Published in final edited form as:
Schizophr Res. 2011 January ; 125(1): 88–92. doi:10.1016/j.schres.2010.09.025.

PAOPA, a potent analogue of pro-leu-glycinamide and allosteric modulator of the dopamine D_2 receptor, prevents NMDA receptor antagonist (MK-801)-induced deficits in social interaction in the rat: Implications for the treatment of negative symptoms in schizophrenia

Bailee Dyck[1], **Kelly Guest**[1], **Christal Sookram**[1], **Dipannita Basu**[1], **Rodney Johnson**[2], and **Ram K. Mishra**[1]

[1]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada

[2]Department of Medicinal Chemistry, University of Minnesota, MI, USA

1. Introduction

Schizophrenia is broadly characterized by the presence of positive symptoms (e.g. delusions and hallucinations) negative symptoms (e.g. social withdrawal, and poor volition) and cognitive deficits (e.g. memory, attention, and reasoning and problem solving deficits) (Crow 1980;Nuechterlein et al. 2004). Research on the pathophysiology of schizophrenia has implicated decreases in glutamate signaling and NMDA receptor hypofunctionality as a causative factor in this disease (Laruelle et al. 2003). A number of studies have demonstrated that treatment with phencyclidine (PCP), ketamine, or dizocilpine (MK-801) can produce symptoms similar to the positive, negative and cognitive symptoms of schizophrenia when given to healthy control subjects, and can exacerbate these symptoms in patients with schizophrenia (Siegel 1978;Snyder 1973). Unlike amphetamine, a dopamine (DA) agonist that is able to mimic the positive symptoms of schizophrenia, only PCP and MK-801 can induce key negative symptoms such as social withdrawal (Guy and Gardner 1985;Lisman et al. 2008;Rung et al. 2005;Sams-Dodd 1996). MK-801, an analogue of PCP, is one of the most potent non-competitive antagonists of the NMDA receptor, binding to a site located with the NMDA receptor ion channel and blocking Ca^{2+} flow, thus disturbing glutamatergic neurotransmission (Rung et al, 2005). Treatment with MK-801 has been shown to induce a variety of symptoms reflective of schizophrenia, including disrupted

Corresponding Author: Dr. Ram K. Mishra, Department of Psychiatry and Behavioural Neurosciences, Health Sciences Center Room 4N78, McMaster University, 1200 Main Street West, Hamilton, ON, Canada, L8S 3Z5. mishrar@mcmaster.ca. Telephone: 1-905-525-9140 x22396. Fax: 1-905-522-8804.

7. Conflict of Interest: All authors declare that they have no conflicts of interest.

6. Contributors: Dr. Ram K. Mishra and Dr. Rodney Johnson designed the study and wrote the protocol, and Dr. Ram K. Mishra and Dr. Bailee Dyck managed the literature searches and analyses. Dr. Bailee Dyck, Kelly Guest, Christal Sookram, and Dipannita Basu undertook the statistical analyses and wrote the first draft of the manuscript. Dr. Bailee Dyck, Kelly Guest and Christal Sookram contributed to running the experiments. All authors contributed to and have approved the final manuscript.

sensorimotor gating (prepulse inhibition), increased locomotor activity and disrupted cognitive function, including deficits in rule acquisition and attentional set-shifting (Bast et al. 2000;BELL 1965;Malhotra et al. 1997;Manahan-Vaughan et al. 2008;Rung et al. 2005;Sams-Dodd 1996;Sams-Dodd 1998;Snyder 1973;Stefani and Moghaddam 2005). Furthermore, MK801 induces social withdrawal (Rung et al, 2005, Sams-Dodd et al, 2004, Snigdha et al, 2008), serving as a strong model of the negative symptoms of schizophrenia.

