Learning under stress in the adult rat is differentially affected by ‘juvenile’ or ‘adolescent’ stress

Michael Tsoory and Gal Richter-Levin
Department of Psychology and The Brain & Behavior Research Center, University of Haifa, Mount Carmel, Haifa, Israel

Abstract
Epidemiological studies suggest that childhood trauma is associated with a predisposition to develop both mood and anxiety disorders, while trauma during adolescence is associated mainly with anxiety disorders. We studied in the rat the long-term consequences of ‘juvenile’ stress, namely stress experienced in a period in which substantial remodelling occurs across species in stress-sensitive brain areas involved in emotional and learning processing. In adulthood, ‘juvenile’ stressed rats exhibited reduced exploration in a novel setting, and poor avoidance learning, with 41% learning mainly to escape while 28% exhibited learned helplessness-like behaviours. In adult rats that underwent ‘adolescent’ stress, learned helplessness-like behaviours were not evident, although decreased exploration and poor avoidance learning were observed. This suggests that in the prepubertal phase juvenility may constitute a stress-sensitive period. The results suggest that juvenile stress induces lasting impairments in stress-coping responses. The ‘juvenile’ stress model presented here may be of relevance to individuals’ reported predisposition to anxiety and depression following childhood trauma, and their increased susceptibility only to anxiety disorders following adolescent stress.

Received 26 January 2005; Reviewed 9 March 2005; Revised 16 August 2005; Accepted 21 August 2005; First published online 2 December 2005

Key words: Adolescent, juvenile, rat, stress.

Introduction
Mood and anxiety disorders are associated with multiple endocrine and anatomical changes in neuronal circuits that are critically involved in modulating stress responses, emotional processing, and learning (Arborelius et al., 1999). Epidemiological studies indicate that early-life stress (ELS) is predominantly associated with higher prevalence of both mood and anxiety disorders, particularly depression and PTSD (Agid et al., 1999; Arborelius et al., 1999; Briere and Elliott, 1994; Draijer and Langeland, 1999; Furukawa et al., 1999; Heim and Nemeroff, 2001; Heim et al., 2004; Maughan and McCarthy, 1997; Weiss et al., 1999). While the long-term effects of ELS are well recognized, conflicting findings exist regarding the significance of the developmental stage at the onset of the adversity. Heim and colleagues (Heim and Nemeroff, 2001; Heim et al., 2004) argued that any form of pre-pubertal stress may be classified as ELS. Others maintain that younger children suffer more severe consequences (Beitchman et al., 1992). Some studies claim that no systematic relationship exists between the victim’s age at the time of the emotional assault and the degree of disturbance (Kiser et al., 1991).

A potentially stress-sensitive period may be juvenility and/or early adolescence. Across species the juvenile brain is ‘in transition’, differing markedly both anatomically and neurochemically from that of newborns, weanlings, or adults. During this period substantial remodelling occurs in brain areas that are involved in emotional and learning processing such as the prefrontal cortex (PFC), the hippocampus, and the amygdala (for a review see Spear, 2000). Pre-teens and adolescent humans have enhanced stress perception and responses. Stressful life events during this period were associated with later socio-emotional maladaptive behaviours (Spear, 2000) and were suggested to represent a significant risk factor for the later development of stress-related
psychopathologies (Heim and Nemeroff, 2001; Heim et al., 2004).

Most common rodent ELS models focus on the perinatal pre-weaning period and involve some form of maternal deprivation or separation (Kehoe et al., 1998; Levine et al., 1991; Ogawa et al., 1994; Plotzky et al., 1993; Rosenfeld et al., 1992; van Oers et al., 1998; Vazquez et al., 1996; von Hoersten et al., 1993), producing acute and long-term effects that vary with the pups’ age (Levine, 1994). For example, prolonged early maternal separation attenuated rates of synaptic development in the hippocampus, which was evident only after sexual maturation (Andersen and Teicher, 2004). However the brain’s development continues well after the pre-weaning period, and substantial maturation processes like myelination continue with varying dynamics well into puberty (Hamano et al., 1998). The ongoing maturational changes render the post-weaning brain susceptible to the harmful effects of stress. Prolonged post-weaning isolation rearing compromises the development and function of central aminergic neurotransmission, which is associated with altered behaviours in adulthood (Lapiz et al., 2001; Muchimapura et al., 2002, 2003).

Furthermore, in this early pre-weaning period (i.e. 3–14 days) that most ELS studies focus on, substantial differences exist between rodents and primates in the course of neural development of the limbic system and the hypothalamic–pituitary–adrenal (HPA) axis. At this stage the rat’s HPA axis is characterized by a silent hypo-responsive period (Vazquez et al., 1996). In humans there is no conclusive evidence for a hypo-responsive period during the course of development of the HPA axis (Gunnar and Donzella, 2002). It was suggested that the age of 3–14 days in the rat roughly corresponds to the 23rd week of gestation in humans (Fitzgerald and Anand, 1993).

To model in the rat the detrimental effects of childhood and early-adolescent emotional trauma observed in humans, we focused on the juvenile and early adolescent period in the rat ontogeny, i.e. 27–35 days. Juvenile rats resemble children and pre-teens in several behavioural features such as maternal care independence, and increased play-behaviours with peers, which diminish with the beginning of puberty (Spear, 2000). Moreover, as in human neural development, the juvenile rat HPA axis is fully developed, while other key brain areas involved in both emotional and learning processes, like the PFC, hippocampus, and amygdala-based neurocircuits, are still undergoing significant maturation processes (Spear, 2000). Using immunoreactivity to synaptophysin, Andersen and Teicher (2004) demonstrated the over-production and pruning trajectory that occurs in the rat’s hippocampus (CA1, CA3), amygdala, and PFC between pre-puberty and adulthood. The HPA axis reaches its developmental asymptote around the juvenile period (Vazquez, 1998; Walker et al., 1986). Juvenile rats’ stress response lasts twice as long as in adults, indicating a slower shut-off of the HPA axis and suggesting less centrally mediated feedback from various forebrain limbic areas at this developmental stage (Romeo et al., 2004).

