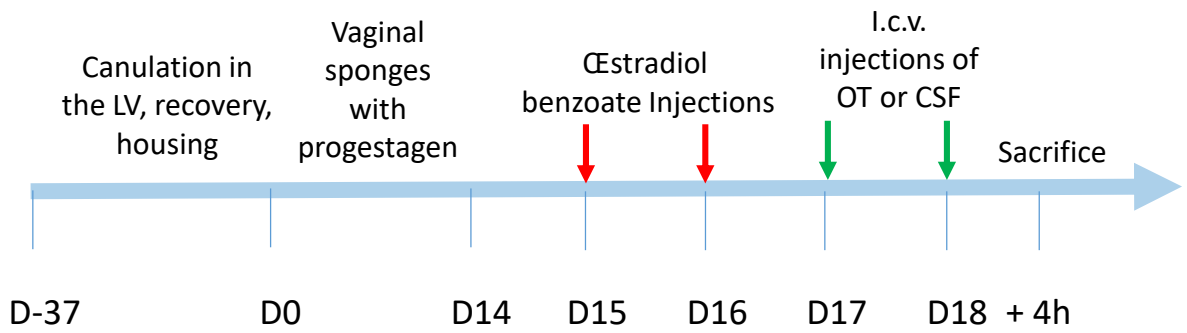
(C). Frontal section of the dHipp showing DCX immunolabeling in the dentate gyrus (scale bar: 25 µm) and higher magnification of DCX immunolabeling in the control and OT groups (scale bar: 500µm). DG: dentate gyrus.

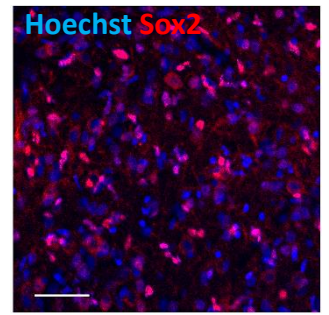
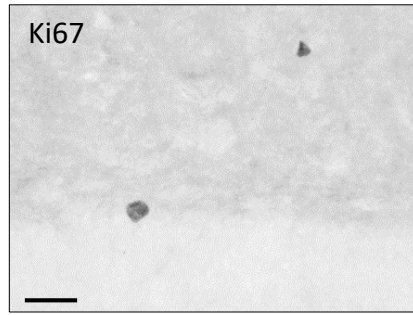
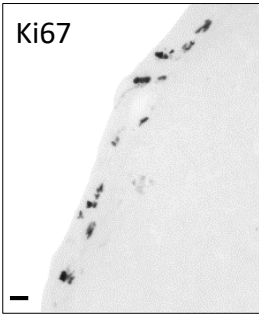
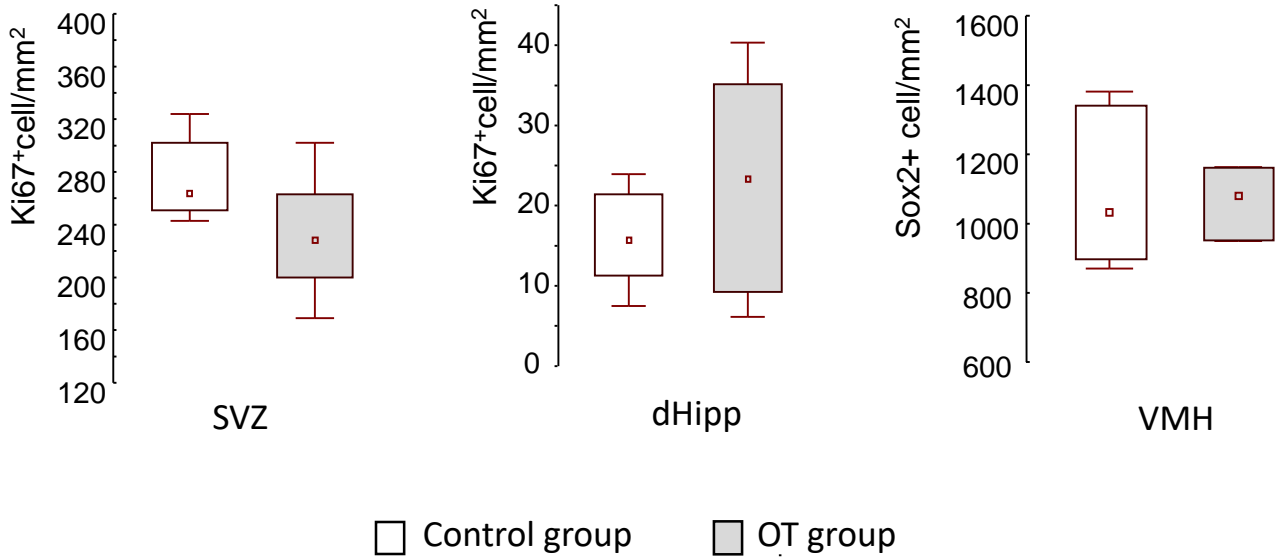
(D). Results are presented as median and interquartiles of the number of DCX+ cells per mm<sup>2</sup> in the dHipp. No significant difference was observed between OT and control groups.