Previous research from our lab has demonstrated that the endogenous brain tripeptide PLG and its analog PAOPA (Figure 1) modify dopaminergic neurotransmission by acting as allosteric modulators of the DA D_2 receptor (Johnson et al. 1986;Mishra 1983;Mishra et al. 1983;Mishra et al. 1997;Verma et al. 2005;Chiu et al. 1981;Chiu et al. 1983;Raghavan et al. 2009;Srivastava et al. 1988;Verma et al. 2005). These compounds have been shown to increase agonist binding to DA D_2 receptors without affecting antagonist binding, and prevent conversion of high-affinity state DA receptors (D_2^{High}) to their low-affinity state (D_2^{Low}) (Mishra et al. 1990;Srivastava et al. 1988;Verma et al. 2005). Furthermore, PLG and PAOPA have been shown to potentiate rotational behaviour in the 6-hydroxy dopamine lesion rat (Mishra et al. 1997;Ott et al. 1996;Smith and Morgan 1982), and inhibit neuroleptic drug-induced vacuous chewing in rat models of human tardive dyskinesia (Castellano et al. 2007;Sharma et al. 2003). Given the interaction between PAOPA and the DA D_2 receptor, and the effects of PAOPA in preclinical animal models, the objective of this study was to investigate whether this potent analog of PLG has an effect on MK-801-induced social withdrawal in the rat.

2. Methods

2.1 Animals

Age-matched male Sprague Dawley rats (225 – 272 g), Charles River Canada, St. Constant, QC, Canada) were tested in accordance with the Canadian Council for Animal Care guidelines. Animals were housed individually in standard cages on a 12 hr light cycle in a room maintained at 22°C with 50% humidity with access to food and water *ad libitum*.

2.2 Drugs and Administration Schedule

(+)-MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine maleate salt) was purchased from Sigma-Aldrich (Oakville, ON, Canada). PAOPA was custom synthesized at the University of Minnesota as previously described (Yu et al. 1988). All drugs were dissolved in 0.9% saline. MK-801 was injected at 0.5 mg/kg, and PAOPA was injected at 1 mg/kg. Four groups of rats were utilized and received daily injections intraperitoneally (I.P.) for 7 days as follows: **Group A** (n=10) served as a control and was injected with saline; **Group B** (n=10) received MK-801; **Group C** (n=10) received PAOPA; and **Group D** (n=10) received PAOPA followed 30 minutes later by MK-801.

2.3 Social Interaction

The following method was adopted from File (File 1980) and Sams-Dodd (Sams-Dodd 1998). Animals were tested 24 hrs after the last drug injection during the light cycle. Social behavior was recorded in a black Plexiglass arena with dimensions 100 cm × 100 cm × 40 cm for a 5 min period via a ceiling mounted video camera. One rat was randomly marked with non-toxic black marker for identification during analysis. Total time spent in interaction was recorded (ms) for each rat and subdivided into active interaction (sniffing, following, crawling under or over, grooming, and aggressive behaviour) or passive interaction (close proximity). Members of each pair were not familiar with one another, with each pair used only once per test. Recordings were analyzed by a blinded observer.

2.4 Statistical Analysis

The amount of time spent in total interaction, in active or passive interaction, and the number of active and passive interaction episodes, was recorded and analyzed by means of one way analyses of variance (ANOVAs) with Tukey's post-hoc test.

3. Results

3.1 Sub-chronic treatment with MK-801 decreases social interaction in rats

Sub-chronic (7 day) treatment with MK-801 significantly decreased social behavior, as reflected by decreases in total time spent in interaction ($F(3,26) = 21.92$, $^{***}p < 0.0001$; post-hoc, $^{***}p < 0.001$), time spent in passive interaction ($F(3,25) = 8.464$, $^{***}p < 0.004$; post-hoc, $^{***}p < 0.001$), and the number of passive interaction episodes ($F(3,26) = 7.648$, $^{***}p = 0.009$; post-hoc, $^{*}p < 0.05$) (FIGURE 2A-E). Interestingly, no significant reductions in the amount of time spent in active interaction ($F(3,27) = 10.29$, $^{***}p$ 0.0001; post-hoc, $p > 0.05$) or the number of active interaction episodes were observed ($F(3,28) = 8.621$, $^{***}p = 0.0003$; post-hoc, $p > 0.05$) (FIGURE 2B, D). This evidence further validates that sub-chronic treatment with MK-801 reliably models negative symptoms of schizophrenia as demonstrated through social withdrawal.

