RESEARCH ARTICLE

Positive or negative allosteric modulation of metabotropic glutamate receptor 5 (mGluR5) does not alter expression of behavioral sensitization to methamphetamine [v1; ref status: indexed, http://f1000r.es/o0]

Peter R Kufahl, Natali E Nemirovsky, Lucas R Watterson, Nicholas Zautra, M Foster Olive

Department of Psychology, Arizona State University, Tempe, AZ, 85287-1104, USA

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Abstract

We investigated the role of metabotropic glutamate receptor type 5 (mGluR5) in methamphetamine-induced behavioral sensitization. The mGluR5 positive allosteric modulator (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB) and negative allosteric modulator fenobam were tested in separate experiments. Sprague-Dawley rats were repeatedly injected with 1 mg/kg methamphetamine or saline, and then given a locomotor challenge test using a dose of 0.5 mg/kg methamphetamine. Prior to the challenge test session, rats were injected with CDPPB, fenobam, or a vehicle. Doses from previous studies showed reduced drug-conditioned behavior; however in this study neither CDPPB nor fenobam pretreatment resulted in an altered expression of behavioral sensitization, indicating a lack of mGluR5 involvement in sensitized methamphetamine-induced locomotion. Additionally, the high dose (30 mg/kg) of fenobam resulted in decreased methamphetamine-induced locomotion in rats regardless of drug exposure history, which suggests evidence of nonspecific behavioral inhibition.

Article Status Summary

Referee Responses

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1 **David Triggle**, SUNY at Buffalo USA

2 **Bianca Jupp**, University of Cambridge UK

3 **Sharon Rosenzweig-Lipson**, IVS Pharma Consulting LLC USA

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Corresponding author: Peter R Kufahl (peter.kufahl@asu.edu)

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Introduction

Compulsive drug use and associated maladaptive behaviors are cardinal features of methamphetamine (METH) addiction, and have been strongly associated with the neurochemical consequences of repeated METH abuse[1–3]. Among the various neurotransmitter systems affected by METH exposure is the glutamate system, where long-lasting drug-induced changes are suspected factors underlying craving and persistent vulnerability to relapse[4]. Due to their dual roles in mediating glutamatergic synaptic plasticity and control of synaptic glutamate release, the metabotropic glutamate receptors (mGluRs) have emerged as therapeutic targets of interest in the study of drug addiction[5]. Antagonizing the excitatory post-synaptic metabotropic glutamate receptor 5 (mGluR5) has been recently shown to attenuate the reinforcing effects of METH on a progressive ratio schedule, as well as attenuating drug-seeking behavior in rats previously trained to self-administer METH[6]. Selective stimulation of mGluR5 has been found to improve the rate of extinction learning in rats previously conditioned to the reinforcing effects of cocaine. This study investigated the role of mGluR5 in the behavioral changes induced by repeated exposure to METH, using positive and negative allosteric modulators of mGluR5 function in separate experiments.

The consequences of chronic METH abuse are often studied in the rat model of behavioral sensitization, where chronic METH injections reliably induce an elevated locomotor response to a subsequent METH challenge, relative to rats with no prior history of METH exposure[8–11]. Through their interactions with the dopaminergic projections of the medial forebrain, mGluRs have been found to have roles in both the development and expression of psychostimulant sensitization[12]. mGluR5 has been associated with the locomotor response and reinforcement attributes of psychostimulants since mice lacking this receptor were found not to respond to or self-administer cocaine as wild-type mice[13]. While antagonism of group I mGluRs, which includes mGluR5, in subsequent experiments has generally failed to convincingly affect locomotor sensitization to cocaine[14], the effects of positive allosteric modulation on psychostimulant sensitization have so far remained untested. We evaluated the effect of the mGluR5 positive allosteric modulator (PAM) 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB) and the mGluR5 negative allosteric modulator (NAM) fenobam on the expression of behavioral sensitization to METH. We utilized doses of CDPPB that have been shown to improve extinction learning after METH [30 mg/kg[15]], and cocaine [60 mg/kg[7]], self-administration training, and doses of fenobam (10–30 mg/kg) that have effectively reduced drug-seeking in METH-trained rats in our laboratory[16].

Methods and materials

Subjects

Eighty-eight male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA), weighing 250–275 g, were pair-housed on arrival in a humidity-controlled colony room and maintained in a reversed light/dark cycle with free access to food and water throughout the experiment. All experimentation was conducted during the dark phase of the light/dark cycle. All procedures were conducted with the approval of the Institutional Care and Use Committee at Arizona State University and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (National Research Council)[17].

