informa
healthcare

Progesterone treatment decreases traumatic brain injury induced anxiety and is correlated with increased serum IGF-1 levels; prefrontal cortex, amygdala, hippocampus neuron density; and reduced serum corticosterone levels in immature rats

B Baykara[1], I Aksu[2], E Buyuk[3], M Kiray[2], AR Sisman[4], B Baykara[5], A Dayi[2], A Tas[2], D Ozdemir[6], MN Arda[7], N Uysal[2]

[1]*Dokuz Eylul University, Medical Faculty, High School of Physical Therapy and Rehabilitation,* [2]*Dokuz Eylul University, Medical Faculty, Physiology,* [3]*Division of Reproductive Endocrinology & Infertility Department of Obstetrics, Gynecology & Women's Health, Albert Einstein College of Medicine/Montefiore Medical Center, New York, USA,* [4]*Dokuz Eylul University, Medical Faculty, Biochemistry,* [5]*Dokuz Eylul University, Medical Faculty, Department of Child and Adolescent Psychiatry,* [6]*Dokuz Eylul University, Medical Faculty, Pediatrics,* [7]*Dokuz Eylul University, Medical Faculty, Neurosurgery, Balcova, 35340, Izmir, Turkey*

Accepted January 21, 2013

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

Abstract

Traumatic brain injury (TBI) may cause neuropsychiatric problems, such as anxiety disorder, that have negative effects on cognitive functions and behavior. We investigated the effects of progesterone on traumatic brain injury induced anxiety in 7-day-old rat pups subjected to contusion injury. Progesterone treatment decreased TBI induced anxiety and serum corticosterone levels, and increased serum IGF-1 levels. Moreover, progesterone treatment increased amygdala, prefrontal cortex and hippocampal neuron density. We found a negative correlation between serum corticosterone levels and anxiety tests, and a positive correlation between serum IGF-1 levels and anxiety tests. In addition, progesterone treatment decreased serum corticosterone compared to the controls and sham. Our results indicate that single dose progesterone may be effective for treating anxiety caused by TBI.

Key words: amygdala, anxiety, brain injury, corticosterone, hippocampus, IGF-1, prefrontal cortex

Traumatic brain injury (TBI) is a leading cause of death and disability in childhood (Adelson and Kochanek 1998). Its long-term sequelae often are more devastating for children owing to their developmental level. During the first 2 years after birth, the brain is more susceptible to damage owing to rapid brain growth during this period (Dobbing and Sands 1979). TBI causes long term functional impairment and decreases quality of life (Hawthorne et al. 2009, Ozdemir et al. 2005, 2012). Psychiatric disorders such as depression, anxiety disorders and personality changes also occur following TBI (Schwarzbold et al. 2008).

Anxiety is worry about the future with the feelings of nonspecific threat (Rosen and Schulkin 1998). Anxiety is associated with the amygdala, hippocampus and prefrontal cortex (LeDoux 1998, Uysal et al. 2011). Experimental TBI may trigger anxiety-like behaviors in rats (Baykara et al. 2012,

Correspondence: Nazan Uysal, M.D., Dokuz Eylul University Medical School, Department of Physiology, 35340 Balcova, Izmir, Turkey. Tel: 00-90-232-4124460, Fax: 00-90-232-4124459. E-mail: nazan.uysal@deu.edu.tr
© 2013 The Biological Stain Commission
Biotechnic & Histochemistry 2013, **88(5): 250–257.**

DOI:10.3109/10520295.2013.769630

RIGHTSLINK

Jones et al. 2008); however, the mechanisms of TBI induced anxiety are not fully understood. TBI may cause anxiety-like behaviors by causing structural changes in the brain (Kwon et al. 2011). It also causes functional changes in the hypothalamus-pituitary-adrenal axis (HPA) as a result of increased serum cortisol levels (Woolf et al. 1990).

Progesterone is a neurohormone with neuroprotective effects; it has been used to attenuate the effects of TBI in humans and animal head trauma models (Wright et al. 2007). Progesterone inhibits voltage-gated calcium channels and reduces excitotoxicity (Luoma et al. 2011). Progesterone has long been known to have anxiolytic activity in both animals and humans (Frye et al. 2004, Kroboth and McAuley 1997), although the underlying mechanisms have not been fully elucidated.

We showed earlier that decreased levels of circulating insulin-like growth factor-1 (IGF-1) were correlated with hippocampal neuron density, and impaired learning and memory in immature rats with TBI (Ozdemir et al. 2012). Rubovitch et al. (2010) demonstrated that TBI caused spatial memory deficits that were prevented by IGF-1 administration and that blocking the IGF-1 receptor signaling in TBI mice did not augment the spatial memory deficit. Also, TBI causes alterations in HPA axis functions and increases cortisol levels (Woolf et al. 1990). Progesterone treatment increases serum IGF-1 (El-Bakri et al. 2004) and decreases serum cortisol levels (Selman et al. 1997). It is not known, however, how progesterone affects TBI induced anxiety. Therefore, we investigated the effects of progesterone treatment on TBI induced anxiety in immature rats.

