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Combination of Stressors in the Critical Periods of Development Increases Resistance to Inflammatory Pain Stress in Adult Rats

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We report here studies of the long-term effects of combined stress in the prenatal and prepubertal periods of development on measures of tonic inflammatory pain in the formalin test and the severity of depression-like behavior, and also the stress reactivity of the hormonal response in adult rats. In addition, the effects of the serotonin (5-HT) reuptake inhibitor fluoxetine and the 5-HT_{1A} receptor agonist buspirone, given chronically to stressed mothers during pregnancy, on various types of adaptive behavior impaired by prenatal stress were assessed in rats of both sexes. The results showed that in rats of both sexes prenatal stress increased the pain response organized at the spinal and supraspinal levels of the central nervous system and that fluoxetine and buspirone normalized responses. Stress during the prepuberal period of development eliminated the effects of prenatal stress on the inflammatory pain responses integrated at the supraspinal level in adult rats; in these conditions, fluoxetine and buspirone had no effects, in contrast to their antinociceptive actions on the pain response integrated at the spinal level. Stress at prepubertal age eliminated sex-related differences seen in depression-like behavior in prenatally unstressed and prenatally stressed rats given physiological saline. Control adult females and adult females exposed to prenatal stress in the prepubertal period showed increases in the plasma corticosterone level after forced swimming as compared with the basal hormone level, though there were no significant differences in the level of stress reactivity of the hormonal response after forced swimming. Thus, the conditions for stress actions increasing stress resistance in adult rats were identified. Stress in the critical period of development formed a phenotype with increased stress resistance to inflammatory pain, which was seen in responses organized at the supraspinal level in adult individuals.

Keywords: stress in the prenatal and prepubertal periods, adult male and female rats, inflammatory pain, depressive behavior, corticosterone.

Stressful events during the early period of individual development impair the functional activity of the hypothalamo-hypophyseal-adrenal system (HHAS) and the morphofunctional development of the brain and, as a result, alter the formation of behavior [1, 2]. Support for this has been obtained from extensive data obtained both in humans [3, 4] and in animal experiments [1, 2] addressing the short- and long-term influences of stress in the prenatal period of development on psychoemotional behavior. Stress in the prepubertal period of development, a characteristic feature of which is high plasticity producing elevated sensitivity to internal and external stimuli [5], is regarded, along with stress in the perinatal period, as a risk factor for the development of anxious and depressive states and derangement of the functional activity of the HHAS [6–8]. Attention has been paid to sex-related differences in adult animals in the effects of stress at prepubertal age [9] on behavior. Stress in animal studies is often imposed prenatally (application of stress to mothers during gestation), by neonatal separation from mothers, chronic moderate stress, and unfavorable life conditions [7, 10, 11]. The effects of stress in early life have been studied mainly in terms of the stress resistance to the emotional and social types of stress [11]. Most studies have addressed the adverse consequences of stress in the criti-

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cal periods of development, which include the prenatal and prepubertal periods [8, 9, 12]. One of the most important targets for prenatal stress is the 5-HT_{1A} receptor [13]. We have previously shown that repeated injections of the serotonin (5-HT) reuptake inhibitor fluoxetine and the 5-HT_{1A} receptor agonist buspirone to pregnant female rats stressed during pregnancy normalizes adaptive behavior in young offspring after disruption by prenatal stress [14]. Both substances act via 5-HT_{1A} receptors, which are involved in psychoemotional behavior and nociception and influence 5-HT levels in the brain [15]. During the prenatal period, serotonin operates as a developmental signal and is responsible, in tight interaction with the HHAS, for the high level of neuroplasticity that allows brain cells to respond to changes in the environment by correcting the development of the central nervous system and adapting to them [16]. These observations attracted our attention to the serotoninergic system in studies of the effects of combined stressors in pre- and postnatal development on adaptive behavior in rats at different age periods.

Recent years have seen increasing data on the possible favorable influence of repeated stress during the critical periods of development on stress sensitivity in adult age [17, 18], with great attention being paid to sex-related differences. The variability of the results obtained in different experimental conditions, including the type and sequence of stressors and their intensity and duration, as well as the animals' sex and age at the time of exposure to stress and during assessment of its sequelae, has held back studies of the mechanisms of this phenomenon. Identification of the various stress influences, combinations of which in the critical periods of development increase adaptation to future stresses, are of current relevance, as are studies in individuals of both sexes, as existing data have been obtained mainly in males [11]. Published data, including different individual features of the development of stress resistance to identical stressor conditions, led to the hypothesis that individuals subjected to stressful events in the critical periods of life can be susceptible or resistant to stress in later ontogeny [17, 19, 20]. Particular attention in this hypothesis is paid to the conditions in which stress is imposed at the early stages of life and stress in the adult stages. Positive results can be expected when these conditions coincide, i.e., resistance to stress; in the opposite situation, the body is susceptible to extreme loads, which leads to the development of anxious-depressive signs and a variety of diseases.

