Global Veterinaria 14 (6): 790-799, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.06.9545

Gender Differences in the Prevalence of Depression (Persolt Swimming Test) in Sciatic Nerve Injury Model Wistar Rats

Bachir Ali Rachedi, Asma Fraia, Hacène Frih,
Moncef Zaafour, Selma Guernine and Samir Djemli

Department of Biology, Faculty of Science,
University Badji Mokhtar, Annaba, BP 12 El Hadjar, Algeria

Abstract: Study of gender differences in the prevalence of depression (Persolt swimming test) in sciatic nerve injury model Wistar rats.A sciatic nerve ligation is performed surgically in two groups of 08 male rats and 08 female rats. The same number in males and females is considered as a control. The investigations concerning the degree of depression are obtained through the Forced Swimming Test Porsolt (FST), Plus maze test and sucrose intake. At the same time, a comparative study between the two sexes.Results: A significant increase in the level of depression is obtained resulting in male rats that had undergone ligation of the sciatic nerve. Regarding the comparison between males and females rats, some level of depression is recorded in female, while among their female depression levels are very low. This was explained by hormonal differences between the sexes. Conclusion: The sciatic nerve ligation causes depression in the rat. In the FST also we found significant differences between male and female rats to depression, suggesting the key role of sex steroids.

Key words: Depression · Sciatic nerve injury · Neuroactive forced swimming test · Gender differences steroids

INTRODUCTION

Pain is defined by IASP (International Association for the Study of Pain) as an unpleasant sensation or emotional perception, following tissue damage.

Inflammatory pain is often caused by activation of the immune system to injuries and / or other tissue infections. By strengthening sensory sensitivity after tissue injury, it promotes the recovery of the body part affected by creating an unpleasant hypersensitivity environment that discourages physical contact and motion, reducing the risk of further damage [1]. On the site of the lesion mast cells, neutrophils, macrophages, T cells and Schwann cells interact and contribute to the sensitization of nociceptors [2, 3]. Mast cells release their products rich in histamine, cytokines and proteases that will help raise awareness of nociceptors and attract other cells to the site of injury. Thus, mast cells appear to play an important role in the initiation of neuropathic pain [4]. Neutrophils will secrete cytokines (including TNF alpha) [5], Reactive Oxygen Species (ROS) and chemokines. Depletion of circulating neutrophils is signaled to prevents neuropathic pain [4]. At the same time or after the recruitment of macrophages, T cells infiltrate the damaged nerves [2]. They are able to increase their painful hypersensitivity [6, 7]. The resident immune cells of the spinal cord and glial cells play a major role in the development of pain after peripheral nerve injury. Oligodendrocytes are closely connected with neurons, astrocytes, microglia and endothelial cells. They can respond and secrete different molecules. These cells are also susceptible to inflammation and to the elevation of glutamate (Glu) levels, observed, for example, when a lesion of the peripheral nerve. According to Echeverry *et al*. [8] in case of a lesion of a peripheral nerve, microglia undergoes changes that lead to an "on" state. These glial cells are capable, under different conditions to stimulate, produce and release a variety of effector molecules intercellular belonging to different molecular classes including pro-inflammatory molecules: cytokines, chemokines and growth factors [9, 10]. Following peripheral nerve injury, astrocyte activation results in the release of different molecules such that the extracellular glutamate concentration is increased. Indeed, the uptake of glutamate is no longer guaranteed because of the decreased expression of GLT-1 transporter by astrocytes

Corresponding Author: Frih Hacène, Department of Biology, Faculty of Science, University Badji Mokhtar, Annaba, BP 12 El Hadjar, Algeria. Tel: +213661399053.

[11]. Moreover, astrocytes may also modulate their glutamate release [12]. Following peripheral nerve injury, there is the release of different molecules proalgesic by injured or activated cells. In addition to histamine, bradykinin, ATP, serotonin or prostaglandins, cytokines play as well at the periphery role in the spinal cord by acting directly or indirectly on the primary sensory neurons or secondary [3].

Cytokines are polypeptides mediators of immunity and inflammation. They participate in a variety of biological functions in several systems, including the CNS. The presence of inflammatory cytokines in the brain was originally assigned to an exclusive synthesis by myeloid-monocytic infiltrating cells (since the nerve cells were not supposed to produce and use these molecules). Studies on cells in culture showed TNF synthesis capacity by glial cells, astrocytes and mainly by microglia [13]. Different cytokine receptors are present in the brain or in the hypothalamus itself or in the brain structures directly connected to the hypothalamus [14]. Some of them are pro-inflammatory (TNF, IL-1β and IL-6), others have anti-inflammatory activity, eg, IL-10. The involvement of cytokines in pain was demonstrated for the first time in 1988 [15], but as evidenced by inhibition experiments of their activity, cytokines do not participate in acute pain [16]. Proinflammatory cytokines are involved in the phenomena of hypersensitivity to pain. They act both in the periphery and centrally. Their role in the periphery has been studied in particular skin-nerve preparations [17]. The expression of these three (03) cytokines is also increased in the bone marrow [18]. Injected intrathecally, they increase the activity of neurons in the dorsal horn and induce animal painful hypersensitivity [19]. Clinical studies have also shown that there is an imbalance between cytokines pro- and anti-inflammatory drugs in patients with painful neuropathy. This imbalance would be a predisposing factor for the onset of chronic pain in cases of neuropathy [20-22].