Material and methods

All experiments were performed in accordance with the guidelines of the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University School of Medicine. Wistar albino rats with dated pregnancies were maintained at the same center and housed in individual cages with free access to water and laboratory chow. Twenty-eight litters delivered spontaneously were reared with their dams until the start of experimentation at 7 days postnatal age. All rats were maintained on a constant 12 h light/dark cycle at constant room temperature (21° C) and 60% humidity. Rats were divided into four groups: 1) control (n = 7), 2) sham (n = 7), 3) TBI (n = 7), 4) TBI + progesterone (n = 7).

We used a modification of an established percussion trauma model for immature rats to model infant and early childhood head trauma (Bittigau et al. 1999). The contusing device consisted of a 40 cm long hollow tube perforated at 1cm intervals to prevent air compression. The device was positioned perpendicular to the surface of the skull and guided a falling weight onto a circular footplate resting on the skin over the parietal bone. Trauma was transferred through the skin and parietal bone to the parietal cortex (closed trauma model). The trauma was applied to the right parietal cortex, which is the dominant hemisphere (Bittigau et al. 1999). The center of the footplate was positioned stereotaxically 3 mm anterior and 2 mm lateral to the lambda and was fixed in place under ether anesthesia. A force of 160 gcm produced by a 10 g weight was selected to produce brain contusion. Experiments were carried out between 9.00 and 11.00 AM in a sound-attenuated and air-regulated experimental room. All pups were kept on a heating pad until they were returned to their mothers 4 h after the trauma or sham surgery. Progesterone (8 mg/kg) was injected intraperitoneally immediately after induction of TBI. Low doses (8–16/mg/kg) of progesterone increase cognitive functions and have neuroprotective effects (Goss et al. 2003). The TBI and sham groups received saline only and lived with their mothers until postnatal day 21.

The remaining rats were weaned on day 21 and their anxiety levels were evaluated using the open field and elevated plus maze tests on day 22 (Denenberg and Victor 1969, Pellow et al. 1985). Recording and analysis of anxiety levels were performed using the HVS-image video tracking system (HVS2020, Hampton, England).

For the open field test, the field consisted of a cubicle with a base 1 m^2 and walls 50 cm high, with a video camera installed 2.5 m above the apparatus. Each rat was placed in the center of the open field and locomotor activity (ambulation) was measured for 5 min in a sound-proof illuminated observation room. The light in the room was 100 lux.

The elevated plus maze consisted of a 5×5 cm central platform with two open arms (50 cm long, 10 cm wide and 0.5 cm high) and two closed arms (same dimensions as the open arms, but with walls 40 cm high that were elevated 50 cm above the ground. Rats were placed on the platform facing the open arm and were observed for 5 min. The total number of entries into the open and closed arms, and the time spent in the open and closed arms were recorded.

On postnatal day 27, following light ether anesthesia, blood was drawn from all animals and the brains removed. Brain tissues were fixed in 10% formalin in phosphate buffer for 24 h. The brain was sectioned coronally into sequential 5 μm sections

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK

using a microtome (Leica RM2255, Cologne, Germany). Estimation of the number neurons for each sample was accomplished using three coronal sections through the prefrontal cortex that corresponded to plates 9, 11, and hippocampus and amygdala that corresponded to plates 21, 23, 25 in the rat atlas of Paxinos and Watson (1982). All sections were stained by cresyl violet. Cresyl violet stains Nissl substance so that neuron cell numbers can be counted. Sections were deparaffinized and hydrated after which they were immersed in cresyl violet solution for 20 min. Sections then were dipped in 96% ethanol followed by treatment with xylene for 20 min and mounted in Entellan. The images were analyzed using a computer assisted image analyzer system consisting of a microscope (Olympus CX-41 Tokyo, Japan) equipped with a high-resolution video camera (Olympus DP71, Japan). The neurons in these regions were counted at 40 × magnification using a 15800 μm^2 counting frame. The counting frame was placed randomly three times on the image analyzer system monitor and the number of neurons in each section was counted; the average of all three counts was calculated. The neurons in the amygdala, prefrontal cortex and hippocampus were counted in CA1, CA2, CA3 and gyrus dentatus (GD) regions and neuron density was calculated.

IGF-1 protein levels in the serum were measured using a commercially available ELISA kit specific for rat IGF-1 with assay sensitivity <5 pg/ml and range 62.5–4000 pg/ml (EK0377; Boster Immunoleader, Wuhan, China) according to the manufacturer's instructions. For visualization of the IGF-1 expression, immunohistochemistry was performed using an anti-IGF-1 antibody. The sections were incubated overnight with anti- IGF-1 antibody (1:100; Bioss, bs-0014R, Woburn, MA), then for another 30 min with the biotinylated mouse secondary antibody. The bound secondary antibody was then amplified with Histostain® plus bulk kit (Invitrogen, 85-9043, Camarillo, CA). The antibody-biotin-avidin-peroxidase complexes were visualized using DAB. The sections were finally mounted onto lysine-coated slides.

Serum corticosterone levels were measured using original Siemens kits and the Siemens Immulite 2000 XPi device with the chemiluminescence immunoassay protocol (Siemens Healthcare Diagnostics S.L, Munich, Germany).

