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1 **Title: Role of the medial prefrontal cortex in the effects of rapid acting**

2 **antidepressants on decision-making biases in rodents**

3 **Running title: Rapid antidepressant effects on decision-making biases**

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Abstract

Major Depressive Disorder is a significant and costly cause of global disability. Until the discovery of the rapid acting antidepressant (RAAD) effects of ketamine, treatments were limited to drugs that have delayed clinical benefits. The mechanism of action of ketamine is currently unclear but one hypothesis is that it may involve neuropsychological effects mediated through modulation of affective biases (where cognitive processes such as learning and memory and decision-making are modified by emotional state). Previous work has shown that affective biases in a rodent decision-making task are differentially altered by ketamine, compared to conventional, delayed onset antidepressants. This study sought to further investigate these effects by comparing ketamine with other NMDA antagonists using this decision-making task. We also investigated the subtype selective GluN2B antagonist, CP-101,606 and muscarinic antagonist scopolamine which have both been shown to have RAAD effects. Both CP-101,606 and scopolamine induced similar positive biases in decision-making to ketamine, but the same effects were not seen with other NMDA antagonists. Using targeted medial prefrontal cortex (mPFC) infusions, these effects were localised to the mPFC. In contrast, the $GABA_A$ agonist, muscimol, induced general disruptions to behaviour. These data suggest that ketamine and other RAADs mediate a specific effect on affective bias which involves the mPFC. Non-ketamine NMDA antagonists lacked efficacy and we also found that temporary inactivation of the mPFC did not fully recapitulate the effects of ketamine, suggesting a specific mechanism.

Introduction

Major Depressive Disorder (MDD) is a prevalent psychiatric disorder, affecting over 300 million people globally[1]. It is the leading worldwide cause of disability, and, until recently, pharmacological treatments were limited to drugs that take weeks to improve symptoms and subjective reporting of mood[2]. The discovery of the rapid acting antidepressant (RAAD) effects of ketamine, an NMDA receptor antagonist, has rejuvenated the field by demonstrating that subjective changes in mood in depressed patients can be seen less than 2 hours following administration and are sustained for at least 7 days in some patients[3]. Although this RAAD has been shown repeatedly[4,5, 6,7,8], the mechanism underlying this effect is unclear, and better understanding could be critical for the development of new, fast-acting treatments.

Patients with MDD exhibit affective biases, whereby impairments in emotional processing leads to reduced positive and/or enhanced negative biases in multiple cognitive domains, including attention, memory, emotional interpretation and decision-making[9,10,11]. In humans, acute (and chronic) treatment with conventional antidepressants induces positive biases in emotional memory and recognition in healthy controls[12,13,14] and patients[15], despite a lack of subjectively reported change in mood. It has been suggested that similar affective biases can also be measured in non-human animals using learning and memory tasks[16] and in decision making under ambiguity (first demonstrated by Harding et al.[17] using a judgement bias task). For review and more detailed discussion of translational studies of affective biases see Robinson and Roiser[18]. Judgement bias tasks (also known as cognitive bias tasks, or ambiguous cue interpretation tasks) were first developed as a cognitive test to measure animal affect (see reviews by Mendl et al.[19] and Roelofs et al.[20]). In the task, animals are trained to associate the presentation two distinct reference cues with two differently valenced outcomes (e,g. positive: reward/high reward, or negative/less positive: punishment/low reward). After training, individuals are presented with untrained, ambiguous cue(s), and responses to these are measured to see whether they respond with a positive or

62 negative bias (more responses matching the positive or negative choice respectively). A

63 recent systemic review and meta-analysis across judgement bias tasks in animals has

64 shown that across 20 published research articles, pharmacological manipulations to induce

65 changes in affective state overall did alter decision making about ambiguous cues as

66 predicted[21], demonstrating the validity of these types of tasks. In previous work in rodents in

67 our lab using a reward-based judgement bias task (first reported by Hales et al.[22]), decision

68 making biases were differentially altered by conventional, delayed acting antidepressants

69 versus the RAAD ketamine[24]. In this task, where reference cues are associated with more or

70 less positive outcomes[22-24], we found that an acute, low dose of ketamine, but not acute

71 treatment with another NMDA receptor antagonist, PCP, immediately induced more

72 optimistic decision making, the direction that would be induced by a more positive affective

73 state, whereas acute treatment with conventional antidepressants had no effect on bias[24].

74 However, when given chronically, the conventional antidepressant fluoxetine did induce a

75 positive bias[24], but only over a timescale similar to the drugs' efficacy in patients, as

76 measured by self-reported improvements in symptoms and mood[25]. The same pattern was

77 also seen in this task with negative affective states, where a chronic stress manipulation, but

78 not an acute stressor, induced more pessimistic decision making at later timepoints[22].

79 The aim of this study was to build upon these findings by testing a selection of other drugs

80 that act via NMDA receptor antagonism: lanicemine, a low-trapping NMDA receptor channel

81 blocker developed for the treatment of MDD, but failed to show efficacy in clinical trials[26];

82 memantine, an Alzheimer's medication that is a moderate affinity, non-competitive NMDA

83 receptor antagonist, but also lacked antidepressant efficacy in clinical trials[5,27]; and MK-801,

84 a potent, non-competitive NMDA receptor antagonist that has shown RAAD efficacy in

85 animal models[28]. We also tested other compounds that have been shown to have RAAD in

86 human clinical trials: the GluN2B subunit selective NMDA receptor antagonist CP-101,606[29],

87 and the acetylcholine muscarinic receptor antagonist scopolamine[30]. We also tested

88 additional doses of ketamine and PCP to ensure we had examined effects across a wider

89   range of receptor occupancy and in line with doses commonly used in preclinical animal

90   models used to study depression[31]. To investigate the mechanism underlying the rapid

91   positive change in decision-making bias we tested local administration of drugs shown to

92   cause this effect directly into the prefrontal cortex (PFC), a brain area thought to be critical in

93   the mechanism of RAAD of ketamine[32,33] and previously shown to modulate learning biases

94   in rodents[34].

Materials and Methods

Animals and apparatus

Three cohorts of male Lister Hooded rats (each cohort n=16) were used (Envigo, UK). Rats were pair-housed with environmental enrichment, consisting of a red 3 mm Perspex house (30x10x17cm), a large cardboard tube (10cm diameter), a wood chew block (9x2.5x2.5cm) and a rope tied across the cage lid (the rope was not present in cages for cohort 3 post-surgery to avoid any possibility of implanted cannula getting caught). Animals were kept under temperature (19-23°C) and humidity (45-65%) controlled conditions on a 12-h reverse lighting cycle (lights off at 08:00h). Water was available *ad libitum* in the home cage, but rats were maintained at no less than 90% of their free-feeding body weight, matched to a standard growth curve, by restricting access to laboratory chow (LabDiet, PMI Nutrition International) to ~18g per rat per day. All procedures were carried out under local institutional guidelines (University of Bristol Animal Welfare and Ethical Review Board) and in accordance with the UK Animals (Scientific Procedures) Act 1986. Rats weighed 270-305 g (cohort 1) / 250-295 g (cohort 2) / 240-290 g (cohort 3) at the start of training, and 400-465 g (cohort 1) / 360-460 g (cohort 2) / 320-380 g (cohort 3) by the start of experimental manipulations. During experiments all efforts were made to minimise suffering including using a low stress method of drug administration[35], and at the end of experiments rats were killed by giving an overdose of sodium pentobarbitone (200mg/kg). Behavioural testing was carried out between 0800 and 1800h, using standard rat operant chambers (Med Associates, Sandown Scientific, UK) as previously described[22,24]. Operant chambers (30.5x24.1x21.0cm) used for behavioural testing were housed inside a light-resistant and sound-attenuating box. They were equipped with two retractable response levers positioned on each side of the centrally located food magazine. The magazine had a house light (28V, 100mA) located above it. An audio generator (ANL-926, Med Associates, Sandown Scientific, UK) produced tones that were delivered to each chamber via a speaker positioned

121   above the left lever. Operant chambers and audio generators were controlled using K-Limbic

122   software (Conclusive Solutions Ltd., UK).

123   *Judgement bias training*

124   Animals were trained and tested using a high versus low reward version of the judgement

125   bias task as previously reported[22,24]. Rats were first trained to associate one tone (2kHz at

126   83dB rats, designated high reward) with a high value reward (four 45mg reward pellets;

127   TestDiet, Sandown Scientific, UK) and the other tone (8kHz at 66dB, designated low reward)

128   with a low value reward (one 45mg reward pellet) if they pressed the associated lever (either

129   left or right, counterbalanced across rats) during the 20s tone (see Figure 1 for a detailed

130   depiction of the task). Unless otherwise specified in Table S1, response levers were

131   extended at the beginning of every session and remained extended for the duration of the

132   session (maximum one hour for all session types). All trials were self-initiated via a head

133   entry into the magazine, followed by an intertrial interval (ITI), and then presentation of the

134   tone. Pressing the incorrect lever during a tone was punished by a 10s timeout, as was an

135   omission if the rat failed to press any lever during the 20s tone. Lever presses during the ITI

136   were punished by a 10s timeout. During a timeout, the house light was illuminated, and

137   responses made on levers were recorded but had no programmed consequences.

138   Animals underwent a graduated training, and were required to meet criteria for at least two

139   consecutive days before progressing to the next stage. Training stages were as follows:

140   1) Magazine training: tone played for 20s followed by release of one pellet into

141   magazine. Criteria:.20 pellets eaten for each tone frequency.

142   2) Tone training: response on lever during tone rewarded with one pellet. Only one tone

143   frequency, and one lever available per session. Criteria: >50 trials completed.

144   3) Discrimination training: response on correct corresponding lever only during tone

145   rewarded with one pellet. Both tones played (pseudorandomly) and both levers

146   available. Criteria: >70% accuracy for both tones, <1:1 ratio of correct:premature

7

147   responses and no significant difference on any behavioural measures analysed over

148   three sessions.

149   4) Reward magnitude training: As for discrimination training but 2kHz tone now

150   rewarded with four pellets, 8kHz tone rewarded with one pellet. Criteria: as for

151   discrimination training but with >60% accuracy for both tones.

152   All training sessions consisted of a maximum of 100 trials. Table S1 contains full details of

153   training stages and criteria used. Rats were considered trained when they maintained stable

154   responding for three consecutive days. This was after a maximum of 29 sessions for cohort

155   1, 25 sessions for cohort 2, and 25 sessions for cohort 3 (see Table S1 for details of session

156   numbers for each training stage).

157   *Judgement bias testing*

158   Baseline sessions (100 trials: 50 high and 50 low reward tones; presented pseudorandomly,

159   for details see Table S1) were conducted on Monday and Thursday. Probe test sessions

160   (120 trials: 40 high reward, 40 low reward, and 40 ambiguous midpoint tones that were 5kHz

161   at 75dB; pseudorandomly, for details see Table S1) were conducted on Tuesday and Friday.

162   The midpoint tone was randomly reinforced whereby 50% of trials had outcomes as for the

163   high reward tone, and 50% had outcomes as for the low reward tone. This was to ensure a

164   specific outcome could not be learnt, and to maintain responding throughout the experiments

165   (see Figure 1 and Table S1 for a detailed description of how this was implemented). Cohort

166   1 were used to test the effect of acute systemic treatments with putative RAAD and other

167   NMDA receptor antagonists. Cohort 2 were made up of two groups of eight rats that had

168   previously been used as control animals in another experiment (data not shown) and were

169   then used for the extension of doses of ketamine and PCP. Cohort 3 were used for mPFC

170   infusion experiments. For further details of the different treatments received by each cohort

171   see Table S2.

172 *Study 1: the effect of acute, systemic treatments with RAADs and NMDA receptors*

173 *antagonists on judgement bias.*

174 **Experimental design**: Each study used a within-subject fully counterbalanced drug

175 treatment schedule (see Table S2 for details of individual treatments). The study design

176 followed the same procedures as used in our earlier work characterising the effects of

177 ketamine in the JBT[24]. We also included a replication study with systemic ketamine in our

178 infusion cohort in order to confirm similar systemic effects before proceeding to the infusion

179 studies. Each animal received all doses for any given treatment in a counter-balanced

180 design with drug doses separated by a minimum of 72 hrs and at least a one-week drug free

181 period between different treatments. There is the potential for compensatory changes to

182 develop due to repeated testing and the drug treatments, but these are minimised by

183 managing washout periods and also recording and analysing the animals' baseline data in

184 between drug studies. We are aware of the increasing evidence that ketamine, and

185 potentially the other treatments tested, can have long lasting effects[36] which may not fully

186 reverse over this dosing schedule. The counterbalanced design does mitigate the risks of

187 any bias of these schedules on the results but there may be carryover effects which could

188 influence the main findings. We carry out analysis of the between treatment baseline

189 sessions (data shown in Table S3-S6) and these analyses do not suggest that the

190 behavioural parameters we measure were affected for any of the cohorts over time. All drugs

191 were given by intraperitoneal injection using a low-stress, non-restrained technique[35].