The achenal axis (HP is sensitive to the complex action of cytokines, whether produced in the CNS or transported through the bloodstream due to the permeability of the blood-brain barrier in the hypothalamic region. IL-1, IL-6, interferon and TNF have a promoting action on this axis. The release by the adrenal glucocorticoids inhibits the inflammatory response and helps contain the immune response [23], the release of CRH, is stimulated by the proinflammatory cytokines IL-1β, IL-6 and IFN alpha, but inhibited by the anti-inflammatory cytokine TGFá. The activation of the HPA axis by cytokines is also accompanied by effects on the metabolism of certain neurotransmitters (GABA, 5HT ...) [24], as the adrenal are another site of interaction between the HPA axis and cytokines [25].

The interactions between CNS, stress and the immune system are better known, especially the role played by cytokines on the cells as well as liaison and mediation actors between the two systems [26]. Therefore, oxidative stress is associated with neuropathic pain as a result of an injury to a peripheral nerve [27]. The activation of Nicotinamide-Adenine-Dinucleotide-Phosphate (NADPH)-oxidase (NOX) is an important intracellular source of free radicals leading to the activation of a variety of signaling events resulting in the expression of cytokines pro -inflammatory, hypertrophy, gene transcription and cell migration [28]. In cases of neuropathic pain with a lesion of a peripheral nerve, NADPH oxidase can be hyperactivated [28, 29], causing deleterious effects (damage to DNA and protein). The Nitric Oxide Synthase (NOS) are potentially involved in the maintenance of neuropathic pain [27]. In addition, a significant increase in NO levels was measured in the sciatic nerve ligated [30].

There are more than several animal models of neuropathic pain, especially in rats, mostly based on the production of a lesion of a peripheral nerve (mostly the sciatic) by physical or chemical trauma [31, 32]. This model was launched in 1988 [33].

It is in this context that fits our work, which is conducted on rats who underwent ligation of the sciatic nerve. As based on the Forced Swim Test (FST), we looked at first possible relations between ligation of the sciatic nerve and the incidence of depression and in further investigations, the possibility of a difference in the degree of depression between males and neuropathic rats females.

MATERIALS AND METHODS

Biological Material: The biological material we used in our study is Wistar rats from the Pasteur Institute of Algiers. The animals, which weigh between 200 and 250 grams, were acclimated to standard conditions of temperature and humidity. The breeding was conducted in polyethylene cages, lined with litter material of wood chips. The hygiene and prophylaxis were observed.

Parcelling Animals: Our study aims to study the incidence of depression in rats underwent ligation of the sciatic nerve and the identification of possible differences between male and female rats.

For this purpose, we defined four (04) experimental batches as follows:

- Male control (n = 08),
- Female control (n = 08),
- LIG male (n = 08),
- LIG female (n = 08).

Sciatic Nerve Ligation «Sciatic Nerve Injury»: The rat model *Ratus ratus* Wistar strain underwent ligature of the sciatic nerve while the sural nerve left intact [34]. The results lesions of hypersensitivity is marked in the lateral region of the leg that is innervated by the sural nerve was spared. The non-operated side of the rat was used as control.

The rats suffered surgery "sciatic nerve ligation" (*sciatic nerve injury*). Following this operation, the subjects received a treatment with anti-Cyclin at a dose of 0.3 ml per for five (05) days by intraperitoneal injection, after a period of complete rest for ten (10) days.

Experimental Protocol
Anesthesia:

- Rats anesthetized by intraperitoneal injection of a ketamine 0.3ml / 100g + a drop orally Largactil (chlorpromazin).
- Animals placed in a calm and quiet place until fully anesthetized.
- Verification of rats reflexes by pinching the tip of the tail and legs with a pair of tweezers to ensure the immobility of animals before any surgery.
- Shaving drapes (using an electric razor) slightly below the knee area to the hip area.
- Application of an ophthalmic ointment to the eyes of animals using a cotton swab.
- The animal was placed on its right side (or left) and placement of the left hind limb (or right) on a small platform in order to keep it high. Leg fixation with tape.
- Disinfection of the operative field with alternating scrubs of ethanol and betadine outside the surgical site.

Surgery:

- Location knee with the thumb of the left hand and use a scalpel to make an application. A few cm incision in the proximal longitudinal direction of the knee.
- Open the skin by blunt dissection using the tip of a pair of sterilized scissors.
- Separation of the muscular layer by dissection just next to the clearly visible blood vessel, close to the femur (thigh bone). The muscle layers are easily separated without bleeding, revealing the sciatic nerve right or left below the muscle.

It should be noted that in case of bleeding due to damage of a blood vessel near the knee, they must be sterilized using a cotton bud body to absorb the blood by pressing until the bleeding stops.

- Rat under a stereo microscope to gently separate the muscles with a pair of tweezers sterilized to clearly visualize the sciatic nerve.
- Identification of the area and the collateral saphenous branches of the sciatic nerve knowing that the sural nerve is the smallest of the three branches.
- Creation of a surgical tight knot around the sciatic nerve. If the first node is a member of the tight contractions will be observed.
- Cut the suture ends with a pair of micro scissors and gently closing the muscle layer. Adding a drop of lidocaine on the wound and suture with surgical knots.

