

Original Research Article

Postmenopausal status exacerbates stress-related neurological disorders in rats

ABSTRACT

Background: Postmenopause is a physiological state often associated with neuronal disorders (NDs). Nowadays, dynamic society leads to many types of stress which are related with NDs. Thus, since the worldwide population is aging and consequently presents more postmenopausal (PM) women.

Aims: The present study aimed to evaluate the effects of social instability stress (SIS) and immobilization stress (IS) in PM conditions.

Place and Duration of Study: the study took place in the Laboratory of Animal Physiology of the University Yaoundé I (Cameroon) for 3 months from September to February.

Study design: an 84-day ovariectomized model was used to induce a PM syndrome.

Methodology: SIS and IS were induced during PM. Behavioral status, biochemical and histopathological analyses were carried out. This study showed that although SIS and IS paired or not do not induce observable effects in sham-operated like in ovariectomized rats.

Results: SIS combined with IS potentialized NDs induced by low estrogen level. Furthermore, ovariectomized stressed rats are more depressed and anxious than the control. They presented hippocampal inflammation, oxidative stress, and memory impairment owing to low levels of ACh, and reduced neuronal expression in hippocampus. Overall, PM makes worse NDs linked to stress in rats.

Conclusion: stress promoted oxidative stress, neuroinflammation, and neuronal death leading to greater affective and cognition disorders when estrogen levels are low. Thus, is a need to focus on the management of stress during menopausal life.

Keywords: Postmenopause, stress, neuronal disorders, estrogen level, ovariectomy

1. INTRODUCTION

In women, aging is closely linked to menopause, a physiological state due to ovarian insufficiency. Estrogen depletion related to this ovarian shortage induced among others climacteric symptoms (hot flashes, night sweats, sleep problems, anxiety, depression, low libido) and symptoms of vulvovaginal including vaginal dryness, dysbiosis, and dyspareunia. Physicians and researchers are viewing practical steps to ease lives, address symptoms, and afford primary prevention advice for chronic conditions such as osteoporosis, cardiovascular and neurodegenerative diseases [1,2]. Neuronal loss, linked or not with menopause, is a worldwide health problem with a huge clinical care problem. In PM women, evidence showed that there is an emphasis on the prevalence of Alzheimer's disease and the decrease of steroid hormones. Indeed, estrogens show protective activity on brain

functions such as cognition [3]. The causes of neuronal death in PM conditions includes apoptosis, neuroinflammation, and high reactive oxygen species in hippocampal regions [4-17].

As well as menopause, changes in hippocampal genomic expression are commonly related to stress. Indeed, stress seems to be a catch-all concept and is a natural biological reaction to external stimulation. The term 'stress' refers to a vague feeling of unease and the difficult situations that cause it [8,9]. Based on its impact, stress can be classified as eustress, which is the positive way experienced by people with prominent arousal, and distress with unpleasant effects on both physical and psychological health [10]. Stress can also be classified according to its etiology. Indeed, they are social, physical, and psychological stress, which affects the predefined body's steady state called homeostasis [11]. Stress is multifactorial and can be due to many factors. COVID-19, sedentary, socio-economic issues, and mental health disorders are among the most important causes of stress in today's society [12-14].

Menopausal women are exposed to social and physical stress during menopause. Indeed, clinical investigation reported that socio-economic level is positively correlated with attitude regarding menopause. Furthermore, women usually complain about asthenia and slowed physical activities during the menopause stage [15-17]. It is well known that stress can promote neuronal impairment in healthy people, but few studies have focused on the impacts of stress on physiological pathways in menopausal women [18-20]. Thus, this study aimed to assess the effects of stress in PM conditions. Herein, the effects of social instability stress (SIS) combined or not with immobilization stress (IS) were assessed in a PM model of ovariectomized rats. We hypothesize that social instability stress associated with immobilization stress is followed by a worsening of neuronal impairment linked to the postmenopause stage as well as memory dysfunction and psychological disturbances.

2 MATERIAL AND METHODS

2.1. Experimental protocol

Forty female rats aged 8-10 weeks were randomized into two sets, a set of 20 rats sham-operated and a set of 20 rats ovariectomized using a dorsal approach according to the protocols described by Mengue et al. [21] and Minami et al. [22]. After 2 weeks of healing of the injured dorsal region, sets of rats were divided into 4 groups, including a control, a group of immobilized animals (IS), a group of animals with social instability stress (SIS), and a group stress up with the 2 types of stress (SIS + IS). The immobilization stress (IS) was induced for 70 days after 14 days of healing. Social instability stress was induced from day 69 to day 84. Behavioral tests were carried out 2 weeks before sacrifice: - Novel Object Recognition test (NOR) for the assessment of memory - Caudal Tail Suspension (CTS) and Sucrose Preference tests (SP) for the assessment of depressive behavior - Novelty Suppressed Feeding (NSF) and Open Arena tests (OA) for the evaluation of anxiety. Animals were sacrificed under anesthesia after 12 hours of non-hydric fasting. Blood was collected in dry, labeled test tubes for centrifugation at 4°C and 3000 rpm for 15 minutes. The serum obtained was used to assess corticosterone and estradiol levels. After the opening of the skull, the hippocampal region from each rat was isolated. 0.4 grams of the left hemisphere of each hippocampal region was homogenized in Tris-HCl buffer. After centrifugation (3000 rpm at 4°C for 25 minutes) the supernatant was collected and stored at -20°C for the determination of levels or activity of oxidative stress parameters (MDA, GSH, nitrites, and catalase), the assessment of concentrations of some inflammatory markers (IL-6, TNF- α , and IF- γ), the evaluation of some neuromodulator's levels (GABA, serotonin (5-

HT) and acetylcholine (ACh)). The right hemisphere of the hippocampal region and the cerebral cortex were fixed for histopathological analyses (Figure 1).

