Research report

Effect of ‘chronic’ versus ‘acute’ ketamine administration and its ‘withdrawal’ effect on behavioural alterations in mice: Implications for experimental psychosis

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1. Introduction

Schizophrenia is a chronic debilitating psychiatric disorder affecting as many as 1% of the population worldwide [36]. The key factor that limits research on schizophrenia has been the lack of suitable animal model which mimics the broad clinical symptoms. Various drugs that are being used to model schizophrenia are unable to simulate the broad range of symptoms observed clinically. Earlier, drugs like amphetamine, which modulates the synaptic dopamine release, was used quite successfully in bringing forth the concept of dopamine as one of the prominent players in the pathophysiology of schizophrenia [5]. Though used extensively in modelling psychosis, amphetamine did not induce the so-called negative symptoms of schizophrenia which includes emotional withdrawal and depressive effects [37]. In contrast, NMDA receptor antagonists, such as MK-801 and phencyclidine (PCP), were reported to induce a wider spectrum of behavioural responses in healthy human volunteers that resemble positive, negative, and cognitive schizophrenia-like symptoms [21]. Evidences from pharmacological, post-mortem, clinical and animal studies implicated a significant role of the glutamatergic NMDA receptors in schizophrenia [21,22,32]. Thus, development of a schizophrenic animal model based on the glutamatergic receptor dysfunction is believed to provide a behavioural assay framework that are robust and quantifiable and isomorphic to certain symptoms in humans for investigating mechanisms of action of antipsychotic drugs as well as to understand the neuropsychological basis of the disease.

Ketamine, another non-competitive NMDA receptor antagonist, is known for its strong psychotomimetic effects in humans and rodents [23] and exaggerates psychosis in schizophrenic patients [24]. This drug has also been shown to induce behavioural alterations and impair cognition in rodents [19]. Ketamine, like PCP, has been used in the past to derive schizophrenic animal models. However, the appropriate effective dose, withdrawal effect, and the treatment regimen necessary to reproduce the classical symptoms are still not well-defined in rodents. Owing to its wide availability in the market as a dissociative anaesthetic agent,
use of ketamine would be a cost effective method for simulating particular domains of schizophrenic symptoms in rodents for high-throughput antipsychotic drug screening. From this perspective, we focussed our interest on studying the acute as well as chronic effects of ketamine administration on the development of a psychosis model.

Hence, the aim of the present investigation was to study some key behavioural alterations in mice after acute and chronic ketamine treatment and also to study the effects of typical and atypical antipsychotics in these behavioural measures to validate it. Further, the persistence of behavioural effects after the withdrawal of ketamine was also characterized.

2. Materials and methods

2.1. Drugs

Ketamine (as injectable vials) was purchased from Ranbaxy, India. Clozapine, haloperidol and risperidone and all other compounds were procured from Sigma–Aldrich, UK, unless otherwise specified.

2.2. Animals

Experimental protocols were approved by our Institutional Ethical Committee following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which complies with International norms of Indian National Science Academy (INSA). Male Swiss mice weighing 20–25 g were used in the study. All animals were housed in polyethylene cage in environmentally controlled rooms (temperature 24–27 °C and humidity 60–65% with 12:12 light:dark cycle). Food was provided in the form of dry pellets and water was given ad libitum.

2.3. Treatment schedule and behaviour observations

Clozapine, haloperidol and risperidone, were dissolved in minimal acetic acid and titrated to pH 6.0 using NaOH. Mice were randomly selected and distributed into groups of 8 animals (n = 8).

Behaviour assays selected for the entire study were: (a) hyperlocomotor activity (representing positive symptoms); (b) forced swim test (representing negative symptoms) and (c) passive avoidance test (representing cognitive symptoms) [17,35]. The methods are described below in details.

The experiments were carried out in three phases as follows:

**Phase 1: Acute study:**

(a) In order to identify the optimum dose of ketamine that produces maximum effect on locomotor activity of mice, graded doses of ketamine 50–200 mg/kg were injected intraperitoneally to the animals. Locomotor activity analysis was initially used as an end-point assay to determine the optimum ketamine concentration to be used subsequently for the entire acute phase study. Drug administration was generally done 30 min prior to the subsequent locomotor activity tests for dosage screening.

(b) Graded doses of standard typical antipsychotic drug, haloperidol (0.1–0.5 mg/kg, i.p.) and atypical antipsychotic drugs, clozapine (5–10 mg/kg, i.p.) and risperidone (0.01–0.025 mg/kg, i.p.), were tested along with ketamine, to identify the optimum dose of above drugs which counters the effect of ketamine-induced symptoms. All the drugs to be tested were administered 30 min prior to ketamine injection at the dose effective in locomotor activity test.

