Prepubertal chronic stress and ketamine administration to rats as a neurodevelopmental model of schizophrenia symptomatology

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Abstract
Increased vulnerability to psychiatric disorders, such as schizophrenia, has been associated with higher levels of stress. In the early development of the central nervous system, changes in function of glutamatergic N-Methyl-D-aspartate (NMDA) receptors can possibly result in the development of psychosis, cognitive impairment and emotional dysfunction in adulthood. Thus, in this study we examined the behavioural consequences of the exposure of male rats to chronic stress (postnatal days 30–60) and ketamine administration (postnatal days 41–45); both during a sensitive developmental time window. We found that the locomotor activity of both ketamine and ketamine+chronic stress groups was significantly higher compared with that of the control rats. In contrast, the locomotor activity of the chronic stress group was significantly lower compared with all other groups. Examining anhedonia in the sucrose preference test we found a significantly decreased sucrose intake in both ketamine+chronic stress and the chronic stress groups compared with the control rats. No significant differences were observed in sucrose intake between the control and the ketamine group. The object recognition test revealed that the attention to the novel object was significantly impaired in the ketamine+chronic stress group. Similarly, the ketamine+chronic stress group showed the poorest learning ability in the eight-arm radial maze, starting on the 8th day. Finally, throughout the different pre-pulse intensities, the ketamine+chronic stress group showed impaired PPI compared with all other groups. The results indicate that the combination of prepubertal onset of chronic stress and ketamine may serve as a valid novel animal model for schizophrenia-like symptoms.

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Introduction
Early life physical and sexual abuse carries with it a life-long behavioural burden with corresponding psychopathological implications (Felitti et al., 1998; Heim and Nemeroff, 2001).

The organism responds to stressful experiences by releasing catecholamines that increase heart rate and blood pressure. Chronic stress elevates catecholamines release that increases heart rate and blood pressure and can cause pathophysiological changes in the neural system (McEwen, 1998).

Increased vulnerability to psychiatric disorders, such as schizophrenia, has been associated with higher levels of stress (Finlay and Zigmond, 1997), and diathesis-stress models, which were originated in the field of schizophrenia, were subsequently applied to other forms of psychopathology (Corcoran et al., 2003). Prolonged exposure to stressors may result in permanent changes in the activity of the hypothalamic–pituitary–adrenal (HPA) axis (Walker and Diforio, 1997). Thus, it has been suggested that schizophrenia may be associated with HPA dysfunction that includes pathology in the neural target of...
glucocorticoids (Corcoran et al., 2003). Moreover, the time of exposure to stress along the developmental trajectory seems to play an important predispositional role in the pathophysiology of schizophrenia (National Institute of Mental Health–National Advisory Mental Health Council, 2008). The two hit hypothesis, specifically, establishes the background for stress investigations focusing on a developmental perspective (Maynard et al., 2001). In our previous studies (Avital and Richter-Levin, 2005; Avital et al., 2006), we have postulated that the exposure to stress during a sensitive developmental window, such as prepuberty (First hit), may serve as the ‘predisposing factor’, and that re-exposure to adverse conditions (Second hit) may correspond to the ‘onset factor’. Taken together, it is plausible that chronic exposure to stress during the prepubertal period may facilitate a latent vulnerability to psychosis and schizophrenia via HPA activation. Indeed, stress has been introduced as a ‘triggering’ element in the etiologic models of the disorder (Dohrenwend and Egri, 1981; Phillips et al., 2007).

In the early development of the central nervous system, changes in function of glutamatergic N-methyl-D-aspartate (NMDA) receptors can possibly result in development of psychosis, cognitive impairment and emotional dysfunction in adulthood (Duncan and Duncan, 2009; Kegeles et al., 2000). Bubenikova-Valesova et al. (2008) have shown that administration of antagonists of the NMDA receptors in rats produced several adaptation mechanisms which correlate with symptoms of schizophrenia in human patients. Moreover, systemic administration of an NMDA receptor antagonist produces an impairment in a variety of learning and memory paradigms (Castellano et al., 2001) such as passive avoidance and spatial learning (Heale and Harley, 1990; Wozniak et al., 1990). Ketamine affects glutamatergic activity by blocking NMDA receptors (Keilhoff et al., 2004). Repeated injection of ketamine at sub-anesthetic doses was found to induce psychoses in remitted schizophrenia patients (Lahti et al., 1995). Accordingly, an NMDA antagonist such as ketamine is a suitable means for the dissection of the molecular mechanism of pathophysiological changes in schizophrenia.

