Title: Role of the medial prefrontal cortex in the effects of rapid acting antidepressants on affective biases in a rodent decision-making task

Running title: Rapid antidepressant effects on affective bias

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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. 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 recapitulate the effects of ketamine, suggesting a specific mechanism. Furthermore, this operant behavioural task provides a sensitive method to better understand the neuropsychological effects of RAADs.

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. In previous work in rodents, affective biases in a decision-making task were differentially altered by conventional, delayed acting antidepressants versus the RAAD ketamine[16]. In this reward-based judgement bias task, which measures biased processing of ambiguous information associated with more or less positive outcomes, we found that an acute, low dose of ketamine, but not acute treatment with another NMDA receptor antagonist, PCP, immediately induced a positive bias, whereas acute treatment with conventional antidepressants had no effect on bias[16]. However, when given chronically, the conventional antidepressant fluoxetine did induce a positive bias[16], but over a timescale that more closely reflects subjective changes in patients.

The aim of this study was to build upon these findings by testing a selection of other drugs that act via NMDA receptor antagonism: lanicemine, a low-trapping NMDA receptor channel

62 blocker developed for the treatment of MDD, but failed to show efficacy in clinical trials[17];
63 memantine, an Alzheimer's medication that is a moderate affinity, non-competitive NMDA
64 receptor antagonist, but also lacked antidepressant efficacy in clinical trials[5,18]; and MK-801,
65 a potent, non-competitive NMDA receptor antagonist that has shown RAAD efficacy in
66 animal models[19]. We also tested other compounds that have been shown to have RAAD in
67 human clinical trials: the GluN2B subunit selective NMDA receptor antagonist CP-101,606[20],
68 and the acetylcholine muscarinic receptor antagonist scopolamine[21]. We also tested
69 additional doses of ketamine and PCP to ensure we had examined effects across a wider
70 range of receptor occupancy and in line with doses commonly used in preclinical animal
71 models used to study depression[22]. To investigate the mechanism underlying the rapid
72 positive change in decision-making bias we tested local administration of drugs shown to
73 cause this effect directly into the prefrontal cortex (PFC), a brain area thought to be critical in
74 the mechanism of RAAD of ketamine[23,24] and previously shown to modulate learning biases
75 in rodents[25].

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 (see Supplementary Materials and Methods) and 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. During experiments all efforts were made to minimise suffering including using a low stress method of drug administration[26], 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[16,27] (and see Supplementary Materials and Methods).

Judgement bias training

Animals were trained and tested using a high versus low reward version of the judgement bias task as previously reported[16]. Rats were first trained to associate one tone (2kHz at 83dB rats, designated high reward) with a high value reward (four 45mg reward pellets; TestDiet, Sandown Scientific, UK) and the other tone (8kHz at 66dB, designated low reward) with a low value reward (one 45mg reward pellet) if they pressed the associated lever (either left or right, counterbalanced across rats) during the 20s tone. Table S1 contains a summary of training stages and criteria used. Rats were considered trained (29/23-25/25 total sessions for cohorts 1/2/3 respectively, see Table S1 for sessions for each training stage) when they maintained stable responding for three consecutive days.

Judgement bias testing

Baseline sessions (100 trials: 50 high and 50 low reward tones; presented pseudorandomly) were conducted on Monday and Thursday. Probe test sessions (120 trials: 40 high reward, 40 low reward, and 40 ambiguous midpoint tones that were 5kHz at 75dB; pseudorandomly) were conducted on Tuesday and Friday. The midpoint tone was randomly reinforced so that a specific outcome could not be learnt, and to maintain responding throughout the experiments. Cohort 1 were used to test the effect of acute systemic treatments with putative RAAD and other NMDA receptor antagonists. Cohort 2 were made up of two groups of eight rats that had previously been used as control animals in another experiment (data not shown) and were then used for the extension of doses of ketamine and PCP. Cohort 3 were used for mPFC infusion experiments. For further details see Table S2.

Study 1: the effect of acute, systemic treatments with RAADs and NMDA receptors antagonists on judgement bias.