(c) Ketamine, at a concentration determined using the above locomotor activity analysis, was administered 24 h prior to the forced swim test. The details of the test are provided below.

(d) In the passive avoidance test for memory dysfunction, ketamine was administered 30 min before the acquisition trial of the passive avoidance test. The effects of this treatment were assessed 24 h later in the retention trial.

**Phase 2: Chronic study:**

The mice were pre-treated with either vehicle or antipsychotic drug 30 min prior to the administration of ketamine (100 mg/kg/day i.p.), for 10 days. As controls, mice were injected with minimal acetic acid solution (vehicle) in parallel. Thus, the eight treatment groups were vehicle + saline (control), vehicle + ketamine (ket), haloperidol (hal); haloperidol + ketamine (Halket), clozapine (Cloz); clozapine + ketamine (Cloket), risperidone (ris), risperidone + ketamine (Risket).

(a) The test for locomotor activity was performed on 1st and 7th day of ketamine treatment. The animals were treated with the effective dose of hal/clo/ris, 30 min prior to ketamine administration and subjected to locomotor observations 30 min later.

(b) The forced swim test was observed 24 h after the 10th day treatments of both ketamine and standard antipsychotic treatments.

(c) Passive avoidance test was carried out on the 9th and 10th day before subjecting the mice to the drug treatment.

**Phase 3: Withdrawal study:**

For the withdrawal studies, ketamine treatments were withdrawn after the 10th day and the mice were subjected to behavioural observations till the 21st day.

(a) The test for locomotor activity was carried out on 14th and 21st day.

(b) Forced swim test for the enhanced immobility was carried out on the 15th and 20th day.

(c) Passive avoidance test for cognitive dysfunction was conducted on 13th and 14th day.

(d) All behaviour observations were performed during 9.00–13.00 h.

2.4. Behavioural assessment

2.4.1. Locomotor activity

Gross open field activity [10,38] was studied using Digiscan Infrared Photo-cell system (Omnitech Electronics, Columbus, OH, USA) in 42 cm × 42 cm × 30 cm plexiglass arena, fitted into infrared beam containing metallic grid. The number of interruptions of the infrared beams along the spatial dimensions of the monitor by the animals was interpreted as horizontal activity and stereotypy counts. Prior to the experiment, both the control and the treated animals were habituated in the experimental cage for 15 min. After the initial habituation period, the activities of the animals were studied for 2 min at 15 min intervals for duration of 1 h.

All cages were connected with a counting module, which counts the number of interruptions. Horizontal activity refers to the total number of beam interruptions that occurred in the horizontal sensor in duration of 2 min.

2.4.2. Forced swimming test

Forced swimming test, as described by Porsolt et al. [34] in mice is a measure of despair behaviour. In brief, mice were placed individually in glass cylinders (20 cm height, 10 cm diameter) containing 10 cm depth of water at 25 °C. After 5 min, the animals were removed from water, dried and returned back to their home cages. They were again placed in the cylinder 24 h later and after the initial 1 min acclimatization period, the total duration of immobility was measured for 5 min. Mice which were floating motionless were considered to be immobile and the duration of swimming was measured by a digital counter.

2.4.3. Passive avoidance test

The mice were subjected to a single trial passive avoidance test as described earlier [8]. Briefly, each experimental mouse was placed in the lighted compartment of an automated shuttle box (Columbus Instruments, OH, USA) controlled by a software program PACS 30. An automated guillotine door separated the light compartment from the dark compartment. After acclimatization period of 30 s in the lighted compartment, the guillotine door was automatically opened. As soon as the animal entered the dark compartment, the door was shut automatically and the subject received a low intensity foot shock (0.5 mA; 10 s). Infrared sensors monitored the transfer of the animal from one compartment to another and were recorded as transfer-latency time (TLT) in seconds. The 1st trial, was conducted for acquisition and the 2nd trial for retention testing. There was a 24 h interval between the 1st trial and the 2nd trial. The learning ability of the animal was determined from an increase in the TLT during the 2nd trial (retention) as compared to the 1st trial (acquisition).

3. Statistical analysis

Data were expressed as mean ± S.E.M. Analysis was performed with STATISTICA version 7.0 software. P < 0.05 was considered to be statistically significant.

