Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

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Abstract

To examine the effects of exposure to post-weaning pre-puberty (juvenile) stress on the emotional and cognitive abilities in response to exposure to stress in adulthood, we first exposed rats to a platform stress at the age of 28 days. Two months later rats were exposed to acute swim stress. Rats exposed to both stressors showed a higher level of anxiety (as measured both in open field and startle response tests) than controls or rats exposed to either the juvenile or the adulthood stressor.

In the Morris water-maze, rats that were exposed to both juvenile and adulthood stress performed better than the other groups.

In a second experiment we verified that the effect of the juvenile stress was indeed age-dependent. One group was exposed at the age of 26-28 days and again at the age of 60 days (juvenile + adulthood stress); the other group was exposed to the first stressor at the age of 60-62 days and to the second at the age of 90 days (adulthood60 + adulthood90 stress). Juvenile+adulthood stress had a significantly greater effect than exposure to stress twice in adulthood, on anxiety level and on the performance in the water maze. Finally, in the third experiment we found that the juvenile + adulthood stress group swam faster and tended to explore the central area more than the other groups – a finding that could explain their better performance on the first trial of the spatial task. These results indicate that an exposure to a relatively brief juvenile stressful experience has profound and long-lasting effects on the ability to cope with stress in adulthood.

Keywords: Juvenile; rat; PTSD; anxiety; spatial learning.
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1. Introduction

The degree of subsequently experienced trauma may be related to the child's age at the onset of abuse, although controversial views have been noted in the literature (Browne and Finkelhor, 1986). Some researchers claim that younger children are more vulnerable to trauma due to their impressionability (Browne and Finkelhor, 1986) and show greater symptomology as a result of abuse (Beitchman et al., 1991). Others claim that young children's naivete' serves as a protective device, especially if children are unaware of the social stigma associated with the type of victimization they have suffered (Browne and Finkelhor, 1986). Still others (e.g., Kiser et al., 1991) argue that the age of onset of the abuse has no systematic relationship with the degree of disturbance. A possible explanation for these controversial findings is that new symptoms may emerge as a child matures, even though the full extent of detrimental effects may not be evident (Beitchman et al., 1991).

The vast majority of the animal research into early life trauma effects on affective symptoms in adulthood has focused on maternal deprivation paradigms. In this very early post-natal period, the rodent brain is remarkably different from the post-weaning pre-puberty and the post-puberty brain, both in structure and function (Vazquez, 1998). For example, in the rodent the early postnatal limbic-hypothalamic-pituitary-adrenal (HPA) axis is remarkably different from that in the adult, both in structure and function (Vazquez, 1998). The HPA-axis in the developing animal has a limited response to acute challenges between days 3 and 14 of life. These first two weeks pre-weaning are characterized by a “silent period” during which the animal is hyporesponsive to stress (stress hyporesponsive period – SHRP). This may in fact lead under extreme conditions to a far more significant stress response when the
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat animal fails swiftly to terminate glucocorticoids secretion. Several hypotheses have been proposed to explain the quiescent state; Immaturity of brain, pituitary and adrenal elements, or excessive feedback inhibition are common explanations (Vazquez et al., 1996).

The period of postnatal development, for which most early-experience research has been conducted with rat pups (i.e., 3 to 14 days postnatal), has been suggested to correspond roughly to the 23rd week of gestation in humans (Fitzgerald and Anand, 1993).

Here we focused on the effects of exposure to post-weaning pre-puberty (named "Juvenile") stress on rats’ ability to cope with stress in adulthood. At this age the pups already present a more independent behavior and are not yet sexually mature. Furthermore, the post-weaning pre-puberty period in the rat, a life period not widely targeted before, may resemble human childhood also in that the HPA axis is fully developed but key brain areas such as the hippocampus are not.

The hippocampus is rich with corticosteroid receptors that play a role in modulating the basal tone of the HPA and in the magnitude and duration of stress responses (Vazquez et al., 1996). Moreover, the rat hippocampal formation continues to develop until the rat is sexually mature (Martin and Berthoz, 2002). The delayed anatomical development of the hippocampus appears to influence the physiological properties of its neurons, in particular the efficacy of inhibitory synaptic transmission. Using paired pulses, Michelson and Lothman (1989) found that excitatory transmission between CA3 and CA1 reached adult levels by PND 14, while full inhibitory responses were not seen until PND 28. Nurse and Lacaille (1999) further
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State that the GABAB-mediated inhibitory transmission is not fully developed until PND 35-45.

