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# Environmental Enrichment Reverses Behavioral Alterations in Rats Prenatally Exposed to Valproic Acid: Issues for a Therapeutic Approach in Autism

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Environmental enrichment has been repeatedly shown to affect multiple aspects of brain function, and is known to improve cognitive, behavioral, and histopathological outcome after brain injuries. The purpose of the present experiments was to determine the effect of an enriched environment on behavioral aberrations observed in male rats exposed to valproic acid on day 12.5 of gestation (VPA rats), and proposed on the basis of etiological, anatomical, and behavioral data as an animal model of autism. Environmental enrichment reversed almost all behavioral alterations observed in the model. VPA rats after environmental enrichment (VPA-E) compared to VPA rats reared in standard conditions have higher sensitivity to pain and lower sensitivity to nonpainful stimuli; stronger acoustic prepulse inhibition; lower locomotor, repetitive/stereotypic-like activity, and enhanced exploratory activity; decreased anxiety; increased number of social behaviors; and shorter latency to social explorations. In comparison with control animals (Con), VPA-E rats exhibited increased number of pinnings in adolescence and social explorations in adulthood, and were less anxious in the elevated plus maze. Similar differences in social behavior and anxiety were observed between control rats exposed to environmental enrichment (Con-E) and control group reared in standard conditions. These results suggest that postnatal environmental manipulations can counteract the behavioral alterations in VPA rats. We propose environmental enrichment as an important tool for the treatment of autism spectrum disorders.
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## INTRODUCTION

Hebb (1949) noted that rats, which he had brought home for several weeks as pets for his children and later returned to the laboratory, showed better problem-solving ability than rats that had remained in the laboratory. This pioneering observation generated a large number of studies, which have shown significant differences in animals' behavior associated with enriched living conditions. Environmental enrichment in rats leads to better performance in various learning tasks (eg Greenough *et al*, 1972; Mohammed *et al*, 1990; Nilsson *et al*, 1999; Rampon *et al*, 2000), enhanced social play behavior (Morley-Fletcher *et al*, 2003), increased exploration, lower anxiety, and ameliorated plasma corticosterone response to stress (Levine, 1962, 1967). Experiments have shown that improvements in behavioral performances were accompanied with changes in various neurochemical and anatomical features in rat brains. These changes include, for example, thicker cortex; increased dendritic spine density, and branching in the cerebral cortex (eg Volkmar and Greenough, 1972; Diamond *et al*, 1964; Diamond, 1967; Greenough *et al*, 1973), hippocampus (Rampon *et al*, 2000), striatum (Comery *et al*, 1995), and cerebellum (Floeter and Greenough, 1979); reduced apoptosis (Young *et al*, 1999); increased expression of neurotrophic factors (Torasdotter *et al*, 1996, 1998; Young *et al*, 1999; Olsson *et al*, 1994; Ickes *et al*, 2000); and enhanced neurogenesis (Kempermann *et al*, 1997; Nilsson *et al*, 1999).

The broad picture regarding enrichment effects on normal animals conduced to the hypothesis that environmental enrichment might also be used to overcome cognitive impairments in brain-damaged animals. Since the 1960s there have been several dozens of investigations, the overwhelming majority of which show beneficial effects of environmental enrichment following damage to the brain (Schwartz, 1964; Will *et al*, 1976, 1977; Dalrymple-Alford and Kelche, 1985; Will and Kelche, 1990; Puurenen *et al*, 1997; Johansson, 1996; Fernandez *et al*, 2004). There have also been several reports that have shown that environmental enrichment, in addition to being beneficial in its own right, can optimize other treatments designed to restore function following brain damage, for example,

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neural implants (Kelche *et al*, 1998), tissue grafts (Mattsson *et al*, 1997), or drug treatments (van Rijzingen *et al*, 1996). Similarly, extensive research on humans suggests that exercise and behavioral stimulation can maintain or improve brain plasticity, benefit overall health and cognitive function (reviewed by Cotman and Berchtold, 2002), and prevent some personality aberrations (Raine *et al*, 2003). It has even been suggested that environmental enrichment might be a basis for the treatment of specific neurodevelopmental disorders (Guilarte *et al*, 2003).

Autism is a neurodevelopmental disorder characterized by impairment in social interaction, verbal and nonverbal communication, and restricted, repetitive, and stereotyped patterns of behavior, interests, and activities (American Psychiatric Association, 1994). The past 50 years have seen a myriad of interventions targeted at people with autism. Attempts to develop drugs that specifically improve social and communicative functioning have failed; however, newer medications were shown to reduce many of the behavioral aberrations observed in autistic patients (reviewed by Buitelaar, 2003). Several reports have also suggested efficacy of early intensive behavioral therapy in attenuation or reversal of the core autistic symptoms (Lovaas, 1987; Harris *et al*, 1991; Birnbauer and Leach, 1993; Scheinkopf and Siegel, 1998; Ozonoff and Cathcart, 1998). The gains of the behavioral therapy appear to sustain over time (McEachin *et al*, 1993).

On the basis of collected pathological data suggesting a high incidence of autism among thalidomide victims (Miller and Strömland, 1993; Strömland *et al*, 1994), a new rodent model of autism has been created (Rodier *et al*, 1996, 1997). Rats exposed to valproic acid on day 12.5 of gestation (VPA rats) exhibit several anatomical abnormalities in the brainstem and cerebellum (Rodier *et al*, 1996, 1997; Ingram *et al*, 2000), resembling those found at autopsy and in brain imaging studies of autistic patients (Bauman and Kemper, 1994; Gaffney *et al*, 1988; Hashimoto *et al*, 1995; Courchesne, 1997). We previously presented evidence that administration of VPA on day 12.5 of gestation also has long-term effects on postnatal behaviors in male rats, which include lower sensitivity to pain and higher sensitivity to nonpainful stimuli; diminished acoustic prepulse inhibition; locomotor and repetitive, stereotypic-like hyperactivity combined with lower exploratory activity; decreased number of social behaviors; and increased latency to social behaviors (Schneider *et al*, 2001; Schneider and Przewlocki, 2005). Similar behavioral abnormalities have been observed in autistic persons (Militerni *et al*, 2000; Ayres and Tickle, 1980; Allen and Courchesne, 2001; McAlonan *et al*, 2002; De Moura-Serra, 1990; Pierce and Courchesne, 2001; American Psychiatric Association, 1994). Autistic patients also exhibit enhanced reaction to stress (Tordjman *et al*, 1997, 1999a, b; Maher *et al*, 1975; Jansen *et al*, 2003).