4. Results

4.1. Locomotor activity

4.1.1. Effect of graded doses of ketamine on locomotor activity response

In order to identify the most efficacious dose for producing hyperactivity response, a study was performed using graded doses of ketamine (50–200 mg/kg, i.p.) as described in Section 2. A two-factor interaction ‘Treatment × Hours’ was significant (P < 0.001) in a two-way ANOVA followed by Bonferroni post hoc tests. Mice were treated with various doses of ketamine (test group) and saline (control group) at the 15 min time point of the experiment (Fig. 1). A single intraperitoneal administration of ketamine (50 mg/kg), produced a significant increase (∼3.6-fold) in horizontal activity
(HA) at 30 min ($P<0.001$) and 45 min ($P<0.001$) in comparison to the control set. However, the activity declined at 60 min and remained insignificant at all other times points. Ketamine at a dose of 100 mg/kg, i.p. showed a significant ($∼2$–$4$-fold) increase of HA at 30 min ($P<0.001$) and persisted significantly till 90 min. In contrast, the ketamine at the dose of 200 mg/kg showed an initial, insignificant hypolocomotory effect followed by an enhancement of about 1.6-fold HA at 60 min ($P<0.05$), 75 min ($P<0.001$) and 90 min ($P<0.001$). From the above observations, it became apparent that an acute dose of 100 mg/kg ketamine produced the most significant and prolonged effect on the hyperactivity of the animals. Hence, 100 mg/kg was selected as the most effective dose for further experiments.

4.1.2. Effect of graded doses of typical and atypical antipsychotics on ketamine-induced hyperlocomotor activity

In order to verify that the ketamine-induced hyperlocomotor activity is significant and acts through dopaminergic mechanism-based actions as published previously, varied concentration of typical and atypical antipsychotics were used with an expectation that these drugs would block the ketamine induced effect and also, allowing us to determine the optimum concentration to be used in subsequent experiments. Hence, the animals were treated with different concentrations of three neuroleptics—haloperidol, clozapine and risperidone, separately, along with ketamine and the effects on hyperlocomotor activity on these mice were compared with the non-neuroleptic drug treated controls (Fig. 2).

Various concentrations of haloperidol (0.1–0.5 mg/kg, i.p.) were administered along with ketamine (100 mg/kg, i.p.) and the locomotor activity was observed at the 60 min time interval (Fig. 2). As shown in Fig. 2, pre-treatment with haloperidol at a dose of 0.1 mg/kg, i.p. had no effect ($∼2.5$%) on the ketamine induced hyperactivity, whereas 0.25 mg/kg haloperidol showed a about 70% decrease ($P<0.001$) in the horizontal activity counts as compared to the ketamine-only controls. Similar decrease ($∼75$%) of horizontal activity was observed with haloperidol at a higher dose of 0.5 mg/kg ($P<0.001$). Therefore, 0.25 mg/kg of haloperidol was found to be the optimum concentration to block the ketamine-induce hyperlocomotor activity.

Similarly, the effect of clozapine was also studied at two different doses of 5 and 10 mg/kg, i.p. As shown in Fig. 2, the higher dose was found to be more effective (62%) in reducing the ketamine-induced horizontal activity counts ($P<0.001$), as compared to lower dose which showed only 34% protection. On the other hand, risperidone, another atypical antipsychotic drug, showed 40% 0.01 mg/kg ($P<0.05$) and 60% protection at 0.025 mg/kg ($P<0.01$). Thus the higher dose was selected as the optimum dose for studying risperidone mediated effects. The data were analysed by one-way ANOVA followed by Neumann–Keul post hoc test.

Our results indicate that the ketamine (100 mg/kg) induced hyperlocomotor activity and stereotype counts could be blocked by an optimum dose of haloperidol (0.25 mg/kg, i.p.), clozapine (10 mg/kg, i.p.) and (risperidone 0.025 mg/kg, i.p.). These doses were selected for further studies.

4.1.3. Effect of typical and atypical antipsychotics on chronic ketamine administration on locomotor activity

In order to study the chronic behavioural effects of ketamine on the locomotor activity and to verify the induction of behavioural supersensitivity as observed in the case of other NMDA receptor antagonists, we pre-treated the mice with 100 mg/kg ketamine for 10 days and then studied the blocking effects of antipsychotics on ketamine challenged mice.