192 Ketamine[¥] (Sigma-Aldrich, UK), scopolamine[§] (Tocris, UK), lanicemine[¥] (Sigma Aldrich, UK),

193 memantine[¥] (Tocris, UK), MK-801[§] (Tocris, UK) and PCP[¥] (Sigma Aldrich, UK) were

194 dissolved in 0.9% sterile saline and given 30[§] or 60[¥] minutes prior to testing. CP-101,606

195 (Experiment 1: Sigma Aldrich, UK; Experiment 2: Boehringer Ingelheim GmbH) was

196 dissolved in 5% DMSO, 10% cremaphor and 85% sterile saline and given 60 minutes prior

197 to testing. Drug doses were selected based on previous rodent behavioural studies[24,37].

198 Doses for ketamine and PCP were chosen to extend the range of doses tested in this task

199   e.g. higher doses of ketamine and lower doses of PCP were used than previously[24]. For all

200   studies, the experimenter was blind to drug dose. The order of testing for each cohort is

201   displayed in Table S2.

202   *Study 2: mPFC cannulation and infusions*

203   **mPFC cannulation**: To localize the site and mechanism of action of RAAD drugs, rats were

204   implanted with mPFC guide cannula. Rats were anesthetised with isoflurane/O2 and

205   secured in a stereotaxic frame. Bilateral 32-gauge guide cannulae (Plastics One, UK) were

206   implanted in the mPFC according to the stereotaxic coordinates: anteroposterior +2.7mm,

207   lateral ±0.75mm and dorsoventral −2.0mm from bregma[38]. The cannulae were secured to

208   the skull with gentamicin bone cement (DePuy CMW, UK) and stainless steel screws

209   (Plastics One, UK). Animals received long acting local anaesthetic during surgery, and after

210   surgery the animals were housed individually for 2-3 hours then allowed 10-13 days

211   recovery in normal paired housing conditions. Following the recovery period, rats underwent

212   one week of baseline sessions to re-establish performance. Following this, one week of

213   probe testing was carried out to check that judgement of the ambiguous tone had not altered

214   after surgery. Based on this, another two weeks of probe testing (4 test sessions) was then

215   conducted.

216   **Systemic ketamine:** Following this, an acute systemic treatment with ketamine was given

217   as a positive control manipulation to ensure that bias could still be manipulated post-surgery.

218   This study was a within-subject fully counterbalanced design, with two treatments (see Table

219   S2, top row of section 3), with the experimenter blind to drug dose. Ketamine (1.0 mg/kg,

220   Sigma Aldrich, UK) was dissolved in 0.9% sterile saline vehicle (0.0 mg/kg) and was given

221   by intraperitoneal injection using a low-stress, non-restrained technique[35] 60 minutes prior to

222   testing.

223   **Infusion Procedure:** Rats were then used for mPFC infusion experiments. For details of the

224   infusion procedure. Rats were habituated to the infusion procedure during one session

225 where animals were lightly restrained and the cannula dummy removed and then replaced.

226 In a second habituation session animals were gently restrained while the cannula dummy

227 was removed and a 33-gauge bilateral injector extending 2.5mm beyond the length of the

228 guide cannula was inserted into the mPFC. This was left in place for two minutes, but no

229 infusion occurred. During experimental infusions, the rats were gently restrained while the

230 cannula dummy was removed and the injector inserted. The injector was left in place for

231 1 min prior to infusions of vehicle or drug (1.0µl total volume) over 2 minutes. The injector

232 was left in place for a further 2 minutes to allow diffusion of the drug into the tissue

233 surrounding the injector, and then the injector was removed and the dummy replaced. The

234 ambiguous probe test session occurred 5 minutes after the dummy was replaced.

235 **Infusion experiments:** In the first infusion experiment vehicle (sterile phosphate-buffered

236 saline (PBS); 0.0µg/µl), ketamine (1.0µg/µl), muscimol (0.1µg/µl) or scopolamine (0.1µg/µl),

237 all dissolved in sterile PBS, were infused intracerebrally into mPFC 5 minutes before testing.

238 Following this, CP-101,606 (1.0µg/µl in the first study, 3.0µg/µl in the second study) was

239 dissolved in 10% 2-hydroxypropyl-cyclodextrin and 90% PBS and tested. All experiments

240 used a within-subject fully counterbalanced design for drug treatments, with the

241 experimenter blind to treatment. Drug doses were chosen based on the results from acute,

242 systemic treatments (see Table S2).

243 **Histology:** Following the completion of mPFC infusions, rats were killed and brains were

244 fixed and processed for histology. Rats were anesthetised with a lethal dose of sodium

245 pentobarbitone (0.5ml Euthatal, 200mg/ml, Genus Express, UK) and perfused via the left

246 ventricle with 0.01M PBS followed by 4% paraformaldehyde (PFA). The brains were

247 removed and post-fixed in 4% PFA for 24 hours. Prior to being cut, brains were transferred

248 to 30% sucrose in 0.1M PBS and left for 2 days until brains were no longer floating. Coronal

249 sections were cut at 40µm on a freezing microtome and stained with Cresyl Violet. Locations

250 of the injector tip positions in the mPFC were mapped onto standardised coronal sections of

251 a rat brain stereotaxic atlas[38] (Figure 3).

Data and statistical analysis

253   Sample size was estimated based on our previous studies using the JBT[22,24] but with a more

254   conservative effect size as we were looking at acute rather than chronic effects and

255   expected to see greater variation in mPFC infusion studies. Changes in judgement bias

256   should occur without effects on other variables and therefore strict inclusion criteria were

257   established to reduce any potential confound in the data analysis. Only animals which

258   maintained more than 60% accuracy for each reference tone, and less than 50% omissions

259   were used for analysis.

260   Cognitive bias index (CBI) was used as a measure of judgement bias in response to the

261   midpoint tone. CBI was calculated by subtracting the proportion of responses made on the

262   low reward lever from the proportion of responses made on the high reward lever. This

263   created a score between -1 and 1, where negative values represent a negative bias and

264   positive values a positive bias. Change from baseline in CBI was then calculated for all

265   experimental manipulations as follows: vehicle (0.0mg/kg) probe test CBI − drug dose probe

266   test CBI. This was calculated to take into account individual differences in baseline bias, and

267   to make directional changes caused by drug treatments clearer. To provide a value for

268   vehicle probe test sessions for this measure, the population average for the vehicle

269   (0.0mg/kg) probe test was taken away from each individual rats' CBI score for this dose.

270   This allowed this measure to be analysed with repeated measures analysis of variance

271   (rmANOVA) with drug dose as the within-subjects factor for drug studies with more than two

272   treatments, or paired samples t-test for studies with only two treatments. The raw data for

273   CBI is included for all drug treatments in Figure S1-S2.

274   Response latency and accuracy, omissions and premature responses were also analysed

275   (see Table S8 for details of these). These measures were analysed with rmANOVAs with

276   drug dose and tone as the within-subjects factors. Paired t-tests were performed as post-hoc

277   tests if significant effects were established. Huynh-Feldt corrections were used to adjust for

278   violations of the sphericity assumption, and Sidak correction was applied for multiple

279   comparisons. All statistical tests were conducted using SPSS 24.0.0.2 for Windows (IBM

280   SPSS Statistics) with α=0.05. Results are reported with the ANOVA F-value (degrees of

281   freedom, error) and *p*-value as well as any post-hoc *p*-values. All graphs were made using

282   Graphpad Prism 7.04 for Windows (Graphpad Software, USA).

Results

Study 1: The effect of acute, systemic treatment with RAADs and selected NMDA receptor antagonists

CP-101,606: One animal was excluded in experiments 1 and 2 as accuracy criteria was not met on the vehicle drug dose. In the initial dose response study, CP-101,606 treated animals did not overall show any change in CBI (no main effect of drug dose ($F_{2.237,31.323}$=0.811, p=0.495). Due to the possibility that there might be small change in CBI for the highest dose (3.0mg/kg; visual inspection of the data and one sample t-test (not corrected for multiple comparisons): p=0.038; Figure 2A), we then tested a higher dose of CP-101,606 (6.0mg/kg) in the second experiment. This dose (6.0mg/kg) resulted in a positive bias relative to vehicle treatment (paired samples t-test: p=0.027; Figure 2A). In experiment 1, 3.0mg/kg CP-101,606 also caused a decrease in response latency (main effect of drug dose: $F_{3,42}$=4.858, p=0.005, post-hoc: p=0.027; Table S7). There were no effects on other behavioural measures in experiment 1 (Table S7). In experiment 2, CP-101,606 (6.0mg/kg) caused response latencies to decrease (main effect of drug dose: $F_{1,14}$=27.396, p<0.001; Table S7). This dose had no effect on accuracy for the reference tones (Table S7), but did increase premature responses (paired samples t-test: p=0.001), and reduced omissions (main effect of drug dose: $F_{1,14}$=10.506, p=0.006; Table S7).

Scopolamine: The highest dose tested (0.3mg/kg) had to be excluded from the analysis as most rats did not complete sufficient trials. Scopolamine (0.1mg/kg) induced a positive bias (main effect of drug dose: $F_{2,30}$=6.739, p=0.004, post-hoc: p=0.035; Figure 2B). This dose of scopolamine (0.1mg/kg) also increased response latencies (main effect of drug dose: $F_{2,30}$=17.263, p<0.001, post-hoc: p=0.001; Table S7), increased premature responding (main effect of drug dose: $F_{1.355,20.330}$=4.387, p=0.039, post-hoc: p=0.047; Table S7), and increased omissions for all tones (significant drug dose*tone interaction: $F_{2.343,35.150}$=4.739, p=0.011, main effect of drug dose: $F_{2,30}$=24.257, p<0.001, post-hoc: ps<0.001; Table S7). The lower dose also caused response latencies to increase (post-hoc: p<0.001; Table S7), accuracy to

310 increase (main effect of drug dose: $F_{1.605,24.069}$=8.558, p=0.003, post-hoc: p=0.002; Table

311 S7), and omissions to increase for all tones (post-hoc: ps≤0.019; Table S7).

312 **Ketamine:** In the rats who had undergone mPFC cannulation surgery, ketamine (1.0mg/kg)

313 caused a positive change in CBI (paired samples t-test: p=0.033; Figure 2C), as has been

314 seen previously[24]. Ketamine did not alter any other behavioural measures (Table S7).

315 **Lanicemine:** None of the doses of lanicemine tested caused a change in CBI (Figure 2D).

316 This drug also had no effect on any other behavioural measures (Table S7).

317 **Memantine:** Memantine did not cause any change in CBI at the doses tested (Figure 2E).

318 There was also no effect on other behavioural measures (Table S7).

319 **MK-801:** MK-801 did not change CBI (Figure 2F). The highest dose of MK-801 tested

320 (0.03mg/kg) decreased response latencies (main effect of drug dose: $F_{2,30}$=3.843, p=0.033;

321 Table S7). There was no effect on accuracy for the reference tones, percentage omissions

322 or premature responding.

323 **High-dose ketamine:** In experiment 2 (25mg/kg ketamine) one rat was excluded for failure

324 to complete sufficient trials. In experiments 1 and 2, ketamine (10mg/kg and 25mg/kg

325 respectively) did not change CBI (Figure 3A). In both experiments these higher doses did

326 alter all other behavioural measures. There was an increase in response latency across all

327 three tones for both 10mg/kg (drug dose*tone interaction: $F_{2,30}$=7.323, p=0.003, post-hoc:

328 ps<0.001 for all tones; Figure 3C), and 25mg/kg ketamine (drug dose*tone interaction:

329 $F_{2,28}$=4.686, p=0.018, post-hoc: ps≤0.002 for all tones; Figure 3C). Both doses decreased

330 premature responses (paired samples t-tests: 10mg/kg – p=0.005, 25mg/kg – p=0.006;

331 Figure 3E). Ketamine also improved accuracy in experiment 1 (10mg/kg: main effect of drug

332 dose: $F_{1,15}$=8.774, p=0.010; Figure 3B) and for the low reward tone in experiment 2

333 (25mg/kg: drug dose*tone interaction: $F1,14$ = 5.513, p=0.034, post-hoc: p=0.033; Figure

334 3B). In both experiments, there was an increase in omissions for all three tones (experiment

335 1, 10mg/kg: drug dose*tone interaction: $F_{1.401,21.021}$=5.662, p=0.018, post-hoc: high reward

15

336   tone – $p=0.015$, midpoint tone: $p=0.003$, low reward tone: $p=0.010$; experiment 2, 25mg/kg:

337   drug dose*tone interaction: $F_{1.368,19.150}=11.964$, $p=0.001$, post-hoc: high reward tone –

338    $p=0.003$, midpoint tone – $p<0.001$, low reward tone – $p=0.001$; Figure 3D).