The present study aimed to elucidate environmental enrichment effects on behavioral alterations described in the model. We therefore selected five behavioral patterns corresponding to those frequently observed in autism and reported to be disturbed in VPA male rats to examine this issue: (1) nociception and mechanical allodynia, (2) sensorimotor gating measured by acoustic prepulse inhibition, (3) locomotor, repetitive/stereotypic-like, and exploratory activity, (4) social behaviors, and (5) anxiety.

## MATERIALS AND METHODS

### Animals

Female Wistar rats (IF PAS breeding colony) with controlled fertility cycle were mated overnight and the morning when spermatozoa were found was designated as the first day of gestation. Females received a single intraperitoneal injection of 600 mg/kg sodium valproate on day 12.5 after conception. Control females were injected with physiological saline at the same time. Sodium valproate (Sigma) was dissolved in saline at a concentration of 250 mg/ml. Administration of this dose to rats during embryogenesis has been shown to result in a maximum level of total VPA (900 μg/ml) in maternal plasma in less than 1 h, with a mean plasma elimination half-life of 2.3 h (Binkerd *et al*, 1988). Valproate-treated females and their offspring were healthy and number of animals per litter was normal compared to controls. Females were housed individually and were allowed to raise their own litters. The litters were not culled. Five (24 male rats) out of 13 (49 male rats) litters from control females and five (24 male rats) out of 13 (46 male rats) litters from valproate-treated females were subjected to multisensory stimulation protocol on postnatal days (PNDs) 7–21. The offspring were weaned on PND 22 and rats of either sex were housed separately. Experiments were carried out on male offspring of the females described above in two developmental periods: prepubertal-adolescence (E 30–50, 120–150 g at the beginning of the tests) and adulthood (E 90–120, 280–350 g at the beginning of the tests). Behavioral tests were conducted in the same sequence (nociception and tactile threshold—sensorimotor gating—locomotor and exploratory activity—social behavior—anxiety) in both developmental periods on groups of 5–8 animals. The animals were kept five to a cage ($60 \times 38 \times 20$ cm), except for the animals subjected to environmental enrichment on PNDs 22–35 kept as a cohort of 12 in a large aquarium ($60 \times 60 \times 40$ cm), with a controlled temperature of $21 \pm 1^{\circ}$C, and light conditions (lights on at 08:00 hours, lights off at 20:00 hours, reversed in social behavior tests in adulthood). Rats had free access to food (standard laboratory pellets) and water. All the experiments were performed in the light phase between 09:00 and 15:00 hours, except social behavior tests in adult rats, which were performed in the dark phase at the same time. All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals, and were approved by the Local Bioethics Committee.

### Environmental Enrichment

We used both pre- and postweaning environmental enrichment in VPA (VPA-E) and control rats (Con-E). Combining of these procedures has previously been shown to induce the strongest stimulatory effect on development of rats (Krech *et al*, 1960; Ivinskis and Homewood, 1980).

### Multisensory Stimulation Between PNDs 7–21

The developmental stage of the rat brain at day 7 of life is considered to be comparable to that of the human brain on the day of birth (Dobbing, 1974, 1981; Hagberg *et al*, 1997).

Thus, rats were subjected to intensive multisensory stimulation from PND 7 to 21. The procedure comprised removal of the dam from the home cage, placement of the pups individually on surfaces differing in temperature and structure (eg wood, glass, paper towels), and training of righting reflex (righting itself on all 4 ft from a supine position), negative geotaxis (completing a 180° turn when placed in a head-down position on a 25–35° inclined surface and in a box with changing slant), and swimming (in an aquarium filled with water, 28–29°C). The whole procedure lasted 25 min every day. The pups were intensively handled during that time. Cage cleaning took place once a week.

### Enriched Environment on PNDs 22–35

After weaning, rats subjected to environmental enrichment were housed as a cohort of 12 with the opportunity to play and explore in a large aquarium ($60 \times 60 \times 40$ cm) with approximately 2 cm of wood shavings covering the floor. The aquarium was filled with toys (eg wheels, ladders, plastic tunnels, wooden shelters, mazes made of colored building blocks, swings, wheel-runners) that were changed every 2 days when cage cleaning took place.

### Behavioral Tests

*Nociception and tactile threshold.* Nociceptive effects were evaluated using a tail flick test. Tactile threshold was determined by the von Frey test. The tail flick test was carried out using a tail flick analgesia meter (TF- 01, Porfex, Poland). An animal was gently restrained by hand, and radiant heat was directed onto its tail. The cutoff time was 9 s. Tail flick measurements were taken three times at 30 s intervals. Tactile threshold was assessed using calibrated von Frey filaments (Stoelting, Chicago, IL, USA) presented serially to the hind paw in ascending order of strength (0.4–26 g), and the mechanical threshold was determined as a rapid withdrawal of the paw immediately after application of the stimulus.