Comparing between the consequences of the exposure to stress in adulthood as a function of whether or not the animals were previously exposed to stress at the proposed age may enable us to suggest that exposure to juvenile trauma may contribute to increased vulnerability to stress-related disorders in adulthood.

In the first experiment, we tested the effects of exposure to a stressor in adulthood on the rats’ ability to perform in a spatial learning task in the Morris water-maze, as a function of whether or not they were exposed to a stressor early in their life. As a juvenile stress, we applied the elevated platform stress (PL), which is similar to a protocol named small platform stress (Pokk and Zharkovsky, 1998; Pokk and Vali, 2001). The PL protocol comprises several factors of stress like novelty, restraint, uncontrollability, and unpredictability. As adulthood stress, we utilized the acute swim stress (ASS) which is a commonly used stress protocol in adult rats (Burgin et al., 1996; Avital et al., 2001). In addition, we used the open field and startle response tests to assess anxiety level alterations.

In the second experiment, we aimed to examine whether the effects observed in the first experiment were qualitative (developmental) or quantitative. Explicitly, was the impact of exposure to juvenile stress due to the developmental period within which it was applied, or were the effects due to repeated exposure to the stressor? Therefore, in this experiment, we compared the juvenile + adulthood stress to a double exposure to stress during adulthood. To facilitate direct comparison between the two exposure protocols, the same stressor (PL) was applied in all stages of this experiment.
Finally, in the third experiment we attempted to evaluate potential mechanisms that may contribute to the better performance of the juvenile + adulthood stress group in the first trial of the spatial task, compared with the controls.

2. Materials and Methods

2.1 Animals

88 Male Wistar rats (eight rats per group in both experiments) weighing between 45 and 49 g (age 24 days) were purchased from Harlan (Jerusalem, Israel) and were given four days of acclimation. Rats were housed four per cage in 75.0 × 55.0 × 15.0 cm Plexiglas cages in temperature-controlled (23 ± 1°C) animal quarters on a 12:12 light-dark cycle (lights on 0700h – 1900h). They had ad lib access to standard Purina Rat Chow pellets and water. All rats were weighed once a week.

2.2 General Procedure

The general procedure of experiment 1 is detailed in Fig.1a. In experiment 2 (Fig. 1b) several modifications were introduced compared with the first one. In addition to the groups we examined in the first experiment, in the second experiment we examined the effects of double exposure to stress in adulthood. Moreover, we intensified the juvenile stress protocol: exposure to the platform stress was repeated for three consecutive days (at the age of 26-28 days). Furthermore, in the second experiment rats were trained in a massed training protocol (see below: massed spatial training - experiment 2). Finally, in experiment 3 (Fig. 1c) we focused on the better performance on the first trial of the spatial learning task, observed in the juvenile + adulthood stress group. Therefore, we recorded the swim pattern and speed.
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat of the juvenile + adulthood stress and the control groups.

2.3 Behavior

2.3.1 Elevated-Platform Stress (PL)

Following four days of habituation to the housing conditions, individual rats (age 28 days) in the juvenile and the juvenile + adulthood stress groups are placed for 30 min on an elevated black platform (12.0 × 12.0 cm) whose top lies 10.0 cm above the water surface. The platform is located in the middle of a water pool. Rats are subjected to this stress three times, separated by 1h in a resting cage, before being returned to their home cage. In experiments 2 and 3, rats are subjected to this stress during three consecutive days (age 26-28 days).

2.3.2 Acute swim stress (ASS)

Individual rats (age 12 weeks) exposed to adulthood stress are made to swim for 15 min in a circular water tank (diameter: 0.5m, height: 0.5m). Water depth is 40.0 cm, and temperature is maintained at 23 ± 1 °C. Following exposure to the ASS, rats are allowed to dry (for 1h) in a resting cage prior to commencing the post-stress tests (Avital et al., 2001).

2.3.3 Open-Field Test

The open field may be seen as a non-conditioned anxiety test based on the creation of a conflict between the exploratory drive of the rat and its innate fear of exposure to an open area (Angrini et al., 1998; Broadhurst, 1975).