339   **Low dose PCP:** Doses of PCP (0.03, 0.1, 0.3mg/kg) that were lower than those previously

340   tested[24] did not cause any change in CBI (Figure 2G). There was also no effect on any other

341   behavioural measures (Table S7).

342   **Analysis of performance split over session:** In addition to the analyses above we also

343   compared performance for the first and last 20 probe trials in order to check whether

344   animals' performance changed within a session during these randomly reinforced trials.

345   Analysis of the data for doses of ketamine (1.0mg/kg), CP101606 (6.0mg/kg) and

346   scopolamine (0.1mg/kg) which change CBI did not find any evidence of differences across

347   the session between vehicle or drug treatments based on this analysis (see Figure S3).

348

349   *Study 2: mPFC infusions of drugs shown to cause positive judgement biases*

350   Two rats were excluded in cohort 3: one rat did not meet accuracy criteria for any probe (or

351   baseline) session following the second drug infusion; and after the end of testing another

352   animal was found to have an incorrect cannula placement. Therefore, both were excluded

353   retrospectively from the entire study. Compared to pre-surgery performance, the CBI of rats

354   became more negative after surgery, and this was stable across testing over three weeks

355   (main effect of week: $F_{3,42}=6.335$, $p=0.001$, post-hoc: ps≤0.011; Figure 4A). There were no

356   differences in response latencies, premature responses, accuracies for reference tones or

357   omissions before compared to after surgery (Table S7). The change in CBI occurred before

358   infusions and seemed to be a response to the surgical intervention potentially causing a

359   more negative affective state. We found no evidence of tissue damage in the area

360   surrounding the cannula post-mortem, so it is unlikely that this was a result of trauma. We

361   think it is not surprising that undergoing surgery and having to adapt to intracerebral cannula

362 could cause a permanent negative change in affect. It is exactly this sort of affective state

363 change that judgement bias assays have been developed to detect (for example see

364 Bethell[39], and Baciadonna & McElligott[40] for reviews summarising how judgement bias tasks

365 can be used as measure of animal welfare).

366 In the first infusion experiment, ketamine (1.0μg/μl), muscimol (0.1μg/μl) and scopolamine

367 (0.1μg/μl) all induced positive biases (main effect of drug dose: $F_{3,36}$=7.241, p=0.001; post-

368 hoc: ketamine – p=0.012, muscimol – p=0.001, scopolamine – p=0.032 Figure 4C). The

369 effect of PFC infusion of ketamine or scopolamine was specific to CBI, as these drugs had

370 no effect on other behavioural measures (Figure 4D-G), unlike muscimol infusions which

371 caused changes to all other behavioural measures. There was an increase in response

372 latency (drug dose*tone interaction: $F_{6,72}$=4.181, p=0.001) for the high reward (post-hoc:

373 p<0.001) and midpoint tone (p=0.028; Figure 4E), and a large increase in premature

374 responses to over 100% (main effect of drug dose: $F_{1.151,13.809}$=33.784, p<0.001, post-hoc:

375 p<0.001; Figure 4G). Muscimol also caused accuracy to decrease (main effect of drug dose:

376 $F_{1.181,14.172}$=43.775, p<0.001, post-hoc: p≤0.001; Figure 4D). For the low reward tone, this

377 reduction was so great that rats were no longer performing any better than chance (one-

378 sample t-test against a test value of 50%: p=0.197; Figure 4D). Omissions increased

379 following muscimol infusion (main effect of drug dose: $F_{1.338,16.057}$=10.418, p=0.003, post-hoc:

380 p=0.007; Figure 4F).

381 In the experiments testing the effect of CP-101,606 mPFC infusion, in experiment 1 the

382 lower dose (1.0μg/μl) did not alter CBI (Figure 5A), but in experiment 2, the higher dose

383 (3.0μg/μl) induced a positive bias (paired samples t-test: p=0.043; Figure 5A). In experiment

384 1, CP-101,606 (1.0μg/μl) caused an increase in response latency (main effect of drug dose:

385 $F_{1,12}$=5.064, p=0.044; Figure 5C) but had no other behavioural effects (Figure 5B,D,E). In

386 experiment 2, 3.0μg/μl CP-101,606 did not have any effects on other behavioural measures

387 (Figure 5B-E).

Discussion

389 As previously shown[24], low dose ketamine (1.0mg/kg) had a specific effect on decision-

390 making biases, inducing a positive change in CBI following acute administration. This effect

391 of ketamine was dose dependent, with higher doses having general effects on task

392 performance without changing CBI. The effects of ketamine were recapitulated to some

393 extent by the GluN2B antagonist, CP-101,606 and muscarinic antagonist, scopolamine, but

394 both also had more general effects on other behavioural measures following systemic

395 administration. All three treatments have previously been reported to have RADD effects in

396 clinical trials[3,29,30], whilst the other NMDA antagonists tested here did not[5,26-28], and these

397 also failed to induce a change in bias. The mPFC infusions suggest that this brain region is

398 central to the effects of ketamine, scopolamine and CP-101,606. Interestingly, mPFC

399 infusions more specifically altered bias, suggesting other brain regions may contribute to the

400 systemic effects on other behavioural measures. The importance of the mPFC in modulating

401 RAAD effects in neuropsychological tasks is consistent with previous findings in our learning

402 and memory bias assay, the affective bias test[34]. Inactivation of the mPFC with muscimol did

403 positively change bias but animals also exhibited large changes in other behavioural

404 measures. This suggests that the RAADs can modulate activity in this brain region in a more

405 specific way than muscimol, which results in a relatively specific effect on biases in decision-

406 making.

407 For lanicemine and memantine, the lack of any behavioural effects means there is a

408 possibility that the doses tested were too low. For both treatments the range of doses tested

409 covers the doses that are equivalent to those used humans in clinical trials (lanicemine: 50,

410 100mg[26], equivalent to approximately 0.75, 1.5mg/kg; memantine: 5-20mg[5], equivalent to

411 approximately 0.07-0.3mg/kg), paralleling our effective dose of ketamine (1.0mg/kg, similar

412 to the 0.5mg/kg dose used by Zarate et al.[3]). Although higher doses may yield behavioural

413 effects, these are likely to be due to much higher levels of receptor occupancy than those

414 relevant to the antidepressant effects and may also arise from non-specific actions at other

415   receptors. When testing lower doses of PCP (another NMDA receptor antagonist not known

416   to show RAAD) than previously used[24], we also failed to see any change in CBI. Conversely,

417   when we tested higher doses of ketamine than those we had previously[24], doses that are

418   often used to demonstrate antidepressant effects in other preclinical models used to study

419   depression such as the forced swim test (FST)[41], we failed to see any change in bias,

420   instead only seeing non-specific changes in other behavioural measures. The behavioural

421   profile seen with these higher doses of ketamine (increased response latency and omissions

422   and decreased premature responding) suggests that these doses may be causing locomotor

423   depression or reducing motivation to respond. Higher doses of ketamine have not been

424   found to have antidepressant effects in clinical trials and these data also suggest that rodent

425   studies using these higher doses may not be looking at specific effects. It may be that the

426   lower 1.0mg/kg dose of ketamine can specifically alter decision making biases because they

427   target a specific population and hence modulate a specific circuit. Some studies have

428   suggested that ketamine may act via disinhibition of GABAergic interneurons leading to a

429   glutamate burst which then activates prefrontal glutamate neurons[42]. Overall, the results

430   from systemic administration of different NMDA receptor antagonists lends support to our

431   interpretation that this reward-based judgement bias task can specifically dissociate between

432   drugs that do show RAAD, and those that do not, despite them having similar pharmacology.

433   The difference in specificity on behavioural effects, whereby ketamine (1.0mg/kg) only

434   positively changes decision-making bias, but both CP-101,606 and scopolamine have other

435   non-specific effects, suggests that 1.0mg/kg ketamine is able to relatively selectively

436   modulate affective bias. The changes in response latencies, omissions and premature

437   responses caused by CP-101,606 and scopolamine suggest that these drugs may also be

438   having effects on other cognitive processes, such as motivation. However, the direction of

439   changes for these drugs are in opposite directions (decreases in response latency and

440   omissions for CP-101,606 but increases in these for scopolamine) despite them both

441   causing positive changes in CBI. This, combined with the lack of change in accuracy for the

442   reference tones, suggest that these non-specific effects cannot fully explain the change in

443   decision-making bias.

444   The neurobiology underlying the relative specificity of ketamine, CP-101,606 and

445   scopolamine in being able to immediately alter decision-making bias, in contrast to the other

446   NMDA receptor antagonists tested that have not shown these effects, are likely to be due to

447   differences in their mechanisms of action. Our findings add weight to the strong body of

448   evidence suggesting that NMDA receptor antagonism is important for short-term, RAAD

449   effects of these drugs[43], but suggests that specific modulation of either a specific subtype of

450   the receptor or a sub-population of neurons may be involved. CP-101,606 is selective for the

451   GluN2B NMDA receptor subunit, whilst it has been shown that scopolamine, and more

452   recently ketamine, cause a glutamate burst via blockade of NMDA receptors specifically on

453   GABA interneurons that leads to increased mechanistic target of rapamycin complex 1

454   signalling, brain-derived neurotrophic factor release and synaptic changes in the PFC[42,44-46].

455   Further studies would be required to test whether these mechanisms also drive these drugs

456   effects on affective bias.

457   The infusion studies localise the site of action of this rapid change in decision-making bias to

458   the mPFC, corresponding with brain imaging studies in humans that have also shown

459   ketamine-dependent changes in prefrontal glutamatergic neurotransmission[32,33]. This also

460   matches with previous rodent studies, where using the affective bias test, it has been shown

461   that whilst ketamine does not induce positive biases in learning, it can remediate previously

462   acquired negative biases, an effect which also localises to the mPFC[34]. For CP-101,606 and

463   scopolamine, unlike when given systemically, intracerebral mPFC infusion did not cause any

464   non-specific behavioural changes on the task. This could suggest that these non-specific

465   effects are driven by off-target effects of drug binding in other brain areas, or in the case of

466   scopolamine, the periphery. The localisation of the positive modulation of decision making

467   caused by these drugs to the mPFC provides further support for the hypothesis that this

468   might be mediated through burst firing in the prefrontal cortex, an effect that has recently

20

469   been shown to cause the activation of downstream pathways thought to be important in the

470   RAAD effects of both ketamine and scopolamine[42,44-46].

471   Interestingly, both GABA$_A$ receptor agonism (musciol infusion), and NMDA receptor

472   antagonism (ketamine infusion) in the mPFC caused the same qualitative, but not

473   quantitative behavioural change in judgement bias (a positive shift but of different

474   magnitudes), mirroring findings seen previously with intra-infralimbic infusions of muscimol

475   and (R)-CPP on the five choice serial reaction time task, where both drugs increased

476   impulsive responding but by different amounts[47]. It has been suggested that the functional

477   effects of NMDA receptor antagonism may be due to excess extracellular glutamate[48,49].

478   However, the pronounced, non-specific behavioural effects on other measures seen

479   following muscimol infusion suggests that mechanism of action of the other infusion drugs is

480   more refined than global inhibition of neurotransmission in the mPFC. Previous work in

481   humans and rodents has shown that subcortical and limbic brain regions, such as the

482   amygdala, are important in the neurocircuitry of MDD / depression-related behaviour[50-52],

483   and a recent study suggests that ketamine may play a critical role in restoring dysfunctional

484   connectivity in these circuits[33]. Furthermore, in rodents, a recent study found that

485   optogenetic activation of pyramidal mPFC neurons containing dopamine receptor D1 caused

486   RAAD-like responses in the forced swim test, and that blockade of these receptors

487   prevented the RADD effects of ketamine[53]. In order to further our understanding of this

488   mechanism, it will be important to investigate the effects of these drugs on different neuronal

489   subtypes within the mPFC, as well as investigating the wider circuitry that is altered by these

490   drugs.