Sterling and Eyer introduced the term allostasis that refer to the active process by which the body responds to daily events and maintains homeostasis (Sterling and Eyer, 1988). Because chronically increased allostasis can lead to pathophysiology, the term allostatic load or overload was introduced by McEwen and Minsky to refer to the wear and tear that results from either too much stress or from inefficient management of allostasis, such as not turning off the neuro-endocrine response when it is no longer needed (McEwen, 2007).

In this study we aim to examine the long term behavioural consequences of the exposure of male rats to chronic stress during a sensitive developmental time window and, in addition, the interaction between chronic stress and ketamine administration (‘two hit’ approach).

Materials and methods

Animals

Forty male Wistar rats, age 24 d (purchased from Harlan, Israel), were included in the study. Rats were housed four per cage (30 cm × 30 cm × 18 cm) with sawdust bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments, unless otherwise specified. A standard 12 h dark/light cycle was maintained (lights were on between 06:00 hours and 18:00 hours) and room temperature was kept at 23±1 °C. The cages system maintained the humidity at 67%.

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the European Communities Council Directive. The study protocol was approved by the Institutional Animal Care and Use Committee and adhered to the guidelines of the society for Neuroscience. All efforts were made to minimize animal suffering.

Procedures

Wistar rats were randomly assigned into four groups (n=10 in each): (i) saline, (ii) ketamine, (iii) chronic stress and (iv) ketamine+chronic stress. The experimental procedures are described in Fig. 1.

Chronic stress procedure

The stress procedure was comprised of swim stress, elevated platform stress and restraint stress, as specified below. Rats were randomly exposed to the different stressors during post-natal days (PND) 30–60 (4 wk), for five consecutive days (per week). Each day during this period, rats were exposed only to one of the three stressors.

Swim stress

Rats were made to swim for 10 min in a square water tank (38 × 30 cm, water depth: 60 cm). Water
temperature maintained at 23±1 °C (Avital and Richter-Levin, 2005).

Elevated platform stress

Rats were placed on a platform (10 cm diameter) with a top that elevated 50 cm above the surface. They were subjected to this stress three times for 30 min, with a 1 h interval spent in a resting cage before returning to their home cages (Avital and Richter-Levin, 2005).

Restraint stress

Rats were placed in a radial-shaped metal net (6 cm height), which allowed for a small amount of lateral movement. They were subjected to the restrainer in the same routine as in the elevated platform stress.

Ketamine injections

Rats were injected with 30 mg/kg Ketamine intraperitoneally (i.p.) at a volume of 1 ml/100 g daily for five consecutive days (1 h post exposure to stress), on PND 41–45 (Becker and Grecksch, 2004). Naive rats were injected, with the same volume and injection regimen, with saline (saline group).

Behavioural tests

At PND 100–125, all rats were behaviourally tested, as described below.

Motor monitor

The test took place in a Plexiglas chamber (45 cm L×24 cm W×40 cm H) surrounded by a beam-break system on the outer side; the motor monitor was placed in a sound-proof ventilated box (70×40×50 cm) with 8 lux light. On PND 100, rats were placed for 5 min in the chamber and activity was measured by velocity, distance and total beam breaks (Stanford et al., 2002). The performance was calculated by the Kinder Scientific program (Campden, UK).