We have previously observed that stress in the perinatal period increases the ability to counter stress in male rats at prepubertal age [21]. In this study we provided the first report of the use of combined stress applied to female rats during pregnancy, this being a risk factor for depression in the offspring, and inflammatory pain stress in neonates, as encountered in the neonatal clinic. The results indicated that in adult males which had experienced these events at perinatal age, the intensity of the tonic pain response was significantly lower than that in control males. Behavioral studies on animals define the need for studies of the mechanisms of this paradoxical sign at the molecular level [22].

The aim of the present work was to study the effects of combined prenatal stress and stress at prepubertal age on the intensity of the tonic inflammatory pain response, the extent of depression-like behavior, and the stress reactivity of the hormonal response in prenatally stressed adult rats. We have previously demonstrated that stress at perinatal age can alter the activity of antidepressants [21], so the present study also addressed the effects of the serotonin (5-HT) reuptake inhibitor fluoxetine and the 5-HT_{1A} receptor agonist buspirone given to stressed mothers during pregnancy on certain types of adaptive behavior impaired by prenatal stress in offspring of both sexes.

Methods. Experiments were carried out on the offspring of Wistar rats obtained from the biocollection of the Pavlov Institute of Physiology, Russian Academy of Sciences (St. Petersburg) and kept at the laboratory animal house. All procedures with animals were carried out in compliance with the principles of the Basel Declaration and the recommendations of the ARRIVE guidelines [23]. The experimental protocols were approved by the Committee for Humane Treatment of Animals, Pavlov Institute of Physiology. All animals were kept in standard conditions (free access to water and food, 12-h day, light on at 08:00). Before pregnancy, females ($n = 50$) and males ($n = 30$) were placed in standard cages (five females and three males in each cage). Pregnancy was determined from vaginal smears, the presence of spermatozoids in smears defining day 0 of pregnancy. From day 9 of pregnancy to birth, females received daily i.p. injections of fluoxetine (10 mg/kg, Sigma), buspirone (3.5 mg/kg, Sigma), or physiological saline; control animals subjected only to manual handling remained untreated. We have previously shown that prenatal stress induces significantly greater increases in inflammatory pain responses in the formalin test ($p < 0.05$) and depression-like behavior in the forced swimming test ($p < 0.001$) in adult rats as compared with pain responses and depression-like behavior in prenatally nonstressed adult rats born to females given physiological saline during pregnancy [24], so controls in the present study were prenatally unstressed rats not given physiological saline. Drug doses were tested in our previous studies [24] and corresponded to published data [25]. From day 15 of pregnancy to birth, females given drugs or physiological saline were subjected to immobilization stress in the morning and evening for 60 min in bright light by being placed in cylinders severely restricting movement. Stress in pregnant females was applied using a model of prenatal stress for offspring: stress in the last week of pregnancy in females inducing extreme increases in the corticosterone level in the fetus lead subsequently to the development of depression-like behavior in the offspring [7]. Administration of the antidepressant fluoxetine or the anxiolytic buspirone to stressed pregnant females eliminated the adverse effects of stress [21]. Birth of

offspring was checked every 4 h (from 08:00 to 20:00). On the day after birth, four individuals of different sexes were left in each litter. Rat pups were in the nest with the mother to age 30 days. Animals of different sexes were then transferred to different cages. The study included the following groups of rats: prenatally unstressed (controls), prenatally stressed and given physiological saline, prenatally stressed and given fluoxetine, and prenatally stressed animals given buspirone.

Rats of both sexes exposed to the treatments listed above and control animals were used in two series of experiments. In series I, rats (females, $n = 38$; males, $n = 38$) at age 90 days were used for assessment of the intensity of the inflammatory pain response in the formalin test (s.c. injection of 2.5% formalin solution, 50 μl, into the left hindpaw) and, three days later, the extent of depression-like behavior in the forced swimming test. In series II (females, $n = 44$; and males, $n = 40$) at age 25 days were subjected to inflammatory pain stress (s.c. injection of 10 μl of 2.5% formalin solution into the footpad of the left hindlimb) and, three days later, forced swimming stress. On reaching age 90 days, adaptive behavior was assessed in these animals as in those of series I.