All statistical procedures were performed by SPSS software for Windows, Version 11.0 (SPSS, Chicago, IL). Differences between groups were analyzed using one-way-ANOVA with Bonferroni post-hoc test when a statistically significant difference was found between groups. Correlations among groups were calculated using Pearson correlation analysis. Results are presented as mean ± S.E.M. Multiple comparisons were performed and to prevent an alpha (type 1) error, ($p/n = 0.05/10$); $p<0.005$ was considered statistically significant (Aickin et al. 1996).

Results

In the open field test, the moving times and the number of total entries into center cells were less in TBI rats compared to sham operated and control groups ($p<0.005$ for both) (Fig. 1A). TBI rats were less active in the open field test compared to both sham operated and control groups ($p<0.005$ for both). In the progesterone treatment group, TBI rats were more active and ambulated more in the center of the open field compared to both sham operated and control groups ($p<0.005$ for both) (Fig. 1B). Compared to both sham operated and control groups, TBI rats entered less frequently and spent less time in the open arms of elevated plus maze test ($p<0.0001$ for both), and the anxiety levels of TBI rats were lower after progesterone treatment ($p<0.0001$ for both) (Fig. 2A, B).

Both the contra- and ipsilateral brain regions were affected by TBI. The cause of the effect in the

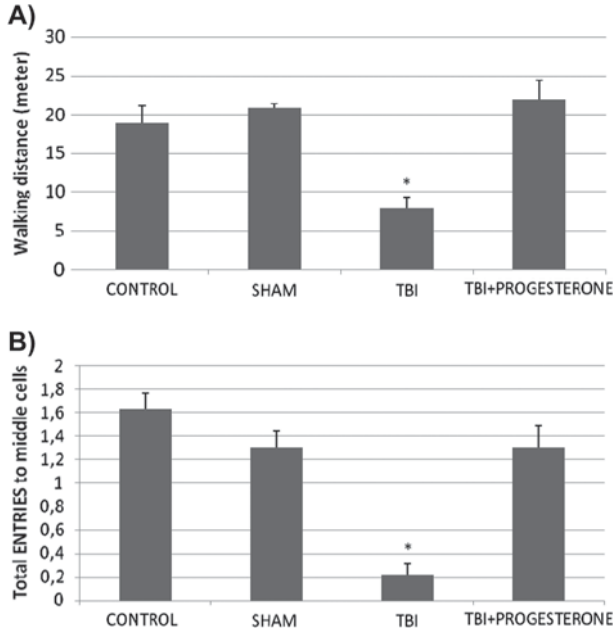


Fig. 1. Open field test results. A) Walking distance in open field arena. B) Total entries to the middle cells in the open field arena; *$p<0.005$ compared to other groups. TBI, traumatic brain injury; TBI + progesterone, TBI with progesterone treatment.

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK

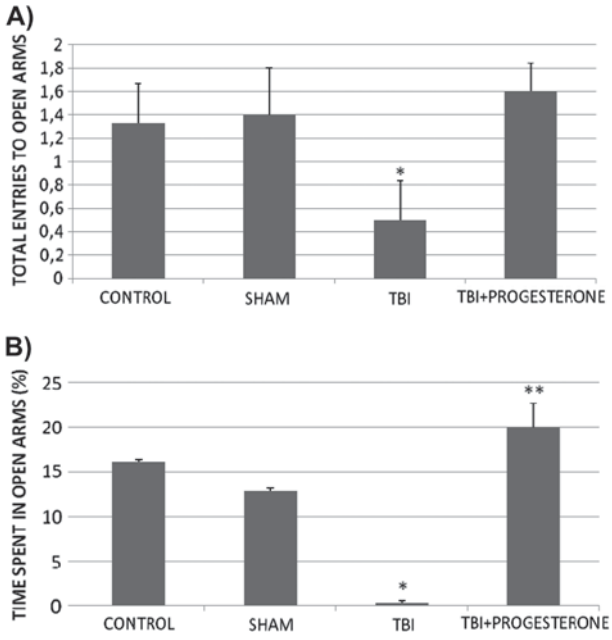


Fig. 2. Elevated plus maze test results. A) Total entries to open arms. B) Time spent in open arms. *$p < 0.0001$ compared to other groups; **$p < 0.0001$ compared to TBI and sham groups. TBI, traumatic brain injury; TBI + progesterone, TBI with progesterone treatment.

contralateral brain regions has been suggested to be due to a contra-coup contusion (Tong et al. 2002). The density of both contralateral and ipsilateral hemisphere neurons were significantly decreased in the amygdala, prefrontal cortex and hippocampus in TBI rats compared to sham and control groups (all regions $p < 0.0001$). Progesterone treatment increased neuron density in all brain regions compared to the TBI group (all regions $p < 0.003$) (Fig. 3A, B).

TBI rats had lower serum IGF-1 and higher corticosterone levels than control and sham treated animals ($p < 0.0001$ for both). Progesterone treatment increased serum IGF-1 and decreased corticosterone levels in progesterone treated TBI rats compared to TBI rats ($p < 0.0001$ for both). Also, progesterone treatment decreased serum corticosterone levels compared to control and sham animals ($p < 0.0001$ for both) (Table 1). Representative photographs of IGF-1 expression show that IGF-1 reactivity was reduced in all regions of the brains of TBI rats. Progesterone treatment increased IGF-1 reactivity (Fig. 4A, B).