491

492   *Final conclusions*

493   This study adds to the evidence that the neuropsychological effects of ketamine are

494   potentially important in its RAAD in patients with MDD, and that these effects in altering

495   affective biases, both in decision-making as demonstrated here, as well as in learning and

496   memory occur at time points (one hour) before major plastic changes arise. It will be

497   important to investigate the neurobiological effects of not just the immediate, RAAD of

498   ketamine, but also the sustained effects by examining how affective biases are altered at

499   longer time points. Furthermore, investigation of the wider circuits involved in this RAAD

500   efficacy will be crucial in revealing the mechanism underlying these actions, which will be

501   important for the development of novel therapeutics. Ketamine (at 1.0mg/kg) seems to have

502   very specific effects on affective bias, which we can capitalise on to better understand the

503   circuits that contribute to these modulations of affective biases that are potentially very

504   important in the cause, perpetuation and treatment of MDD. More detailed circuit analyses

505   are needed including undertaking studies in other brain regions to determine whether

506   ketamine's effects are specific to the mPFC.

516

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518   0033-z)

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Figure Legends

Figure 1 – *Schematic of the judgement bias task and trial structure.*

In the judgement bias task (JBT), rats are trained to associate one tone frequency (2kHz) with a high value reward: i.e. if the rat presses the correct lever (shown as the left lever in (A), but counterbalanced across rats in a cohort) they receive a high value reward (four reward pellets). They also learn to associate a second tone frequency (8kHz) with receiving a low value reward (one reward pellet; shown in (A) as pressing the right lever during the tone). Judgement bias, or decision making about an ambiguous cue, which is known to be influenced by affective state, can be probed by presenting an ambiguous tone that has a mipdoint frequency between the two reference cues (5kHz), and recording which lever the rat presses. If the rat is expecting the more positive outcome (indicative of an optimistic judgement bias), then they will more often choose the large reward lever, but if the rat is in a more negative affective state, they will expect the less positive outcome and more often choose the low reward lever, a pessimistic judgement bias. During the task, tones are presented within discrete trials, the format of which is depicted as a flow chart in (B). The task is self-initiated, and so each trial begins only once the rat makes a nosepoke entry into the magazine port. This is followed by a 5 second inter-trial interval (ITI), during which time the rat has to wait and refrain from making a lever press response. If the rat does press a lever, they are punished with a 10 second timeout (TO). The tone cue is presented for a maximum of 20 seconds following the ITI, or until the rat makes a lever press response. The outcome following each lever press depends on which tone was played, and which lever was pressed. Correct lever presses to either reference tone (high or low tones) results in the corresponding reward being delivered to the magazine, whilst incorrect lever presses results in a 10 second TO. This TO also occurs if the rat fails to make any lever press during the 20 second tone presentation (an omission). During TOs, lever presses and magazine entries are recorded but have no consequences, meaning the rat has to wait to be able to begin the next trial. When the midpoint tone is presented, 50% of the time this tone is "classified" by

31

704 the software as having the same response properties as the high reward tone. I.e., if the rat

705 makes a high reward lever press during a midpoint tone presentation classified in this way,

706 then they will receive a four pellet reward, but will experience the 10 second TO if they make

707 a low reward lever press. Similarly, if the midpoint tone is "classified" as having the same

708 response properties as the low reward tone, then a high reward lever press would result in a

709 TO, whilst a low reward lever press would result in delivery of the small reward. In this way,

710 each lever is only every associated with the same reward outcome (i.e. four pellets for the

711 high reward lever), but the midpoint tone becomes randomly reinforced, and so rats will

712 maintain responding for this tone across multiple trials within a session, whilst being unable

713 to learn a specific reward contingency to associate with the midpoint tone.

714

715 **Figure 2** – *The effect of acute treatment with rapid acting antidepressant drugs and NMDA*

716 *receptor antagonists on judgement bias of the midpoint ambiguous tone.*

717 Ketamine (0.0, 1.0 mg/kg; n = 13), scopolamine (0.0, 0.03, 0.1 mg/kg; n = 16), CP-101,606

718 (Expt 1: 0.0, 0.3, 1.0, 3.0 mg/kg, n = 15; Expt 2: 0.0, 6.0 mg/kg, n = 15), lanicemine (0.0, 0.3,

719 1.0, 3.0 mg/kg; n = 16), memantine (0.0, 0.1, 0.3, 1.0 mg/kg; n = 16) and MK-801 (0.0, 0.01,

720 0.03 mg/kg; n = 16 were administered acutely by intraperitoneal injection prior to testing on

721 the judgement bias task. (A) Replicating previous studies, ketamine (1.0 mg/kg) positively

722 changed CBI. (B) Scopolamine (0.1 mg/kg) also caused a positive change from baseline in

723 CBI. (C) In experiment 1, there was no overall effect of CP-101,606 on change in CBI. A

724 positive change was seen in experiment 2 with a higher 6.0 mg/kg dose. (D-G) Lanicemine,

725 memantine, MK-801 and low doses of PCP did not induce a change in CBI for the midpoint

726 tone at the doses tested. Data shown and represent mean ± SEM (bars and error bars)

727 overlaid with individual data points for each rat. Dashed line (panel C) indicates separate,

728 counterbalanced experiments. $*p < 0.05$; $^{\#}p < 0.05$ for a one-sample t-test for 3.0 mg/kg CP-

729 101,6060 only (comparison to a test-value of zero representing a change in CBI for that drug

730   only from baseline). CP-101,606, ketamine, lanicemine, memantine, PCP: 60 min pre-

731   treatment; scopolamine, MK-801: 30 min pre-treatment

732

733   **Figure 3** – *Behavioural data from the judgement bias task following acute treatment with*

734   *high doses of ketamine.*

735   Acute doses of ketamine (Expt 1: 0.0, 10.0 mg/kg, n = 16; Expt 2: 0.0, 25.0 mg/kg, n = 16)

736   were administered by intraperitoneal injection to measure their effect on judgement bias. (A)

737   Neither high dose of ketamine caused a change in interpretation of the midpoint tone. (B)

738   Both doses of ketamine increased accuracy for the low tone. (C) Both doses of ketamine

739   increased response latencies across all three tones. (D) Omissions were increased across

740   all three tones following both ketamine doses. (E) High doses of ketamine (10.0, 25.0 mg/kg)

741   decreased premature responding. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Data represent mean ±

742   SEM (panels B-E) with individual data points overlaid for each rat (panel A). Dashed lines

743   indicate separate, counterbalanced experiments. 60 min pre-treatment. HT - high reward

744   tone; MT - midpoint tone; LT - low reward tone.

745

746   **Figure 4** – D*ata from mPFC cannulated rats on the judgement bias task.*
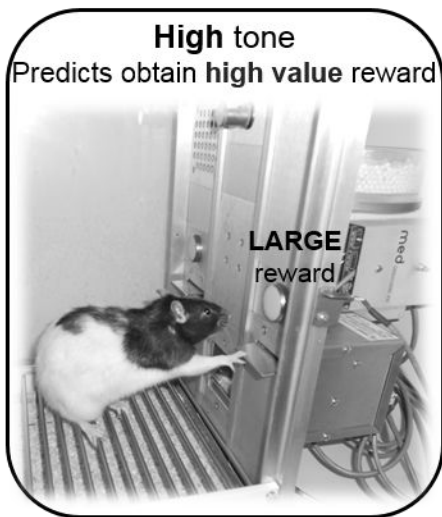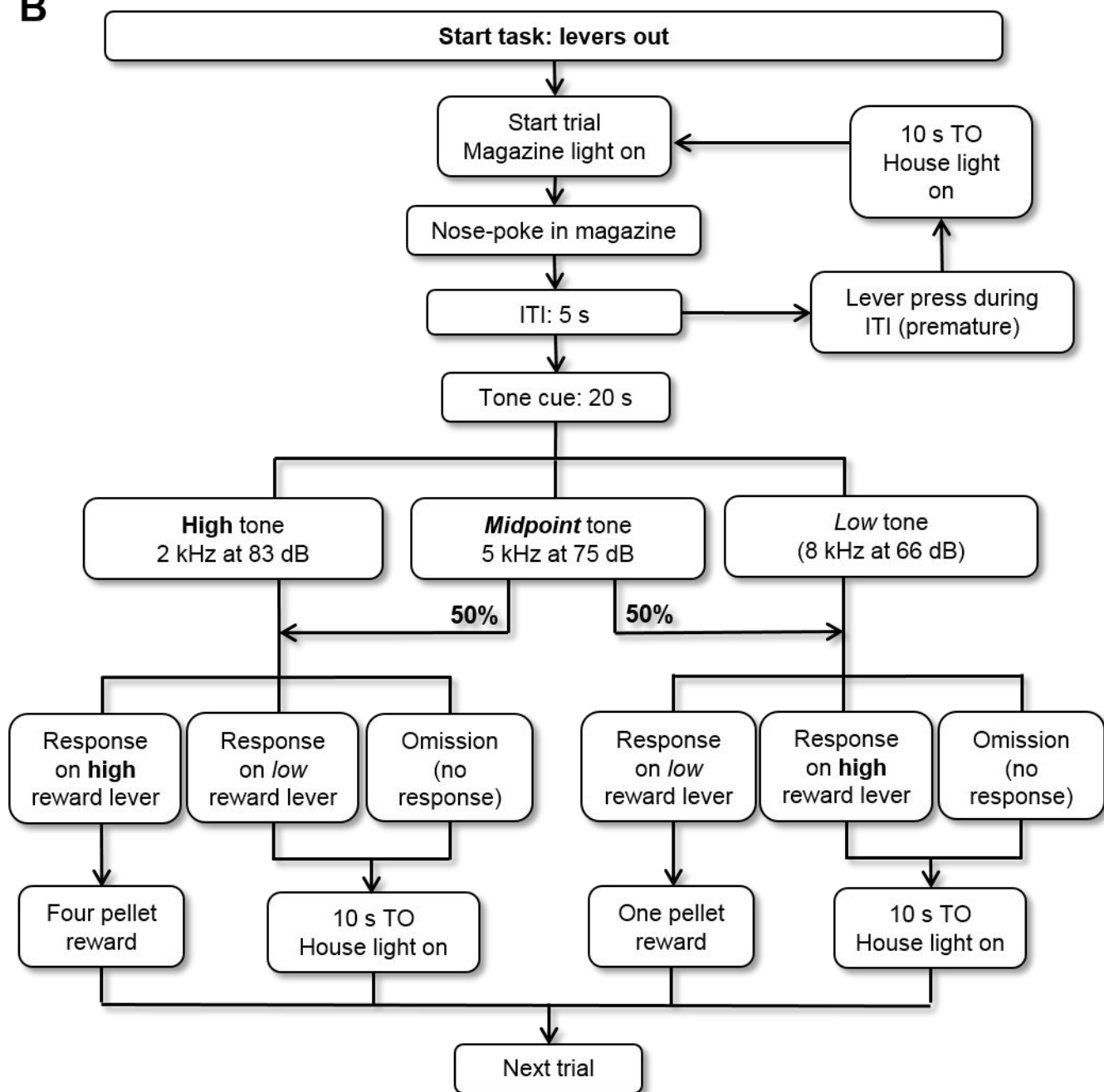
747   Probe tests with no experimental manipulation were conducted before and after mPFC

748   cannulation surgery to ensure that the surgery itself did not effect performance in the

749   judgement bias task. (A) Cognitive bias index became more negative in the probe tests

750   conducted after surgery. (B) The location of the injector placement was confirmed post-

751   mortem and black dots represent the location of the cannula tip as assessed from Cresyl

752   violet-stained brain sections. Coronal sections are +3.7 mm to +2.5mm relative to bregma

753   (Paxinos and Watson, 1998). (C-G) In the first infusion experiment, ketamine (Ket; 1.0 µg/µl)

754   muscimol (Mus; 0.1 µg/µl), scopolamine (Sco; 0.1 µg/µl) or vehicle (Veh; 0.0 µg/µl; n = 13),

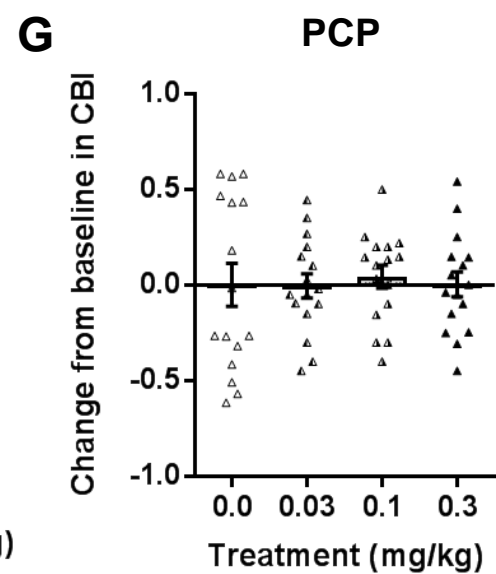755   were administered by intracerebral infusion into the mPFC to measure the effect on