The PFC is substantially remodelled, diminishing in volume, during this period in both humans and rats (Genazzani et al., 1997; Insel et al., 1990; Jernigan et al., 1991; Kalsbeek et al., 1988; Sowell et al., 1999; Van Eden et al., 1990). Similarly, hippocampal pyramidal neurons, for example, undergo NMDA receptor pruning processes between the ages of 28 and 60 d (Insel et al., 1990). The underdeveloped hippocampus lacks full inhibitory responses between CA3 and CA1 subfield (Michelson and Lothman, 1989) and GABAB-mediated inhibitory transmission (Nurse and Lacaille, 1999). On the behavioural level, PFC and hippocampal-dependent behaviours, like the T-maze discrimination task, are also compromised (Bronstein and Spear, 1972). The juvenile rat amygdala is distinct from that of both younger and older rats. Juvenile and adolescent rats are markedly more sensitive to seizures induced by electrical stimulation of the amygdala (Terasawa and Timiras, 1968) and exhibit less stress-induced c-fos activity in certain amygdala nuclei (Kellogg, 1998). Juvenile rats differ from adults also in the altered behavioural profile following pre-weaning exposure to stress, suggesting differential effects of corticosterone elevation on the developing hippocampus (Frisone et al., 2002).

Exposure to stressors during the juvenile period has often been reported to produce more pronounced effects than exposure at earlier or later ages. Prominent effects of social isolation during adolescence were reported on object exploration (Einon and Morgan, 1977) and fluid intake (McGivern et al., 1996). Deprivation from social play behaviours during juvenility disrupted social and non-social behaviours in adulthood and was associated with dysregulation of the endogenous opioid systems during juvenility (Van den Berg et al., 1999a–c, 2000).

Juvenile stress exposure was suggested to affect limbic system development, leading to enduring effects on stress coping in adulthood. Adult rats exposed to chronic variable stress in juvenility (21–32 d) had an enhanced acoustic startle response, similar to patients with PTSD (Maslova et al., 2002).
Avital and Richter-Levin (2005) showed that acute juvenile stress may produce increased vulnerability to stressful events in adulthood, resulting in an augmented response to adverse experiences. The combination of exposure to stress at juvenility and re-exposure in adulthood, at 60 or 90 d of age, increased anxiety levels, as measured in the open field and by startle response tests, and affected water maze performance not only in comparison with control rats but also in comparison with rats that were exposed and then re-exposed to the stress protocol only in adulthood, at 60 and 90 d. This suggested that the significant anxiogenic effect observed may be attributed to the juvenile stress exposure.

Accordingly, the present study examined the consequences of juvenile stress alone on learning under stressful conditions in adulthood. Furthermore, to test for a ‘sensitive period’ during this post-weaning pre-pubertal phase we compared the effects of exposure to stress at juvenility (28 d) and adolescence (34 d).

Our study also addressed the issue of analysing animals’ responses individually in order to form ‘behavioural profiles’ of altered responses in a manner that resembles clinical diagnosis (Cohen et al., 2003, 2004). A person is diagnosed as suffering from a certain stress-related psychopathology only if he or she exhibits a cluster of symptoms from well-defined symptom clusters over a certain period of time; yet animal studies include the entire exposed population as the study population, although in practice both control and stressed animals display a diverse range of responses. To approximate animal models to contemporary clinical standards we applied a ‘human diagnostic strategy’ of clustering symptoms on the rats’ responses in adulthood, which allowed the discrimination of different patterns of altered responses; animals were segregated according to the pattern by which their coping with learning the aversive task was disrupted.

Methods

Study design

To evaluate the consequences of exposure to stress in juvenility or adolescence on coping with stress in adulthood, rats were randomly assigned to one of four groups as follows: exposure to a short-term stress paradigm at either juvenility, 27–29 d (JUV28) or adolescence, 33–35 d (ADL34). In adulthood, at ~8.5 wk old, their exploratory behaviour was assessed prior to and 24 h following an additional stressful challenge, namely learning the two-way shuttle avoidance task.

Their exploratory behaviour prior to the aversive avoidance learning as well as their performance in the two-way shuttle avoidance task were compared with control rats (Control) that were not exposed to adverse conditions prior to the two-way shuttle avoidance task in adulthood. Their exploratory behaviour was assessed in the open-field test 24 h after the adulthood stressful challenge and compared with naive rats (Naive) that were not exposed to any adverse conditions at any age.

Materials and methods

Animals

A total of 82 male Sprague-Dawley rats (Harlan Laboratories Jerusalem), 22 d old on delivery, weighing 35–49 g, were maintained for the entire duration of the experiment on a 12-h light-dark cycle (lights on at 07:00 hours). Room temperature was 22 ± 2°C, with four rats per cage (35 × 60 × 18 cm) on sawdust bedding, with water and solid food pellets (Teklad Global Diet 2018S, Harlan Teklad Ltd, WI, USA) provided ad libitum.

Behavioural procedures

Stress procedure. The stress procedure was 3 d in tandem exposure to stressors, with a different protocol each day, as detailed below.

Day 1 – Forced swim

Ten minutes forced swim in a circular water tank (diameter 0.5 m, height 0.5 m, water depth 0.4 m), water temperature 22 ± 2°C (adapted from Avital et al., 2001; Hall et al., 2001).

