

Stress-induced changes of interleukin-1 β within the limbic system in the rat

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The aim of this study was to investigate the influence of two periods of life, namely P28 and P360, on the changes in interleukin-1beta (IL-1 β) immunoreactivity (-ir) in the hippocampus (CA1, CA3, DG) and amygdala (central-CeA, medial-MeA) caused by acute and repeated open field (OF), or by forced swim (FS) exposition. Rats were divided into groups: non-stressed, exposed to acute (one-time for 15 min) and chronic stressors (21 days for 15 min daily). We found IL-1 β -ir in the control group to be higher in P360 than in P28. In P28, under OF and FS exposure, IL-1 β -ir in the CeA remained unaltered but increased in the MeA and in the hippocampus after acute and chronic stress. In P360 no changes were observed in the IL-1 β -ir level after acute and chronic stimulation. These data demonstrate that only the levels of IL-1 β -ir in juvenile rat brains are affected by FS and OF. Additionally, there was no significant difference between FS and OF stimulation in IL-1 β -ir. (Folia Morphol 2009; 68, 3: 119–128)

Key words: IL-1 β , hippocampus, amygdala, open field, forced swim

INTRODUCTION

The structures of the limbic system are implicated in many functions which are altered by adverse experience and play an important role within the neural circuitry controlling responses to stressor stimulation [3, 7, 9, 12, 26, 32, 42, 45].

Repeated stress, caused, for example, by involvement of endogenous inflammatory cytokines like interleukin-1beta (IL-1 β), leads to plastic changes in neurons of the CNS [2, 17, 36].

IL-1 β is a pleiotropic, proinflammatory cytokine, produced within the central nervous system in response to neuron damage or stress stimuli [15, 43]. In the brain, IL-1 β serves numerous diverse functions, forcing neurochemical, neuroendocrine, neuroimmune, and behavioural changes [23, 34, 36, 40]. Endogenous IL-1 β plays a major physiological role in the normal, healthy brain [40]. It is synthesized in glia (microglia and astrocytes) and neu-

rons, both of which respond to stress factors [20, 30, 33, 40, 46].

There is evidence that IL-1 β is one of the key mediators linking immune signals associated with stressors to the hypothalamic-pituitary-adrenocortical (HPA) function [16, 34]. Stress-induced changes in IL-1 β have been localized in various brain regions [10, 25, 37, 40]. Exposure to acute stressors such as immobilization, tailshock, and footshock has been shown to induce IL-1 β expression in some regions of the limbic system, while other stressors — restraint, social isolation, predator exposure, and forced swim — have no effect [34]. This disparity of findings has led to considerable controversy regarding the ability of stressors to induce IL-1 β expression.

Open field (OF) and forced swim (FS) tests are deemed aversion stimuli; hence they are used as experimental models for the assessment of despair/depression-like behaviour [13, 29, 44, 50]. Through

the application of the tests, it is possible to create conditions for neurogenic stress response, and thereby to evoke neuronal activity. The OF test corresponds to a psychological, emotional stressor whereas the FS test, to a psychophysical stressor [8, 11, 52].

Contrary to the diverse stressors investigated in various periods of life (predominantly in adults), the influence of mild stressors in the OF and FS tests on IL-1 β expression in the juvenile and aged limbic system structures needs to be explored further.

Considering the psychophysiological changes in juvenile and aged rats, our aim was to assess the influence of age (juvenile: P28 and aged: P360) on producing IL-1 β under acute and chronic stressors in FS and OF tests in chosen structures of the amygdala (central CeA and medial MeA nuclei) and hippocampus (CA1, CA3, DG).

MATERIAL AND METHODS

Animals

The material consisted of 26 Wistar male rats. The animals were divided into two age groups — juvenile: P28, and aged: P360 days old (P — post-natal day). Each age group comprised 3 non-stressed control rats and 5 experimental animals exposed to acute or chronic stressors. At the beginning of the chronic stress stimulation, the P28 group were seven days old (P7). The rats were kept singly in plastic cages and were given free access to water and food pellets. The care and treatment of the rats were in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the Local Ethical Committee of the Medical University of Gdańsk.

The animals from the experimental groups were exposed to the stressors in the OF or FS tests.