*Sensorimotor gating—prepulse inhibition.* A startle apparatus (Columbus Instruments, Ohio) consisted of three plastic, transparent cages, equipped with movable platform floor attached to a sensor, which recorded vertical movements of the floor. A loudspeaker was suspended above all cages, and the cages were placed in a soundproof cabinet. A transient force resulting from up-and-down movements of the floor, evoked by a startle reaction to acoustic stimuli, was recorded by PC using a recording window of 200 ms measured from the onset of acoustic stimulus. The amplitude of a startle response was defined as a difference between the average force detected within a recording window and the force measured immediately before the stimulus onset. The threshold was set at 10 g (adolescent males) and 20 g (adult rats), and allowed for correct evaluation of the maximum response in all the animals tested. The experiment started with an adaptation period during which the animals were placed in experimental cages for 5 min and exposed to a 68 dB background white noise. Following habituation, each rat was confronted with two types of acoustic stimuli: pulse alone trials in which acoustic stimuli of 120 dB (4000 Hz, duration of 40 ms) were applied, and prepulse + pulse trials in which a tone of 120 dB (4000 Hz, duration of 40 ms) was preceded by a prepulse of 75 dB (4000 Hz, duration of 20 ms) applied 30–100–300 ms earlier, in a series of 10-pulse stimuli alone and three series of 10-prepulse + puls stimuli with trials separated by an interstimulus interval of 10 s, applied in a pseudo-random order. Prepulse inhibition was calculated as percentage of inhibition of the startle amplitude evoked by pulse alone: $((\text{pulse}-\text{prepulse})/\text{pulse}) \times 100$.

*Locomotor, repetitive/stereotypic-like, and exploratory activity.* The locomotor activity of rats was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, USA), linked online to an IBM-PC compatible computer. Each cage ($43 \times 44$ cm) was equipped with 15 infrared emitters located on the $x$ and $y$ axes, and with an equivalent number of receivers on the opposite walls of the cage. The behavior of rats was analyzed using Auto-track software (Columbus Instruments, USA). The locomotor activity was defined as a breakage of three consecutive photo-beams. Time of repetitive/stereotypic-like activity was defined as the sum of time intervals (1/10th of a second) in which there was a movement but an animal did not cross three consecutive photo-beams. The animal would have to repeatedly break and make the same three beams for the time interval (1/10th of a second) to be recorded as time of repetitive/stereotypic-like activity. The number of repetitive/stereotypic-like movements was defined as the number of repeated breaks of the same beam in 1/10th of a second. Locomotor and stereotypic-like behavioral patterns were assessed by comparing changes in mean activity across a 60-min testing session divided into two 30-min intervals.

The exploratory activity was assessed in a small, open field. The apparatus consisted of a wooden rectangular box measuring $66 \times 57 \times 40$ cm ($l \times w \times h$) with two holes in the shorter and three in the longer walls of the box located regularly in each wall. Number of rearings and hole-pokings (nose of an animal put inside the hole) were measured during a 3-min time session. Background noise was produced by a radio.

*Social behavior.* The test area for adolescent rats consisted of an acrylic plastic circular cage measuring $38 \times 26$ cm (diameter × height) with approximately 2 cm of wood shavings covering the floor. Adult rats were tested in an aquarium measuring $60 \times 40 \times 40$ cm ($l \times w \times h$) with approximately 2 cm of wood shavings covering the floor. The test cage and aquarium were illuminated by a 40 W red light bulb mounted 60 cm above them. Background noise was produced by a radio.

*Social (play) behavior.* The test was performed under dim light/unfamiliar conditions, which means that the animals were tested under red light in a novel test cage. On the day of the experiment, the animals were socially isolated in cages measuring $43 \times 28 \times 15$ cm ($l \times w \times h$) for 3.5 h prior to the experiment. This isolation period has been shown to produce a half maximal increase in the amount of social play (Niesink and Van Ree, 1989). The test consisted in placing two animals from the same group but different litters (VPA *vs* VPA, Con *vs* Con, VPA-E *vs* VPA-E, Con-E *vs*

Con-E) into the test cage for 15 min. Pairs were tested in a randomized order for groups, and the animals did not differ by more than 15 g in body weight. Animals were tested between day 30 and 35 of life. Behavior was assessed for a pair of animals, so behavior of the individual animal was not analyzed. Latency to pinning (one of the animals is lying with its dorsal surface on the floor of the test cage with the other animal standing over it), total duration, and frequency of pinning were measured. Latency to social behavior unrelated to social play behavior (following or approaching the test partner, mounting or crawling over the test partner, sniffing or grooming any part of the body of the test partner), its total duration and frequency were also measured.

*Social behavior in adulthood.* At 3 weeks before social behavior was tested, the animals were housed under reversed light conditions (lights off at 08:00 hours, lights on at 20:00 hours). At 1 week before the experiment, rats from both groups were socially isolated. This isolation period has previously been shown to produce a maximal increase in social behavior (Niesink and Van Ree, 1982). The stimulus animals were housed in groups of five per cage. All animals were placed individually in the test cage two times daily for 5 min for 2 days prior to the experimentation day in order to reduce the stress due to the novel environment. Animals were tested between day 80 and 90 of life. The test consisted in placing one isolated animal (from VPA, Con, VPA-E, or Con-E group) and one stimulus animal into the test cage for 10 min. Animals were tested in randomized order for groups, and the weight differences between test partners were kept as small as possible. Behavior was assessed for an individual animals (VPA, Con, VPA-E, or Con-E). Latency to social behavior, total duration and frequency of social exploration, and contact were measured including the following behaviors: sniffing or licking any part of the body of the conspecific except the anogenital area, crawling, or mounting (standing on hind legs and putting one or two forepaws on the back of conspecific, or climbing over the conspecific), and approaching or following the conspecific. Anogenital investigation (sniffing or licking the anogenital area of the other rat) was measured separately.

*Elevated plus-maze.* The elevated plus-maze was made of wood and consisted of two opposite open arms ($50 \times 10$ cm), and two opposite arms enclosed with 40-cm high walls. The maze was elevated 50 cm above the floor. Behavior was tested in a dimly lit room with a 40 W bulb hung 60 cm above the central part of the maze. Each rat was placed for 5 min in a pretest arena ($60 \times 60 \times 35$ cm, constructed of the same material) prior to exposure to the maze. This step facilitates exploratory behavior. An investigator sitting approximately 2 m apart from the apparatus observed the rats. The experimental procedure was similar to that described by Pellow *et al* (1985). Immediately after the pretest, exposure rats were placed in the center of the elevated plus-maze facing one of the open arms. During the 5-min test period, the following measurements were taken: the number of entries into the open and closed arms and the time spent in the open and closed arms. From those measures, the following variables were defined: % time spent in the open arms compared to time spent in both open and enclosed arms, and % open arms entries compared to both open and enclosed arms entries. An entry was defined as entering into one arm with all four paws. The maze was cleaned after each trial.

