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Progesterone treatment normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus after traumatic brain injury

Cindy K. Barha¹, Tauheed Ishrat⁴, Jonathan R. Epp², Liisa A. M. Galea^{1,2,3}, and Donald G. Stein⁴

¹Department of Psychology

²Graduate Program in Neuroscience

³Brain Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

⁴Department of Emergency Medicine, Brain Research Laboratory, Emory University School of Medicine, Atlanta, Georgia, USA

Abstract

Traumatic brain injury (TBI) increases cell death in the hippocampus and impairs hippocampus-dependent cognition. The hippocampus is also the site of ongoing neurogenesis throughout the lifespan. Progesterone treatment improves behavioral recovery and reduces inflammation, apoptosis, lesion volume, and edema, when given after TBI. The aim of the present study was to determine whether progesterone altered cell proliferation and short-term survival in the dentate gyrus after TBI. Male Sprague-Dawley rats with bilateral contusions of the frontal cortex or sham operations received progesterone or vehicle at 1 and 6 hours post-surgery and daily through post-surgery Day 7, and a single injection of bromodeoxyuridine (BrdU) 48 hours after injury. Brains were then processed for Ki67 (endogenous marker of cell proliferation), BrdU (short-term cell survival), doublecortin (endogenous marker of immature neurons), and Fluoro-Jade B (marker of degenerating neurons). TBI increased cell proliferation compared to shams and progesterone normalized cell proliferation in injured rats. Progesterone alone increased cell proliferation in intact rats. Interestingly, injury and/or progesterone treatment did not influence short-term cell survival of BrdU-ir cells. All treatments increased the percentage of BrdU-ir cells that were co-labeled with doublecortin (an immature neuronal marker in this case labelling new neurons that survived 5 days), indicating that cell fate is influenced independently by TBI and progesterone treatment. The number of immature neurons that survived 5 days was increased following TBI, but progesterone treatment reduced this effect. Furthermore, injury increased cell death and progesterone treatment reduced cell death to levels seen in intact rats. Together these findings suggest that progesterone treatment after TBI normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus.

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*Correspondence should be addressed to: Liisa A. M. Galea, Ph.D., Professor, University of British Columbia, 2136 West Mall, Vancouver, B. C. V6T 1Z4; Phone: (604) 822-6536, Fax: (604) 822-3697, lgalea@psych.ubc.ca.

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Keywords

Traumatic brain injury; progesterone; hippocampal neurogenesis

1. INTRODUCTION

Progesterone, a naturally occurring steroid hormone, is present in both male and female brains. Its actions in the central nervous system are not limited to the control of neuroendocrine regulation and reproduction (Baulieu and Schumacher, 2000, Birzniece, et al., 2006); it influences several neural survival functions including neuronal and glial differentiation, hippocampal neurogenesis, and synaptic stability (Ghoumari, et al., 2005, Smith, et al., 1987, Tsutsui, et al., 2004, Zhang, et al., 2010). There are now more than 160 publications showing that progesterone is an effective neuroprotective agent against a number of different insults including blunt and penetrating traumatic brain injury (TBI) (De Nicola, et al., 2009, Garcia-Estrada, et al., 1999, Labombarda, et al., 2010, Schumacher, et al., 2007, Stein, 2007, Stein and Sayeed, 2010, Stein and Wright, 2010), diffuse TBI (O'Connor, et al., 2007), stroke (Ishrat, et al., 2010, Sayeed and Stein, 2009), anoxic brain injury, glutamate toxicity (Ogata, et al., 1993), and spinal cord injury (Labombarda, et al., 2010) in both males and females. Progesterone exerts its beneficial effects in the central nervous system via multiple pathways that are not always dependent on the activation of the classical intranuclear progesterone receptor (Bottino, et al., 2010, Falkenstein, et al., 2000, Losel, et al., 2003). These effects include, among others, protecting the blood-brain barrier, reducing cerebral edema, decreasing the inflammatory cascade, and decreasing cellular necrosis and apoptosis (Djebaili, et al., 2005, Gibson, et al., 2005, He, et al., 2004, Ishrat, et al., 2010, Majewska, 1992, Mani, 2006, Roof, et al., 1997, Smith and Woolley, 2004).

Treatment with progesterone following TBI also improves functional outcome. For example, cognitive deficits, a hallmark of TBI, are attenuated following progesterone treatment (Roof, et al., 1994). Systemic treatment with progesterone facilitates recovery of spatial learning and memory, which involves the hippocampus, in brain-injured male rats compared to placebo-treated male rats (Djebaili, et al., 2004, Roof, et al., 1994, Shear, et al., 2002). The neuroprotective properties of progesterone in response to TBI are also seen in humans. Two recently completed phase IIa single-center clinical trials found a beneficial effect of progesterone as a treatment in moderate to severe TBI in male and female adults, with fewer mortalities and better functional outcome seen in progesterone-treated patients (Wright, et al., 2007, Xiao, et al., 2008). These encouraging findings have led to two phase III double-blind randomized clinical trials now enrolling patients (ProTECT III: NCT00822900; SyNAPSe: NCT01143064).

Hippocampal neurons may be the most vulnerable to TBI-induced degeneration and are among the first population of neurons in the brain to die following experimental TBI (Ariza, et al., 2006, Kotapka, et al., 1991). The dramatic upregulation in apoptotic and necrotic cell death following TBI can contribute to cognitive deficits, and drugs that prevent cell death also reduce cognitive impairment (Sinson, et al., 1997).

Progenitor cells in the dentate gyrus of the hippocampus retain the ability to proliferate into neurons during adulthood in most mammalian species studied including humans (Eriksson, et al., 1998, Gould, et al., 1999, Gould, et al., 1997, Gould, et al., 2001, Huang and Sato, 1998, Lavenex, et al., 2000). Hippocampal neurogenesis consists of at least four processes: cell proliferation, cell migration, cell differentiation, and cell survival. Cell proliferation in the dentate gyrus is the production of new cells from the division of progenitor cells within the subgranular zone. Daughter cells migrate from the subgranular zone to the inner layers

of the granule cell layer while undergoing a process of fate determination, differentiating into either neurons or glial cells (Brown, et al., 2003, Cameron, et al., 1993). These new cells continue to mature over a period of weeks to months resulting in new functionally mature neurons (Esposito, et al., 2005, van Praag, et al., 2002). Cell survival is the number of new cells that survive to maturity with the majority of these new cells being neurons (Cameron, et al., 1993).

In addition to its effects on cell death and hippocampus-dependent learning and memory, TBI also affects different aspects of adult hippocampal neurogenesis (Chirumamilla, et al., 2002, Gao, et al., 2009, Lu, et al., 2005, Richardson, et al., 2007). Different experimental models of TBI, including lateral fluid percussion and controlled cortical impact (CCI) injury, induce a 3- to 4-fold number of BrdU-ir cells in the dentate gyrus as early as 2 days postinjury that peaks within the first 7 days following injury (Dash, et al., 2001, Emery, et al., 2005, Kernie, et al., 2001, Lu, et al., 2005, Lu, et al., 2003, Rice, et al., 2003, Sun, et al., 2005, Yoshimura, et al., 2003) and returns to baseline levels by 35 days post-injury (Kleindienst, et al., 2005). However it is important to note that in the majority of these studies cell proliferation was not independently assessed as multiple BrdU injections were given and thus the number of BrdU-ir cells were an index of both proliferating cells and the short-term survival of newly-produced cells. The capacity of TBI to increase cell proliferation and subsequent neurogenesis in the dentate gyrus may not necessarily be beneficial and could actually contribute to injury-induced cognitive deficits. Some authors have suggested that too much or too little neurogenesis may interfere with proper functioning of the hippocampus, as predicted by computer modelling (Butz, et al., 2008). Indeed, prolonged and continuous seizures lead to an increase in neurogenesis which can then directly contribute to cognitive decline seen after the seizures (Jessberger, et al., 2007). Here we offer the hypothesis that the long-term beneficial effects of progesterone on cognitive outcome after TBI are associated with progesterone's influence on modulating potentially pathological neurogenesis.

The aim of the present study was to determine the effects of progesterone treatment on cell death, cell proliferation and immature neuron survival in the hippocampus after TBI. We tested the idea that TBI would increase cell proliferation, the survival of immature neurons and cell death and that progesterone treatment would reduce this TBI-induced augmentation.