Sucrose preference

The test took place in home cages. During the acclimatization period (1 wk, PND 100–106), rats were allowed to consume 1% (w/v) sucrose/water solution or tap water, in order to overcome neophobia, 4 h per day only, and for the remaining time they were deprived of water. On the 6th day we assessed the amount of liquid consumed, so as to detect whether there are general differences in liquid consumption (i.e. 1% (w/v) sucrose/water solution and tap water). On the 8th day (PND 107) we inserted, through the metal mesh top cover of the cage, two drinking bottles, identical to the home cage water bottles. The bottles, one containing a 10% sucrose solution and the other tap water, were weighed just before the test and immediately following its completion, after 4 h. The relative positioning of the bottles providing sucrose and
water was counterbalanced between tests to prevent the development of side preference. Initial relative positioning of the bottles was counterbalanced between groups (Avital et al., 2011).

Object recognition

The test took place in a black lustreless Perspex box (50×50 cm). The procedure was modified from Karasawa et al. (2008) and Lebrun et al. (2000). Rats were acclimated to the test arena during two successive days (PND 108–109), i.e. they were allowed to explore the arena (without any objects) twice a day, for 5 min with 1 h interval. On the third day (PND 110), rats were allowed to explore the arena for 5 min, with the presence of two identical objects (two multi-colored squared Lego blocks 7×7 cm). Following the first exploration session, rats were removed and placed in home cages. Subsequently, after 1 h, rats were placed back in the arena for the test trial, but this time with two un-identical objects, (one object was replaced by a new one) for 5 min. Every trial was videotaped by a Panasonic cctv camera and post-recording analysed with Ethovision XT software (Nuldus, The Netherlands).

Eight-arm radial maze

Rats were familiarized with the maze for three days (PND 111–113) before training: On the first day they were placed in the maze for 20 min with scattered food rewards (45 mg food treats). On the second and third days, they were placed in the maze for 15 min and were able to consume the reward only from the food cups at the end of the arms. In the learning phase, rats were given daily training sessions (one trial per session) over a 12 d period (PND 114–125), so they would learn to visit each arm only once within a trial. A trial starts with the rat placed in the central area. A trial ends when one of the following conditions is reached: (i) the rat has visited all eight arms, (ii) the rat has made 16 visits, or (iii) the trial lasted for more than 10 min.

Pre-pulse inhibition (PPI)

The session (80 trials) started with 5 min acclimatization period with a 57 dB background noise level that was delivered continuously throughout the test session. To evaluate the startle response, the first and last ten trials consisted of single 40 ms 120 dB ‘pulse-alone’ startle stimuli. These trials were used to obtain a measure of habituation in response to repeated delivery of the startling stimuli (inter-trial interval 1 min). The middle 60 trials consisted of random delivery of ten ‘no stimuli’ trials, during which no stimuli was delivered, ten ‘pre’ stimuli (at 59, 61, 65, 69, 73, 78 or 85 dB), and forty ‘pre pulse’ trials. The latter trials, consisted of a single 120 dB pulse preceded (80 ms interval) by a 20 ms ‘pre pulse’ of 2, 4, 8, 12, 16, 21 or 28 dB above background (i.e. 59, 61, 65, 69, 73, 78 or 85 dB) (Avital et al., 2011). The PPI test conducted on PND125, 1 hr post eight-arm radial maze last training session.

Data analysis

Data were analysed for statistical significance using two-way ANOVA, with treatment (saline or ketamine) and condition (no stress or chronic stress) as between subject’s factors, followed by Student’s t-test for independent variables as post-hoc test. In order to examine differences between the various groups in the sucrose preference and the PPI tests, data was analysed using three-way ANOVA for mixed design, with treatment and condition as between subject’s factors and sucrose concentration/PPI pre-intensity as within subject’s factor. A result was significant when p<0.05. All tests were calculated as two-tailed with SPSS V17.0. Results are presented as means±standard error of the means (S.E.M.).

Results

Measuring activity by distance made in the motor monitor test, a two-way ANOVA revealed a significant main effect for treatment (saline or ketamine) (F(1, 36) =263.93, p<0.0001) but not for condition (no stress or chronic stress) (F(1, 36)<1). A significant treatment×condition interaction (F(1, 36)=56.04, p<0.0001) was found. Post hoc t-tests revealed that the locomotor activity of ketamine was significantly higher (p<0.0001) compared with the saline treated rats. While the exposure to chronic stress has led to decreased activity in saline treated group, interacting with ketamine treatment, chronic stress has yielded a significant increase in locomotor activity (p<0.0001) (Fig. 2).

