	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

Antipsychotic-like profile of CIQ isomers in animal models of schizophrenia

Pushparaj Gawai[a], Rohit Upadhyay[a], Sukanya G. Gakare[a], Lopmudra Sarode[a], Shashank Dravid[b] and Rajesh R. Ugale[a]

Earlier, we have shown the efficacy of racemic (±) CIQ, a positive allosteric modulator of GluN2C/2D receptor against MK-801 induced impairment of prepulse inhibition as well as working memory. The present study investigated the antipsychotic-like profile of different CIQ (±, +, −) isomers against schizophrenia-like symptoms in series of behavioural animal models like apomorphine climbing, social isolation behaviour and NMDA receptor antagonist MK-801 induced cognitive deficits. Further, we also tested CIQ (±, +, −) isomers in neurodevelopmental model against MK-801induced deficits using open field test, Y-maze test and novel object recognition test. CIQ (±, +, −) isomers decreased climbing behaviour, increased social interaction and improved the MK-801 induced deficits in working memory in Y-maze. Further, CIQ (±, +) but not CIQ (−) improved the recognition memory in novel object recognition test as well as reduced hyperlocomotion and stereotyped behaviour. We conclude that CIQ (±, +) but not CIQ (−) exhibit the significant antipsychotic-like profile. ***Behavioural Pharmacology*** **XXX: 000–000 Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.**

Behavioural Pharmacology 2019, **XXX**:000–000

Keywords: (±) CIQ, (+) CIQ, (−) CIQ GluN2C/2D, MK-801, neurodevelopmental model, NMDA hypofunction, schizophrenia

[a]Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India and [b]Department of Pharmacology, Creighton University, Omaha, Nebraska, USA

Correspondence to Rajesh R. Ugale, PhD, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur 440033, India
E-mail: ugale.rajesh@gmail.com

Received 17 July 2018 **Accepted** 25 October 2019

Introduction

Schizophrenia (SZ) is characterised by a complex array of positive symptoms (hallucinations and delusional behaviours), negative symptoms (anhedonia, social withdrawal and apathy) and cognitive dysfunctions (diminished capacity for learning, memory and executive functions) (Cioffi, 2013). It is now well established that SZ involves the hyperdopaminergic ~~and/or~~ hypoglutamatergic transmission (Tanaka *et al.*, 2006). In rodents, systemic administration of N-methyl-D-aspartate receptor (NMDAR) blockers, such as ketamine, phencyclidine and MK-801 produces behavioural hyperactivity, stereotypy, impairments in spatial memory, sensorimotor gating, etc. (Ellison, 1995). Perinatal NMDAR antagonist treatment in rodents is useful model to study SZ, since it is based on both the neurodevelopmental (Kirch, 1993; McGrath *et al.*, 2003; Rapoport *et al.*, 2005; Rapoport *et al.*, 2012) and NMDAR hypofunctioning (Javitt and Zukin, 1991; Krystal *et al.*, 1994) hypotheses of the disease. It has been reported that NMDAR antagonist causes long-term behavioural and neurochemical alterations that are relevant to the symptoms of SZ (du Bois and Huang, 2007). Report suggests that MK-801 impairs cognitive flexibility and working memory in rat pups resembling cognitive disturbance relevant to neurodevelopmental symptoms of SZ (Stefani and Moghaddam, 2005).

Moreover, NMDA receptor antagonists MK-801, ketamine, and dextromethorphan also reduce the apomorphine-induced climbing behaviour (a positive symptom in SZ) (Kim *et al.*, 1996; Lisman *et al.*, 2008). On the other hand, social interaction test is used to assess the negative symptoms of SZ (Geyer and Moghaddam, 2002; Mouri *et al.*, 2007). Reports suggest that imbalance in the functions of D2 receptors and NMDA glutamate receptors within the striatum play a major role in the pathophysiology of SZ (Carlsson and Carlsson, 1990; Starr, 1995; Laruelle *et al.*, 2003). NMDARs are densely present in striatum and localized on both presynaptic dopamine (DA) terminals as well as γ-aminobutyric acid (GABA) interneurons and found to inhibit presynaptic DA release through local feedback regulation (Wu and Parent, 2000). Dysfunction or blockade of NMDARs particularly on GABA interneurons is shown to produce DA hyperactivity similar to that observed in SZ (Balu, 2016). Further, it has been postulated that glutamatergic neurotransmission may modulate DA function at the postsynaptic level (Svensson *et al.*, 2003). On the other hand, the alterations in the expression of NR2 subunits in the prefrontal cortex is considered as potential indicator of deficits in NMDAR-mediated neurotransmission associated with hypofunction of the frontal lobes in schizophrenics (Akbarian *et al.*, 1996). Reports also suggest that GluN2D subunit is involved in enhanced DAergic transmission and increase in locomotor activity (Hagino *et al.*, 2010; Yamamoto *et al.*, 2015). Animal studies including knockouts suggest that GluN2C-containing receptors may have preferential involvement in working memory and

DOI: 10.1097/FBP.0000000000000532

<zdoi;10.1097/FBP.0000000000000532>

	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

associative contextual fear working memory and other SZ-related behaviours (Hillman *et al.*, 2011; Hillman, 2012; Khlestova *et al.*, 2016).

A novel structural class of NMDAR GluN2C/2D potentiator, CIQ exist in three isoforms such as, (±) CIQ (3-Chlorophenyl) [3,4-dihydro-6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-2(1*H*)-isoquinolinyl] methanone, (+) CIQ:(*R*)-(3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (*2-R*) and (–) CIQ: (S)-(3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (*2-S*) (Mullasseril *et al.*, 2010; Santangelo Freel *et al.*, 2014). CIQ selectively potentiate GluN2C/N2D containing NMDA receptors expressed in Xenopus laevis oocytes, but not through other NMDA receptor subtypes or AMPA or kainate receptors (Mullasseril *et al.*, 2010). Further, (±) and (+) CIQ exhibits higher potency as compared to (–) CIQ towards GluN2C/2D receptors, as observed in BHK cells as well as oocytes (Santangelo Freel *et al.*, 2014). CIQ modestly inhibits nicotinic receptor and exhibit affinity towards κ-opioid receptor, 5-hydroxytryptamine (5-HT) 2A, 5-HT2B, 5-HT6 and norepinephrine transporter. However, the functional effect of CIQ binding with these targets remains unknown. Our previous study has shown that systemic administration of (±) CIQ isomer prevent MK-801-induced deficit in prepulse inhibition (PPI) and partially attenuated MK-801- and methamphetamine-induced hyperlocomotion and stereotyped behaviours. Moreover, (±)CIQ also reversed the MK-801-induced working memory deficit in Y-maze. These results indicate the antipsychotic-like profile of (±) CIQ isomer at least against positive and cognitive symptoms (Suryavanshi *et al.*, 2014).

Therefore, in the present study, we observed the effects of different isomers of CIQ (i.e. ±, +, –) in series of behavioural animal models of SZ. We have included apomorphine-induced climbing behaviour to mimic positive symptoms of SZ. Social isolation paradigm was used to produce the negative symptoms, whereas cognitive symptoms were observed by MK-801 induced hyperlocomotion and stereotypy as well as deficits in working and recognition memory. In addition, CIQ (i.e. ±,+, –) isomers were also tested using neurodevelopmental model.

Material and methods

Animals and housing

Male Swiss albino mice (25–30 g; 5–6 weeks) were housed under controlled room temperature (25 ± 2°C) and maintained at 12:12 hours light/dark cycle (light on at 07:00 hours).

Food and water were available *ad libitum*. Animals were procured from National Institute of Nutrition, Hyderabad, India. Experimental procedures were approved by the Institutional Animal Ethical Committee (Sanction letter No: IAEC/UDPS/2014/9; IAEC/UDPS/2015/12) and executed strictly according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

Drug solutions and administrations

CIQ (±, +, –) isomers were generously gifted by Prof. S. Dravid, Creighton University, Omaha, NE, USA. Vehicle for intracerebroventricular (*icv*) administration of CIQ isomers was prepared in 1% (v/v) DMSO and 99% (v/v) PEG-400. Apomorphine was procured from Tocris Biosciences, New Delhi (India), and dissolved in saline containing 0.1% ascorbic acid just before the experiment and admininstered via subcutaneous (*s.c.*) route. MK-801 hydrogen maleate (M107; Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in sterile isotonic saline and administered via intraperitoneal (*i.p.*) injection. Doses of CIQ (±, +, –) isomers were selected based on the earlier studies performed in our lab.

