Group II metabotropic glutamate (mGlu2/3) receptors

Imre, Gábor

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Dose-response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity

Gabor Imre, Dirk S. Fokkema, Johan A. Den Boer, Gert J. Ter Horst.

Brain Research Bulletin (2006), 69; 338-345
Chapter 2

ABSTRACT

The present dose-response study sought to determine the effects of subanesthetic dosages (4-16 mg/kg) of ketamine on locomotion, sensorimotor gating (PPI), working memory, as well as c-fos expression in various limbic regions implicated in the pathogenesis of schizophrenia. In addition, we examined whether ketamine-induced locomotion was influenced by the dark/light cycle. We found that ketamine increased locomotor activity in a dose dependent manner, but found no influence of the dark/light cycle. Additionally, ketamine dose-dependently interrupted PPI, resulting in prepulse facilitation at doses of 8 and 12 mg/kg. The dose of 12 mg/kg also induced impairments in working memory assessed by the discrete-trial delayed-alternation task. C-fos expression indicated that the dose-dependent behavioral effects of ketamine might be related to changes in the activity of limbic regions, notably hippocampus and amygdala.
INTRODUCTION

A widely accepted and utilised psychopharmacological model of schizophrenia is based on the effect of non-competitive NMDA receptor antagonists, such as phencyclidine (PCP), dizocilpine (MK801) and ketamine (Javitt and Zukin, 1991; Olney et al., 1999). A subanesthetic dose of ketamine produces several behavioural abnormalities in rodents such as disruption of sensorimotor gating (PPI) and working memory, which are analogous to the effects of ketamine in humans and mimic the symptoms of schizophrenic patients (Jentsch and Roth, 1999; Krystal et al., 1994; 2002; Mansbach and Geyer, 1991; Verma and Moghaddam, 1996). Such experimentally induced deficits provide a model with significant face, predictive and construct validity (Jentsch and Roth, 1999). Ketamine and other NMDA antagonists also increase locomotor activity in rodents, but not in humans (Jentsch and Roth, 1999). Thus, the relationship between rodent hyperlocomotion and schizophrenic symptoms lacks a proper degree of face validity (Schulz et al., 2001b). However, because locomotor activity is associated with limbic-striatal function, ketamine-induced hyperlocomotion might be indirectly linked to schizophrenia since the latter is generally associated with limbic abnormalities (Takahata and Moghaddam, 2003).

The mechanism by which ketamine produces its adverse behavioural effects, at least partly, have been attributed to the blockade of NMDA receptors located on inhibitory GABAergic neurons limbic and subcortical brain regions (Duncan et al., 1998a; Moghaddam and Adams, 1997; Nakao et al., 2003). This disinhibitory action leads to increase in the neuronal activity and excessive glutamate and dopamine release in the prefrontal cortex and limbic striatal regions (Duncan et al., 1998a; Gao et al., 1998; Gass et al., 1993; Lorrain et al., 2003a; Moghaddam and Adams, 1997). In these studies, however, a higher dose range (10-35 mg/kg) was used, compared to the studies in which the behavioural effects of ketamine (2.5–12 mg/kg) were investigated (Mansbach and Geyer, 1991; Pallares et
al., 1995; Silvestre et al., 1997; Swerdlow et al., 1998). Although Nisizawa et al. (2000) found a strong correlation between ketamine-induced locomotor responses and cell activity measured by c-fos expression, this study investigated only posterior cortical areas using considerably high doses of ketamine (20-50 mg/kg). Thus, the question arises which are the links between behavioural changes (hyperlocomotion and cognitive deficit) and brain activity induced by the lower doses of ketamine.

In Experiment 1, subanesthetic doses of ketamine (4-16 mg/kg) were used 1) to characterise the nature and time course of its effect on the locomotion 2) to define neuroanatomical regions where this treatment regime would affect neuronal activity assessed by c-fos expression 3) and to investigate the correlation between behavioural changes and c-fos expression. Since rats are nocturnal animals, locomotor activity is more abundant in the dark phase of the dark/light cycle (Westenbroek et al., 2003), Nevertheless, behavioural studies investigating the locomotor activating effects of stimulants have been conducted during the light phase (i.e. the sleeping period) or do not mention this experimental conditions. Thus, we also compared the effects of ketamine on locomotion under different light conditions.