Open field test

The open field test (OF) procedure applied to this experiment has been described in our previous study [1, 22]. The open field area constituted a wooden white box (100 × 100 × 40 cm), illuminated by a 500 W halogen light. Each animal was gently placed in the centre of the box to provoke stress reaction.

Forced swim test

The forced swim (FS) stressor was performed according to the method of Porsolt et al. [35] in a vertical glass cylinder (45 cm high, 20 cm in diameter), filled with 22/23°C water. The depth of the water was

chosen so that the animals had to swim or float without their hind limbs or tail touching the bottom.

Experimental procedure

The rats were exposed to the open field test or forced swim test between 9:00 a.m. and 2:00 p.m. Each rat underwent single acute (lasting 15 min) or repeated (during 21 days for 15 min daily) testing. After each experiment the animals were placed back in their home cages. The control animals remained in their home cages until perfusion. Ninety minutes after administering the tests all rats were deeply anesthetized with lethal doses of Nembutal (80 mg/kg of body weight).

The anesthetized animals were perfused transcardially with 0.9% saline solution and heparin, followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4). The brains were postfixed in 4% paraformaldehyde for 3–4 hours and then kept in 0.1 M phosphate buffer containing 10% sucrose (overnight at 4°C) and then 30% sucrose (until sunk). Coronal serial sections of the brain were cut 40 μ m thick using a JUNG 1800 cryostat (Leica, Germany). IL-1 β expression in the brain was examined with the use of immunohistochemical analyses.

Immunohistochemistry

The sections were subsequently stained with the use of single and double immunohistochemical (IHC) staining methods. The free-floating sections were blocked with 10% Normal Goat Serum (NGS), containing 0.3% Triton X-100, for 2 hours and then incubated with primary polyclonal rabbit anti-IL-1 β antibody (Endogen; dilution 1:100) or a mixture of primary polyclonal rabbit anti-IL-1 β antibody (Endogen; dilution 1:100) and monoclonal mouse anti-NeuN antibody (Chemicon; dilution 1:500) in 10% NGS for 48 hours at 4°C. After multiple rinses in phosphate buffered saline pH 7.4 (PBS), the sections were incubated (2–3 hours at room temperature) with appropriate secondary antibodies: Cy3-conjugated goat anti-rabbit (Jackson ImmunoResearch; dilution 1:600) or a mixture of: Cy3-conjugated goat anti-rabbit (Jackson ImmunoResearch; dilution 1:600) and Alexa Fluor 488-conjugated goat-anti-mouse (Molecular Probes; dilution 1:150).

The immunohistochemically stained slides were examined with an Eclipse 600 fluorescent microscope (Nikon, Japan) with confocal system Radiance 2100 (Bio-Rad, UK), equipped with a Krypton/Argon laser. The confocal microscopy images were obtained using 40 \times and 60 \times objective lenses. The optimal