One hour post adulthood stress (experiment #1 - following ASS; experiment #2 - following PL stress), the open-field test was carried out according to methods described previously (Carli et al., 1989; Lemoine et al., 1990). Briefly, the open field consists of a wooden box 90.0 × 90.0 × 38.0 cm positioned in a dimly lit room. The
walls are painted black, the floor is white and divided by 1cm-wide black lines into 25 squares $17.0 \times 17.0$ cm. Rats are placed at the corner of the open field. For the following 3 min, the number of line crossings in the central and the peripheral areas are manually recorded. The total line crossings represent the activity level of the rat, whereas the ratio between line crossings in the peripheral area and the total line crossings represents an anxiety index (Avital et al., 2001). All rats are tested between 1100h and 1700h by the same experimenter.

2.3.4 The water-maze task

The water-maze (Morris, 1984) consists of a circular pool of water (1.7m in diameter with a rim 0.5m high) painted black. Water depth is 30.0 cm, and temperature is maintained at $23 \pm 1 \, ^\circ\text{C}$. The only means of escape from swimming is a hidden black platform ($12.0 \times 12.0$ cm) whose top lies 3.0 cm beneath the water surface. The maze itself is featureless and the only obvious landmarks are those outside the water-maze, in the surrounding environment. Rats are placed in the maze and allowed to swim freely for a maximum of 1 min or until they reach the hidden platform. At the end of each trial rats are either led to or left on the platform for 15 s. Each trial begins from a different starting point in random order. The experimenter measures the escape latency with a stopwatch. All rats are tested between 1100h and 1700h by the same experimenter.

2.3.5 Water-maze spaced spatial training (experiment 1)

In a spatial learning task that is carried out one day after the open-field test, rats are given one block of three trials per day (with inter-trial interval of one minute) for six consecutive days (Richter-Levin and Segal, 1991). On the seventh day and for three days thereafter (four trials per day, with inter-trial-interval of one minute), the escape
platform is positioned at a new location and the rats are trained to find this new location (reversal task).

2.3.6 Water-maze massed spatial training (experiment 2)

The difference in watermaze performance in the first experiment was mainly during the first training day. To focus on this difference in performance we have employed in the second experiment a more intensive, single day protocol, as described previously (Akirav et al., 2001). In the massed spatial learning task conducted one day after the open-field test, rats were given a total of 12 trials with inter-trial intervals of one and four minutes, alternately. A criterion for good performance (>15 sec to allocate the escape platform) was defined, as described before (Akirav et al. 2001) which is based on the maze size and the average swim speed.

2.3.7. Tracing system (experiment 3)

To analyze the differences in path length and swim speed, we used the Water-maze 3.1, which is a computer-based system to analyze Morris water-maze trials online (Grossmann and Skinner, 1996).

2.3.8 The startle response test

The acoustic startle response test is used extensively to index fear and anxiety in rodents (e.g., Faraday and Grunberg, 2000) and humans (e.g., Riba et al., 2001). Previous studies have shown that the amplitude of the acoustic startle response is increased by the presentation of aversive stimuli, e.g., noise (Vazquez et al., 1996), and by Corticotropin Releasing Factor (CRF) infusion (Lee and Davis, 1997; Liang et al., 1992).

The startle response (Patrick, 1994) was measured by an automated JR. Startle box (Hamilton-Kinder, U.S.A.) that was positioned in a dimly lit room. Immediately after exposure to the water-maze spatial learning, rats are habituated for
30 min to the startle test room before being placed in the chamber. All rats are tested between 1100h and 1700h by the same experimenter. Rats are subjected to eight tones (40.0 ms 115 dB noise stimulus), separated by 1 min. The maximum startle response for each tone is measured.

2.4 Statistical Analysis

Results are presented as means ± standard error of the means (SEM). Two-way mixed analysis of variance (ANOVA) as well as one-way ANOVA followed by Tukey post hoc multiple comparison tests were used as indicated. In addition, a Student’s t-test for unpaired samples and Pearson’s correlation coefficient were calculated. All tests were two-tailed and a $P$ value of less than 0.05 was considered statistically significant. All tests were done with use of SPSS 11.0.

2.5 Approval

The experiments were approved by the institutional Animal Care and Use Committee, and adequate measures were taken to minimize pain or discomfort, in accordance with the guidelines laid down by the NIH in the USA regarding the care and the use of animals for experimental procedures.