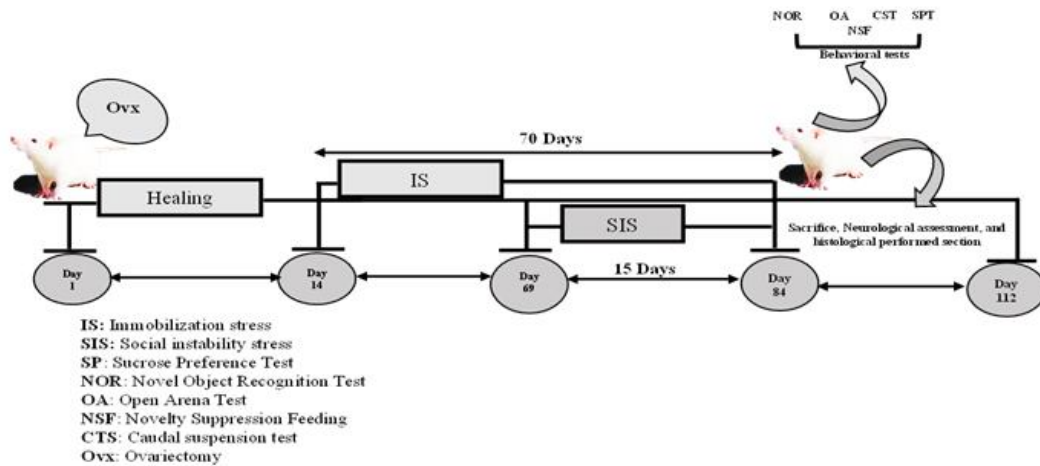


Figure 1: Experimental paradigm

2.2. Social and Physical stress induction

Social stress was induced in rats 69 days after surgery and for 15 days daily by a social instability model. Social instability stress (SIS) was induced according to the protocol described by Koert et al. [23]. Each rat was removed from its familiar cage (A) for 1 hour or cohabited with another rat to be introduced into another cage (B) with a new partner. Once the 1 hour had elapsed, the rat was reintroduced into its cage A for the rest of the day. Physical stress was induced by immobilization. The immobilization stress (IS) was performed using the cage volume reduction method according to the protocols described by Marmonti et al. [24] and Marmonti et al. [25]. Two weeks after surgery, rats from the non-immobilized groups were introduced 2 by 2 into plastic cages with a volume of 8038.40 cm³. Rats in the immobilized groups were placed in the same number of animals in plastic cages with a volume reduced by 80%, which means a volume of 1607.68 cm³.

2.3. Behavioral tests

The Novel Object recognition test (NOR) was first used by Ennaceur and Delacour in 1988 to assess episodic memory. NOR is based on the natural tendency of rodents to preferentially explore a new object over a familiar one [26]. Novelty-suppressed feeding test (NSF) was carried out to assess anxiety in rodents. NSF was performed according to the protocol of Blasco-Serra et al. [27]. The assessment is done by measuring the latency time for an animal to approach and eat a portion of familiar food in a new aversive environment after non-hydric fasting of 24 h. Open arena test (OA) was used to assess rodents' locomotor activity, exploration, and emotional reactivity [28]. The caudal tail suspension test (CTS) was performed according to the prototype described by Cryan et al. [29]. CTS was set up to evaluate depression. The sucrose preference test (SP) was used here to determine hedonic behavior, one of the main features of major depression in humans. The rats were trained to consume a 1% sucrose solution before the start of protocol (30).

2.4. Biochemical analysis

Blood samples were used to determine estradiol and corticosterone serum levels. The Elabscience ELISA commercial diagnostic kit was used to measure seric levels of estradiol and corticosterone. Enzyme-linked immunosorbent assay system was used to measure hippocampal interleukin 6 (IL-6), Tumor Necrosis Factor- α (TNF- α), and interferon- γ (IF- γ) levels using commercial diagnostic ELISA kits Elabscience. The levels of serotonin (5-HT), - γ -amino butyric acid (GABA), and acetylcholine (ACh) were determined using commercial diagnostic ELISA kits Elabscience. Malondialdehyde (MDA) and reduced glutathione (GSH) in brain homogenate were determined using the procedure described by Wilbur [32] and Ellman [33], respectively, while the nitrites content was determined using the method described by Green et al. [34].

2.5. Histopathological analysis of the brain and neuron counting

The hippocampal and cortex region was fixed (2 weeks) in 10 % buffered formalin, then trimmed and dehydrated in alcohol of croissant gradient (70 %, 80 %, 90 %, and 100 % (3 baths)). Following alcohol dehydration, tissues were clarified in 2 baths of xylene (1h30 min per bath) and impregnated in liquid paraffin at 60 °C (for 5 hours). The number of hippocampal neurons in the CA1 and CA3 regions were assessed on microphotography obtained using a light microscope (Leitz Wetzlar Germany 513) connected with a digital camera Celestron 44421 linked to a computer. The images captured by the software DCM35 were transferred and analyzed using the software Image J version 2-20160205.