In a second study (Experiment 2) the dose-response characteristic of ketamine-evoked (4-12 mg/kg) cognitive deficits were assessed using two behavioural measures which are likely to have relevance to clinical symptomology: the prepulse inhibition of the startle reflex paradigm (PPI) and the discrete-trial delayed alternation task. PPI refers to the phenomenon that a weak stimulus (prepulse) reduces the behavioural response to a second, more intense, stimulus. PPI provides an operational measure of sensorimotor gating both in human and rodents (Geyer et al., 2001). The discrete-trial delayed alternation task is a working memory-related paradigm. Performance of this task depends on the functional integrity of the prefrontal cortex, and it is sensitive to the acute effects of psychostimulants (Aultman and Moghaddam, 2001).
MATERIALS AND METHODS

Experiment 1

ANIMALS

Twenty-five male Wistar rats (Harlan, The Netherlands) weighing 248±8 g (5-6 weeks of age) at the start of the experiments were individually housed in plexiglas cages (45×28×20 cm) on a 12/12 h light/dark cycle (lights on at 7:00). After the second open field test, the light/dark cycle was reversed (lights on at 19:00) and animals were allowed to acclimate to it for a period of 1 week. A piece of PVC tube (d=8 cm, l=17 cm) was provided in the home cage as a shelter. Food (standard rat chow, Hopefarms) and water were available ad libitum. All animals were handled and weighed daily to minimise stress during the experiment. The Animal Ethics Committee of the University of Groningen approved the protocol (FDC: 2935).

LOCOMOTOR BEHAVIOUR

Open field tests (OF) were performed under both day-light (sleep period) and red-light (active period) condition in order to compare the effect of ketamine (Sigma, Germany) on locomotion in the inactive period with the active period of the animals. The open field consisted of a circular black arena with a diameter of 1 m. Rats were placed in the centre of the field at certain time points i.e. at 0, 20, 40, 60 and 90 min post saline or ketamine (4, 8, 12, 16 mg/kg) injection (s.c.) and observed for a period of 5 min. This test was repeated 4 times, each performed 1 week apart; every rat served as its own control therefore each received a saline injection followed by a ketamine challenge under both normal- and reversed-light condition (Table 1). Five animals received only saline during this protocol in order to get control brain activity for c-fos comparison. Locomotor behaviour was recorded with a videotracking system (Etho Vision, 1.96, Noldus Information Technology, Wageningen, The Netherlands). The distance moved within the arena was analysed.

<table>
<thead>
<tr>
<th>1st day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
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<td>2nd OF ketamine</td>
<td>3rd OF saline</td>
<td>4th OF ketamine</td>
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<td>acclimatization</td>
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<td>normal light</td>
<td>normal light</td>
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</tr>
</tbody>
</table>

Table 1. Experimental protocol for open field-locomotor behaviour.

IMMUNOHISTOCHEMISTRY

The rats were euthanized 2 h after the last ketamine injection using sodium pentobarbital anesthesia (1 ml, 6%) and perfused with 50 ml heparinised saline and 300 ml of 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed and postfixed in the same fixative overnight at 4°C. Following an overnight cryoprotection in a 30% sucrose solution, serial 40 μm coronal sections of the cerebrum were made with a cryostat microtome and collected in 0.02 M potassium phosphate saline buffer (KPBS). Fos immunostaining was performed as described previously (Westenbroek et al., 2003). Briefly, after rinsing with 0.3% H₂O₂, for 10 min the sections were thoroughly washed with KPBS and incubated with rabbit anti-Fos antibody (1:10000; Oncogene Research Products, San Diego, CA, USA) diluted in 0.02 M KPBS with 0.25% Triton X-100 and 2% normal goat serum for 72 h at 4°C. After washing, the sections were subsequently incubated for 2 h with biotinylated goat-anti-rabbit IgG (1:1000 in 0.02 M KPBS)
and avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA). After thorough washing, the peroxidase reaction was developed with DAB-nickel solution and 0.1% H$_2$O$_2$. Sections were washed for 15 min in buffer and mounted with gelatine solution and air-dried, dehydrated in graded alcohol and xylol solutions and then coverslipped with DePeX mounting medium (BDH).