3. Results

3.1 Experiment 1:

Activity level was measured as the total line crossings in the open field (Fig. 2a). One-way ANOVA revealed a significant treatment effect [$F(3,28) = 15.54$, $P<0.0001$]. Post hoc Tukey test revealed a significant decrease in activity level of the juvenile stress group ($P<0.01$), the adulthood stress group ($P<0.001$), and the juvenile + adulthood stress group ($P<0.001$), compared with the control group.
The ratio between the number of line crossings in the peripheral part of the open field and the total line crossings is considered a measure of anxiety (Fig. 2b). One-way ANOVA revealed a significant treatment effect \[F(3,28) = 21.72, P<0.0001\]. Post hoc Tukey test revealed that exposure to stress in adulthood significantly increased anxiety level compared with the control group \((P<0.001)\) and the juvenile stress group \((P<0.01)\). Juvenile + adulthood exposure to stress increased anxiety levels further, and the anxiety index was significantly higher than in the control group \((P<0.001)\), the juvenile stress group \((P<0.001)\), and the adulthood stress group \((P<0.05)\).

Beginning 24h after exposure to stress in adulthood, all groups were trained for six days on a spatial learning task (platform was positioned in q1) in the Morris water-maze (Fig. 3). Two-way mixed ANOVA with group as a between-subjects factor and days as a within-subjects factor revealed a significant main effect of days \([F(3,101)=145.69, P<0.0001]\), group \([F(3,28)=27.27, P<0.0001]\), and the interaction between the two factors \([F(10,101)=3.36, P<0.001]\). Importantly, post hoc Tukey test revealed that the juvenile + adulthood stress group performed significantly better than the other groups \((P<0.0001)\) across the first four days of training. However, this difference tended to disappear over days (on the 5th and the 6th days), when rats from the other groups improved their performance.

On the seventh day, and for three days thereafter, the escape platform was positioned in a new location (q3) and the rats were trained to find the new platform location (Fig. 4). One-way ANOVA revealed a significant treatment effect of first day’s performance
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat [F(3,28)=13.488, P<0.0001]. The juvenile + adulthood and the juvenile stress groups performed significantly better than either the controls (P<0.001) or the adulthood stress group (P<0.05). The adulthood stress group performed significantly better than the control group (P<0.01).

Rats were then tested for startle response (Fig. 5). One-way ANOVA revealed a significant treatment effect [F(3,28) = 17.686, P<0.0001]. Post hoc Tukey test revealed that exposure to stress in adulthood significantly increased startle response compared with the control group (P<0.01). However, the combination of juvenile and adulthood exposure to stress significantly increased startle response compared with all groups (P<0.001).

Finally, to assess the relationship between the differences observed in the first trial performance in the water-maze and the anxiety level measured in the startle response test, we calculated a Pearson correlation. A significant negative correlation was found between these two factors [r_p= - 0.623, P<0.0001], i.e., better performance in the first trial was associated with higher anxiety level.

3.2 Experiment 2:

The activity level was measured as the total line crossings in the open field (Fig. 6a). One-way ANOVA revealed a significant treatment effect [F(5,42) =20.58, P<0.0001]. Post hoc Tukey test revealed a significant decrease in activity level in all experimental groups compared with the control group (P<0.001).

Using the ratio between the number of line crossings in the peripheral part of the open field and the total line crossings as a measure of anxiety (Fig. 6b), one-way ANOVA revealed a significant treatment effect [F(5,42) = 97.57, P<0.0001]. Post hoc Tukey test revealed that exposure to juvenile stress significantly increased anxiety.
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat level compared with the control group (\(P<0.001\)). In addition, exposure to stress in adulthood significantly increased anxiety level compared with the control group (\(P<0.001\)) and the juvenile stress group (\(P<0.001\)). Juvenile + adulthood exposure to stress increased anxiety levels further, and the anxiety index was significantly higher than in the control group (\(P<0.001\)), the juvenile stress group (\(P<0.001\)), the adulthood stress group (\(P<0.001\)), double exposure to adulthood stress (\(P<0.001\)), and adulthood stress group (\(P<0.01\)).