In the formalin test, which is widely used for assessment of the antinociceptive actions of drugs on the intensity of inflammatory pain, the number of bends and shakes (a reaction integrated at the spinal level) and the duration of licking reactions (organized at the supraspinal level) in response to s.c. injection of formalin into the left hindpaw were assessed in the tonic phase of the biphasic behavioral response [26]. In the forced swimming test, each rat was placed in a cylinder (diameter 25 cm, height 60 cm water temperature 24–25°C) containing water for 5 min and the time spent by the rat in the immobile state (duration of immobility, a measure of depression-like behavior) was measured. Blood was collected from female rats in series II by decapitation 30 min after the forced swimming test. Females were in diestrus on the day of the experiment. Basal corticosterone levels were determined using females ($n = 43$) subjected to all the treatments as females of series II, with collection of blood at 09:00. Blood was centrifuged and plasma was stored at –20°C. Plasma corticosterone contents were determined by immunoenzyme analysis using reagents from Xema-Medica Co. (cat. No. K210R).

Data were analyzed using a three-factor model for analysis of variance (ANOVA). The factors were: series (I, II), sex (male, female), and action (prenatally unstressed / physiological saline + prenatal stress / fluoxetine + prenatal stress / buspirone + prenatal stress. ANOVA was followed by multiple a posteriori comparisons using the Bonferroni method. Dependent variables were: bends + shakes, duration of licking, and duration of immobility. The strength of the link between a factor and the dependent variable was measured in terms of the value η^2 (partial eta squared). Corticosterone was analyzed by two-factor analysis of variance (ANOVA). The factors were: series (basal, series II) and action prenatally unstressed / physiological saline + prenatal stress / fluoxetine + prenatal stress / buspirone + prenatal stress). ANOVA was followed by multiple a posteriori comparisons using the Bonferroni method. Data are presented as means ± standard errors. Significant differences were identified at a level of 5%. Data were analyzed in SPSS.

Results. *In the formalin test,* assessment of bends and shakess in the second tonic phase of the response revealed significant series ($F(1,144) = 42.238$, $***p < 0.001$, $\eta^2 = 0.222$) and treatment ($F(3,144) = 18.384$, $***p < 0.001$, $\eta^2 = 0.277$) effects. Licking duration in the second tonic phase of the response showed significant series ($F(1,444) = 148.796$, $***p < 0.001$, $\eta^2 = 0.508$) and treatment ($F(3,144) = 8.227$, $p < 0.001$, $\eta^2 = 0.146$), sex ($F(1,14) = 5.481$, $*p = 0.021$, $\eta^2 = 0.037$) effects, along with a significant interaction between series and treatment ($F(3,144) = 3.317$, $*p = 0.022$, $\eta^2 = 0.065$).

The Bonferroni test showed that in series I, prenatal stress increased the pain response in terms of indicators of both the spinal and supraspinal levels in adult males ($p < 0.01$ in both cases) (Fig. 1, *A*, *C*) and in females at the supraspinal level ($p = 0.004$) (Fig. 1, *D*) as compared with identical values for the inflammatory pain response in prenatally unstressed adult rats. Fluoxetine decreased measures of the pain response in males at the spinal ($p = 0.042$) and supraspinal ($p = 0.021$) levels (Fig. 1, *A*, *C*), and in females only at the supraspinal level ($p = 0.034$) (Fig. 1, *D*) as compared with measures of the response in prenatally stressed rats. Buspirone decreased measures of the pain response in males at the spinal ($p = 0.043$) and supraspinal ($p = 0.030$) levels (Fig. 1, *C*) and in females at the spinal ($p = 0.021$) and supraspinal ($p = 0.018$) levels (Fig. 1, *B*, *D*) as compared with measures of the response in prenatally stressed rats. Thus, repeat administration of fluoxetine and buspirone to pregnant females in series I decreased the inflammatory pain response organized at the spinal and supraspinal levels, which was augmented by prenatal stress, in the adult offspring.