In order to avoid differences in staining intensities, sections were stained according to the same procedure. The c-fos labelled cells were quantified using computerized image analysis system by an observer who was blind to group assignment. The area of the region of interest (ROIs) was digitized using a Sony (SONY Corporation, Tokyo, Japan) chargecoupled device digital camera mounted on a LEICA Leitz DMRB microscope (Leica Qwin version 2.3, Leica Microsystems Imaging Solutions, Wetzlar, Germany). The number of immunopositive nuclei was quantified in a single focus plane (at ×100) for each ROIs, which were outlined with digital pen and each digitized image was individually set at a threshold to subtract the background optical density. The average of immunoreactive cells was expressed as number of positive nuclei/0.1 mm$^2$.

Experiment 2

ANIMALS

Twenty-four male Wistar rats (Harlan, The Netherlands) weighing 210±5 g (4-5 weeks of age) at the start of the experiments were individually housed in plexiglas cage (45×28×20 cm) with a 12/12 h light/dark cycle (lights on at 7:00). A piece of PVC tube (d=8 cm, l=17 cm) was provided in the home cage as a shelter. All animals were handled and weighed daily to minimise handling stress during the experiment. Food was available ad libitum two weeks following arrival, after which they were placed on restricted diet of 15 g per day per rat. The rats had ad libitum access to water throughout the duration of the experiment. The Animal Ethics Committee of the University of Groningen approved the protocol (FDC: D4116A).

PREPULSE INHIBITION OF THE STARTLE REFLEX (PPI)

After 1 week of acclimatization PPI tests were conducted using a TSE Startle Response Measuring System (Technical and Scientific Equipment GmbH, Bad Homburg, Germany). The rats were restrained in a small cage (27×10×12.5 cm) and placed on a transducer platform that registers movement. Acoustic stimuli were generated by means of high-quality high-linearity speakers situated on both sides of the cage. The whole setup was operated in a sound-attenuating isolation chamber equipped with a ventilation fan and house light. An IBM-compatible computer with TSE Startle Response software and control interface was used to present stimuli and record data. Following saline or ketamine (4, 8 and 12 mg/kg s.c.) injection, the rats were placed in the startle box for a 5-minute acclimatization period with a 70 dB background noise. This noise continued throughout the session.

The test session consisted of four trial types: (1) SP alone: rats were exposed to 120 dB 40-ms startle pulse; (2) PP+SP: startle pulse preceded 100 ms earlier by 85 dB 20-ms prepulse (3) PP alone: prepulse alone; (4) no-stimulus: background noise only. One session consisted of 35 trials, with 3 consecutive sp alone trials in the beginning and 32 subsequent trials (each trial types 8 times) presented in a random order, with 15 s average (range 10-20 s) intertrial interval.

PPI was defined as the percentage reduction in mean startle response magnitude (SRM) in the presence of the prepulse compared to mean SRM in the absence of the prepulse: $100\%-\frac{100\%}{\text{mean SRM PP+SP trials/ mean SRM SP alone trials}}$. The 3 SP alone trials, which were presented at the beginning of the test session to achieve a relatively stable level of startle reactivity for the remainder of the session (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus, {Geyer et al., 1990}), were not included in the calculation of PPI values.
Ketamine model of schizophrenia

**DISCRETE PAIRED-TRIAL DELAY T-MAZE TASK**

The test was conducted in a T-maze that was made of wood and the arms were covered with grey plastic. The main alley, 80×20×13 cm, was connected to two goal arms, each 40×20×13 cm. Two sliding doors were manually operated to close off either goal arm. At the end of each goal arm, a 3-cm high piece of metal blocked the food reward from view. During the inter-trial and the intra-trial periods, animals were confined in a holding box (20×20×13 cm) which was separated from the main alley by a sliding door. The experiments were performed in the light period between 8:00 a.m. and 12:00 a.m. The testing room was illuminated by three 25-W lamps positioned around the maze.

