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Postmenopausal Status Exacerbates Stress-Related Neurological Disorders in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNYS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors OPE, BAGR and TYC managed performed the statistical analysis and the analyses of the study. Authors BCD and DDPD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed at assessing the effects of social instability stress (SIS) and immobilization stress (IS) in postmenopausal (PM) conditions. Postmenopause is a physiological state often associated with mild neuronal disorders (NDs) such as anxiety and irritability. The aging

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of the worldwide population, coupled with multiple stressors, can lead to significant public health concerns, including severe NDs.
Place and Duration of Study: Tests and analyses were carried out within the confines of the Laboratory of Animal Physiology of the University of Yaoundé I (Cameroon) for 3 months.
Study Design: To induce PM syndrome, an 84-day ovariectomized Wistar rat model was used.
Methodology: Random sampling of 4 groups of 6 rats, each aged between 8 and 10 weeks, was used. SIS and IS were induced during PM installation. Behavioral status, biochemical, and histopathological analyses were carried out.
Results: SIS combined with IS exacerbates NDs induced by low estrogen levels. Furthermore, ovariectomized stressed rats are more depressed and anxious than the control. They presented severe hippocampal inflammation, oxidative stress, and memory impairment owing to low levels of ACh and reduced neuronal expression in the hippocampus. Overall, PM status makes NDs linked to stress worse in rats.
Conclusion: Stress promotes oxidative stress, neuroinflammation, and neuronal death, leading to greater affective and cognition disorders when estrogen levels are low. Thus, there is a need to focus on the management of stress during menopausal life.

Keywords: Postmenopause; stress; neuronal disorders; estrogen level; ovariectomy.

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

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, and low libido) and symptoms of vulvovaginal diseases, including vaginal dryness, dysbiosis, and dyspareunia [1,2]. Physicians and researchers are taking practical steps to ease lives, address symptoms, and afford primary prevention advice for chronic conditions such as osteoporosis, cardiovascular and neurodegenerative diseases [3,4]. 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 [5,6]. The causes of neuronal death in PM conditions include apoptosis, neuroinflammation, and high reactive oxygen species in hippocampal regions [7-10].

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 [11,12]. Based on its impact, stress can be classified as eustress, which is the positive way it is experienced by people with prominent arousal, or distress, which has unpleasant effects on both physical and psychological health [13]. Stress can also be classified according to its etiology. Indeed, they are social, physical, and psychological stresses, that affect the predefined body's steady state called homeostasis [14]. Stress is multifactorial and can be due to many factors. COVID-19, sedentary behavior, socio-economic issues, and mental health disorders are among the most important causes of stress in today's society [15-17].

Menopausal women are exposed to social and physical stress during menopause. Indeed, a clinical investigation reported that socio-economic level is positively correlated with attitudes regarding menopause. Furthermore, women usually complain about asthenia and slowed physical activity during the menopause stage [18-20]. 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 [21-24]. 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. MATERIALS AND METHODS

2.1 Experimental Protocol

Forty-eight female rats aged 8–10 weeks were randomly assigned to one of two distinct sets: twenty-four rats were sham-operated, and the same number were ovariectomized using a dorsal approach, following the protocols described by Mengue et al. [24] and Minami et al. [25]. 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 stressing with the 2 types of stress (SIS + IS). 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 two 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 of each rat was isolated. 0.4 grams of the left hemisphere of each hippocampal region were 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), and the evaluation of some neuromodulators' levels (GABA, serotonin (5-HT), and acetylcholine (ACh)). The right hemisphere of the hippocampal region and the cerebral cortex were fixed for histopathological analyses (Fig. 1).

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. [26]. 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 hour had elapsed, the rat was reintroduced into 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. [27] and Marmonti et al. [28]. Two weeks after surgery, rats from the non-immobilized groups were introduced 2 by 2 into plastic cages with a volume of 8038.40 cm^3. Rats in the immobilized groups were placed in the same number of plastic cages with a volume reduced by 80%, which means a volume of 1607.68 cm^3.