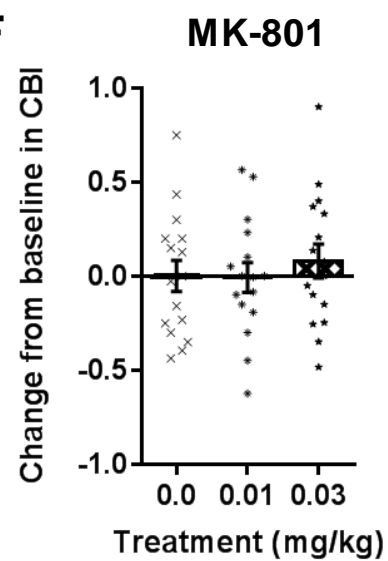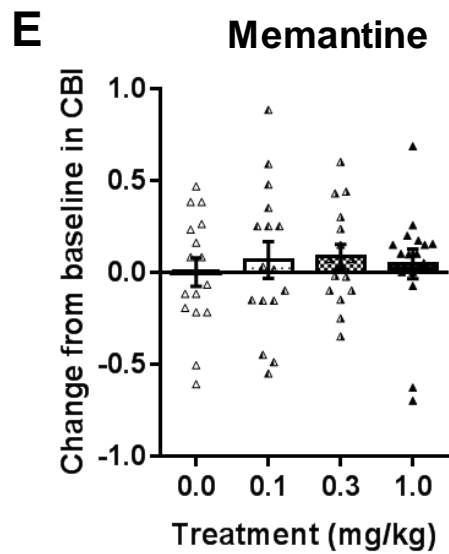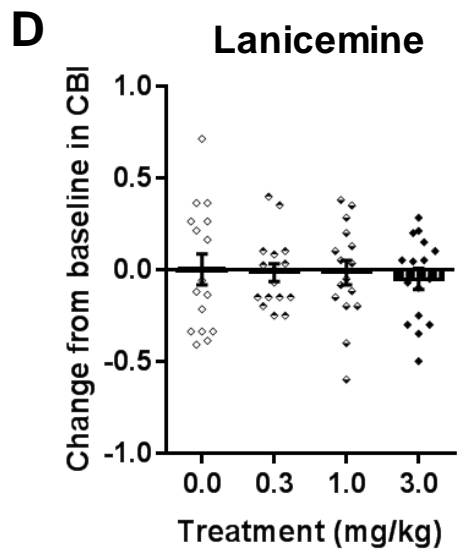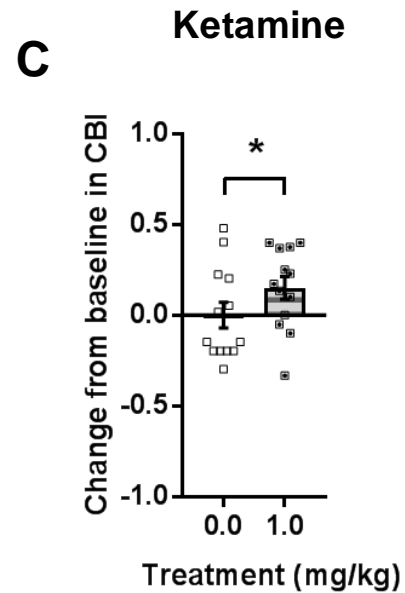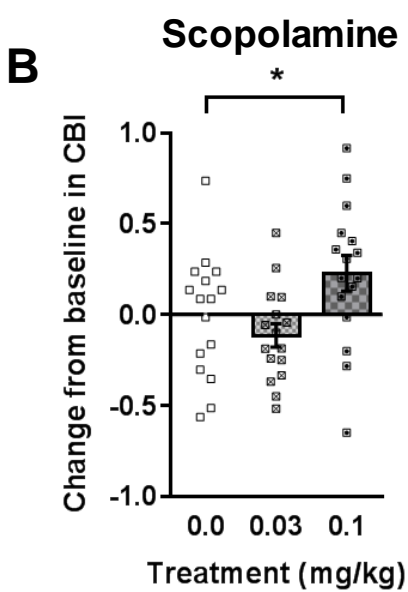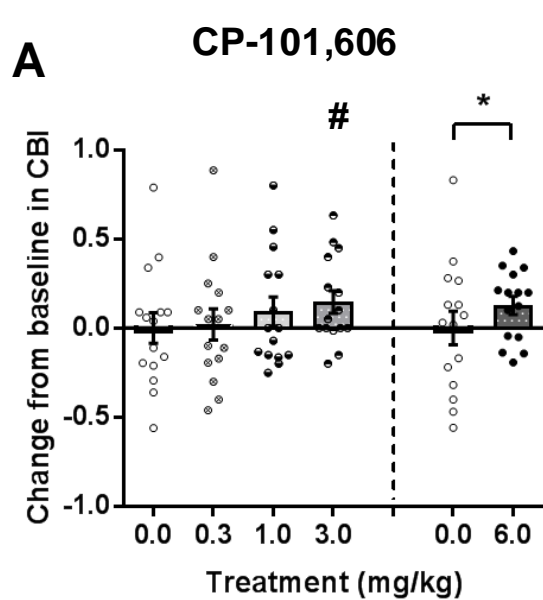756   judgement bias. (C) Ketamine, muscimol and scopolamine all caused a positive change in

757   cognitive bias index (CBI) for the midpoint tone. (D) Muscimol decreased accuracy for both

758   reference tones. (E) Muscimol increased response latencies for the high and midpoint tones.

759   (F) For the high and low tones, muscimol increased omissions. (G) Muscimol also increased

760   premature responding. Data represent mean ± SEM (panels A, C-G) with individual data

761   points overlaid for each rat (panel A,C). Black dashed line (panel f) represents 50% accuracy

762   depicting performance at chance. 5 min pre-treatment. ***$p < 0.001$, *$p < 0.05$. HT - high

763   reward tone; MT - midpoint tone; LT - low reward tone.

764

765   **Figure 5** – *Behavioural data from the judgement bias task following mPFC infusions of CP-*

766   *101,606.*

767   CP-101,606 (Expt 1: 00.0, 1.0 µg/µl, n = 13; Expt 2: 0.0, 3.0 µg/µl, n = 12) was administered

768   by intracerebral infusion in the mPFC to measure the effect on judgement bias. (A) The

769   higher dose of CP-101,606 (3.0 µg/µl) caused a positive change from baseline in CBI. (B)

770   Accuracy was not altered by either dose of CP-101,606. (C) In experiment 1, CP-101,606

771   (1.0 µg/µl) increased response latency for the midpoint tone. (D/E) There was no effect of

772   either dose on omissions or premature responding, *$p < 0.05$. Data represent mean ± SEM

773   (panels B-E) with individual data points overlaid for each rat (panel A). Dashed lines indicate

774   separate, counterbalanced experiments. 5 min pre-treatment. HT - high reward tone; MT -

775   midpoint tone; LT - low reward tone.

A

High tone
Predicts obtain **high value** reward
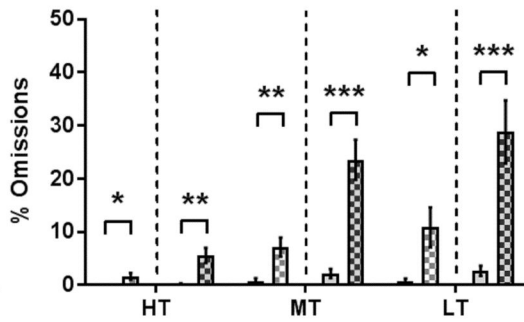
LARGE reward

Low tone
Predicts obtain *low value* reward

small reward

Ambiguous midpoint tone
Which lever does the rat choose?

small reward **?** **LARGE** reward

B

Start task: levers out

Start trial
Magazine light on

10 s TO
House light on

Nose-poke in magazine

ITI: 5 s → Lever press during ITI (premature)

Tone cue: 20 s

High tone
2 kHz at 83 dB

Midpoint tone
5 kHz at 75 dB

Low tone
(8 kHz at 66 dB)

50% **50%**

Response on **high** reward lever

Response on *low* reward lever

Omission (no response)

Response on *low* reward lever

Response on **high** reward lever

Omission (no response)

Four pellet reward

10 s TO House light on

One pellet reward

10 s TO House light on

Next trial

Supplementary Information for: **Role of the medial prefrontal cortex in the effects of rapid acting antidepressants on decision-making biases in rodents**

Title: Role of the medial prefrontal cortex in the effects of rapid acting antidepressants on decision-making biases in rodents

Running title: Rapid antidepressant effects on decision-making biases

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Table S1 - *Training stages and required performance criteria for the judgement bias task.*

Stage	Description	Criteria	Sessions required to meet criteria		
			Cohort 1	Cohort 2	Cohort 3
1 – Magazine training	Tone (2 kHz only for half the session followed by 8 kHz only for the rest of the session, order counterbalanced across rats) played for 20 s followed by release of one pellet into magazine; 10 s ITI. No levers available.	20 pellets eaten for each tone frequency	1	1	1
2 – Tone training	Response on lever during tone (2 kHz or 8kHz only, order counterbalanced across rats) rewarded with one pellet. Lever corresponding to that tone frequency available only.	> 50 trials completed for two consecutive sessions on each tone frequency	4	4	4
3 – Discrim-ination training	Response on correct corresponding lever only during tone (either 2 kHz or 8 kHz presented pseudorandomly) rewarded with one pellet. Both levers available. Incorrect or omitted trials were repeated (i.e. same tone frequency played) until a correct response occurred.	> 70% accuracy for both tones, no significant differences on analysed behavioural measures over three sessions and < 1:1 ratio of correct:premature responses	15	10	10
4 – Reward magnitude training	As Stage 3 but response on correct corresponding lever only rewarded with four pellets for high reward tone and one pellet for low reward tone. Both levers available.	As for Stage 3 but with > 60% accuracy for both tones (to allow for biases in responding to reference tones caused by the difference in associated reward magnitude).	9	8-10	10
5 – Baseline session	Same format as reward magnitude training sessions.	Animals had to show equivalent baseline session performance to pre-drug study baseline sessions (measured by no significant differences pre- and post- on behavioural measures.	-	-	-
6 – Probe sessions	For reference tones (2 of 8 kHz) response on correct corresponding lever during the tone rewarded with either 4 pellets (2 kHz) or 1 pellet (8 kHz) reward. For ambiguous midpoint tone (5 kHz), random reinforcement was used whereby outcomes for 50% of the trials followed 2 kHz tone trials, whilst the other 50% followed 8 kHz tone trials (see Supplementary Figure XX for further details). There were no repeated trials following incorrect or omissions.	< 60% accuracy for both reference tones, < 50% omissions	-	-	-

For all training stages, trial structure was as depicted in Figure S1 (except for magazine training which excludes any form of lever press response). Training stages 1-4 were conducted once per day, Monday to Friday, and consisted of a maximum of 100 trials, or lasted for 60 minutes. Where both tones were played (stages 3-4) tone type was equally spilt

across the session (50 trials per tone). Baseline sessions also consisted of 100 trials, and were conducted Monday to Friday during baseline weeks between drug studies, and on Monday and Thursday during drug studies. Probe sessions consisted of 120 trials: 40 of each reference tone (2 and 8 kHz) and 40 midpoint tones (5 kHz). Pseudorandom tone presentation was achieved by splitting each training/baseline (probe) session into blocks of 10 (12) trials, within which there were 5 (4) presentations of each tone frequency. Within a block, there could only be a maximum of n-1 consecutive tone presentations (i.e. in baseline sessions, a maximum of 4 consecutive trials of either 2 or 8 kHz). Omitted trials (no lever press during tone presentation; possible in stages 2-4) were punished with a 10 second timeout where the house light was turned on, and the animal was unable to initiate another trial. Incorrect trials (wrong lever for the tone presented; possible in stages 3-4) were also punished with a 10 second timeout with the house light on. Premature trials (lever press during the ITI; possible during stage 2-4) were similarly punished with a 10 second timeout with the house light on. Each new trial had to be self-initiated by the animal by making an entry into the magazine (this was signalled by the magazine light being turned on, and was switched off once animals made the magazine nose poke). Baseline session were the same format as reward magnitude training sessions, with animals required to repeat incorrect or omitted trials. Midpoint tones during the probe session were reinforced as follows: to program random reinforcement within the constraints of the software, the ambiguous midpoint tone was made up of two copies of the 5 kHz tone (75 dB), each of which was programmed to be classed as "correct" for one of the two lever press responses. This meant that the outcome associated with each of the two ambiguous tones could be programmed to be the same as one of the reference tones, hence resulting in random reinforcement. I.e., 50% of the time lever presses for the midpoint tone had outcomes that were the same as the high reward tone (4 pellets or a timeout), whilst 50% of the time lever presses had outcomes that were the same as the low reward tone (timeout or 1 pellet). Responses to either of the "two" midpoint tones were analysed together.