Intracerebroventricular cannula implantation surgery

Mice were anaesthetised with thiopentone sodium, 45 mg/kg, *i.p.* and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, California, USA). A guide cannula prepared in-house was stereotaxically implanted into the right lateral ventricle as per the coordinates, –0.8 mm posterior, +1 mm midline to lateral and 2.3 mm ventral with respect to bregma (Paxinos and Franklin, 2001) and secured into the skull using mounting screws and dental cement (DPI-RR Cold Cure, acrylic powder, Dental Product of India, Mumbai, India). A stainless steel dummy cannula was used to occlude the guide cannula, when not in use. The animals were then allowed to recover for a week following surgery and daily treated with 0.2 ml of the antibiotic Sodium Cefotaxime (262 mg/ml, *i.p.*). Drug infusion was performed using a Hamilton syringe connected to an internal cannula (30 gauge) by polyethylene tubing and a volume of 1 µl was administered over a period of 2 minutes into the right lateral ventricle. The injection cannula was left in place for about 1 minute before being slowly withdrawn to avoid backflow. Dilute India ink was injected (2 µl, *icv*) at the end of an experiment. Animals were euthanized with pentobarbitone (60 mg/kg, *i.p.*) overdose and immediately brains were dissected to assure the cannula placement. Data of animals showing a uniform distribution of ink into lateral ventricles were used for statistical analysis.

Apomorphine-induced climbing behaviour

Apomorphine induces a peculiar climbing behaviour characterized by initial rearing and then full-climbing activity in mice (Costall *et al.*, 1978). The ability of a drug to antagonize apomorphine-induced climbing behaviour in the mouse has been correlated with neuroleptic activity. Mouse was placed individually in cylindrical wire mesh cage (height 13 cm, diameter 14 cm, mesh size 3 mm)

	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

for 60 minutes to acclimatize with the new environment. The climbing behaviour was scored at 5 minutes intervals for a period of 20 minutes as follows: 0 = four paws on the floor, 1 = one paw on the wall of cage, 2 = two paws on the wall of cage, 3 = three paws on the wall of cage, 4 = four paws on the wall of cage. Climbing scores across each time interval were then summed and expressed as cumulative climbing index, thus providing a maximum possible climbing index of 20 (Ugale *et al.*, 2004).

Locomotor activity and stereotypy

Locomotor activity was recorded in a 25.4 × 25.4 cm open field test (OFT) area made of acrylic white wall and divided into 24 sections (V.J. Instruments, India). For the OFT, mouse was placed in the center of the arena and number of square crossed by the four paws (locomotor activity) was recorded manually for 10 minutes (Taksande *et al.*, 2009). The arena was cleaned with 70% alcohol to eliminate the odour. The following behaviours were adjudged as stereotype behaviour, that is, head swings, 360° circular turn, rearing or wall leaping, rapid circular movements, digging and grooming (Suryavanshi *et al.*, 2014). Stereotype behaviour was recorded followed by locomotor activity in the same animal groups.

Social isolation-induced behaviour

Three-week-old mice were randomly divided into two groups: socially isolated (SI) and group-housed (GH). Mice in the SI group were individually housed in wire-topped opaque polypropylene cages (20 × 12 × 10 cm), while mice in the GH group continued to be housed under normal conditions (five per cage) in wire-topped clear plastic cages (34 × 22 × 15 cm). Mice were subjected to social interaction test after 4 weeks of social isolation. Social interaction test was carried out to investigate the habituation response to a novel mouse. A male resident mouse was housed alone in a home cage (34 × 22 × 15 cm high) for 2 days before test and then a novel male mouse was introduced into the cage for 5 minutes per trial. Test mouse was exposed to the same novel mouse in four trials with an inter-trial interval of 30 minutes. The time spent during social interaction (close following, inspection, anogenital sniffing and other social body contact) was calculated (Koike *et al.*, 2009).

Spontaneous alternation in Y-maze

A custom made Y-maze with three identical wood arms (40 × 4.5 × 12 cm, 120° apart) was placed at the centre of a room under dim light condition. The walls of each arm had a distinct design to provide visual cues. Each mouse was placed at the end of one arm facing the center and allowed to explore the maze for 8 minutes. Arm entries were scored manually. A mouse was excluded from analysis, if it did not have new entries for a period of 2 minutes or had less than 12 arm entries during the 8 minutes procedure. The arena was cleaned with 70% alcohol to eliminate the odour. Successful alternation was defined as a visit to an arm that has not been visited during the previous two-arm entries. Procedure for spontaneous alternation in the Y-maze was performed as previously described (Suryavanshi *et al.*, 2014). Percent alternation was calculated as

$$\%\ \text{Alternation} = \frac{(\text{Number of successful alternations})}{\text{Total arm entries} - 2} \times 100$$

Novel object recognition

Novel object recognition (NOR) test was performed in a square open field (25.4 × 25.4 × 17.8 cm). Short-term memory (STM) and long-term memory (LTM) were assessed in mice as previously described (Hillman *et al.*, 2011). The NOR task was performed in three phases: habituation, training and testing. During acclimation, mice were handled 1–2 minutes a day for 3 days. On days 2 and 3 of handling, mouse was placed in the experimental apparatus for 10 minutes to acclimate with the environment. On days 4 and 5 of training, mouse was placed in the chamber with two identical objects and allowed to explore for 10 minutes. On day 5, 30 minutes after training, a familiar object was exchanged with a novel object and mouse was allowed to explore the experimental apparatus for 10 minutes. Time spent by the animal near the novel and familiar objects within a 1 cm radius was recorded to assess the STM. Location of the novel object was counter balanced with half of the animals in each group exposed to the novel object on the left side of the chamber and the other half exposed to the right side of the chamber. For LTM assessment, a second novel object was presented 24 hours after training and location of the novel object was counter balanced in the chamber. All objects were cleaned with 70% ethanol between trials to eliminate potential olfactory cues or preference for each object. Relative exploration time was recorded and expressed as a discrimination index (DI).

$$\text{DI} = \left[\left(\begin{array}{l} \text{time spent with novel object} - \\ \text{time spent with familiar object} \end{array} \right) / \left(\begin{array}{l} \text{time spent with novel object} + \\ \text{time spent with familiar object} \end{array} \right) \right] \times 100$$

Experimental design

Apomorphine induced climbing behaviour in mice

Mice (n = 8–10) were treated with either saline or apomorphine (1 mg/kg, *s.c.*) and placed back in the home cage. After 5 minutes, they were administered with vehicle or (±)/(+)/(−) CIQ via *icv* route (20 or 40 mM/mice). These animals were assessed in circular mesh cage after 15 minutes of CIQ administration.

Hyperlocomotion and stereotypy

Mice (n = 8–10) were treated with either saline or MK-801 (0.15 mg/kg, *i.p.*) 15 minutes before vehicle or

	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

(±)/(+)/(−) CIQ (10 and 20 mM/mice, *icv*) administration to achieve systemic level. After 15 minutes of vehicle or CIQ administration, mice were assessed in OFT for hyperlocomotion and stereotypy behaviour was recorded manually for 10 minutes. The following behaviours were adjudged as stereotype behaviour, that is, head swings, 360° circular turn, rearing or wall leaping, rapid circular movements, digging and grooming. Stereotype behaviour was recorded followed by number of square crossed by the four paws (locomotor activity) in the same animal groups.

Social isolation induced behaviour

The Swiss albino mice (n = 8–10) were SI for 4 weeks. The social interaction was observed after 15 minutes of vehicle or (±)/(+)/(−) CIQ (20 mM/mice) *icv* administration.

Y-maze test

Mice (n = 8–10) were treated either with saline or MK-801 (0.15 mg/kg, *i.p.*) and placed back in the home cage. Mice were then administered with vehicle or (±)/(+)/(−) CIQ via *icv* route (10 or 20 mM/mice) after 15 minutes and placed back in the home cage. These animals were assessed for spontaneous alteration in Y-maze after 15 minutes of CIQ administration.

Novel object recognition test

Groups (n = 8–10) of mice were treated with saline or MK-801 (0.15 mg/kg, *i.p.*) 15 minutes followed by vehicle or (±)/(+)/(−) CIQ (10 and 20 mM/mice, *icv*). NOR test was performed in three phases: habituation, training and testing. STM was assessed after 15 minutes of CIQ injection, whereas LTM was assessed after 24 hours of treatment as mentioned in the procedure and location of the novel object was counter-balanced in the chamber in both STM and LTM.