The Bonferroni test showed that in series II, prenatal stress increased the number of bends and shakess in males ($p = 0.011$) and, at the level of a tendency, in females ($p = 0.063$) (Fig. 1, *A*, *B*) but did not alter the duration of licking in rats of both sexes (Fig. 1, *C*, *D*). Fluoxetine and buspirone decreased the influence of prenatal stress on the pain response in terms of measures of the spinal level in males ($p = 0.020$, $p = 0.002$) (Fig. 1, *A*) and females ($p = 0.006$, $p = 0.002$) (Fig. 1, *B*) as compared with prenatally stressed rats. The Bonferroni test showed the following differences between series I and II in the tonic pain response. Higher values of the response were seen in series II in terms of measures of the spinal level in prenatally unstressed and prenatally stressed males ($p = 0.009$, $p = 0.025$) (Fig. 1, *A*) and prenatally stressed females ($p = 0.007$) (Fig. 1, *B*). It is important to note that in terms of measures of the supraspinal level, higher values were seen in series I in prenatally unstressed males ($p = 0.004$) (Fig. 1, *C*) and females ($p =$

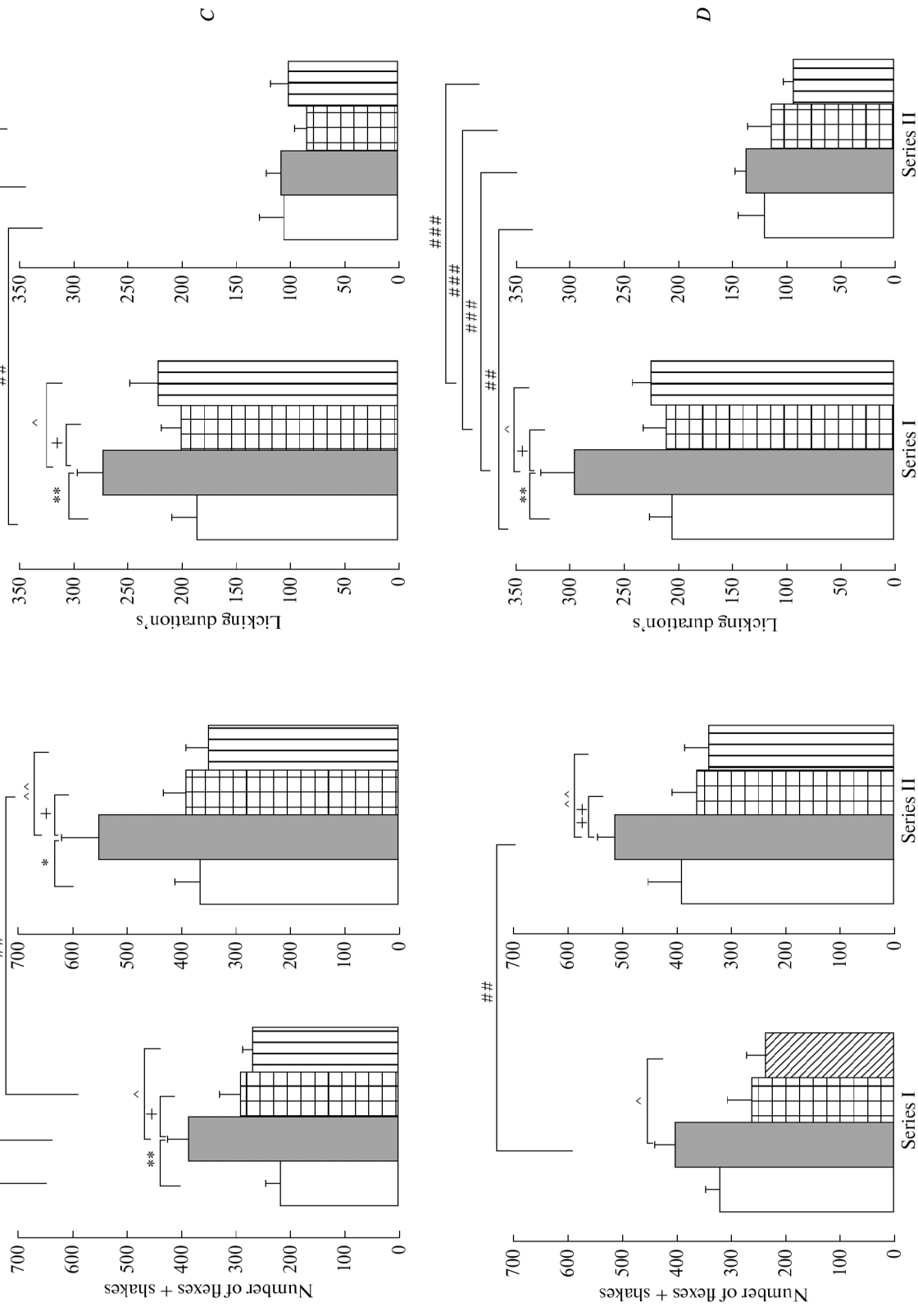

Fig. 1. Influences of prenatal exposures (prenatal administration of fluoxetine and buspirone, prenatal stress) (Series I) and their combinations with stress in the prepubertal period of development (Series II) on the inflammatory pain response in the formalin test in adult male (*A*, *B*) and female (*C*, *D*) rats. Vertical axes show numbers of bends and shakess (spinal level) (*A*, *B*) and the duration of the licking reaction (supraspinal level) (sec) (*C*, *D*). White columns show prenatally unstressed (control) animals, gray columns show prenatally stressed animals given physiological saline, hatched columns show prenatally stressed animals given fluoxetine, and striped columns show prenatally stressed animals given buspirone. Significance: $^{*}p < 0.05$; $^{**}p < 0.01$ – between prenatally stressed animals given physiological saline and controls; $^{+}p < 0.05$, $^{++}p < 0.01$ – between prenatally stressed animals given physiological saline and prenatally stressed animals given fluoxetine; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ – between prenatally stressed animals given physiological saline and prenatally stressed animals given buspirone; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ – between series I and series II prenatally stressed animals given physiological saline and prenatally stressed animals given fluoxetine. Data are presented as mean ± standard error.