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 [29]. The 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. [30]. 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 a non-hydric fast of 24 hours. The Open Arena Test (OA) was used to assess rodents' locomotor activity, exploration, and emotional reactivity [31]. The caudal tail suspension test (CTS) was performed according to the prototype described by Cryan et al. [32]. CTS was set up to evaluate depression [33]. 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 the protocol [34].

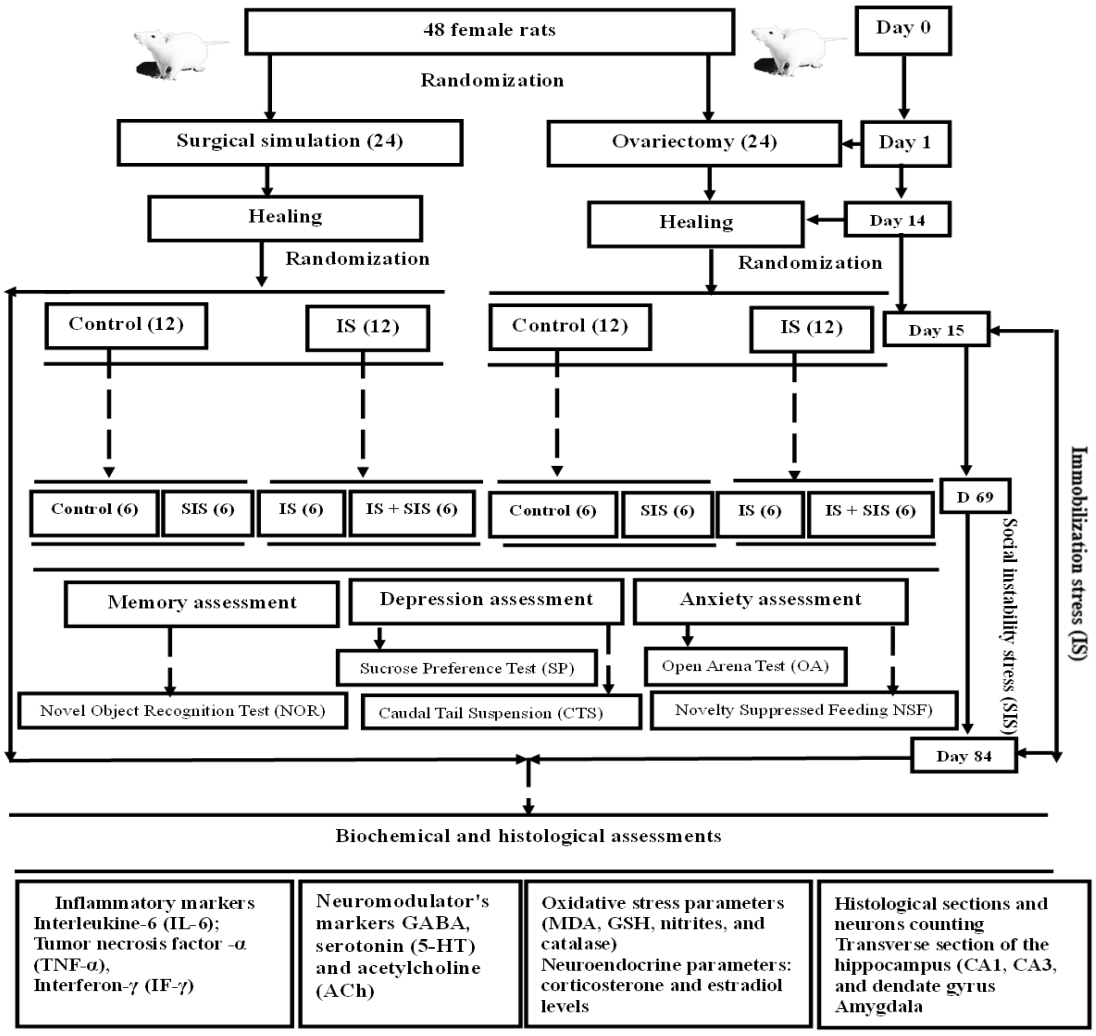

Fig. 1. Experimental paradigm

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 serum levels of estradiol and corticosterone. An 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 from Elabscience. The levels of serotonin (5-HT), -γ-amino butyric acid (GABA), and acetylcholine (ACh) were determined using commercial diagnostic ELISA kits from Elabscience. Malondialdehyde (MDA) and reduced glutathione (GSH) in brain homogenate were determined using the procedures described by Wilbur [35] and Ellman [36], respectively, while the nitrite content was determined using the method described by Green et al. [37].

2.5 Histopathological Analysis of the Brain and Neuron Counting

The hippocampal and cortex regions were 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 (1 hour 30 minutes per bath) and impregnated in liquid paraffin at 60°C for 5 hours. The number of hippocampal neurons in the CA1 and CA3 regions was assessed using microphotography obtained by 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. The variances of groups were found to be homogeneous, and 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 the mean ± standard error of the mean. Differences were considered significant at a probability of $P < .05$.