3.2 Administration of PAOPA attenuates the effects of MK-801 on social behaviour

When administered 30 minutes before MK-801 challenge, PAOPA attenuated the deficits in social interaction induced by the NMDA receptor antagonist (FIGURE 2A-E). More specifically, animals spent significantly more time in total interaction ($^{***}p < 0.001$), active interaction ($^{***}p < 0.001$), and passive interaction ($^{*}p < 0.05$), and had significantly higher numbers of active ($^{**}p < 0.01$) and passive ($^{**}p < 0.01$) interaction episodes relative to rats challenged with MK801. Furthermore, PAOPA alone significantly increased the number of active interaction episodes ($^{*}p < 0.05$) when compared to saline-challenged animals (FIGURE 2D).

4. Discussion

Sub-chronic (7 day) treatment with the non-competitive NMDA receptor antagonist MK-801 induced deficits in social interaction, including decreased total time spent in interaction, decreased time spent in active and passive interaction, and decreased number of active and passive interaction episodes. Pre-treatment with PAOPA 30 minutes before MK-801 challenge attenuated these effects, such that animals displayed normal social behaviour. Furthermore, treatment with PAOPA alone increased the number of passive interaction episodes when compared to control animals challenged with saline. These results are consistent with previous reports on the ability of sub-chronic MK-801 challenge to interrupt normal social behaviour (Rung et al. 2005;Sams-Dodd 2004), and suggest that PAOPA may serve as a novel compound to treat the negative symptoms associated with schizophrenia.

The ability of non-competitive NMDA receptor antagonists, such as MK-801, to induce schizophrenic-like behavior provides evidence for dysregulated glutamatergic neurotransmission in schizophrenia, which involves hypofunctionality of NMDA receptors (Lahti et al, 2001) (Farber 2003;Laruelle et al. 2003). Pertinent to NMDA receptor activity, a high degree of interaction exists between glutamate and DA neurotransmission. Striatal D_2 receptor stimulation results in NMDA deficiency and decreased glutamate transmission, whereas striatal D_1 receptor stimulation increases glutamate transmission (Laruelle et al. 2003). In addition, a significant reduction in DA transmission has been observed in rat prefrontal cortex following sub-chronic administration of MK-801 (Jentsch et al, 1998).

Ketamine and PCP have also been shown to possess similar levels of affinity for NMDA receptors and DA D_2 receptors (Kapur and Seeman 2002).

Current treatment of schizophrenia with antipsychotic drugs (APDs), the majority of which exert their action via dopamine D_2 receptor antagonism, is largely targeted toward treating positive symptoms but has limited ability to treat the negative symptoms of this disorder (Blin 1999). Interestingly, treatment with dopamine agonists have been shown to be effective in treating negative symptoms (Benkert et al. 1995;Olbrich and Schanz 1988;Wetzel et al. 1994). PAOPA allosterically binds the DA D_2 receptor and has been shown to increase the proportion of D_2^{High} states by preventing conversion to the D_2^{Low} state (Mishra et al. 1990;Verma et al. 2005). Our results demonstrate that treatment with PAOPA prevents deficits in social interaction induced by MK-801 and increases social behavior when administered alone. While the exact mechanism by which these behavioural changes occur remains unknown, PAOPA's interaction with the D_2 receptor may induce increased stimulation of this receptor by endogenous DA. Upon increased agonist stimulation, most receptors are translocated from the cellular membrane to the cytosol by endocytosis as a compensatory mechanism to prevent receptor overstimulation (Namkung et al. 2009). This has been demonstrated for D_2 ligands such as quinpirole (Guo et al. 2010) and 2-methoxy-N-propylnorapomorphine (Skinbjerg et al. 2009). Thus, PAOPA's mechanistic action may involve internalization of D_2 receptors due to increased agonist stimulation, thereby compensating for excess endogenous DA in the MK-801-induced schizophrenic-like state.

In conclusion, PAOPA caused significant increases in social interaction and attenuated MK-801-induced social deficits. These results suggest that peptidomimetics such as PAOPA may improve the negative symptoms in schizophrenia.

Acknowledgments

We would like to thank all members of the laboratory group for assisting in the preparation and proof-reading of the manuscript. With regard to these results, Ram Mishra, Rodney Johnson, Bailee Dyck and Dipannita Basu are involved in an intellectual property patent (US patent no. 61/378,599).