= 0.005) (Fig. 1, *D*) and prenatally stressed males and females ($p < 0.001$ in both cases). In addition, in series II, as compared with series I, the magnitude of the pain response at the supraspinal level was smaller in prenatally stressed males given fluoxetine and buspirone ($p < 0.001$ and $p = 0.002$) (Fig. 1, *C*) and females ($p < 0.001$ in both cases) (Fig. 1, *D*), and at the spinal level in prenatally stressed males given fluoxetine ($p = 0.003$) (Fig. 1, *A*). Thus, in series II, the influences of the combinations of stressors were different in terms of their effects on the pain response organized at the spinal and supraspinal levels. Thus, the number of bends and shakess in prenatally stressed rats was increased, and fluoxetine and buspirone normalized this indicator. The duration of licking, a measure of the pain response organized at the supraspinal level, decreased by more than 50% in prenatally unstressed rats and rats with prenatal exposure, with no differences in the response between groups of rats.

In the forced swimming test, the duration of immobility showed significant exposure ($F(3,144) = 51.853$, $^{***}p < 0.001$, $\eta^2 = 0.519$) and sex ($F(1,144) = 7.429$, $^{**}p = 0.007$, $\eta^2 = 0.049$) effects, as well as an interaction between series and sex ($F(1,144) = 9.706$, $^{**}p = 0.002$, $\eta^2 = 0.063$) and a tendency to an interaction between series, sex, and exposure ($F(3,144) = 2.239$, $p = 0.086$, $\eta^2 = 0.045$). The Bonferroni test indicated that in series I and II, prenatal stress increased the duration of immobility in males ($p = 0.002$, $p < 0.001$) (Fig. 2, *A*) and females ($p = 0.004$, $p < 0.001$) (Fig. 2, *B*) as compared with values in prenatally unstressed animals. In series I, fluoxetine and buspirone decreased the duration of immobility in males ($p < 0.001$ in both cases) (Fig. 2, *A*) and females ($p = 0.003$, $p = 0.001$) (Fig. 2, *B*) as compared with these values in prenatally stressed rats. In series II, fluoxetine decreased the duration of immobility in males ($p = 0.02$) and females ($p = 0.006$) (Fig. 2, *A*, *B*), while buspirone decreased this parameter in rats of both sexes ($p = 0.002$ in both cases) (Fig. 2, *A*, *B*). A significant difference in the duration of immobility was seen between series in prenatally stressed females ($p < 0.001$), with greater indictors of depression in series II (Fig. 2, *B*). Sex-related differences were seen only in series I in prenatally unstressed ($p < 0.01$) and prenatally stressed ($p < 0.001$) rats with greater values in males (Fig. 2, *A*, *B*). Thus, in series I, prenatal stress increased the extent of depression-like behavior in adult rats as compared with controls and fluoxetine and buspirone normalized psychoemotional behavior. Stress in the prepubertal period increased the level of depression-like behavior in prenatally stressed females but produced no change in prenatally stressed males. The sex-related differences seen in series I in prenatally unstressed and prenatally stressed rats, with higher levels of depression-like behavior in males, were eliminated by stress in the prepubertal period of development.