Correlation analysis was performed between the measurement results: We found strongly positive correlations between amygdala, prefrontal cortex, and hippocampus neuron numbers and time spent in open arms of elevated plus maze (amygdala, $r = 0.791$, $p = 0.0001$; prefrontal cortex, $r = 0.725$, $p = 0.001$; CA1, $r = 0.818$, $p = 0.0001$; CA3, $r = 0.842$, $p = 0.0001$; GD, $r = 0.741$, $p = 0.0001$). We also found strongly positive correlations between serum IGF-1 levels and time spent in the center of the open field arena ($r = 0.685$, $p = 0.003$), and serum IGF-1 levels and time spent on open arms of elevated plus maze ($r = 0.900$, $p = 0.0001$). There were negative correlations between serum corticosterone levels and time spent in the center of the open field arena ($r = -0.648$, $p = 0.012$), and serum corticosterone levels and time spent on the open arms of the elevated plus maze ($r = -0.880$, $p < 0.0001$).

Discussion

We found that anxiety levels were increased in the TBI rats. TBI rats did not exhibit exploring behaviors, e.g., they spent less time on the open arms of elevated plus maze test instrument, they showed less moving time and entered the center cells of the open field arena less often. Progesterone treatment decreased the anxiety levels of TBI rats. We also found that the numbers of neurons and IGF-1 levels, which decreased with TBI, increased with progesterone treatment. Also, serum corticosterone levels, which increased with TBI, decreased with progesterone treatment.

Cortisol in humans or corticosterone in rodents is secreted by the adrenal glands in response to both physical and psychological stress. Serum and salivary cortisol levels are increased in anxiety disorders (Albanidou-Farmaki et al. 2008). Plasma cortisol levels are increased after mild to moderate TBI (Steinbok and Thompson 1979).

We found that corticosteroid levels were increased by 3 weeks after TBI. Moreover, glucocorticoid receptor expression was increased in TBI rats, which is recognized as an indication of negative feedback (McCullers et al. 2002). It is known that progesterone decreases serum cortisol levels (Selman et al. 1997). We found that serum corticosterone levels were increased as a result of TBI, but that they were decreased by progesterone treatment in the TBI + progesterone group. Also, progesterone treatment decreased serum corticosterone levels in the TBI + progesterone group compared to control and sham groups.

Chronically elevated levels of corticosterone can adversely affect neurogenesis and cause loss of synapses (De Bellis et al. 2000). We observed that neuron density decreased in the amygdala, prefrontal cortex and hippocampus of TBI rats and was associated with high serum corticosterone levels. Progesterone treatment increased neuron density in all brain regions of TBI rats and this finding corresponded to decreased serum corticosterone levels.