5. Role of Funding Source: Funding for this study was provided by the National Institutes of Health Research Grant 2R01-NS020036-20; NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

Bast T, Zhang W, Feldon J, White IM. Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats. Pharmacol Biochem Behav 2000;67:647–658. [PubMed: 11164097]

Bell DS. Comparison of Amphetamine Psychosis and Schizophrenia. Br J Psychiatry 1965;111:701–707. [PubMed: 14337419]

Benkert O, Muller-Siecheneder F, Wetzel H. Dopamine agonists in schizophrenia: a review. Eur Neuropsychopharmacol 1995 5:43–53. [PubMed: 8775758]

Blin O. A comparative review of new antipsychotics. Can J Psychiatry 1999;44:235–244. [PubMed: 10225124]

Castellano JM, Batrynchuk J, Dolbeare K, Verma V, Mann A, Skoblenick KJ, Johnson RL, Mishra RK. MIF-1 and its peptidomimetic analogs attenuate haloperidol-induced vacuous chewing movements and modulate apomorphine-induced rotational behavior in 6-hydroxydopamine-lesioned rats. Peptides 2007;28:2009–2015. [PubMed: 17766011]

Chiu S, Paulose CS, Mishra RK. Neuroleptic drug-induced dopamine receptor supersensitivity: antagonism by L-prolyl-L-leucyl-glycinamide. Science 1981;214:1261–1262. [PubMed: 6117947]

Chiu S, Wong YW, Wan YP, Chiu P, Mishra RK. Are the pharmacological effects of L-prolyl-L-leucyl-glycinamide (PLG) mediated through specific receptor mechanisms? Prog Neuropsychopharmacol Biol Psychiatry 1983;7:739–742. [PubMed: 6141616]

Crow TJ. Positive and negative schizophrenic symptoms and the role of dopamine. Br J Psychiatry 1980;137:383–386. [PubMed: 7448479]

Farber NB. The NMDA receptor hypofunction model of psychosis. Ann N Y Acad Sci 2003;1003:119–130. [PubMed: 14684440]

File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 1980;2:219–238. [PubMed: 6120260]

Guo N, Guo W, Kralikova M, Jiang M, Schieren I, Narendran R, Slifstein M, Abi-Dargham A, Laruelle M, Javitch JA, Rayport S. Impact of d2 receptor internalization on binding affinity of neuroimaging radiotracers. Neuropsychopharmacology 2010;35:806–817. [PubMed: 19956086]

Guy AP, Gardner CR. Pharmacological characterisation of a modified social interaction model of anxiety in the rat. Neuropsychobiology 1985;13:194–200. [PubMed: 2864655]

Johnson RL, Rajakumar G, Mishra RK. Dopamine receptor modulation by Pro-Leu-Gly-NH2 analogues possessing cyclic amino acid residues at the C-terminal position. J Med Chem 1986;29:2100–2104. [PubMed: 2876103]

Kapur S, Seeman P. NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2)receptors-implications for models of schizophrenia. Mol Psychiatry 2002;7:837–844. [PubMed: 12232776]

Laruelle M, Kegeles LS, Abi-Dargham A. Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann N Y Acad Sci 2003;1003:138–158. [PubMed: 14684442]

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 2008;31:234–242. [PubMed: 18395805]

Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 1997;17:141–150. [PubMed: 9272481]

Manahan-Vaughan D, von Haebler D, Winter C, Juckel G, Heinemann U. A single application of MK801 causes symptoms of acute psychosis, deficits in spatial memory, and impairment of synaptic plasticity in rats. Hippocampus 2008;18:125–134. [PubMed: 17924525]

Mishra RK. Modulation of CNS dopamine receptors by peptides. Prog Neuropsychopharmacol Biol Psychiatry 1983;7:437–442. [PubMed: 6141599]

Mishra RK, Chiu S, Chiu P, Mishra CP. Pharmacology of L-prolyl-L-leucyl-glycinamide (PLG): a review. Methods Find Exp Clin Pharmacol 1983;5:203–233. [PubMed: 6136640]

Mishra RK, Marcotte ER, Chugh A, Barlas C, Whan D, Johnson RL. Modulation of dopamine receptor agonist-induced rotational behavior in 6-OHDA-lesioned rats by a peptidomimetic analogue of Pro-Leu-Gly-NH2 (PLG). Peptides 1997;18:1209–1215. [PubMed: 9396063]