Drugs

3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB, custom synthesized by Chemir Analytical Services, Maryland Heights, MO) was suspended in 10% *v/v* Tween 80 via sonication to form a 60 mg/ml concentration for intraperitoneal (i.p.) administration. Fenobam (1-(3-chlorophenyl)-3-3-methyl-5-oxo-4H-imidazol-2-yl) urea (custom synthesized by Chemir Analytical Services) was suspended in 0.3% *v/v* Tween 80 vehicle to form a 30 mg/ml concentration for i.p. administration. (+)Methamphetamine hydrochloride (Sigma Aldrich, St Louis, MO) was dissolved in sterile saline for i.p. administration.

Locomotor testing procedures

Locomotor activity was assessed in a Rotorat System apparatus (Med Associates, Mt. St Albans, VT) that measured rotational ambulation, quantified as quarter turns in both directions, within a bowl-shaped arena (Figure 1A). The rats (*N*=43 in the CDPPB experiment, *N*=45 in the fenobam experiment) were divided into groups where half of the rats received five injections of 1 mg/kg METH dissolved in saline (1 ml/kg, i.p.), separated by 48 hours, and the other half received injections of saline of matching volume (Figure 1B). Each injection was immediately followed by a 90 min locomotor test session. After a 6-day waiting period in the colony room, all rats were given a saline injection (1 ml/kg, i.p.) and subjected to a locomotor test session. The next day, rats were injected with 0 (*N*=7), 30 (*N*=8) or 60 mg/kg (*N*=6–7) CDPPB in one experiment; or 0 (*N*=8), 10 (*N*=8) or 30 mg/kg (*N*=6–7) fenobam in the other experiment, and 30 min later given a challenge dose of 0.5 mg/kg METH and subjected to a 90 min locomotor test session.

Additional experiments were conducted to examine the effects of mGluR5 modulation on baseline locomotion. Rats were acclimated to the apparatus in 90 min sessions for two consecutive days, and on the next day given a 90 min locomotor test session 30 min after treatment with 0, 30 or 60 mg/kg CDPPB in one experiment (*N*=5); or 0, 10 or 30 mg/kg fenobam in another experiment (*N*=5).

Data analysis

Data analysis procedures were performed using Prism 5 (GraphPad, La Jolla, CA). For the sensitization experiments, quarter turn data (in either direction, totaled over 90 min) taken during the five chronic treatment sessions were analyzed using 2-way ANOVA with *METH history* (naïve, METH-treated) as a between-subjects factor and *day* (1, 3, 5, 7 or 9) as a within-subjects factor. Locomotor behavior exhibited during the challenge sessions were quantified as quarter turns and analyzed using 2-way ANOVA with *METH history* and *treatment* (0, 30 or 60 mg/kg for the CDPPB experiment, and 0, 15 or 30 mg/kg for the fenobam experiment) as between-subjects factors. Significant interaction effects were followed by pairwise comparisons (Fisher's LSD tests).