2.6 Data analysis

All statistical analyses were performed with the GraphPad Prism 8.0.1 software. Variances of groups were found to be homogeneous, then comparisons between groups were made using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test. The results were expressed as mean \pm standard error of the mean. Differences were considered significant at the probability $P < .05$.

3. RESULTS

3.1 Effect of social instability stress (SIS) and immobilization stress (IS) on psychological disorders

3.1.1 Effects on anxiety

Open arena parameters such as grooming number and time spent in the center of the arena showed no difference between control and stressed animals regarding sham-operated (Table 1). Besides, in ovariectomized rats, all the parameters of OA showed differences compared to sham control. Furthermore, in estrogen-deficient animals, SIS and IS reduced significantly both lines crossed the number and time spent in the center of the arena. Social instability stress induced significant effects on OA parameters than immobilization stress in ovariectomized rats. The combination of SIS and IS induced remarkable effects on OA parameters compared to ovariectomized control. As against sham-operated rats, ovariectomized animals paired SIS + IS caused a significant ($P < .01$) reduction of GABA concentrations in the hippocampus compared to the control. Furthermore, the social instability stress linked with immobilization stress induced a notable decrease in serotonin (5-HT) ($P < .01$) levels compared to the ovariectomized (Ovx) control.

Table 1: Effects of social instability stress (SIS) and immobilization stress (IS) on OA parameters and GABA and serotonin

Sets	SHAM				Ovx			
	Control	IS	SIS	IS + SIS	Control	IS	SIS	IS + SIS
	Neurotransmitters levels (GABA and serotonin)							
GABA (pg/mL)	46.3 ± 2.5	42.5 ± 2.2	38.6 ± 3.5	38.3 ± 2.5	33.5 ± 1.8 ^a	30.5 ± 1.6 ^b	28.9 ± 2.9 ^y	20.6 ± 2.0 ^{y2}
5-HT (pg/mL)	66.3 ± 2.3	62.3 ± 3.5	62.6 ± 1.3	54.1 ± 1.3 ^a	54.3 ± 1.6 ^a	46.5 ± 1.5 ^y	44.9 ± 3.2 ^{y1}	36.2 ± 1.1 ^{y2}
	Open arena test parameters							
Crossing	49.21 ± 3.43	39.67 ± 0.48	36.19 ± 1.09 ^b	36.25 ± 1.88 ^b	34.21 ± 1.26 ^y	30.50 ± 1.14 ^{y1}	25.21 ± 3.11 ^{y2}	18.80 ± 1.15 ^{y3}
Grooming	6.18 ± 0.63	6.60 ± 0.92	7.40 ± 1.32	8.20 ± 0.58	10.60 ± 0.50 ^b	10.60 ± 0.51 ^b	12.11 ± 0.94 ^y	17.60 ± 1.74 ^{y2}
Time spent in the center of arena (s)	20.75 ± 2.28	19.80 ± 2.20	16.60 ± 1.63	17.40 ± 2.18	11.60 ± 1.74 ^a	11.80 ± 1.15 ^a	10.20 ± 1.24 ^b	6.00 ± 1.12 ^{y1}

Mean ± S.E.M = Mean values ± Standard error of means of six experiments ^a $P < .05$, ^b $P < .01$, ^y $P < .001$ significant from sham-operated control, ¹ $P < .05$, ² $P < .01$, ³ $P < .001$ significant from ovariectomized control.

For the NSF, there were no significant differences in the latency to bite food in a familiar environment in all the groups. Besides, ovariectomy induced after 112 days a significant increase ($P < .001$) in the latency to bite food in an aversive environment compared to sham control. (Figure 2).

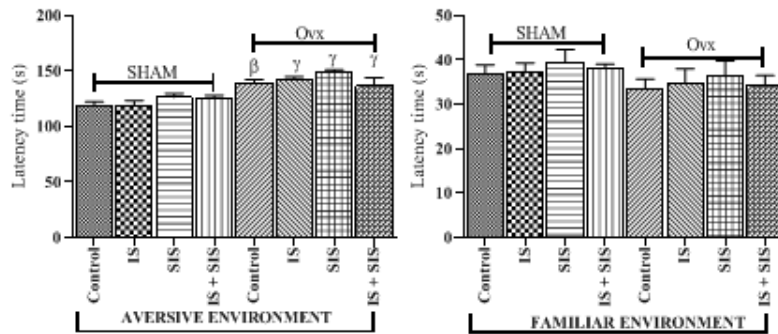


Figure 2: Effects of social instability stress (SIS) and immobilization stress (IS) in NSF
 Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments ^a $P < .05$, ^b $P < .01$, ^c $P < .001$ significant from sham-operated control.