The training protocol was adapted from Aultman and Moghadam (2001). One week after the PPI test, the animals were placed on food restriction and were introduced to the food reward (yoghurt drop, Vitakraft) in their home cage. After that rats were habituated to the T-maze. On the first day of habituation, rats were placed in the maze individually and allowed to explore freely for 5 min. Food rewards were scattered throughout the maze. Subsequently, only the ends of the two goal arms were baited. This procedure continued until the rats ran quickly to both goal arms and consumed the food (2-3 days). This was followed by one week of “forced alternation” training, where rats were allowed to retrieve the reward from only one arm, the other being blocked. Rats were given ten trials each day; on five trials the rat was forced to enter the right goal arm and on five the left, in an alternating pattern. A 10-s inter-trial interval separated these trials.

Following the forced alternation training, the discrete, paired-trials delayed alternation task began. Each trial-pair consisted of a forced trial and a choice trial, separated by a delay interval of 10 s (memory retention). In forced trial the rat had access to only one goal arm, which was baited. Following consumption of the reward, the rat was removed to the holding box for the delay. In choice trial the rat has access to both goal arm, but only the previously blocked arm contained the reward. Correct choice was scored if the rat entered the baited arm; entries into the initially visited forced arm were scored as errors. Trial-pairs were separated by 30s inter-trial interval. Each test block consisted of ten trial pairs. A different, randomly chosen, pattern of forced trials, but no more than two consecutive forced entries in a given direction (e.g. R-L-R-R-L-L-R-L-R-L), was used every day (on a given day the same pattern for all animals). This training continued until the rat successfully performed seven out of ten trials (70%, 7-10 days) for three consecutive days. Upon reaching a stable performance level, the animals randomly received a saline or ketamine injection (8 and 12 mg/kg s.c.). According to the duration of action on the locomotion as established in Experiment 1, ketamine was applied 10 min (8 mg/kg) and 25 min (12 mg/kg) prior to testing in order to avoid the locomotor effects of ketamine which might influence the performance in the T-maze.

**STATISTICAL ANALYSIS**

Statistical analyses were done with SPSS (version 10), and P<0.05 was considered significant. Locomotor activity was analysed with repeated measures ANOVA, with time point as within subject factor and treatment and light condition as between subject factors. T-maze performance was also analysed with repeated measures ANOVA, with ketamine treatment being specified as between subject factor and consecutive days (4 days) as within factor. Sphericity assumed modelling, with Huynh-Feldt adjustment, was applied. C-fos and PPI were analysed with univariate ANOVA with dose as a between subject factor. An LSD post hoc test was used for the pairwise comparisons. Correlative studies between the ketamine induced locomotion under red-light condition and c-fos expression of different brain regions utilised the Kendall rank correlation test.
RESULTS

Experiment 1

Locomotor behaviour

Under both light conditions, at all dosages of ketamine rats displayed the characteristic behavioural responses of side-to-side head rocking, arching of the neck and staggered locomotion. Overall ANOVA showed a significant ketamine main effect ($F(3, 32) = 4.000, P<0.016$). The stimulatory effects of ketamine were dose-dependent to the extent that the higher doses produced a longer duration of the response as illustrated in Fig. 1. There was a significant main effect of time ($F(3, 124) = 46.631, P<0.001$) and time $\times$ ketamine interaction ($F(11, 124) = 5.292, P<0.001$).

As repeated measures ANOVA revealed, when the rats were tested under daylight condition only the highest dose (16 mg/kg) caused significant changes in locomotion during the first trial ($t=0, P<0.002$), which lasted for 20 min ($t=20, P<0.001$) and reached baseline activity after 40 min. The shape of the curve is similar in the 12mg/kg group, however it failed to be significant during the first trial ($t=0, P<0.169; t=20, P<0.019$). At the dose 4 and 8 mg/kg ketamine slightly increased locomotion in the first 5 min and the locomotor activity reached the baseline level again before the second trial.