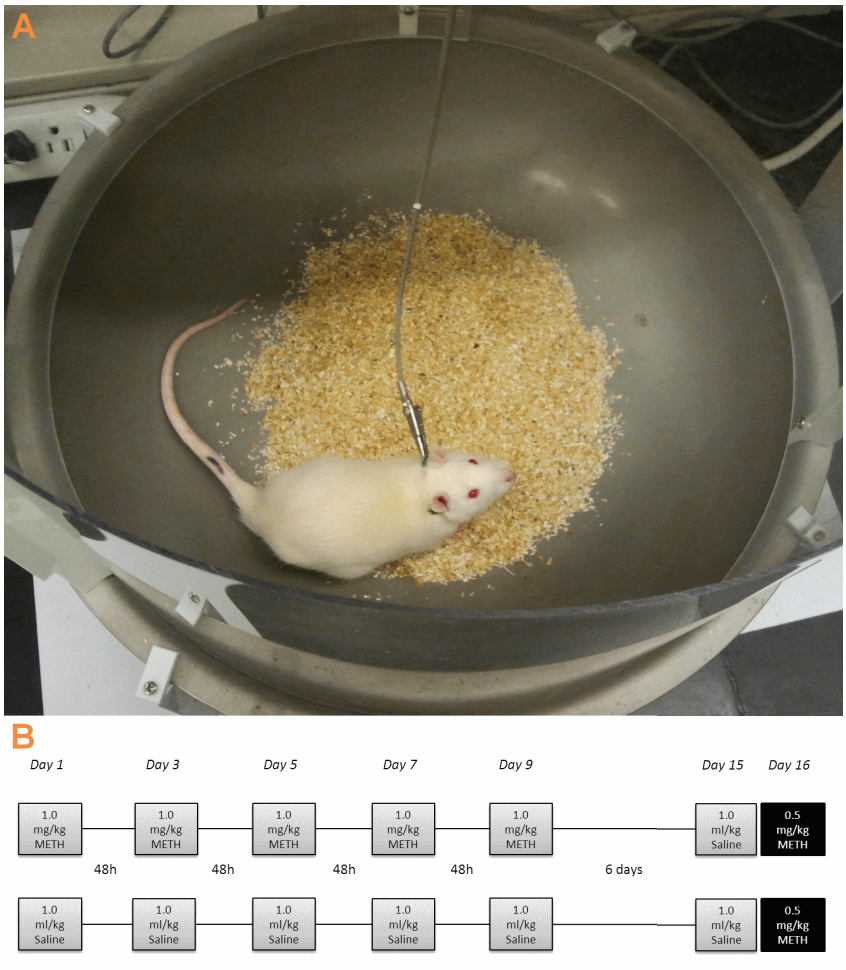

Figure 1. Apparatus and experimental protocol. The locomotor apparatus (**A**) consists of a rotating actuator anchored to a U-shaped bracket over a steel bowl-shaped arena (Med Associates; 18 in top diameter, 6 in bottom diameter, 6 in depth) containing a layer of Sani-chip bedding. The rat is attached to the actuator via 45 cm spring leash terminated with an alligator clip, which is hooked onto a cable tie around the neck for the duration of the test session. The apparatus registers rotational movements as the rat causes the actuator to pivot, accumulated by computer as quarter turns. The experimental procedure (**B**) consisted of three days of acclimation sessions in the locomotor arenas, followed by five injections of METH (1.0 mg/kg, i.p.) or saline separated by 48 hr (Days 1, 3, 5, 7 and 9). After each injection, rats were placed into the locomotor arenas for 90 min and their rotational data were recorded as quarter turns. Rats underwent locomotor testing following a saline injection on Day 15, and these data were balanced between groups assigned to mGluR5 treatment or vehicle treatment. On Day 16, the rats were given an injection of the mGluR5 ligand (CDPPB or fenobam) or vehicle, and tested 30 min later following a probe injection of METH (0.5 mg/kg, i.p.).

In the baseline locomotion experiments, quarter turn data were analyzed using one-way ANOVA with CDPPB or fenobam treatment as the main factor.

Results

Elevated locomotion as a consequence of repeated METH treatment

In the CDPPB experiment, rats treated with repeated METH injections exhibited progressively increasing amounts of quarter turns, as confirmed by a significant main effect of *METH history* ($F_{1,164} = 51.8$, $p < 0.0001$) and a *day* × *METH history* interaction ($F_{4,164} = 3.4$, $p < 0.05$). In these rats, locomotion was significantly elevated from Day 1 levels (2110 ± 284) on Day 5 (3117 ± 401, $p < 0.05$, Fisher's LSD test) and Day 7 (3432 ± 433, $p < 0.01$), but not Day 9 (Figure 2A and Table S1–Table S2). Similarly, in the fenobam experiment, repeated injections of METH but not saline resulted in elevated quarter turns, as confirmed by significant main effects of *day* ($F_{4,172} = 4.1$, $p < 0.005$) and *METH history* ($F_{1,172} = 60.9$, $p < 0.0001$) and a *day* × *METH history* interaction ($F_{4,172} = 6.0$, $p < 0.0005$). In these rats, locomotion was significantly elevated from Day 1 levels (2175 ± 320) on Day 5 (3136 ± 297, $p < 0.05$, Fisher's LSD test), Day 7 (3548 ± 388, $p < 0.01$) and Day 9 (3469 ± 438, $p < 0.05$, Figure 2B and Table S3–Table S4).

Effect of mGluR5 modulation on locomotor sensitization to METH

In the CDPPB experiment, rats with a history of repeated METH treatments exhibited a greater number of quarter turns following a

probe injection of 0.5 mg/kg METH, evidence of locomotor sensitization (Figure 2C and Table S5–Table S6). This elevated response to METH was not attenuated by CDPPB pretreatment, as shown by the existence of a main effect of *METH history* ($F_{1,37}$ = 10.7, $p < 0.005$) but no other main effects or interactions.