Studies of the corticosterone level showed a significant main effect or the series factor ($F(1,75) = 25.1$, $^{***}p < 0.001$, $\eta^2 = 0.25$), and a tendency was seen for the exposure factor ($F(3.75) = 2.6$, $p = 0.056$, $\eta^2 = 0.096$). Post hoc analysis demonstrated a decrease in the corticosterone level in prenatally stressed females given buspirone ($p = 0.011$) as compared with the hormone level in prenatally stressed rats given physiological saline, while fluoxetine produced a change at the level of a tendency ($p = 0.069$). Post hoc analysis showed that the corticosterone level after the forced swimming test increased in prenatally unstressed rats ($p = 0.049$) as compared with the basal hormone level and in prenatally stressed rats given physiological saline ($p = 0.021$) as compared with the basal hormone level, and in prenatally stressed rats given fluoxetine ($p = 0.024$) and prenatally stressed rats given buspirone ($p = 0.003$) as compared with the basal corticosterone levels in rats of these groups. No significant differences were seen in the corticosterone level between prenatally unstressed rats, prenatally stressed rats given physiological saline, prenatally stressed rats given fluoxetine, and prenatally stressed rats given buspirone (Table 1).

Discussion. This study identified the conditions for stress exposures increasing stress resistance in adult life. The combination of prenatal exposures (administration of fluoxetine or buspirone to females stressed during pregnancy) and stress at prepubertal age (inflammatory pain and forced swimming) induced significant decreases in tonic pain inflammatory responses integrated at the supraspinal level in adult rats of both sexes as compared with the corresponding responses in control adults. The combination of stress during the prenatal and pubertal periods of development eliminated the sex-related differences seen in the level of psychoemotional behavior in prenatally unstressed and prenatally stressed rats. In females subjected to the combination of prenatal exposures and stress at prepubertal age, the plasma corticosterone level after forced swimming was significantly greater than the basal hormone level in females of all the groups studied, though no significant differences in the level of stress reactivity of the hormonal response after forced swimming were seen.

Prenatal stress induced increases in the duration of licking in the tonic phase of the formalin test and the duration of immobility (a measure of depression-like behavior) in the forced swimming test in adult rats, which supported our previously published data on adult rats [26]. Extensive published data obtained both from humans [2, 4] and animals [7, 10, 11] point to adverse sequelae in behavior induced by prenatal stress, particularly increases in anxious-depressive behavior. The present study provides the first data showing prenatal fluoxetine-induced decreases in the intensity of the inflammatory pain response and the extent of depression-like behavior in prenatally stressed female adult offspring. These results point to the ability of the antidepressant fluoxetine to induce an antinociceptive effect, which increases the currently small number of contradictory data on the role of fluoxetine in nociception [27, 28]. Chronic administration of buspirone to stressed pregnant females