Under the red-light condition, except the 8 mg/kg dose, every dose increased locomotion significantly in the first trial ($t=0, 4 \text{ mg/kg } P<0.015; 12 \text{ mg/kg } P<0.001 \text{ and } 16 \text{ mg/kg } P<0.001$). During the second trial hyperactivity remained significantly high in groups receiving the 12 mg/kg ($P<0.007$) and 16 mg/kg dose ($P<0.002$). After this excitatory effect the activity fell under the baseline level, which was significant at dose 12 mg/kg and 16 mg/kg ($t=40, P<0.005$ and $P<0.017$ respectively).

Altering the light/dark cycle did not influence the locomotor activity in the open field. There was no main effect of light condition ($F(1, 32) = 1.865$,
P<0.182), nor light condition×ketamine interaction (F (3, 32) =0.19, P<0.996). Because of the hyperlocomotion effect of ketamine is apparent at t=0 and t=20, statistical analysis were repeated for these two time points excluding the results at t=40, t=60 and t=90. In this analysis, similarly to the overall ANOVA, there was ketamine main effect (F (3, 32) =6.166, P<0.002) but no light condition (F (1, 32) =1.322, P<0.259) and no light condition×ketamine interaction (F (3, 32) =0.081, P<0.970); therefore all rats were sacrificed during the red light period for subsequent c-fos determinations.

**Figure 1.** Effect of different doses of ketamine on locomotion (n=5). Hyperlocomotion evoked by ketamine expressed as relative total distance moved (Δcm): difference in total distance moved between ketamine and saline control test.

*P<0.05; significant increased locomotion by ketamine under normal light condition.

*P<0.05; significant increased locomotion by ketamine under reverse light condition.

$P<0.05;$ significant reduction in locomotion following ketamine excitation under reverse light.
C-fos expression was analysed in brain regions which are involved in the pathophysiology of schizophrenia such as prefrontal-, (PFC) retrosplenial- (RS) and entorhinal cortex (EC) and limbic regions (hippocampus, striatum and amygdala). In addition, the hypothalamic paraventricular nucleus (PVN) and dorsomedial nucleus (DMH) was analysed since they are associated with stress responses. The ketamine treated animals showed significantly higher c-fos expression in various brain areas compared to saline-treated controls. As illustrated in Table 2, ketamine dose-independently increased the number of c-fos labelled cells in the PFC ([ACAd]: F (4, 20) = 4.329, P<0.001, 262-312%; [ILA]: F (4, 20) = 20.700, P<0.001, 255-283%; [PL]: F (4, 20) = 10.705, P<0.001, 258-311%), RS (F (4, 20) = 13.711, P<0.001, 340-489%) and NAc (F (4, 20) = 4.411, P<0.05, 176-218%). There was no main effect in the EC (F (4, 20) = 1.882, P<0.155) and CP (F (4, 20) = 2.144, P<0.113). In the amygdala ([CeA]: F (4, 20) = 20.069, P<0.001; [Bla]: F (4, 20) = 6.343, P<0.01), hippocampus ([DG]: F (4, 20) = 4.150, P<0.05; [CA1]: F (4, 20) = 5.409, P<0.01; [CA3]: F (4, 20) = 8.690, P<0.001) and the hypothalamus ([PVN]: F (4, 20) = 25.329, P<0.001; [DMH]: F (4, 20) = 3.179, P<0.05) ketamine induced dose-dependent changes in c-fos expression. In the central amygdala, the CA1 and the CA3 regions the lowest dose (4 mg/kg) of ketamine induced changes in c-fos expression that were significantly higher (901%, 634% and 473% respectively) than other doses (531-636%, 336-364% and 288-333%). In general, increasing doses tend to induce less staining (Fig. 2). However, in the PVN 4 mg/kg induced a significantly lower c-fos response (347%) compared with the higher doses (445-464%). Noteworthy is that with the highest dose, the number of c-fos positive cells was lower than with intermediate doses in most brain areas and did no longer differ significantly from the baseline in hippocampus (147%) basolateral amygdala (164%) and DMH (155%).