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK

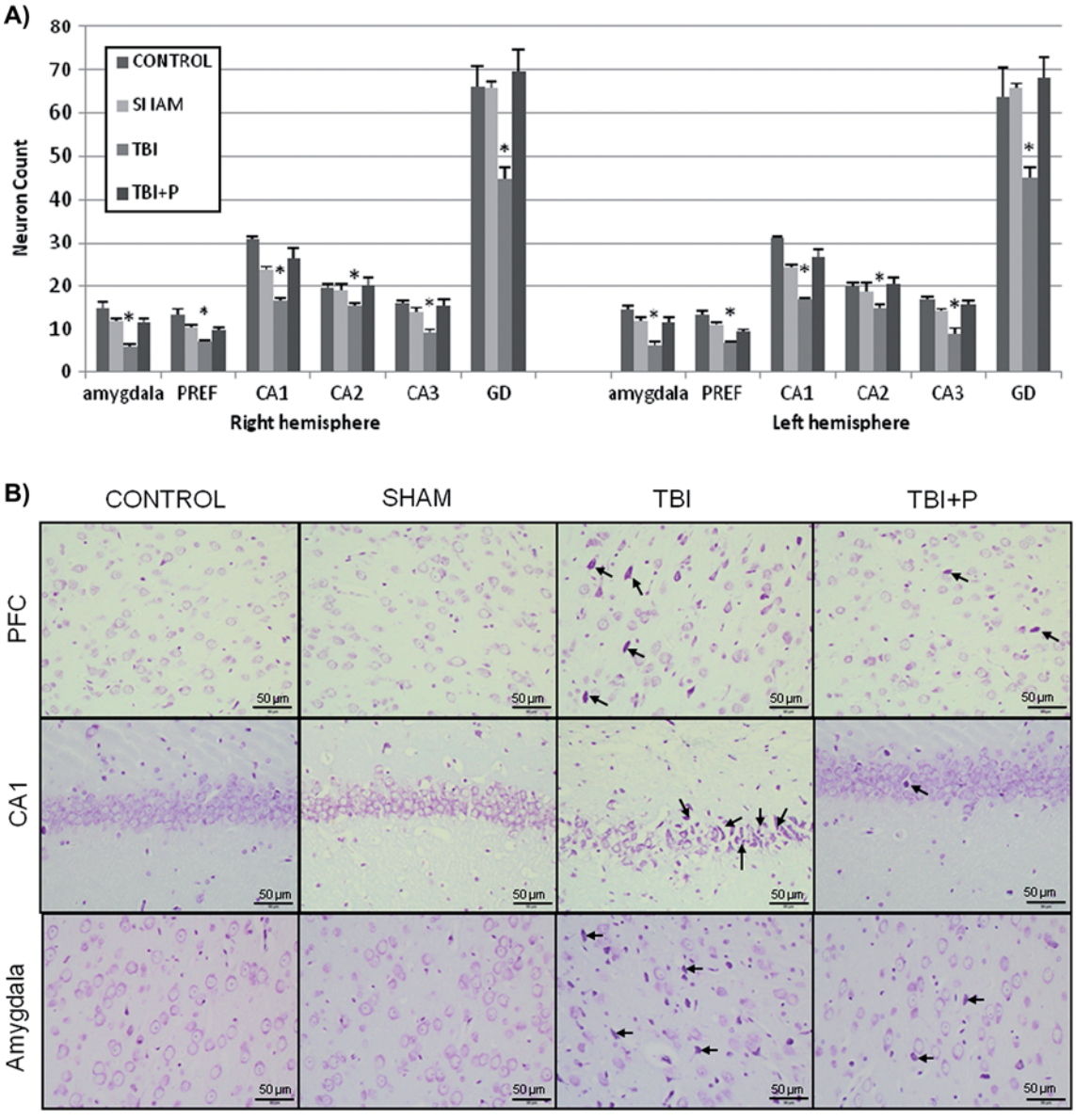


Fig. 3. Neuron density results. A) Neuron density of amygdala, prefrontal cortex and hippocampus; *$p < 0.003$ compared to other groups. B) Representative photomicrographs of cresyl violet stained sections of the amygdala, prefrontal cortex and hippocampus. TBI, traumatic brain injury; TBI + P, TBI with progesterone treatment; PREF, prefrontal cortex; GD, gyrus dentatus.

IGF-1 may play a role in the amelioration of anxiety, depression, and memory deficits (Trejo et al. 2008). It is generally accepted that circulating IGF-1 levels reflect the level of IGF-1in the brain. Administration of exogenous IGF-I improved cognition and reduced anxiety (Trejo et al. 2008). Decreased serum levels of IGF-1 correspond to an increased level of anxiety in humans and rodents (Llorens-Martín et al. 2010, Mitschelen et al. 2011). We showed in an earlier study that decreased serum IGF-1 with decreased prefrontal cortex IGF-1 levels corresponded to increased anxiety in diabetic rats (Aksu et al. 2012). It has been reported that hippocampal IGF-1 levels increased by 3 days after trauma, then normalized by 14 days after trauma (Schober et al. 2012); there are no reports

Table 1. Serum IGF-1 and corticosterone levels

	Corticosterone (µg/dL)	IGF-1 (pg/ml)
Control	1.50 ± 0.09	2036.06 ± 27.42
Sham	1.7 ± 0.09	2058.80 ± 22.89
TBI	2.64 ± 0.14*	1512.99 ± 37.9*
TBI + progesterone	0.99 ± 0.09*	2396.83 ± 25.50

*$p < 0.0001$ compared to other groups.