Day 2 – Elevated platform

Three 30-min trials; inter-trial interval (ITI): 60 min in the home cage. Elevated platform (12 × 12 cm), 70 cm above floor level, located in the middle of a small closet-like room (adapted from Maroun and Richter-Levin, 2003).

Day 3 – Foot shock

A 3-min session of six trials, unconditioned stimulus (US): electric foot shock (1 s, 0.8 mA); ITI: 29 s. Apparatus: a small cube-like chamber (31 × 31 × 31 cm) with a metal-grid floor connected to a computer-controlled electrical shocker device (Solid State Shocker/Scrambler, Model no. 113-33, Lehigh Valley Electronics Ltd, Lehigh Valley, PA, USA).
Protocols were applied simultaneously to rats in all stress groups, so no rat was left isolated in the home cage. On days 1 and 2 at the end of the stress protocol, rats were returned to their home cage and were not handled until the next day. Following the completion of the Foot-shock session, on day 3, rats were returned to their home cage and were not handled until adulthood, except for weekly cage maintenance.

The 3-d in-tandem variable stress protocol, applied at juvenility or adolescence, consisted of stress procedures chosen for their well-documented effects on adult rats.

- **Forced swim**: the forced-swim test procedure, adapted from Porsolt et al. (1977), was shown significantly to increase circulating corticosterone levels (Hall et al., 2001).
- **Elevated platform**: 60 min (Degroot et al., 2004) or even 15 min (Ebner et al., 2004) elevated platform stress significantly activated stress response mechanisms.
- **Foot shock**: even mild foot-shock protocols in adult rats elicit activation of stress response mechanisms throughout the brain (Li and Sawchenko, 1998; Passerin et al., 2000; Pezzone et al., 1992, 1993; Rassnick et al., 1998) in an activity pattern similar to that following many other stress paradigms (Cecchetti et al., 1989; Cullinan et al., 1995; Melia et al., 1994).
- **Variable stress protocols**: were found to produce stronger effects than repeated stress. Variable and repeated stress protocols differed in their effects on the glucocorticoids (Garcia-Vallejo et al., 1998). Adrenal hypertrophy, a sign that tolerance to stress has not developed (Chappell et al., 1986), was evident in rats subjected to variable stress, but not in those subjected to repeated or acute stress (Prieto et al., 2003). In comparison with the effect of a novel stressor, the rat’s ACTH responses to a familiar stressor were blunted (Simpkiss and Devine, 2003).

Romeo et al. (2004) found an enhanced stress response in pre-pubertal male rats. Therefore, it is reasonable to assume that the combination of the stress procedures used in the current study’s variable stress protocol did in fact activate stress-response mechanisms sufficiently.

**Exploratory behaviour assessment in adulthood prior to the stressful challenge**

In adulthood, at ~8.5 wk, the rats’ exploratory behaviour was assessed with the two-way shuttle avoidance apparatus. Rats were first placed in the apparatus, described below, while it was in inoperative mode, and were allowed to explore both compartments for a total of 10 min. Crossing between compartments was measured as an index of exploratory behaviour. If a rat did not visit the adjacent compartment after 5 min it was manually gently directed through the passage door. The same was done if the rat failed to cross back into the adjacent compartment after a further 5 min. A record was kept by an observing experimenter of the rats’ back-and-forth exploratory shuttles. Only voluntary shuttles were counted.

**Assessment of learning under stressful conditions in adulthood**

Immediately following the exploratory behaviour assessment, rats were trained in the two-way shuttle avoidance task in a single 100-trial session.

**Apparatus**

The two-way shuttle avoidance box, placed in a dimly lit, ventilated, sound-attenuated cupboard, is a rectangular chamber (60 × 26 × 28 cm) divided by an opaque partition with a small (10 × 8 cm) passage connecting two equal-sized side-by-side cube-shaped compartments, A and B. The metal-grid floors of both compartments are weight sensitive; micro-switches transmit information about the rat’s location to a computer control and data collection program which controls both conditioned stimulus (CS) presentations (a tone produced by loudspeakers located on the distal walls of the compartments) and electric shock deliveries (electrical shocker device: Solid State Shocker/Distributor, Coulborn Instruments Inc., Lehigh Valley, PA, USA).

**Procedure**

One session of 100 trials of ‘trace conditioning’. CS: 10 s tone presentation; US: immediately following the termination of the CS an electric shock (0.5 mA) was delivered for a maximum of 10 s; ITI: 60 ± 12 s. Rats could produce one of three responses: (1) Avoidance – shuttling to the adjacent compartment upon hearing the CS tone; the tone stopped and an ITI started; the rat avoided the electric shock. (2) Escape – shuttling to the adjacent compartment while the shock was on; the shock was stopped and an ITI started. (3) No-response – failing to move to the adjacent compartment; the ITI commenced at the end of the 10-s foot shock. Thus, the rat was subjected to the full duration of the electric shock.
The open-field test, assessment of exploratory behaviour 24 h following the stressful challenge in adulthood

Twenty-four hours after the two-way shuttle avoidance task, the rats were tested by the open-field test (adapted from Carli et al., 1989 and Lemoine et al., 1990). Briefly, the open field, a square Plexiglas box (50 × 50 × 38 cm), was positioned in a dimly red-lit, ventilated, sound-attenuated cupboard. The walls were painted black, the floor white, and divided by 0.3 cm-wide black lines into 25 squares of 10 × 10 cm. Rats were placed in the corner and allowed to explore the novel environment for 5 min while their behaviour was videotaped. Each of the sessions recorded was later analysed by two independent ‘blind’ experimenters, who charted the exploration course of the rats and counted line crossings to provide an index of exploratory activity. Correlation between the two observers (calculated as Pearson correlation of line crossings) was highly significant (r = 0.93–0.97, p’s < 0.001) and for each rat the line-crossing index was the mean of the two observers’ scoring.