Cohort	# rats	Acute drug treatment	Doses (mg/kg)
1	16	Memantine MK-801 Lanicemine CP-101,606 (Experiment 1) CP-101,606 (Experiment 2) Scopolamine	0.0, 0.1, 0.3, 1.0 0.0, 0.01, 0.03 0.0, 0.3, 1.0, 3.0 0.0, 0.3, 1.0, 3.0 0.0, 6.0 0.0, 0.03, 0.1, 0.3
2	16	Low dose PCP High dose ketamine (Experiment 1) High dose ketamine (Experiment 2)	0.0, 0.03, 0.1, 0.3 0.0, 10.0 0.0, 25.0
3	15[$]	Ketamine (systemic) mPFC infusions (Experiment 1): ketamine, muscimol, scopolamine mPFC infusion: CP-101,606 (Experiment 1) mPFC infusion: CP-101,606 (Experiment 2)	0.0, 1.0 0.0, 1.0, 0.1, 0.1 µg/µl 0.0, 1.0 µg/µl 0.0, 3.0 µg/µl

Table S2 - *Summary of treatments used in the different cohorts.*

[$]Initial total n number for this manipulation is 15 as one rat had to be euthanised after the first infusion habituation session as dummy cannula could not be removed from the guide.

Table S3 – *Data for response latency for baseline weeks between drug studies from the JBT.*

		Day 1		Day 2		Day 3		Day 4		Day 5	
Drug	**Cohort**	HT	LT	HT	LT	HT	LT	HT	LT	HT	LT
Memantine		2.26±0.17	3.68±0.26	2.54±0.15	4.65±0.28	2.71±0.19	5.08±0.30	2.63±0.17	4.59±0.27	2.84±0.21	4.39±0.33
MK-801		2.52±0.19	4.57±0.32	2.92±0.15	5.61±0.28	2.97±0.15	5.35±0.23	2.82±0.16	5.20±0.26	3.11±0.22	5.72±0.29
Lanicemine	1	2.60±0.15	4.81±0.24	2.85±0.14	5.04±0.25	2.81±0.15	4.82±0.21	2.95±0.18	5.26±0.20	3.05±0.34	5.80±0.43
CP-101,606: low		2.58±0.21	4.59±0.26	2.72±0.15	4.94±0.29	2.72±0.20	4.91±0.26	2.48±0.19	4.44±0.17	2.94±0.21	5.42±0.20
CP-101,606: high		2.99±0.23	5.11±0.31	3.15±0.25	5.30±0.29	3.28±0.24	5.84±0.26	3.02±0.21	5.34±0.30	3.68±0.22	5.80±0.29
Scopolamine		2.62±0.22	4.92±0.33	2.97±0.19	5.23±0.30	3.93±0.24	5.69±0.32	3.03±0.22	5.62±0.28	4.32±0.71	6.16±0.30
PCP		2.29±0.13	4.52±0.37	3.07±0.29	6.12±0.43	2.88±0.31	5.74±0.46	2.82±0.29	5.59±0.47	2.84±0.24	5.36±0.46
Ketamine (10)	2	3.54±0.29	5.99±0.44	4.24±0.40	7.17±0.49	3.71±0.20	6.01±0.32	3.73±0.22	6.29±0.41	3.53±0.29	6.12±0.31
Ketamine (25)		2.63±0.26	4.17±0.47	3.21±0.22	5.61±0.35	3.48±0.24	6.42±0.49	3.54±0.20	6.41±0.31	3.62±0.26	5.93±0.28
Post-Surgery		1.86±0.13	3.20±0.26	1.97±0.10	4.06±0.27	2.08±0.11	4.33±0.27	2.00±0.15	4.37±0.24	2.49±0.15	4.61±0.34
Ketamine (1)		2.01±0.15	4.03±0.30	2.29±0.22	4.47±0.42	2.34±0.22	4.32±0.39	2.62±0.21	4.60±0.32	2.41±0.15	4.19±0.31
Infusions 1	3	2.14±0.24	4.33±0.38	2.43±0.27	4.89±0.37	3.02±0.30	5.69±0.41	2.54±0.25	5.19±0.36	2.32±0.20	4.99±0.34
CP-101,606 infusion: low		2.61±0.21	5.16±0.35	2.70±0.19	5.54±0.42	2.84±0.21	5.44±0.29	2.82±0.19	5.68±0.88	2.49±0.18	5.57±0.34
CP-101,606 infusion: high		2.55±0.20	5.03±0.36	2.81±0.28	5.54±0.45	2.83±0.28	5.63±0.40	2.88±0.20	5.80±0.42	2.89±0.15	5.57±0.21

Behavioural data are presented as mean ± SEM. Drug studies are listed in chronological order for each cohort. Ketamine doses are listed in brackets.

Table S4 – *Data for accuracy for baseline weeks between drug studies from the JBT.*

		Accuracy									
		Day 1		Day 2		Day 3		Day 4		Day 5	
Drug	Cohort	HT	LT	HT	LT	HT	LT	HT	LT	HT	LT
Memantine	1	96.2±0.80	79.5±1.40	96.1±0.91	84.8±1.31	97.2±1.00	84.8±1.10	97.8±0.44	83.1±1.83	96.4±1.28	82.4±1.30
MK-801		95.9±1.00	83.4±1.27	95.3±1.22	85.4±2.06	96.9±0.76	86.7±1.11	97.5±0.80	86.0±1.38	97.7±0.51	88.8±1.08
Lanicemine		97.3±0.60	86.4±1.82	98.8±0.48	88.4±1.48	98.0±0.56	88.9±1.68	98.8±0.43	87.9±1.61	96.8±0.69	82.4±1.44
CP-101,606: low		95.8±1.49	82.3±1.25	97.8±0.65	87.8±1.69	98.5±0.42	88.4±1.31	98.2±0.62	90.1±1.49	98.6±0.46	89.3±1.34
CP-101,606: high		97.6±0.73	84.6±1.75	98.0±0.72	86.8±2.04	97.7±0.93	90.2±1.40	97.9±0.56	89.8±1.79	98.6±0.48	88.9±1.09
Scopolamine		97.5±0.57	85.6±1.79	98.3±86.6	86.6±1.70	96.3±1.20	87.8±1.93	98.0±0.57	86.3±1.33	97.1±0.64	87.1±1.22
PCP	2	91.0±3.68	79.3±2.05	93.8±2.03	81.5±2.56	97.1±1.48	82.1±2.07	97.8±1.01	81.6±1.87	96.6±1.78	80.9±2.86
Ketamine (10)		97.3±0.94	82.4±3.36	98.6±0.57	85.9±2.46	99.5±0.35	85.5±1.60	98.9±0.58	85.0±1.86	98.9±0.57	86.7±1.12
Ketamine (25)		93.5±3.66	79.6±3.50	98.1±0.88	84.5±3.28	97.0±1.08	86.5±2.18	98.7±0.79	87.8±1.76	97.6±1.19	85.1±2.23
Post-Surgery	3	92.2±1.47	70.4±1.73	94.9±1.11	85.4±1.75	95.1±1.13	84.9±1.45	93.2±1.29	87.8±1.64	93.1±1.68	86.6±1.93
Ketamine (1)		93.0±2.98	79.1±3.42	93.6±3.03	85.0±2.66	94.2±3.06	86.1±2.62	91.9±2.03	82.1±1.22	93.0±1.45	81.6±1.76
Infusions 1		96.3±1.22	81.7±2.50	96.7±0.89	83.6±2.10	96.0±0.80	86.6±2.23	96.9±0.79	88.6±1.78	96.7±0.45	84.7±1.24
CP-101,606 infusion: low		97.3±0.66	86.8±1.56	94.8±1.41	87.1±1.90	96.7±1.20	85.3±1.92	97.9±0.88	86.2±2.18	95.8±0.98	86.1±2.45
CP-101,606 infusion: high		96.9±0.79	83.6±1.89	96.5±0.90	82.3±1.81	98.0±0.77	84.5±0.90	97.3±0.74	87.0±1.63	97.0±0.81	85.4±1.58

Behavioural data are presented as mean ± SEM. Drug studies are listed in chronological order for each cohort. Ketamine doses are listed in brackets.

Table S5 – *Data for omissions for baseline weeks between drug studies from the JBT.*

		Day 1		Day 2		Day 3		Day 4		Day 5	
Drug	**Cohort**	HT	LT	HT	LT	HT	LT	HT	LT	HT	LT
Memantine	1	0.00±0.00	2.57±0.86	0.66±0.32	2.55±0.82	0.26±0.18	3.84±0.86	0.00±0.00	3.86±1.10	0.42±0.30	4.32±0.96
MK-801		1.36±0.70	3.91±0.95	0.84±0.41	6.30±1.60	0.93±0.43	3.30±1.16	0.68±0.43	2.64±0.93	0.32±0.22	3.93±1.18
Lanicemine		0.38±0.28	1.98±0.52	0.00±0.00	3.13±1.08	0.13±0.13	2.44±0.82	0.28±0.19	3.94±1.17	0.27±0.18	4.34±0.97
CP-101,606: low		0.87±0.44	3.61±1.02	0.53±0.24	3.18±1.03	0.27±0.27	2.50±0.73	0.38±0.27	1.07±0.34	0.14±0.14	3.98±1.05
CP-101,606: high		0.77±0.37	5.77±1.53	0.26±0.17	4.48±1.00	0.24±0.24	4.03±1.14	0.52±0.29	3.67±1.13	0.26±0.18	3.52±0.90
Scopolamine		0.40±0.29	3.10±0.94	0.00±0.00	3.55±0.95	0.26±0.18	3.90±0.75	0.13±0.13	5.07±1.26	0.89±0.45	5.77±1.16
PCP	2	0.97±0.64	3.32±1.35	0.87±0.58	8.05±2.58	0.25±0.25	6.51±2.19	0.54±0.36	5.07±2.04	0.84±0.51	5.87±1.18
Ketamine (10)		0.00±0.00	5.28±1.91	0.54±0.36	7.75±1.90	0.00±0.00	5.35±0.85	0.28±0.28	6.62±2.37	0.00±0.00	3.49±0.89
Ketamine (25)		0.85±0.59	6.00±2.17	0.00±0.00	5.71±1.89	0.81±0.57	4.68±1.46	0.26±0.26	4.89±1.66	0.27±0.27	8.32±2.21
Post-Surgery	3	0.91±0.40	3.50±0.99	0.53±0.44	3.28±0.89	0.64±0.42	2.25±0.41	0.52±0.23	2.87±0.68	0.42±0.23	2.76±0.77
Ketamine (1)		0.00±0.00	5.95±1.94	0.14±0.14	2.03±0.64	0.07±0.07	2.17±0.63	0.13±0.13	3.32±0.68	0.27±0.19	2.33±0.55
Infusions 1		0.00±0.00	3.96±1.43	0.35±0.24	3.17±1.42	0.19±0.19	5.58±1.46	0.00±0.00	3.24±1.24	0.14±0.14	4.12±1.85
CP-101,606 infusion: low		0.16±0.16	4.14±0.90	0.48±0.25	3.53±0.95	0.54±0.37	3.94±1.07	0.16±0.16	5.07±1.26	0.00±0.00	5.01±1.01
CP-101,606 infusion: high		0.83±0.67	4.82±1.41	0.33±0.22	4.01±1.16	0.65±0.37	4.86±1.15	0.16±0.16	4.29±1.28	0.54±0.37	4.25±0.98

Behavioural data are presented as mean ± SEM. Drug studies are listed in chronological order for each cohort. Ketamine doses are listed in brackets.