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 controls. Furthermore, in estrogen-deficient animals, SIS and IS significantly reduced the number and time spent in the center of the arena. Social instability stress induced more 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 controls. As against sham-operated rats, ovariectomized animals paired with SIS and 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) levels ($P < .01$) compared to the ovariectomized (Ovx) 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 the sham control (Fig. 2).

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 the sham control (Fig. 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 the ovariectomized control. As shown in Fig. 3B, SIS ($P < .001$) and pairing SIS with IS ($P < .01$) significantly reduced the sucrose preference index in sham-operated rats compared to the control. The lack of estrogen promotes stress in rats. Hence, ovariectomized animals presented a reduced sucrose preference index compared to the sham control. Furthermore, in SIS + IS ovariectomized rats, the sucrose preference index is significantly low ($P < .01$) compared to the ovariectomized control.

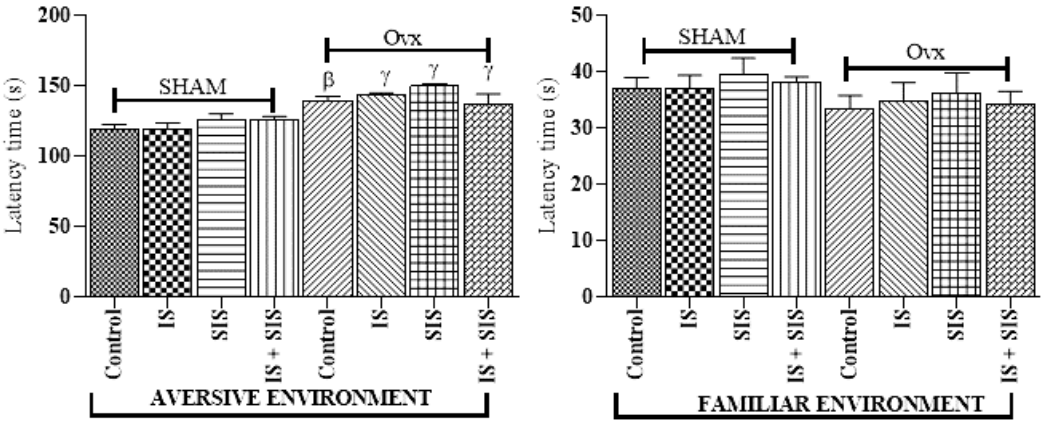

Fig. 2. Effects of social instability stress (SIS) and immobilization stress (IS) in NSF
Mean ± S.E.M = mean values ± Standard error of means of six experiments; $^{\alpha}P < .05$, $^{\beta}P < .01$, $^{\gamma}P < .001$ significant from sham-operated control