As the Kendall rank correlation test showed there was no significant correlation between ketamine-induced hyperlocomotion and c-fos expression (Table 2).
Table 2. C-fos expression in the investigated brain areas Mean number of c-fos positive cell per 0.1 mm$^2$ ± SEM. *P<0.05, **P<0.01; ketamine compared with saline. #P<0.05; 4 mg/kg of ketamine compared with other doses. Kendall rank correlation tests do not show significant correlations between ketamine-induced hyperlocomotion and c-fos expression.

<table>
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<tr>
<th>Brain Area</th>
<th>Saline</th>
<th>4mg/kg</th>
<th>8mg/kg</th>
<th>12mg/kg</th>
<th>16mg/kg</th>
<th>Kendall τ</th>
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<td>116±12*</td>
<td>115±19*</td>
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</table>

Table 2. C-fos expression in the investigated brain areas Mean number of c-fos positive cell per 0.1 mm$^2$ ± SEM. *P<0.05, **P<0.01; ketamine compared with saline. #P<0.05; 4 mg/kg of ketamine compared with other doses. Kendall rank correlation tests do not show significant correlations between ketamine-induced hyperlocomotion and c-fos expression.

Figure 2. Photomicrographs illustrating ketamine-induced c-fos immunoreactivity in the central (CeA) and basolateral (BlA) amygdala.

Scale bar = 50 μm
**Experiment 2**

**Prepulse Inhibition of the Startle Reflex (PPI)**

One-way ANOVA showed a significant main effect of ketamine on PPI ($F(3, 20) = 8.146, P < 0.001$) as well as on the startle magnitude (SRM) in sp alone trials ($F(3, 20) = 6.268, P < 0.001$). As illustrated in Fig. 4, all doses of ketamine significantly reduced PPI ($P < 0.001$) and resulted in prepulse facilitation (PPF) at doses of 8 and 12 mg/kg. There was a significant increase in SRM by 4 mg/kg compared to control ($P < 0.005$), whereas the higher doses did not alter SRM.

![ketamine effect on PPI and startle response](image)

**Figure 3.** Effects of ketamine on the prepulse inhibition (PPI) and the startle response magnitude (SRM). All doses of ketamine significantly impaired the PPI ($n=6$). In the 4 mg/kg condition PPI was reduced significantly concurrent with a significant increase in a SRM. The negative PPI value at the doses of 8 and 12 mg/kg represent prepulse facilitation without significant changes in the SRM. *$P < 0.05$; significant changes in PPI and in SRM compared with control.
**Discrete Paired-Trial Delay T-maze Task**

Injection of saline, after rats reached the criterion, did not influence the number of correct response as illustrated in Fig. 4. Repeated measures ANOVA revealed a main effect of ketamine treatment on the performance ($F (2, 17) = 9.324, P<0.002$). Ketamine at 12 mg/kg significantly reduced the percentage of correct responses compared to control ($P<0.001$). The lower dose of ketamine did not affect the performance.

As mean velocity indicated (measured in the main alley), ketamine had no effects on locomotion during the test in the T-maze (Fig. 4, inset; $F (2, 17) = 0.373, P<0.696$).