2. MATERIALS AND METHODS

2.1 Animals

A total of twenty-four adult male Sprague-Dawley rats (8 to 12 weeks; Charles River Laboratories, Wilmington, MA) weighing 275–300 g were used according to procedures approved by the Institutional Animal Care and Use Committee, Emory University, Atlanta, GA, USA (protocol 164–2008). The rats were handled for at least 5 days before surgery, and were individually housed. Food and water were provided *ad libitum* throughout the experiment and the animals lived in a reversed 12-h light/12-h dark cycle controlled environment. Animals were separated into four groups (n = 6): group I: sham-operated vehicle-treated control (S); II: sham-operated and PROG (16 mg/kg) -treated (SP); group III: controlled cortical impact injury (CCI) + vehicle-treated (L); group IV: CCI + PROG (16 mg/kg) -treated (LP). Sham-operated animals underwent surgery similar to that for animals receiving CCI, but they did not receive craniectomies or cerebral contusions (see below for surgical details). Vehicle-treated animals were just given 22.5% 2-hydroxypropyl- β -cyclodextrin (see below for injection details).

2.2 Procedures

2.2.1 Induction of controlled cortical impact model for TBI—Bilateral CCI to the medial frontal cortex (MFC) was induced by a cortical contusion device as previously described (Cutler, et al., 2006). Rats were anesthetized with isoflurane (5%) (Novaplus™), N₂O (700 cm³/min) and O₂ (300 cm³/min) for 4 min, and mounted in a stereotaxic frame. Under aseptic conditions, a sagittal incision was made in the scalp and the fascia was retracted to expose the cranium. Then, a 6-mm diameter trepan drill was used to open the skull immediately anterior to bregma. Bilateral CCI injuries of the MFC were made with a 5-mm-diameter stainless impactor attached to a computer-controlled piston propelled by compressed air (velocity = 2.25 m/s; depth = 2 mm; duration = 500 ms). After CCI cortical surface haemorrhaging was controlled and the fascia and scalp were sutured. Sham-operated rats were anaesthetized, mounted in the stereotaxic apparatus, and their scalps cut and sutured, but they were not given craniectomy, contusion or neurosteroid treatment. Using a SurgiVet™ (model V3304) pulse oximeter, blood SpO₂ was monitored and maintained at levels ≥ 90%. Body temperature was maintained at 37°C with a homeothermic heating blanket system (Harvard Apparatus, Holliston, MA).

2.2.2 Progesterone (PROG) administration—PROG (P-0130; Sigma-Aldrich Co., St. Louis, MO) was dissolved in 22.5% 2-hydroxypropyl-β-cyclodextrin. The first injection was administered IP (16 mg/kg) at 1 h post-injury to ensure relatively rapid absorption following injury, and the subsequent doses were administered SC (16 mg/kg) at 6 h post-injury for more gradual absorption and then every 24 h with tapering as shown in Table 1. The tapered dose (16 mg/kg) of PROG was determined from previous research results showing that the selected amount provided the maximal protective effects in TBI model (Cutler, et al., 2006). Vehicle injections were given at the same times.

2.2.3 5-Bromo-2-deoxyuridine (BrdU) administration—Bromodeoxyuridine (BrdU) is an exogenous thymidine analogue that is injected and becomes incorporated into cells that are in S-phase (synthesizing DNA) within 2 hours of injection (Packard, et al., 1973). BrdU can be used as a marker of cell proliferation or cell survival depending on the time that has elapsed between injection and perfusion (Barha, et al., 2009). If animals are perfused within 24hrs of injection then BrdU labelling can be used to assess cell proliferation, because it requires approximately 24 h for dividing precursor cells to produce daughter cells in the dentate gyrus (Cameron and McKay, 2001). If animals are perfused days to months after injection then BrdU assesses cell survival. Forty-eight hours after CCI, all the rats received a single IP injection of BrdU (200 mg/kg; Sigma) dissolved in 0.9% saline. On the same day, the dose of PROG was given 2 h prior to the single injection of BrdU. A timeline of the procedure is shown in Figure 1. In the present study, because BrdU was given 6 days prior to perfusion, BrdU-ir cells assessed short-term cell survival.

2.2.4 Tissue processing—Six days following BrdU injection (8 days after brain injury) the rats were deeply anaesthetized with an overdose of Nembutal and then perfused with 0.9% saline followed by 4% formaldehyde. Brains were rapidly removed and post fixed in 4% formaldehyde at 4°C for 2 days. For cryoprotection, the intact brain was transferred to 30% sucrose in phosphate buffered saline (PBS) until sectioning.

2.2.5 Immunohistochemistry—Brains were sliced into 40µm coronal sections throughout the entire rostral caudal extent of the hippocampus using a vibratome (Leica, VT1000s). Every 10th slice was processed for either Ki67, an endogenous protein expressed in all phases of the cell cycle except G₀ and early G₁ and a marker of cell proliferation, or for BrdU, an exogenous marker of DNA synthesis and in the current study a marker of 6-day-old cells (short-term cell survival). In addition, one series of slices were double-stained

for BrdU and doublecortin, a microtubule-associated protein expressed in immature neurons during the first few days to weeks following proliferation (Brown, et al., 2003), to allow phenotyping of the stained cells. Another series of slices was stained with Fluoro-Jade B, a polyanionic fluorescein derivative that specifically binds to degenerating (apoptotic and necrotic) neurons (Schmued and Hopkins, 2000). All immunohistochemistry procedures were performed as previously described (Barha, et al., 2010, Barha and Galea, 2009, Barha, et al., 2009, Epp, et al., 2009, Epp, et al., 2010, Sliwowska, et al., 2010).

Briefly, for Ki67 staining (see Figure 2A), sections were pretreated with 0.6% H₂O₂ for 30 min, rinsed three times in 0.1M PBS and then transferred to a primary antibody solution containing a 1: 1000 rabbit anti-Ki67 monoclonal antibody (Novocastra; Newcastle upon Tyne, United Kingdom), 1 % NGS and 0.5% Triton-X in 0.1M PBS for 16 hr. Tissue was rinsed three times again and then incubated in a secondary antibody solution containing 1:1000 goat anti-rabbit (Vector; Burlington, ON, Canada) in 0.1M PBS for 1 h, followed by another rinse (3X) and incubation in an ABC solution (Vector) for 40 mins. The sections were rinsed again, developed with diaminobenzidine (DAB; Vector) for 5 min and then mounted on gel-coated glass slides. All sections were lightly counterstained with cresyl violet, dehydrated and coverslipped with Permount (Fisher Scientific Canada, Ottawa, ON).

BrdU staining (see Figure 2B) was similar to Ki67 staining but using tris-buffered saline (TBS) for rinsing instead of PBS and with the following modifications: after initial incubation in H₂O₂ for 30 min and the subsequent rinses, tissue was incubated in 2N hydrochloric acid at 37 °C, followed by one rinse in 0.1M borate buffer for 10 min and three rinses in PBS. Tissue was then incubated in a solution containing 3% normal horse serum (NHS) and 0.1% Triton-X in PBS for 30 min, before it was transferred to a primary antibody solution containing 1:200 mouse anti-BrdU (Roche; Mississauga, ON, Canada), 3% NHS and 0.1% Triton-X in PBS for 48 hours. The secondary antibody solution contained 1:100 anti mouse IgG and tissue was incubated in this solution for 4 hours. Incubation time for the ABC solution was 1.5 hrs.

To assess the phenotype of the BrdU-ir cells, double labelling was performed using BrdU and doublecortin (see Figure 2C), a marker for immature neurons in the brain. Tissue was incubated for 48 h in 0.1M TBS containing 3% normal donkey serum (NDS), 0.4% Triton-X, 1:1000 goat anti-doublecortin (Santa Cruz Biotechnology, Santa Cruz, CA). The tissue was rinsed three times and then transferred to a 1:500 dilution of donkey anti-goat Alexa 488 in 0.1M TBS for 18 h. Next, the doublecortin signal was protected by fixing the tissue in 4% formaldehyde for 10 min and then rinsed 3 times in TBS. The tissue was then incubated in a BrdU primary antibody solution containing 1:500 rat anti-BrdU (AbD Serotec, Raleigh, NC), 4% NDS and 0.4% Triton-X for 24 h. Sections were then rinsed 3X in 0.1M TBS before being incubated overnight in 0.1M TBS containing 1:500 donkey anti-rat Alexa 594 (Invitrogen, Carlsbad, CA). Sections were then rinsed and mounted on glass slides and coverslipped with PVA-DABCO.