Experimental design: Each study used a within-subject fully counterbalanced drug treatment schedule (see Table S2 for details of individual treatments). All drugs were given by intraperitoneal injection using a low-stress, non-restrained technique[26]. Ketamine[¥] (Sigma-Aldrich, UK), scopolamine[§] (Tocris, UK), lanicemine[¥] (Sigma Aldrich, UK), memantine[¥] (Tocris, UK), MK-801[§] (Tocris, UK) and PCP[¥] (Sigma Aldrich, UK) were dissolved in 0.9% sterile saline and given 30[§] or 60[¥] minutes prior to testing. CP-101,606 (Experiment 1: Sigma Aldrich, UK; Experiment 2: Boehringer Ingelheim GmbH) was dissolved in 5% DMSO, 10% cremaphor and 85% sterile saline and given 60 minutes prior to testing. Drug doses were selected based on previous rodent behavioural studies[16,28]. Doses for ketamine and PCP were chosen to extend the range of doses tested in this task e.g. higher doses of ketamine and lower doses of PCP were used than previously[16]. For all studies, the experimenter was blind to drug dose. Each dose-response study was separated by at least one week (five sessions) of baseline testing. The order of testing is displayed in Table S2.

Study 2: mPFC cannulation and infusions

To localize the site and mechanism of action of RAAD drugs, rats were implanted with mPFC guide cannula (see Supplementary Materials and Methods for details). Following recovery from surgery, one week of probe testing was carried out to check that judgement of the ambiguous tone had not altered after surgery. Based on this, another two weeks of probe testing (4 test sessions) was then conducted. Following this, an acute systemic treatment with ketamine was given as a positive control manipulation to ensure that bias could still be manipulated in rats post-surgery. Rats were then used for mPFC infusion experiments. For details of the infusion procedure, see Supplementary Materials and Methods.

In the first infusion experiment, vehicle (sterile phosphate-buffered saline (PBS); 0.0μg/μl), ketamine (1.0μg/μl), muscimol (0.1μg/μl) or scopolamine (0.1μg/μl), all dissolved in sterile PBS, were infused intracerebrally into mPFC 5 minutes before testing. Following this, CP-101,606 (1.0μg/μl in the first study, 3.0μg/μl in the second study) was dissolved in 10% 2-hydroxypropyl-cyclodextrin and 90% PBS and tested. All experiments used a within-subject fully counterbalanced design for drug treatments, with the experimenter blind to treatment. Drug doses were chosen based on the results from acute, systemic treatments (see Table S2).

Following the completion of mPFC infusions, rats were killed and brains were fixed and processed for histology (see Supplementary Materials and Methods). The locations of the final injector tip positions in the mPFC were mapped onto standardized coronal sections of a rat brain stereotaxic atlas.

Data and statistical analysis

For sample size justification and exclusion criterion see Supplementary Materials and Methods. Cognitive bias index (CBI) was used as a measure of judgement bias in response to the midpoint tone. CBI was calculated by subtracting the proportion of responses made on

155 the low reward lever from the proportion of responses made on the high reward lever. This
156 created a score between -1 and 1, where negative values represent a negative bias and
157 positive values a positive bias. Change from baseline in CBI was then calculated for all
158 experimental manipulations as follows: vehicle (0.0mg/kg) probe test CBI − drug dose probe
159 test CBI. This was calculated to take into account individual differences in baseline bias, and
160 to make directional changes caused by drug treatments clearer. To provide a value for
161 vehicle probe test sessions for this measure, the population average for the vehicle
162 (0.0mg/kg) probe test was taken away from each individual rats' CBI score for the same
163 session. This allowed this measure to be analysed with repeated measures analysis of
164 variance (rmANOVA) with session as the within-subjects factor for drug studies with more
165 than two treatments, or paired samples t-test for studies with only two treatments.