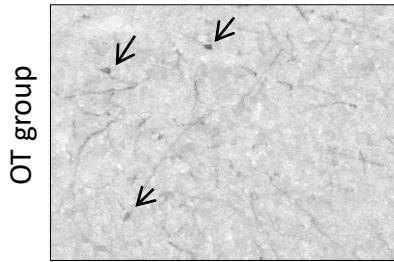
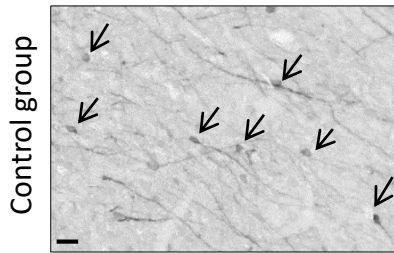
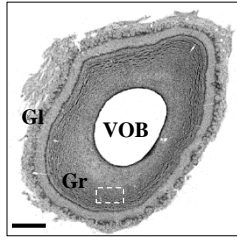
**Figure 4:** Effects of OT i.c.v. infusion on neurogenesis in the VMH.

(A). Photomicrographs showing double immunostaining with DCX (red) and Hoechst (blue) in the VMH in OT and control groups. Scale bars: 50µm.

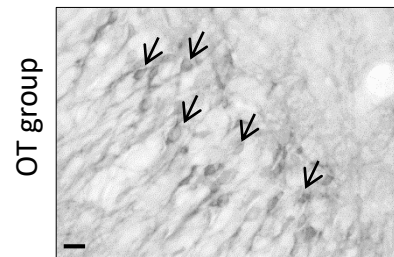
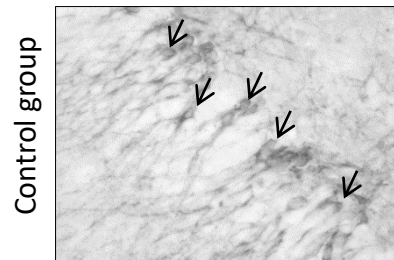
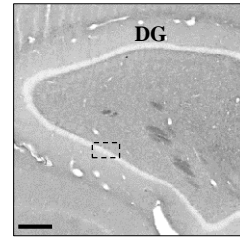
(B). Results are presented as median and interquartiles of the number of DCX+ cells per mm<sup>2</sup> in the VMH. OT infusion induces a significant increase in the number of DCX+ cells per mm<sup>2</sup>.

**A****B**

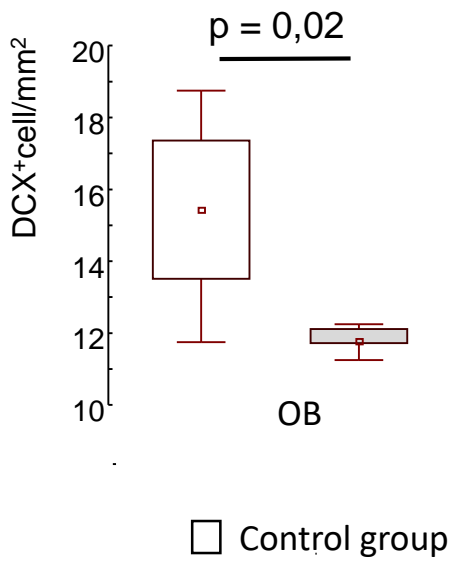
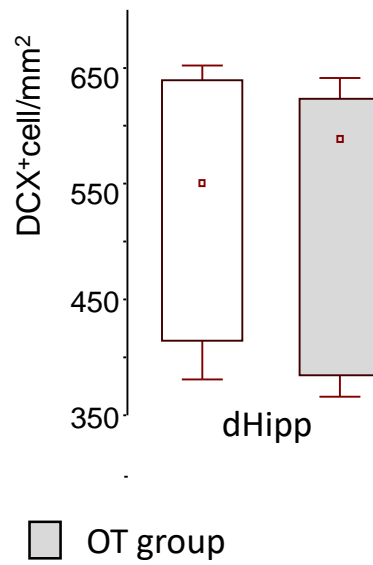
**A****B**

**A**

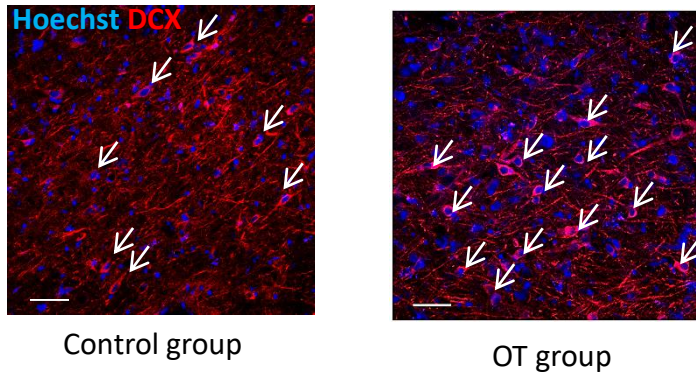
OB

**C**

dHipp

**B****D**

**A**



**B**