To assess degenerating neurons tissue was stained with Fluoro-Jade B (Schmued and Hopkins, 2000). Specifically, tissue was rinsed three times in PBS, then mounted on slides and left to air-dry for 48 h at room temperature. The slides were soaked in 0.06% potassium permanganate for 20 min, followed by soaking for 2 min in deionized water. The slides were then soaked in 0.0004% Fluoro-Jade B solution (Chemicon, Temecula, CA, USA) in 0.1% glacial acetic acid for 30 min, rinsed three times in de-ionized water, rapidly air dried, cleared in xylene, and cover-slipped with Permount.

2.2.6 Cell counting—Counting was done by an experimenter blind to the animal's group designation. BrdU- and Ki67-ir cells were counted in every 10th section throughout the

entire granule cell layer (GCL), including the subgranular zone (SGZ) using a Nikon microscope at 1000x magnification. Cells in the hilus were counted separately to account for potential changes in the blood-brain barrier permeability. Cells were considered to be BrdU-ir if they were intensely stained and exhibited medium round or oval cell body morphology (Cameron, et al., 1993, Ormerod and Galea, 2001, Ormerod, et al., 2004). Overlapping cells were counted only if a clear outline of each cell was discernable. The total number of BrdU- and Ki67-ir cells was estimated by multiplying the total number counted by 10 as previously described (Eadie, et al., 2005, Kronenberg, et al., 2003). The percentage of BrdU/doublecortin double-labelled cells was obtained by selecting 25 BrdU-ir cells arbitrarily per brain from at least five sections and determining the percentage of these cells that also expressed doublecortin on an Olympus epifluorescent microscope at 600x magnification. This method to determine the proportion of new surviving cells that are immature neurons is well-established (Dalla, et al., 2009, Epp, et al., 2011, Kempermann, et al., 2003, Suzuki, et al., 2007). In order to estimate the total number of immature neurons surviving for 6 days in the granule cell layer of the hippocampus, the total number of BrdU-ir cells was multiplied by the percentage of BrdU/doublecortin cells as has been done previously (Brandt, et al., 2010, Ramirez-Rodriguez, et al., 2009).

2.3 Data analyses

Total number of Ki67-ir cells (cell proliferation), and total number of BrdU-ir cells (cell survival) were analyzed using repeated-measures ANOVAs with group (sham, TBI, TBI + PROG, sham + PROG) as the between-subjects factor and region (GCL + SGZ, hilus) as the within-subjects factor. BrdU/doublecortin co-labelling was analyzed with a one-way ANOVA with group as the between-subjects factor. The total number of immature neurons surviving for 6 days in the granule cell layer of the hippocampus was analyzed using a one-way ANOVA with group as the between-subjects factor. The total number of Fluoro-Jade B labelled cells (cell death) in the granule cell layer and in the CA1 region of the hippocampus were analyzed separately using a one-way ANOVA with group as the between-subjects factor. Post-hoc tests utilized the Newman-Keuls procedure unless otherwise indicated. All statistical procedures were set at $\alpha = 0.05$.

3. RESULTS

3.1 Brain injury increases cell proliferation compared to sham operations. Progesterone decreases cell proliferation in TBI rats but increases cell proliferation in intact rats

The total number of Ki67-ir cells in the granule cell layer and the hilus for all groups are shown in Figure 3A. A repeated-measures ANOVA conducted on the total number of Ki67-ir cells in the granule cell layer and the hilus showed a significant interaction between group and region [$F(3, 16) = 8.05, p < 0.002$]. Post-hoc analysis revealed that the sham group had significantly fewer total Ki67-ir cells in the granule cell layer compared to the sham + progesterone group ($p < 0.004$) and the TBI group ($p < 0.03$), but had significantly more Ki67-ir cells compared to the TBI + progesterone group ($p < 0.002$). Furthermore, the TBI + progesterone group had significantly fewer Ki67-ir cells compared to the TBI group ($p < 0.0002$) and the sham + progesterone group ($p < 0.0002$). The sham + progesterone group and the TBI group did not significantly differ from each other ($p = 0.15$). Groups did not differ in the total number of Ki67-ir cells in the hilus (all p 's > 0.55). Significant main effects of group [$F(3, 16) = 13.13, p < 0.0002$] and region [$F(1, 16) = 249.20, p < 0.00001$] were also found.

3.2 All treatments increase the percentage of immature neurons in the granule cell layer

An ANOVA conducted on the percentage of BrdU-ir cells co-expressing doublecortin showed a significant main effect of group [$F(3,15) = 5.74, p < 0.009$; see Table 2], with the

sham group having the lowest percentage of BrdU-ir cells co-expressing doublecortin compared to all other groups (all p 's < 0.02).

3.3 Brain injury and progesterone treatment do not influence short-term cell survival of BrdU-ir cells but did alter the estimated number of immature neurons

The total number of BrdU-ir cells in the granule cell layer and the hilus for all groups is shown in Figure 3B. A repeated-measures ANOVA conducted on the total number of BrdU-ir cells in the granule cell layer and the hilus did not find a significant interaction between group and region [$F(3, 22) = 0.11$, $p = 0.95$], or significant main effect of group [$F(3, 22) = 0.75$, $p = 0.54$]. As expected, a significant main effect of region was seen [$F(1, 22) = 343.48$, $p = 0.00001$], with a higher number of BrdU-ir cells seen in the granule cell layer compared to the hilus.

We multiplied the total number of BrdU-ir in the granule cell layer by the percentage of BrdU-ir cells that were co-labelled with doublecortin to obtain a measure of immature neurons that survived 6 days (Brandt, et al., 2010, Ramirez-Rodriguez, et al., 2009). An ANOVA conducted on the total number of immature neurons was not significant [main effect of group: $F(3, 15) = 2.34$, $p = 0.11$; see Figure 3C]. However, *a priori* we expected that TBI and progesterone treatment would influence the total number of immature neurons surviving. *A priori* tests indicate that the TBI group had more doublecortin cells surviving than the sham group ($p < 0.018$). The TBI + progesterone group and the sham + progesterone group did not differ from the sham group ($p < 0.20$), indicating that progesterone treatment reduced the genesis of new neurons in the damaged brain.

3.4 Brain injury increases cell death in the granule cell layer of the dentate gyrus and progesterone treatment normalized cell death back to intact levels

The total number of Fluoro-Jade-ir cells in the granule cell layer and the CA1 region of the hippocampus for all groups are shown in Figure 4. An ANOVA conducted on the total number of Fluoro-Jade-ir cells in the granule cell layer showed a significant main effect of group [$F(3, 22) = 8.12$, $p < 0.001$], with the TBI group containing more Fluoro-Jade-ir cells than the sham group ($p < 0.003$), the sham + progesterone group ($p < 0.002$), and the TBI + progesterone group ($p < 0.04$). An ANOVA conducted on the total number of Fluoro-Jade-ir cells in the CA1 region showed a tendency for a main effect of group [$F(3, 23) = 2.75$, $p < 0.07$]. *A priori* we expected TBI to increase cell death and *A priori* tests indicate that the TBI group has more Fluoro-Jade-ir cells than the sham group ($p < 0.03$) and the sham + progesterone group ($p < 0.02$).

4. DISCUSSION

The results from the present study demonstrate that TBI increased cell proliferation and cell death in the dentate gyrus of the adult male rat, while progesterone treatment normalized the injury-induced levels of both cell proliferation and cell death to sham levels. Progesterone's ability to influence cell proliferation was dependent on whether it was administered to a healthy or injured brain. In the present study neither injury nor progesterone treatment influenced short-term cell survival of 6-day-old BrdU-ir cells. However, all treatments increased the percentage of new cells with an immature neuronal phenotype. Furthermore, only injury increased the number of immature neurons relative to sham-treated rats, and progesterone after injury reduced the number of immature neurons to sham control levels. We also found that TBI increased neuronal death in the dentate gyrus and CA1 region of the hippocampus and that progesterone restored cell death to control levels. To our knowledge this is the first demonstration of how progesterone treatment following TBI can influence adult neurogenesis in the dentate gyrus of male rats.