In the sucrose preference test a two-way ANOVA revealed a significant main effect for condition (no chronic stress or chronic stress) (F(1, 36)=24.65, p<0.0001) but not for treatment (saline or ketamine) (F(1, 36)=2.31, p>0.137) nor for the treatment×condition interaction (F(1, 36)=3.64, p>0.064). Post hoc t-tests revealed that the sucrose intake of the chronic stress, treated with saline or with ketamine, showed a significant anhedonia compared with their
counterpart no chronic stress rats ($p<0.034$, $p<0.0001$, respectively) (Fig. 3).

In the object recognition test (Fig. 4) a two-way ANOVA revealed a significant main effects for condition (no stress or chronic stress) ($F(1, 36)=51.68$, $p<0.0001$), for treatment (saline or ketamine) ($F(1, 36)=5.12$, $p<0.03$) and for the treatment×condition interaction ($F(1, 36)=12.93$, $p<0.001$). Post hoc t-tests revealed that the attention to the novel object was significantly impaired in the chronic stress rats treated with ketamine ($^*p<0.0001$). A tendency towards impaired performance was observed in the chronic stress group treated with saline ($p>0.07$).

In the eight arm radial arm maze task, a three-way ANOVA for mixed-design was performed with treatment and condition as a between-subject factors and the training days as a within-subjects factor. The results revealed a significant main effects for training days ($F(12, 25)=229.67$, $p<0.0001$), for condition (no stress or chronic stress) ($F(1, 36)=484.54$, $p<0.0001$), for treatment (saline or ketamine) ($F(1, 36)=367.76$, $p<0.0001$). A significant interactions were found between condition (no stress or chronic stress) and treatment (saline or ketamine) ($F(1, 36)=5.31$, $p<0.027$), training days and treatment ($F(12, 25)=41.87$, $p<0.0001$), training days and condition ($F(12, 25)=21.23$, $p<0.0001$) and for the treatment×condition×training days ($F(1, 25)=7.06$, $p<0.0001$). Starting on day 5 and onward all experimental groups showed poor learning compared with their counterpart saline treated ($p<0.0001$). The ketamine+chronic stress group showed the poorest performance ($p<0.0001$) starting on day 8 and onward (Fig. 5).

In the PPI a three-way ANOVA for mixed-design was performed with treatment and condition as a between-subject factors and the pre-intensities as a within-subjects factor. The results revealed a significant main effects for pre-intensities ($F(6, 31)=635.83$, $p<0.0001$), for condition (no stress or chronic stress) ($F(1, 36)=674.88$, $p<0.0001$), for treatment (saline or ketamine) ($F(1, 36)=761.37$, $p<0.0001$). A significant interactions were found between condition (no stress or chronic stress) and treatment (saline or ketamine) ($F(1, 36)=301.76$, $p<0.0001$), pre-intensities and treatment ($F(6, 31)=28.14$, $p<0.0001$), pre-intensities and condition ($F(6, 31)=6.34$, $p<0.0001$) and for the treatment×condition×pre-intensities ($F(6, 36)=40.58$, $p<0.0001$). Throughout the different pre-pulse intensities, the PPI of the ketamine+chronic stress group showed the poorest performance ($p<0.0001$).
was significantly impaired compared with all the other groups (\(p<0.0001\); Fig. 6).

**Discussion**

The present study examined the emotional and cognitive developmental consequences of exposure to chronic stress during a sensitive period (PND 30–60), with or without administration of sub-anesthetic dose of ketamine. Our results suggest that prepubertal onset of exposure to ketamine and chronic stress may serve as an animal model for schizophrenia-like symptoms.

Our results show that rats exposed to chronic stress and injected with ketamine, displayed a significantly increased level of locomotor activity in the motor monitor compared with saline treated controls. In contrast, the activity level of rats that were subjected to chronic stress alone was significantly low, a result that can be related to human findings concerning hypo-activity observed in major depression (Ferentinos et al., 2009).