### Statistics

The results were statistically assessed by parametric analysis of variance (ANOVA) with two levels of prenatal status (VPA *vs* Con) and two levels of postnatal environment (enriched *vs* standard) as between-subjects factors, followed by LSD *post hoc* test. In the case of nonhomogenicity, data were analyzed using the Kruskal–Wallis ANOVA on ranks or Chi-squared tests followed by the Mann–Whitney *U*-test. The confidence limit of $p < 0.05$ was considered statistically significant.

## RESULTS

### Nociception and Tactile Threshold (Figure 1)

Environmental enrichment significantly alleviated increased nociceptive threshold in VPA rats measured by tail flick test (Figure 1a). *Post hoc* tests revealed that differences observed between groups in adolescence ($F_{(3, 28)} = 32.21$, $p < 0.001$) and adulthood ($H_{(3, 32)} = 24.26$, $p < 0.001$) resulted from increased nociceptive threshold in VPA group compared to Con ($p < 0.001$ and $p < 0.001$, respectively), Con-E ($p < 0.001$ and $p < 0.001$, respectively), and VPA-E ($p < 0.001$ and $p < 0.001$, respectively) groups. Tactile threshold measured by von Frey filaments significantly differed between groups in adolescence ($F_{(3, 28)} = 15.11$, $p < 0.001$) but not adulthood ($F_{(3, 28)} = 1.13$, n.s.), and was lower in adolescent VPA rats compared to Con ($p < 0.001$), Con-E ($p < 0.001$), and VPA-E ($p < 0.001$) groups (Figure 1b).

### Sensorimotor Gating—Prepulse Inhibition (Figure 2)

Environmental enrichment significantly increased diminished prepulse inhibition in adolescent VPA rats measured for 100 ms prepulse-pulse interval. *Post hoc* tests revealed that differences observed between groups in adolescence ($F_{(3, 36)} = 9.98$, $p < 0.001$) and adulthood ($F_{(3, 36)} = 2.77$, $p < 0.05$) resulted from diminished prepulse inhibition in VPA group compared to Con ($p < 0.001$ and $p < 0.05$, respectively), Con-E ($p < 0.001$ and $p < 0.05$, respectively), and VPA-E ($p < 0.01$ and $p =$ n.s., respectively) groups. Prepulse inhibition in VPA-E adult rats compared to VPA group, being statistically not significant, was much higher. There were no differences between groups in startle amplitude reaction and prepulse inhibition for 30 and 300 ms prepulse-pulse intervals either in adolescence or adulthood (data not shown).

### Locomotor, Repetitive/Stereotypic-Like, and Exploratory Activity (Figures 3 and 4)

Environmental enrichment significantly alleviated increased locomotor and repetitive/stereotypic-like activity in VPA rats. In adolescence, differences observed in locomotor activity in the 0–30 min interval ($F_{(3, 28)} = 2.99$, $p < 0.05$)

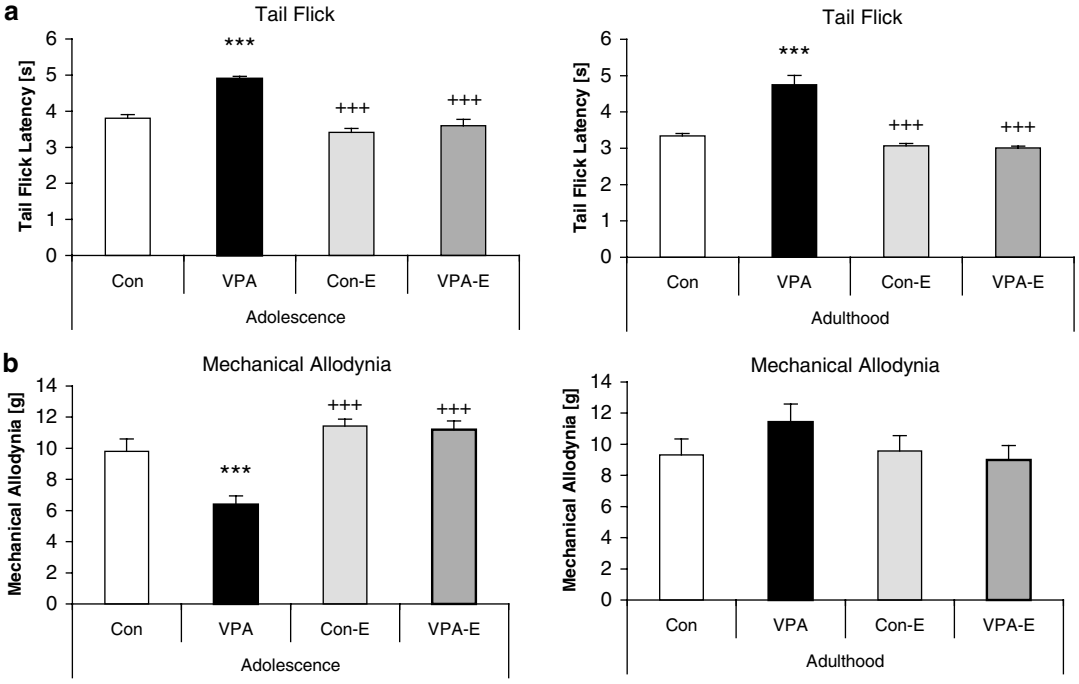


**Figure 1** The effect of environmental enrichment on thermal nociceptive threshold (a) and mechanical allodynia (b) in adolescent and adult rats prenatally exposed to VPA. Data expressed as mean + SEM, $n = 8$ per group. ***$p < 0.001$ *vs* Con, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test, Kruskal–Wallis test + Mann–Whitney *U*-test).