In the fenobam experiment, rats with a history of repeated METH treatments also exhibited elevated quarter turns following the 0.5 mg/kg METH probe (Figure 2D and Table S7–Table S8). Pretreatment with fenobam attenuated the locomotor response to METH, regardless of METH exposure history, as revealed by the presence of main effects of *METH history* ($F_{1,39}$ = 20.1, $p < 0.001$) and *treatment* ($F_{2,39}$ = 6.7, $p < 0.005$), but no *METH history* × *treatment* interaction. However, pretreatment with the large dose of fenobam (30 mg/kg) resulted in significantly reduced METH-induced locomotion in rats with a history of chronic 1 mg/kg METH injections (0 mg/kg fenobam: 1192 ± 105 quarter turns vs. 30 mg/kg fenobam: 597 ± 150 quarter turns, $p < 0.01$, two-sample *t*-test), and produced a trend toward a significant reduction

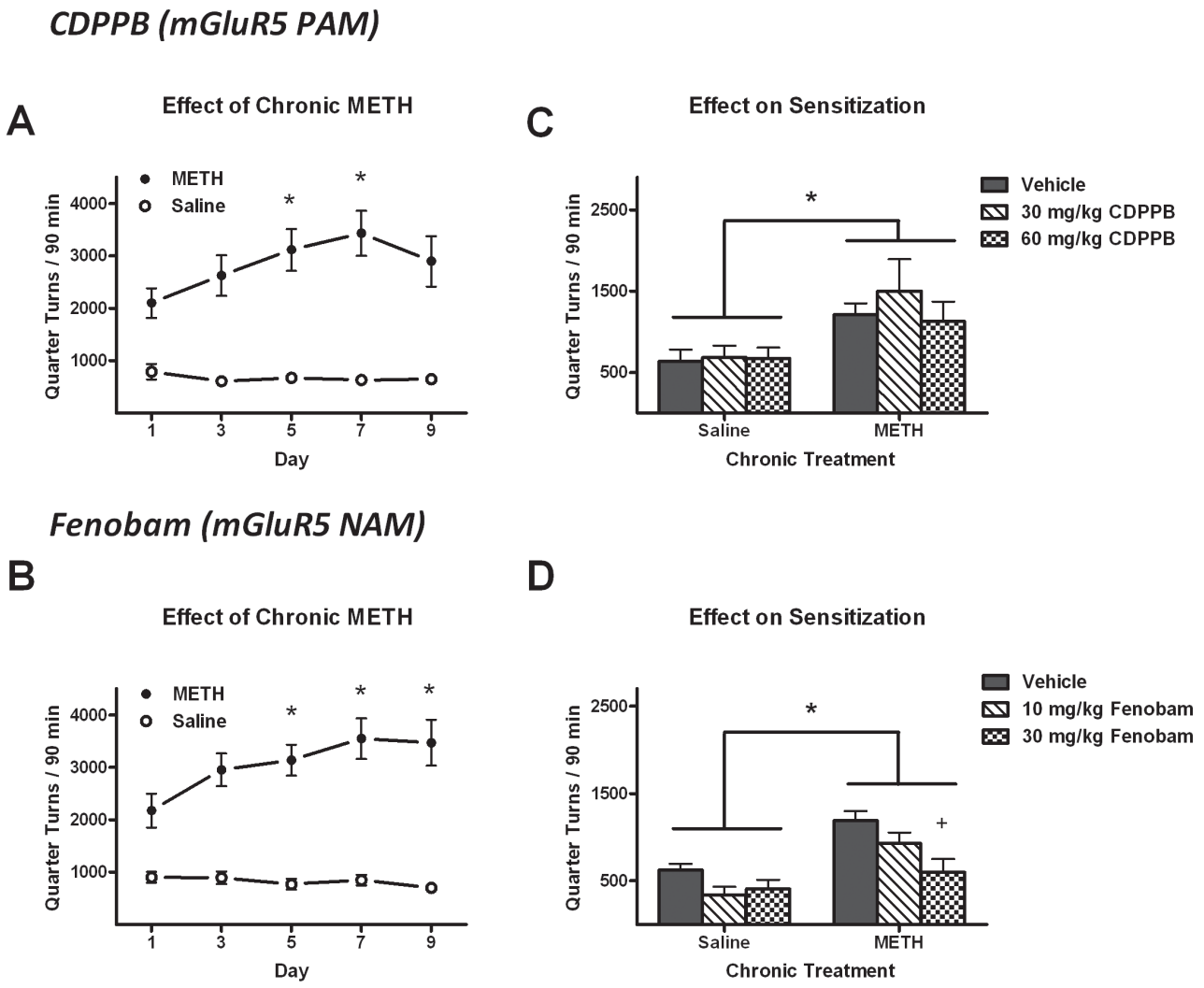

Figure 2. Effects of mGluR5 treatment by CDPPB (top row) or fenobam (bottom row) on locomotion and methamphetamine (METH) behavioral sensitization. In locomotor sessions prior to mGluR5-targeted treatment (**A**-**B**), rats were chronically given 1 mg/kg METH (filled circles) or saline (open circles). In both the CDPPB (**A**) and fenobam (**B**) experiments, the reported quarter turns progressively increased above first-day levels in the METH-exposed groups. **P* < 0.05 different from Day 1 levels. In the subsequent test using 0.5 mg/kg METH in all groups (**C**), rats with a history of chronic METH exposure exhibited elevated locomotor behavior, but CDPPB pretreatment had no effect. In the fenobam experiment (**D**), rats with a history of chronic METH exposure also exhibited elevated locomotor activity, and this behavioral sensitization was not affected by 10 mg/kg fenobam pretreatment. After 30 mg/kg fenobam treatment, the METH-sensitized locomotor response was reduced from the vehicle level. **P* < 0.05 difference between METH history groups, regardless of mGluR5 ligand treatment. +*P* < 0.05 different from vehicle treated group with matching history of METH exposure. *PAM* stands for positive allosteric modulation, and *NAM* stands for negative allosteric modulation.

in rats with a history of saline injections (0 mg/kg fenobam: 622 ± 493 quarter turns vs. 30 mg/kg fenobam: 405 ± 106 quarter turns, P = 0.08).

Effect of mGluR5 modulation on baseline locomotion

All of the tested doses of CDPPB and fenobam had negligible effects on baseline locomotion, measured 30 min after time of injection. Both the 60 mg/kg dose of CDPPB (300 ± 92 quarter turns, vs. 345 ± 43 for the vehicle) and the 30 mg/kg dose of fenobam (389 ± 59 quarter turns, vs. 407 ± 74 for the vehicle) produced slightly attenuated locomotor responses, but no significant effects were revealed by ANOVA in either experiment (Figure 3 and Table S9–Table S10).