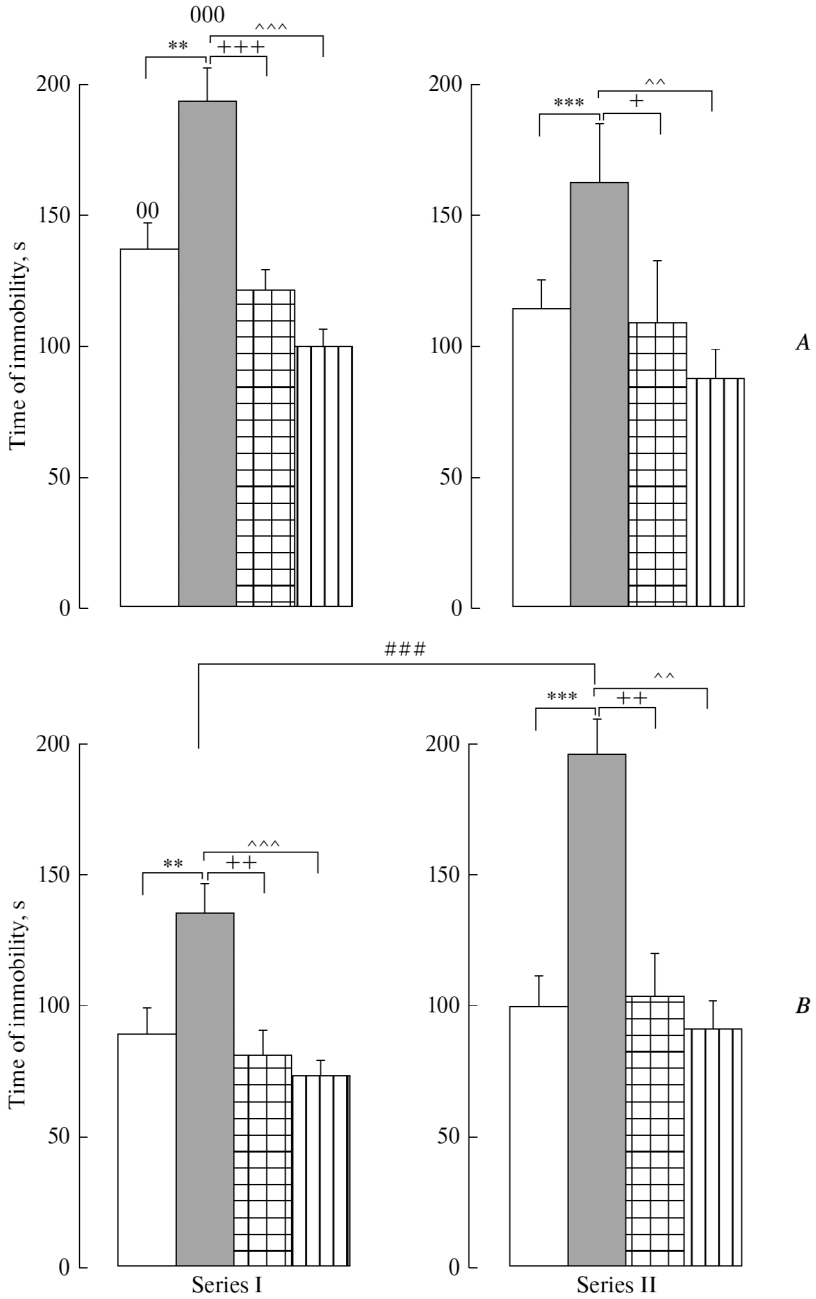

Fig. 2. Influences of prenatal exposures (prenatal administration of fluoxetine and buspirone, prenatal stress) (Series I) and their combinations with stress in the prepubertal period of development (Series II) on the duration of immobility in the forced swimming test in adult male (*A*) and female (*B*) rats. Vertical axes show the duration of immobility (sec). White columns show prenatally unstressed (control) animals, gray columns show prenatally stressed animals given physiological saline, hatched columns show prenatally stressed animals given fluoxetine, and striped columns show prenatally stressed animals given buspirone. Significance: $^{**}p < 0.01$; $^{***}p < 0.001$ – between prenatally stressed animals given physiological saline and controls; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ – between prenatally stressed animals given physiological saline and prenatally stressed animals given fluoxetine; $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ – between prenatally stressed animals given physiological saline and prenatally stressed animals given buspirone; $^{\#\#\#}p < 0.001$ – between series I and series II prenatally stressed animals given physiological saline and prenatally stressed animals given fluoxetine; $^{00}p < 0.01$, $^{000}p < 0.001$ – sex-related difference between prenatally unstressed and prenatally stressed rats in series I. Data are presented as mean ± standard error.

also induced decreases in the measures of adaptive behavior studied here in offspring, which supported our data obtained previously in adult rats [24] and prenatally stressed rats of prepubertal age exposed to buspirone and fluoxetine during the prenatal period of development [14]. Thus, the results obtained here provide evidence that in certain conditions, the antidepressant fluoxetine and the anxiolytic buspirone can induce antidepressant and antinociceptive effects.

Stress in the prepubertal period of development induced an increase in the pain response integrated at the spinal level only in males. The literature contains data on the sequelae of stress in the prepubertal period of development (chronic combined stress or social stress) seen in psychoemotional behavior and the cognitive domain in adult rats [29, 30]. The non-identity of the results is due to a multitude of factors, including the sex difference.

In the present study, our assessment of the intensity of the inflammatory pain response in the formalin test yielded an important fact providing evidence that the result showed a relationship between the effect of the combination of stressors used on the intensity of the response and the level of integration of the pain reaction. Thus, the duration of the licking reaction, integrated at the supraspinal level, decreased (by more than 50%) in all groups of male and female rats subjected to stressors in the pubertal and prepubertal periods of development, which points to an increase in stress resistance to inflammatory pain in adult rats. In these conditions, the effects of fluoxetine and buspirone were not apparent, and no differences in the magnitude of the pain response were seen in rats of different groups, while in rats not subjected to stress at prepubertal age (series I), fluoxetine and buspirone induced antinociceptive effects in prenatally stressed animals of both sexes and suppressed the pronociceptive influence of prenatal stress. An entirely different effect – an increase in the intensity of the pain response – was seen in the number of bends and shakess in males of all study groups and only in prenatally stressed females. Thus, the sequelae of the combination of stressors at different age periods in adult rats were different in reactions integrated at different levels of the CNS: increased sensitivity, i.e., susceptibility, to inflammatory pain stress in reactions at the spinal level and stress resistance in reactions at the supraspinal level. The formalin test is widely used to assess the influences of different substances on the nociceptive system [31]. The results of our study confirm the informativeness of the formalin test for assessing the influences of stressful events on the pain system.