166 Response latency and accuracy, omissions and premature responses were also analysed
167 (see Table S3 for details of these). These measures were analysed with rmANOVAs with
168 session and tone as the within-subjects factors. Paired t-tests were performed as post-hoc
169 tests if significant effects were established. Huynh-Feldt corrections were used to adjust for
170 violations of the sphericity assumption, and Sidak correction was applied for multiple
171 comparisons. All statistical tests were conducted using SPSS 24.0.0.2 for Windows (IBM
172 SPSS Statistics) with $\alpha=0.05$. Results are reported with the ANOVA F-value (degrees of
173 freedom, error) and *p*-value as well as any post-hoc *p*-values. All graphs were made using
174 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 session. Despite a lack of main effect when comparing all drug doses, CP-101,606 treated animals showed a positive bias for the highest dose (3.0mg/kg; one sample t-test: p=0.038; Figure 1A). In the second experiment, CP-101,606 (6.0mg/kg) resulted in a positive bias relative to vehicle treatment (paired samples t-test: p=0.027; Figure 1A). In experiment 1, 3.0mg/kg CP-101,606 also caused a decrease in response latency for the midpoint tone (main effect of session: $F_{3,42}$=4.858, p=0.005, post-hoc: p=0.048; Table 1), with a trend towards the same effect for the low tone (post-hoc: p=0.076; Table 1). There were no effects on other behavioural measures in experiment 1 (Table 1). In experiment 2, CP-101,606 (6.0mg/kg) caused response latencies to decrease for all three tones (main effect of session: $F_{1,14}$=27.396, p<0.001, post-hoc: ps≤0.005; Table 1). This dose had no effect on accuracy for the reference tones (Table 1), but did increase premature responses (paired samples t-test: p=0.001), and reduced omissions (main effect of session: $F_{1,14}$=10.506, p=0.006; Table 1) for the midpoint (p=0.008) and low tones (p=0.024; Table 1).

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 session: $F_{2,30}$=6.739, p=0.004, post-hoc: p=0.035; Figure 1B). This dose of scopolamine (0.1mg/kg) also increased response latencies for all three tones (main effect of session: $F_{2,30}$=17.263, p<0.001, post-hoc: ps≤0.011; Table 1), increased premature responding (main effect of session: $F_{1.355,20.330}$=4.387, p=0.039, post-hoc: p=0.047; Table 1), and increased omissions for all tones (significant session*tone interaction: $F_{2.343,35.150}$=4.739, p=0.011, main effect of session: $F_{2,30}$=24.257, p<0.001, post-hoc: ps<0.001; Table 1). The lower dose also caused response latencies to increase for all tones (post-hoc: ps≤0.001; Table 1), accuracy to increase for the low tone only (main effect of session:

$F_{1.605,24.069}$=8.558, p=0.003, post-hoc: p=0.004; Table 1), and omissions to increase for all tones (post-hoc: ps≤0.019; Table 1).

Ketamine: In the rats who had undergone mPFC cannulation surgery, ketamine (1.0mg/kg) caused a positive change in CBI (paired samples t-test: p=0.033; Figure 1C), as has been seen previously[16]. Ketamine did not alter any other behavioural measures (Table 1).

Lanicemine: None of the doses of lanicemine tested caused a change in CBI (Figure 1D). This drug also had no effect on any other behavioural measures (Table 1).

Memantine: Memantine did not cause any change in CBI at the doses tested (Figure 1E). There was also no effect on other behavioural measures (Table 1).

MK-801: MK-801 did not change CBI (Figure 1F). The highest dose of MK-801 tested (0.03mg/kg) decreased response latency for the midpoint tone (main effect of session: $F_{2,30}$=3.843, p=0.033, post-hoc: p=0.013; Table 1) and also had a tendency to cause premature responses to increase (trend towards main effect of session: $F_{1.565,23.480}$=2.915, p=0.085, post-hoc: p=0.039; Table 1). There was no effect on accuracy for the reference tones or percentage omissions.