Post-Surgery Period:

- Verification of the sufficiency of eye ointment.
- Rat placed in a clean cage under a paper towel in a comfortable posture to room temperature.
- Water and food are easily accessible for the animal operated.
- Intra-peritoneal injection of the antibiotic in a 0.3 ml dose for five (05) days after surgery.
- Conduct a battery of behavioral tests (neuropathic pain and anxiety and depression measures) after ten (10) days of the surgery.

Methods
Elevated Plus Maze (EPM) Test: The elevated plus maze test [35-37] is used to measure the degree of anxiety in rodents. It is composed of four arms (50 × 10cm), two open arms perpendicular to two closed arms with 40cm high walls of Plexiglas. The intersection of the four arms (central area) is a square of 10 × 10cm. The apparatus was elevated of 50 cm from the ground. The test was performed for 5 min by placing the animal in the central

area facing an open arm. Since the rate fears the empty and high spaces, his exploration of open arms shows a less anxious behavior. On the contrary, the more the animal remains in the closed arms, his behavior are known to be anxious. The 5 min sequences were recorded by a video camera to measure the following parameters: Open arms duration, closed arms duration, Open arms entries, closed arms entries and Freezing duration.

Forced Swimming Test (FST): The forced swim test [36-39] is a behavioral test of inducing despair in rats by placing the animal 15 min in a glass aquarium 54 cm height (34 × 60cm). This dimension ensures that the rat can´t escape by climbing to the edges of the device. The aquarium is filled with water (26°C) to a height of 40 cm, in order to ensure that the rat will not use his legs to keep the surface and thus force him to swim. The procedure of FST in rats occurs in two phases: the pre-test and test, separated by an interval of 24 hours. During the pre-test, the rat was placed for 15 min. At the end of session, the animal is immobile. The next day, the animal plunged into the aquarium for 5 min. The swimming session on each day was videotaped for behavioral analysis. The time of immobility, swimming and climbing are calculated.

Sucrose Preference Test (SPT): All the rats were submitted to 48 h of forced exposure to 1% sucrose solution in order to habituate to it [37], during which sucrose solution was the only fluid available for consumption, followed by two days of free access to food and water. After this, the rats were submitted to water deprivation for 16 "h" prior to performing the sucrose preference test; baseline test at day zero. The sucrose preference test was performed in the rat's home cage: two pre-weighted bottles, one containing tap water and another containing 1% sucrose solution, were presented to each rat. The bottles were weighed again after 1 "h" and the weight difference was considered to be the rat intake from each bottle. The sum of water and sucrose intake was defined as total intake and the sucrose preference was expressed as the percentage of sucrose intake from the total intake following the formula: % sucrose preference= sucrose intake X100/total intake.

Statistical Analysis: Data are presented as mean ± SEM. Data were analyzed by one-way ANOVA and Newman and Keuls as the post hoc test. Results were considered significant at p <0.05. Graph Pad Prism 5 for windows version 5.01 was used to do the analysis.

RESULTS

The following histograms represent the test results of forced swimming where the analysis of variance was conducted by the ANOVA1 and followed by a Newman-Keuls test, in case of significant differences between the different groups.

Forced Swiming Test:

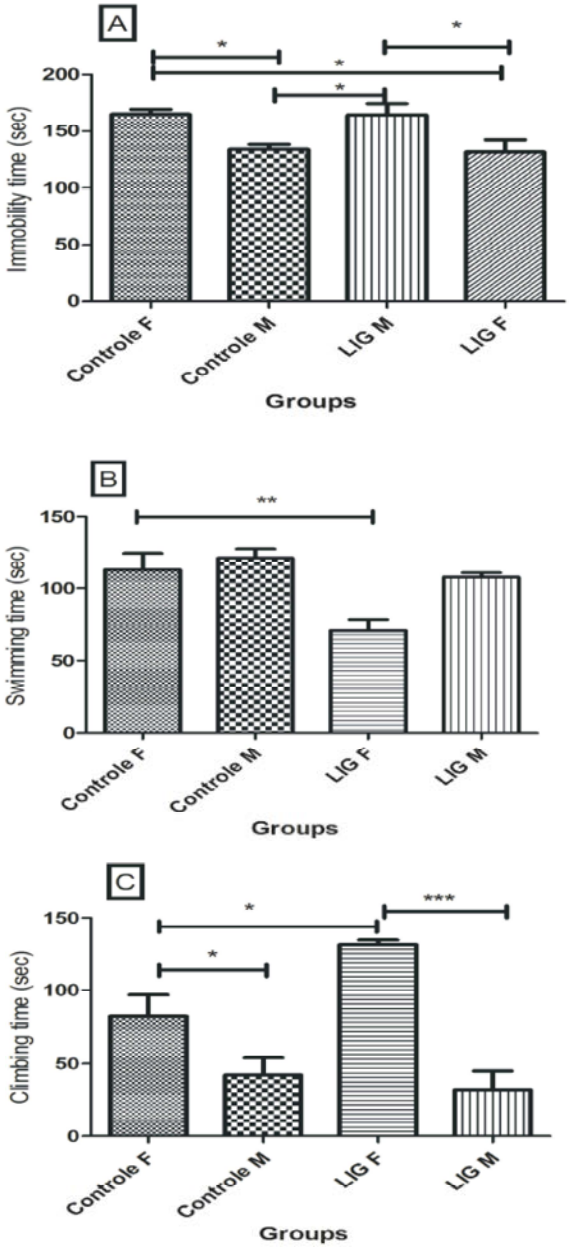


Fig. 1: Settings forced swimming in male animals and females ligated and unligated. (A: swimming time, B: immobility time, C: climbing time)

Regarding immobility time (Fig. 1B), it was observed a very highly significant (F = 21.93, R square = 0.7327, p <0.0001). The immobility time of the ligated rats was significantly higher than that of control rats (control M vs Lig M). The control rats have a significantly immobility time lower than that of control rats (control M vs control F). The immobility time is significantly reduced in rats than in controls control ligated rats (control M vs Lig F). We note further that the immobility time is higher in ligated females rats than in ligated males rats (Lig M vs Lig F).