We are unable to compare our results with published data, as we found no reports using inflammatory pain stress as the stressor at early age in combination with any other kind of stress, though inflammatory pain is often encountered in the neonatal clinic. How does the combination of stressors in the critical periods of individual development induce increases in inflammatory pain responses integrated at the spinal level and decrease them at the supraspinal level in rats in the adult state? We can only address this question with a suggestion: the increase in the number of bends + shakes induced by the combination of stressors is due to the excitatory glutamatergic system, while the decrease in the duration of licking is due to enhanced inhibitory GABAergic influences at the supraspinal level. In fact, formalin-induced pain activates intense reactions consisting of bending, shaking, and licking the limb damaged by the inflammatory agent, accompanied by the dominant action of the excitatory glutamatergic system at the spinal level [32]. The mechanisms of formation of the nociceptive arousal in response to formalin injections operate at different levels of the CNS, including the cerebral cortex [33]. Existing data indicate that the monoaminergic system descending from raphe nuclei regulate the nociceptive signal at the level of the posterior horns of the spinal cord; both afferent pathways from spinal cord neurons to the raphe nuclei and efferent pathways from the raphe nuclei to spinal neurons have been demonstrated [34]. The serotoninergic, HHAS, glutamatergic, and GABAergic systems are known to be involved in nociception and to be subject to the influences on stress [35–38]. Stress deranges the balance of the processes of excitation and inhibition in the CNS and the functional activity of the HHAS, which influences the pain control systems of the top-down serotoninergic system and, thus, the neurophysiological mechanisms underlying pain perception. Considering the important role of metabotropic and inotropic receptors of both the glutamatergic [39] and GABAergic [35] types in the influences of stress on nociception and the mechanism of cotransmission of glutamate with serotoninergic and GABAergic neurons [40], it can be suggested that these factors are involved in the differences in the effects of stress at different periods of development on inflammatory pain responses integrated at the spinal and supraspinal levels of the CNS in rats seen here.

It should be noted that the influence of prepubertal stress apparent as an increase in the intensity of the pain response at the spinal level was seen in prenatally unstressed males, prenatally stressed males given physiological saline, and prenatally stressed males given fluoxetine, while in females this was seen only in the group subjected to prenatal stress and given physiological saline. However, no sex-related differences were seen in the intensity of the pain response in either series of experiments.

In adult prenatally stressed rats both subjected and not subjected to stress at prepubertal age, the extent of depression-like behavior was increased as compared with the level of depression-like behavior in control rats in the corresponding groups. Chronic administration of fluoxetine and buspirone to stressed pregnant females normalized this type of behavior in the adult offspring of both sexes. The difference between series I and II consisted of an increase in the duration of immobility only in prenatally stressed females. Despite the absence in series I of significance in the duration of immobility in prenatally stressed drug-treated males and females, the extent of depression-like behavior in females given both fluoxetine and buspirone was lower than that in males. Sex-related differences seen in the extent of depression-like behavior in prenatally unstressed and prenatally stressed rats given physiological saline (series I) were eliminated by stress at prepubertal age (series II). The literature contains data showing that females at prepubertal age are more sensitive to stress than males [30], though these data apply only to social

stress. Thus, the results obtained in studies of inflammatory pain responses and the extent of depression-like behavior provide evidence of the complexity of the mechanisms of the long-term influences of combined stressors at different critical periods of development on the hormonal and neurotransmitter systems. Although the mechanism of this important adaptive manifestation has been studied at the molecular genetic level, many questions remain unanswered, including the question: at which stage of development are impairments to the neurohumoral regulation induced by stress at early age converted to an adaptive process?

Thus, the data obtained here allowed the conditions of stress exposures increasing stress resistance in adult rats to be identified. Stress at the critical periods of development was shown to form a phenotype with increased stress resistance on exposure to inflammatory pain, which was seen in responses organized at the supraspinal level in adult individuals. The influence of stress on depression-like behavior depended on sex. The importance of a positive result for the interaction of two adverse stressors is clear: stress resistance increases, providing an opportunity to withdraw antidepressants which have become ineffective. The novel data obtained here extend our understanding of susceptibility and resistance of individuals subjected to stressor events during the critical periods of life to stress imposed in later ontogeny [17, 19, 20] and have practical value.

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