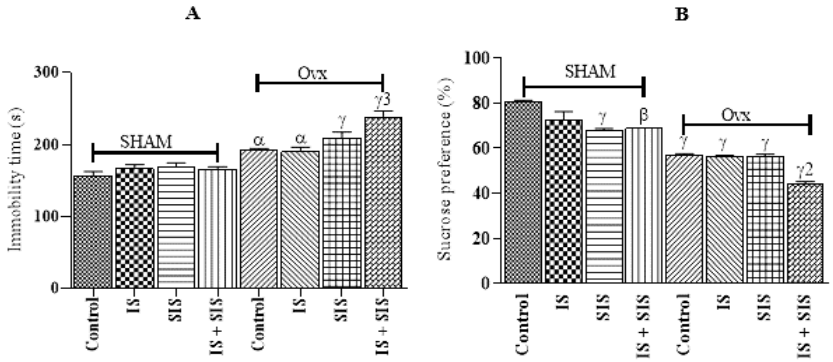

Fig. 3. Effects of social instability stress (SIS) and immobilization stress (IS) in CTS and SP
Mean ± S.E.M = mean values ± Standard error of means of six experiments, $^{\alpha}P < .05$, $^{\beta}P < .01$, $^{\gamma}P < .001$ *significant from sham-operated control,* $^{2}P < .01$, $^{3}P < .001$ *significant from ovariectomized control*

Fig. 4 shows stress-related effects on neuronal expression in the 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 nuclei compared to controls. In the SIS + IS ovariectomized animal, there is a noticeable presence of hyperchromatic nuclei, 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 ($P < .001$).

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 the 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 significantly increased 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 the sham control. Furthermore, the social instability stress linked with immobilization stress induced a greater decrease in ACh ($P < .001$) levels compared to the control (Fig. 5D).

3.2.2 Effects on hippocampal oxidative stress

Pairing SIS with IS induced a significant decrease in catalase activity ($P < .01$) and nitrite levels ($P < .001$) compared to the control. Whereas ovariectomy reduced CAT activity, GSH, and nitrite levels 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 the ovariectomized control (Table 2).

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 the 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 noticeably higher than in the ovariectomized 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$^{\alpha}$	30.5 ± 1.6$^{\beta}$	28.9 ± 2.9$^{\gamma}$	20.6 ± 2.0$^{\gamma 2}$
5-HT (pg/mL)	66.3 ± 2.3	62.3 ± 3.5	62.6 ± 1.3	54.1 ± 1.3$^{\alpha}$	54.3 ± 1.6$^{\alpha}$	46.5 ± 1.5$^{\gamma}$	44.9 ± 3.2$^{\gamma 1}$	36.2 ± 1.1$^{\gamma 2}$
Open arena test parameters								
Crossing	49.21 ± 3.43	39.67 ± 0.48	36.19 ± 1.09$^{\beta}$	36.25 ± 1.88$^{\beta}$	34.21 ± 1.26$^{\gamma}$	30.50 ± 1.14$^{\gamma 1}$	25.21 ± 3.11$^{\gamma 2}$	18.80 ± 1.15$^{\gamma 3}$
Grooming	6.18 ± 0.63	6.60 ± 0.92	7.40 ± 1.32	8.20 ± 0.58	10.60 ± 0.50$^{\beta}$	10.60 ± 0.51$^{\beta}$	12.11 ± 0.94$^{\gamma}$	17.60 ± 1.74$^{\gamma 2}$
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$^{\alpha}$	11.80 ± 1.15$^{\alpha}$	10.20 ± 1.24$^{\beta}$	6.00 ± 1.12$^{\gamma 1}$

Mean ± S.E.M = mean values ± Standard error of means of six experiments, $^{\alpha}P < .05$, $^{\beta}P < .01$, $^{\gamma}P < .001$ significant from sham-operated control, $^{1}P < .05$, $^{2}P < .01$, $^{3}P < .001$ significant from ovariectomized control