Ethical approval

All procedures were performed in accordance with the institutional guiding principals for the care and use of animals, and were approved by the institutional ethical committee.

Statistical analysis

The results are expressed as mean ± S.E.M. Differences between groups in exploratory behaviours and total responses in the two-way shuttle avoidance task were determined by ANOVA. Differences between groups in the learning process of the two-way shuttle avoidance task were determined by repeated-measures ANOVA, and an overall mixed ANOVA design.

Differences between groups in frequencies of responses in the two-way shuttle avoidance task and in the prevalence of animals classified into qualitatively distinct avoidance-learning categories were determined by χ² non-parametric analysis.

Classification of animals into qualitatively distinct avoidance-learning categories using cut-off behavioural criteria (CBC) analyses

We analysed the rats’ behaviours while learning the two-way shuttle avoidance task in adulthood in a manner that allowed identification of distinct altered response patterns. This was done by a two-step procedure, detailed below (Cohen et al., 2003, 2004).

Step I. Prior to attempting to distinguish the differentially ‘affected’ subgroups, we performed a preliminary assessment of the overall response of the stressed, exposed population, in order to demonstrate that the stress procedure did in fact significantly affect both the juvenile and adolescent stress groups, compared with controls. Reduced exploratory behaviour in a novel setting, and impaired learning of the two-way shuttle avoidance task in adulthood, both reflect increased anxiety and persistent behavioural changes associated with the exposure to stress (for review see Cohen et al., 2004).

Step II. The ‘cut-off behavioural criteria’ applied to stress-exposed rats. Having established that the stressor had an effect on the exposed rats, we further analysed the patterns of altered responses to form classification criteria based on integrating different levels of responses. In a similar manner to that used in clinical diagnosis procedures to form psychopathological symptom clusters, sets of classification criteria, representing inclusion and exclusion criteria, produced distinct patterns of altered responses in the two-way shuttle avoidance task.

To maximize the accuracy of the animals’ classifications, and to minimize the likelihood of including ‘false-positives’, each animal had to meet both sets of criteria, inclusion and exclusion, to be defined as ‘affected’. Conversely, to be considered to have hardly responded at all, animals had to meet the criteria for ‘unaffected’ behaviours. The validity of the criteria was affirmed by ascertaining that the vast majority of unexposed control animals accorded with the ‘unaffected’ category and only a minority with the ‘affected’. The classification process and criteria are described in details in the Results section below.

Results

Step I

Ascertaining our zero-hypothesis, i.e. demonstrating that exposure to stress in juvenility or adolescence exerts significant overall behavioural effects on exposed rats as a group, compared with unexposed rats.

Exploratory behaviour prior to the stressful challenge in adulthood

Young adult rats from both JUV28 (n = 25) and ADL34 (n = 14) groups exhibited significantly less exploratory behaviour in a novel setting, namely the shuttle box, prior to avoidance learning, while young adult
rats from the Control group (n = 43) engaged in exploratory behaviour.

ANOVA of number of exploratory shuttles revealed a significant main effect for groups [F(2, 79) = 63.57, p < 0.000]. Scheffé post-hoc analysis showed significant differences (p < 0.000) between Control rats (11.65 ± 0.66) and both JUV28 rats (1.08 ± 0.43) and ADL34 rats (0.57 ± 0.25) (Figure 1).

Two-way shuttle avoidance learning in adulthood

Discernible differences were observed in rats’ coping with the avoidance task between and within groups as detailed below for each of the possible behavioural responses.

Avoidance responses. Significant differences (p < 0.001) were observed in avoidance shuttle responses between Controls (n = 26) and both JUV28 (n = 29, p < 0.000) and ADL34 (n = 14, p < 0.014) rats while learning the two-way shuttle avoidance task (Figure 2a). Repeated-measures analysis for avoidance shuttle responses per block of 10 trials revealed a significant main effect for blocks [F(4, 285) = 14.53, p < 0.0001] but not for groups [F(2, 66) = 1.12, n.s.]. ANOVA per block showed that the JUV28 rats exhibited no-response behaviour significantly more than Controls in all blocks (1–10), and significantly more than ADL34 rats in blocks 6–8. ADL34 rats differed significantly from Controls only in block 1.

Significant differences (p < 0.000) were also observed in overall no-response between JUV28 (n = 32, p < 0.000) and Control (n = 27) rats in the two-way shuttle avoidance task. ANOVA for total avoidance shuttle responses revealed a significant main effect for groups [F(2, 70) = 14.36, p < 0.0001]. Scheffé post-hoc analysis showed that Control rats (52.78 ± 4.35) differed significantly from both JUV28 rats (20.41 ± 3.76, p < 0.000) and ADL34 rats (29.86 ± 6.81, p < 0.017) (Figure 2b).

Escape responses. No significant differences were observed in escape shuttle responses between Control (n = 26), JUV28 (n = 29) and ADL34 (n = 14) rats while learning the two-way shuttle avoidance task (Figure 2c). Repeated-measures analysis for escape shuttle responses per block of 10 trials revealed a significant main effect for blocks [F(4, 285) = 14.53, p < 0.0001] but not for groups [F(2, 66) = 1.12, n.s.]. No significant differences were observed either in overall escape shuttle responses between Control (n = 27), JUV28 (n = 32), and ADL34 (n = 14) rats [F(2, 70) = 1.12, n.s.] in the two-way shuttle avoidance task (Figure 2d).