Progesterone normalizes the injury-induced increase in cell proliferation and progesterone alone increases cell proliferation

Bilateral controlled cortical impact to the medial frontal cortex increased the number of cells proliferating in the dentate gyrus of adult male rats 8 days after injury compared to sham-operated controls. Exogenous progesterone treatment augmented the number of new cells proliferating in sham-operated controls but decreased the number of new cells proliferating in brain-injured rats. This observation indicates a differential neurogenic response to progesterone in the healthy versus the injured brain. Although perhaps counterintuitive, we think it is possible that proliferating cells may actually interfere with hippocampal function until they mature and make appropriate connections. In fact many studies have found that high rates of cell proliferation are not necessarily beneficial for hippocampus-dependent learning (Epp and Galea, 2009) and can directly contribute to impairments in learning (Jessberger, et al., 2007). This may be because new cells that are not yet connected to the existing circuitry could “add noise” and interfere with the proper functioning of the dentate gyrus. Furthermore, the findings in the present study are consistent with progesterone’s protective effects via neurogenesis in a focal ischemia model of injury. Zhang et al. (2010) found that progesterone treatment reduced cell proliferation induced by ischemia but increased 28-day survival of neurons. Recently, Chrousos (2010) pointed out that the idea that steroid hormones have to have uniform actions in various tissues is overly simplistic. He suggests that the response to steroid exposure depends on the environmental context of the cells and the state of the individual cells (i.e. healthy or injured), among many other factors that can determine cellular response to steroid hormones.

Progesterone reduces the injury-induced increase in cell death in the dentate gyrus

We found that bilateral injury to the medial frontal cortex increased the number of degenerating neurons in the granule cell layer of the dentate gyrus and to a lesser extent, in the CA1 region of the hippocampus. Importantly, progesterone treatment did not influence cell death in sham-operated rats, but after cortical contusion injury, it decreased the number of dying cells in the dentate gyrus, indicating that progesterone’s neuroprotective effects are more obvious in the injured brain. It is important to note that Fluoro-Jade B stains degenerating apoptotic and necrotic mature neurons. Therefore the cells that are stained with Fluoro-Jade B in this experiment are of a different, much older population than Ki67-ir cells and BrdU-ir cells (which are very immature neurons) and thus in our study, most of the Fluoro-Jade B-ir cells existed well before the bilateral controlled cortical impact.

Cell death is a prominent feature seen following TBI in the cortex, cerebellum, thalamus, and hippocampus. The hippocampus is particularly vulnerable to TBI. Bilateral loss of hippocampal neurons is seen in 85% of fatal head injuries in humans (Kotapka, et al., 1992). Within the hippocampus, the CA1, CA3, and dentate gyrus subregions show differing degrees of neuronal death after controlled cortical impact injury. A higher number of degenerating neurons are seen in the dentate gyrus, and fewer in the CA1 region. This is consistent with our other findings (Anderson, et al., 2005, Ariza, et al., 2006, Gao, et al., 2008, Pulella, et al., 2006, Saatman, et al., 2006). The results of our experiment support the regional distinction in TBI-induced pathology as cellular death was significantly increased by TBI in the dentate gyrus, but less so in the CA1 region. Newborn immature neurons in the dentate gyrus of the hippocampus are the most vulnerable to TBI (Gao, et al., 2008), as seen in the present study, where the cortical contusions increased cell proliferation but did not affect 6-day-old BrdU cells in the dentate gyrus. The effects of a cortical contusion on hippocampal neurogenesis could be due to the inhibitory effects of diaschisis, and may involve many different secondary pathways elicited by the traumatic injury, like increases in inflammation (Rola, et al., 2006), which lead to disruptions in the neurogenic microenvironment of the dentate gyrus. The cortical impact, penetrating about 2mm into the

brain also produces both proximal and distal effects on the brain vasculature leading to ischemic injury in underlying tissue such as the dorsal hippocampus. This can produce a cascade of inflammation, excitotoxicity, secondary neurodegeneration and gliosis—all of which can damage or kills vulnerable hippocampal neurons.

Traumatic brain injury and progesterone did not influence short-term survival of new cells

TBI and/or progesterone treatment did not increase short-term (6-day) survival of BrdU-ir cells. However, TBI and/or progesterone treatment did increase the percentage of new cells that expressed doublecortin compared to sham control rats. This finding is consistent with previous work showing that TBI increases long-term (> 21 days) survival of new neurons in the dentate gyrus (Dash, et al., 2001, Sun, et al., 2010, Sun, et al., 2005). In the current study we found that TBI increased the number of immature neurons surviving in the granule cell layer compared to shams. We also observed that the number of immature neurons in rats given progesterone treatment after TBI was not different from sham controls, indicating that progesterone after TBI normalized the number of immature neurons. Thus far we have examined only the survival of 6-day-old cells. It is possible that the effects of TBI and/or progesterone on survival of new neurons can be seen at longer time points, as reported in a study conducted by Zhang et al. (2010) in which 28-day survival of neurons was assessed.

Seven days of progesterone treatment increases cell proliferation and neuronal phenotype in male rats

We found that 7 days of progesterone treatment increased both cell proliferation and the percentage of new cells expressing a neuronal phenotype, but not short-term survival of immature neurons in male rats. In agreement with the current study, *in vitro* work found that progesterone enhanced cell proliferation (Liu, et al., 2009, Wang, et al., 2005).

In contrast, a recent study by Zhang et al. (2010) did not see an increase in a mixture of cell proliferation and 2 day cell survival following progesterone treatment in healthy adult male mice, but we found that 8 progesterone injections (tapered dose: 16 mg/kg to 4 mg/kg) over 7 days led to an increase in cell proliferation in the dentate gyrus. This regimen of progesterone has previously been shown to provide maximal protective effects in a TBI model (see Table 1 and Figure 1; Cutler, et al., 2006). Thus the Zhang et al. study differs from ours in the dose of progesterone given (4 mg/kg vs tapered schedule), number of progesterone injections (3 vs 8), the method used to detect cell proliferation (BrdU vs Ki67), and the species tested (mouse vs rat). Importantly, Zhang et al. gave multiple injections of BrdU over 2 days, which would have resulted in labelling a heterogeneous population of cells of both proliferating and short-term survival (see Taupin, 2007), and would not have been a pure measure of cell proliferation unlike in our study.

In the present study we saw a TBI effect on cell proliferation, using Ki67, 8 days after contusion, but we did not observe a TBI effect on BrdU-ir cells surviving 8 days after TBI that were labelled 2 days after the injury. It is important to note that in the present study, Ki67-ir and BrdU-ir cells were labeling very different populations of cells. Ki67 labelled a population proliferating at the time of death which was 8 days after TBI and BrdU was labeling cells that proliferated 2 days after TBI and survived a further 6 days. A single pulse of BrdU is incorporated into approximately 50% of the Ki67-ir cell population in the dentate gyrus (Kee, et al., 2002, Leuner, et al., 2009). This difference between the total number of cells labelled by BrdU and Ki67 is due to the fact that Ki67 is expressed throughout the cell cycle, except G0, whereas BrdU is only incorporated during the S-phase.

Progesterone receptors and potential role in hippocampal neurogenesis

The reduction in the injury-induced increase in cell proliferation and cell death by progesterone treatment may occur through many possible mechanisms. Progesterone exerts its influence on the central nervous system through classical progesterone receptor (PR) mediated pathways and alternative non-genomic mechanisms. Two major classical PR exist: PRB and PRA (Boonyaratanakornkit, et al., 2008). Several splice variants of each of the classical PRs have also been identified in several species (Zhu, et al., 2003, Zhu, et al., 2003). Additionally two main putative membrane-bound PRs have been identified in recent years, the seven transmembrane domain G protein-coupled receptor 7Tmpr (Zhu, et al., 2003, Zhu, et al., 2003) and the single transmembrane domain 25-Dx PR membrane components, the PGRMCs 1 and 2 (Guenoun, et al., 2008, Meffre, et al., 2007, Meffre, et al., 2007). The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury (Meffre, et al., 2005).

PRs are found throughout the brain (for review see Brinton, et al., 2008) and PRB and PRA are expressed throughout the hippocampus of both pre- and postsynaptic neurons (Foy, et al., 2010) throughout the hippocampus. Although PRs have been located in the subgranular zone of the dentate gyrus (Waters, et al., 2008), colocalization of PRs and progenitor cells that also reside in the subgranular zone has yet to be determined. However, progesterone-induced increase in cell proliferation is dependent on the ERK/MAPK signalling pathway and involves activation of the membrane PR, PGRMC1, as depleting PGRMC1 levels via siRNA reduces the progesterone-induced increase in cell proliferation (Liu, et al., 2009).