Neurodevelopmental model

Effect of CIQ (±, +, −, icv) on neonatal MK-801 treated mice

Separate groups (n = 8–10) of mice pups were treated with saline or MK-801 (0.25 mg/kg, *s.c.*) on postnatal day (PD) 5 to PD10. Behavioural assessments were carried out at young stage (65th day) after 15 minutes of vehicle or (±)/(+)/(−) CIQ (10 or 20 mM/mice) *icv* administration. Locomotion and stereotypy were assessed in OFT whereas, working memory (Y-maze) and recognition memory (NOR) were observed in different groups.

Data analysis

The results were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The data of mice weight were analysed with two-way ANOVA followed by post-hoc Bonferroni's multiple comparison test. The significance level of $P < 0.05$ was considered statistically significant. All data are presented as mean ± SEM.

Results

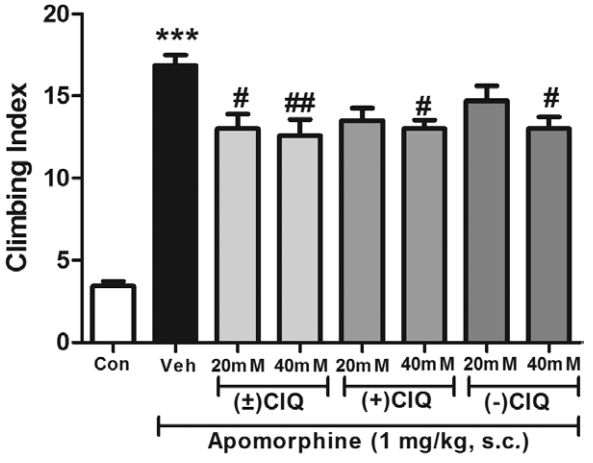
CIQ (±, +, −) decreased the apomorphine-induced climbing behaviour in mice

One way ANOVA showed significant effect of CIQ treatment on apomorphine-induced climbing behaviour [$F(7, 54) = 27.96$, $P < 0.0001$, n = 8–10 per group; Fig. 1). Post-hoc test revealed that apomorphine (1 mg/kg, *s.c.*) significantly increased the climbing index as compared to the control group ($P < 0.001$). On the contrary, effect of apomorphine was significantly attenuated by both the doses (i.e. higher dose 40 mM) of (±) CIQ ($P < 0.01$), (+) and (−) CIQ ($P < 0.05$ each) as well as lower dose (20 mM) of (±) CIQ ($P < 0.05$). As shown in Table 1, (±), (+), (−) CIQ alone (in present doses) did not produce any effect ($P > 0.05$). F1 T1

CIQ (± and +) attenuated the MK-801 induced hyperlocomotion and stereotyped behaviours in open field test

CIQ (± and +) administration had significant effect on MK-801 (0.15 mg/kg, *i.p.*) induced hyperlocomotion [$F(7, 79) = 36.68$, $P < 0.0001$, n = 8–10 per group; Fig. 2a] and stereotyped behaviours [$F(7, 79) = 9.22$, $P < 0.0001$, n = 8–10 per group; Fig. 2b]. Post-hoc test suggest MK-801 induced significant increase in locomotion ($P < 0.01$) and stereotyped behaviour ($P < 0.001$). (+) CIQ at 10 as well as 20 mM ($P < 0.001$ each) significantly prevented the MK-801 induced hyperlocomotion. Similarly, (±) CIQ at 20 mM ($P < 0.05$) but not at 10 mM ($P > 0.05$) attenuated the MK-801 induced hyperlocomotion and stereotype behaviour. (+) CIQ at 10 as well as 20 mM ($P < 0.05$ and $P < 0.01$, respectively) significantly prevented the MK-801 induced stereotypy. However, (−) CIQ does not show any significant effect at 10 or 20 mM doses F2

Fig. 1



Dose related effect of CIQ isomers (±,+, −) on apomorphine induced climbing behaviour in mice. Each bar represents mean ± SEM of climbing index (n = 8–10 per group). ***P < 0.001 vs. control, #P < 0.05, ##P < 0.01 vs. veh + apomorphine treated group.

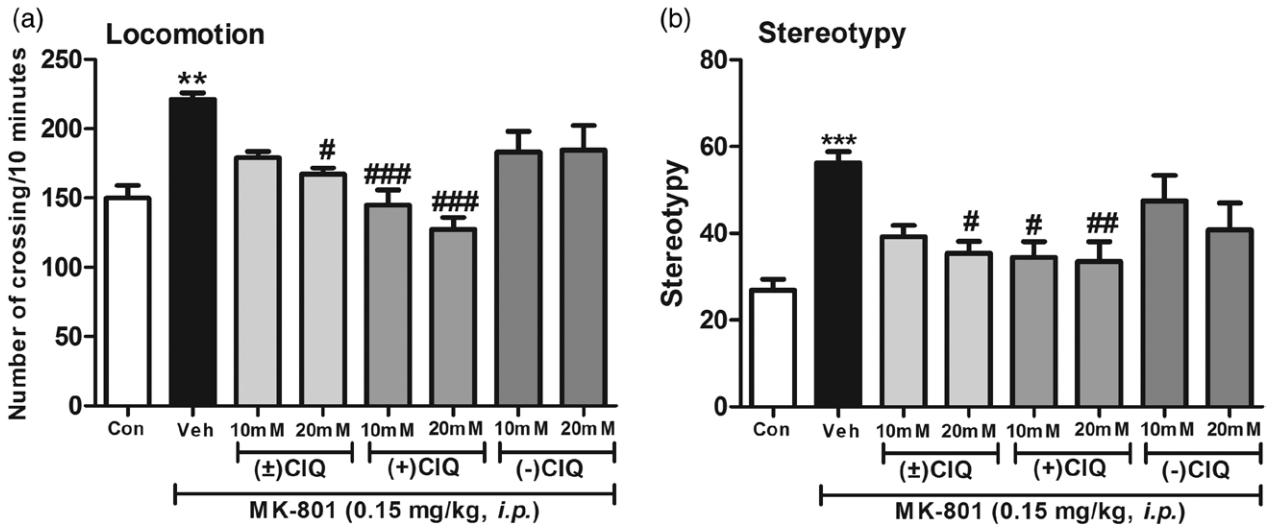
	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

Table 1 Dose-dependent effect of CIQ (±, +, –, *icv*) isomers on positive and cognitive symptoms of schizophrenia in acute and neurodevelopmental study

Treatment and doses	Control	(±)CIQ 20 mM	(±)CIQ 40 mM	(+)CIQ 20 mM	(+)CIQ 40 mM	(–)CIQ 20 mM	(–)CIQ 40 mM
Climbing index	3.4 ± 0.4	3.2 ± 0.86	4.4 ± 1.02	3.8 ± 0.96	3.6 ± 0.74	3.5 ± 0.85	3.9 ± 0.36
Treatment and doses	Control	(±)CIQ 10 mM	(±)CIQ 20 mM	(+)CIQ 10 mM	(+)CIQ 20 mM	(–)CIQ 10 mM	(–)CIQ 20 mM
Locomotion	165 ± 8.50	160 ± 9.66	174.5 ± 6.66	178.5 ± 5.33	171.5 ± 5.52	173.75 ± 9.04	156.75 ± 4.05
Stereotypy	30.66 ± 1.60	31.5±3.26	33.33 ± 2.33	32.75 ± 7.69	31.5 ± 1.43	33.25 ± 2.76	33.75 ± 3.23
% alternations (Y-maze)	67.83 ± 4.10	66.52 ± 2.32	65.66 ± 2.29	72.99 ± 1.87	74.12 ± 2.79	71.33 ± 0.70	72.19 ± 0.27
STM	15.07± 2.49	16.77 ± 1.79	16.04 ± 3.44	14.95 ± 2.30	16.3 ± 1.57	19.89 ± 2.08	15.32 ± 2.69
LTM	18.61 ± 4.03	19.51 ± 4.01	19.81 ± 3.04	17.92 ± 2.16	18.32 ± 3.75	19.19 ± 1.93	18.73 ± 5.37
Neurodevelopmental study							
Locomotion	138.5 ± 6.64	140.5 ± 6.95	135.5 ± 5.92	168 ± 8.89	164.4 ± 3.28	173.2 ± 7.99	169 ± 10.08
Stereotypy	53.5 ± 3.56	57.33 ± 3.92	52.5 ± 3.92	52.8 ± 5.51	49.6 ± 3.96	19.75 ± 1.27	22 ± 4.03
% alternations	73.83 ± 3.40	70 ± 4.54	72.5 ± 2.26	72.99 ± 1.87	74.12 ± 2.79	71.33 ± 0.70	72.19 ± 0.27
STM	39.52 ± 3.07	37.6 ± 2.3	38.01 ± 3.35	44.97 ± 4.25	45.22 ± 3.32	39.89 ± 2.08	40.32 ± 2.22
LTM	41.26 ± 2.45	36.57 ± 2.66	39.28 ± 4.71	38.18 ± 3.14	37.92 ± 4.58	37.19 ± 1.93	40.71 ± 3.54

LTM, long term memory; STM, short term memory.