The three-factor interaction ‘Treatment × Days × Hours’ (Fig. 3) showed a significant difference between control and ketamine groups in the RM-ANOVA. On comparing the mean HA counts of ketamine group with control, it was found that both groups differ at each time point as well as in terms of days, as shown in Fig. 3. Acute treatment with ketamine showed a significant increase of about $∼2$–$4$-fold in the HA counts at 30 min ($P<0.05$), 60 min ($P<0.01$) and 90 min ($P<0.05$). Further, chronic administration of ketamine for 7 days generated a supersensitive ($P<0.01$) hyperactivity response (378–453%) starting at 30 min and persisted till the 90 min. To study the persistence of the drug effects even after drug withdrawal, ketamine challenge was stopped after 10th day as described in Section 2 and the locomotor activity was monitored till the 21st day (i.e. 11 days post-drug). The effect persisted even after 4 days (i.e. day 14 of experiment) of withdrawal and showed a significant difference (56–112%) at 30 min ($P<0.05$), 60 min ($P<0.01$) and 90 min ($P<0.01$). Moreover, 11 days after the drug withdrawal (i.e. day 21 of experiment) the ketamine treated group showed a significant difference ($∼1.5$–$1.6$-fold) in locomotor activity at 90 min ($P<0.01$) in comparison to the control.
The experiment on locomotor activity was further validated using typical and atypical antipsychotics drugs as described below.

(a) Study with haloperidol

To verify whether the blockade of ketamine induced behavioural activity is mediated by dopaminergic receptors, we pre-treated the animals with haloperidol before ketamine administration and observed the behavioural changes. A group with only haloperidol pre-treatment was used to study the per se effects of the drug. As evident from Fig. 3a, the mean values of hal + ket treated group showed a consistent time-dependent reduction in HA counts within the group. In addition, the ketamine group pre-treated with acute dose of haloperidol significantly reduced (59–87%) the ketamine induced increase in HA at 30 min ($P < 0.01$) and 60 min ($P < 0.01$) suggesting a blockade of ketamine induced activity. Similarly, chronic haloperidol treatment also reduced (48–62%) HA counts at 60 min ($P < 0.01$) and 90 min ($P < 0.01$) in comparison to the ketamine group indicating a dopaminergic correlation with development of behavioural supersensitivity. The reduction in activity ($\sim 54\%$) as observed in hal + ket groups persisted even after 4 days of drug withdrawal and showed significant difference at 30 min ($P < 0.05$) and 90 min ($P < 0.05$) when compared to the ketamine groups in post-drug withdrawal period. However, the hal + ket group when compared to the control, did not demonstrate any significant ($P > 0.05$) changes throughout the experimental study, which might signify a restoration of baseline activity in the ketamine induced groups.

Acute haloperidol group of mice showed initial reduction in locomotor activity ($P < 0.01$) when compared to saline treated control but no significant difference in HA counts was observed at later time points with respect to the control, probably due to the blockade of dopaminergic receptors. Since the dose selected for experimental study was low, therefore no significant difference was observed after 7th day chronic treatment of haloperidol. However, during post-withdrawal period, the hal groups showed increased HA counts on 14th day at 60 min ($P < 0.01$) and 90 min ($P < 0.01$) with respect to the control. This increased locomotor activity, however, did not persist till 21st day and remained statistically insignificant at all time points.

(b) Study with clozapine

Similar to our findings in the case of haloperidol, pre-treatment with clozapine (10 mg/kg, i.p.) in the acute studies also showed a significant reduction (43–87%) in HA at 30 min ($P < 0.01$) and 60 min ($P < 0.05$) when compared to ketamine groups on the first day of experiment (Fig. 3b), which is possibly due to its antagonistic activity towards D2 receptor. Our studies further confirm the preventive effect of clozapine pre-treatment on development of behavioural supersensitivity induced by chronic ketamine treatment. The protective effect was significant (24–53%) on the 7-day chronic treatment of clozapine along with ketamine at 30 min ($P < 0.05$) and 90 min ($P < 0.05$) time points. The locomotor reduction (10–49%) was also observed after the withdrawal of drugs on 14th day at 90 min ($P < 0.05$) and also on 21st day (28–58%) at 30 min ($P < 0.05$) and 90 min ($P < 0.01$). However, when compared to control the clo + ket group remained insignificant throughout the experimental study. The effect of clozapine did not show significant difference on HA in both acute and chronically treated group, whereas a significant increase ($\sim 42\%$) in HA was observed after 4 days of clozapine withdrawal at 60 min ($P < 0.01$) and 90 min ($P < 0.01$). Such effects, however, did not persist till the 11th day after withdrawal.
Ketamine could induce immobility in mice similar to PCP [29,30], and to analyse the effect of ketamine on immobility duration, we carried out whether it can also be suppressed by antipsychotics. In order to investigate this, we conducted a study involving chronic treatment of ketamine and its withdrawal, and the effect on depression-like behaviour of animals.