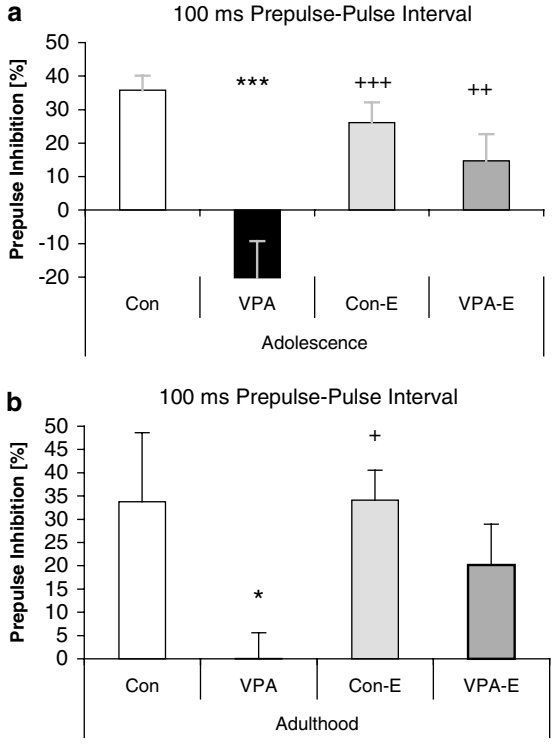


**Figure 2** The effect of environmental enrichment on acoustic prepulse inhibition in adolescent (a) and adult (b) rats prenatally exposed to VPA. Data expressed as mean + SEM, $n = 10$ per group. *$p < 0.05$, ***$p < 0.01$ *vs* Con, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test).

resulted from the increased distance traveled by VPA rats compared to Con ($p < 0.05$) and approaching significance differences to Con-E ($p < 0.06$) and VPA-E ($p < 0.06$) groups, and for the 30–60 min interval ($F_{(3,28)} = 3.39$, $p < 0.05$) resulted from the increased distance traveled by VPA rats compared to Con ($p < 0.05$), Con-E ($p < 0.05$), and VPA-E ($p < 0.05$) groups (Figure 3a). Differences observed between groups in the 0–30 min interval, both in time and number of repetitive/stereotypic-like movements ($F_{(3,28)} = 4.93$, $p < 0.01$, and $F_{(3,28)} = 3.79$, $p < 0.05$, respectively) resulted from increased time and number of repetitive/stereotypic-like movements in VPA rats compared to Con ($p < 0.01$ and $p < 0.01$, respectively), Con-E ($p < 0.01$ and $p < 0.05$, respectively), and VPA-E ($p < 0.01$ and $p < 0.05$, respectively) groups (Figure 3b and c). In adulthood, differences between groups were observed only in the 30–60 min interval and only for time ($F_{(3,28)} = 3.41$, $p < 0.05$) and number ($\chi^2 = 9$, df = 3, $p < 0.03$) of repetitive/stereotypic-like movements (Figure 3b and c). They resulted from increased time and number of repetitive/stereotypic-like movements in VPA rats compared to Con ($p < 0.01$ and $p < 0.05$, respectively) and VPA-E ($p < 0.01$ and $p < 0.05$, respectively) groups, with results approaching significance for duration of repetitive movements compared to Con-E group ($p < 0.08$).

Environmental enrichment significantly increased *exploratory activity* of VPA rats measured in the small open field (Figure 4). In adolescence, differences observed between groups in rearing ($H_{(3,32)} = 16.95$, $p < 0.001$) and hole-poking behavior ($H_{(3,32)} = 9.27$, $p < 0.05$) resulted from lowering of these parameters in VPA group compared to Con ($p < 0.001$ and $p < 0.05$, respectively), Con-E ($p < 0.01$ and $p =$ n.s., respectively), and VPA-E ($p < 0.001$ and