Progesterone's effects on hippocampal morphology and function

The effects of progesterone on learning and memory are complex and depend on timing of administration. Progesterone treatment prior to training impairs spatial working memory and footshock avoidance (Bimonte-Nelson, et al., 2004, Farr, et al., 1995, Orr, et al., 2009) while post-training treatment with progesterone can improve spatial reference memory, object recognition and Y-maze inhibitory avoidance (Harburger, et al., 2008, Johansson, et al., 2002). Furthermore progesterone rapidly decreases the estradiol-induced increase in neuronal spine and synapse density and synaptic proteins such as syntaxin, synaptophysin, and spinophilin in the CA1 region of the hippocampus (Choi, et al., 2003, Gould, et al., 1990, Woolley and McEwen, 1993). Microtubule-associated protein 2 (MAP2) is involved in the assembly and stabilization of microtubules and is involved in the growth of the dendritic spines of neurons (Leclerc, et al., 1996). Interestingly both estradiol and progesterone increase protein levels of MAP2 in the hippocampus (Reyna-Neyra, et al., 2002). Thus, taken together with the results of this experiment showing that progesterone affects some aspects of neurogenesis in the hippocampus, we believe that progesterone likely works through a number of mechanisms to alter hippocampal morphology and function.

The role of neurosteroids on TBI

Progesterone can work directly and indirectly to exert its effects on neuroprotection. Importantly, treatment with progesterone or its metabolite, allopregnanolone, decreases infarct size, improves functional recovery, reduces inflammatory response, reduces edema, and protects blood brain barrier integrity following brain injury (Djebaili, et al., 2005, Gibson, et al., 2009, Ishrat, et al., 2010, Ishrat, et al., 2009, Sayeed, et al., 2006, Wang, et al., 2009, Wright, et al., 2001). Depending on the outcome variable of interest, allopregnanolone may be a more potent inducer of neuroprotection than progesterone (Djebaili, et al., 2005, Sayeed and Stein, 2009). Interestingly, allopregnanolone also increases progenitor cell proliferation (Brinton and Wang, 2006, Wang, et al., 2008). Importantly these two

neurosteroids do not work through the same receptor. Unlike progesterone, allopregnanolone does not bind to the intracellular membrane-bound progesterone receptor, the sigma-1 receptor, and the putative progesterone dx-25 receptor (Monnet, et al., 1995, Rupprecht, et al., 1993). Allopregnanolone does act on the GABA-A receptor and it may be through this action that it is exerting its effects (Ghoumari, et al., 2003).

Translocator protein (TSPO) is an 18 kDA protein with a high affinity for binding cholesterol and is found mainly in the outer mitochondrial membrane. Tissues rich in steroidogenesis throughout the body, including the brain, express TSPO (Papadopoulos, et al., 1997) and its expression is increased in the brain after various insults including TBI (for review see Chen and Guilarte, 2008). TSPO is involved in multiple functions, including regulating apoptosis, cell proliferation, and mitochondrial function (for review see Batarseh and Papadopoulos, 2010, Chen and Guilarte, 2008, Rupprecht, et al., 2010). Furthermore, TSPO is a key component in steroidogenesis, as it binds cholesterol via steroidogenic acute regulatory protein (StAR) (Lacapere and Papadopoulos, 2003) and then facilitates the transport of cholesterol from the outer to the inner mitochondrial membrane (Papadopoulos, et al., 1997). Within the inner mitochondrial membrane, the side chain cleavage cytochrome P-450 enzyme converts the cholesterol to pregnenolone, which is further converted to many neurosteroids including allopregnanolone (Brown and Papadopoulos, 2001). It may be through this pathway in neurosteroid synthesis that TSPO and TSPO selective ligands influence neuroprotection.

5. CONCLUSIONS

Severe TBI is often very debilitating and leads to alterations in hippocampal function and structure. Progesterone is an effective neuroprotective agent that improves functional outcome following TBI. The results of our study extend the evidence for the beneficial effects of progesterone following TBI, and show that progesterone administration following contusion reduces cell proliferation, number of immature neurons, and cell death in the dentate gyrus of male rats. These findings are particularly interesting in light of recent studies reporting that high levels of cell proliferation in the dentate gyrus correlate with impairments in hippocampus-dependent learning and memory, further indicating that the beneficial effects seen with progesterone treatment on cognitive functioning following TBI may be associated with the ability of progesterone to reinstate neurogenic homeostasis in the hippocampus.

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References

1. Anderson KJ, Miller KM, Fugaccia I, Scheff SW. Regional distribution of fluoro-jade B staining in the hippocampus following traumatic brain injury. *Exp Neurol*. 2005; 193:125–130. [PubMed: 15817271]
2. Ariza M, Serra-Grabulosa JM, Junque C, Ramirez B, Mataro M, Poca A, Bargallo N, Sahuquillo J. Hippocampal head atrophy after traumatic brain injury. *Neuropsychologia*. 2006; 44:1956–1961. [PubMed: 16352320]

3. Barha, CK.; Barker, JM.; Brummelte, S.; Epp, JR.; Galea, LA. Regulation of adult hippocampal neurogenesis in the mammalian brain. In: Pfaff, DA.; AP; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. Academic Press; San Diego: 2009. p. 2165-2197.
4. Barha CK, Brummelte S, Lieblich SE, Galea LA. Chronic restraint stress in adolescence differentially influences hypothalamic-pituitary-adrenal axis function and adult hippocampal neurogenesis in male and female rats. *Hippocampus*. 2010
5. Barha CK, Galea LA. Motherhood alters the cellular response to estrogens in the hippocampus later in life. *Neurobiol Aging*. 2009
6. Barha CK, Lieblich SE, Galea LA. Different forms of oestrogen rapidly upregulate cell proliferation in the dentate gyrus of adult female rats. *J Neuroendocrinol*. 2009; 21:155–166. [PubMed: 19076272]
7. Batarseh A, Papadopoulos V. Regulation of translocator protein 18 kDa (TSPO) expression in health and disease states. *Mol Cell Endocrinol*. 2010; 327:1–12. [PubMed: 20600583]
8. Baulieu EE, Schumacher M. Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Hum Reprod*. 2000; 15(Suppl 1):1–13. [PubMed: 10928415]
9. Bimonte-Nelson HA, Singleton RS, Williams BJ, Granholm AC. Ovarian hormones and cognition in the aged female rat: II. progesterone supplementation reverses the cognitive enhancing effects of ovariectomy. *Behav Neurosci*. 2004; 118:707–714. [PubMed: 15301598]
10. Birzniece V, Backstrom T, Johansson IM, Lindblad C, Lundgren P, Lofgren M, Olsson T, Ragagnin G, Taube M, Turkmen S, Wahlstrom G, Wang MD, Wihlback AC, Zhu D. Neuroactive steroid effects on cognitive functions with a focus on the serotonin and GABA systems. *Brain Res Rev*. 2006; 51:212–239. [PubMed: 16368148]
11. Boonyaratankornkit V, Bi Y, Rudd M, Edwards DP. The role and mechanism of progesterone receptor activation of extra-nuclear signaling pathways in regulating gene transcription and cell cycle progression. *Steroids*. 2008; 73:922–928. [PubMed: 18321550]
12. Bottino MC, Cerliani JP, Rojas P, Giulianelli S, Soldati R, Mondillo C, Gorostiaga MA, Pignataro OP, Calvo JC, Gutkind JS, Panomwat A, Molinolo AA, Luthy IA, Lanari C. Classical membrane progesterone receptors in murine mammary carcinomas: agonistic effects of progestins and RU-486 mediating rapid non-genomic effects. *Breast Cancer Res Treat*. 2010
13. Brandt MD, Maass A, Kempermann G, Storch A. Physical exercise increases Notch activity, proliferation and cell cycle exit of type-3 progenitor cells in adult hippocampal neurogenesis. *Eur J Neurosci*. 2010; 32:1256–1264. [PubMed: 20950279]
14. Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, Nilsen J. Progesterone receptors: form and function in brain. *Front Neuroendocrinol*. 2008; 29:313–339. [PubMed: 18374402]
15. Brinton RD, Wang JM. Preclinical analyses of the therapeutic potential of allopregnanolone to promote neurogenesis in vitro and in vivo in transgenic mouse model of Alzheimer's disease. *Curr Alzheimer Res*. 2006; 3:11–17. [PubMed: 16472197]
16. Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol*. 2003; 467:1–10. [PubMed: 14574675]
17. Brown RC, Papadopoulos V. Role of the peripheral-type benzodiazepine receptor in adrenal and brain steroidogenesis. *Int Rev Neurobiol*. 2001; 46:117–143. [PubMed: 11599298]
18. Butz M, Teuchert-Noodt G, Grafen K, van Ooyen A. Inverse relationship between adult hippocampal cell proliferation and synaptic rewiring in the dentate gyrus. *Hippocampus*. 2008; 18:879–898. [PubMed: 18481284]
19. Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J Comp Neurol*. 2001; 435:406–417. [PubMed: 11406822]
20. Cameron HA, Woolley CS, McEwen BS, Gould E. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience*. 1993; 56:337–344. [PubMed: 8247264]
21. Chen MK, Guilarte TR. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. *Pharmacol Ther*. 2008; 118:1–17. [PubMed: 18374421]