Discussion

As expected, rats repeatedly injected with 1 mg/kg METH exhibited greater locomotor activity than the saline-treated rats, and demonstrated more activity during the latter sessions than the initial session. Treatment with CDPPB did not significantly alter METH-induced rotational locomotion, and treatment with fenobam only significantly reduced rotational locomotion at its highest dose (30 mg/kg). Neither CDPPB nor fenobam significantly attenuated the baseline locomotor activity of drug-naïve animals, although the small effect found for 30 mg/kg fenobam in that experiment (Figure 3B) could explain the moderate reduction of quarter turns exhibited by METH-challenged rats (Figure 2D) as a non-specific phenomenon. Thus, locomotor effects of mGluR5 modulation were largely absent at the dose ranges that have been shown in earlier studies to reduce operant behavior motivated by METH or cocaine training[7,15,16,18,19].

These largely negative findings indicate that the maintenance of behavioral sensitization is likely mediated by neurobiological substrates other than mGluR5. These data are also in agreement with previous observations that mGluR5 function does not appear critical for the expression of locomotor sensitization to cocaine[14,20], and extends them to include METH sensitization. Furthermore, the contribution of mGluR5 to initial locomotor responses to injected psychostimulants[13] appears to be replaced by other neurochemical substrates with chronic drug exposure.

While mGluR5 is an important therapeutic target in researching treatments for addiction to psychostimulants as well as other abused substances, there is building evidence that the role of this receptor in drug-related behaviors changes with increasing exposure. A recent study of rats chronically exposed to METH sufficient to induce measurable conditioned place preference found a reduction of surface expression of mGluR5 in the medial prefrontal cortex[21], an area known to contribute to the expression of behavioral sensitization[4]. The current findings using the behavioral sensitization model therefore suggest that the changes in the degree to which mGluR5 mediates drug-stimulated and drug-conditioned behavior previously shown to occur with chronic cocaine exposure might also take place in rats with a history of chronic METH exposure. The possibility of the changing roles among the various mGluR subfamilies as a result of drug exposure merits further studies utilizing animal models of METH-induced activity and motivated behavior.