3.1.2 Effects on depression

Ovariectomy induced a significant increase ($P < .05$) in the immobility time during the caudal tail suspension test (CTS) compared to sham control (Figure 3A). By the way, pairing SIS with IS in ovariectomized rats induced a significant ($P < .001$) increase in the immobility time during CTS compared to ovariectomized control. As shown in figure 3B, SIS ($P < .001$) and pairing SIS with IS ($P < .01$) reduced significantly the sucrose preference index in sham-operated rats compared to the control. The lack of estrogen promotes the action of stress. Hence, ovariectomized animals presented a reduced sucrose preference index compared to sham control. Furthermore, in SIS + IS ovariectomized rats, the sucrose preference index is significantly low ($P < .01$) compared to ovariectomized control.

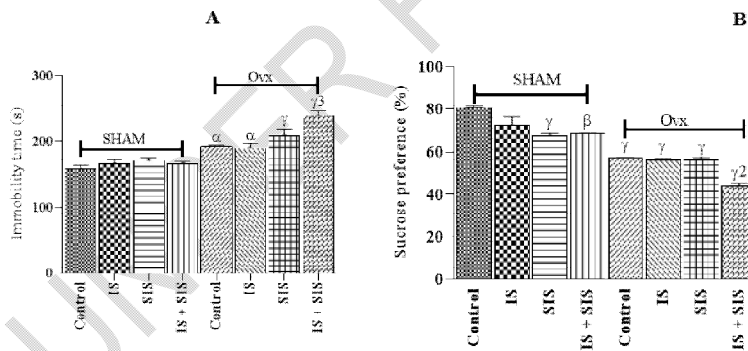


Figure 3: Effects of social instability stress (SIS) and immobilization stress (IS) in CTS and SP

Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments ^a $P < .05$, ^b $P < .01$, ^c $P < .001$ significant from sham-operated control, ² $P < .01$, ³ $P < .001$ significant from ovariectomized control.

Figure 4 shows stress-related effects on neurons expression in amygdala. The sham-operated control presented a normal structure of neurons with well differentiated nuclei surrounded by cytoplasm. Sham-operated rats exposed to both SIS and IS presented hyperchromatic nucleus compared to control. In the SIS + IS ovariectomized animal, there is a noticed presence of hyperchromatic nucleus, neuronal cytoplasmic shrinkage and neuronal death. Histomorphometric analysis of the amygdala shows a considerable increase in the number of neurons with hyperchromatic nuclei in animals stressed with both social and immobilization stress ($P < .001$).

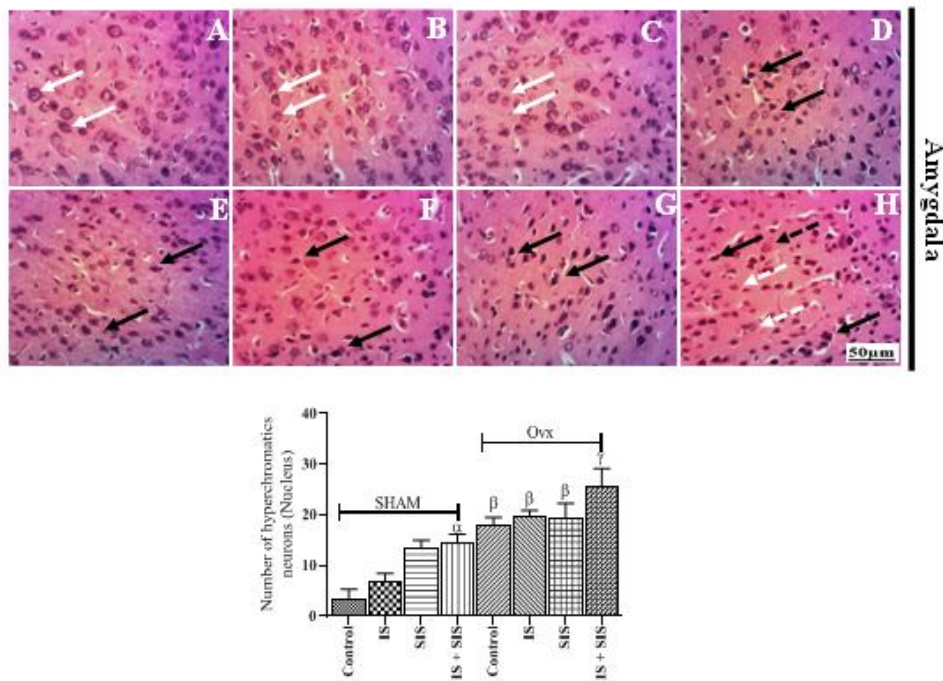


Figure 4: Effects of social instability stress (SIS) and immobilization stress (IS) on neuronal expression in the amygdala (H-E, 200X).

A = Sham-operated control, B = Sham-operated + IS, C = Sham-operated + SIS, D = Sham-operated + IS + SIS, E = Ovariectomized control, F = Ovariectomized + IS, G = Ovariectomized + SIS, H = Ovariectomized + IS + SIS. White arrow: normal neuron; black arrow: Hyperchromatic nucleus; broken line black arrow: neuronal cytoplasmic shrinkage; broken line white arrow: neuronal death. Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments: ^a $P < .05$, ^b $P < .01$, ^c $P < .001$ significant from sham-operated control.