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

	Parameters groups	MDA (µmol/g of tissue)	GSH (µmol/g of tissue)	CAT (µmol/g of tissue)	Nitrites (µmol/g of tissue)
	Control	10.78 ± 1.32	17.75 ± 1.53	58.18 ± 2.88	0.12 ± 0.01
	IS	13.00 ± 1.35	14.23 ± 0.42	40.60 ± 5.71$^{\alpha}$	0.10 ± 0.00
SHAM	SIS	15.33 ± 1.08	13.82 ± 0.79	37.65 ± 5.17$^{\alpha}$	0.08 ± 0.00$^{\gamma}$
	IS + SIS	16.58 ± 2.42	13.76 ± 0.92	35.58 ± 3.95$^{\alpha}$	0.07 ± 0.00$^{\gamma}$
	Control	21.60 ± 0.60$^{\gamma}$	13.01 ± 0.50$^{\alpha}$	32.25 ± 1.74$^{\beta}$	0.07 ± 0.00$^{\gamma}$
	IS	21.26 ± 0.60$^{\gamma}$	12.38 ± 0.81$^{\alpha}$	33.25 ± 3.15$^{\beta}$	0.06 ± 0.00$^{\gamma}$
Ovx	SIS	23.21 ± 1.44$^{\gamma}$	8.17 ± 0.87$^{\gamma 2}$	26.25 ± 2.25$^{\gamma 1}$	0.05 ± 0.00$^{\gamma}$
	IS + SIS	30.42 ± 2.06$^{\gamma 2}$	7.34 ± 0.79$^{\gamma 3}$	20.49 ± 0.82$^{\gamma 2}$	0.05 ± 0.00$^{\gamma}$

Mean ± S.E.M = mean values ± Standard error of means of six experiments, $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$, $^{\gamma}P < 0.001$ significant from sham-operated control; $^{1}P < .05$, $^{2}P < .01$, $^{3}P < .001$ significant from 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 ± 7.2	256.2 ± 4.5	266.6 ± 8.7	292.8 ± 4.7	299.7 ± 7.6$^{\alpha}$	295.5 ± 4.8$^{\alpha}$	300.1 ± 6.4$^{\alpha}$	364.9 ± 17.6$^{\gamma 3}$
IL-6 (pg/mL)	39.2 ± 3.7	41.3 ±3.5	39.8 ± 2.9	45.9 ± 3.6	54.6 ± 3.6$^{\alpha}$	57.3 ± 1.1$^{\beta}$	58.6 ± 2.54$^{\beta}$	70.6 ±1.86$^{\gamma 2}$
TNF-α (pg/mL)	181.0 ± 2.4	225.2± 5.7α	197.1 ± 1.9$^{\gamma}$	268.0 ± 9.3$^{\gamma}$	276.1 ± 8.2$^{\gamma}$	273.2 ± 9.8$^{\gamma}$	254.1 ± 12.1$^{\gamma}$	327.2 ± 13.0$^{\gamma 2}$

Mean ± S.E.M = mean values ± Standard error of means of six experiments, $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$, $^{\gamma}P < 0.001$ significant from sham-operated control; $^{1}P < .05$, $^{2}P < .01$, $^{3}P < .001$ significant from ovariectomized control