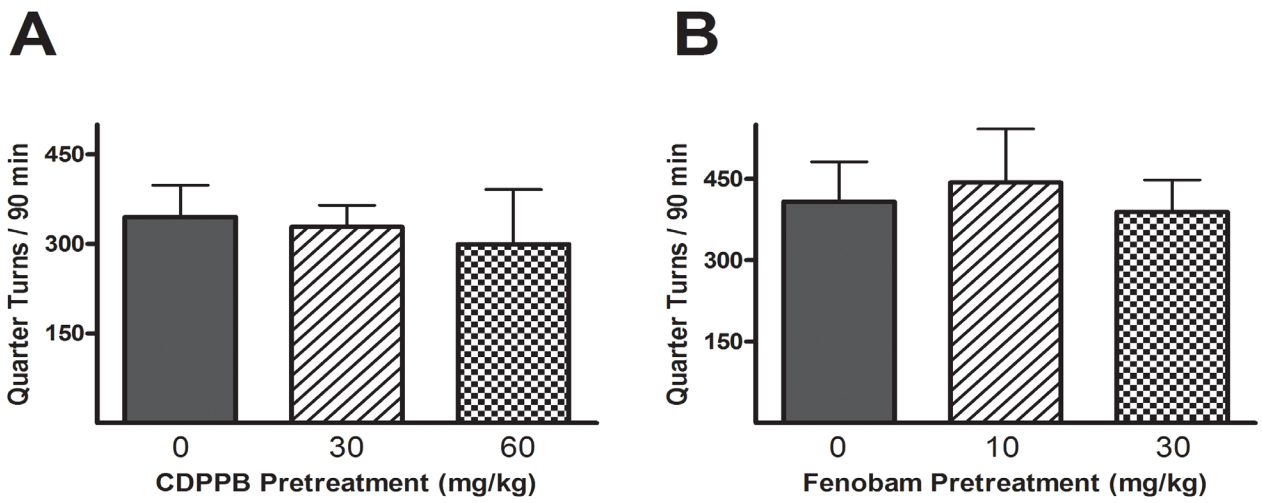

Figure 3. Effects of mGluR5 treatment on baseline locomotion in previously drug-naïve rats. CDPPB (**A**) or fenobam (**B**) was injected 30 min prior to locomotor testing. No significant effects were reported from the quarter turns collected over 90 min sessions.

Author contributions

PRK and MFO conceived of the study and designed the experiments. PRK, NEN, LRW and NZ carried out the research. PRK and MFO prepared the initial draft of the manuscript and all further revisions. All authors approved of the final manuscript for publication.

Competing interests

No relevant competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Supplementary tables

Table S1. CDPPB experiment – locomotor response (total quarter turns over 90 min) after chronic METH treatments. In locomotor sessions prior to mGluR5-targeted treatment, rats were chronically given 1 mg/kg METH i.p. In this experiment, the reported quarter turns progressively increased above first-day levels.

Rat	Day of treatment (1 mg/kg METH)				
	1	3	5	7	9
203	2419	2269	3200	4701	1648
205	3840	3197	2640	6428	1867
213	2436	1520	3379	1243	2273
234	585	990	913	950	577
238	2119	1539	1046	2845	1151
242	1487	1825	1215	1412	1606
244	987	1063	3221	3230	1475
201	2907	2145	3695	5875	4264
207	1454	1568	3963	3442	2566
211	3581	2512	3086	3152	5037
215	1534	1727	3699	1804	1655
232	726	1229	1567	1737	1492
236	3436	7602	6724	7647	7239
246	2275	2439	6851	5386	4959
248	818	2449	1101	1434	2336
253	1016	1599	1306	1097	3678
254	415	3854	1492	4504	2005
255	4608	5091	3499	3836	3150
256	1672	1794	5353	4008	9378
257	1160	2158	5724	1625	1425
258	4639	6600	1770	5712	1024

Table S2. CDPPB experiment – locomotor response (total quarter turns over 90 min) after saline treatments. In locomotor sessions prior to mGluR5-targeted treatment, rats were chronically given 1 ml/kg saline i.p. The reported quarter turns did not significantly change from first-day levels.

Rat	Day of treatment (saline)				
	1	3	5	7	9
202	397	248	181	301	359
206	2964	247	1240	969	1621
214	342	408	1202	539	557
235	644	1205	750	858	653
237	668	919	863	983	675
241	295	516	890	634	646
212	423	607	322	442	289
243	420	557	331	449	683
204	448	321	435	367	288
208	923	940	730	855	1098
216	2078	1246	1651	960	1563
231	653	895	711	604	494
233	1265	640	803	917	612
245	1488	1151	817	820	1138
247	477	549	723	1160	885
251	74	178	381	214	424
252	67	26	77	124	128
271	316	797	454	391	298
272	202	202	190	226	136
275	1288	495	642	1063	495
263	959	681	941	576	681
264	922	490	421	347	445

Table S3. Fenobam experiment – locomotor response (total quarter turns over 90 min) after chronic METH treatments. In locomotor sessions prior to mGluR5-targeted treatment, rats were chronically given 1 mg/kg METH i.p. In this experiment, the reported quarter turns progressively increased above first-day levels.