Twenty-four hours after exposure to stress in adulthood, all groups were trained for a massed spatial learning task in the Morris water-maze (Fig. 7). Two-way ANOVA with group as a between-subjects factor and trials as a within-subjects factor revealed a significant main effect of trials [\(F(7,307)=101.97, P<0.0001\)], group [\(F(5,41)=14.08, P<0.0001\)], and the interaction between the two factors [\(F(37,307)=3.36, P<0.001\)]. Interestingly, post hoc Tukey test revealed that the juvenile + adulthood stress group performed better significantly than all other experimental groups (\(P<0.0001\)) across all trials.

Rats were then tested for startle response (Fig. 8). One-way ANOVA revealed a significant treatment effect [\(F(5,42) = 137.93, P<0.0001\)]. Post hoc Tukey test revealed that exposure to stress in adulthood significantly increased startle response compared with the control group (\(P<0.001\)) and the juvenile stress group (\(P<0.001\)). However, juvenile + adulthood exposure to stress further increased the startle response. It was significantly higher than in the control (\(P<0.001\)), the juvenile stress (\(P<0.001\)), the adulthood stress (\(P<0.001\)), double exposure to adulthood stress (\(P<0.001\)), and the adulthood (90 days) stress (\(P<0.001\)) groups.

### 3.3 Experiment 3:
Twenty-four hours after the exposure to stress in adulthood, rat's path length and average swim speed were recorded during a one-minute swim trial (without a platform) in the water-maze.

A t-test for independent samples revealed a significant increase in both path length and the average swim speed (Fig. 9a) of the juvenile + adulthood stress group compared with controls \( t(6)=4.96, P<0.01 \), on the first trial performance. There was no significant difference in the maximal swim speed between the control and the juvenile + adulthood stress groups (15.01±3.6 and 16.18±3.39, respectively).

The representative exploration patterns of the control and the juvenile + adulthood stress groups, with reference to peripheral versus central areas of the water-maze, are given in figures 9b,c.

**4. Discussion**

The results of the present study demonstrate that even a brief exposure to juvenile stress can modulate the ability to cope with stress in adulthood, as was observed up to two months later.

The emotional consequence of exposure to juvenile stress was examined in both the open-field and the startle response tests. Single or double exposure to stress in adulthood increased anxiety level, but the exposure to the combination of juvenile and adulthood stress increased anxiety further. The activity level of all stress groups significantly decreased compared with the control group, a finding that excludes the possibility that the differential increase in anxiety level in the adulthood and juvenile + adulthood stress groups may be related to differences in general activity. Similar findings were reported for chronic isolation-reared rats (21-77 days) that showed
increased startle reactivity measured at the end of the isolation period (Varty et al.,
2000). Recently, Maslova et al. (2002) found that exposure to chronic variable stress
(CVS) during the pre-pubertal period of life (21-32 days) produced long-lasting
effects on startle response, comparable to that seen in our juvenile stress group. The
long-lasting effects of CVS experienced in prepubertal life appears to produce startle
response changes similar to those seen in patients with PTSD.

Similar to our finding in the open field, male rats that were exposed to chronic
variable social stress for 28 days at the onset of adolescence showed low locomotor
activity in a novel environment in adulthood, compared with the controls (Kabbaj et
al., 2002). Previously, Marks-Kaufman and Lewis (1984) reported that rats that were
raised in isolation (from weaning until the age of 60 days) were less active in the open
field than animals raised in groups. Early housing experience was also found to
modify later morphine consumption and physical dependence. Animals raised in
isolation exhibited a trend to start drinking morphine sooner and experienced less
severe withdrawal symptoms following naloxone administration than group-raised
animals (Marks-Kaufman and Lewis, 1984). The present results indicate that even a
relatively brief exposure to juvenile stress may lead to long lasting impairments in
coping abilities in adulthood.

The effects of exposure to stress in adulthood on the performance of rats in a spatial
learning task in the Morris water-maze, as a function of exposure to juvenile stress
was also tested. There is abundant evidence that depending on the severity and
context, stress can either improve or impair performance in the water-maze task.
Intense arousal and corticosterone were reported to enhance performance on a spatial
learning task in the Morris water-maze
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat (Vazquez, 1998). Similarly, in the first experiment, the adulthood stress group preformed significantly better than the juvenile stress group and the control group on the first day. Interestingly, rats exposed to the combination of juvenile + adulthood stress performed significantly better than all other groups. This difference tended to disappear over days, when rats from the other groups improved their performance. A significant negative correlation was found between the performance on the first day in the water-maze and the anxiety level measured in the startle response test. Therefore, the differences in performance observed in the first day may be related to the differences in anxiety level.