3.2 Effects on memory dysfunction

3.2.1 Effects on NOR and Ach level

Over the 112 days of ovariectomy, a significant increase in exploration time for object A ($P < .01$) and the opposite result for object B ($P < .001$) were observed in NOR for Ovx rats compared to sham control. Besides, the lack of estrogen increased the discrimination index ($P < .001$) in rats compared to Sham-operated animals. However, in ovariectomized rats, IS like SIS increased significantly the exploration time for object A ($P < .01$), and their association induced not only a greater augmentation of exploration time ($P < .001$) but also a significant decrease ($P < .05$) of discrimination index compared to the Ovx control. The two

types of stress individually reduced significantly the acetylcholine (ACh) levels in the hippocampus in both sham-operated and ovariectomized rats compared to sham control. Furthermore, the social instability stress linked with immobilization stress induced a greater decrease of ACh ($P < .001$) levels compared to the control (Figure 5D).

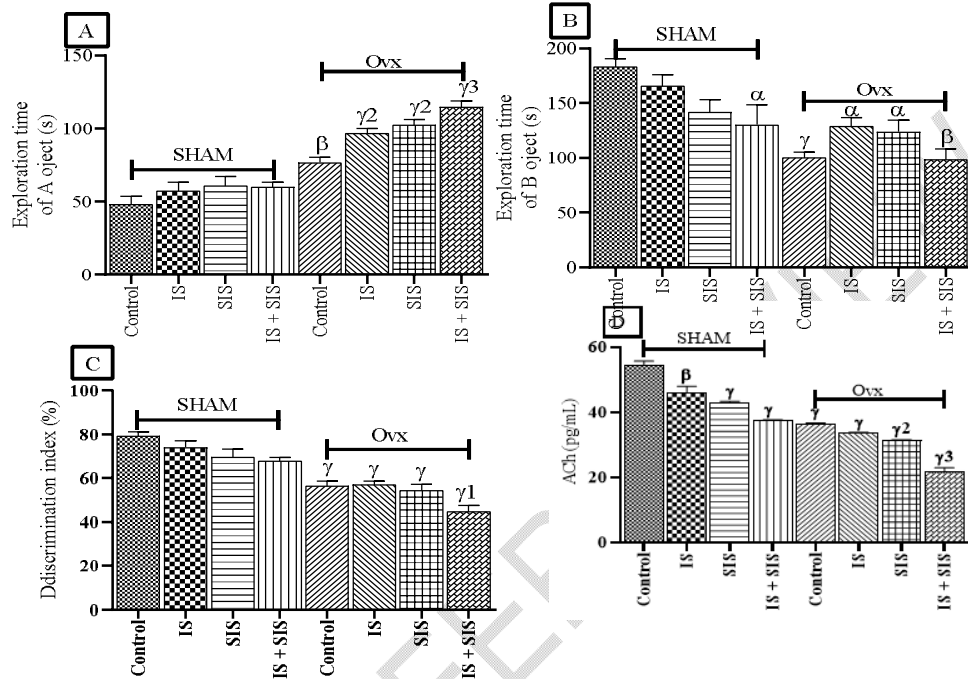


Figure 5: Effects of social instability stress (SIS) and immobilization stress (IS) in NOR
Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments ^a $P < .05$, ^b $P < .01$, ^γ $P < .001$ significant from sham-operated control. ¹ $P < .05$, ² $P < .01$, ³ $P < .001$ significant from ovariectomized control.

3.2.2 Effects on hippocampal oxidative stress

Pairing SIS with IS induced a significant decrease in catalase activity ($P < .01$) and nitrites level ($P < .001$) compared to the control. Whereas ovariectomy reduced CAT activity, GSH, and nitrites activity compared to sham control. In the PM conditions, the combination of two types of stress induced a significant decrease in CAT activity ($P < .01$) and GSH level ($P < .001$) compared to ovariectomized control (Table 2).

Table 2: Effects of social instability stress (SIS) and immobilization stress (IS) on some markers of hippocampal oxidative status

Parameters		MDA ($\mu\text{mol/g}$ of tissue)	GSH ($\mu\text{mol/g}$ of tissue)	CAT ($\mu\text{mol/g}$ of tissue)	Nitrites ($\mu\text{mol/g}$ of tissue)
Groups					
SHAM	Control	10.78 \pm 1.32	17.75 \pm 1.53	58.18 \pm 2.88	0.12 \pm 0.01
	IS	13.00 \pm 1.35	14.23 \pm 0.42	40.60 \pm 5.71 ^a	0.10 \pm 0.00
	SIS	15.33 \pm 1.08	13.82 \pm 0.79	37.65 \pm 5.17 ^a	0.08 \pm 0.00 ^y
	IS + SIS	16.58 \pm 2.42	13.76 \pm 0.92	35.58 \pm 3.95 ^a	0.07 \pm 0.00 ^y
Ovx	Control	21.60 \pm 0.60 ^y	13.01 \pm 0.50 ^a	32.25 \pm 1.74 ^b	0.07 \pm 0.00 ^y
	IS	21.26 \pm 0.60 ^y	12.38 \pm 0.81 ^a	33.25 \pm 3.15 ^b	0.06 \pm 0.00 ^y
	SIS	23.21 \pm 1.44 ^y	8.17 \pm 0.87 ^{y2}	26.25 \pm 2.25 ^{y1}	0.05 \pm 0.00 ^y
	IS + SIS	30.42 \pm 2.06 ^{y2}	7.34 \pm 0.79 ^{y3}	20.49 \pm 0.82 ^{y2}	0.05 \pm 0.00 ^y

Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments ^aP < 0.05, ^bP < 0.01, ^yP < 0.001 significant from sham-operated control; ¹P < .05, ²P < .01, ³P < .001 significant from ovariectomized control.