Rat	Day of treatment (1 mg/kg METH)				
	1	3	5	7	9
362	315	1314	1818	1068	966
364	1691	1869	4040	3447	2381
366	3813	2074	3556	6491	7163
368	1261	2087	926	1961	2489
377	1888	3952	4491	3738	3905
383	1547	1065	3203	3511	2747
385	1989	1586	2476	3679	2865
387	1214	1960	536	1807	963
352	1983	1325	1693	1853	1865
354	2966	2963	4444	4726	5932
356	7984	5835	6043	6727	7125
358	1798	4432	3827	7331	6979
371	2167	2344	2538	2110	3273
373	2342	3220	1545	2069	2442
375	1796	3876	2117	3638	2653
381	1863	2059	3483	3319	3158
313	676	3157	2552	2467	5972
314	1868	5270	5345	2352	5141
315	3195	2660	3308	6766	951
316	1600	6267	3301	3516	3549
317	1741	3105	3223	1767	717
318	2154	2530	4528	3704	3091

Table S4. Fenobam experiment – locomotor response (total quarter turns over 90 min) after saline treatments. In locomotor sessions prior to mGluR5-targeted treatment, rats were chronically given 1 ml/kg saline i.p. The reported quarter turns did not significantly change from first-day levels.

Rat	Day of treatment (1 mg/kg saline)				
	1	3	5	7	9
351	979	1042	670	763	727
357	2092	2047	1343	1656	1664
361	418	369	348	387	433
367	1309	1444	1751	1440	1480
372	345	244	486	430	359
374	1120	1177	847	1412	1195
384	1307	613	878	598	730
386	1216	1368	939	1246	633
353	852	701	466	528	636
355	452	452	320	1445	1010
363	735	1092	1185	1084	733
365	1308	2251	2095	1649	1018
376	1406	748	1147	1024	1078
378	1146	762	816	948	599
382	540	191	393	438	567
388	1338	1233	970	1146	678
311	225	378	219	390	362
312	192	255	152	297	161
323	959	1028	941	576	681
324	922	490	421	347	445
331	316	797	454	391	298
332	202	202	190	226	136
335	1288	1623	642	1063	495

Table S5. CDPPB (0, 30, 60 mg/kg) effects on METH locomotor response (total quarter turns over 90 min) – rats with histories of saline injections. In the Day 16 tests using 0.5 mg/kg METH in all groups, rats with a history of chronic saline injections exhibited elevated locomotor behavior, but CDPPB pretreatment had no effect.

Rat	CDPPB	Quarter turns
202	0	910
206	0	215
214	0	363
235	0	952
237	0	1001
241	0	871
212	0	135
243	30	1495
204	30	885
208	30	129
216	30	692
231	30	281
233	30	744
245	30	683
247	30	539
251	60	1117
252	60	358
271	60	668
272	60	127
275	60	1113
263	60	681
264	60	622

Table S6. CDPPB effects on METH locomotor response (total quarter turns over 90 min) – rats with histories of METH injections. In the Day 16 tests using 0.5 mg/kg METH in all groups, rats with a history of chronic METH exposure exhibited elevated locomotor behavior, but CDPPB pretreatment had no effect.

Rat	CDPPB	Quarter turns
203	0	1425
205	0	1767
213	0	1112
234	0	933
238	0	1100
242	0	653
244	0	1475
201	30	542
207	30	1674
211	30	1325
215	30	1701
232	30	904
236	30	1858
246	30	3808
248	30	210
253	60	345
254	60	397
255	60	1675
256	60	1414
257	60	1252
258	60	1662

Table S7. Fenobam (0, 10, 30 mg/kg) effects on METH locomotor response (total quarter turns over 90 min) – history of saline injections. In the Day 16 tests using 0.5 mg/kg METH in all groups, rats with a history of chronic saline injections exhibited elevated locomotor behavior, but fenobam pretreatment had no effect.