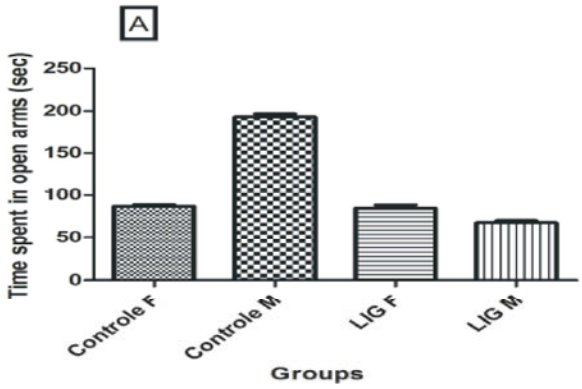
The test Newmen-Keurls and revealed a significant increase in immobility time in females compared to males and before and after ligation.

As to the swimming time (Fig. 1A), the analysis of the results showed significant differences between male and female animals and that, before and after ligation. It is worth mentioning a very highly significant (F = 33.77; R square = 0.8021, p <0.0001) between the control males rats and ligated females rats (control M vs Lig F), between male animals ligated and ligated females (control M vs Lig M).

Newman-Keurls test and certify a significant increase in swimming time in males compared to females before and after ligation.

Analysis of results for the climbing time we find reveals a significant increase in climbing time (Fig. 1 C) in females compared to males. A significantly lower level (F = 11.56, R square = 0.6012, p <0.0001) is reported in rats compared to controls rats controls (control F vs control M) and female control and ligated males (control F vs Lig M). The climbing time is significantly reduced in the control rats compared to that of ligated rats (control M vs Lig F). Finally, the escalation time is significantly higher in the ligated rats compared to ligated rats (Lig F vs Lig M).

The Elevated Plus Maze (EPM) Test:



Continued

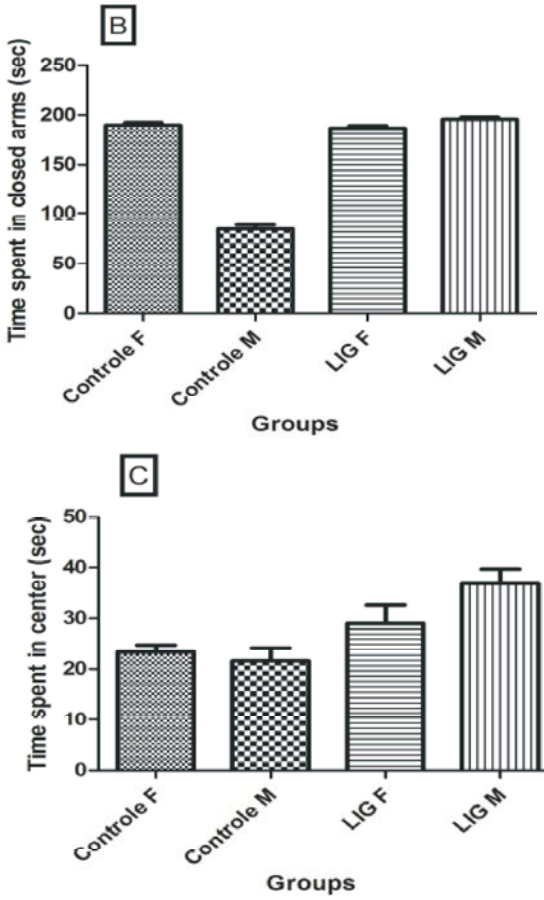


Fig. 2: Elevated Plus Maze (EPM) test parameters in male rats and female ligated and unligated (A: time spent in the open arms, B: time spent in the closed arms, C: time spent in the center of the device).

Time Spent in the Closed Arms: The ANOVA1 for time spent in the closed arms revealed a significant effect of sciatic nerve ligation (F = 320.3, R Square = 0.9717, p <0.0001).

The Newman-Keuls test watch that the level of anxiety is higher among females than among controls their male counterparts. This is revealed by the time spent in the closed arms greater in females (control M vs control M : t = 35.40, p <0.05). The results also show that the ligature of the sciatic nerve causes an increase in the level of anxiety, as shown in the time spent in the closed arms higher in rats after sciatic nerve ligation (control M vs LIG M : t = 37.40, p <0.05).

Time Spent in the Open Arms: The ANOVA1 for time spent in the open arms revealed a significant effect of sciatic nerve ligation (F = 353.8, R Square = 0.9743, p < 0.0001).