High-dose ketamine: In experiment 2 (25mg/kg ketamine) one rat was excluded for failure to complete sufficient trials. In experiments 1 and 2, ketamine (10mg/kg and 25mg/kg respectively) did not change CBI (Figure 2A). In both experiments these higher doses did alter all other behavioural measures. There was an increase in response latency across all three tones for both 10mg/kg (session*tone interaction: $F_{2,30}$=7.323, p=0.003, post-hoc: ps<0.001 for all tones; Figure 2B), and 25mg/kg ketamine (session*tone interaction: $F_{2,28}$=4.686, p=0.018, post-hoc: ps≤0.002 for all tones; Figure 2B). Both doses decreased premature responses (paired samples t-tests: 10mg/kg – p=0.005, 25mg/kg – p=0.006; Figure 2C). Ketamine also improved accuracy for the low reward tone only in experiment 1 (10mg/kg: main effect of session: $F_{1,15}$=8.774, p=0.010, and trend towards session*tone interaction: $F1,15$=3.665, p=0.075, post-hoc: p=0.022; Figure 2D) and experiment 2

(25mg/kg: trend towards main effect of session: $F_{1,14}$=4.500, p=0.052, and session*tone interaction: $F1,14$ = 5.513, p=0.034, post-hoc: p=0.033; Figure 2D). In both experiments, there was an increase in omissions for all three tones (experiment 1, 10mg/kg: session*tone interaction: $F_{1.401,21.021}$=5.662, p=0.018, post-hoc: high reward tone – p=0.015, midpoint tone: p=0.003, low reward tone: p=0.010; experiment 2, 25mg/kg: session*tone interaction: $F_{1.368,19.150}$=11.964, p=0.001, post-hoc: high reward tone – p=0.003, midpoint tone – p<0.001, low reward tone – p=0.001; Figure 2E).

Low dose PCP: Doses of PCP (0.03, 0.1, 0.3mg/kg) that were lower than those previously tested[16] did not cause any change in CBI (Figure 1G). There was also no effect on any other behavioural measures (Table 1).

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

Two rats were excluded in cohort 3: one rat did not meet accuracy criteria for any probe (or baseline) session following the second drug infusion; and after the end of testing another animal was found to have an incorrect cannula placement. Therefore, both were excluded retrospectively from the entire study. Compared to pre-surgery performance, the CBI of rats became more negative after surgery, and this was stable across testing over three weeks (main effect of week: $F_{3,42}$=6.335, p=0.001, post-hoc: ps≤0.011; Figure 3A). There were no differences in response latencies, premature responses, accuracies for reference tones or omissions before compared to after surgery (Table 1).

In the first infusion experiment, ketamine (1.0µg/µl), muscimol (0.1µg/µl) and scopolamine (0.1µg/µl) all induced positive biases (main effect of session: $F_{3,36}$=7.241, p=0.001; post-hoc: ketamine – p=0.012, muscimol – p=0.001, scopolamine – p=0.032 Figure 3C). The effect of PFC infusion of ketamine or scopolamine was specific to CBI, as these drugs had no effect on other behavioural measures (Figure 3D-G), unlike muscimol infusions which caused changes to all other behavioural measures. There was an increase in response latency

(session*tone interaction: $F_{6,72}$=4.181, p=0.001) for the high reward (post-hoc: p<0.001) and midpoint tone (p=0.028; Figure 3D), and a large increase in premature responses to over 100% (main effect of session: $F_{1.151,13.809}$=33.784, p<0.001, post-hoc: p<0.001; Figure 3E). Muscimol also caused accuracy to decrease for both reference tones (main effect of session: $F_{1.181,14.172}$=43.775, p<0.001, post-hoc: ps≤0.001; Figure 3F). For the low reward tone, this reduction was so great that rats were no longer performing any better than chance (one-sample t-test against a test value of 50%: p=0.197; Figure 3F). Omissions for the high and low reward tones increased following muscimol infusion (main effect of session: $F_{1.338,16.057}$=10.418, p=0.003, post-hoc: high tone – p=0.010, low tone – p=0.012; Figure 3G).