Fig. 2



Effect of CIQ isomers (±, +, –) on MK-801 induced hyperlocomotion (a) and stereotypy behaviours (b). Each bar represents mean ± SEM of number of crossing (a) and stereotypy (b) (n = 8–10 per group). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. veh+MK-801.

in hyperlocomotion and stereotyped behaviour. Further, (±), (+) and (–) CIQ alone in the above doses did not altered locomotion or stereotype behaviour ($P > 0.05$; Table 1).

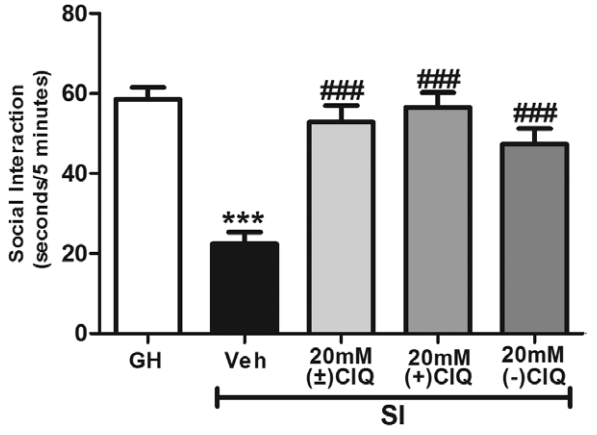
CIQ (±, +, –) isomers improved the social interaction in socially isolated mice

Social isolation of mice for 4 weeks induced social withdrawal in mice. One-way ANOVA showed significant effect of CIQ (±, +, –) treatment on the social interaction [F (4, 34) = 17.26, $P < 0.0001$, n = 8–10 per group; F3 Fig. 3]. Post-hoc test revealed that social interaction was decreased ($P < 0.001$) in SI mice as compared to GH mice. CIQ isomers (±, +, –) at 20 mM significantly increased social interaction time in SI mice ($P < 0.001$ each) as compared to vehicle-treated SI mice.

CIQ (±, +, –) improved the MK-801 induced working memory deficit in Y-maze test

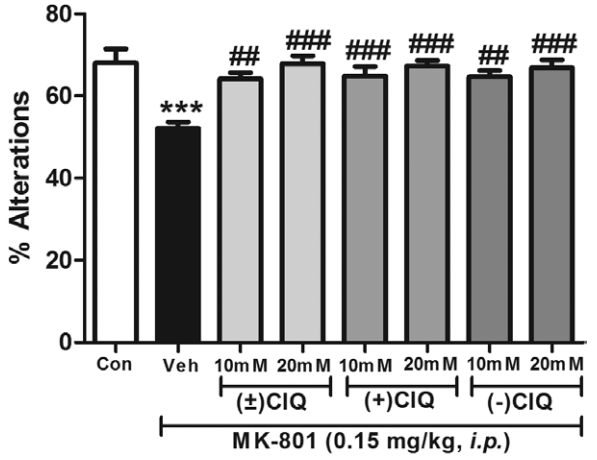
CIQ (±, +, –) isomers (10 and 20 mM/mice, *icv*) attenuated MK-801 (0.15 mg/kg, *i.p.*) induced deficit in the Y-maze spontaneous alternation test (one-way ANOVA, $F(7, 79) = 6.62$, $P < 0.001$, n = 8–10 per group; Fig. 4). F4 Post-hoc Bonferroni's multiple comparison test reveals that, MK-801 (0.15 mg/kg, *i.p.*) significantly reduced spontaneous alternation in mice as compared to vehicle-treated group ($P < 0.001$). Further, (±), (+), (–) CIQ at 20 mM ($P < 0.001$ each) and 10 mM ($P < 0.01$, $P < 0.001$, $P < 0.01$, respectively) significantly reduced the MK-801-induced deficit in spontaneous alternation in the Y-maze test. However, (±), (+) and (–) CIQ alone in the above doses did not alter spontaneous alternation as compared to control group ($P > 0.05$; Table 1).

Fig. 3



Effect of CIQ isomers (±, +, – 20 mM) on social interaction in socially isolated mice. Each bar represents mean ± SEM of social interaction (n = 8–10 per group). ***$P < 0.001$ vs. GH, ###$P < 0.001$ vs. respective SI. GH, group-housed mice; SI, socially isolated mice.

Fig. 4



CIQ isomers (±, +, –) improved the MK-801-induced deficit in working memory. Each bar represents mean ± SEM of % alternations (n = 8–10 per group). ***$P < 0.001$ vs. control, ##$P < 0.01$, ###$P < 0.001$ vs. veh + MK-801.

CIQ (± and +) improved the MK-801 induced deficit in recognition memory (A: short-term memory, B: long-term memory) in novel object recognition test

CIQ (±, +) at 10 and 20 mM/mice, *icv* significantly prevented the MK-801 (0.15 mg/kg, *i.p.*) induced deficit in STM as well as LTM in NOR test. One-way ANOVA showed significant effect of CIQ treatment on DI in STM and LTM [STM, $F(7, 63) = 8$, $P < 0.0001$; Fig. 5a and LTM, $F(7, 63) = 15.63$, $P < 0.0001$, n = 8–10 per group; Fig. 5b]. MK-801 significantly reduced DI in STM ($P < 0.001$) and LTM ($P < 0.001$) as compared to the respective control group. We found that, (+) CIQ ($P < 0.001$) as well as (±) CIQ ($P < 0.01$) significantly prevented the MK-801 induced deficit in STM at both 10 and 20 mM doses. Similarly, (+) CIQ isomer at 10 and 20 mM ($P < 0.001$ each) and (±) CIQ isomer at 10 mM ($P < 0.05$) and 20 mM ($P < 0.01$) prevented the MK-801 induced deficit in LTM in the NOR test. We also observed that (+) CIQ 10 as well as 20 mM showed significant difference ($P < 0.001$ each) than (±) CIQ respective dose treated group both in STM as well as LTM. However, (–) CIQ did not show any significant effect at both doses. (±), (+) and (–) CIQ alone in present doses did not affected recognition memory ($P > 0.05$; Table 1).

Neurodevelopmental study

Effect of neonatal exposure of MK-801 on body weight

The somatic growth of the each animal was evaluated by measuring body weight weekly until the behavioural study has been performed. The application of two-way ANOVA showed significant interaction between the treatment and time period [$F(8,72) = 5.982$, $P < 0.000$; Fig. 6]. Statistical analysis revealed a main effect of MK-801 treatment [$F(1,72) = 200.7$, $P < 0.0001$] and time period [$F(8,72) = 90.03$, $P < 0.0001$] on body weight. The post-hoc Bonferroni's multiple comparison test revealed that the neonatal administration of mice with MK-801 (0.25 mg/kg, *s.c.*) from PD5 to PD10 resulted in a significant decrease in body weight on second week ($P < 0.05$), third week ($P < 0.01$), fourth week ($P < 0.05$) and fifth week onwards ($P < 0.001$) as compared to control mice.