3.2.3 Effects on hippocampal neuroinflammation

In sham-operated animals, pro-inflammatory cytokines cerebral levels including interleukin-6 (IL-6) and interferon gamma (IF- γ) presented no observable differences in both control and stressed rats (Table 3). However, IS alone ($P < .05$) or paired with SIS ($P < .001$) induced a significant increase in the hippocampus level of tumor necrosis factor-alpha (TNF- α) in sham-operated animals compared to the control. Besides, ovariectomy resulted in a significant increase in IL-6 ($P < .05$), TNF- α ($P < .001$), and IF- γ ($P < .05$) compared to Sham-operated control. Paired with IS, SIS induced a significant increase of all the pro-inflammatory cytokines investigated in the brain in ovariectomized rats. Indeed, in IS + SIS ovariectomized rats, the levels of IL-6 ($P < .01$), IF- γ ($P < .001$), and TNF- α ($P < .01$) were noticeable than in ovariectomized control.

Table 3: Effects of social instability stress (SIS) and immobilization stress (IS) on some pro-inflammatory cytokines

Sets	SHAM				Ovx			
	Control	IS	SIS	IS + SIS	Control	IS	SIS	IS + SIS
IF- γ (pg/mL)	254.1 \pm 7.2	256.2 \pm 4.5	266.6 \pm 8.7	292.8 \pm 4.7	299.7 \pm 7.6 ^a	295.5 \pm 4.8 ^a	300.1 \pm 6.4 ^a	364.9 \pm 17.6 ^{y3}
IL-6 (pg/mL)	39.2 \pm 3.7	41.3 \pm 3.5	39.8 \pm 2.9	45.9 \pm 3.6	54.6 \pm 3.6 ^a	57.3 \pm 1.1 ^b	58.6 \pm 2.54 ^b	70.6 \pm 1.86 ^{y2}
TNF- α (pg/mL)	181.0 \pm 2.4	225.2 \pm 5.7 ^a	197.1 \pm 1.9 ^y	268.0 \pm 9.3 ^y	276.1 \pm 8.2 ^y	273.2 \pm 9.8 ^y	254.1 \pm 12.1 ^y	327.2 \pm 13.0 ^{y2}

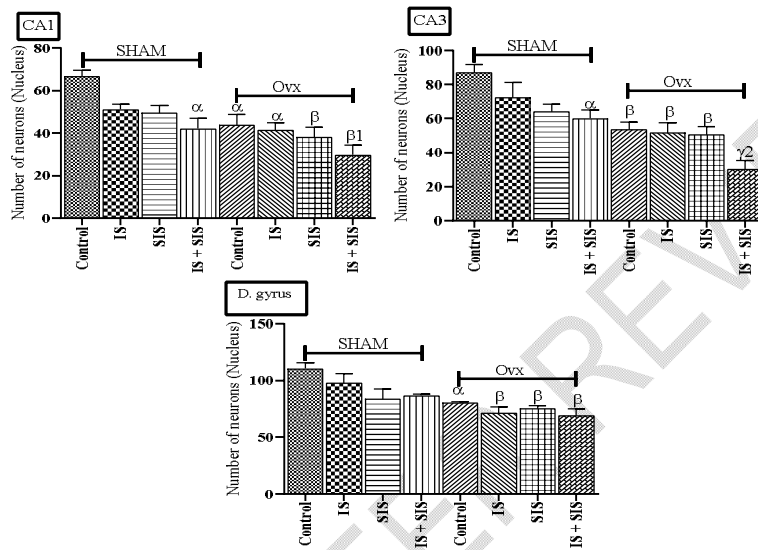
Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments ^aP < 0.05, ^bP < 0.01, ^yP < 0.001 significant from sham-operated control; ¹P < .05, ²P < .01, ³P < .001 significant from ovariectomized control.

3.2.4 Effects on neuronal expression in the hippocampus

Photomicrographs of a transverse section of the hippocampus showed that sham-operated control presented a normal pyramidal layer molded of densely packed rounded neurons. SIS paired with IS induced a low neuronal death in sham-operated rats, namely shrunken cells and nuclei with acidophilically stained cytoplasm compared to control in hippocampal regions. Ovariectomy induced neuronal loss in all the hippocampal regions investigated. Ovariectomized rats all showed disorganized pyramidal layers, shrunken cells, hyperchromatic nuclei, and oligodendrocyte necrosis in CA1, CA3, and DG. Social instability stress and immobilization stress induced a massive neuronal death in ovariectomized rats

than in all the other groups. All observations of photomicrographs of the hippocampus are confirmed by the histomorphometry of CA1, CA3, and DG. Indeed, SIS paired with IS induced a significant decrease ($P < .05$) of neuron numbers in both CA1 and CA3 regions in sham-operated animals compared to the control. Regarding ovariectomized rats the combination of SIS and IS induced a significant diminution of neuron numbers in CA1 and CA3 compared to ovariectomized control.