**Figure 4.** Effects of ketamine on working memory. After reaching the criterion (7 correct choices out of 10 for 3 consecutive days) animals (n=6-7) received the ketamine injection 10 min (8 mg/kg) and 25 min (12 mg/kg) prior to test respectively on the 4th day. At the dose of 12 mg/kg ketamine significantly impaired the performance. (*$P<0.05$). During the test, ketamine had no longer effect on locomotion as indicated by mean velocity.
DISCUSSION

The hyperlocomotion evoking effects of NMDA antagonists have been well characterized (de Leonibus et al., 2001; Jentsch and Roth, 1999; Lorrain et al., 2003a). Our findings replicated and extended these previous reports by demonstrating that ketamine increased the locomotion dose-dependently as that the effect of the highest doses (12-16 mg/kg) lasted at least 25 min, whereas the non-significant effect of the lower doses (4-8 mg/kg) was confined to the first 5 min. In addition, we showed that this ketamine-induced hyperlocomotion is not influenced by the light/dark cycle. With an exception of the lowest dose (4 mg/kg) which could induce significant hyperactivity during the animals’ active phase, but not in day-light condition, the locomotor behaviour was similar irrespectively of light condition for every dosage of ketamine. This implies that the light cycle was irrelevant for the locomotor activity in the present setup and therefore the further behavioural tests were conducted under day-light condition.

In accordance with the literature (Mansbach and Geyer, 1991; Swerdlow et al., 1998; Verma and Moghaddam, 1996), ketamine induced a dose-dependent deficit in PPI and working memory. In fact at the doses of 8 and 12 mg/kg ketamine elicited prepulse facilitation (PPF) without affecting the startle response magnitude (SRM), while 4 mg/kg increased SRM resulting in reduction of PPI. Additionally, at the dose of 12 mg/kg ketamine impaired working memory assessed by discrete-trial delayed alternation task, whereas the lower dose (8 mg/kg) had no effect on the performance.

PPI is regulated by highly interconnected cortico-limbic systems and is therefore particularly sensitive for disruption of the homeostasis of this network (Swerdlow et al., 2001). Thus, low doses of ketamine, which are not able to induce hyperlocomotion or working memory deficit, could produce significant disruption in PPI resulting in PPF. Under normal conditions PPF occurs either at low (0-30 ms) or large (>800 ms) prepulse-startle interval (PSI) (Reijmers and
Peeters, 1994a-b) and this facilitation can be enhanced by ketamine (Mansbach and Geyer, 1991). Moreover, 10 mg/kg ketamine was shown to evoke PPF at the interval 100 ms when normally prepulse inhibition is observed (de Bruin et al., 1999). Based on these findings it has been suggested that PPI and PPF are two different mechanisms, where the facilitatory process normally is suppressed by the concurrent inhibition and that ketamine could selectively disrupt the inhibitory mechanisms, thereby unmasking the facilitatory process (Mansbach and Geyer, 1991). It can be argued that in the present experiment at the doses of 8 and 12 mg/kg ketamine produced maximal reduction of the inhibitory processes which in turn manifested as PPF.

As previously reported, ketamine could produce working memory deficit in spatial delayed alternation task at the dose of 20-30 mg/kg but not 10 mg/kg (Verma and Moghaddam, 1996). Here we demonstrated that dose of 12 mg/kg ketamine is also able to impair working memory. It is likely that methodological differences account for this inconsistency of dose response. The main disadvantage of the traditional delayed-alternation task is the overtraining, which in turn requires long inter-trial delays (up to several minutes) in order to observe treatment effects on the performance that may not represent working memory deficit but impaired task acquisition. Another confound of this task is that the performance of the animal continuously improves with training and therefore it might be difficult to reach a stable baseline performance. In contrast, in the discrete-trial delayed alternation task animals reach a steady, delay dependent performance as that the rats performed significantly worse at the longer delay interval than short retention time supporting the hypothesis that this task assesses working memory (Aultman and Moghaddam, 2001). Thus, the discrete-trial delayed alternation task provide more sensitive measurement for the acute effects of psychostimulants on working memory which might explain that such a low dose of ketamine was found to impair the percentage of correct responses in this task. It should be noted that the T-maze tests were performed only at a delay interval of 10 s. Therefore, we
cannot rule out the possibility that 8 mg/kg or lower doses may lead to working memory deficit at longer memory retention.