CIQ (±,+) prevents hyperlocomotion and stereotyped behaviour induced by neonatal exposure of MK-801 in mice

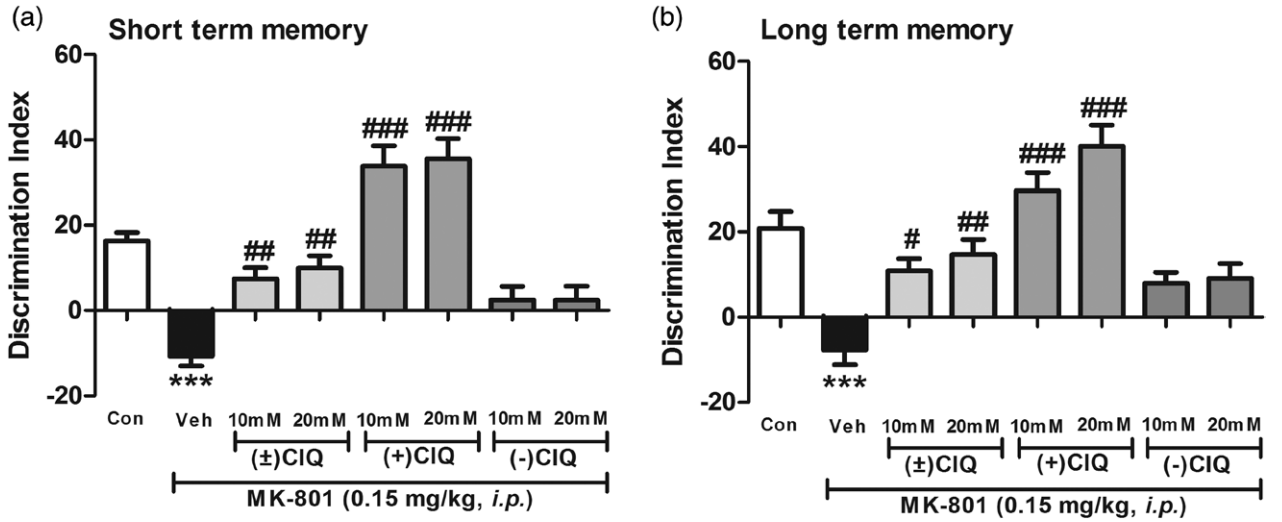
Similar to above results, CIQ (±, +) prevents the MK-801 induced hyperlocomotion and stereotyped behaviour as well in neurodevelopmental model. Pups were administered with MK-801 (0.25 mg/kg, *s.c.*) from PD5–10. Locomotion and stereotype behaviour was observed in young mice (65th day) in OFT for 10 minutes. One-way ANOVA showed significant effect of CIQ (±, +) treatment on the locomotion [$F(7, 62) = 19.65$, $P < 0.0001$, Fig. 7a] and stereotypy [$F(7, 62) = 40.10$, $P < 0.0001$, Fig. 7b]. Post-hoc Bonferroni's multiple comparison test revealed that neonatal mice when exposed to MK-801, shows significant increase in locomotion and stereotyped behaviour ($P < 0.001$). CIQ (±, +) but not CIQ (–) ($P > 0.05$) significantly decreased locomotion at 10 mM ($P < 0.01$, $P < 0.001$, respectively) and 20 mM ($P < 0.001$, $P < 0.001$, respectively) as well as reduced stereotyped behaviour at 10 mM ($P < 0.001$ each) and 20 mM ($P < 0.001$ each). However, the effects of (±), (+), (–) CIQ alone on locomotion and stereotype behaviour in present doses were not different from the control group ($P > 0.05$; Table 1).

CIQ (±, +, –) improved deficit in working memory induced by neonatal exposure of MK-801 in Y-maze

Application of one-way ANOVA showed that all the CIQ (±, +, –) isomers prevented MK-801 induced deficits in

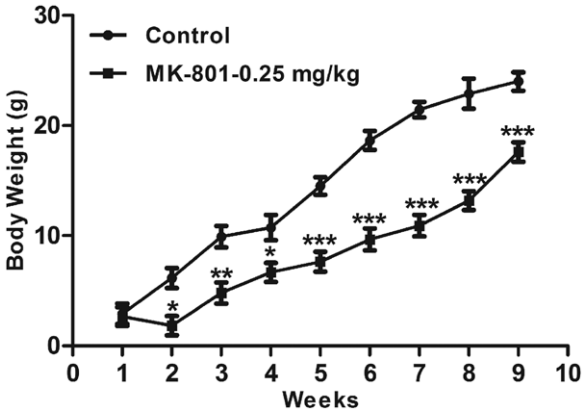
	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

Fig. 5



CIQ isomers (± and +) reversed MK-801 induced deficits in short term memory (STM, a) as well as long term memory (LTM, b) in novel object recognition (NOR) test. Each bar represents mean ± SEM of discrimination index (n = 8–10 per group). ***$P < 0.001$ vs. vehicle, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. veh + MK-801.

Fig. 6



Age dependent effect of MK-801 on body weight: Pups were pre-treated with either control (saline) or MK-801 (0.25 mg/kg/day, s.c.) from PD5 to PD10 and weight taken weekly up to behavioural study. Each line represents the mean ± SEM (n = 8–10 per group). *$P <$ 0.05, **$P < 0.01$, ***$P < 0.001$ vs. control.

working memory [$F(7, 63) = 4.987$, $P < 0.001$, n = 8–10 per group; Fig. 8]. MK-801 treatment showed significant deficit in working memory (decreased spontaneous alteration) in Y-maze test ($P < 0.001$), whereas, all the isomers at 10 and 20 mM, that is, CIQ (±) ($P < 0.05$ and $P < 0.01$, respectively), (+) CIQ ($P < 0.01$ and $P < 0.001$, respectively) and also CIQ (–) isomer ($P < 0.05$ each) significantly improved the MK-801 induced deficits in working memory. (±), (+) and (–) CIQ alone in present doses did not showed any effect on working memory ($P > 0.05$; Table 1).

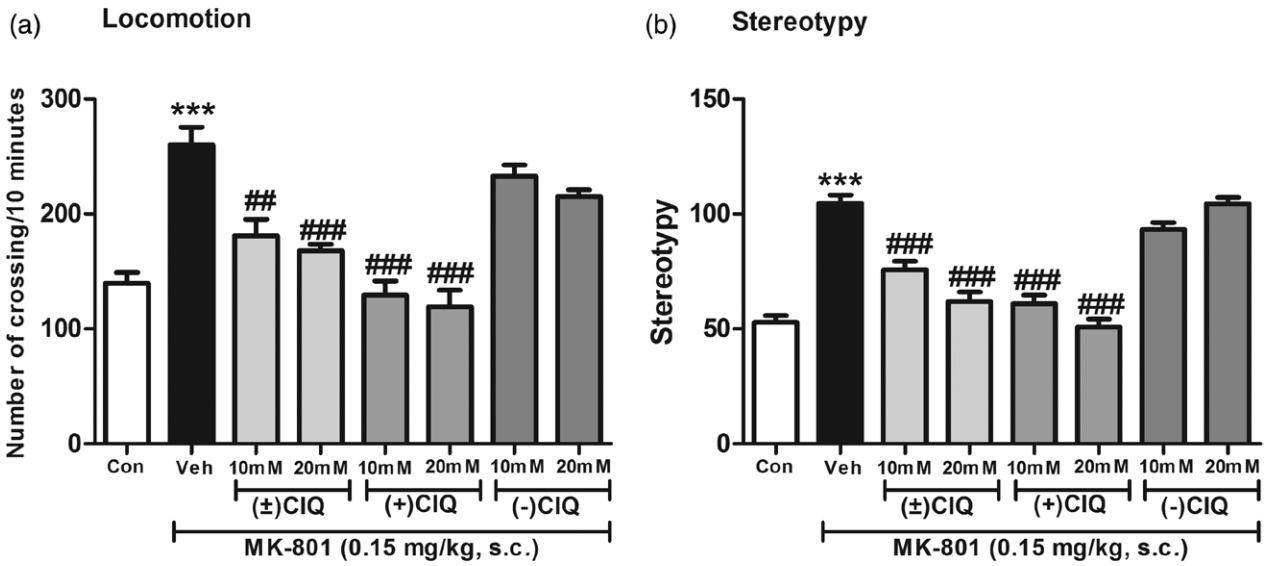
CIQ (± and +) improved deficits in recognition memory induced by neonatal exposure of MK-801 in novel object recognition

CIQ (± and +) but not CIQ (–) significantly reduced the neonatal MK-801 induced deficit in STM and LTM at 10 and 20 mM, *icv*. One-way ANOVA revealed the main effect of CIQ (± and +) treatment on DI in STM and LTM [STM: $F(7, 55) = 16.34$, $P < 0.0001$; Fig. 9a and LTM: $F(7, 55) = 29.59$, $P < 0.001$, n = 8–10 per group; Fig. 9b). Post-hoc test revealed that, MK-801 significantly reduced exploration time to novel object as compared to familiar object (decreased DI) in LTM as well as STM (LTM: $P <$ 0.001 and STM, $P < 0.001$). Further, (±) CIQ and (+) CIQ at 10 mM ($P < 0.01$, $P < 0.001$, respectively) and at 20 mM ($P < 0.01$, $P < 0.001$, respectively) reduced the MK-801 induced deficit in STM in the NOR test. Similarly, in case of LTM, (±) CIQ and (+) CIQ both at 20 mM ($P < 0.001$ each) and at 10 mM ($P < 0.01$each) significantly reduced the MK-801-induced deficits. However, we did not observe significant effect of (–) CIQ at both10 or 20 mM doses on STM or LTM. (±), (+) and (–) CIQ alone in present doses did not affected recognition memory ($P > 0.05$; Table 1).