No-response. Significant differences (p < 0.001) were observed in no-response between Controls (n = 26) and both JUV28 (n = 29, p < 0.000) and ADL34 (n = 14, p < 0.014) rats while learning the two-way shuttle avoidance task (Figure 2e). Repeated-measures analysis for no-response per block of 10 trials revealed a significant main effect for blocks [F(4, 271) = 34.21, p < 0.0001] and for groups [F(2, 66) = 9.71, p < 0.001]. ANOVA per block showed that the JUV28 rats exhibited no-response behaviour significantly more than Controls in all blocks (1–10), and significantly more than ADL34 rats in blocks 6–8. ADL34 rats differed significantly from Controls only in block 1.

Significant differences (p < 0.000) were also observed in overall no-response between JUV28 (n = 32) and Control (n = 27) rats in the two-way shuttle avoidance task (p < 0.000). ANOVA for total no-response revealed a significant main effect for groups [F(2, 70) = 10.13, p < 0.000]. Scheffé post-hoc analysis showed that JUV28 rats (29.91 ± 6.48) differed significantly from Control rats (1 ± 0.62, p < 0.000). JUV28 rats also marginally (p < 0.069) differed from ADL34 rats (n = 14) (11.07 ± 2.90) (Figure 2f).

Exploratory behaviour in the open-field test 24 h after the stressful challenge in adulthood

Twenty-four hours after the two-way shuttle avoidance learning task, marked differences were found in rats’ exploratory behaviour in the open-field test. Young adult rats from the JUV28 (n = 12) and ADL34
(n = 14) groups did not differ significantly. Both groups exhibited considerably less exploratory behaviour in the open-field test than young adult rats from both the Control (n = 12) and Naive (n = 10) groups. Control rats, which underwent their first stressful experience in adulthood (in the two-way shuttle avoidance task), exhibited exploratory behaviour no different from that of the Naive rats. ANOVA of total number of line crossings in 5 min in the open field revealed a significant main effect for groups [F(3, 44) = 12.55, p < 0.001]. Scheffé post-hoc analysis found significant differences (p < 0.01) between Control rats (121.75 ± 10.61) and both JUV28 (45.33 ± 10.24) and ADL34 rats (52.71 ± 10.17). Naive rats (111.40 ± 7.31) also differed significantly (p < 0.01) from both JUV28 (45.33 ± 10.24) and ADL34 rats. No difference was found between JUV28 and ADL34 rats or between Naive and Control rats (Figure 3).

Step II
After establishing that juvenile or adolescent exposure to stress had an enduring effect (Step I), we further analysed the animals’ responses in the two-way shuttle avoidance task based on their total avoidance responses and total no-response frequencies. Total escape responses were not included as these were not found to distinguish groups. We compared the distribution of both total avoidance responses and total no-response across groups.

Distribution of total avoidance responses across groups
Pearson χ² analysis showed a significant difference among groups with respect to the distribution of total avoidance responses within each group [χ²(8) = 22.49, p < 0.004; ϕ = 0.551; r = 0.390, p < 0.004] (Table 1a).

Distribution of total no-response across groups
Pearson χ² analysis showed a significant difference among groups with respect to the distribution of total no-response within each group [χ²(8) = 16.71, p < 0.033; ϕ = 0.475; r = 0.336, p < 0.033] (Table 1b).

After establishing the relationships between the distribution of both total avoidance responses and total no-response and the age of exposure to stress, we proceeded to set our cut-off criteria.

Total avoidance responses cut-off. The ‘unexposed to early stress’ Control group 25th percentile was taken as the total avoidance cut-off criterion (34 total avoidance responses).

Total no-response cut-off. Only in JUV28 group rats did the total no-response extend from 0 to 100. Their distribution pattern was apparently bi-modal, with nearly a third of the animals not responding more than 61% while the remaining majority failed to respond less than 40%. Thus, a cut-off criterion of the value of 60% total no-responses was defined.

These criteria enabled us to distinguish three qualitatively distinct avoidance-learning categories: (1) Rats that performed more than 34 total avoidance shuttle responses and failed to respond <60% were termed ‘Good learners’. (2) Rats that performed less than 34 total avoidance shuttle responses but failed to respond <60% were termed ‘Bad learners’. (3) However, rats that performed less than 34 total avoidance shuttle responses but also failed to respond >60% were termed ‘Learned helplessness’.

Pearson χ² analysis showed a significant difference among groups with respect to the proportion of Good learners, Bad learners, and Learned helplessness rats within each group [χ²(4) = 18.48, p < 0.001; ϕ = 0.503; r = 0.356, p < 0.001] (Figure 4).

In the Control group 75% were categorized as Good learners and the remaining 25% as Bad learners. None of the rats in the Control group were categorized as Learned helplessness.

In the ADL34 group, only 50% were categorized as Good learners, while the remaining 50% were categorized as Bad learners. As in the Control group, none of the rats in the ADL34 group were categorized as Learned helplessness.

However, in the JUV28 group just 29% were categorized as Good learners, 42% were categorized as Bad learners, and 29% were categorized as Learned helplessness.

It is noteworthy that the proportion of Bad learners increased from 25% in the Control group to 50% in the ADL34 stress group to ~70% (including the Learned helplessness rats) in the JUV28 stress group. Furthermore, Learned helplessness rats were found only in the JUV28 stress group (constituting nearly a third of that population) and did not appear in either the ADL34 stress or Control group.