Mishra RK, Srivastava LK, Johnson RL. Modulation of high-affinity CNS dopamine D2 receptor by L-pro-L-leu-glycinamide (PLG) analogue 3(R)-(N-L-prolylamino)-2-oxo-1-pyrrolidineacetamide. Prog Neuropsychopharmacol Biol Psychiatry 1990;14:821–827. [PubMed: 1981396]

Namkung Y, Dipace C, Javitch JA, Sibley DR. G protein-coupled receptor kinase-mediated phosphorylation regulates post-endocytic trafficking of the D2 dopamine receptor. J Biol Chem 2009;284:15038–15051. [PubMed: 19332542]

Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. Schizophr Res 2004;72:29–39. [PubMed: 15531405]

Olbrich R, Schanz H. The effect of the partial dopamine agonist terguride on negative symptoms in schizophrenics. Pharmacopsychiatry 1988;21:389–390. [PubMed: 2907647]

Ott MC, Mishra RK, Johnson RL. Modulation of dopaminergic neurotransmission in the 6-hydroxydopamine lesioned rotational model by peptidomimetic analogues of L-prolyl-L-leucyl-glycinamide. Brain Res 1996;737:287–291. [PubMed: 8930377]

Raghavan B, Skoblenick KJ, Bhagwanth S, Argintaru N, Mishra RK, Johnson RL. Allosteric modulation of the dopamine D2 receptor by Pro-Leu-Gly-NH2 peptidomimetics constrained in

either a polyproline II helix or a type II beta-turn conformation. J Med Chem 2009;52:2043–2051. [PubMed: 19271750]

Rung JP, Carlsson A, Ryden MK, Carlsson ML. (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:827–832. [PubMed: 15916843]

Sams-Dodd F. Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. Behav Pharmacol 1996;7:3–23. [PubMed: 11224390]

Sams-Dodd F. Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. Neuropsychopharmacology 1998;19:18–25. [PubMed: 9608573]

Sams-Dodd F. (+) MK-801 and phencyclidine induced neurotoxicity do not cause enduring behaviours resembling the positive and negative symptoms of schizophrenia in the rat. Basic Clin Pharmacol Toxicol 2004;95:241–246. [PubMed: 15546479]

Sharma S, Paladino P, Gabriele J, Saeedi H, Henry P, Chang M, Mishra RK, Johnson RL. Pro-Leu-glycinamide and its peptidomimetic, PAOPA, attenuate haloperidol induced vacuous chewing movements in rat: A model of human tardive dyskinesia. Peptides 2003;24:313–319. [PubMed: 12668218]

Siegel, RK. NIDA Res Monogr. 1978. Phencyclidine and ketamine intoxication: a study of four populations of recreational users; p. 119-147.

Skinbjerg M, Namkung Y, Halldin C, Innis RB, Sibley DR. Pharmacological characterization of 2-methoxy-N-propylnorapomorphine's interactions with D2 and D3 dopamine receptors. Synapse 2009;63:462–475. [PubMed: 19217026]

Smith JR, Morgan M. The effects of prolyl-leucyl-glycine amide on drug-induced rotation in lesioned rats. Gen Pharmacol 1982;13:203–207. [PubMed: 6124481]

Snyder SH. Amphetamine psychosis: a “model” schizophrenia mediated by catecholamines. Am J Psychiatry 1973;130:61–67. [PubMed: 4345465]

Srivastava LK, Bajwa SB, Johnson RL, Mishra RK. Interaction of L-prolyl-L-leucyl glycinamide with dopamine D2 receptor: evidence for modulation of agonist affinity states in bovine striatal membranes. J Neurochem 1988;50:960–968. [PubMed: 2892892]

Stefani MR, Moghaddam B. Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. Biol Psychiatry 2005;57:433–436. [PubMed: 15705361]

Verma V, Mann A, Costain W, Pontoriero G, Castellano JM, Skoblenick K, Gupta SK, Pristupa Z, Niznik HB, Johnson RL, Nair VD, Mishra RK. Modulation of agonist binding to human dopamine receptor subtypes by L-prolyl-L-leucyl-glycinamide and a peptidomimetic analog. J Pharmacol Exp Ther 2005;315:1228–1236. [PubMed: 16126839]