The Newman-Keuls test was reported time spent in open arms greater in males than in controls their female counterparts (control F vs Control M : t = 34.81, p <0.05). Similarly it is reported a significant decrease in the time spent in the open arms after sciatic nerve ligation (control M vs LIG M : t = 71.18, p <0.05).

Time Spent in the Center: The ANOVA1 for time spent in the center revealed a significant effect of sciatic nerve ligation (F = 6.592, R Square = 0.4139, p <0.0001).

The Newman-Keuls test for time spent in the center mainly reveals a significant increase in time spent at the center after sciatic nerve ligation (control M vs LIG M: t = 5.701, p <0.05).

Preference Sucrose Intake:

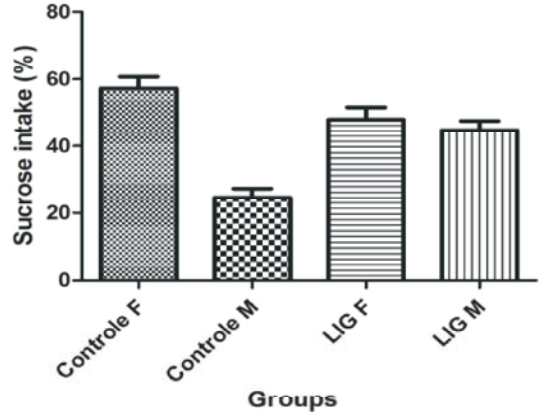


Fig. 3: Sucrose intake in rats males and females who have undergone or not the sciatic nerve ligation.

The preference for the sweet ANOVA1 revealed a significant effect of sciatic nerve ligation (F = 18,36; R Square = 0,6630, p <0.0001).

The Newman-Keuls test for preference shows that sweet sweet consumption level is significantly lower in males than in controls their female counterparts (Control M vs Control F: t = 10,17 ; p <0.05). It also appears that male rats who underwent ligation of the sciatic nerve had significantly higher sugar consumption levels (Control M vs LIG M t = 7,275, p <0.05).

DISCUSSION

Our aims and objectives is to investigate the effect of the sciatic nerve ligation of the degree of depression animals. It also includes a comparative study between males and females. Our results show that in controls the degree of depression in females is more important than in males, this is revealed by the immobility assessment time that are greater in females than in males. We attribute these variations in the degree of depression at the level of fluctuation of different sex steroids in females and males. Gender differences exist in the prevalence of depressive disorders. Some studies suggest that women respond better than men to action inhibitors of serotonin reuptake (SSRI) suggest that gonadal hormones modulate mood and drug response [40]. Significant gender differences were regularly reported because its impact on the prevalence and morbidity risk is twice as high among young adult women. The symptoms of depression are also sexual and disturbances in the circulating levels of ovarian hormones product would produce a behavioral depression [41]. In addition, behavioral changes associated with the estrous cycle, clearly showed a reduction of immobility and a higher active behaviors during proestrus [42]. From these observations, it would appear that estrogen would have a depressing effect [43]. Stocco (2008) suggest that this enzyme could be activated by various factors such as acute stress. The concentrations of estradiol in adult females and prepubertal rats could be related to the reduction of immobility and its increase observed during the test, revealing activation of the serotonin system [45]. The administration of 17ß-estradiol in female or male rats increases swimming and decreases immobility in the FST. Estradiol facilitates the effect of SSRI, such as fluoxetine, the antagonist of the 5-HT1A receptor and blocks the antidepressant effect of estradiol in the FST [46, 47]. Also, it is well known that estrogens also have anti-inflammatory properties. Studies in rats and mice have demonstrated the ability of estradiol to reduce tissue damage while decreasing the expression of Th1 cytokines involved in acute inflammatory response, such as Macrophage Migration Inhibitory Factor (MIF), TNFa, IL-1β and IFN-γ [43, 48].

Progesterone is a sex steroid. When synthesized by the nervous system from pregnenolone [49], it seems with neurosteroid metabolites (3α-hydroxy-5α-pregnan-20-one or allopregnenolone) have an antidepressant-like effect in the swim forced [50]. It also seems to provide the body with a protective barrier in response to constantly changing environment. This protection would be through the production of neuroactive steroids such as progesterone and its metabolites. It has been shown that these products are heavily involved in the installation of anxiety [51, 52] and depression.

By cons, testosterone would anxiolytic and antidepressant effects [53, 54]. Recent reports show that in adult males, stress increases testosterone possibly by stimulating the secretion of gonadotropin.

However current data argue against this observation since the levels of this hormone are similar between the control animals and those exposed to FST.

During inflammation, the circulating cytokines suppress reproductive function by activating the secretion of hypothalamic CRH and therefore inhibiting ovarian and testicular steroidogenesis [55]. Activation of pro-inflammatory cytokines by altering neurotransmission of serotonin, is a component of the depression. But, the anti-inflammatory anti inflammatory cytokines regulate the inflammatory response by inhibiting the production and action of inflammatory cytokines [56].

In males, we note that the sciatic nerve ligation causes increased levels of depression. This is revealed in our results in increased immobility time in the ligated males. The level of depression in males ligated would be the consequence of surgery that consisted in the sciatic nerve ligation. Many studies have examined the relationship between depression and inflammation [57] and between anxiety and inflammation [58]. In addition, the limited research on the state of anxiety and inflammation gave a mixed model results. Apparently, there is a two-dimensional relationship between the CNS and the immune system. More recently, research has shown that the steroid may also bind to receptors of specific neurotransmitters and neuronal excitability change [59]. The molecules steroids that act as neuromodulators are then called neurosteroids.