Discussion
The current study’s results demonstrate long-term effects of juvenile stress on adulthood in coping with stressful experiences; young adult rats exposed to juvenile stress (27–29 d) exhibited significantly reduced exploratory behaviour in a novel setting and poor avoidance learning. Furthermore, we were able to distinguish two behavioural profiles of impaired avoidance learning among the juvenile stress-exposed rats. The first, termed ‘Learned helplessness-like
Figure 2. Adulthood two-way shuttle avoidance task: Across-group comparison of avoidance responses (a, b), escape responses (c, d), and no-response (e, f), while learning the two-way shuttle avoidance task. In adulthood, at age ~8.5 wk, both JUV28 (n = 29) and ADL34 (n = 14) were significantly more impaired in learning the two-way shuttle avoidance task than Control rats (n = 26), as indicated by significantly fewer avoidance responses (p < 0.001) (a, b) and significantly more no-response (p < 0.001) (e, f). No significant differences were detected between the groups’ escape responses (c, d). (a) Avoidance responses throughout learning the two-way shuttle avoidance task. Significant differences (p < 0.000) were observed between groups’ avoidance shuttle responses while learning the two-way shuttle avoidance task. Control rats differed significantly from both JUV28 (p < 0.000) and ADL34 (p < 0.014) rats (** Control > JUV28, p < 0.00; # Control > ADL34, p < 0.05; ## Control > ADL34, p < 0.00; ~ Control > ADL34, p < 0.06). (b) Overall avoidance shuttle responses in the two-way shuttle avoidance task. Significant differences (p < 0.000) were also observed between groups’ overall avoidance shuttle responses in the two-way shuttle avoidance.
behaviour’, is characterized by poor avoidance learning with a high frequency of no-response trials. The second impaired avoidance learning profile, termed ‘Bad learners’, is characterized by poor avoidance learning but with a low frequency of no-response trials. ‘Good learners’ were rats that performed more than 34 avoidance trials and had a low frequency of no-response trials. Nearly a third of the juvenile stress rats were characterized by learned helplessness-like behaviour, and ~40% were characterized as Bad learners. Thus, just over two thirds of the rats exposed to juvenile stress exhibited maladaptive behaviours in adulthood.

We found an interactive effect of exposure to stress early in life (juvenile or adolescent) and in adulthood on anxiety levels. Exploratory behaviour measured in the open field 24 h after the avoidance task (i.e. adulthood stress) was markedly reduced in both juvenile and adolescent stressed animals. However, adult rats that were not subjected to any ELS, but encountered it while learning the avoidance task in adulthood, did not differ from Naive rats. This effect supports our recent findings demonstrating that the combination of exposure to stress in juvenility and re-exposure in adulthood increases anxiety levels not only in comparison with Control rats but also in comparison with rats that were exposed twice to stress in adulthood (Avital and Richter-Levin, 2005). These results indicate that exposure to a relatively short-term stress protocol, especially during the juvenile period, has enduring effects on stress responses in adulthood, compromising the ability to learn under stress.

Our findings are supported by studies showing that reduction in exploratory behaviour in a novel setting following exposure to stress is a commonly used and pharmacologically validated index for heightened anxiety and activation of stress mechanisms in rats (for examples see Gregus et al., 2005; Kalynchuk et al., 1997; Ramos and Mormede, 1998). Open-field exploration, measured as number of squares crossed, was highly and particularly correlated with other accepted behavioural anxiety indices, like latency to move in the open-field test and latency to float in the forced-swim test, as well as decreased central amygdala fos activation (Campbell et al., 2003).

Poor performance in the two-way shuttle avoidance task, as observed in this study, does not necessarily

---

task. Control rats differed significantly from both JUV28 (p < 0.000) and ADL34 (p < 0.017). (c) Escape responses throughout learning the two-way shuttle avoidance task. No significant differences were observed between groups in rats’ escape shuttle responses while learning the two-way shuttle avoidance task. (d) Overall escape shuttle responses in the two-way shuttle avoidance task. No significant differences were observed between groups in rats’ overall escape shuttle responses in the two-way shuttle avoidance task. (e) No-response throughout learning the two-way shuttle avoidance task. Significant differences (p < 0.001) were observed between groups in rats’ no-response while learning the two-way shuttle avoidance task. Control rats differed significantly from JUV28 rats (p < 0.000), but differed only marginally from ADL34 rats (p < 0.066). JUV28 and ADL34 rats did not differ significantly (** JUV28 > Control, p < 0.00; # JUV28 > ADL34, p < 0.05; ## JUV28 > ADL34, p < 0.00; $$ ADL34 > Control, p < 0.00). (f) Overall no-response in the two-way shuttle avoidance task. Significant differences (p < 0.000) were observed between groups’ overall no-responses in the two-way shuttle avoidance task. JUV28 rats differed significantly from Control rats (p < 0.000). JUV28 rats also marginally differed (p < 0.069) from ADL34 rats. Control and ADL34 rats did not differ significantly (** JUV28 > Control, p < 0.00; ~ JUV28 > ADL34, p < 0.069).
imply learning deficits but rather may indicate enhanced anxiety. Numerous studies demonstrated the impeding effects of stress on cognitive functions like operant learning, and were associated primarily with limbic system dysfunctions (for reviews see Brown et al., 1999; McEwen, 1999; McEwen and