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK

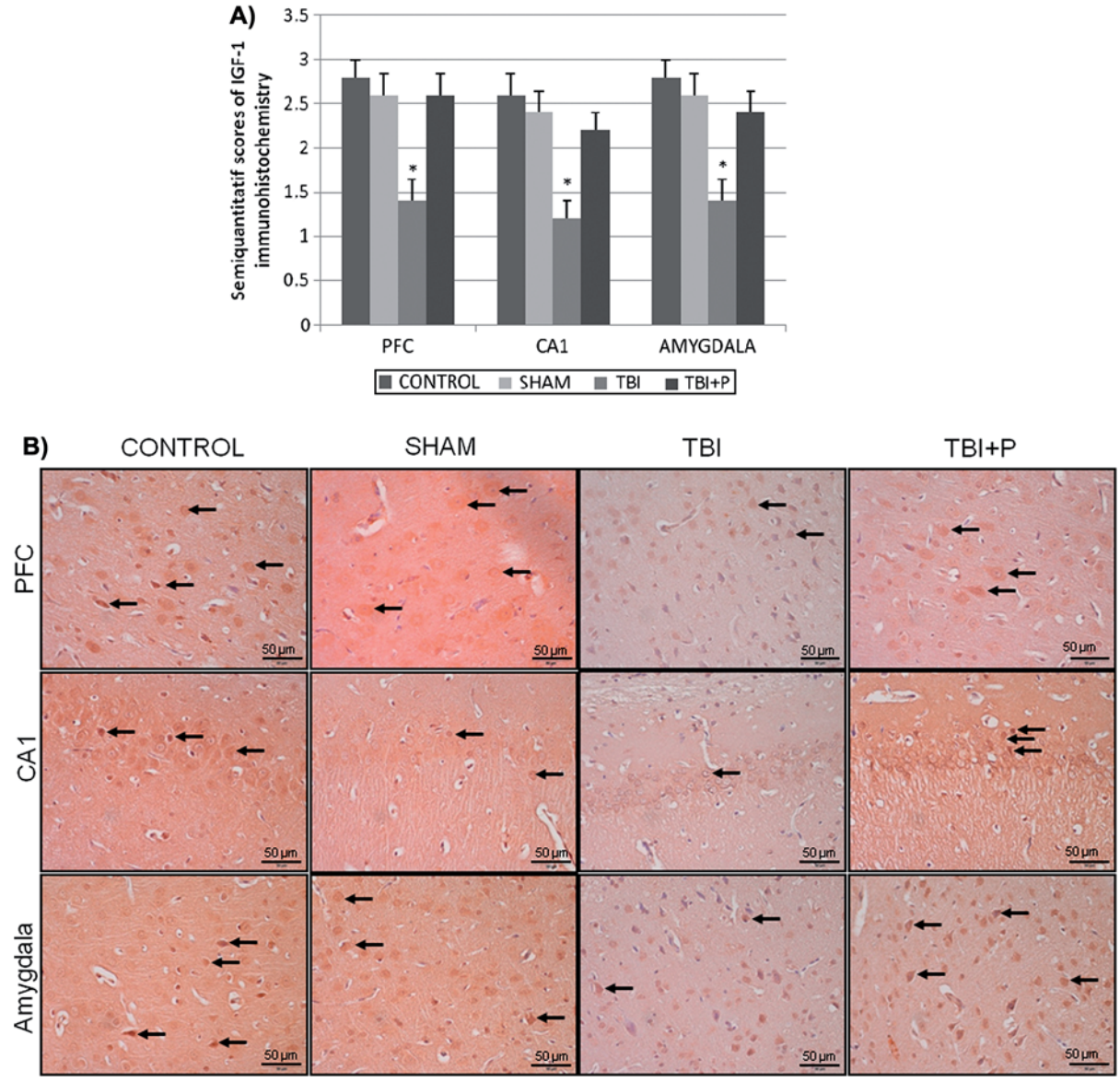


Fig. 4. IGF-1 + immune staining results. A) Quantification of IGF-1 immunostaining. B) IGF-1 + immunostained cells in amygdala, prefrontal cortex and hippocampus; $^{*}p < 0.0001$ compared to other groups.

concerning IGF-1 levels after that. We sacrificed animals 20 days after TBI. IGF-1 immunostaining decreased in the prefrontal cortex, amygdala and hippocampus of TBI rats. In addition, progesterone treatment restored IGF-1 levels in prefrontal cortex, amygdala and hippocampus.

Glucocorticoids inhibit liver and serum IGF-1 (Luo and Murphy 1989). We found that serum IGF-1 levels were lower in TBI rats because of higher serum corticosterone levels. Moreover, progesterone treatment increased serum IGF-1 levels, which corresponded to decreased serum corticosterone levels in TBI rats. High levels of corticosterone during the development of the dopaminergic system can cause permanent behavioral changes by increasing dopamine transmission in the mesocorticolimbic regions of the brain (Jahng et al. 2010). The mesocortical dopamine neurons that originate from the midbrain ventral tegmental area and project to the prefrontal cortex have been implicated by many studies to be involved in the emotional responses of fear and anxiety (Pezze and Feldon 2004). TBI increases the dopaminergic activity in mesocorticolimbic regions of immature rats (Walter et al. 2004). The amygdala and prefrontal cortex regulate the secretion of dopamine from the mesocortical projection system (Goldstein et al. 1996). IGF-1 protects human and rodent neuron cultures against dopamine induced neurocytotoxicity (Offen et al. 2001).

It is known that TBI causes behavioral and neuroendocrine differences in anxiety disorders (Kwon

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK

et al. 2011). We found that progesterone treatment decreased TBI induced anxiety in immature rats. Progesterone treatment decreases anxiety in both animals and humans (Frye et al. 2004, Kroboth and McAuley 1997). Anxiety characteristics are closely correlated with natural fluctuations in progesterone levels during the ovarian cycle (Schmidt et al. 1994). We found that progesterone treatment decreased corticosterone and increased IGF-1 levels after TBI.

Anxiety is associated with recruitment of at least a part of the "fear network," which involves the hippocampus, amygdala and prefrontal cortex (LeDoux 1998). Morphological studies have revealed an increased volume of bilateral amygdala in anxiety disorder patients (Bellis et al. 2000). Prefrontal cortex inhibits autonomous and endocrine responses to stress, and reduces anxiety (Porritt et al. 2005). The amygdala, hippocampus and prefrontal cortex use sensory information to evaluate extinguished fear-conditioned stimuli and form a key circuit for maintaining an appropriate behavioral response. Consistent with this, we found a strongly positive correlation between anxiety tests and amygdala, hippocampus and prefrontal cortex neuron numbers.

TBI increases serum corticosterone levels, decreases serum IGF-1 levels and increases the anxiety levels of rat pups. Progesterone treatment decreases serum corticosterone, increases serum IGF-1 levels and decreases anxiety levels. Further studies are needed to elucidate the role of IGF-1 on TBI induced changes in dopamine metabolism and the interactions among the amygdala, prefrontal cortex and hippocampus for processing emotional inputs.

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

Acknowledgment

This work was supported by Dokuz Eylul University Research Foundation Grant no: 2012.KB.SAG.070.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Adelson PD, Kochanek PM (1998) Head injury in children. *J. Child Neurol.* 13: 2–15.

Albanidou-Farmaki E, Poulopoulos AK, Epivatianos A, Farmakis K, Karamouzis M, Antoniades D (2008) Increased anxiety level and high salivary and serum cortisol concentrations in patients with recurrent aphthous stomatitis. *Tohoku J. Exp. Med.* 214: 291–296.