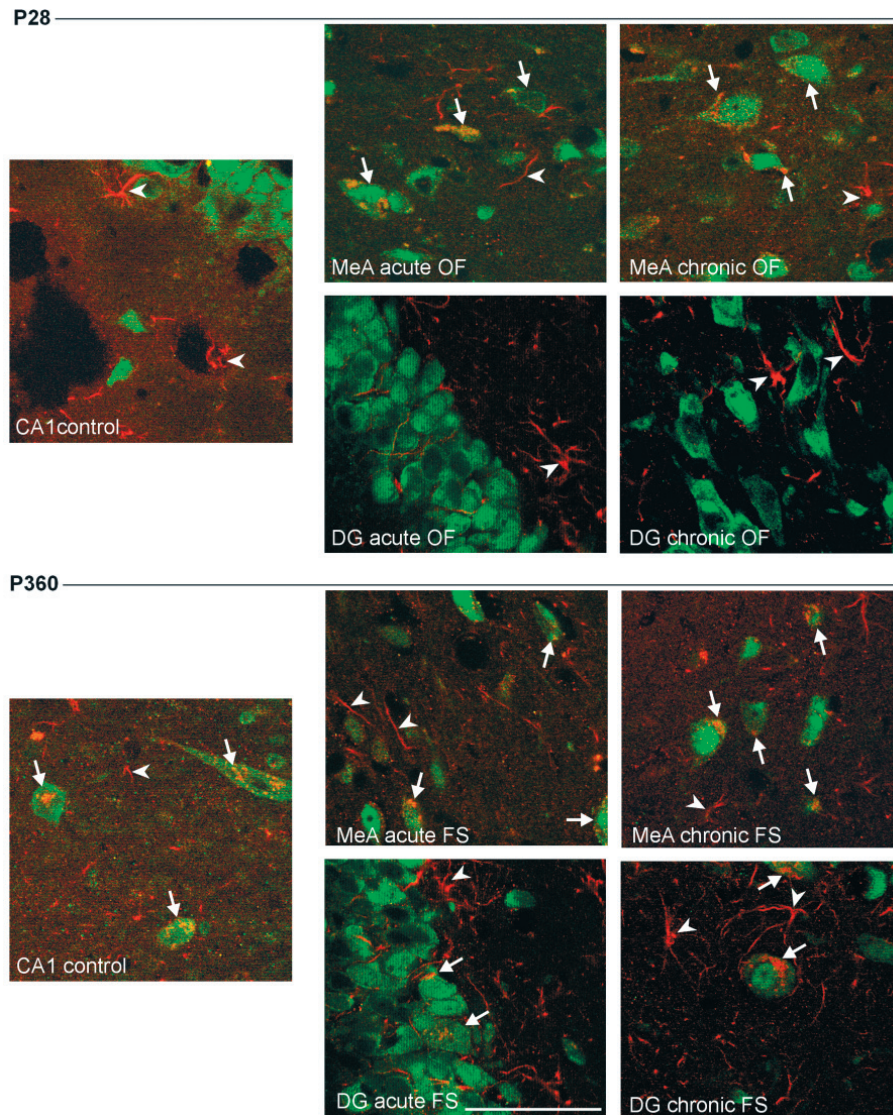


Figure 1. IL-1 β immunoreactive glia (red) (arrow heads) and neurons (green) and double immunostaining with IL-1 β /NeuN (arrow) in studied structures of the limbic system in the control, and after acute and chronic stress in P28 and P360; FS — forced swim test; OF — open field test; CA1 — hippocampal region; DG — hippocampal region; MeA — medial amygdaloid nucleus. Scale bar 50 μ m.

iris was used for each magnification, and only the sections completely stained with fluorescence were taken into account.

Semiquantitative analysis

The number of cells in the amygdala (CeA and MeA nuclei) and hippocampal regions (CA1, CA3 and DG) was estimated semiquantitatively, and classified into the following: \pm very low, + low, ++ moderate, +++ high.

RESULTS

Analysis of double-stained sections (IL-1 β with NeuN) revealed that IL-1 β immunoreactivity ap-

peared predominantly in the glia in both juvenile and aged rats. Moreover, IL-1 β immunoreactive neurons were observed more frequently in aged than in juvenile animals in the control group, and the same result was obtained under stress stimulation in all the investigated structures (Fig. 1).

IL-1 β -ir was detected in the juvenile as well as in the aged rats of the control group; its content, however, depended on the age of the animals and the investigated structures (Tables 1, 2; Figs. 2–5). A very low number of IL-1 β immunoreactive cells were found in P28 in the CeA and MeA nuclei of the amygdala, and a relatively small quantity of these cells were also observed in the hippocampal regions

Table 1. Semiquantitative data concerning IL-1 β -immunoreactivity in the amygdala (CeA, MeA) and hippocampus (CA1, CA3, DG) in P28 in rats

Limbic region	Non-stressed (control)	Acute stress		Chronic stress	
		FS	OF	FS	OF
CeA	±	±	±	±	±
MeA	±	+	+	+	+
CA1	+	++	++	++	++
CA3	+	++	++	++	++
DG	+	++	++	++	++

Number of immunoreactive cells: ± very low; + low; ++ moderate; OF — open field; FS — forced swim

Table 2. Semiquantitative data concerning IL-1 β -immunoreactivity in the amygdala (CeA, MeA) and hippocampus (CA1, CA3, DG) in P360 in rats

Limbic region	Non-stressed (control)	Acute stress		Chronic stress	
		FS	OF	FS	OF
CeA	+	+	+	+	+
MeA	+	+	+	+	+
CA1	+++	+++	+++	+++	+++
CA3	+++	+++	+++	+++	+++
DG	+++	+++	+++	+++	+++

Number of immunoreactive cells: + low; +++ high; OF — open field; FS — forced swim

(CA1, CA3, DG). In the case of the P360 group, a low number of IL-1 β -ir cells was noted in CeA and MeA, but the level of IL-1 β -ir in the hippocampus (CA1, CA3, DG) was high (Tables 1, 2; Figs. 2–5).