I



UNDER REVIEW

II

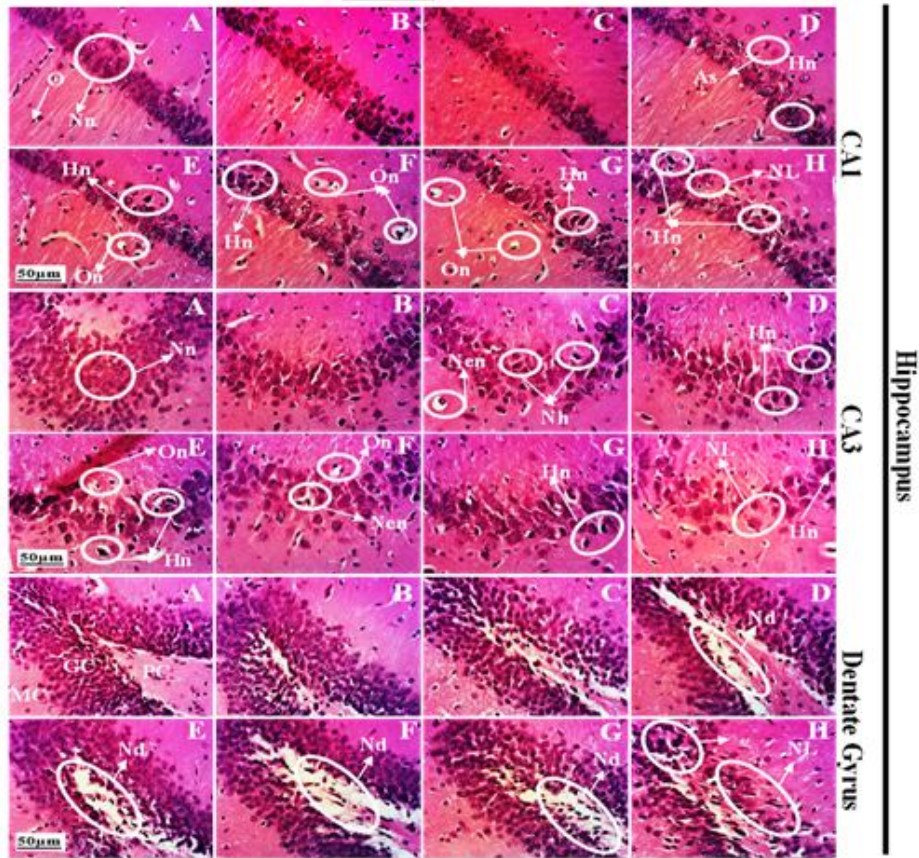


Figure 6: Effects of social instability stress (SIS) and immobilization stress (IS) on neuronal expression in the hippocampus (H-E, 200X).

Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments, ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < .001$ significant from sham-operated control; ¹ $P < .05$, ² $P < .01$, significant from ovariectomized control. A = Sham-operated control, B = Sham-operated + IS, C = Sham-operated + SIS, D = Sham-operated + IS + SIS, E = Ovariectomized control, F = Ovariectomized + IS, G = Ovariectomized + SIS, H = Ovariectomized + IS + SIS. CA1/3: Cornu Ammonis 1/3; DG: Dentate gyrus; Nn = Normal neuron; O = Oligodendrocyte; As = acidophilically stained cytoplasm; On = Oligodendrocyte necrosis; Hn = Hyperchromatic nucleus; Gc = Granular cell layer, Pc = Polymorphic cell layer, Mc = Molecular layer; Nd = Neuronal degeneration, NL = Neuronal loss.

3.3 Effects on neuroendocrine system

Figure 7 presents the effects of social instability stress (SIS) and immobilization stress (IS) on estradiol and corticosterone levels in the serum. Only SIS induced a significant increase ($P < .05$) in corticosterone levels in sham-operated rats compared to the control. Concerning estradiol concentration, neither IS nor SIS induced observable changes in sham-operated animals compared to control. Ovariectomy induced a significant reduction ($P < .01$) in estradiol levels in rats compared to the control. There are no observable changes in estradiol levels owing to SIS and IS paired or not in ovariectomized rats, whereas SIS combined with

IS increased significantly ($P < .05$) corticosterone level in ovariectomized rats compared to control.

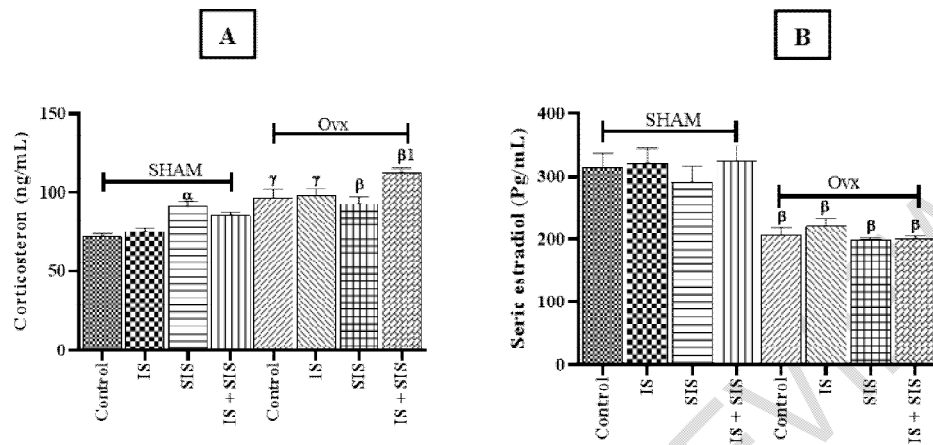


Figure 7: Effects of social instability stress (SIS) and immobilization stress (IS) on neuroendocrine system

Mean \pm S.E.M = Mean values \pm Standard error of means of six experiments, $^{\alpha}P < .05$, $^{\beta}P < .01$, $^{\gamma}P < .001$ significant from sham-operated control; $^{\beta 1}P < .05$, significant from ovariectomized control.