Aickin M, Gensler H (1996) Adjusting for multiple testing when reporting research results: the Bonferroni vs. Holm methods. *Am. J. Pub. Health* 86: 726–728.

Aksu I, Ates M, Baykara B, Kiray M, Sisman AR, Buyuk E, Baykara B, Cetinkaya C, Gumus H, Uysal N (2012) Anxiety correlates to decreased blood and prefrontal cortex IGF-1 levels in streptozotocin induced diabetes. *Neurosci. Lett.* Nov 2. doi:pii: S0304–3940(12)01413-9. 10.1016/j.neulet.2012.10.045.

Baykara B, Cetin F, Baykara B, Aksu I, Dayi A, Kiray M, Sisman AR, Ozdemir D, Arda MN, Uysal N (2012) Anxiety caused by traumatic brain injury correlates to decreased prefrontal cortex VEGF immunoreactivity and neuron density in immature rats. *Turk. Neurosurg.* doı: 10.5137/1019-5149.JTN.5633-11.1.

Bittigau P, Sifringer M, Pohl D, Stadthaus D, Ishimaru M, Shimizu H, Ikeda M, Lang D, Speer A, Olney JM, Ikonomidou C (1999) Apoptotic neurodegeneration following trauma is markedly enhanced in the immature brain. *Ann. Neurol.* 45: 724–735.

Brummelte S, Galea L (2010) Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience* 168: 680–690.

De Bellis MD, Casey BJ, Dahl RE, Birmaher B, Williamson DE, Thomas KM, Axelson DA, Frustaci K, Boring AM, Hall J, Ryan ND (2000) A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol. Psych.* 48: 51–57.

Denenberg, Victor H. (1969). "Open-field behavior in the rat: what does it mean"? *Ann. NY Acad. Sci.* 159: 852–859.

Dobbing J, Sands J (1979) Comparative aspects of the brain growth spurt. *Early Hum. Dev.* 3: 79–83.

El-Bakri NK, Islam A, Suliman I, Lindgren U, Winblad B, Adem A (2004) Ovariectomy and gonadal hormone treatment: effects on insulin-like growth factor-1 receptors in the rat brain. *Growth Horm. IGF Res.* 14: 388–393.

Frye CA, Walf AA, Rhodes ME, Harney JP (2004) Progesterone enhances motor, anxiolytic, analgesic, and antidepressive behavior of wild-type mice, but not those deficient in type 1 5α-reductase. *Brain Res.* 1004: 116–124.

Goldstein LE, Rasmusson AM, Bunney BS, Roth RH (1996) Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J. Neurosci.*16: 4787–4798.

Goss CW, Hoffman SW, Stein DG (2003) Behavioral effects and anatomic correlates after brain injury: a progesterone dose-response study. *Pharmacol. Biochem. Behav.* 76: 231–242.

Hawthorne G, Gruen RL, Kaye AH (2009) Traumatic brain injury and long-term quality of life: findings from an Australian study. *J. Neurotrauma* 26: 1623–1633.

Jahng JW, Ryu V, Yoo SB, Noh SJ, Kim JY, Lee JH (2010) Mesolimbic dopaminergic activity responding to acute stress is blunted in adolescent rats that experienced neonatal maternal separation. *Neuroscience* 171: 144–152.

Jones NC, Cardamone L, Williams JP, Salzberg MR, Myers D, O'Brien TJ (2008) Experimental traumatic brain injury induces a pervasive hyperanxious phenotype in rats. *J. Neurotrauma* 25: 1367–1374.

Klur S, Muller C, Pereira de Vasconcelos A, Ballard T, Lopez J, Galani R, Certa U, Cassel JC (2009)