Since the hyperlocomotion effect of ketamine might affect the performance of the animals in the T-maze, tests were conducted after the peak effect of ketamine on locomotion i.e. 10 (8 mg/kg) and 25 (12 mg/kg) min respectively. The lack of significant difference in the mean velocity between the ketamine- and saline-treated groups in the present study suggest that ketamine-induced impairment of the discrete-trial delayed alternation task cannot be attributed primarily to interference by locomotor activity; rather, a disruption in associative function might have occurred.

Ketamine and other NMDA antagonists have been consistently reported to induce c-fos expression in the cortical areas (prefrontal and retrosplenial cortex), nucleus accumbens (NAc), amygdala and the hypothalamus (Duncan et al., 1998a; Gao et al., 1998; Gass et al., 1993; Sharp, 1997; Szakacs et al., 2003). Similarly, in the present study ketamine increased the number of c-fos positive cells within these areas irrespectively of doses. In contrast to these studies, we also found significant ketamine-evoked c-fos expression in the subregions of the hippocampus (CA1, CA3 and DG) which was dose-dependent to the extent that the 4mg/kg ketamine produced higher expression compared to the other doses, whereas increasing doses gradually decreased this expression. Similar c-fos pattern was observed in the central nucleus of amygdala (CeA). Nevertheless, the c-fos expression in all brain areas investigated in this study did not correlate to the dose-dependent effect of ketamine on the locomotion.

Our finding concerning the inverse dose-dependent c-fos induction in the hippocampus and CeA is in accord with earlier report demonstrating that dizocilpine (MK801) up to 2 mg/kg increased c-fos in the hippocampus and then c-fos level declined from a dosage of 4 mg/kg (Ahn et al., 2002). The former effect may be due to the indirect activation of excitatory neurotransmitter systems as a consequence of reduced activity of GABA-containing neurons, whereas the
latter effect is possibly a direct NMDA receptor blockade on excitatory neurons which might also lead to reduction in c-fos induced by its own activating effect. NMDA receptors on hippocampal inhibitory neurons were found to be more sensitive to an NMDA antagonist, in comparison to NMDA receptors on CA1 pyramidal neurons (Grunze et al., 1996). Thus, at a low dose of ketamine, a preferential reduction of inhibitory tone would be expected resulting in high c-fos expression in the hippocampus which can be reduced by blocking the excitatory neurons at higher dosages.

According to the model of Grace (2000), glutamatergic afferents from the amygdala and the hippocampus provide a gating influence over the information flow from the prefrontal cortex at the level of the NAc. Furthermore, among the multiple limbic regions that regulate PPI (Swerdlow et al., 2001), the hippocampus and the amygdala were found to contribute to the sensorimotor gating deficit produced by NMDA antagonists (Bakshi and Geyer, 1998). On the other hand, NAc is considered as a pivotal structure in regulating the locomotor-activating effects (de Leonibus et al., 2001) whereas PFC plays a vital role in working memory deficit evoked by these drugs (Verma and Moghaddam, 1996). Taken together it can be argued that the inverse c-fos expression observed in the hippocampus and the CeA might indicate a dose-dependent ketamine-evoked deficit in the hippocampal and amygdala gating mechanism on the information flow between the PFC and Nac, which contributes to the profound effects of the highest doses of ketamine on the locomotion as well as on the cognitive function.

In conclusion, at a dose range of 4-16 mg/kg ketamine induced hyperlocomotion in a dose dependent manner and this was not influenced by the dark/light cycle. Ketamine also produced dose-dependent sensorimotor gating deficit with a maximal reduction in PPI manifested as PPF at the dose 8 and 12 mg/kg. In addition, 12 mg/kg ketamine impaired working memory deficit assessed by discrete-trial delayed alternation test. As c-fos expression indicated,
ketamine increased cell activity in cortical areas, NAc and the hypothalamus irrespectively of dosage while in the hippocampus and amygdala an inverse dose-dependent c-fos induction was observed. These latter finding might indicate that systemic ketamine dose dependently interrupts the neurotransmission within these two structures and, as a consequence, disrupts the information flow between other interconnected limbic regions such as PFC and NAc, which in turn lead to a profound behavioural effects evoked by higher dosages.