Anhedonia is often characterized as negative symptom in schizophrenia (Shankman et al., 2010). In the sucrose preference test, we found that rats exposed to chronic stress showed decreased preference for sucrose compared with the saline treated. Though not as overwhelmingly as in the chronic stress group, ketamine-injected rats exposed to chronic stress also showed lower sucrose consumption compared with the saline treated rats. This might be viewed as a moderating effect of ketamine on anhedonia, Thus, taken together with the robust hyperactivity observed in the ketamine-injected chronic stress group, these results may indicate that chronic stress by itself led to major depression-like symptoms, while chronic stress with ketamine led to psychotic depression-like symptoms.

In the cognitive symptoms domain, both in psychotic depression and in schizophrenia (Devulapalli et al., 2008; Faraone et al., 2000; Tolin et al., 2006), memory deficits were reported (Karasawa et al., 2008; Lebrun et al., 2000; Shalev and Kafkafi, 2002). In the radial arm maze test, rats in the chronic stress+ketamine group showed an utterly poor performance compared with the saline treated group. The chronic stress group also showed a decrease in performance compared with the saline treated rats; this could also be related to anhedonia, resulting in lack of motivation to find the food reward (despite the food limitation prior to the test). Either way, these results further reflect the difference between major depression and schizophrenia, since major depression is less likely to be characterized with poor cognitive performance (Bubenikova-Valesova et al., 2008; Castellano et al., 2001). Hence, these results further support our suggested model that represents, most-likely, a behavioural impairment similar to negative signs of schizophrenia.

The cognitive functioning of schizophrenic patients is linked closely to attention abilities (Ross et al., 2006). Disruption of cortical interneurons was previously suggested to underlie prefrontal cortex dependent functioning, such as attention (Gonzalez-Burgos et al., 2011; Uhlhaas and Singer, 2006). Rats treated with ketamine and exposed to chronic stress showed a severe deficiency of overt attention in the object recognition test. While overt attention is measured as it is worded, behaviourally, covert attention is measured via assessment of cortical inhibition. Cortical inhibition deficit has been suggested as one potential
pathophysiological mechanism in schizophrenia (Wobrock et al., 2008). PPI refers to the inhibition of the startle reflex by a weak pre-pulse presented prior to the startling stimulus. We previously proposed that this type of startle reflex reflects a neurophysiological measure of sensorimotor gating as attention related process (Avital et al., 2011). Deficits in the habituation to a startle reflex have been quantified in schizophrenia patients and thus have been suggested to be putative biomarkers of a tendency to develop schizophrenia (Ludewig et al., 2002). In the PPI test we found a significant impairment in the ability to execute PPI in rats that were exposed to the combination of chronic stress and ketamine. These results, along with the results of the object recognition test, serve as a convergent validity for our suggested animal model of schizophrenia-like symptoms. We postulate that the cortical inhibition deficit can be attributed to both the effects of chronic stress and the transient blockade of NMDA receptors, during a sensitive time window such as periadolescence. Recently it has been shown (Thomases et al., 2013) that indeed a transient exposure to the NMDA receptor antagonist MK-801 during the periadolescent period resulted in a long lasting frequency-dependent disinhibition of prefrontal processing of ventral hippocampal inputs in adulthood. Such susceptibility to MK-801-induced enduring prefrontal cortex (PFC) disruption was not observed in adult-exposed animals, further supporting the idea that periadolescence is a sensitive period for the functional maturation of prefrontal inhibitory control. Moreover, both the PFC and the hippocampus are known to be susceptible to glucocorticoids (Avital et al., 2006), and therefore the exposure to chronic stress during periadolescence period may also contribute to the cortical inhibition deficits.

Limited to the periadolescence period, overall we observed that adding ketamine to chronic stress, both occurring during a sensitive developmental time window, had an impact similar to that of positive and negative symptomatology of schizophrenia. Ketamine injected rats that were exposed to chronic stress showed hyper-activity alongside low sucrose preference, poor memory performance, attention deficits in the object recognition and impaired pre-pulse inhibition.

This manifestation of symptoms succeeds in modeling rather profoundly the human cluster of schizophrenia, both negative and positive symptomatology.

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Statement of Interest

None.

References