RIGHTSLINK

Hippocampal-dependent spatial memory functions might be lateralized in rats: an approach combining gene expression profiling and reversible inactivation. *Hippocampus* 19: 800–816.
Kroboth PD, McAuley JW (1997) Progesterone: does it affect response to drug? *Psychopharmacol. Bull.* 33: 297–301.
Kwon SK, Kovesdi E, Gyorgy AB, Wingo D, Kamnaksh A, Walker J, Long JB, Agoston DV (2011) Stress and traumatic brain injury: a behavioral, proteomics, and histological study. *Front. Neurol.* 2: 1–14.
LeDoux J (1998) Fear and the brain: where have we been, and where are we going? *Biol. Psych.* 44: 1229–1238.
Llorens-Martín M, Torres-Alemán I, Trejo JL (2010) Exercise modulates insulin-like growth factor 1-dependent and -independent effects on adult hippocampal neurogenesis and behaviour. *Mol. Cell Neurosci.* 44: 109–117.
Luo JM, Murphy LJ (1989) Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I abundance in the intact rat. *Endocrinology* 125: 165–171.
Luoma JI, Kelley BG, Mermelstein PG (2011) Progesterone inhibition of voltage-gated calcium channels is a potential neuroprotective mechanism against excitotoxicity. *Steroids* 76: 845–855.
McCullers DL, Sullivan PG, Scheff SW, Herman JP (2002) Traumatic brain injury regulates adrenocorticosteroid receptor mRNA levels in rat hippocampus. *Brain Res.* 947: 41–49.
Mitschelen M, Yan H, Farley JA, Warrington JP, Han S, Hereñú CB, Csiszar A, Ungvari Z, Bailey-Downs LC, Bass CE, Sonntag WE (2011) Long-term deficiency of circulating and hippocampal insulin-like growth factor I induces depressive behavior in adult mice: a potential model of geriatric depression. *Neuroscience* 185: 50–60.
Offen D, Shtaif B, Hadad D, Weizman A, Melamed E, Gil-Ad I (2001) Protective effect of insulin-like-growth-factor-1 against dopamine-induced neurotoxicity in human and rodent neuronal cultures: possible implications for Parkinson's disease. *Neurosci. Lett.* 16: 129–132.
Ozdemir D, Tugyan K, Uysal N, Sonmez U, Sonmez A, Acikgoz O, Ozdemir N, Duman M, Ozkan H (2005) Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats. *Neurosci. Lett.* 385: 234–239.
Ozdemir D, Baykara B, Aksu I, Kiray M, Sisman AR, Cetin F, Dayi A, Gurpinar T, Uysal N, Arda MN (2012) Relationship between circulating IGF-1 levels and traumatic brain injury-induced hippocampal damage and cognitive dysfunction in immature rats. *Neurosci. Lett.* 507: 84–89.
Paxinos G, Watson C (1982) *The Rat Brain in Stereotaxic Coordinates* Academic Press, New York. Plates 9, 11, 21, 23, 25.
Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14: 149–167.
Pezze MA, Feldon J (2004) Mesolimbic dopaminergic pathways in fear conditioning. *Prog. Neurobiol* .74: 301–320.
Porritt MJ, Batchelor PE, Howells DW (2005) Inhibiting BDNF expression by antisense oligonucleotide infusion causes loss of nigral dopaminergic neurons. *Exp. Neurol.*192: 226–234.
Rosen JB, Schulkin J (1998) From normal fear to pathological anxiety. *Psychol. Rev* .105: 325–350.
Rubovitch V, Edut S, Sarfstein R, Werner H, Pick CG (2010) The intricate involvement of the insulin-like growth factor receptor signaling in mild traumatic brain injury in mice. *Neurobiol. Dis.* 38: 299–303.
Schmidt PJ, Purdy RH, Moore PH Jr, Paul SM, Rubinow DR (1994) Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects. *J. Clin. Endocrinol. Metab.* 79: 1256–1260.
Schober ME, Ke X, Xing B, Block BP, Requena DF, McKnight R, Lane RH (2012) Traumatic brain injury increased IGF-1B mRNA and altered IGF-1 exon 5 and promoter region epigenetic characteristics in the rat pup hippocampus. *J. Neurotrauma* 29: 2075–2085.
Schwarzbold M, Diaz A, Martins ET, Rubino A, Amate LN, Thais ME, Queued J, Howl A, Linares MN, Waltz R (2008) Psychiatric disorders and traumatic brain injury. *Neuropsych. Dis. Treat.* 4: 797–816.
Selman PJ, Mol JA, Rutteman GR, van Garderen E, van den Ingh TS, Rijnberk A (1997) Effects of progestin administration on the hypothalamic-pituitary-adrenal axis and glucose homeostasis in dogs. *J. Reprod. Fertil.* Suppl. 51: 345–354.
Stein DG (2008) Progesterone exerts neuroprotective effects after brain injury. *Brain Res. Rev.* 57: 386–397.
Steinbok P, Thompson G (1979) Serum cortisol abnormalities after craniocerebral trauma. *Neurosurgery* 5: 559–565.
Tong W, Igarashi T, Ferriero DM, Noble LJ (2002) Traumatic brain injury in the immature mouse brain: characterization of regional vulnerability. *Exp. Neurol.* 176: 105–116.
Trejo JL, Llorens-Martin MV, Torres-Aleman I (2008) The effects of exercise on spatial learning and anxiety-like behavior are mediated by an IGF-I-dependent mechanism related to hippocampal neurogenesis. *Mol. Cell Neurosci.* 37: 402–411.
Uysal N, Sisman AR, Dayi A, Aksu I, Cetin F, Gencoglu C, Tas A, Buyuk E (2011) Maternal exercise decreases maternal deprivation induced anxiety of pups and correlates to increased prefrontal cortex BDNF and VEGF. *Neurosci. Lett.* 505: 273–278.
Walter B, Brust P, Füchtner F, Müller M, Hinz R, Kuwabara H, Fritz H, Zwiener U, Bauer R (2004) Age-dependent effects of severe traumatic brain injury on cerebral dopaminergic activity in newborn and juvenile pigs. *J. Neurotrauma* 21: 1076–1089.
Woolf PD, Cox C, Kelly M, Nichols D, McDonald JV, Hamill RW (1990) The adrenocortical response to brain injury: correlation with the severity of neurologic dysfunction, effects of intoxication, and patient outcome. *Alcohol Clin. Exp. Res.* 14: 917–921.
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 (2007) ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. *Ann. Emerg. Med.* 49: 391–402.

Biotech Histochem Downloaded from informahealthcare.com by Dokuz Eylul Univ. on 09/13/13
For personal use only.

RIGHTSLINK