RESEARCH ARTICLE

Positive or negative allosteric modulation of metabotropic glutamate receptor 5 (mGluR5) does not alter expression of behavioral sensitization to methamphetamine [version 1; peer review: 1 approved, 2 approved with reservations]

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

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.

Keywords
methamphetamine, sensitization, mGluR5, allosteric modulator
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 abuse1-3. 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 relapse4. 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 addiction3. Antagonizing the excitatory postsynaptic 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 METH4. 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 exposure5-11. 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 sensitization12. 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 mice13. While antagonism of group I mGluRs, which includes mGluR5, in subsequent experiments has generally failed to convincingly affect locomotor sensitization to cocaine14, 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], self-administration training, and doses of fenobam (10–30 mg/kg) that have effectively reduced drug-seeking in METH-trained rats in our laboratory16.

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 (naive, 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).
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 ($2110 \pm 284$) on Day 5 ($3117 \pm 401$, $p < 0.05$, Fisher’s LSD test) and Day 7 ($3432 \pm 433$, $p < 0.01$), 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 ($2175 \pm 320$) on Day 5 ($3136 \pm 297$, $p < 0.05$, Fisher’s LSD test), Day 7 ($3548 \pm 388$, $p < 0.01$) and Day 9 ($3469 \pm 438$, $p < 0.05$, 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
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 cocaine13,14,15,16,17,18.

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 cocaine14,15,16,17,18, and extends them to include METH sensitization. Furthermore, the contribution of mGluR5 to initial locomotor responses to injected psychostimulants13 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 cortex19, an area known to contribute to the expression of behavioral sensitization20. 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](image_url)

**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.

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  | 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  | 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          |

References

1. McLellan AT, Lewis DC, O’Brien CP, et al.: Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. JAMA. 2000; 284(13): 1689–95. PubMed Abstract | Publisher Full Text

2. WHO. Amphetamine-type stimulants: a report from the WHO meeting on amphetamines, MDMA and other psychostimulants. W. Substance Abuse Dept., Editor, World Health Organization (WHO): Geneva 1996. Reference Source

3. Barr AM, Panenka WJ, MacEwan GW, et al.: The need for speed: an update on methamphetamine addiction. J Psychiatry Neurosci. 2006; 31(5): 301–13. PubMed Abstract | Free Full Text

4. Tzschentke TM, Schmidt WJ: Glutamatergic mechanisms in addiction. Mol Psychiatry. 2003; 8(4): 373–82. PubMed Abstract | Publisher Full Text

5. Gass JT, Olive MF: Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol. 2006; 71(1): 218–65. PubMed Abstract | Publisher Full Text

6. Gass JT, Osborne MP, Watson NL, et al.: mGluR5 antagonism attenuates methamphetamine reinforcement and prevents reinstatement of methamphetamine-seeking behavior in rats. Neuropharmacology: 2009; 34(4): 820–33. PubMed Abstract | Publisher Full Text | Free Full Text

7. Cleva RM, Hicks MP, Gass JT, et al.: mGluR5 positive allosteric modulation enhances extinction learning following cocaine self-administration. Behav Neurosci. 2011; 125(1): 10–9. PubMed Abstract | Publisher Full Text | Free Full Text

8. Fujii K, Kojii Y, Hiroaka S, et al.: Differential regulation by stimulants of neocortical expression of m1 receptors: coro, and homer1 mRNA in the rats treated with repeated methamphetamine. Synapse. 2003; 49(3): 143–9. PubMed Abstract | Publisher Full Text

9. Ohmori T, Abeakawa T, Koyama T; Environment modifies the expression of behavioral sensitization produced by methamphetamine: behavioral and neurochemical studies. Behav Pharmacol. 1995; 6(2): 133–142. PubMed Abstract | Publisher Full Text

10. Ohmori T, Abeakawa T, Koyama T: Scopolamine prevents the development of sensitization to methamphetamine. Life Sci. 1995; 56(14): 1223–9. PubMed Abstract | Publisher Full Text

11. Ohmori T, Abeakawa T, Koyama T; Opioids potentiate and scopolamine prevents the development of sensitization to methamphetamine. J Addict Res Ther. 2011; 2(4): 873–4. PubMed Abstract | Publisher Full Text | Free Full Text

12. Vezina P, Kim HJ: Metabotropic glutamate receptors and the generation of locomotor activity: interactions with midbrain dopamine. Neurosci Biobehav Rev. 1999; 23(4): 577–89. PubMed Abstract | Publisher Full Text

13. Chiamulera C, Epping-Jordan MP, Zocchi A, et al.: Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. Nat Neurosci. 2001; 4(9): 873–4. PubMed Abstract | Publisher Full Text

14. Dravolina OA, Danysh W, Bespalov AV: Effects of group I metabotropic glutamate receptor antagonists on the behavioral sensitization to motor effects of cocaine in rats. Psychopharmacology (Berl). 2006; 187(4): 397–404. PubMed Abstract | Publisher Full Text

15. Kufal PR, Hood LE, Nemirovsky NE, et al.: Positive Allosteric Modulation of mGluR5 Accelerates Extinction Learning but Not Relearning Following Methamphetamine Self-Administration. Front Pharmacol. 2012; 3: 194. PubMed Abstract | Publisher Full Text | Free Full Text

16. Watterson LR, Kufal PR, Nemirovsky NE, et al.: Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a metabotropic glutamate receptor 5 negative allosteric modulator. Psychopharmacology (Berl). 2013; 225(1): 151–9. PubMed Abstract | Publisher Full Text | Free Full Text

17. Council NR Guide for the care and use of laboratory animals. 8 ed, Washington, DC: National Academies Press 2011. PubMed Abstract

18. Gass JT, Olive MF: Positive allosteric modulation of mGluR5 receptors facilitates extinction of a cocaine contextual memory. Biol Psychiatry. 2009; 65(8): 717–20. PubMed Abstract | Publisher Full Text | Free Full Text

19. Widholm JJ, Gass JT, Cleva RM, et al.: The mGluR5 Positive Allosteric Modulator CDPPB Does Not Alter Extinction or Contextual Reinstatement of Methamphetamine-Seeking Behavior in Rats. J Addict Res Ther. 2011; S1(4). PubMed Abstract | Publisher Full Text | Free Full Text

20. Herzog V, Schmidt WJ: Effects of MPEP on locomotion, sensitization and conditioned reward induced by cocaine or morphine. Neupharmacology 2004; 47(7): 973–84. PubMed Abstract | Publisher Full Text

21. Herrold AA, Voigt RM, Napier TC; Brain region-selective cellular redistribution of mGluR5 but not GABAB receptors following methamphetamine-induced associative learning. Synapse. 2011; 65(12): 1333–43. PubMed Abstract | Publisher Full Text
Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 06 June 2013

https://doi.org/10.5256/f1000research.864.r987

© 2013 Rosenzweig-Lipson S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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

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.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and 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.

Reviewer Report 14 May 2013

https://doi.org/10.5256/f1000research.864.r952

© 2013 Jupp B. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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

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.

**Competing Interests:** No competing interests were disclosed.

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

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com