22. Chirumamilla S, Sun D, Bullock MR, Colello RJ. Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. *J Neurotrauma*. 2002; 19:693–703. [PubMed: 12165131]
23. Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z, McEwen BS. Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (*Macaca mulatta*). *Endocrinology*. 2003; 144:4734–4738. [PubMed: 12960039]
24. Chrousos GP. Stress and sex versus immunity and inflammation. *Sci Signal*. 2010; 3:pe36. [PubMed: 20940425]
25. Cutler SM, Vanlandingham JW, Stein DG. Tapered progesterone withdrawal promotes long-term recovery following brain trauma. *Exp Neurol*. 2006; 200:378–385. [PubMed: 16797538]
26. Dalla C, Papachristos EB, Whetstone AS, Shors TJ. Female rats learn trace memories better than male rats and consequently retain a greater proportion of new neurons in their hippocampi. *Proc Natl Acad Sci U S A*. 2009; 106:2927–2932. [PubMed: 19188598]
27. Dash PK, Mach SA, Moore AN. Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. *J Neurosci Res*. 2001; 63:313–319. [PubMed: 11170181]
28. De Nicola AF, Labombarda F, Deniselle MC, Gonzalez SL, Garay L, Meyer M, Gargiulo G, Guennoun R, Schumacher M. Progesterone neuroprotection in traumatic CNS injury and motoneuron degeneration. *Front Neuroendocrinol*. 2009; 30:173–187. [PubMed: 19318112]
29. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma*. 2005; 22:106–118. [PubMed: 15665606]
30. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. *Neuroscience*. 2004; 123:349–359. [PubMed: 14698743]
31. Eadie BD, Redila VA, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J Comp Neurol*. 2005; 486:39–47. [PubMed: 15834963]
32. Emery DL, Fulp CT, Saatman KE, Schutz C, Neugebauer E, McIntosh TK. Newly born granule cells in the dentate gyrus rapidly extend axons into the hippocampal CA3 region following experimental brain injury. *J Neurotrauma*. 2005; 22:978–988. [PubMed: 16156713]
33. Epp JR, Galea LA. Hippocampus-dependent strategy choice predicts low levels of cell proliferation in the dentate gyrus. *Neurobiol Learn Mem*. 2009; 91:437–446. [PubMed: 19185612]
34. Epp JR, Haack AK, Galea LA. Task difficulty in the Morris water task influences the survival of new neurons in the dentate gyrus. *Hippocampus*. 2009
35. Epp JR, Scott NA, Galea LA. Strain differences in neurogenesis and activation of new neurons in the dentate gyrus in response to spatial learning. *Neuroscience*. 2010
36. Epp JR, Scott NA, Galea LA. Strain differences in neurogenesis and activation of new neurons in the dentate gyrus in response to spatial learning. *Neuroscience*. 2011; 172:342–354. [PubMed: 20955769]
37. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med*. 1998; 4:1313–1317. [PubMed: 9809557]
38. Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J Neurosci*. 2005; 25:10074–10086. [PubMed: 16267214]
39. Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones—a focus on rapid, nongenomic effects. *Pharmacol Rev*. 2000; 52:513–556. [PubMed: 11121509]
40. Farr SA, Flood JF, Scherrer JF, Kaiser FE, Taylor GT, Morley JE. Effect of ovarian steroids on footshock avoidance learning and retention in female mice. *Physiol Behav*. 1995; 58:715–723. [PubMed: 8559781]
41. Foy MR, Baudry M, Akopian GK, Thompson RF. Regulation of hippocampal synaptic plasticity by estrogen and progesterone. *Vitam Horm*. 2010; 82:219–239. [PubMed: 20472141]

42. Gao X, Deng-Bryant Y, Cho W, Carrico KM, Hall ED, Chen J. Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury. *J Neurosci Res.* 2008; 86:2258–2270. [PubMed: 18381764]
43. Gao X, Enikolopov G, Chen J. Moderate traumatic brain injury promotes proliferation of quiescent neural progenitors in the adult hippocampus. *Exp Neurol.* 2009; 219:516–523. [PubMed: 19615997]
44. Garcia-Estrada J, Luquin S, Fernandez AM, Garcia-Segura LM. Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury. *Int J Dev Neurosci.* 1999; 17:145–151. [PubMed: 10221674]
45. Ghoumari AM, Baulieu EE, Schumacher M. Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. *Neuroscience.* 2005; 135:47–58. [PubMed: 16054770]
46. Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. *J Neurochem.* 2003; 86:848–859. [PubMed: 12887683]
47. Gibson CL, Constantin D, Prior MJ, Bath PM, Murphy SP. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. *Exp Neurol.* 2005; 193:522–530. [PubMed: 15869954]
48. Gibson CL, Coomber B, Rathbone J. Is progesterone a candidate neuroprotective factor for treatment following ischemic stroke? *Neuroscientist.* 2009; 15:324–332. [PubMed: 19359672]
49. Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci.* 1999; 2:260–265. [PubMed: 10195219]
50. Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci.* 1997; 17:2492–2498. [PubMed: 9065509]
51. Gould E, Vail N, Wagers M, Gross CG. Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. *Proc Natl Acad Sci U S A.* 2001; 98:10910–10917. [PubMed: 11526209]
52. Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci.* 1990; 10:1286–1291. [PubMed: 2329377]
53. Guennoun R, Meffre D, Labombarda F, Gonzalez SL, Deniselle MC, Stein DG, De Nicola AF, Schumacher M. The membrane-associated progesterone-binding protein 25-Dx: expression, cellular localization and up-regulation after brain and spinal cord injuries. *Brain Res Rev.* 2008; 57:493–505. [PubMed: 17618691]
54. Harburger LL, Pechenino AS, Saadi A, Frick KM. Post-training progesterone dose-dependently enhances object, but not spatial, memory consolidation. *Behav Brain Res.* 2008; 194:174–180. [PubMed: 18687366]
55. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol.* 2004; 189:404–412. [PubMed: 15380490]
56. Huang S, Sato S. Progenitor cells in the adult zebrafish nervous system express a Brn-1-related POU gene, tai-ji. *Mech Dev.* 1998; 71:23–35. [PubMed: 9507055]
57. Ishrat T, Sayeed I, Atif F, Hua F, Stein DG. Progesterone and allopregnanolone attenuate blood-brain barrier dysfunction following permanent focal ischemia by regulating the expression of matrix metalloproteinases. *Exp Neurol.* 2010; 226:183–190. [PubMed: 20816826]
58. Ishrat T, Sayeed I, Atif F, Stein DG. Effects of progesterone administration on infarct volume and functional deficits following permanent focal cerebral ischemia in rats. *Brain Res.* 2009; 1257:94–101. [PubMed: 19135987]
59. Jessberger S, Nakashima K, Clemenson GD Jr, Mejia E, Mathews E, Ure K, Ogawa S, Sinton CM, Gage FH, Hsieh J. Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. *J Neurosci.* 2007; 27:5967–5975. [PubMed: 17537967]
60. Johansson IM, Birzniece V, Lindblad C, Olsson T, Backstrom T. Allopregnanolone inhibits learning in the Morris water maze. *Brain Res.* 2002; 934:125–131. [PubMed: 11955475]