Influence of animal age and stress duration on the IL-1 β -ir content in the amygdala

In the group of juvenile rats (P28) submitted to acute FS and OF stress stimulation, the amount of IL-1 β immunoreactivity in the CeA did not significantly differ, and did not change under chronic exposure to stressors, whereas the content of IL-1 β -ir in the MeA increased under acute and repeated FS and OF tests (Table 1; Fig. 2).

The differences in the levels of IL-1 β -ir in the CeA and MeA nuclei between individuals of the aged (P360) rats after both acute and long-term stimulation were insignificant (Table 2; Fig. 3).

There was no disparity in the IL-1 β -ir level between the juvenile and aged animals under acute and long term stress stimulation (Tables 1, 2; Figs. 2, 3).

Influence of animal age and stress duration on the IL-1 β -ir content in the hippocampus

The study showed an increase of IL-1 β -immunoreactivity in the juvenile (P28) rats in the hippocampal

CA1, CA3, and DG regions under both acute and chronic FS and OF (Table 1; Fig. 4). In the aged (P360) rats, there were no differences of IL-1 β -ir content in these regions after acute or repeated stimulation (Table 2; Fig. 5).

The comparison of the IL-1 β -ir contents in the CA1, CA3, and DG hippocampal regions in the juvenile and the aged animals under acute and chronic FS and OF exposure did not reveal any differences (Tables 1, 2; Figs. 4, 5).

DISCUSSION

In our study all the investigated structures of the limbic system showed the most intense IL-1 β immunoreactivity in the glia cells and in scattered neurons. The expression of IL-1 β in neurons increased with age and under stress conditions. According to Kwon et al. [24], expression of IL-1 β in neurons under chronic stress appears to be related to neuronal injury rather than neuronal protection or physiological function. The results of our research may confirm that IL-1 β is a contributor to neurodegenerative disorders caused by age and by neuronal injury after chronic stress.

In the control groups, IL-1 β -ir appeared both in the juvenile and in the aged animals, but a higher expres-