4. DISCUSSION

Worldwide, the number of older adults is growing rapidly. Definitely, the number of people aged 80 increased more than threefold in 2019, and the life expectancy will increase to 77.1 years by 2050, according to the United Nations [35]. Over 50% of women are expected to break the 90-year barrier by 2030. Menopause is the period in a woman's life when her menses stop as a result of the drop and loss of 'ovarian reproductive function'. Thus, 50 % of the worldwide women is expected to leave both menopausal symptoms due to estrogen depletion by 2030 [36,37]. Estrogen fluctuation accompanying the menopause transition leads to a more vulnerable status that exposes women to mental health problems [18]. Stress nowadays has a notable ubiquity and menopausal women are not spared. However, the impact it has on postmenopausal disorders are not really documented.

This study aimed to evaluate different types of stress common to menopausal women on the brain and psychological disorders. Herein, both physical and social instability stress were induced in 84-days ovariectomized rats. Results showed that the social instability stress (SIS) linked to immobilization (IS) induced anxiogenic behavior more in ovariectomized than sham-operated animals as reflected by OA parameters as well as GABA and serotonin levels assessment. Indeed, estrogens are known as potent modulators of neuronal physiology, although the exact mode of action by which estrogen exerts its diverse effects is not clear [39]. Nevertheless, it is almost certain that GABAergic and serotonergic neurons are involved in estrogens modulators effects. Indeed, GABAergic neuron activation in the amygdala is linked to serotonergic signaling and thus leads to psychological disorders like anxiety and depression [40,41]. In the current study, neuron viability in the amygdala as well as parameters of CTS and SP in ovariectomized rats stress-up with both SIS and IS compared to sham animals. Furthermore, although ovariectomy induced depression-related symptoms in rats, stress worsened those symptoms. The role of the amygdala in the experience of emotional states and stress is well established [42]. Basolateral amygdala

neurons are commonly linked to psychological disorders such as depression in animals and humans. Networks from the amygdala to neuroendocrine pathways are documented. Precisely, they are linked to hypothalamic activation for neuroendocrine response to stress [43]. Social instability stress paired with immobility stress in the present study increased corticosterone levels in ovariectomized rats. Thus, stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the corticosterone response on the paraventricular nuclei. HPA axis launch reduced the effectiveness of the steroid negative feedback on the arcuate nucleus of the hypothalamus [44] The present study showed no significant difference in estradiol levels in ovariectomized rats since the hypothalamic-pituitary-ovary (HPO) axis is disrupted. Ovariectomy-induced neuroinflammation in this study noticed by the increase of IL-6, IF- γ , and TNF- α I levels in the hippocampus. The increase of hippocampal pro-inflammatory cytokines is more noticeable in stressful ovariectomized rats. In fact, estrogen decline in menopause may cause neuroinflammation by reducing intracellular Mg²⁺ in nervous cells and increasing oxygen reactive species (ROS) [45]. Effectively, there is a noticeable hippocampal oxidative stress in stress up ovariectomized rats. Thus, menopausal common stress factors like socio-economic status and menopause increased pro-oxidative status, which induced neuroinflammation in PM conditions. Neuroinflammation usually leads to neuronal loss [46]. In this study, estrogen depletion induced neuron necrosis in hippocampal regions including CA1, CA3, and dentate gyrus (DG). Indeed, it is well documented that drastic estrogen lowering is linked to memory dysfunction [47,48]. Memory impairment is confirmed in ovariectomized rats by the reduction of ACh levels and a low discrimination index in novel object recognition test (NOR). Ovariectomized rats exposed to both stresses presented most expressive memory impairment than sham-operated animal.

5. CONCLUSION

Overall, the current study shows that social instability stress (SIS) like immobilization stress (IS) increased neuronal impairment and psychological disorders in PM conditions. Indeed, SIS and IS combined induced anxiety, depression, neuroinflammation, oxidative stress, and memory dysfunction in 84-day ovariectomized rats. Hence, the management of psychological stress like physical stress should be well-addressed in PM women. This fact is owing to the drastic suppression of hormonal production and the psychosocial context.

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ABBREVIATIONS

5-HT: Serotonin

Ach: Acetylcholine

CA1/3: Cornu Ammonis 1/3

CAT: Catalase

CTS: Caudal Tail Suspension test

DG: Dentate Gyrus

GSH: Reduced glutathione

IS: Immobilization Stress

MDA: Malondialdehyde

NDs: Neuronal disorders

NOR: Novel Object Recognition test

NSF: Novelty-Suppressed Feeding test

OA: Open Arena test

PM: Postmenopausal

SIS: Social Instability Stress

SP: Sucrose Preference test

UNDER PEER REVIEW