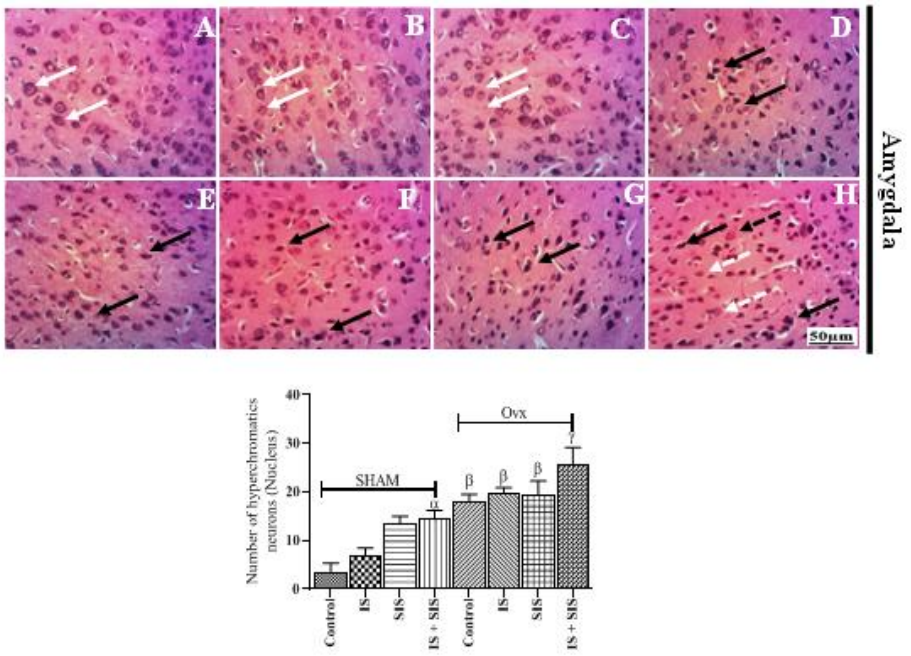

Fig. 4. Effects of social instability stress (SIS) and immobilization stress (IS) on neuronal expression in the amygdala (H-E, 200X)

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

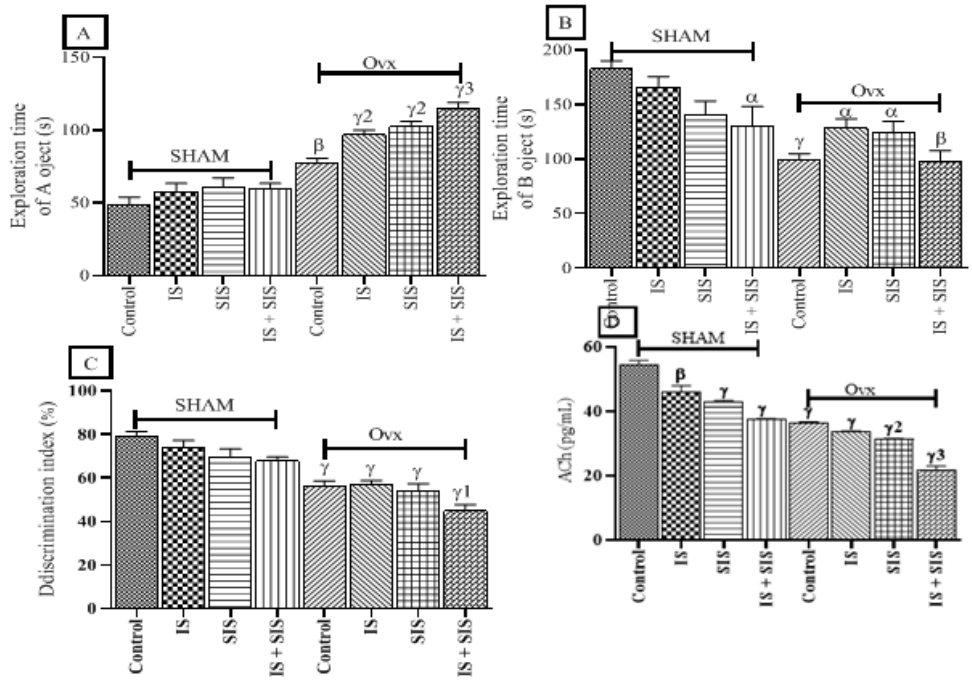

Fig. 5. Effects of social instability stress (SIS) and immobilization stress (IS) in NOR

Mean ± S.E.M = mean values ± Standard error of means of six experiments; $^{\alpha}P < .05$, $^{\beta}P < .01$, $^{\gamma}P < .001$ significant from sham-operated control. $^{1}P < .05$, $^{2}P < .01$, $^{3}P < .001$ significant from ovariectomized control

3.2.4 Effects on neuronal expression in the hippocampus

Photomicrographs of a transverse section of the hippocampus showed that the sham-operated control presented a normal pyramidal layer molded of densely packed and rounded neurons. SIS paired with IS induced 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 more 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 the ovariectomized control.