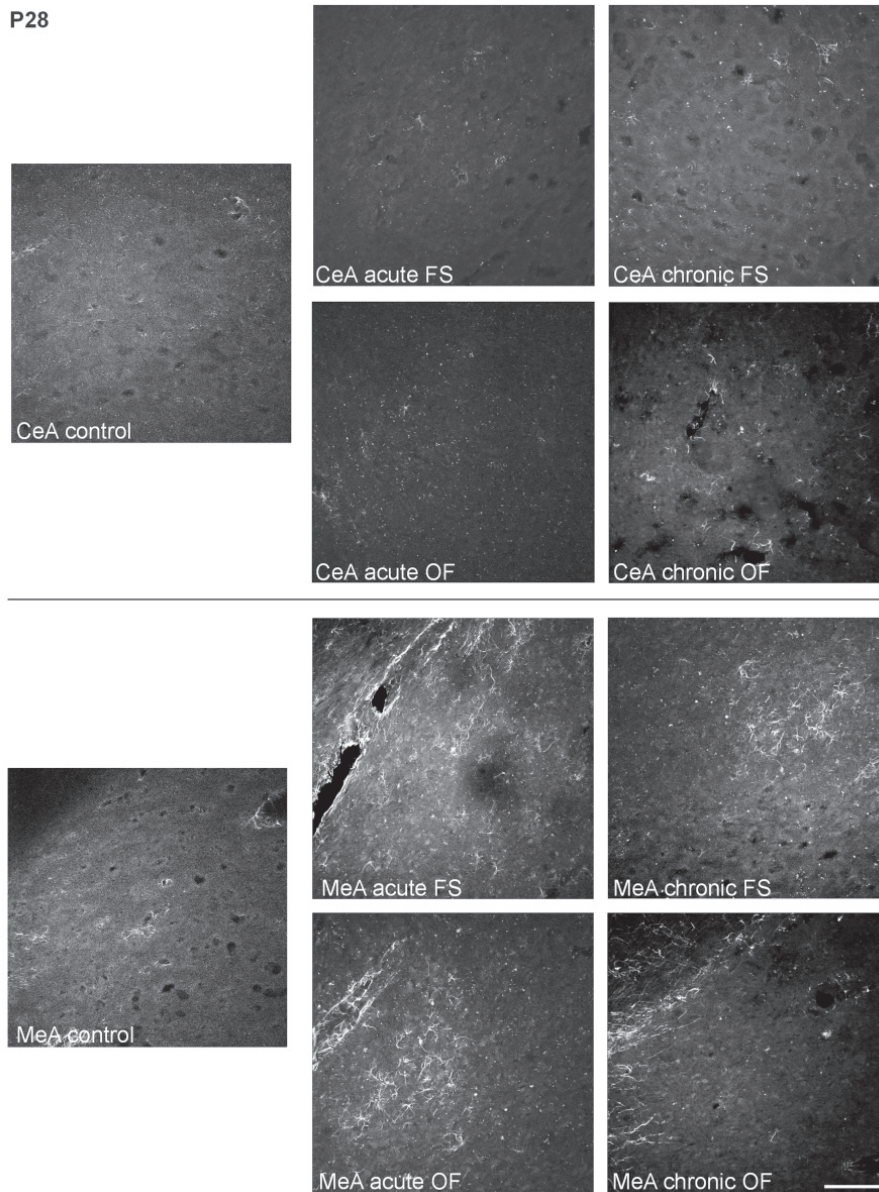


Figure 2. IL-1 β immunoreactivity in the studied nuclei of amygdala in P28 in control animals, and those exposed to acute and chronic open field (OF) test or forced swim (FS) test; CeA — central nucleus; MeA — medial nucleus. Scale bar: 100 μ m.

sion of IL-1 β was found within the investigated amygdaloid nuclei and hippocampal regions in the aged control rats than in the juvenile individuals. It has been reported by several authors [21, 23, 28, 48], and demonstrated in our study, that among the changes which occur in the brain during the aging process, an increase in brain concentrations of proinflammatory cytokines, like IL-1 β , is quite considerable [6]. Based on the findings, it can be concluded that IL-1 β is engaged in natural age-related processes (neurodegeneration) in the amygdala and hippocampus.

Compared with the control group, in the juvenile rats acute and chronic exposure to FS and OF stressors appeared to increase the level of IL-1 β -ir in the amygdaloid MeA nucleus, but had no effect in the CeA, whereas in the hippocampus we observed an increase of IL-1 β -ir in the investigated regions after both kinds of stimulation.

Our findings provide evidence for amygdala and hippocampal functions in anxiety-related behaviour, and indicate that these regions are differentially regulated by stress conditions [49].