Substantial evidence supports the existence of reciprocal relationships of immune regulation by the HPA axis, with a focus on the hypothalamic CRH neuron, shown as a main junction between peripheral events and the responses of the central nervous system. In this regard, if the glucocorticoid secretion is physiologically active in response to an inflammatory process, in order to prevent substantial damage to tissue of the immune response, a number of experiments indicate that other reaction mediators inflammatory / immune, including cytokines, may activate the HPA axis by the neuron hypothalamic CRH [60]. In fact, the depression has been associated with increased circulating levels of the pro-inflammatory cytokine [61].

In females, the sciatic nerve ligation does not seems to cause a remarkable difference in the level of depression in control animals. We believe in fact that ligation increases the degree of depression, however this increase was not observed in this case due to the hyperdepressif state control animals. This we have already explained by the role played by sex steroids and partly by estrogen on depressive state.

We wish to draw attention to the fact that changes of immobility time observed during the FST are inversely proportional to swim time observed. As has been reported in several studies, this means that the nerve pathway is responsible for the occurrence of depression in our study through 5HT, as reported by Albert and François in 2010. In the same vein, we argue that the activation of the 5-hydroxytryptamine (5HT), reduced the immobility by increasing swimming.

During the Forced Swim Test, antidepressants, producing a noradrenergic and dopaminergic predominant elevation reduced the immobility by increased climbing time [63].

CONCLUSION

In this study, it appears that the rats suffered sciatic nerve ligation have a higher degree of depression. This is clearly visible in male subjects, where the immobility time is higher ligated rats compared to their not ligated. We explained the occurrence of this depression by inflammation occurred following surgery. This inflammation will spark changes in the immune system with an overproduction of proinflammatory cytokines and that these would depressinogenic as reported in several studies.

The FST also revealed a gender difference related to the state of depression. Indeed, the immobility time measured in controls female is higher than that measured at their male. This means that controls are more depressed females than males. The explanation for this difference is, in our view, to the hormonal differences between males and females. Involvement of sex steroids in the mood changes has been much documented. Thus, it has been attributed to testosterone antidepressant role. Other authors attribute, by cons, of estradiol depressinogenic effects. Another current research considers progesterone and some of its derivatives as substances with antidepressant-like effects. According to these authors, these derivatives of progesterone, such as pregnenolones are considered neuroactive steroids when they are in the brain.

REFERENCES

1. Woolf, C.J., 2010. What is this thing called pain ? J. Clin. Invest., 120: 3742-3744.
2. Marchand, F., M. Perretti and S.B.McMahon, 2005. Role of the immune system in chronic pain. Nat. Rev. Neurosci., 6(7): 521-532.

3. Moalem, G. and D.J. Tracey, 2006. Immune and inflammatory mechanisms in neuropathic pain. Brain Res. Rev., 51(2): 240-264.
4. Zuo, Y., N.M. Perkins, D.J. Tracey and C.L. Geczy, 2003. Inflammation and hyperalgesia induced by nerve injury in the rat: a key role of mast cells. Pain, 105(3): 467-479.
5. Leung, L. and C.M. Cahill, 2010. TNF-alpha and neuropathic pain-a review. J. Neuroinflammation, 7: 27.
6. Moalem, G., K. Xu and L. Yu, 2004. T lymphocytes play a role in neuropathic pain following peripheral nerve injury in rats. Neuroscience, 129(3): 767-777.
7. Sommer, C. and M. Kress, 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett., 361: 184-187.
8. Echeverry, S., X.Q. Shi and J. Zhang, 2008. Characterization of cell proliferation in rat spinal cord following peripheral nerve injury and the relationship with neuropathic pain. Pain, 135: 37-47.
9. Eddleston, M. and L. Mucke, 1993. Molecular profile of reactive astrocytes--implications for their role in neurologic disease. Neuroscience, 54: 15-36.
10. Yeh, T.H., Lee, Y. Da, S.M. Gianino and D.H. Gutmann, 2009. Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)- regulated glial proliferation. Glia, 57: 1239-1249.
11. Sung, B., G. Lim and J. Mao, 2003. Altered expression and uptake activity of spinal glutamate transporters after nerve injury contribute to the pathogenesis of neuropathic pain in rats. J. Neurosci, 23: 2899-2910.
12. Araque, A., V. Parpura, R.P. Sanzgiri and P.G. Haydon, 1999. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci, 22: 208-215.
13. Holsboer, F., 1999. Neuroendocrine studies and antidepressant drug development. Seishin Shinkeigaku Zasshi, 101: 711-716.
14. Vitkovic, L., J. Bockaert and C. Jacque, 2000. Inflammatory cytokines: neuromodulators in normal brain? J. Neurochem, 74(2): 457-71.
15. Ferreira, S.H., B.B. Lorenzetti, A.F. Bristow and S. Poole, 1988. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature, 334: 698-700.
16. Ramer, M.S., E.J. Bradbury and S.B. McMahon, 2001. Nerve growth factor induces $P2X_3$ expression in sensory neurons. J. Neurochem, 77(3): 864-875.
17. Opree, A. and M. Kress, 2000. Involvement of the proinflammatory cytokines tumor necrosis factor-alpha, IL-1 beta and IL-6 but not IL-8 in the development of heat hyperalgesia: effects on heat-evoked calcitonin gene-related peptide release from rat skin. J. Neurosci, 20(16): 6289-6293.
18. Winkelstein, B.A., M.D. Rutkowski, S.M. Sweitzer, J.L. Pahl and J.A. DeLeo, 2001. Nerve injury proximal or distal to the DRG induces similar spinal glial activation and selective cytokine expression but differential behavioral responses to pharmacologic treatment. J. Comp. Neurol., 439(2): 127-139.
19. Reeve, A.J., S. Patel, A. Fox, K. Walker and L. Urban, 2000. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. Eur. J. Pain, 4(3): 247-257.
20. Koch, A., K. Zacharowski, O. Boehm, M. Stevens, P. Lipfert, H.J. Von Giesen, A. Wolf and R. Freynhagen, 2007. Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. Inflamm. Res., 56(1): 32-7.
21. Uceyler, N., J.P. Rogausch, K.V. Toyka and C. Sommer, 2007. Differential expression of cytokines in painful and painless neuropathies. Neurology, 69(1): 42-9.
22. Uceyler, N. and C. Sommer, 2008. Cytokine regulation in animal models of neuropathic pain and in human diseases. Neurosci Lett, 437: 194-198.
23. Cartmell, T., G. Luheshi and N. Rothwell, 1999. Brain sites of action of endogenous interleukin-1 in the febrile response to localized inflammation in the rat. J. Physiol. (Lond), 518: 585 94 585-94.
24. Dunn, A., J. Wang and T. Ando, 1999. Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress. Adv. Exp. Med. Biol., 461: 117-27.
25. Marx, C., M. Ehrhart-Bornstein, W. Scherbaum and S. Bornstein, 1998. Regulation of adrenocortical function by cytokines-relevance for immune-endocrine interaction. Horm. Metab. Res., 30(6-7): 416-20.
26. Thurin, J.M., 1996. Une vie sans soi. Clinique et interprétation des maladies psychosomatiques. Frison Roche, pp: 237.