4.2. Forced swim test

We further compared the effects of chronically and acute ketamine administration on depression-like behaviour of animals in a forced swim test [29,30,34]. We wanted to find out whether ketamine could induce immobility in mice similar to PCP [29,30], an indicator of depressive behaviour, particularly anhedonia and whether it can also be suppressed by antipsychotics. In order to analyse the effect of ketamine on immobility duration, we carried out a two-factor interaction 'Treatment versus Days' in the two-way ANOVA, followed by Bonferroni post hoc tests. The results (Fig. 4) revealed that acute administration of ketamine at 100 mg/kg alone or in combination with antipsychotics, did not induce any significant change in the immobility response of mice as compared to the vehicle-treated group. The enhancement of immobility was also not observed after 5 days of chronic ketamine treatment (data not shown). However, repeated ketamine treatment (100 mg/kg/day i.p. for 10 days) significantly (P < 0.01) enhanced the immobility duration (~48.3%) with respect to the control. The effect persisted even after 5 days (~124%, P < 0.001) and 10 days (~111%, P < 0.001) of drug withdrawal.

In order to further validate our model and to verify whether this immobility enhancement is linked to serotonergic hyperactivation, as observed with PCP by others [30], we carried out the studies with ketamine in combination with antipsychotics (Fig. 4) and are described as follows:

(c) Study with risperidone

Similar to the observation on the effect of haloperidol and clozapine above, acute studies with risperidone also showed a significant reduction (~40–68%) in HA at 30 min (P < 0.01) and 60 min (P < 0.05) when compared to the ketamine groups (Fig. 3c). In the chronic studies, the protective effect (~47–57%) of risperidone on behavioural hypersensitivity was also evident as shown in Fig. 3c. The effect was significant at 30 min (P < 0.05) and 90 min (P < 0.05) time points. Similar to the effects of other antipsychotics studied above, significant reduction (~20%) of the locomotor activity was also observed after the withdrawal of drugs (i.e. on 14th day) at 90 min (P < 0.05) and also on 21st day at 30 min (P < 0.05) and at 90 min (P < 0.01). However, when compared to the control, the ris + ket group remained insignificant throughout the experimental study.

The effect of risperidone showed no significant difference on HA in both acute and chronic treated groups. However, a significant increase (~70%) in HA was observed after 4 days of risperidone withdrawal at 60 min (P < 0.01) and 90 min (P < 0.01). But, this effect did not persist till the 11th day of the post-withdrawal period.

Hence, our results above confirm that ketamine treatment induces abnormal locomotor hyperactivity on acute as well as chronic treatment and the effect persists even after 11 days of drug withdrawal. Both, typical as well as atypical antipsychotics, block the ketamine induced activity as well as prevent development of behavioural supersensitivity in mice after chronic treatment. Further, an interesting finding is that antipsychotic drugs alone showed enhanced locomotor activity after withdrawal, which was observed in all classes of antipsychotics.

4.2. Forced swim test

We further compared the effects of chronically and acute ketamine administration on depression-like behaviour of animals in a forced swim test [29,30,34]. We wanted to find out whether ketamine could induce immobility in mice similar to PCP [29,30], an indicator of depressive behaviour, particularly anhedonia and whether it can also be suppressed by antipsychotics. In order to analyse the effect of ketamine on immobility duration, we carried out a two-factor interaction 'Treatment versus Days' in the two-way ANOVA, followed by Bonferroni post hoc tests. The results (Fig. 4) revealed that acute administration of ketamine at 100 mg/kg alone or in combination with antipsychotics, did not induce any significant change in the immobility response of mice as compared to the vehicle treated group. The enhancement of immobility was also not observed after 5 days of chronic ketamine treatment (data not shown). However, repeated ketamine treatment (100 mg/kg/day i.p. for 10 days) significantly (P < 0.01) enhanced the immobility duration (~48.3%) with respect to the control. The effect persisted even after 5 days (~124%, P < 0.001) and 10 days (~111%, P < 0.001) of drug withdrawal.