Discussion

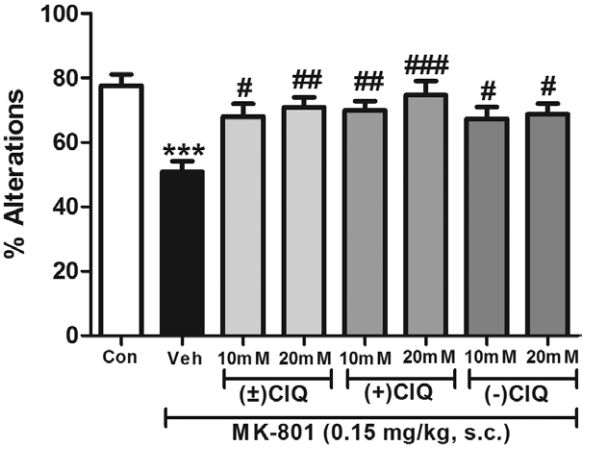
Earlier, we have shown that, (±) CIQ reduces MK-801related deficit in PPI and partially attenuated MK-801- and methamphetamine-induced hyperlocomotion and stereotyped behaviours as well as reversed the MK-801-induced working memory deficit in Y-maze (Suryavanshi *et al.*, 2014). In the present study, we have found similar results with CIQ (±, +) isomers. CIQ (±, +, –) isomers decreased climbing behaviour, increased social

Fig. 7



Effect of CIQ (±, +, –) on hyperlocomotion (a) and stereotyped (b) behaviour induced by neonatal exposure of MK-801. Each bar represents mean ± SEM (n = 8–10 per group). ***$P < 0.001$ vs. control, ##$P < 0.01$, ###$P < 0.001$ vs. veh + MK-801.

Fig. 8



Effect of CIQ (±, +, –) on working memory deficits induced by neonatal exposure of MK-801. Each bar represents mean ± SEM of % alternations (n = 8–10 per group). ***$P < 0.001$ vs. control, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. veh + MK-801.

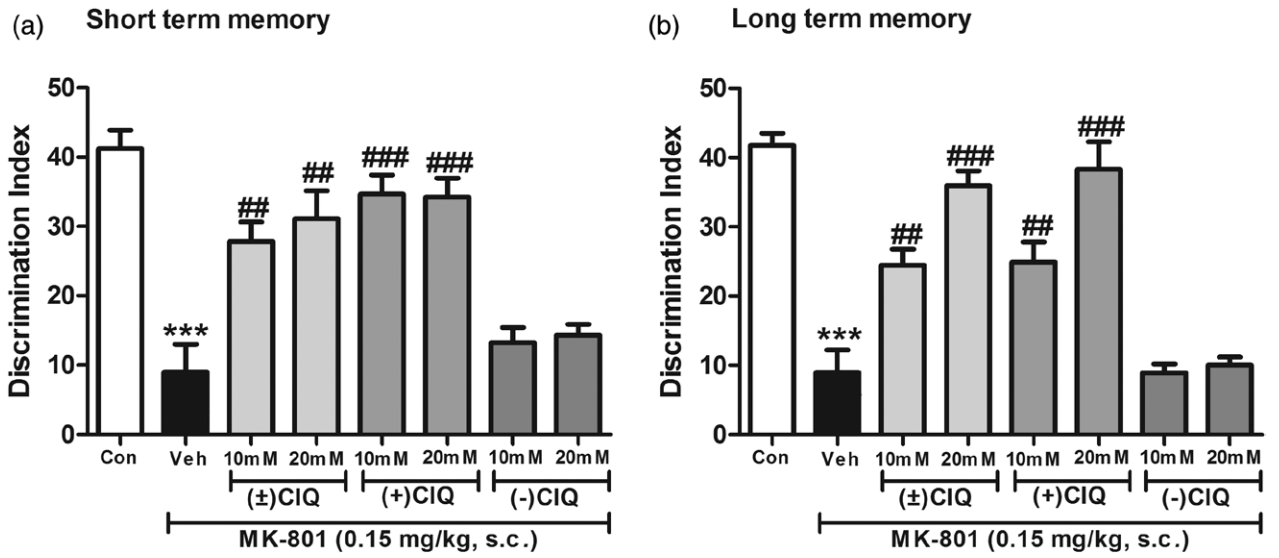
interaction and improved the MK-801 induced deficits in working memory with Y-maze. However, CIQ (±, +) but not CIQ (–) improved the recognition memory in NOR test as well as reduced hyperlocomotion and stereotyped behaviour at least with the doses used in the present study. Rather, ceiling effect was observed with (–) CIQ in higher dose. The reason behind the ineffectiveness of (–) CIQ could not be established. Present study has not investigated the involvement of GluN2C/2D receptors in the antipsychotic like actions of CIQ (±, +, –) isomers. However, as stated earlier (±) and (+) CIQ exhibits higher potency as compared to (–) CIQ towards GluN2C/2D receptors, as observed in BHK cells as well as oocytes (Santangelo Freel *et al.*, 2014).

We found that all the CIQ (±, +, –) isomers significantly attenuated the DA agonist i.e. apomorphine-induced climbing behaviour. However, higher doses (20 and 40 mM) were required to reduce the apomorphine climbing behaviour as compared to doses (10 and 20 mM) required against MK-801 induced behaviours, which may be attributed to indirect action of CIQ on D2 receptor-mediated behaviour. Our earlier study had shown that CIQ attenuated the methamphetamine-induced psychotic symptoms (Suryavanshi *et al.*, 2014). Researchers have shown dual effect of CIQ, such that it decreases DA exclusively involving the cholinergic interneurons, whereas potentiates phasic release of DA by GluN2D-containing NMDARs independently of cholinergic interneurons in the striatum (Zhang *et al.*, 2014). In the present study, (±) and (+) CIQ but not (–) CIQ attenuated MK-801 induced hyperlocomotion and stereotypy which is regarded as a surrogate for positive symptoms of SZ (Adell *et al.*, 2012).

Deficits in social interaction reflect abnormalities in social cognition and are indicative of the negative symptomatology of SZ (Wilson and Terry Jr, 2010). However, the exact mechanism for negative symptoms are not clear; it has been proposed that the frontal cortex plays an important role for negative symptom in SZ (Semkovska *et al.*, 2001). In the present study, (±, +, –) CIQ, significantly

	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

Fig. 9



Effect of CIQ (±, +, –) on recognition memory induced by neonatal exposure of MK-801 in short term memory (STM, a) as well as long term memory (LTM, b) in novel object recognition (NOR) test. Each bar represents mean ± SEM of discrimination index (n = 8–10 per group). ***$P < 0.001$ vs. control, ##$P < 0.01$, ###$P < 0.001$ vs. veh + MK-801.

increased the social interaction in SI mice indicating its beneficial effect in relieving the negative symptoms. It was proposed that negative symptoms and cognitive deficits present in SZ do not seem to result from an excessive DAergic transmission at D2 receptors (Adell *et al.*, 2012). Rather, glutamatergic dysfunction of SZ may be the driver of psychosis, negative symptoms, and cognitive deficits (Burkett and Young, 2012). Further studies are required in this direction to investigate the role of hypoglutamatergic functioning on negative symptoms of SZ.

Further, (±, +, –) CIQ isomers significantly prevented the deficits in spontaneous alteration, indicative of its potential to relieve the cognitive symptoms such as working memory deficit. NOR is widely accepted model because it requires no external motivation, reward or punishment (Silvers *et al.*, 2007). In the present study, (±) as well as (+) CIQ attenuated deficits in STM and LTM in NOR induced by MK-801. However, (–) CIQ did not affected recognition memory in NOR paradigm.