Table 1a. Distribution of total avoidance grouped frequencies across groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Indices</th>
<th>0–20</th>
<th>21–40</th>
<th>41–60</th>
<th>61–80</th>
<th>81–100</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>JUV28</td>
<td></td>
<td>19</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>19</td>
<td>0%</td>
<td>21.88%</td>
<td>15.63%</td>
<td>3.13%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>63.33%</td>
<td>53.85%</td>
<td>31.25%</td>
<td>7.69%</td>
<td>0%</td>
<td>43.24%</td>
<td></td>
</tr>
<tr>
<td>Total subjects</td>
<td>9.46%</td>
<td>21.88%</td>
<td>15.63%</td>
<td>3.13%</td>
<td>0%</td>
<td>100.00%</td>
<td></td>
</tr>
<tr>
<td>ADL34</td>
<td></td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>7</td>
<td>0%</td>
<td>14.29%</td>
<td>28.57%</td>
<td>14.29%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>50.00%</td>
<td>7.14%</td>
<td>25.00%</td>
<td>35.71%</td>
<td>7.14%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Total subjects</td>
<td>15.63%</td>
<td>25.00%</td>
<td>35.71%</td>
<td>7.14%</td>
<td>0%</td>
<td>100.00%</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>4</td>
<td>0%</td>
<td>14.29%</td>
<td>28.57%</td>
<td>14.29%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>14.29%</td>
<td>17.86%</td>
<td>25.00%</td>
<td>35.71%</td>
<td>7.14%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Total subjects</td>
<td>5.41%</td>
<td>25.00%</td>
<td>35.71%</td>
<td>7.14%</td>
<td>0%</td>
<td>100.00%</td>
<td></td>
</tr>
</tbody>
</table>

Table 1b. Distribution of total no-response grouped frequencies across groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Indices</th>
<th>0–20</th>
<th>21–40</th>
<th>41–60</th>
<th>61–80</th>
<th>81–100</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>JUV28</td>
<td></td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>20</td>
<td>62.50%</td>
<td>9.38%</td>
<td>3.13%</td>
<td>3.13%</td>
<td>21.88%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>34.48%</td>
<td>50.00%</td>
<td>100.00%</td>
<td>100.00%</td>
<td>87.50%</td>
<td>43.24%</td>
<td></td>
</tr>
<tr>
<td>Total subjects</td>
<td>27.03%</td>
<td>40.50%</td>
<td>13.50%</td>
<td>13.50%</td>
<td>9.46%</td>
<td>43.24%</td>
<td></td>
</tr>
<tr>
<td>ADL34</td>
<td></td>
<td>11</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>11</td>
<td>78.57%</td>
<td>21.43%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>18.97%</td>
<td>50.00%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>18.92%</td>
</tr>
<tr>
<td>Total subjects</td>
<td>14.86%</td>
<td>40.50%</td>
<td>13.50%</td>
<td>13.50%</td>
<td>9.46%</td>
<td>43.24%</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>27</td>
<td>96.43%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Within group</td>
<td>46.55%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>37.84%</td>
</tr>
<tr>
<td>Total subjects</td>
<td>36.49%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>37.84%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>58</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>74</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>83.78%</td>
<td>8.11%</td>
<td>1.35%</td>
<td>1.35%</td>
<td>10.81%</td>
<td>100.00%</td>
<td></td>
</tr>
</tbody>
</table>

...
Sapolsky, 1995; Shors, 2004). The two-way shuttle avoidance task is a higher-order operant learning task, characterized as dependent on the function of the hippocampus (Becker et al., 1997; Schwegler et al., 1981) and amygdala (Savonenko et al., 2003), as well as basal forebrain areas (Miyamoto et al., 1985) that are involved in regulating stress responses. Learning and performance in the two-way shuttle avoidance task were associated with regulation of stress-response mechanisms. High rates of no-response (‘escape failures’) in the two-way shuttle avoidance task were observed following both negligible and high doses of injected corticosterone (Kademian et al., 2005). Brush (2003) showed that ‘low-avoidance’ phenotype was best characterized in terms of high levels of state/trait anxiety, and suggested that selection for a difference in avoidance learning does not involve the fundamental neurobiological substrates of associative or instrumental learning, but results from differences in factors related to the affective domain (state and/or trait anxiety) that influenced performance. Asai et al. (2004) reported that ‘high/low-avoidance’ Hatano rats differed in their stress response activation pattern of the HPA axis. ACTH-induced adrenal response of corticosterone release was higher in ‘low-avoidance’ rats than in ‘high-avoidance’ rats. Previous work with these rats had established that the differences between the ‘high/low avoidance’ strains were not associated with locomotion activity (Ohta et al., 1999).

Both the ELS protocol (Juvenile or Adolescent) and the two-way shuttle avoidance task involved applying electric foot shocks. Thus, the possibility of impaired performance due to familiarity with the stressor should be considered. However, we have previously addressed this issue and found that the impact of similar or different juvenile and adulthood stress was the same (Avital and Richter-Levin, 2005). Furthermore, familiarity with a stressor was found

![Avoidance learning categories and criteria](image)

**Figure 4.** Distribution of avoidance categories across groups. Significant differences ($p<0.001$) were found between the JUV28 ($n=32$), ADL34 ($n=14$), and Control ($n=27$) groups in distribution of avoidance categories. The proportion of Good learners fell from 75% in the Control group to 50% in the ADL34 stress group to only 31% in the JUV28 stress group, whereas the proportion of Bad learners increased from 25% in the Control group to 50% in the ADL34 stress group and to almost 70% (including the Learned helplessness rats) in the JUV28 stress group. Furthermore, Learned helplessness rats were found only in the JUV28 stress group; they did not appear in either the ADL34 stress group or in the Control group. AV, total number of avoidance shuttles; NO, total number of no-responses.
to moderate the stress response rather than to enhance it. When rats were exposed to a familiar acute stress, ACTH response was blunt compared with the response to a novel acute stress (Simpkiss and Devine, 2003). It is also reasonable to assume that in the present study familiarity with the US was masked by differences in foot-shock parameters between the ELS (0.8 mA, 1 s) and the shuttle avoidance task (0.5 mA, max. 10 s) which were also presented in a completely different context (apparatus, room; cued or non-cued).