Table S6 – *Data for premature responding for baseline weeks between drug studies from the JBT.*

| Drug	Cohort	Day 1	Day 2	Day 3	Day 4	Day 5
Premature						
Memantine		7.71±1.56	6.38±0.97	6.19±0.90	7.02±0.83	6.30±0.73
MK-801		8.03±1.00	6.57±0.69	5.77±1.13	5.76±0.84	5.56±1.01
Lanicemine	1	6.38±0.81	5.60±0.83	5.57±0.93	5.03±0.71	8.72±1.37
CP-101,606: low		6.58±1.06	5.13±0.91	4.21±0.48	6.07±0.95	4.00±0.69
CP-101,606: high		5.05±0.63	4.91±0.77	3.62±0.79	4.26±0.560	3.41±0.46
Scopolamine		5.81±1.15	4.71±0.83	4.05±0.72	3.26±0.62	4.80±0.91
PCP		5.81±1.20	4.39±0.95	2.73±0.94	3.86±1.13	4.17±0.49
Ketamine (10)	2	12.0±2.15	6.55±1.62	3.66±0.96	3.13±0.49	3.44±0.88
Ketamine (25)		11.76±1.39	6.20±1.24	5.25±0.98	6.45±1.09	6.84±1.73
Post-Surgery		39.4±3.86	19.9±2.16	14.1±1.88	13.8±1.98	13.0±1.89
Ketamine (1)		14.0±4.41	11.8±3.03	12.3±3.00	13.0±1.89	12.3±2.00
Infusions 1	3	11.2±1.98	6.27±0.74	6.46±1.11	7.26±1.25	6.58±1.64
CP-101,606 infusion: low		6.74±0.87	6.39±1.03	4.86±0.82	4.89±0.80	5.86±0.98
CP-101,606 infusion: high		5.31±0.76	3.85±0.72	5.38±0.78	4.98±1.00	4.35±0.75

Behavioural data are presented as mean ± SEM. Drug studies are listed in chronological order for each cohort. Ketamine doses are listed in brackets.
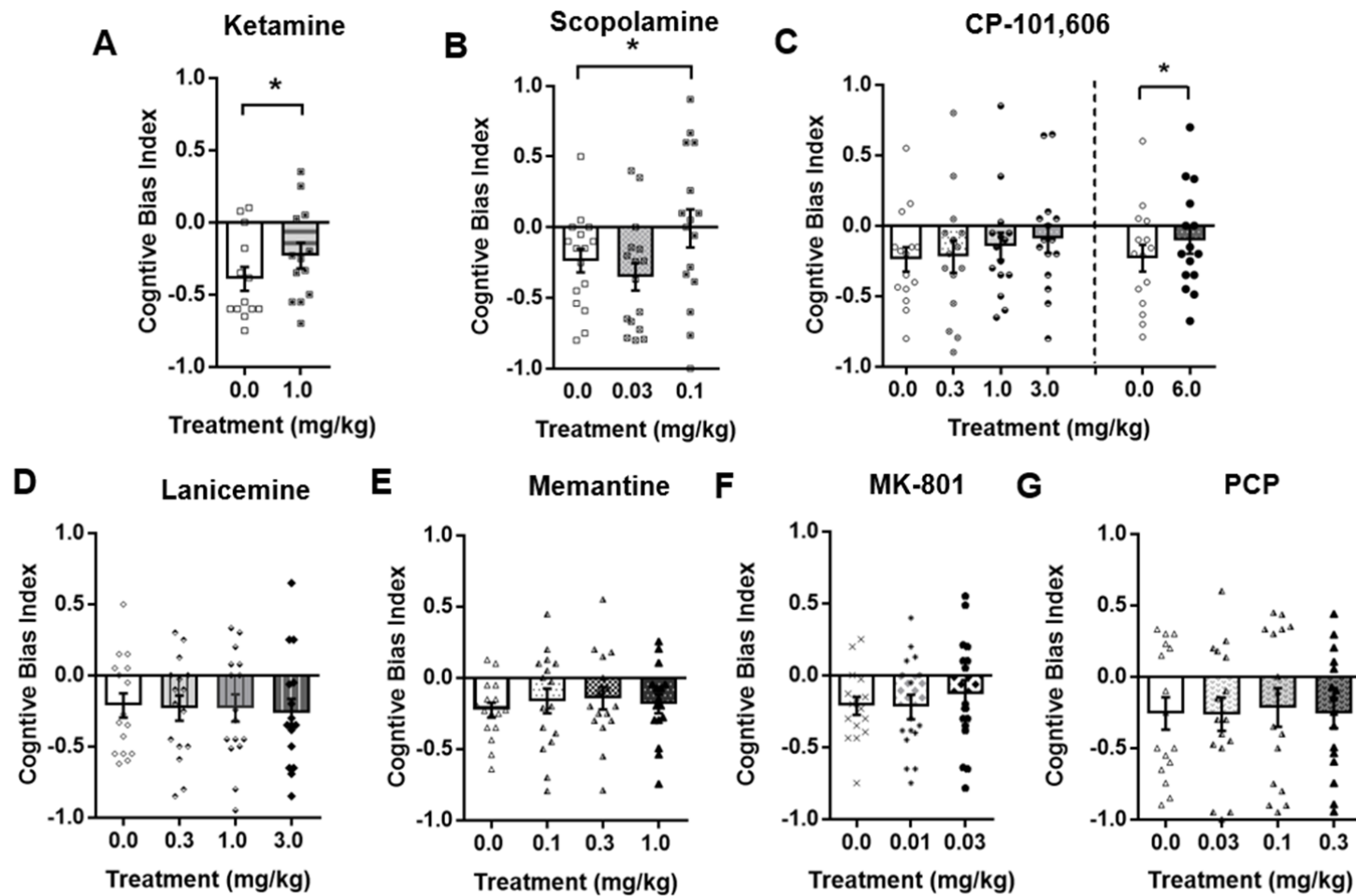
Figure S1 – *The effect of acute treatment with rapid acting antidepressant drugs and NMDA receptor antagonists on judgement bias of the midpoint ambiguous tone displayed as CBI.*

This figure shows data from Figure 1 displayed as cognitive bias index (CBI) scores. Ketamine (0.0, 1.0 mg/kg; n = 13), scopolamine (0.0, 0.03, 0.1 mg/kg; n = 16), CP-101,606 (Expt 1: 0.0, 0.3, 1.0, 3.0 mg/kg, n = 15; Expt 2: 0.0, 6.0 mg/kg, n = 15), lanicemine (0.0, 0.3, 1.0, 3.0 mg/kg; n = 16), memantine (0.0, 0.1, 0.3, 1.0 mg/kg; n = 16) and MK-801 (0.0, 0.01, 0.03 mg/kg; n = 16 were administered acutely by intraperitoneal injection prior to testing on the judgement bias task. (A) Replicating previous studies, ketamine (1.0 mg/kg) caused CBI to shift in the positive direction. (B) Scopolamine (0.1 mg/kg) changed CBI scores to near zero, a positive shift. (C) CBI became moved in a positive direction after a 6.0 mg/kg dose of CP-101,606. (D-G) Lanicemine, memantine, MK-801 and low doses of PCP did not alter CBI for the midpoint tone at the doses tested. Data shown and represent mean ± SEM (bars and error bars) overlaid with individual data points for each rat. Dashed line (panel C) indicates separate, counterbalanced experiments. *$p < 0.05$. CP-101,606, ketamine, lanicemine, memantine, PCP: 60 min pre-treatment; scopolamine, MK-801: 30 min pre-treatment.
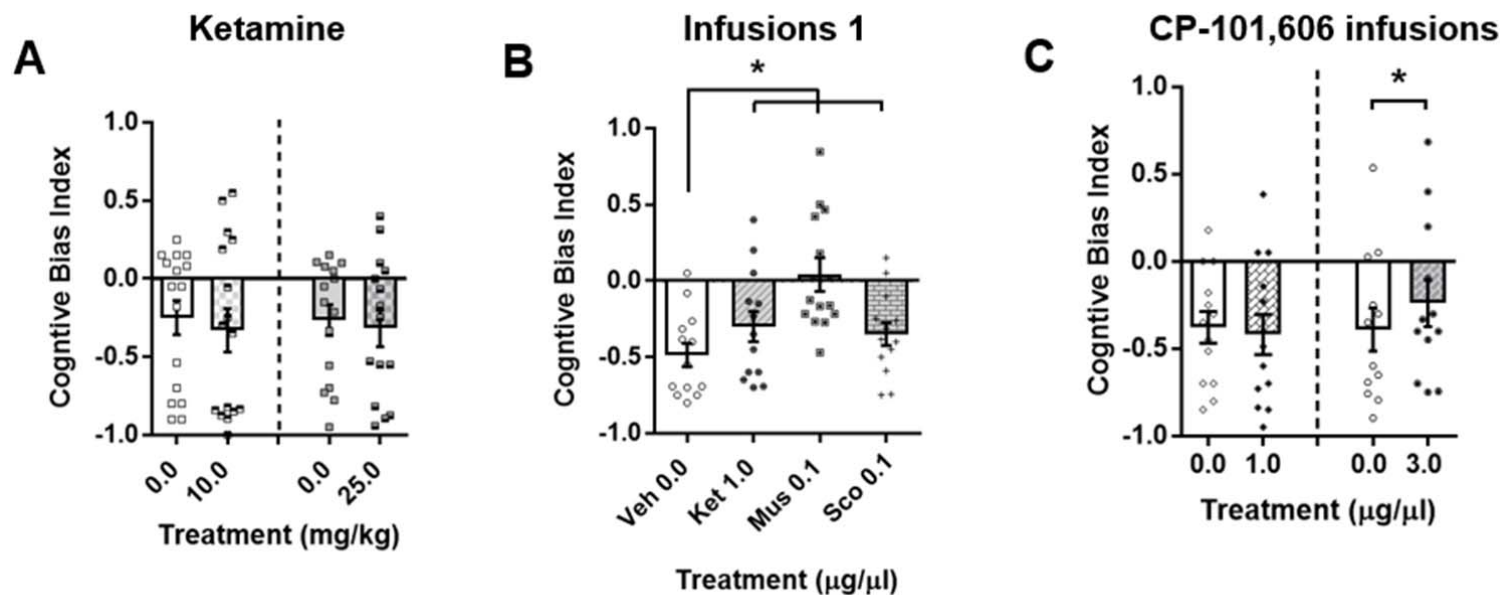
Figure S2 – *The effect of acute treatment with high doses of ketamine, and mPFC infusions of rapid acting antidepressants on judgement bias of the midpoint ambiguous tone displayed as CBI.*

This figure shows data from Figures 2-4 displayed as cognitive bias index (CBI) scores. (A) From Figure 2: ketamine (Expt 1: 0.0, 10.0 mg/kg, n = 16; Expt 2: 0.0, 25.0 mg/kg, n = 16) were administered by intraperitoneal injection to measure their effect on judgement bias. Neither dose had any effect on CBI. (B) From Figure 3:) In the first infusion experiment, ketamine (Ket; 1.0 µg/µl) muscimol (Mus; 0.1 µg/µl), scopolamine (Sco; 0.1 µg/µl) or vehicle (Veh; 0.0 µg/µl; n = 13), were administered by intracerebral infusion into the mPFC to measure the effect on judgement bias. All infuions caused positive changes in CBI. (C) From Figure 4: CP-101,606 (Expt 1: 00.0, 1.0 µg/µl, n = 13; Expt 2: 0.0, 3.0 µg/µl, n = 12) was administered by intracerebral infusion in the mPFC to measure the effect on judgement bias. Only the higher dose (3.0 mg/kg) caused CBI to become more positive. Data shown and represent mean ± SEM (bars and error bars) overlaid with individual data points for each rat. Dashed line (panel C) indicates separate, counterbalanced experiments. *p < 0.05. Ketamine (systemic): 60 min pre-treatment; infusions: 5 min pre-treatment.