61. Kee N, Sivalingam S, Boonstra R, Wojtowicz JM. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. *J Neurosci Methods*. 2002; 115:97–105. [PubMed: 11897369]
62. Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development*. 2003; 130:391–399. [PubMed: 12466205]
63. Kerner SG, Erwin TM, Parada LF. Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. *J Neurosci Res*. 2001; 66:317–326. [PubMed: 11746349]
64. Kleindienst A, McGinn MJ, Harvey HB, Colello RJ, Hamm RJ, Bullock MR. Enhanced hippocampal neurogenesis by intraventricular S100B infusion is associated with improved cognitive recovery after traumatic brain injury. *J Neurotrauma*. 2005; 22:645–655. [PubMed: 15941374]
65. Kotapka MJ, Gennarelli TA, Graham DI, Adams JH, Thibault LE, Ross DT, Ford I. Selective vulnerability of hippocampal neurons in acceleration-induced experimental head injury. *J Neurotrauma*. 1991; 8:247–258. [PubMed: 1803033]
66. Kotapka MJ, Graham DI, Adams JH, Gennarelli TA. Hippocampal pathology in fatal non-missile human head injury. *Acta Neuropathol*. 1992; 83:530–534. [PubMed: 1621508]
67. Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G. Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol*. 2003; 467:455–463. [PubMed: 14624480]
68. Labombarda F, Gonzalez Deniselle MC, De Nicola AF, Gonzalez SL. Progesterone and the spinal cord: good friends in bad times. *Neuroimmunomodulation*. 2010; 17:146–149. [PubMed: 20134188]
69. Lacapere JJ, Papadopoulos V. Peripheral-type benzodiazepine receptor: structure and function of a cholesterol-binding protein in steroid and bile acid biosynthesis. *Steroids*. 2003; 68:569–585. [PubMed: 12957662]
70. Lavenex P, Steele MA, Jacobs LF. The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. *Eur J Neurosci*. 2000; 12:643–648. [PubMed: 10712644]
71. Leclerc N, Baas PW, Garner CC, Kosik KS. Juvenile and mature MAP2 isoforms induce distinct patterns of process outgrowth. *Mol Biol Cell*. 1996; 7:443–455. [PubMed: 8868472]
72. Leuner B, Glasper ER, Gould E. Thymidine analog methods for studies of adult neurogenesis are not equally sensitive. *J Comp Neurol*. 2009; 517:123–133. [PubMed: 19731267]
73. Liu L, Wang J, Zhao L, Nilsen J, McClure K, Wong K, Brinton RD. Progesterone increases rat neural progenitor cell cycle gene expression and proliferation via extracellularly regulated kinase and progesterone receptor membrane components 1 and 2. *Endocrinology*. 2009; 150:3186–3196. [PubMed: 19359388]
74. Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M. Nongenomic steroid action: controversies, questions, and answers. *Physiol Rev*. 2003; 83:965–1016. [PubMed: 12843413]
75. Lu D, Mahmood A, Qu C, Goussev A, Schallert T, Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. *J Neurotrauma*. 2005; 22:1011–1017. [PubMed: 16156716]
76. Lu D, Mahmood A, Zhang R, Copp M. Upregulation of neurogenesis and reduction in functional deficits following administration of DETA/NONOate, a nitric oxide donor, after traumatic brain injury in rats. *J Neurosurg*. 2003; 99:351–361. [PubMed: 12924710]
77. Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol*. 1992; 38:379–395. [PubMed: 1349441]
78. Mani SK. Signaling mechanisms in progesterone-neurotransmitter interactions. *Neuroscience*. 2006; 138:773–781. [PubMed: 16310962]
79. Meffre D, Delespierre B, Guezou M, Leclerc P, Vinson GP, Schumacher M, Stein DG, Guennoun R. The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury. *J Neurochem*. 2005; 93:1314–1326. [PubMed: 15934950]

80. Meffre D, Delespierre B, Gouezou M, Schumacher M, Stein DG, Guennoun R. 3beta-Hydroxysteroid dehydrogenase/5-ene-4-ene isomerase mRNA expression in rat brain: effect of pseudopregnancy and traumatic brain injury. *J Steroid Biochem Mol Biol*. 2007; 104:293–300. [PubMed: 17428656]
81. Meffre D, Pianos A, Liere P, Eychenne B, Cambourg A, Schumacher M, Stein DG, Guennoun R. Steroid profiling in brain and plasma of male and pseudopregnant female rats after traumatic brain injury: analysis by gas chromatography/mass spectrometry. *Endocrinology*. 2007; 148:2505–2517. [PubMed: 17303653]
82. Monnet FP, Mahe V, Robel P, Baulieu EE. Neurosteroids, via sigma receptors, modulate the [3H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci U S A*. 1995; 92:3774–3778. [PubMed: 7731982]
83. O'Connor CA, Cernak I, Johnson F, Vink R. Effects of progesterone on neurologic and morphologic outcome following diffuse traumatic brain injury in rats. *Exp Neurol*. 2007; 205:145–153. [PubMed: 17362936]
84. Ogata T, Nakamura Y, Tsuji K, Shibata T, Kataoka K. Steroid hormones protect spinal cord neurons from glutamate toxicity. *Neuroscience*. 1993; 55:445–449. [PubMed: 8104320]
85. Ormerod BK, Galea LA. Reproductive status influences cell proliferation and cell survival in the dentate gyrus of adult female meadow voles: a possible regulatory role for estradiol. *Neuroscience*. 2001; 102:369–379. [PubMed: 11166123]
86. Ormerod BK, Lee TT, Galea LA. Estradiol enhances neurogenesis in the dentate gyri of adult male meadow voles by increasing the survival of young granule neurons. *Neuroscience*. 2004; 128:645–654. [PubMed: 15381292]
87. Orr PT, Lewis MC, Frick KM. Dorsal hippocampal progesterone infusions enhance object recognition in young female mice. *Pharmacol Biochem Behav*. 2009; 93:177–182. [PubMed: 19477194]
88. Packard DS Jr, Menzies RA, Skalko RG. Incorporation of thymidine and its analogue, bromodeoxyuridine, into embryos and maternal tissues of the mouse. *Differentiation*. 1973; 1:397–404. [PubMed: 4802502]
89. Papadopoulos V, Amri H, Boujrad N, Cascio C, Culty M, Garnier M, Hardwick M, Li H, Vidic B, Brown AS, Reversa JL, Bernassau JM, Drieu K. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids*. 1997; 62:21–28. [PubMed: 9029710]
90. Pulella R, Raber J, Pfankuch T, Ferriero DM, Claus CP, Koh SE, Yamauchi T, Rola R, Fike JR, Noble-Haeusslein LJ. Traumatic injury to the immature brain results in progressive neuronal loss, hyperactivity and delayed cognitive impairments. *Dev Neurosci*. 2006; 28:396–409. [PubMed: 16943663]
91. Ramirez-Rodriguez G, Klempin F, Babu H, Benitez-King G, Kempermann G. Melatonin modulates cell survival of new neurons in the hippocampus of adult mice. *Neuropsychopharmacology*. 2009; 34:2180–2191. [PubMed: 19421166]
92. Reyna-Neyra A, Camacho-Arroyo I, Ferrera P, Arias C. Estradiol and progesterone modify microtubule associated protein 2 content in the rat hippocampus. *Brain Res Bull*. 2002; 58:607–612. [PubMed: 12372566]
93. Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H, Bullock MR. Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. *Exp Neurol*. 2003; 183:406–417. [PubMed: 14552881]
94. Richardson RM, Sun D, Bullock MR. Neurogenesis after traumatic brain injury. *Neurosurg Clin N Am*. 2007; 18:169–181. xi. [PubMed: 17244562]
95. Rola R, Mizumatsu S, Otsuka S, Morhardt DR, Noble-Haeusslein LJ, Fishman K, Potts MB, Fike JR. Alterations in hippocampal neurogenesis following traumatic brain injury in mice. *Exp Neurol*. 2006; 202:189–199. [PubMed: 16876159]
96. Roof RL, Duvdevani R, Braswell L, Stein DG. Progesterone facilitates cognitive recovery and reduces secondary neuronal loss caused by cortical contusion injury in male rats. *Exp Neurol*. 1994; 129:64–69. [PubMed: 7925843]
97. Roof RL, Hoffman SW, Stein DG. Progesterone protects against lipid peroxidation following traumatic brain injury in rats. *Mol Chem Neuropathol*. 1997; 31:1–11. [PubMed: 9271001]

98. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov.* 2010; 9:971–988. [PubMed: 21119734]
99. Rupprecht R, Reul JM, Trapp T, van Steensel B, Wetzel C, Damm K, Zieglgansberger W, Holsboer F. Progesterone receptor-mediated effects of neuroactive steroids. *Neuron.* 1993; 11:523–530. [PubMed: 8398145]
100. Saatman KE, Feeko KJ, Pape RL, Raghupathi R. Differential behavioral and histopathological responses to graded cortical impact injury in mice. *J Neurotrauma.* 2006; 23:1241–1253. [PubMed: 16928182]
101. Sayeed I, Guo Q, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med.* 2006; 47:381–389. [PubMed: 16546625]
102. Sayeed I, Stein DG. Progesterone as a neuroprotective factor in traumatic and ischemic brain injury. *Prog Brain Res.* 2009; 175:219–237. [PubMed: 19660659]
103. Schmued LC, Hopkins KJ. Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res.* 2000; 874:123–130. [PubMed: 10960596]
104. Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacol Ther.* 2007; 116:77–106. [PubMed: 17659348]
105. Shear DA, Galani R, Hoffman SW, Stein DG. Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury. *Exp Neurol.* 2002; 178:59–67. [PubMed: 12460608]
106. Sinson G, Perri BR, Trojanowski JQ, Flamm ES, McIntosh TK. Improvement of cognitive deficits and decreased cholinergic neuronal cell loss and apoptotic cell death following neurotrophin infusion after experimental traumatic brain injury. *J Neurosurg.* 1997; 86:511–518. [PubMed: 9046309]
107. Sliwowska JH, Barker JM, Barha CK, Lan N, Weinberg J, Galea LA. Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. *Stress.* 2010; 13:301–313. [PubMed: 20536332]
108. Smith SS, Waterhouse BD, Chapin JK, Woodward DJ. Progesterone alters GABA and glutamate responsiveness: a possible mechanism for its anxiolytic action. *Brain Res.* 1987; 400:353–359. [PubMed: 2880640]
109. Smith SS, Woolley CS. Cellular and molecular effects of steroid hormones on CNS excitability. *Cleve Clin J Med.* 2004; 71(Suppl 2):S4–10. [PubMed: 15379294]
110. Stein DG. Sex differences in brain damage and recovery of function: experimental and clinical findings. *Prog Brain Res.* 2007; 161:339–351. [PubMed: 17618989]
111. Stein DG, Sayeed I. Is progesterone worth consideration as a treatment for brain injury? *AJR Am J Roentgenol.* 2010; 194:20–22. [PubMed: 20028900]
112. Stein DG, Wright DW. Progesterone in the clinical treatment of acute traumatic brain injury. *Expert Opin Investig Drugs.* 2010; 19:847–857.
113. Sun D, Bullock MR, Altememi N, Zhou Z, Hagood S, Rolfe A, McGinn MJ, Hamm R, Colello RJ. The effect of epidermal growth factor in the injured brain after trauma in rats. *J Neurotrauma.* 2010; 27:923–938. [PubMed: 20158379]
114. Sun D, Colello RJ, Daugherty WP, Kwon TH, McGinn MJ, Harvey HB, Bullock MR. Cell proliferation and neuronal differentiation in the dentate gyrus in juvenile and adult rats following traumatic brain injury. *J Neurotrauma.* 2005; 22:95–105. [PubMed: 15665605]
115. Suzuki S, Gerhold LM, Bottner M, Rau SW, Dela Cruz C, Yang E, Zhu H, Yu J, Cashion AB, Kindy MS, Merchenthaler I, Gage FH, Wise PM. Estradiol enhances neurogenesis following ischemic stroke through estrogen receptors alpha and beta. *J Comp Neurol.* 2007; 500:1064–1075. [PubMed: 17183542]
116. Taupin P. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. *Brain Res Rev.* 2007; 53:198–214. [PubMed: 17020783]
117. Tsutsui K, Sakamoto H, Shikimi H, Ukena K. Organizing actions of neurosteroids in the Purkinje neuron. *Neurosci Res.* 2004; 49:273–279. [PubMed: 15196775]

118. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature*. 2002; 415:1030–1034. [PubMed: 11875571]
119. Wang J, Jiang C, Li X, Liu C, Cheng N, Hao Y. The protective mechanism of progesterone on blood-brain barrier in cerebral ischemia in rats. *Brain Res Bull*. 2009; 79:426–430. [PubMed: 19477244]
120. Wang JM, Johnston PB, Ball BG, Brinton RD. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. *J Neurosci*. 2005; 25:4706–4718. [PubMed: 15888646]
121. Wang JM, Liu L, Irwin RW, Chen S, Brinton RD. Regenerative potential of allopregnanolone. *Brain Res Rev*. 2008; 57:398–409. [PubMed: 17931704]
122. Waters EM, Torres-Reveron A, McEwen BS, Milner TA. Ultrastructural localization of extranuclear progesterin receptors in the rat hippocampal formation. *J Comp Neurol*. 2008; 511:34–46. [PubMed: 18720413]
123. Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol*. 1993; 336:293–306. [PubMed: 8245220]
124. Wright DW, Bauer ME, Hoffman SW, Stein DG. Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats. *J Neurotrauma*. 2001; 18:901–909. [PubMed: 11565602]
125. Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, Lowery DW, Patel MM, Denson DD, Gordon AB, Wald MM, Gupta S, Hoffman SW, Stein DG. ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. *Ann Emerg Med*. 2007; 49:391–402. 402, e391–392. [PubMed: 17011666]
126. Xiao G, Wei J, Yan W, Wang W, Lu Z. Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. *Crit Care*. 2008; 12:R61. [PubMed: 18447940]
127. Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO, Moskowitz MA. FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. *J Clin Invest*. 2003; 112:1202–1210. [PubMed: 14561705]
128. Zhang Z, Yang R, Cai W, Bai Y, Sokabe M, Chen L. Treatment with progesterone after focal cerebral ischemia suppresses proliferation of progenitor cells but enhances survival of newborn neurons in adult male mice. *Neuropharmacology*. 2010; 58:930–939. [PubMed: 20079361]
129. Zhang Z, Yang R, Zhou R, Li L, Sokabe M, Chen L. Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. *Hippocampus*. 2010; 20:402–412. [PubMed: 19475650]
130. Zhu Y, Bond J, Thomas P. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progesterin receptor. *Proc Natl Acad Sci U S A*. 2003; 100:2237–2242. [PubMed: 12601167]
131. Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. Cloning, expression, and characterization of a membrane progesterin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci U S A*. 2003; 100:2231–2236. [PubMed: 12574519]

Research Highlights

- Progesterone improves outcome following traumatic brain injury in humans and rodents
- Traumatic brain injury increased cell proliferation and cell death in hippocampus
- Progesterone normalized these levels in hippocampal neurogenesis
- Improved cognitive outcome could be related to progesterone effects on neurogenesis

Figure 1.
Depicts the procedural timeline used in this experiment.

Figure 2.

Panel A shows Ki67-ir cells in the granule cell layer. Panel B shows BrdU-ir cells in the granule cell layer. Panel C shows a BrdU (red) and doublecortin (green) double labelled cell in the granule cell layer. Photomicrographs are magnified 1000x. 1 cm scale bar = 10 μ m.

Figure 3.

A) Mean (+ SEM) total number of Ki67-ir cells in the granule cell layer (GCL) and hilus of the dentate gyrus. The sham group had significantly fewer Ki67-ir cells compared to the sham + progesterone group and the traumatic brain injury (TBI) group and had significantly more Ki67-ir cells compared to the TBI + progesterone group. The TBI + progesterone group had significantly fewer Ki67-ir cells than the TBI group and the sham + progesterone group. There were no significant differences between groups in the hilus. B) Mean (+ SEM) total number of BrdU-ir cells in the GCL and hilus of the dentate gyrus. Groups did not differ in the total number of BrdU-ir cells in the GCL or the hilus. C) Mean (+ SEM) total number of immature neurons surviving for 6 days. The TBI group had significantly more immature neurons compared to the sham group. All other groups did not differ from the sham group.

a denotes significantly different from the sham group ($p < 0.05$), **b** denotes significantly different from the TBI group and the sham + progesterone group ($p < 0.05$).

Figure 4.

Mean (+ SEM) total number of Fluoro-Jade B labelled cells in the granule cell layer (GCL) of the dentate gyrus and the CA1 region of the hippocampus. The traumatic brain injury (TBI) group had significantly more Fluoro-Jade B labelled cells in the GCL compared to the sham group, the sham + progesterone group, and the TBI + progesterone group. The TBI group tended to have more Fluoro-Jade B labelled cells in the CA1 region compared to the sham group and the sham + progesterone group. **a** denotes significantly different from the TBI group ($p < 0.05$).

Table 1

Post-injury progesterone treatment schedule:

	Days 1–5	Day 6	Day 7
Progesterone	16 mg/kg	8 mg/kg	4 mg/kg
Vehicle	22.5% HBC	22.5% HBC	22.5% HBC

Table 2

Mean (+ SEM) percentage of BrdU/Doublecortin double-labelled cells in the granule cell layer with the sham group having the lowest percentage of double-labelled cells (doublecortin and BrdU).

Group	% of BrdU/DCX
Sham	73.11% $\pm$ 4.74
Sham + Progesterone	88.00% $\pm$ 2.53*
TBI	89.00% $\pm$ 3.42*
TBI + Progesterone	89.60% $\pm$ 2.04*

* indicates significantly different from sham group.