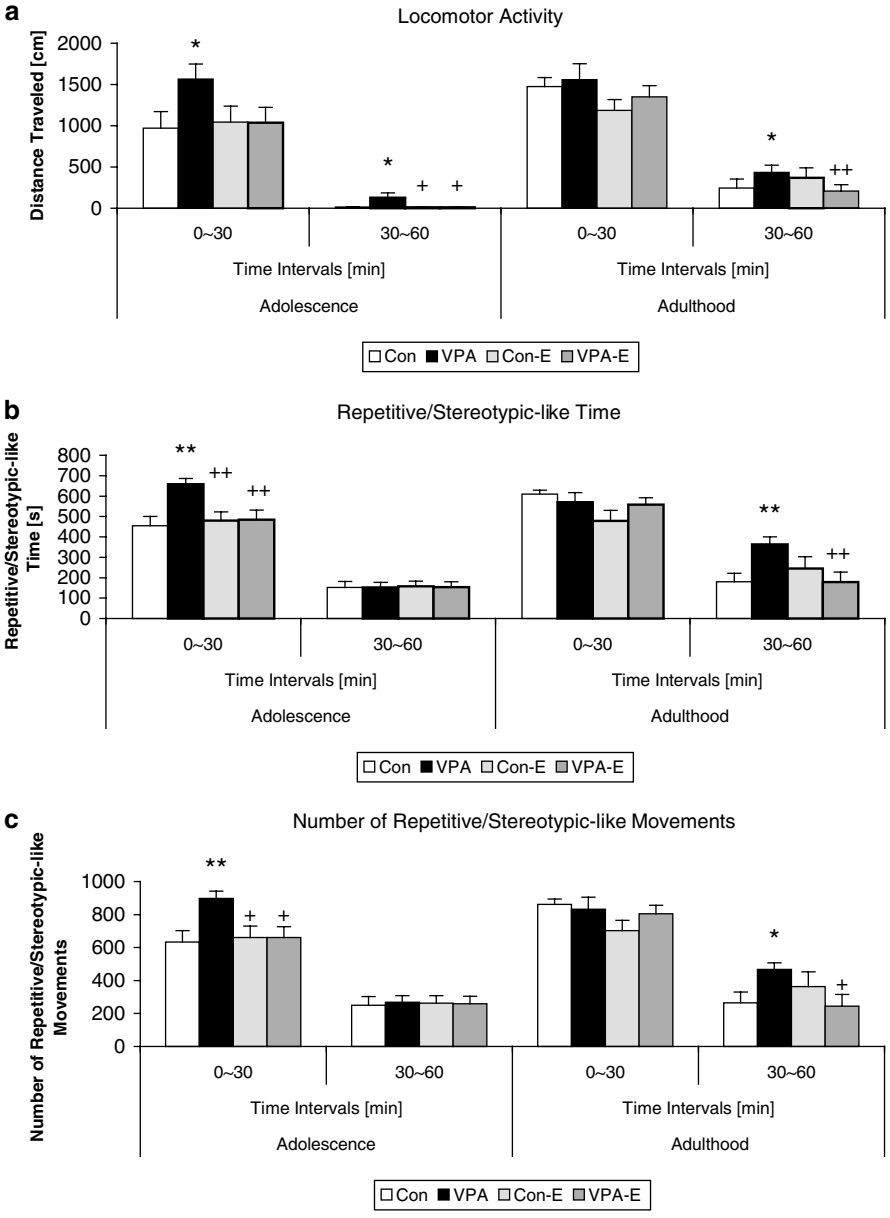


**Figure 3** The effect of environmental enrichment on locomotor and repetitive/stereotypic-like behaviors measured across 30-min time blocks in a 60-min session in auto track cages in adolescence and adulthood of rats prenatally exposed to VPA. Data are expressed as mean + SEM, $n = 8$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* Con, $^{+}p < 0.05$, $^{++}p < 0.01$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test, Chi-squareed + Mann–Whitney *U*-test).

$p < 0.05$, respectively). In adulthood, differences observed between groups in rearing ($F_{(3,28)} = 7{,}54$, $p < 0.001$) and hole-poking behavior ($F_{(3,28)} = 9.54$, $p < 0.001$) resulted from lowering of these parameters in VPA group compared to Con ($p < 0.01$ and $p < 0.001$, respectively), Con-E ($p < 0.001$ and $p < 0.001$, respectively), and VPA-E ($p < 0.01$ and $p < 0.01$, respectively) groups, with approaching significance increase in hole-poking behavior in Con-E and VPA-E groups compared to Con ($p < 0.07$, either) group.

## Social Behavior (Figures 5 and 6)

*Social (play) behavior.* Environmental enrichment significantly increased number of pinnings both in VPA and Con group. Differences between groups in this parameter ($F_{(3,20)} = 16.10$, $p < 0.001$) resulted from decrease in a number of pinnings in VPA rats compared to Con ($p < 0.01$), Con-E ($p < 0.001$), and VPA-E ($p < 0.001$), and increase in number of pinnings in Con-E ($p < 0.01$) and VPA-E (approaching significance, $p < 0.07$) groups compared to Con (Figure 5a). Latency to pinning and duration of pinning did not differ between groups (Figure 5b), as well as latency to social nonplay behavior and duration of social nonplay behavior (data not shown).

*Social behavior in adulthood.* Environmental enrichment significantly increased number of social explorations and decreased latency to social behavior both in VPA and Con

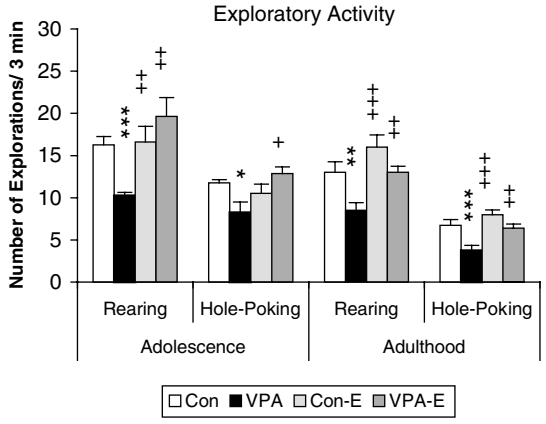


**Figure 4** The effect of environmental enrichment on exploratory behaviors (rearing, hole poking) measured in a 3-min test in the small open field in adolescent and adult rats prenatally exposed to VPA. Data expressed as mean + SEM, $n = 8$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ *vs* Con, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test, Kruskal–Wallis test + Mann–Whitney *U*-test).

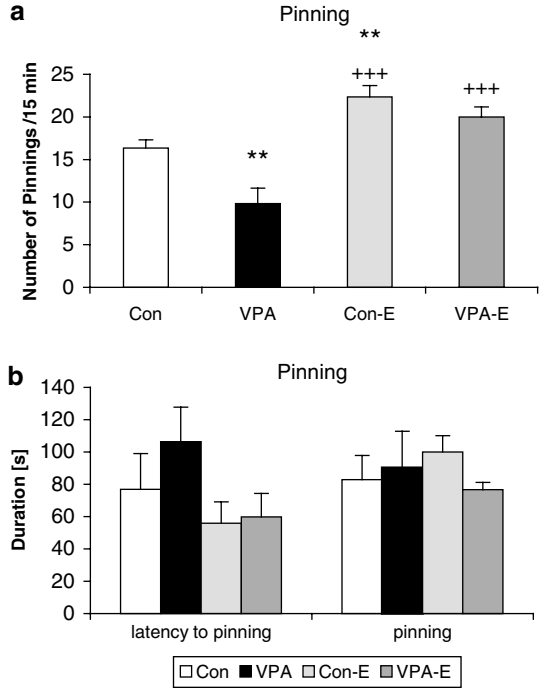


**Figure 5** The effect of environmental enrichment on number of pinnings (a), and latency to and duration of pinning (b) measured during 15-min test in adolescent rats prenatally exposed to VPA. Data are expressed as mean + SEM, $n = 6$ pairs/group. $^{**}p < 0.01$ *vs* Con, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test).