3.3 Effects on Neuroendocrine System

Fig. 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 the 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 levels in ovariectomized rats compared to the 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 [38]. 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 in and loss of 'ovarian reproductive function'. Thus, 50% of women worldwide are expected to experience both menopausal symptoms by 2030 [39-41]. Estrogen fluctuation accompanying the menopause transition leads to a more vulnerable status that exposes women to mental health problems [21]. Stress nowadays has a notable ubiquity, and menopausal women are not spared. However, the impact it has on postmenopausal disorders is not really documented.

This study aimed to evaluate the effects of 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-day ovariectomized rats. Results showed that 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 assessments. 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 [42]. Nevertheless, it is almost certain that GABAergic and serotoninergic neurons are involved in estrogen modulators effects. Indeed, GABAergic neuron activation in the amygdala is linked to serotonergic signaling and thus leads to psychological disorders like anxiety and depression [43,44].

In the current study, neuron viability in the amygdala as well as parameters of CTS and SP in ovariectomized rats were stressed 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 [45]. 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 responses to stress [46]. 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 in the paraventricular nuclei. HPA axis launch reduced the effectiveness of the steroid's negative feedback on the arcuate nucleus of the hypothalamus. This observation is in line with the results of De Nicola et al. [47].

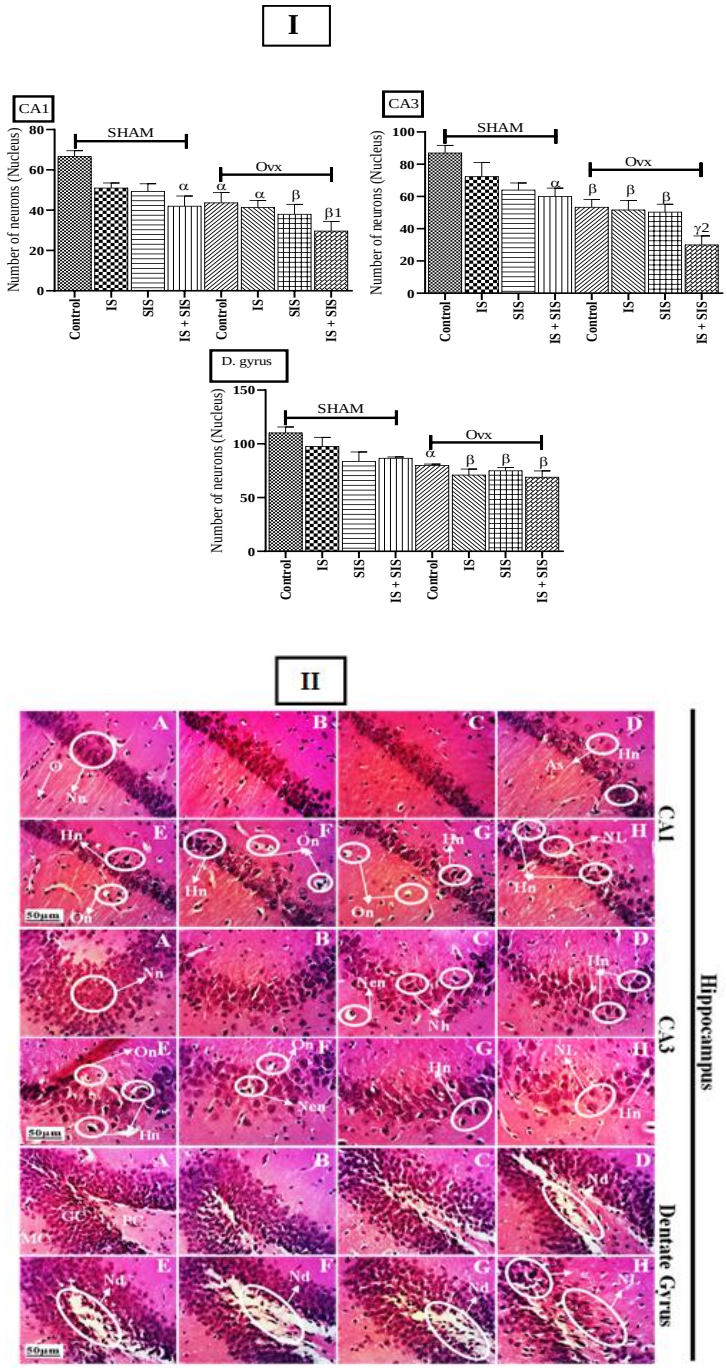

Fig. 6. Effects of social instability stress (SIS) and immobilization stress (IS) on neuronal expression in the hippocampus (H-E, 200X)

Mean ± S.E.M = mean values ± Standard error of means of six experiments, $^{\alpha}P$ <0.05, $^{\beta}P$ <0.01, $^{\gamma}P$ < .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