Wetzel H, Hillert A, Grunder G, Benkert O. Roxindole, a dopamine autoreceptor agonist, in the treatment of positive and negative schizophrenic symptoms. Am J Psychiatry 1994;151:1499–1502. [PubMed: 7916543]

Yu KL, Rajakumar G, Srivastava LK, Mishra RK, Johnson RL. Dopamine receptor modulation by conformationally constrained analogues of Pro-Leu-Gly-NH2. J Med Chem 1988;31:1430–1436. [PubMed: 3385734]

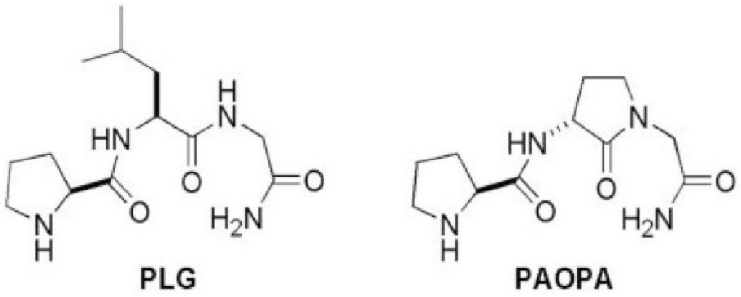

Figure 1.
Schematic structure diagram of PLG and its analogue, PAOPA.

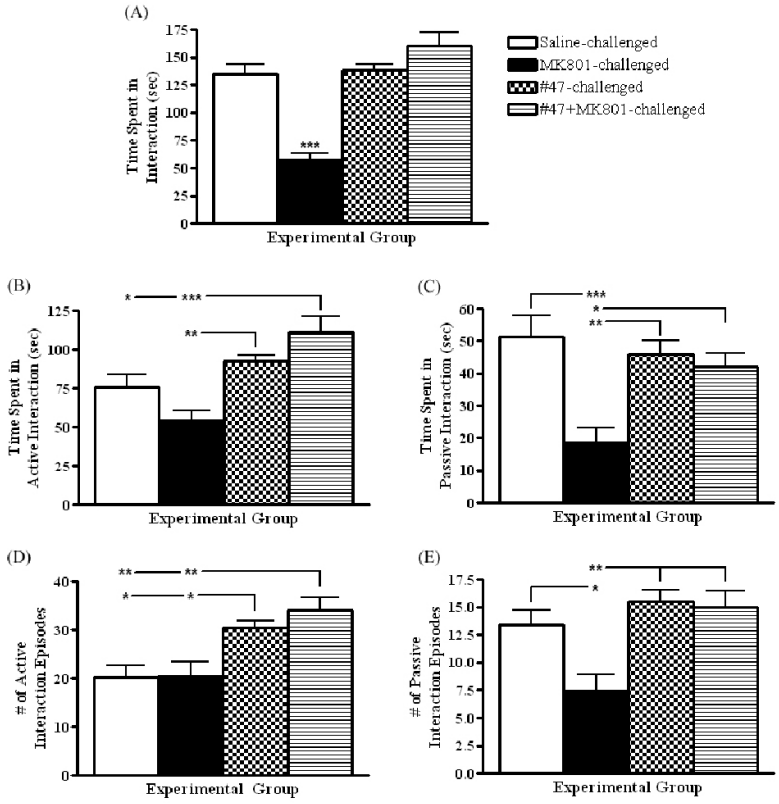

Figure 2.

Graphs depicting social behaviour (mean ± SEM) in saline-, MK-801-, MK-801+PAOPA (#47)-, and PAOPA (#47)-injected animals after 7 days of drug challenge. **(A)** Total time spent in interaction: $F(3,26) = 21.92$, $^{***}p < 0.0001$; **(B)** time spent in active interaction: $F(3,27) = 10.29$, $^{***}p$ 0.0001; **(C)** time spent in passive interaction: $F(3,25) = 8.464$, $^{***}p < 0.004$; **(D)** number of active interaction episodes: $F(3,28) = 8.621$, $^{***}p = 0.0003$; and **(E)** number of passive interaction episodes: $F(3,26) = 7.648$, *** $p = 0.009$. Post-hoc: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N=10/group.