In order to further validate our model and to verify whether this immobility enhancement is linked to serotonergic hyperactivation, as observed with PCP by others [30], we carried out the studies with ketamine in combination with antipsychotics (Fig. 4) and are described as follows:

(a) Haloperidol: In consistence with the previous findings with PCP [10], haloperidol administration tends to worsen the negative symptoms. Pre-treatment with haloperidol did not ameliorate the ketamine induced enhanced immobility. In contrast the symptoms were further aggravated on 10th day (P < 0.05) and persisted even after withdrawal i.e. on 15th (P < 0.001) as well as 20th day (P < 0.001) when compared with control. Chronic administration of haloperidol, however, had no statistically significant effect in terms of immobility duration when compared to control on the 10th day. But showed a significant increase in the immobility duration after 5 days (P < 0.001) and 10 days (P < 0.001) of the drug withdrawal. This could be due to hyper-activation of serotonergic control over excessive DA release in response to chronic antipsychotic blockade of DA receptors.

(b) Clozapine: Pre-treatment with clozapine (Fig. 4) for 10 days was effective in reducing the ketamine induced enhanced immobility (~28–42%) significantly (P < 0.05) and showed complete restoration of baseline activity after drug withdrawal on 15th (P < 0.001) and 20th day (P < 0.001). In comparison with the control, clo + ket group showed statistically insignificant difference on all days observed. However, chronic treatment of clozapine showed enhanced immobility on the 10th day (P < 0.001) when compared to the ketamine induced group (ket).
to our findings, in the acute trials above, the ketamine treated group showed reduced performance (Fig. 6) as no significant difference was observed in transfer-latency time when compared to the latency time in acquisition trial. The results were similar even in our chronic hal+ket group as haloperidol did not ameliorate the memory deficit induced by ketamine administration. In contrast, clozapine (P < 0.01) and risperidone (P < 0.001) significantly reversed the ketamine-induced deficit in the passive avoidance performance. Clozapine showed ~1.9-fold and risperidone showed 1.7-fold increase in latency time in comparison to ketamine groups as shown in Fig. 6. A significantly (P < 0.001) increased TLT was also observed in mice treated with haloperidol, clozapine and risperidone treated groups confirming that the changes in TLT was not due to the non-specific effects of the antipsychotic drugs alone.

Though, acute and chronic ketamine shows impairment in memory in the passive avoidance task, the deficit did not persist till the 13th and 14th day of the experiment during the post-withdrawal study as the ketamine treated mice showed significantly increased TLT (P < 0.001) in comparison to the acquisition TLT (data not shown).

5. Discussion

Schizophrenia is a heterogeneous chronic disease characterized by severe behavioural perturbations. Our goal was to characterize a ketamine-induced working model in mice with respect to some selected behaviour phenotypes that correlate with certain sections of symptoms observed in schizophrenia. Since there are no consensus animal models available for schizophrenia so far, we revisited the idea of using ketamine as an inducer of psychotic behavioural parameters and attempted to define a relationship between the period of drug administration, effect on the behavioural modulations and the persistence of the drug effect in the post-drug withdrawal period. In this study, we have investigated the effects of chronic ketamine administration in mice using some key behavioural parameters with relevance to schizophrenic disorder and simultaneously compared the consequences with acute treatment of ketamine.Further, we have also studied the persistence of these behavioural abnormalities after ketamine withdrawal to assess the appropriateness of using the model as a drug screening tool.

Our findings in the acute studies with ketamine at various graded doses showed increased locomotor activity and stereotypy counts. Though the degree of hyperactivity induced is nearly compared to the control; however, the effects did not last after the withdrawal of drugs i.e. on 15th and 20th day.

(c) Risperidone: Similar to clozapine, our finding with chronic pre-treatment of risperidone for 10 days (Fig. 4) showed a significant reduction of about 24% (P < 0.05) in the ketamine induced enhanced immobility duration, this protective effect (~28–37%) persisted even after 5 days (P < 0.001) and 10 days (P < 0.001) of the drug withdrawal. The ris+ket group showed statistically insignificant difference on the 10th and 20th day, which implies restoration of the activity to baseline. However, in our chronic risperidone group, we did not find any statistical difference in comparison to control on the 10th day, but a significant difference in immobility duration was observed after drug withdrawal on the 15th (P < 0.001) and 20th day of experiment (P < 0.001) in comparison to control.

From the above experiments, it can be concluded that chronic ketamine administration induces enhanced immobility in mice, similar to PCP, which gets reversed upon administration of clozapine and risperidone, which further confirms the serotonergic involvement in ketamine induced enhanced immobility duration.