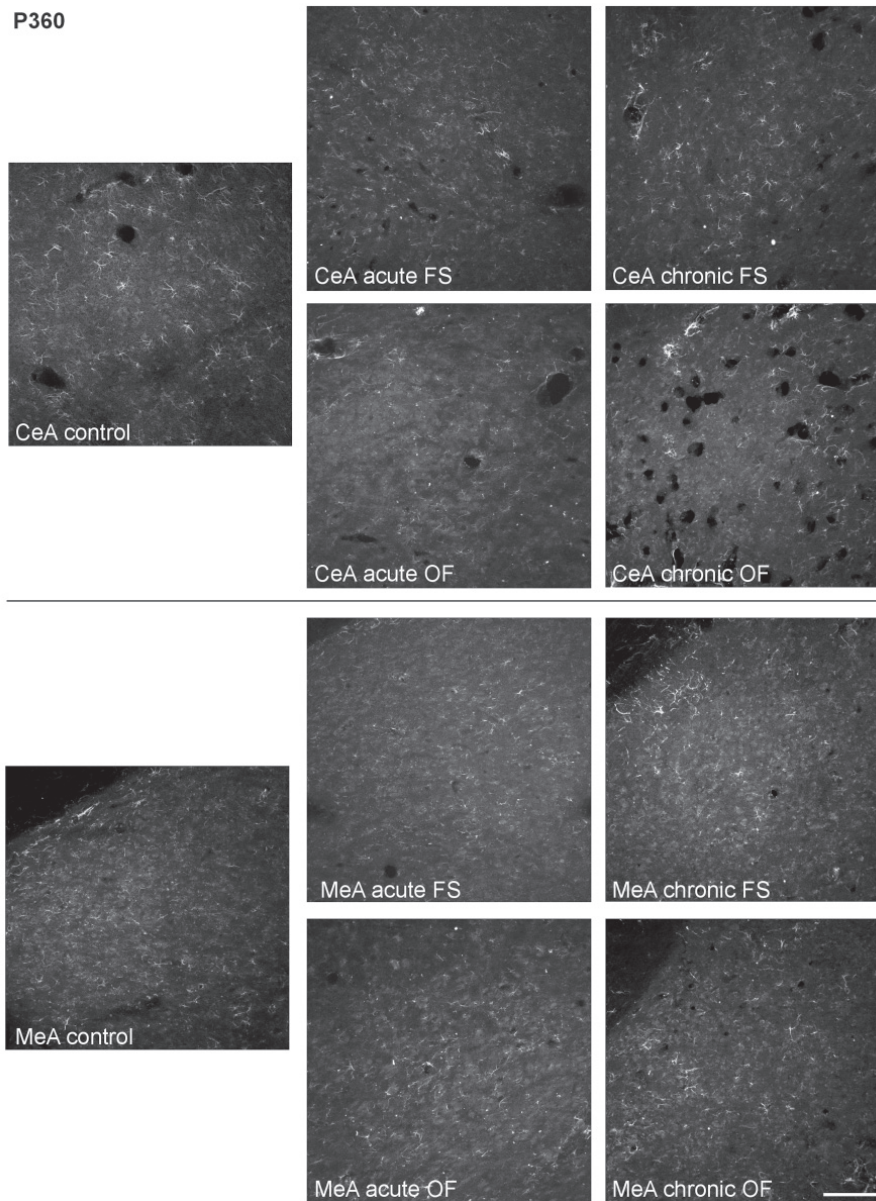


Figure 3. IL-1 β immunoreactivity in the studied nuclei of amygdala in P360 in control animals, and those exposed to acute and chronic open field (OF) or forced swim (FS) tests; CeA — central nucleus; MeA — medial nucleus. Scale bar: 100 μ m.

It is known that CeA and MeA play a key role in the regulation of stress response by HPA axis activity [5, 18, 19]. The activation of MeA and/or CeA depends on the kind of stress stimulation [4, 8]. Dayas et al. [9] also observed that the medial, rather than central, amygdala is critical to hypothalamic activation during emotional stress response. The increase of IL-1 β -ir in the MeA nucleus in juvenile rats may indicate intensive and long lasting emotional reactions after exposure to acute and chronic FS and OF.

It is widely accepted that the hippocampus is sensitive to different stressors [27]. Romeo et al. [38] found that juvenile rats might be especially sensi-

tive to stress, exhibiting a significantly prolonged hormonal stress response. Other researchers reported increased IL-1 β levels in the hippocampus under different acute and repeatable stressors in juvenile animals [24, 47, 48]. Murray and Lynch [31] observed that rats under mild stress showed an increase in circulating corticosterone which correlated with increased IL-1 β concentration. In our study, hippocampal response of juvenile rats to acute and repeatable exposure to FS or OF resembled that which had been found by the above authors. It is thought that immune response via activation of the stress system stimulates secretion of IL-1 β in juvenile animals [14, 39, 51].