27. Tanabe, M., Y. Nagatani, K. Saitoh, K. Takasu and H. Ono, 2009. Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice. Neuropharmacology, 56(3): 702-708.
28. Kim, D., B. You, E.K. Jo, S.K. Han, M.I. Simon and S.J. Lee, 2010. NADPH oxidase 2- derived reactive oxygen species in spinal cord microglia contribute to peripheral nerve injury induced neuropathic pain. Proc. Natl. Acad. Sci. USA, 107(33): 14851-14856.
29. Ibi, M., K. Matsuno, D. Shiba, M. Katsuyama, K. Iwata, T. Kakehi, T. Nakagawa, K. Sango, Y. Shirai, T. Yokoyama, S. Kaneko, N. Saito and C. Yabe-Nishimura, 2008. Reactive oxygen species derived from NOX1/NADPH oxidase enhance inflammatory pain. J. Neurosci, 28(38): 9486-9494.
30. Naik, A.K., S.K. Tandan, Dudhgaonkar, S.P. Jadhav, S.H. Kataria, M.V.R. Prakash and D. Kumar, 2006. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-L-cysteine in rats. Eur. J. Pain, 10(7): 573-579.
31. McMahon, S.B., W.B. Cafferty and F. Marchand, 2005. Immune and glial cell factors as pain mediators and modulators. Exp. Neurol., 192: 444-462.
32. Ueda, H., 2008. Peripheral mechanisms of neuropathic pain - involvement of lysophosphatidic acid receptor-mediated demyelination. Mol. Pain, 4: 11.
33. Bennett, G.J. and Y.K. Xie, 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain, 33(1): 87-107.
34. Zaafour, M., A. Fraia, H. Frih, S. Guernine, S. Djemli and B. Ali Rachedi, Assessment of steroids changes (testosterone and oestradiol) after BCG inoculation in sciatic nerve injury model (male Wistar rat). Global Veterinaria (in press).
35. Porsolt, R.D., M. LePichon and M. Jalfre, 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature, 266(5604): 730-732.
36. Frih, H., R. Djenidi, B. Ali Rachedi, N. Frih, A. Tahraoui and M. Bairi, 2010. [Ketoconazole antagonizes the immune and gonadotropic systems in the forced swimming test in male Wistar rats]. [Article in French] Le kétoconazole antagonise les effets immuno-gonadotropes au test de la nage forcée chez le rat mâle Wistar. Canadian Journal of Physiology and Pharmacology, 88(7): 733-744.
37. Fraia A., B. Ali Rachedi, S. Zouiche, S. Djemli and H. Frih, 2015. Polyphenon E Could Improve Negative Changes Caused by Chronic Mild Stress in Male Wistar Rats. Global Veterinaria, 4(4): 478-489.
38. Porsolt, R.D., G. Anton, N. Blavet and M. Jalfre, 1978. Behavioral despair in rats: A new model sensitive to antidepressant treatment. Eur. J. Pharmacol., 47(4): 379-391.
39. Porsolt, R.D., A.G. Bertin, N. Blavet, M. Deniel and M. Jalfre, 1979. Immobility induced by the FST in rodents: effects of agents wich modify central catecholamines and serotoninergic activity. Eur. J. Pharm., 57: 201-210.
40. Frye, C.A. and A.A. Walf, 2002. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav, 41(3): 306-15.
41. Frye, C.A. and A.A. Walf, 2009. Depression-like behavior of aged male and female mice is ameliorated with administration of testosterone or its metabolites. Physiol. Behav., 97: 266-269.
42. Contreras, C., L. Martínez-Mota and M. Saavedra, 1998. Desipramine restricts estral cycle oscillations in swimming. Prog. Neuropsychopharmacol. Biol. Psychiatry, 22(7): 1121-8.
43. Verdu, E.F., Y. Deng, P. Bercik and S.M. Collins, 2002. Modulatory effects of estrogen in two murine models of experimental colitis. Am. J. Physiol. Gastrointest Liver Physiol., 283(1): G27-36.
44. Stocco, C., 2008. Aromatase expression in the ovary: hormonal and molecular regulation. Steroids, 73(5): 473-87.
45. Estrada-Camarena, E., A. Fernández-Guasti and C. López-Rubalcava, 2006. Participation of the 5-HT1A receptor in the antidepressant-like effect of estrogens in the forced swimming test. Neuropsychopharmacology, 31: 247-55.
46. Estrada-Camarena, E., A. Fernández-Guasti and C. López-Rubalcava, 2004. Interaction between estrogens and antidepressants in the forced swimming test in rats. Psychopharmacology, 173: 139-45.
47. Martínez-Mota, L., J. Cruz Martínez, M. Márquez-Baltazar and A. Fernández-Guasti, 2008. Estrogens participate in the antidepressant-like effect of desipramine and fluoxetine in male rats. Pharmacol. Biochem. Behav., 88(3): 332-40.