4.3. Passive avoidance test

In order to verify the effect of ketamine on the cognitive behaviour of mice, passive avoidance test was selected as a representative behavioural phenotype of the psychotic disorders. In the acute studies (single dose of drug administration), ketamine treated mice were unable to perform in the passive avoidance task and showed 79% deficit in memory retention in comparison to the control (Fig. 5). In control mice, a robust increase of about 4.5-fold (P < 0.001) in the transfer-latency time (TLT) was observed during the retention trial when compared to the acquisition trial. This increase in TLT was obliterated in the ketamine treated group.

Further, upon treatment with haloperidol and clozapine prior to ketamine administration, the mice showed improved latencies (~1.5-fold) in clozapine treated groups but not in haloperidol administered animals (Fig. 5). Since passive avoidance test is based on the animal’s ability to move to the darker chamber, hence it is of utmost importance that the animal should possess sound locomotor activity. To nullify the interference of increased locomotor activity following acute ketamine administration, we carried out passive avoidance experiment in our chronic groups, 24 h after the last injection on the 9th (acquisition trial) and 10th day (retention trial). Similar
the same at all doses, we found a dose dependent effect in the persistence of hyperactivity duration, i.e. the mice treated with the lowest dose (50 mg/kg) was active for a very short duration, roughly 30 minutes, whereas the one treated with 100 mg/kg showed a sustained hyperactivity for more than an hour. A higher dose of 200 mg/kg caused an initial reduction in locomotor activity followed by a gradual rise in the activity after 60 minutes. This increased level of activity was sustained till 90 minutes of the total experimental duration (Fig. 3). The hyperlocomotory activity observed here are believed to be the result of dopamine agonistic action induced by ketamine. However, the effects observed with further higher doses of ketamine (200 mg/kg) which caused initial hypolocomotion followed by locomotor activation, is thought to be synergistically induced by norepinephrine and serotonin pathways as well [16]. However, since 100 mg/kg ketamine treatment showed more consistent hyperactivity in the animals, the dose was selected for further investigation of mice behaviour. Furthermore, the degree of hyperactivity response observed in chronic treatment schedule was significantly greater and more persistent than the response after acute administration. Also, the hyperactivity further got exacerbated when the treatment was prolonged up to 10 days and the mice remained in an active state for 10 days after ketamine withdrawal. As schizophrenia is a chronic psychiatric disorder, there is a need for a chronic model to study the unremitted changes associated with this disorder. The persistence of symptoms even up to 10 days more after withdrawal of the drug (Fig. 3) in our study may enable us to achieve this goal. The day dependent response also showed that after each dose of ketamine administration, the mice developed behavioural super sensitivity (Fig. 3), similar to those reported after repeated PCP administration [10], which was successfully blocked after repeated pre-treatment with both typical and atypical antipsychotic drugs. The mechanism by which ketamine produces this adverse behavioural effects, at least partially, have been attributed to the blockade of NMDA receptors located on inhibitory GABAergic neurons in the limbic and subcortical brain regions [9, 26, 27]. This disinhibitory action has been reported to increase the neuronal activity and excessive glutamate and dopamine release in the limbic striatal regions [9, 12, 13, 25, 26].

An interesting finding here was the persistence of locomotor hyperactivity of mice even after 4 and 10 days of withdrawal of ketamine, which was not observed in antipsychotic pre-treated group. An increase in locomotor activity was also noticed in the group which were treated with antipsychotics only i.e. per se group after 4 days of withdrawal. This phenomenon might be might be attributed to the excessive DA neurotransmitter release during the chronic blockade of dopaminergic receptors by the neuroleptics [2, 31] and due to the compensatory activation of dopaminergic neurons subsequent to receptor blockade [6]. However these hyperactivity effects did not persist for a longer duration.

The enhancement of immobility after chronic administration of PCP has been used previously as a model for the negative symptoms of psychosis, such as flattening of affect and avolition [30]. In this study, we have found that chronic administration of ketamine for 10 days induced enhancement of the immobility duration of mice in the FST paradigm. This effect was not observed after acute or a 5-day sub-chronic treatment. Further, this enhancement of immobility was found to persist for 10 days after the withdrawal of the drug. As expected, the effect of ketamine on enhancing the immobility was found to be attenuated by pre-treatment with clozapine, and risperidone. In contrast, haloperidol failed to block the ketamine-induced symptoms, supporting the hypothesis that typical antipsychotic drugs are ineffective in improving the negative symptoms of psychosis. Our observations, demonstrating exacerbation of negative symptoms of schizophrenic symptomatology, is consistent with previous observations of PCP induced effects in animal models of schizophrenia [29, 30]. Since 5HT-2A blockers could reverse the immobility induced by ketamine, it has been thought to have been mediated by 5-HT2A receptor activation [30]. Further, we have observed that haloperidol per se does not cause any significant changes in immobility duration during chronic treatment but it significantly enhanced the immobility duration after its withdrawal. It may be possible that increased dopaminergic neurotransmission after withdrawal from chronic blockade might activate the serotonergic control and thus hyper activation of serotonergic system to produce enhanced immobility [1]. Further, a recent work implicates a decrease in the expression of myelin/oligodendrocyte related genes in the mouse brain following chronic haloperidol administration but not clozapine administration [28].