The exact age at the time of exposure to stress may be a critical factor in shaping the impaired coping with stress in adulthood. We found a significant relationship between age at exposure to stress and the proportion of Good learners, Bad learners, and rats exhibiting Learned helplessness-like behaviours. Whereas among the juvenile-stressed rats two types of impaired avoidance learning were observed, among the adolescent-stressed rats (33–35 d) only Bad learners were observed, comprising 50% of the animals, and none conformed to the Learned helplessness profile.

The maturational processes that take place in juvenility are common to a variety of species and occur in several brain areas that mediate the age-specific neurobehavioural and physiological characteristics, including the PFC, hippocampus, and amygdala neurocircuits, all critically involved in emotional and learning processes (Spear, 2000, 2004). Human juvenility is recognized as a stressful and challenging stage, in which the overwhelming maturational changes may lead to significant levels of stress (Petersen et al., 1993), which have been suggested to play an important role in the increasing prevalence of stress-related psychopathologies during the adolescent period (Bogerts, 1989; Compas et al., 1993; Lipska and Weinberger, 1993; Petersen et al., 1996; Spear, 2000).

The maturational processes that occur during juvenility in the rat brain have also been suggested as critical factors in susceptibility to stress-induced dysfunctions (Einon and Morgan, 1977; McGivern et al., 1996; Spear, 2000, 2004). These include substantial changes in neural mechanisms regulating emotional and learning processing within the PFC (Genazzani et al., 1997; Insel et al., 1990; Jernigan et al., 1991; Kalsbeek et al., 1988; Sowell et al., 1999; Van Eden et al., 1990), hippocampal formation (Insel et al., 1990; Michelson and Lothman, 1989; Nurse and Lacaille, 1999), and the amygdala (Kellogg, 1998; Terasawa and Timiras, 1968).

Here juvenile stress was found to result in two distinct behavioural profiles of impaired coping with stress in adulthood, namely Bad learners and Learned helplessness-like behaviours. It is tempting to suggest that the Bad learners’ profile may resemble symptoms of anxiety-related psychopathologies (i.e. heightened anxiety impeding coping with stressful situations and aversive learning) while the Learned helplessness profile may correspond with depressive symptoms. Both mood and anxiety disorders, such as depression and generalized anxiety disorder (GAD) or PTSD, are more prevalent in humans with a history of childhood trauma or child abuse (Agid et al., 1999; Arborelius et al., 1999; Weiss et al., 1999). When the same stress protocol was applied to adolescent rats, only the Bad learners’ profile was evident among young adult rats, suggesting that in the post-weaning pre-puberty phase the juvenile age may be a particularly sensitive period in the development of stress-response neurocircuits. These findings support the findings of Maslova et al. (2002) and Avital and Richter-Levin (2005) regarding the long-term effects of juvenile stress.

The different outcome of juvenile and adolescent stress may be interpreted in more than one way. As the brain matures it may become less susceptible to stress. In support of this possibility we recently reported that the impact of juvenile and adulthood stress was significantly more severe than that of exposure to stress twice in adulthood at the same intervals (Avital and Richter-Levin, 2005). Another possibility is that juvenile and adolescent exposures to stress differ qualitatively in their impact. Depending on the brain developmental trajectory, different systems or regions may be more vulnerable in juvenility than in adolescence. Substantial differences in the rat brain do exist at these ages. For example, at age 30 d the rapid rate of myelination terminates and the maturation of the cholinergic system begins and lasts until age 60 d, while at age 35 d the maturation of the dopaminergic system is in progress (Kaufmann, 2000). Clearly, further investigation is required, and our juvenile/adolescent model is suggested as useful to verify these possibilities.

Some epidemiological studies support the view that ELS at different ages may result in the development of different psychopathologies. Childhood trauma (under the age of 12 yr) was found to increase the risk of developing major depression, while trauma during adolescence was associated with a greater predisposition to PTSD (Maercker et al., 2004), suggesting challenging maturing mechanisms of emotional or mood regulation at different developmental phases (Heim and Nemeroff, 2001; Maercker et al., 2004; Pynoos et al., 1999).
The present juvenile stress model, combined with the cut-off behavioural criteria-based analyses of the altered stress response in adulthood, may be used as an animal model for the emergence of stress-related disorders in humans who have suffered trauma early in life, and facilitate the investigation of the relevant underlying mechanisms associated with factors related to both vulnerability and resilience in the different psychopathologies.

**Limitations**

One must take care not to be too literal in interpreting animal models. They are not to be taken as accurately reflecting a human disorder, but merely as approximating certain aspects of it. Stress-related psychopathologies involve so much more than a physiological imbalance or change in behaviour alone, which this animal model is unable to approximate. It would be presumptuous to assume that the ‘criteria’ applied in this study in fact reflect behavioural-physiological parameters in the life of the rat that are commensurate with the criteria for PTSD, GAD, or depression in humans.

**Acknowledgments**

This project was supported by a 2002 NARSAD Independent Investigator award to G.R-L.

**Statement of Interest**

None.

**References**


Li HY, Sawchenko PE (1998). Hypothalamic effector neurons and extended circuitries activated in ‘neurogenic’ stress: a comparison of footshock effects exerted acutely, chronically, and in animals with controlled glucocorticoid levels. Journal of Comparative Neurology 393, 244–266.


Maslova LN, Bulygina VV, Markel AL (1999). Late maturation of GABA(B) synaptically transmission in area CA1 of the rat hippocampus. Neuropharmacology 38, 1733–1742.