Furthermore, during the first day of a reversal-learning task in experiment 1, the control group exhibited the worst level of performance, whereas rats exposed to juvenile or juvenile + adulthood stress performed significantly better than both the control and the adulthood stress groups. This result may be an indication that spatial memory for the previous location of the platform (learned during the first phase of the experiment) was most efficient in the control group compared with all stress groups. Retaining the memory of the previous location of the platform can be expected to interfere at first with the shift to learning the new platform location.

To focus on the difference in performance during the first day we have employed in the second experiment a single day, one hour massed training protocol.

In both the first and the second experiment rats exposed to juvenile + adulthood stress performed better mainly during the first trial of the water-maze task. Therefore in the third experiment we examined the path length and the averaged swim speed on a single (1st) trial and found that both measures were increased following the exposure to juvenile + adulthood stress. Interestingly, it seems that the stressed rats tended to
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swim more in the central part than in the peripheral part of the water-maze, compared with controls. Such a pattern of behavior during the first trial could explain the faster level of acquisition of the water-maze task, observed in these animals, since this pattern of behavior increases the chance of locating the hidden platform. The different swim patterns of control and juvenile+adulthood stress rats in the water-maze are opposite to those observed in the open-field and may be explained by the different nature of these two tests: compared to the water-maze, the open-field considered as a non-conditioned anxiety test based on the creation of a conflict between the exploratory drive of the rat and its innate fear of exposure to an open area (Angrini et al., 1998; Broadhurst, 1975).

A repeated exposure to adulthood stress yielded a higher startle response compared to a single adulthood exposure. However, it should be noted that the exposure to juvenile+adulthood stress yielded a significantly more robust increase in startle response, a significantly higher anxiety index in the open field test, as well as an effect on water maze performance, thus supporting the notion that the juvenile period is a particularly sensitive period and that an exposure to stress during this period may lead to adverse consequences in adulthood.

In sum, exposure to juvenile stress enhanced anxiety level in response to exposure to stress in adulthood, as measured both in an open-field and a startle box. Similarly, exposure to juvenile stress accentuated the effects of the adulthood stress on performance in a spatial learning task in the Morris water-maze.

These results indicate that exposure to a relatively brief stressful experience during the post-weaning pre-puberty period has profound and long-lasting effects on the ability to cope with stress in adulthood.
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This model may now be used to enhance the search for the neurobiological and endocrine mechanisms associated with stress-related syndromes.

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**Statement of Interest:** None
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Figure Legends

Figure 1. A flow chart describing the chronology of exposure to stress and the behavioral tests. The juvenile + adulthood stress group was exposed to all stages, as depicted in the chart. Other groups were exposed only to those stages indicated below.

(A) Experiment 1 - Four groups were tested:

1) Control - Naive rats (n=8).

2) Juvenile stress - Rats were exposed to an elevated platform (PL) at the age of 28 days (n=8).

3) Adulthood stress - Rats were exposed to acute swim stress (ASS) at the age of 90 days (n=8).

4) Juvenile + Adulthood stress - Rats were exposed both to PL (at the age of 28 days) and ASS (at the age of 90 days) (n=8).

* All rats were similarly examined from the age of 90 days in a battery of tests including the open-field, the water-maze, and finally, the startle response test.

(B) Experiment 2 - Rats were exposed to two stressors, one month apart. They were exposed either to a Juvenile + Adulthood or to Adulthood (60) + Adulthood (90) stress. Other groups were exposed only to those stages indicated below.

The following groups were compared:

1) Control - Naive rats (n=8).

2) Juvenile stress - Rats were exposed to PL at the age of 26-28 days (n=8).

3) Adulthood (60) stress - Rats were exposed to PL at the age of 60 days (n=8).

4) Juvenile + Adulthood stress - Rats were exposed to PL both at the age of 26-28 and at the age of 60 days (n=8).
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5) Adulthood (90) stress - Rats were exposed to PL at the age of 90 days (n=8).

6) Adulthood (60)+(90) stress - Rats were exposed to PL at the age of 60-62 days and again at the age of 90 days (n=8).