Haloperidol treatment has been reported to exacerbate pre-existing negative symptoms and our results support these observations; the severity of these deficits has been correlated with white matter pathology in schizophrenia. The importance of dopamine D2 receptors in the oligodendrocyte function and myelination and its blockade by haloperidol can be a possible cause for exacerbation of negative symptoms of schizophrenia. The failure of clozapine to induce similar changes in myelin/oligodendrocyte related gene expression may be due to its lower affinity for the D2 class of dopamine receptors than haloperidol [28]. Clozapine per se did not show any significant enhancement of immobility after withdrawal. However, risperidone follows a similar pattern of withdrawal as with haloperidol, but the underlying mechanism is not clearly understood. Differences in binding affinities towards D2 receptors between risperidone and clozapine might partially contribute to the differential effects of these two drugs. Also, clozapine has been known to have 5HT-1A agonistic properties whereas risperidone display 5HT-1A antagonistic properties. Hence, this difference in action of clozapine and risperidone, as observed here is probably due to the synergistic effects of divergent cellular pathways and this has also been highlighted by other works as well [9]. Cognitive impairments such as deficits in attention, executive function, working (short-term) memory, and long-term memory, are core symptoms in patients with schizophrenia [15]. Among these, learning and memory impairments are known to be particularly severe [39], and they are suggested to be major determinants of the amount of disability patients with schizophrenia experience in social and occupational functioning and in independent living [14]. The passive avoidance test has been used previously to evaluate the effects of antipsychotic drugs (e.g., haloperidol, olanzapine and clozapine) on learning and memory function in rodents [17, 18, 35]. Our findings also depict the memory impairing properties of ketamine, which has been suggested to be mediated by the activation of dopaminergic systems associated with D2 receptors through facilitating the dopamine release from the presynaptic terminal. The involvement of dopaminergic mechanism in the impairment of passive avoidance performance shown in this study is consistent with several previous reports [3, 11, 17]. Furthermore, it has also been reported that ketamine activates dopaminergic neurons in the nucleus accumbens [20] and that the administration of dopamine into the nucleus accumbens disrupts the acquisition of the passive avoidance task [3]. These findings also support a role for dopaminergic mechanisms in the ketamine-induced disruptive effect. The reversal of ketamine induced memory effects by clozapine and risperidone, and not by haloperidol, in the chronic studies might relate to their affinities towards D2 receptors and 5HT receptors.

Another consideration in the interpretation of our data is the possibility that ketamine might induce behavioural and cognitive effects via other non-NMDA, neurotransmitter systems. However, ketamine’s affinity for the intrachannel site within the NMDA receptor is several-fold higher than its affinities for monoamine transporter sites [40], the sigma receptor [33], the mu opiate recep-
tor [41] and acetylcholinesterase [7]. Moreover, opiate, cholinergic, and monoamine receptor antagonists do not block ketamine and PCP induced behavioural effects [4], providing an indirect evidence that ketamine’s behavioural effects might be mediated by its interaction with the PCP site.

Thus, in the present study, we have demonstrated that ketamine induced chronic model mimics the core behavioural deficits in schizophrenia including the positive, negative and cognitive symptoms and these effects persists even after the drug withdrawal period. The reversal of the positive symptoms by typical and atypical antipsychotics and the negative as well as the cognitive symptoms by atypical antipsychotic drugs only, validate this model. This work also supports ketamine induced chronic paradigm instead of the acute-models, to be a better tool in understanding the molecular mechanisms of psychiatric disorders like schizophrenia. Moreover, these studies contribute towards establishing the relationship between the optimum dosage of ketamine administration and phenotypic end-point assays to ‘fine-tune’ the mice-model, which can be utilized for evaluating novel antipsychotic agents. Further studies with brain neurotransmitters systems in this model may provide an additional insight into the development of the behavioural alterations and may also help in elucidating the mechanism of actions of the drugs in more details.

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