**Table S7 –** *Data for behavioural measures from the JBT.*

Drug / Session	Dose (mg/kg) / Week	Response latency			% Accuracy		% Omissions			% Premature
		HT	MT	LT	HT	LT	HT	MT	LT	
Ketamine	0.0	1.62 ± 0.12	3.23 ± 0.22	3.80 ± 0.36	97.88 ± 0.62	83.06 ± 1.96	0.00 ± 0.00	0.58 ± 0.30	1.15 ± 0.36	12.44 ± 1.94
	1.0	1.77 ± 0.13	3.38 ± 0.29	4.05 ± 0.44	97.69 ± 9.57	83.54 ± 2.48	0.20 ± 0.20	0.38 ± 0.26	0.78 ± 0.34	14.09 ± 3.07
CP-101,606	0.0	2.19 ± 0.18	3.74 ± 0.39	4.26 ± 0.32	97.82 ± 0.77	84.87 ± 1.74	0.38 ± 0.26	0.88 ± 0.41	1.28 ± 0.55	8.34 ± 1.24
	0.3	2.43 ± 0.21	3.85 ± 0.36	4.54 ± 0.34	97.54 ± 0.91	85.44 ± 2.39	0.00 ± 0.00	0.82 ± 0.38	1.15 ± 0.47	7.42 ± 1.23
	1.0	2.21 ± 0.15	3.48 ± 0.26	4.46 ± 0.29	98.33 ± 0.72	83.49 ± 2.16	0.00 ± 0.00	0.69 ± 0.41	0.83 ± 0.47	8.80 ± 1.26
	3.0	*1.97 ± 0.17	*2.88 ± 0.23	*3.72 ± 0.27	95.83 ± 1.14	82.45 ± 2.62	0.00 ± 0.00	0.17 ± 0.17	0.33 ± 0.23	12.44 ± 2.58
CP-101,606	0.0	3.05 ± 0.24	4.52 ± 0.27	5.38 ± 0.33	98.25 ± 0.51	86.57 ± 1.70	0.70 ± 0.39	2.50 ± 0.82	2.81 ± 0.80	4.24 ± 2.58
	6.0	***2.32 ± 0.13	***3.52 ± 0.17	***4.10 ± 0.19	97.64 ± 0.87	84.37 ± 1.98	0.00 ± 0.00	**0.00 ± 0.00	**0.72 ± 0.32	**11.75 ± 1.80
Scopolamine	0.0	2.28 ± 0.17	3.84 ± 0.24	4.42 ± 0.31	97.80 ± 0.64	86.56 ± 1.84	0.16 ± 0.16	0.31 ± 0.21	1.09 ± 0.51	6.20 ± 0.70
	0.03	***4.10 ± 0.34	***5.52 ± 0.38	***6.51 ± 0.40	**99.50 ± 0.27	**94.01 ± 1.40	*7.17 ± 2.17	***17.18 ± 3.38	**18.02 ± 3.60	7.31 ± 1.55
	0.1	**4.26 ± 0.38	**5.54 ± 0.37	**6.27 ± 0.51	95.00 ± 1.76	85.26 ± 3.93	*22.85 ± 3.79	***27.98 ± 3.91	**35.92 ± 6.05	*14.52 ± 3.85
Lanicemine	0.0	2.42 ± 0.22	3.80 ± 0.31	4.72 ± 0.45	96.60 ± 0.82	86.24 ± 1.81	0.00 ± 0.00	3.16 ± 2.21	2.93 ± 1.89	7.17 ± 1.11
	0.3	2.23 ± 0.17	3.60 ± 0.24	4.40 ± 0.29	98.13 ± 0.63	85.01 ± 1.89	0.00 ± 0.00	0.31 ± 0.21	1.26 ± 0.95	9.48 ± 1.36
	1.0	2.40 ± 0.22	4.20 ± 0.32	4.79 ± 0.40	96.41 ± 1.58	85.45 ± 2.15	0.00 ± 0.00	3.54 ± 1.99	4.03 ± 2.76	6.68 ± 1.03
	3.0	2.55 ± 0.21	3.89 ± 0.28	4.81 ± 0.37	98.58 ± 0.57	86.74 ± 1.71	0.16 ± 0.16	1.56 ± 1.25	2.81 ± 1.72	8.18 ± 1.67
Memantine	0.0	2.38 ± 0.15	4.12 ± 0.21	4.94 ± 0.23	97.77 ± 0.60	83.96 ± 1.52	0.19 ± 0.19	1.16 ± 0.44	2.07 ± 1.03	7.98 ± 0.93
	0.1	2.48 ± 0.18	4.15 ± 0.23	4.65 ± 0.30	97.45 ± 0.82	84.75 ± 1.81	0.16 ± 0.16	2.30 ± 0.85	1.09 ± 0.45	8.45 ± 1.25
	0.3	2.42 ± 0.18	3.84 ± 0.25	4.53 ± 0.30	97.49 ± 0.65	81.21 ± 1.79	0.16 ± 0.16	0.63 ± 0.36	2.34 ± 0.84	9.25 ± 1.40
	1.0	2.40 ± 0.16	3.90 ± 0.23	4.50 ± 0.28	97.66 ± 0.58	84.98 ± 1.64	0.00 ± 0.00	1.56 ± 0.75	2.03 ± 1.25	8.38 ± 1.24
MK-801	0.0	2.51 ± 0.15	4.16 ± 0.28	4.72 ± 0.30	96.39 ± 1.00	85.23 ± 1.78	0.16 ± 0.16	1.12 ± 0.40	1.31 ± 0.54	6.66 ± 0.77
	0.01	2.25 ± 0.14	3.80 ± 0.21	4.39 ± 0.27	96.71 ± 0.78	83.05 ± 1.64	0.16 ± 0.16	0.47 ± 0.25	1.09 ± 0.51	8.39 ± 1.72
	0.03	*2.34 ± 0.13	*3.44 ± 0.29	*4.07 ± 0.35	97.19 ± 0.88	81.19 ± 2.15	0.00 ± 0.00	1.41 ± 0.56	1.88 ± 0.74	10.73 ± 1.64
PCP	0.0	2.23 ± 0.17	3.88 ± 0.23	4.96 ± 0.42	98.28 ± 0.50	84.89 ± 1.88	0.00 ± 0.00	0.47 ± 0.25	0.47 ± 0.34	6.46 ± 0.98
	0.03	2.22 ± 0.18	3.99 ± 0.29	5.11 ± 0.42	98.59 ± 0.56	87.44 ± 1.70	0.00 ± 0.00	1.25 ± 0.60	2.03 ± 1.08	5.72 ± 0.75
	0.1	2.25 ± 0.18	3.81 ± 0.29	4.60 ± 0.30	99.07 ± 0.31	86.37 ± 1.87	0.31 ± 0.21	0.94 ± 0.39	1.41 ± 0.94	5.63 ± 0.89
	0.3	2.24 ± 0.20	3.93 ± 0.37	4.99 ± 0.44	98.75 ± 0.51	86.78 ± 2.34	0.57 ± 0.57	1.34 ± 0.73	4.11 ± 1.70	6.77 ± 1.41
Pre-Surgery		2.05 ± 0.14	3.72 ± 0.22	4.47 ± 0.33	95.80 ± 0.69	83.07 ± 2.41	0.51 ±0.30	1.50 ± 0.48	2.54 ± 0.77	14.80 ± 2.30
Post-Surgery	1	1.99 ± 0.15	3.82 ± 0.23	4.64 ± 0.36	95.89 ± 1.00	85.62 ± 1.95	0.00 ± 0.00	0.93 ± 0.31	2.18 ± 0.59	11.88 ± 1.53
	2	1.81 ± 0.12	3.81 ± 0.31	4.29 ± 0.44	97.17 ± 0.71	84.15 ± 1.45	0.17 ± 0.11	0.67 ± 0.24	2.25 ± 0.62	13.33 ± 1.44
	3	1.77 ± 0.16	3.43 ± 0.24	4.19 ± 0.40	96.58 ± 0.59	81.16 ± 2.43	0.08 ± 0.08	1.00 ± 0.28	1.25 ± 0.39	11.97 ± 1.70

Behavioural data are presented as mean ± SEM. Cells marked in bold indicate a significant difference for that measure, for that tone (compared to vehicle 0.0 mg/kg session for that drug) from a significant dose*tone interaction. Cells highlighted in grey indicate where a main effect of dose was found, indicating a difference for that drug treatment not specific to tone. * / light grey shading: $p<0.05$, ** / medium grey shading: $p<0.01$, *** / dark grey shading: $p<0.001$.

Table S8 – *Description of statistical analysis for other behavioural measures*

Behavioural measure	Analysed for:	Description	Statistical analysis
Response latency	Each tone	Time between presentation of the tone and response on the lever (correct lever for high and low reward tones, either lever for midpoint tone)	Two-way repeated measures ANOVA with tone and session as within-subjects factors
Accuracy	Reference tones (high and low tones)	Number of correct responses made divided by the total number of responses made (correct + incorrect) for that tone	
Percentage omissions	Each tone	Number of trials where no lever press occurred during 20 s tone presentation divided by total completed trials for that tone	
Percentage of premature responses	Whole session	Number of trials where a response was made in the 5 s inter-trial interval divided by total completed trials	Repeated measures ANOVA with session as the within-subjects factor

This table details the other behavioural measures (apart from cognitive bias index) that were analysed for each experimental manipulation and are presented in Table 1. ANOVA – analysis of variance.
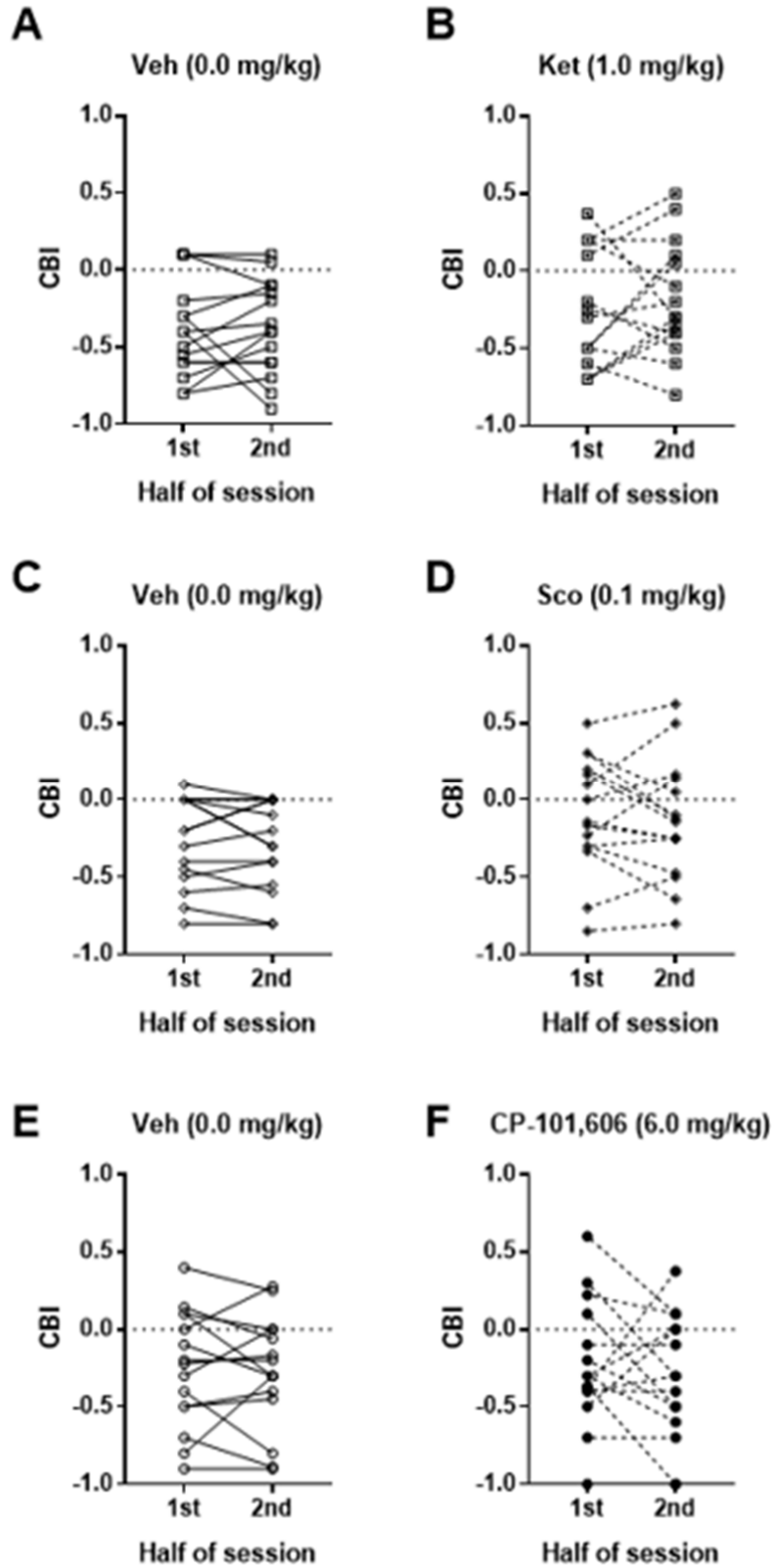
Figure S3 – *CBI analysed by session split in half for systemic drugs that caused a positive change in bias.*

Ketamine (0.0, 1.0 mg/kg; n = 13), scopolamine (0.0, 0.03, 0.1 mg/kg; n = 16), CP-101,606 (Expt 2: 0.0, 6.0 mg/kg, n = 15) were administered acutely by intraperitoneal injection prior to testing on the judgement bias task. Data from these drug studies for doses that showed a positive change in judgement bias (ketamine: 1.0 mg/kg; scopolamine: 0.1 mg/kg) and CP-101,606: 6.0 mg/kg) were re-analysed by splitting each session in half, and comparing CBI for the first and last half of the sessions. Vehicle doses for each drug (panels A,C,E) are also shown for comparison. There is no consistent change between CBI scores across the first and second halves of a session for the drugs shown. Data shown are individual data points, linked for each individual rat.