group. Differences between groups in a number of social explorations ($F_{(3,16)} = 18.50$, $p < 0.001$) resulted from decreased number of social explorations in VPA rats compared to Con ($p < 0.05$), Con-E ($p < 0.001$), and VPA-E

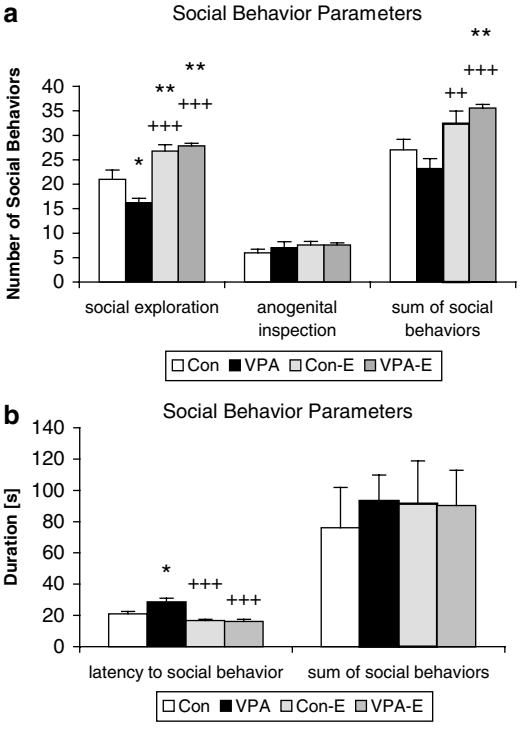


**Figure 6** The effect of environmental enrichment on the number of social explorations, anogenital inspections, and total number of social behaviors (a), and latency to social behavior and duration of social behaviors (b) in adult rats prenatally exposed to VPA measured during a 10-min test. Data are expressed as mean + SEM, $n = 5$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* Con, $^{++}p < 0.01$, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test).

($p < 0.001$) groups, and in a sum of social behaviors ($F_{(3,16)} = 7.64$, $p < 0.01$) resulted from increased number of social behaviors in Con-E and VPA-E groups compared to VPA group ($p < 0.01$ and $p < 0.001$, respectively), and in VPA-E group compared to Con group ($p < 0.01$), and approaching significance increase in Con-E group compared to Con ($p < 0.07$). Differences in latency to social behavior ($F_{(3,16)} = 12.75$, $p < 0.001$) resulted from longer latency in VPA group compared to Con ($p < 0.05$), Con-E ($p < 0.001$), and VPA-E ($p < 0.001$) groups (Figure 6b). There were no significant differences in the number of anogenital inspections and total duration of social behaviors.

## Elevated Plus-Maze (Figure 7)

Environmental enrichment significantly decreased anxiety in elevated plus-maze both in VPA and Con group. Differences between groups in time spent in the open arms in adolescence ($H_{(3,32)} = 17.59$, $p < 0.001$) and adulthood ($H_{(3,32)} = 22.27$, $p < 0.001$) resulted from a shorter time spent in the open arms in VPA group compared to Con ($p < 0.001$ and $p < 0.001$, respectively), Con-E ($p < 0.001$ and $p < 0.001$, respectively), and VPA-E ($p < 0.001$ and $p < 0.001$, respectively), and a longer time in adult Con-E and VPA-E groups compared to Con ($p < 0.05$, either) (Figure 7a).

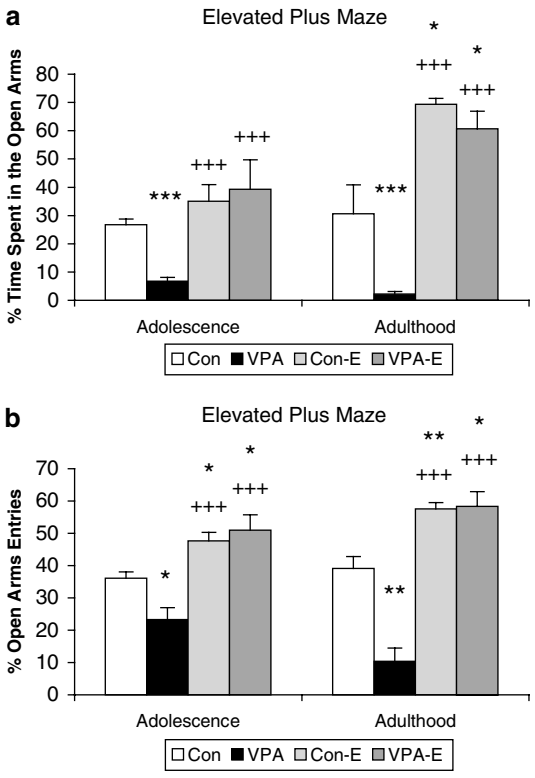


**Figure 7** The effect of environmental enrichment on anxiety measured as time spent in the open arms (a), and open arms entries (b) in relation to the sum of time spent both in the open and enclosed arms, and to the sum of the open and enclosed arms entries, assessed in a 5-min test in elevated plus-maze. Data are expressed as mean + SEM, $n = 8$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ *vs* Con, $^{+++}p < 0.001$ *vs* VPA. Con—control group, VPA—valproic acid group, Con-E—control group after environmental enrichment, VPA-E—valproic acid group after environmental enrichment (ANOVA + LSD test, Kruskal–Wallis test + Mann–Whitney *U*-test).

Differences in the number of the open arms entries in adolescence ($F_{(3,32)} = 13.33$, $p < 0.001$) and adulthood ($H_{(3,32)} = 21.93$, $p < 0.001$) resulted from a lower number of open arms entries in VPA group compared to Con ($p < 0.05$ and $p < 0.01$, respectively), Con-E ($p < 0.001$ and $p < 0.001$, respectively), and VPA-E ($p < 0.001$ and $p < 0.001$, respectively) groups, and from an increased number of open arms entries in Con-E and VPA-E groups compared to Con in adolescence ($p < 0.05$, either) and adulthood ($p < 0.001$ and $p < 0.05$, respectively) (Figure 7b).