48. Houdeau, E., R. Moriez, M. Leveque, C. Salvador-Cartier, A. Waget, L. Leng, L. Bueno, R. Bucala and J. Fioramonti, 2007. Sex steroid regulation of macrophage migration inhibitory factor in normal and inflamed colon in the female rat. Gastroenterology, 132(3): 982-993.
49. Craft, R.M., J.S. Mogil and A.M. Aloisi, 2004. Sex differences in pain and analgesia: the role of gonadal hormones. Eur. J. Pain, 8(5): 397-411.
50. Andrade, S., S.L. Silveira, B.D. Arbo, B.A. Batista, R. Gomez, H.M. Barros and M.F. Ribeiro, 2010. Sex-dependent antidepressant effects of lower doses of progesterone in rats. Physiol Behav., 99(5): 687-90.
51. Frih, H., A. Latreche, B. Ali Rachedi, R. Djenidi, L. Sahraoui and A. Tahraoui 2012. Evolution of Elevated Plus Maze Test (Anxiety) and Porsolt Swimming Test Depression) Parameters in Wistar Female Rats Treated with Low Dose of Toluene from the 4th to 14 thday of Pregnancy: Implication of Progesterone to Protect GABAergic route. Journal of Depression and Anxiety, 1(2): 1.
52. Neumann, I.D., A. Wigger, G. Liebsch, F. Holsboer and R. Landgraf, 1998. Increased basal activity of the hypothalamo–pituitary–adrenal axis during pregnancy in rats bred for high anxiety-related behaviour. Psychoneuroendocrinology, 23: 449-463.
53. Carrier, N. and M. Kabbaj, 2012.Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. Horm Behav., 61(5): 678-85.
54. Carrier, N., S.K. Saland, F. Duclot, H. He, R. Mercer and M. Kabbaj, 2014. The Anxiolytic and Antidepressant-like Effects of Testosterone and Estrogen in Gonadectomized Male Rats. Biological Psychiatry, 12.024.
55. Martínez-Mota, L., R.E. Ulloa, J. Herrera-Pérez, R. Chavira and A. Fernández-Guasti, 2011. Sex and age differences in the impact of the forced swimming test on the levels of steroid hormones. Physiology & Behavior, 104: 900-905.
56. Baumann, H. and J .Gauldie, 1994. The acute phase response. Immunol Today, 15(2): 74-80.
57. Dantzer, R., J.C. O'Connor, G.G. Freund, R.W. Johnson and K.W. Kelley, 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci., 9(1): 46-56.
58. Kubzansky, L.D. and I. Kawachi, 2000. Going to the heart of the matter: do negative emotions cause coronary heart disease ? J. Psychosom. Res., 48(4-5): 323-37.
59. Rupprecht, R., 2003. Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. Psychoneuroendocrinology, 28(2): 139-168.
60. Sapolsky, R.M., L.M. Romero and A.U. Munck, 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. Endocr. Rev., 21(1): 55-89.
61. Zorrilla, E.P., L. Luborsky, McKay, Jr, R. Rosenthal, A. Houldin, A. Tax, R. McCorkle, D.A. Seligman and K. Schmidt, 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav. Immun., 15(3): 199-226.
62. Albert, P.R. and B.L. François, 2010. Modifying 5-HT1A receptor gene expression as a new target for antidepressant therapy. Front Neurosci., 4: 35.
63. Kawaura, K., S. Honda, F. Soeda, T .Shirasaki and K.A. Takahama, 2010. Novel antidepressant-like action of drugs possessing GIRK channel blocking action in rats. Yakugaku Zasshi, 130(5): 699-705.