In the experiments testing the effect of CP-101,606 mPFC infusion, in experiment 1 the lower dose (1.0μg/μl) did not alter CBI (Figure 4A), but in experiment 2, the higher dose (3.0μg/μl) induced a positive bias (paired samples t-test: p=0.043; Figure 4A). In experiment 1, CP-101,606 (1.0μg/μl) caused an increase in response latency for the midpoint tone only (main effect of session: $F_{1,12}$=5.064, p=0.044, post-hoc: p=0.019; Figure 4B) but had no other behavioural effects (Figure 4C-E). In experiment 2, 3.0μg/μl CP-101,606 did not have any effects on other behavioural measures (Figure 4B-E).

Discussion

As previously shown, low dose ketamine (1.0mg/kg) had a specific effect on decision-making biases, inducing a positive change in CBI following acute administration. This effect of ketamine was dose dependent, with higher doses having general effects on task performance without changing CBI. The effects of ketamine were recapitulated to some extent by the GluN2B antagonist, CP-101,606 and muscarinic antagonist, scopolamine, but both also had more general effects on other behavioural measures following systemic administration. All three treatments have previously been reported to have RADD effects in clinical trials[3,20,21], whilst the other NMDA antagonists tested here did not[5,17,18], and these also failed to induce a change in bias. The mPFC infusions suggest that this brain region is central to the effects of ketamine, scopolamine and CP-101,606. Interestingly, mPFC infusions more specifically altered bias, suggesting other brain regions may contribute to the systemic effects on other behavioural measures. The importance of the mPFC in modulating RAAD effects in neuropsychological tasks is consistent with previous findings in our learning and memory bias assay, the affective bias test[25]. Inactivation of the mPFC with muscimol did positively change bias but animals also exhibited large changes in other behavioural measures. This suggests that the RAADs can modulate activity in this brain region in a more specific way than muscimol, which results in a relatively specific effect on biases in decision-making.

For lanicemine and memantine, the lack of any behavioural effects means there is a possibility that the doses tested were too low. However, for both of these the range of doses tested covers the doses that are equivalent to those used humans in clinical trials (lanicemine: 50, 100mg[17], equivalent to approximately 0.75, 1.5mg/kg; memantine: 5-20mg[5], equivalent to approximately 0.07-0.3mg/kg), paralleling our effective dose of ketamine (1.0mg/kg, similar to the 0.5mg/kg dose used by Zarate et al.[3]). Furthermore, when testing lower doses of PCP (another NMDA receptor antagonist not known to show RAAD) than previously used[16], we also failed to see any change in CBI. Conversely, when we tested

297 higher doses of ketamine than those we had previously, doses that have been shown to
298 have antidepressant effects in other preclinical models used to study depression[29], we failed
299 to see any change in bias, instead only seeing non-specific changes in other behavioural
300 measures. This lends support to our interpretation that this reward-based judgement bias
301 task can specifically dissociate between drugs that do show RAAD, and those that do not,
302 despite them having similar pharmacology.

303 The difference in specificity on behavioural effects, whereby ketamine (1.0mg/kg) only
304 positively changes decision-making bias, but both CP-101,606 and scopolamine have other
305 non-specific effects, suggests that 1.0mg/kg ketamine is able to relatively selectively
306 modulate affective bias. The changes in response latencies, omissions and premature
307 responses caused by CP-101,606 and scopolamine suggest that these drugs may also be
308 having effects on other cognitive processes, such as motivation. However, the direction of
309 changes for these drugs are in opposite directions (decreases in response latency and
310 omissions for CP-101,606 but increases in these for scopolamine) despite them both
311 causing positive changes in CBI. This, combined with the lack of change in accuracy for the
312 reference tones, suggest that these non-specific effects cannot fully explain the change in
313 decision-making bias.