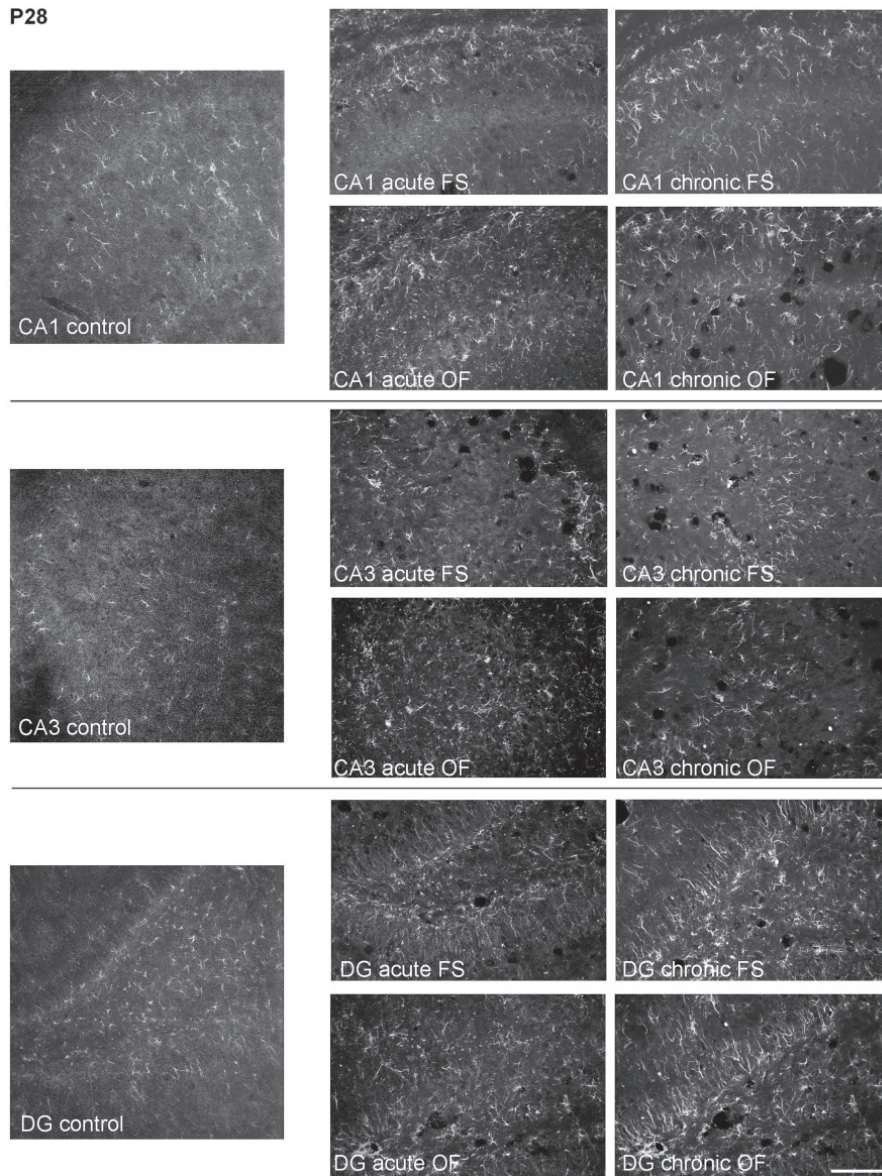


Figure 4. IL-1 β immunoreactivity in the hippocampal regions CA1, CA3, and DG (dentate gyrus) in P28 in the control animals, and those exposed to acute and chronic open field (OF) or forced swim (FS) tests. Scale bar: 100 μ m.

The data gathered during our experiment showed that the number of IL-1 β -ir cells in the investigated nuclei of amygdala and hippocampal regions did not change after acute or repeated FS and OF stress stimulation in the P360 group compared with the unexposed controls. Corresponding findings were recorded by Plata-Salaman et al. [34], who did not observe any discrepancies in the levels of IL-1 β in the hippocampus of adult rats after acute predator exposure and restraint stress. Similarly, Deak et al. [10] demonstrated that single exposure to the forced swim test or novelty stimulation did not change the IL-1 β level in the adult rat hippo-

campus. We proved in our study that the response of the aged animals to FS or OF exposure is much the same as the response of adult rats. These data suggest that FS and OF stressors do not play a role in the increase of IL-1 β expression in the aged rats. It is presumed that a natural stressor may provoke compensatory homeostatic mechanisms, which consequently might prevent neuroinflammatory responses to stress [34, 41]. Therefore P360 aged rats are probably more resistant to stressful stimuli than juvenile animals. It can also be deduced that mild stress does not represent a risk factor for permanent alterations in habituation processes in aged rats [11].