Chronic NMDAR blockade during the critical period of neurodevelopment leads to structural, neurochemical and functional alterations of the brain (Rakic *et al.*, 1994). Neurodevelopmental dysfunction has been proposed to play a critical role in the aetiology of SZ (for review, see Fatemi and Folsom, 2009). Transient exposure to MK-801 during the neonatal period causes behavioural changes in rodents such as hyperlocomotion (Harris *et al.*, 2003; Guo *et al.*, 2010; Kocahan *et al.*, 2013; Furuie *et al.*, 2013) and cognitive deficits (Stefani and Moghaddam, 2005; Uehara *et al.*, 2010; Akillioglu *et al.*, 2012; Nozari *et al.*, 2014; Su *et al.*, 2011). We found that pups treated with MK-801 (PD5–10) showed deficits in young stage (65th day) in spontaneous alternation in Y-maze used for working memory. (±, +, –) CIQ isomers dose-dependently reduced the deficits suggesting that CIQ isomers were effective against neurodevelopmental SZ. However, as seen earlier, CIQ (±, +) but not (–) CIQ attenuated STM and LTM deficits in neonatal MK-801 treated mice. Neonatal blockade of NMDAR causes delayed emergence of hyperdopaminergic activity leading to hyperlocomotion and stereotyped behaviours (Uehara *et al.*, 2010). (±) CIQ and (+) CIQ but not (–) CIQ significantly reversed the hyperlocomotion and stereotyped behaviours induced by neonatal MK-801 exposure. However, we did not require higher dose of CIQ isomers to alleviate the symptoms in neurodevelopmental model.

Thus, inefficiency of (–) CIQ against the deficit in recognition memory in NOR test as well as hyperlocomotion and stereotyped behaviour cannot be explained based on our results. However, report suggests that (–) CIQ exhibit less affinity towards the NR2C/2D receptor subunit (Santangelo Freel *et al.*, 2014). It is also not clear whether CIQ essentially requires GluN2C/2D subunits for its antipsychotic like activity due to unavailability of selective antagonist of GluN2C/2D subunits. Indeed, several reports suggest the involvement of GluN2C/2D subunits in SZ. The genetic deletion of GluN2C in mouse leads to certain characteristic features observed in SZ such as working memory deficit and deficit in associative contextual fear (Hillman *et al.*, 2011). In addition, the efficacy of

D-cycloserine in SZ symptoms has been proposed to arise partly due to its higher efficacy at GluN2C-containing receptors (Dravid *et al*., 2010; Goff, 2012). Further, reduced GluN2C expression was found in the PFC of human SZ patients (Weickert *et al*., 2012) indicative of its importance in cognitive deficits in SZ. GluN2D subunits are highly expressed in prefrontal cortex, hippocampus and ventral tegmentum that participates in circuits involved in SZ-related symptoms (Lisman *et al*., 2010; Duan *et al*., 2015; Griffin, 2015; Ito *et al*., 2015). Researchers suggest that GluN2D-containing NMDARs are necessary for full neuronal activation induced by ketamine and that GluN2D hypofunction potentially contributes to SZ symptoms (Sapkota *et al*., 2015). Additionally, NMDAR inhibition in the reticular nucleus that shows expression of GluN2D and GluN2C subunits (Yamasaki *et al*., 2014), generates telencephalic delta oscillations and potentially SZ-related symptoms (Zhang *et al*., 2009).

Thus, existing literature is insufficient to comment on the probable mechanism of antipsychotic-like activity of CIQ. Although CIQ (±, +) isomers exhibits antipsychotic-like profile, the present study is not eligible to comment on the involvement of NR2C/2D receptors in their effects. Although, report suggests less affinity of (−) CIQ towards the NR2C/2D receptor subunit; the study is restricted to mammalian BHK cells (Santangelo Freel *et al*., 2014). Thus, role of other mechanisms along with NR2C/2D receptors cannot be denied in antipsychotic-like profile of CIQ. Further studies are warranted here.

Conclusion

Thus, (±, +) CIQ isomers exhibits antipsychotic like profile against the positive, negative and cognitive symptoms as well as neurodevelopmental symptoms of SZ in the present study. However, we could not found the beneficial effects of (−) CIQ isomer against the hyperlocomotion and stereotypy, the striatal mediated behaviour. However, these are preliminary pilot studies and needs to be substantiated carefully using further pharmacological and molecular studies.

Acknowledgements

We thank Rashtrasant Tukadoji Maharaj Nagpur University for providing facilities.

Conflicts of interest

There are no conflicts of interest.

References

Adell A, Jiménez-Sánchez L, López-Gil X, Romón T (2012). Is the acute NMDA receptor hypofunction a valid model of schizophrenia? *Schizophr Bull* **38**:9–14.

Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP, *et al.* (1996). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J Neurosci* **16**:19–30.

Akillioglu K, Binokay S, Kocahan S (2012). The effect of neonatal N-methyl-D-aspartate receptor blockade on exploratory and anxiety-like behaviors in adult BALB/c and C57BL/6 mice. *Behav Brain Res* **233**:157–161.

Balu DT (2016). The NMDA receptor and schizophrenia: from pathophysiology to treatment. *Adv Pharmacol* **76**:351–382.

Burkett JP, Young LJ (2012). The behavioral, anatomical and pharmacological parallels between social attachment, love and addiction. *Psychopharmacology (Berl)* **224**:1–26.

Carlsson M, Carlsson A (1990). Schizophrenia: a subcortical neurotransmitter imbalance syndrome? *Schizophr Bull* **16**:425–432.

Cioffi CL (2013). Modulation of NMDA receptor function as a treatment for schizophrenia. *Bioorg Med Chem Lett* **23**:5034–5044.

Costall B, Naylor RJ, Nohria V (1978). Climbing behaviour induced by apomorphine in mice: a potential model for the detection of neuroleptic activity. *Eur J Pharmacol* **50**:39–50.

Dravid SM, Burger PB, Prakash A, Geballe MT, Yadav R, Le P, *et al.* (2010). Structural determinants of D-cycloserine efficacy at the NR1/NR2C NMDA receptors. *J Neurosci* **30**:2741–2754.

du Bois TM, Huang XF (2007). Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. *Brain Res Rev* **53**:260–270.

Duan AR, Varela C, Zhang Y, Shen Y, Xiong L, Wilson MA, Lisman J (2015). Delta frequency optogenetic stimulation of the thalamic nucleus reuniens is sufficient to produce working memory deficits: relevance to schizophrenia. *Biol Psychiatry* **77**:1098–1107.

Ellison G (1995). The N-methyl-D aspartate antagonists phencyclidine, ketamine and dizocilpine as both behavioral and anat omical models of the dementias. *Brain Res Rev* **20**:250–267.

Fatemi SH, Folsom TD (2009). The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull* **35**:528–548.

Furuie H, Yamada K, Ichitani Y (2013). MK-801-induced and scopolamine-induced hyperactivity in rats neonatally treated chronically with MK-801. *Behav Pharmacol* **24**:678–683.

Geyer M, Moghaddam B (2002). Animal models relevant to schizophrenia disorders. *Neuropsychopharmacology* **50**:690–701.

Goff DC (2012). D-cycloserine: an evolving role in learning and neuroplasticity in schizophrenia. *Schizophr Bull* **38**:936–941.

Griffin AL (2015). Role of the thalamic nucleus reuniens in mediating interactions between the hippocampus and medial prefrontal cortex during spatial working memory. *Front Syst Neurosci* **9**:29.

Guo C, Yang Y, Su Y, Si T (2010). Postnatal BDNF expression profiles in prefrontal cortex and hippocampus of a rat schizophrenia model induced by MK-801 administration. *J Biomed Biotechnol* **2010**:783297.

Hagino Y, Kasai S, Han W, Yamamoto H, Nabeshima T, Mishina M, Ikeda K (2010). Essential role of NMDA receptor channel ε4 subunit (glun2d) in the effects of phencyclidine, but not methamphetamine. *Plos One* **5**:e13722.

Harris LW, Sharp T, Gartlon J, Jones DN, Harrison PJ (2003). Long-term behavioural, molecular and morphological effects of neonatal NMDA receptor antagonism. *Eur J Neurosci* **18**:1706–1710.

Hillman B (2012). Studies of GluN2C-containing NMDA receptors in schizophrenia-like behaviors and fear learning: relevance to the glutamate hypofunction hypothesis of schizophrenia. *ProQuest Diss Publ* 1–36. AQ4

Hillman BG, Gupta SC, Stairs DJ, Buonanno A, Dravid SM (2011). Behavioral analysis of NR2C knockout mouse reveals deficit in acquisition of conditioned fear and working memory. *Neurobiol Learn Mem* **95**:404–414.

Ito HT, Zhang SJ, Witter MP, Moser EI, Moser MB (2015). A prefrontal-thalamo-hippocampal circuit for goal-directed spatial navigation. *Nature* **522**:50–55.

Javitt DC, Zukin SR (1991). Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**:1301–1308.

Khlestova E, Johnson JW, Krystal JH, Lisman J (2016). The role of glun2c-containing NMDA receptors in ketamine's psychotogenic action and in schizophrenia models. *J Neurosci* **36**:11151–11157.