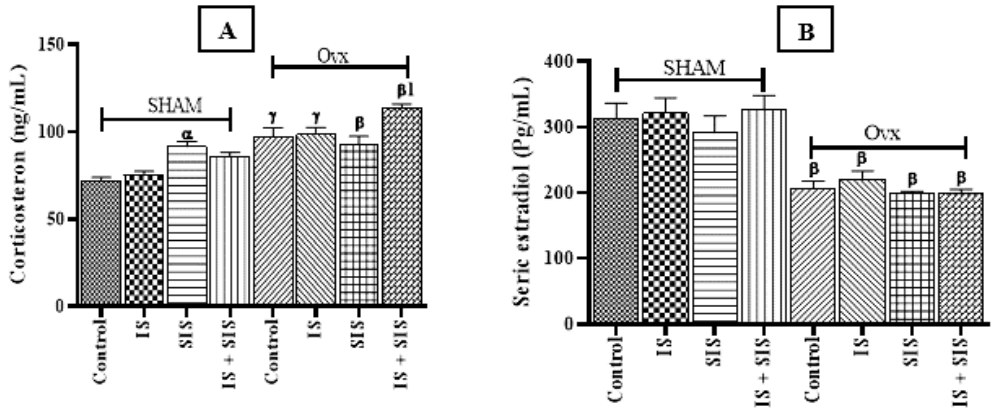

Fig. 7. Effects of social instability stress (SIS) and immobilization stress (IS) on neuroendocrine system

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

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 was noticed by the increase of IL-6, IF-γ, and TNF-α l levels in the hippocampus. The increase in hippocampal pro-inflammatory cytokines is more noticeable in stressed ovariectomized rats. In fact, estrogen decline in menopause may cause neuroinflammation by reducing intracellular Mg2+ in nervous cells and increasing oxygen reactive species (ROS) [48]. Effectively, there is a noticeable hippocampal oxidative stress in stressed-ovariectomized rats. Thus, common menopausal stressors like socio-economic status and menopause increased pro-oxidative status, which induced neuroinflammation in PM conditions. Neuroinflammation usually leads to neuronal loss [49].

In this study, estrogen depletion induced neuron necrosis in hippocampal regions including CA1, CA3, and the dentate gyrus (DG). Indeed, it is well documented that drastic estrogen lowering is linked to memory dysfunction, as reported by Weber et al. and Hampson (2018) [50,51]. Memory impairment is confirmed in ovariectomized rats by the reduction of ACh levels and a low discrimination index in the Novel Object Recognition Test (NOR). Ovariectomized rats exposed to both stresses presented more expressive memory impairment than sham-operated animals. This study highlights that impaired central nervous system in PM conditions are not only attributable to low estrogen level; exposure to stress can also lead to severe NDs in PM.

5. CONCLUSION

Overall, the current study shows that social instability stress (SIS) and immobilization stress (IS) increased neuronal impairment and psychological disorders in PM conditions. Indeed, SIS and IS combined induced severe 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. Further studies are needed to verify stress-related effects on vasomotor symptoms in PM women.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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