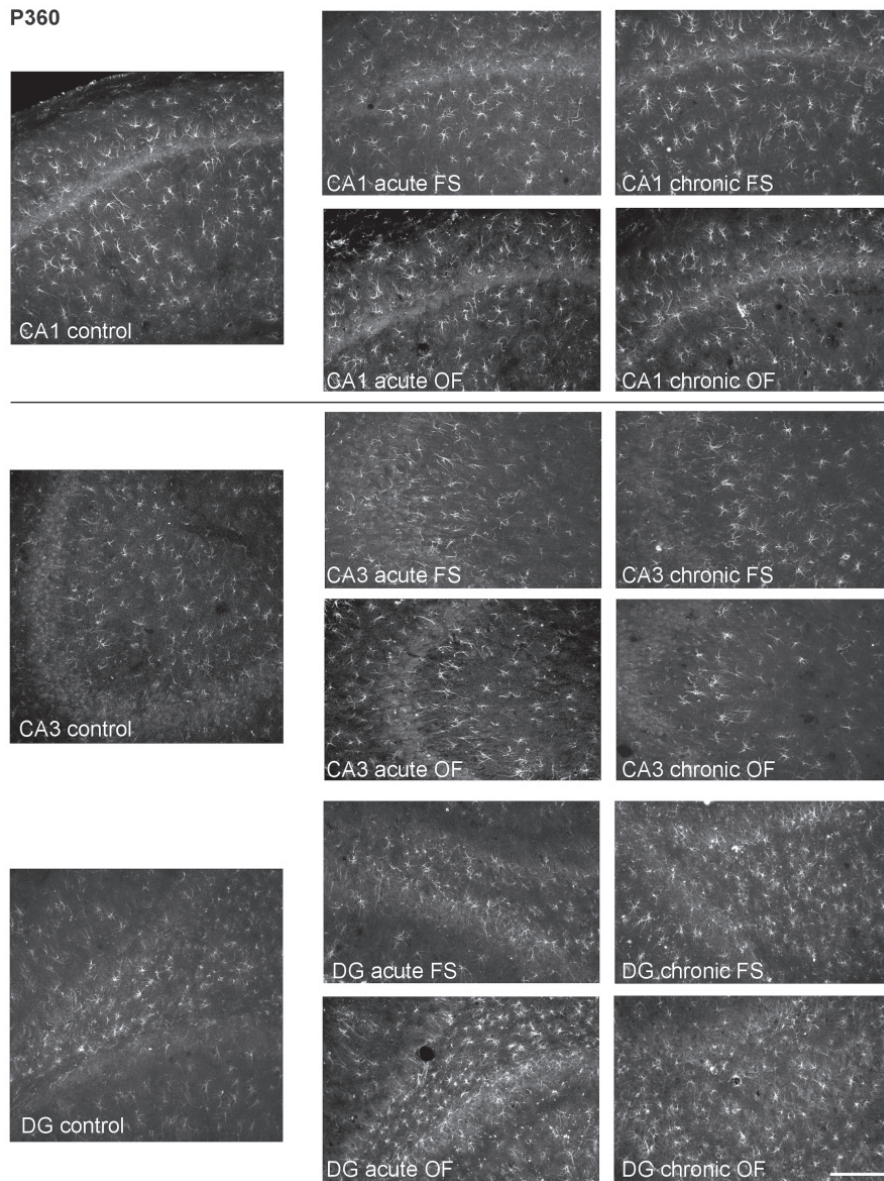


Figure 5. IL-1 β immunoreactivity in the hippocampal regions CA1, CA3, and DG (dentate gyrus) in P360 in the control animals, and those exposed to acute and chronic open field (OF) test or forced swim (FS) test. Scale bar: 100 μ m.

Finally, we observed no fundamental differences in IL-1 β expression in either type (FS or OF) of stress stimulation. This may imply that these types of stressors are of similar intensity and affect the investigated structures of the limbic system in a similar manner. Both stressors are of a neurogenic, psychoemotional nature, and we may conclude, thereby supporting the hypothesis provided by Herman and Cullinan [18], that limbic structures are activated by stressors involving higher-order sensory processing which, in turn, can be manifested in similar IL-1 β levels.

CONCLUSIONS

The levels of IL-1 β in the investigated structures were affected by stress exposition in the juvenile rats only. No fundamental differences in the concentration of IL-1 β -ir were demonstrated in the FS and OF tests.

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