Kim HS, Rhee GS, Jung JY, Lee JH, Jang CG, Park WK (1996). Inhibition by noncompetitive NMDA receptor antagonists of apomorphine-induced climbing behavior in mice. *Life Sci* **58**:1397–1402.

Kirch DG (1993). Infection and autoimmunity as etiologic factors in schizophrenia: a review and reappraisal. *Schizophr Bull* **19**:355–370.

Kocahan S, Akillioglu K, Binokay S, Sencar L, Polat S (2013). The effects of N-methyl-D-aspartate receptor blockade during the early neurodevelopmental period on emotional behaviors and cognitive functions of adolescent Wistar rats. *Neurochem Res* **38**:989–996.

Koike H, Ibi D, Mizoguchi H, Nagai T, Nitta A, Takuma K, *et al.* (2009). Behavioral abnormality and pharmacologic response in social isolation-reared mice. *Behav Brain Res* **202**:114–121.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, *et al.* (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**:199–214.

	11/21/19	4 Color Fig(s):0	15:13	Art: BP-18-95

Laruelle M, Kegeles LS, Abi-Dargham A (2003). Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Ann N Y Acad Sci* **1003**:138–158.

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* **31**:234–242.

Lisman JE, Pi HJ, Zhang Y, Otmakhova NA (2010). A thalamo-hippocampal-ventral tegmental area loop may produce the positive feedback that underlies the psychotic break in schizophrenia. *Biol Psychiatry* **68**:17–24.

McGrath JJ, Féron FP, Burne TH, Mackay-Sim A, Eyles DW (2003). The neurodevelopmental hypothesis of schizophrenia: a review of recent developments. *Ann Med* **35**:86–93.

Mouri A, Noda Y, Hara H, Mizoguchi H, Tabira T, Nabeshima T (2007). Oral vaccination with a viral vector containing abeta cdna attenuates age-related abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. *Faseb J* **21**:2135–2148.

Mullasseril P, Hansen KB, Vance KM, Ogden KK, Yuan H, Kurtkaya NL, *et al.* (2010). A subunit-selective potentiator of NR2C- and NR2D-containing NMDA receptors. *Nat Commun* **1**:90.

Nozari M, Shabani, M, Hadadi M, Atapour N (2014). Enriched environment prevents cognitive and motor deficits associated with postnatal MK-801 treatment. *Psychopharmacology (Berl)* **231**:4361–4370.

Paxinos G, Franklin K (2001). *The Mouse Brain in Stereotaxic Coordinates*. 2. San Diego: Academic Press.

Rakic P, Bourgeois JP, Goldman-Rakic PS (1994). Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. *Prog Brain Res* **102**:227–243.

Rapoport JL, Addington AM, Frangou S, Psych MR (2005). The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry* **10**:434–449.

Rapoport JL, Giedd JN, Gogtay N (2012). Neurodevelopmental model of schizophrenia: update 2012. *Mol Psychiatry* **17**:1228–1238.

Santangelo Freel RM, Ogden KK, Strong KL, Khatri A, Chepiga KM, Jensen HS, *et al.* (2014). Correction to synthesis and structure activity relationship of tetrahydroisoquinoline-based potentiators of glun2c and glun2d containing N-methyl-d-aspartate receptors. *J Med Chem* **57**:4975.

Santangelo Freel RM, Ogden KK, Strong KL, Khatri A, Chepiga KM, Jensen HS, *et al.* (2013). Synthesis and structure activity relationship of tetrahydroisoquinoline-based potentiators of glun2c and glun2d containing N-methyl-D-aspartate receptors. *J Med Chem* **56**:5351–5381.

AQ5

Sapkota S, Vergote D, Westaway D, Jhamandas J, Dixon RA (2015). Synergistic associations of catechol-O-methyltransferase and brain-derived neurotrophic factor with executive function in aging are selective and modified by apolipoprotein E. *Neurobiol Aging* **36**:249–256.

Semkovska M, Bédard MA, Stip E (2001). [Hypofrontality and negative symptoms in schizophrenia: synthesis of anatomic and neuropsychological knowledge and ecological perspectives]. *Encephale* **27**:405–415.

Silvers JM, Harrod SB, Mactutus CF, Booze RM (2007). Automation of the novel object recognition task for use in adolescent rats. *J Neurosci Methods* **166**:99–103.

Starr MS (1995). Glutamate/dopamine D1/D2 balance in the basal ganglia and its relevance to Parkinson's disease. *Synapse* **19**:264–293.

Stefani MR, Moghaddam B (2005). Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. *Biol Psychiatry* **57**:433–436.

Su YA, Wang XD, Li JT, Guo CM, Feng Y, Yang Y, *et al.* (2011). Age-specific effects of early MK-801 treatment on working memory in female rats. *Neuroreport* **22**:402–406.

Suryavanshi PS, Ugale RR, Yilmazer-Hanke D, Stairs DJ, Dravid SM (2014). Glun2c/glun2d subunit-selective NMDA receptor potentiator CIQ reverses MK-801-induced impairment in prepulse inhibition and working memory in Y-maze test in mice. *Br J Pharmacol* **171**:799–809.

Svensson E, Wikström MA, Hill RH, Grillner S (2003). Endogenous and exogenous dopamine presynaptically inhibits glutamatergic reticulospinal transmission via an action of D2-receptors on N-type ca2+ channels. *Eur J Neurosci* **17**:447–454.

Taksande BG, Kotagale NR, Tripathi SJ, Ugale RR, Chopde CT (2009). Antidepressant like effect of selective serotonin reuptake inhibitors involve modulation of imidazoline receptors by agmatine. *Neuropharmacology* **57**:415–424.

Tanaka S (2006). Dopaminergic control of working memory and its relevance to schizophrenia: a circuit dynamics perspective. *Neuroscience* **139**:153–171.

Uehara T, Sumiyoshi T, Seo T, Matsuoka T, Itoh H, Suzuki M, Kurachi M (2010). Neonatal exposure to MK-801, an N-methyl-D-aspartate receptor antagonist, enhances methamphetamine-induced locomotion and disrupts sensorimotor gating in pre- and postpubertal rats. *Brain Res* **1352**:223–230.

Ugale RR, Hirani K, Morelli M, Chopde CT (2004). Role of neuroactive steroid allopregnanolone in antipsychotic-like action of olanzapine in rodents. *Neuropsychopharmacology* **29**:1597–1609.

Weickert CS, Fung SJ, Catts VS, Schofield PR, Allen KM, Moore LT, *et al.* (2013). Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. *Mol Psychiatry* **18**:1185–1192.

Wilson C, Terry AV Jr (2010). Neurodevelopmental animal models of schizophrenia: role in novel drug discovery and development. *Clin Schizophr Relat Psychoses* **4**:124–137.

Wu Y, Parent A (2000). Striatal interneurons expressing calretinin, parvalbumin or NADPH-diaphorase: a comparative study in the rat, monkey and human. *Brain Res* **863**:182–191.

Yamamoto H, Hagino Y, Kasai S, Ikeda K (2015). Specific roles of NMDA receptor subunits in mental disorders. *Curr Mol Med* **15**:193–205.

Yamasaki M, Okada R, Takasaki C, Toki S, Fukaya M, Natsume R, *et al.* (2014). Opposing role of NMDA receptor glun2b and glun2d in somatosensory development and maturation. *J Neurosci* **34**:11534–11548.

Zhang X, Feng ZJ, Chergui K (2014). Allosteric modulation of glun2c/glun2d-containing NMDA receptors bidirectionally modulates dopamine release: implication for Parkinson's disease. *Br J Pharmacol* **171**:3938–3945.

Zhang Y, Llinas RR, Lisman JE (2009). Inhibition of nmdars in the nucleus reticularis of the thalamus produces delta frequency bursting. *Front Neural Circuits* **3**:20.

AUTHOR QUERY FORM

LIPPINCOTT
WILLIAMS AND WILKINS

JOURNAL NAME: FBP

ARTICLE NO: BP-18-95

QUERIES AND / OR REMARKS

QUERY NO.	Details Required	Author's Response
Q1	For indexing purposes, please confirm that author names have been correctly identified as given names (blue) and surnames (red). Color in the byline will not appear on the final published version.	authors names are correct
Q2	Please provide alternate word for 'and/or' in the article.	corrected
Q3	Please provide city name for manufacturer 'V.J. Instruments'.	City name included
Q4	Please provide the volume number for the reference 'Hillman (2012)'.	It is PhD Dissertation Published online by ProQuest Dissertations Publishing, 2012. 3552788
Q5	Please provide in-text citation for reference 'Santangelo Freel et al. (2013)'.	Corrected