* The following groups were examined at the age of 60 days: Juvenile, Adulthood (60) and Juvenile + Adulthood stress groups.

The following groups were examined at the age of 90 days: Adulthood (90) and Adulthood (60)+(90) stress groups.

The control group included 4 control (60) (examined at the age of 60 days) and 4 control (90) (examined at the age of 90 days) rats. Previous verification indicated that there was no differences between those groups and so they were grouped together.

(C) Experiment 3 - The following groups were compared:

1) Control - Naive rats (n=4).

2) Juvenile + Adulthood stress - Rats were exposed to PL at the age of 26-28 days and then to ASS at the age of 60 days (n=4).

Figure 2. In the open field test (Experiment 1) (A) a significant decrease in the total number of crossings was observed in all experimental groups as compared with the controls. (B) The juvenile + adulthood stress group showed the highest anxiety index (** P<0.001; * P<0.05).

Figure 3. In a spaced spatial learning task (Experiment 1), the juvenile + adulthood stress group learned the location of the hidden platform better than the other groups. This difference tended to disappear over days (*P<0.0001).
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Figure 4. On the first day of training in a reversal spatial learning task (Experiment 1) the juvenile + adulthood and the juvenile stress groups have reached the escape platform significantly faster than the control (**P<0.01) and the adulthood stress (*P<0.05) groups. The adulthood stress group has reached the platform significantly faster than the control group (**P<0.01). These differences disappeared from the second day and thereafter.

The results suggest that spatial memory for the previous location of the platform was most efficient in the control group, which was thus more significantly disturbed during the first reversal trials.

Figure 5. In the startle response test (Experiment 1), rats exposed to stress in adulthood had a significantly increased startle response than the control group. However, exposure to the combination of juvenile and adulthood stress significantly increased startle response as compared with all other groups (**P<0.01; **P<0.01).

Figure 6. In the open field test (Experiment 2), (A) a significant decrease in the total number of crossings was observed in all experimental groups.

(B) Exposure to juvenile stress significantly increased anxiety index as compared with the control group. In addition, exposure to stress in adulthood significantly increased anxiety level as compared with both the control and the juvenile stress groups. However, exposure to juvenile + adulthood stress further increased anxiety level, which was significantly higher than in all other groups (**P<0.01; **P<0.01).
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

Figure 7. In a massed spatial learning task (Experiment 2), the juvenile stress group showed poor learning, whereas the juvenile + adulthood stress group have reached the criterion of good performance (>15 sec to reach the escape platform, dushed line) faster than all other experimental groups.

Figure 8. Adulthood exposure to stress significantly increased startle response (Experiment 2). However, exposure to juvenile + adulthood stress further increased startle response in comparison with all other groups (**P<0.01; *P<0.05).

Figure 9. During the first trial in the Morris water-maze, (A) rats exposed to juvenile + adulthood stress swam faster than controls (*P<0.01), and (B) tended to explore the central part of the water-maze more than (C) controls.
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

**Figures**

Figure 1A

- **Behavioral tests:**
  - Open field test
  - Morris Water-maze:
    - Spaced spatial task.
    - Reversal task
  - Startle response test

Days: 0, 10, 20, 28, 30, 40, 50, 90
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Figure 1B

- Juvenile stress
- Adulthood stress (60)
- Juvenile + Adulthood stress
- Adulthood stress (90)
- Adulthood (60)+(90) stress

Behavioral tests:
- Open field test
- **Morris Water-maze:** Massed spatial task.
- Startle response test
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

Figure 1C

Behavioral tests:
- Morris Water-maze:
  One minute swim without platform.
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

Figure 2A

Figure 2B
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

Figure 3
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

Figure 4

Latency in Sec.

- Control
- Juvenile stress
- Adulthood stress
- Juv.+Adu. stress

Day

0 5 10 15 20 25 30 35

1 2 3

*** ** *
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

Figure 5

![Stress Response Graph](image)
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.

**Figure 6A**

![Graph showing total crossings across different groups](Image)

**Figure 6B**

![Graph showing anxiety index across different groups](Image)
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

Figure 7
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat.
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Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

Figure 9A
Exposure to juvenile stress exacerbates the behavioral consequences of exposure to stress in the adult rat

Figure 9B

Figure 9C