## DISCUSSION

The results of the present experiments stay in concordance with our previous reports, which have shown that prenatal exposure to VPA on day 12.5 of gestation has long-term negative effects on postnatal behaviors in male rats (Schneider *et al*, 2001; Schneider and Przewlocki, 2005).

Environmental enrichment reversed almost all behavioral alterations observed in the model. VPA-E rats compared to VPA group have higher sensitivity to pain and lower sensitivity to nonpainful stimuli; stronger acoustic prepulse inhibition; lower locomotor, repetitive/stereotypic-like activity, and enhanced exploratory activity; decreased anxiety; increased number of social behaviors, and shorter latency to social explorations. However, prepulse inhibition, a measure of sensorimotor gating and attentional processes, was still lower, but not statistically significant, in VPA-E group, than the standard results for healthy animals (10 *vs* 35% in adolescence and 15 *vs* 34% in adulthood in our experiments) suggesting, in spite of significantly higher PPI in VPA-E adolescent rats compared to VPA group, rather attenuation than reversal of decreased PPI in VPA-E rats. In comparison with control animals, VPA rats subjected to environmental enrichment exhibited increased number of pinnings in adolescence and social explorations in adulthood, and more numerous entries to open arms and longer time spent in the open arms of the elevated plus-maze, which suggest decreased anxiety. It is important to underline that enrichment exerted a differential influence as a function of prenatal background. Con-E rats differed from control animals only in two parameters: they were less anxious in the elevated plus-maze, and exhibited more pinnings, and social explorations. This profile is in concordance with previous studies, which indicate a weak effect of enrichment on behavioral and physiological parameters in 'naïve' animals (van Praag *et al*, 2000; Schrijver *et al*, 2002). On the basis of the present data, we would suggest that lower anxiety in VPA rats subjected to environmental enrichment in the early stage of life may conduct to increased exploration, normalized locomotor and repetitive/stereotypic activity, and attenuated nociceptive and tactile thresholds, as fear and anxiety have profound influence on these parameters in animals (eg King *et al*, 2003; da Silva Torres *et al*, 2003; Palermo-Neto *et al*, 2003; Knott and Hutson, 1982). Enhanced repetitive/stereotypic-like activity is associated with many neurodevelopmental disorders in humans (eg autism), and often observed under conditions of environmental restriction in animals. Attenuation of repetitive activity in VPA rats after environmental enrichment suggests the efficacy of this behavioral procedure in the prevention of stereotypies. This result is consistent with data from other studies (eg Powell *et al*, 2000). Increased number of pinnings and social explorations observed in enriched animals (both Con-E and VPA-E) in our experiments might also indicate a reduced level of anxiety, as engaging in various forms of social interactions is accompanied by a lower level of attention to environment, and anxious animals display both less play behaviors (Vanderschuren *et al*, 1995) and less social explorations (Haller *et al*, 2003; Haller and Bakos, 2002; File and Hyde, 1978). Unfortunately, the effects exerted by environmental enrichment on social behaviors in animals have been poorly documented. To our knowledge, there are only two studies that have so far evaluated this issue and their results are inconsistent (Renner and Rosenzweig, 1986; Morley-Fletcher *et al*, 2003). In elucidating the attenuating effects of environmental enrichment in VPA rats, the known effects of enrichment on neurogenesis (Kemperman *et al*, 1997; Nilsson *et al*, 1999), neurotrophin gene expression (Torasdotter *et al*, 1996, 1998; Young *et al*, 1999; Olsson *et al*, 1994; Ickes *et al*, 2000), and anatomical features (Diamond *et al*, 1964; Diamond, 1967; Rampon *et al*, 2000; Floeter and Greenough, 1979) should also be taken into account. However, it has been suggested that the most

important single factor in stimulating brain changes in enriched rats is the enforced interaction with the physical environment. Thus, the vital element in enrichment effects may be the cognitive engagement with the environment, confronting the contingencies between responses and the consequences of those responses (Rosenzweig *et al*, 1972; Will *et al*, 2004). Other components of enriched environment, such as physical exercise, may have additive effects with those of training (Will *et al*, 2004).

Establishing what constitutes 'enrichment' for human beings is, of course, more problematic. However, if we treat environmental enrichment as increased interaction with the environment, some of the parallels between the animal enrichment literature and the problems of human brain damage, and neurodevelopmental disorders rehabilitation begin to emerge. Although the brain possesses a relatively constant macrostructural organization, its complex microarchitecture is powerfully shaped by experiences throughout life, which could be used to optimize the function of the remaining intact tissue after acute and congenital brain injuries (Johansson *et al*, 1999; Rosenzweig and Bennett, 1996).

Several reports have suggested efficacy of early intensive behavioral therapy proposed by Lovaas in attenuation or reversal of the core autistic symptoms (Lovaas, 1987; Harris *et al*, 1991; Birnbauer and Leach, 1993; Scheinkopf and Siegel, 1998; Ozonoff and Cathcart, 1998; McEachin *et al*, 1993). However, it must be emphasized that the use of behavioral techniques in autism is not limited to the 'Lovaas method'. Many forms of applied behavioral analysis offer parents and educators a wealth of educative strategies to address problematic behavior as well as to teach appropriate alternative behaviors. The combination of enriched experience with pharmacological treatments may further strengthen these efforts and improve their therapeutic effectiveness.

Of the vast number of animal studies that yield results of interest to human research, studies on the impact of an enriched environment on brain development and behavior can be of enormous interest. What we need now is the development of a sound theory of the effects of specific environmental experiences on neurobehavioural development and a rationale for the external and internal mechanisms that mediate these effects. Studies on the influence of an enriched environment are one of the major attempts to understand the interaction between the environment and the genome in the regulation of the phenotype. At the very least, our work indicates that there are many opportunities for enhancing brain activity and behavior, and that they can have pronounced therapeutic effects on behavioral alteration in the animal model of autism induced by prenatal exposure to VPA. This leads us to a consideration of the relevance of this model to brain damage rehabilitation and behavioral-cognitive therapy attenuation of autistic features in humans.

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