Rat	Fenobam	Quarter turns
351	0	257
357	0	770
361	0	661
367	0	909
372	0	449
374	0	587
384	0	693
386	0	656
353	10	748
355	10	181
363	10	394
365	10	725
376	10	298
378	10	910
382	10	480
388	10	207
311	30	315
312	30	101
323	30	274
324	30	219
331	30	955
332	30	465
335	30	508

Table S8. Fenobam (0, 10, 30 mg/kg) effects on METH locomotor response – history of METH injections. In the Day 16 tests using 0.5 mg/kg METH in all groups, rats with a history of chronic METH exposure exhibited elevated locomotor behavior, and 30 mg/kg but not 10 mg/kg fenobam resulted in reduced quarter turns relative to vehicle-pretreated animals.

Rat	Fenobam	Quarter turns
362	0	1551
364	0	1190
366	0	1111
368	0	611
377	0	1509
383	0	1354
385	0	1050
387	0	1162
352	10	929
354	10	1263
356	10	1084
358	10	1391
371	10	861
373	10	614
375	10	281
381	10	1009
313	30	275
314	30	927
315	30	419
316	30	619
317	30	218
318	30	1129

Table S9. Locomotor response (total quarter turns over 90 min) to CDPPB (0, 30, 60 mg/kg).

Rat	CDPPB	Quarter turns
101	0	304
104	0	171
107	0	490
110	0	353
113	0	407
102	30	353
105	30	401
108	30	198
111	30	384
114	30	307
103	60	650
106	60	120
109	60	245
112	60	199
115	60	285

Table S10. Locomotor response (total quarter turns over 90 min) to Fenobam (0, 10, 30 mg/kg).

Rat	Fenobam	Quarter turns
403	0	365
406	0	577
409	0	584
412	0	226
415	0	286
401	10	317
404	10	468
407	10	339
410	10	274
413	10	817
402	30	478
405	30	465
408	30	274
411	30	219
414	30	508

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Current Referee Status:

Referee Responses for Version 1

Sharon Rosenzweig-Lipson
Pharmacology & Physiology Faculty, IVS Pharma Consulting LLC, East Brunswick, NJ, USA

Approved with reservations: 06 June 2013

Referee Report: 06 June 2013
The present studies investigated the effects of positive and negative allosteric modulation of mGluR5 receptors on methamphetamine sensitization. The authors conclude that “Positive or negative allosteric modulation of metabotropic glutamate receptor 5 (mGluR5) does not alter expression of behavioral sensitization to methamphetamine”. While the data, in part, support those conclusions; the presence of an effect of 30 mg/kg fenobam on methamphetamine sensitization suggests at least some role of mGlur5 NAM activity. Evaluation of an additional NAM or a higher dose of fenobam would allow for a firmer conclusion on this point.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Bianca Jupp
Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, UK

Approved with reservations: 14 May 2013

Referee Report: 14 May 2013
The publication by Kufahl and colleagues presents an investigation into the effect of positive and negative allosteric modulators of mGluR5 on the expression of locomotor sensitization to the psychostimulant methamphetamine, the results of which apparently support previous data regarding a lack of involvement of this receptor in the expression of sensitized locomotion. While the study is well designed, a critical component of the results was omitted making the interpretation of the current data impossible, and severely undermines the author’s conclusions.

Specifically, while the authors methodologically included a saline challenge when assessing the expression of sensitization, they failed to report these results. Without this it is not possible to determine if indeed the increase in locomotor activity observed in the METH pre-treatment group is due to expression of conditioned hyperactivity or locomotor sensitization. I suspect it may be the former due to the apparently reduced locomotor activity (approx 1200) observed during this challenge session even when compared to acute METH (approx 2000). Usually expression of locomotor sensitization is much greater than the final conditioning session. It is therefore unreasonable for the authors to conclude that PAM or NAM of mGluR5 has no effect on expression of sensitization as it is not even clear if the animals are

expressing sensitized behaviour. Inclusion of the saline challenge data will clarify this point.

Have the authors considered using a longer 'waiting' period between development and testing expression? A recent study by Timmer and Steketee, 2012 found that intra-prefrontal cortex injections of the mGluR5 PAM MTEP reduced the expression of locomotor sensitization to cocaine following 21 days but not 7 days. The authors should include this in the discussion of their results.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

David Triggle
School of Pharmacy and Pharmaceutical Sciences, SUNY at Buffalo, Buffalo, NY, USA

Approved: 18 March 2013

Referee Report: 18 March 2013
Although this is a report of primarily negative findings it is not without value and should be published. The premise of the research is reasonable, the methods appropriate and the conclusions appropriate and not overreaching. Essentially, the workers have demonstrated through behavioural studies in rats that allosteric modulation – either positive or negative – of the metabotropic glutamate receptor 5 does not modify methamphetamine-induced behavioural sensitization. This adds to our knowledge of the effects of methamphetamine in its abuse.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.