314 The neurobiology underlying the relative specificity of ketamine, CP-101,606 and
315 scopolamine in being able to immediately alter decision-making bias, in contrast to the other
316 NMDA receptor antagonists tested that have not shown these effects, are likely to be due to
317 differences in their mechanisms of action. Our findings add weight to the strong body of
318 evidence suggesting that NMDA receptor antagonism is important for short-term, RAAD
319 effects of these drugs[30], but suggests that specific modulation of either a specific subtype of
320 the receptor or a sub-population of neurons may be involved. CP-101,606 is selective for the
321 GluN2B NMDA receptor subunit, whilst it has been shown that scopolamine, and more
322 recently ketamine, cause a glutamate burst via blockade of NMDA receptors specifically on
323 GABA interneurons that leads to increased mechanistic target of rapamycin complex 1

signalling, brain-derived neurotrophic factor release and synaptic changes in the PFC[31,32,33,34]. Further studies would be required to test whether these mechanisms also drive these drugs effects on affective bias.

The infusion studies localise the site of action of this rapid change in decision-making bias to the mPFC, corresponding with brain imaging studies in humans that have also shown ketamine-dependent changes in prefrontal glutamatergic neurotransmission[23,24]. This also matches with previous rodent studies, where using the affective bias test, it has been shown that whilst ketamine does not induce positive biases in learning, it can remediate previously acquired negative biases, an effect which also localises to the mPFC[25].

Unlike when given systemically, intracerebral mPFC infusion of CP-101,606 or scopolamine did not cause any non-specific behavioural changes on the task. This could suggest that these effects are driven by off-target effects of drug binding in other brain areas, or in the case of scopolamine, the periphery. The pronounced, non-specific behavioural effects seen following muscimol infusion suggests that mechanism of action of these drugs is more refined than global inhibition of neurotransmission in the mPFC. Previous work in humans and rodents has shown that subcortical and limbic brain regions, such as the amygdala, are important in the neurocircuitry of MDD / depression-related behaviour[35,36,37], and a recent study suggests that ketamine may play a critical role in restoring dysfunctional connectivity in these circuits[24]. Furthermore, in rodents, a recent study found that optogenetic activation of pyramidal mPFC neurons containing dopamine receptor D1 caused RAAD-like responses in the forced swim test, and that blockade of these receptors prevented the RADD effects of ketamine[38]. In order to further our understanding of this mechanism, it will be important to investigate the effects of these drugs on different neuronal subtypes within the mPFC, as well as investigating the wider circuitry that is altered by these drugs.

Final conclusions

This study adds to the evidence that the neuropsychological effects of ketamine are potentially important in its RAAD in patients with MDD, and that these effects in altering affective biases, both in decision-making as demonstrated here, as well as in learning and memory occur at time points (one hour) before major plastic changes arise. It will be important to investigate the neurobiological effects of not just the immediate, RAAD of ketamine, but also the sustained effects by examining how affective biases are altered at longer time points. Furthermore, investigation of the wider circuits involved in this RAAD efficacy will be crucial in revealing the mechanism underlying these actions, which will be important for the development of novel therapeutics. Ketamine (at 1.0 mg/kg) seems to have very specific effects on affective bias, which we can capitalise on to better understand the circuits that contribute to these modulations of affective biases that are potentially very important in the cause, perpetuation and treatment of MDD.

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Figure Legends

Figure 1 – *The effect of acute treatment with rapid acting antidepressant drugs and NMDA receptor antagonists on judgement bias of the midpoint ambiguous tone.*

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) positively changed CBI. (B) Scopolamine (0.1 mg/kg) also caused a positive change from baseline in CBI. (C) In experiment 1, the 3.0 mg/kg dose caused a positive change in cognitive bias index (CBI) for the midpoint tone. A positive change was also seen in experiment 2 with a higher 6.0 mg/kg dose. (D-G) Lanicemine, memantine, MK-801 and low doses of PCP did not induce a change in 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 2 – *Behavioural data from the judgement bias task following acute treatment with high doses of ketamine.*

Acute doses of 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. (A) Neither high dose of ketamine caused a change in interpretation of the midpoint tone. (B) Both doses of ketamine increased accuracy for the low tone. (C) Both doses of ketamine increased response latencies across all three tones. (D) Omissions were increased across all three tones following both ketamine doses. (E) High doses of ketamine (10.0, 25.0 mg/kg)

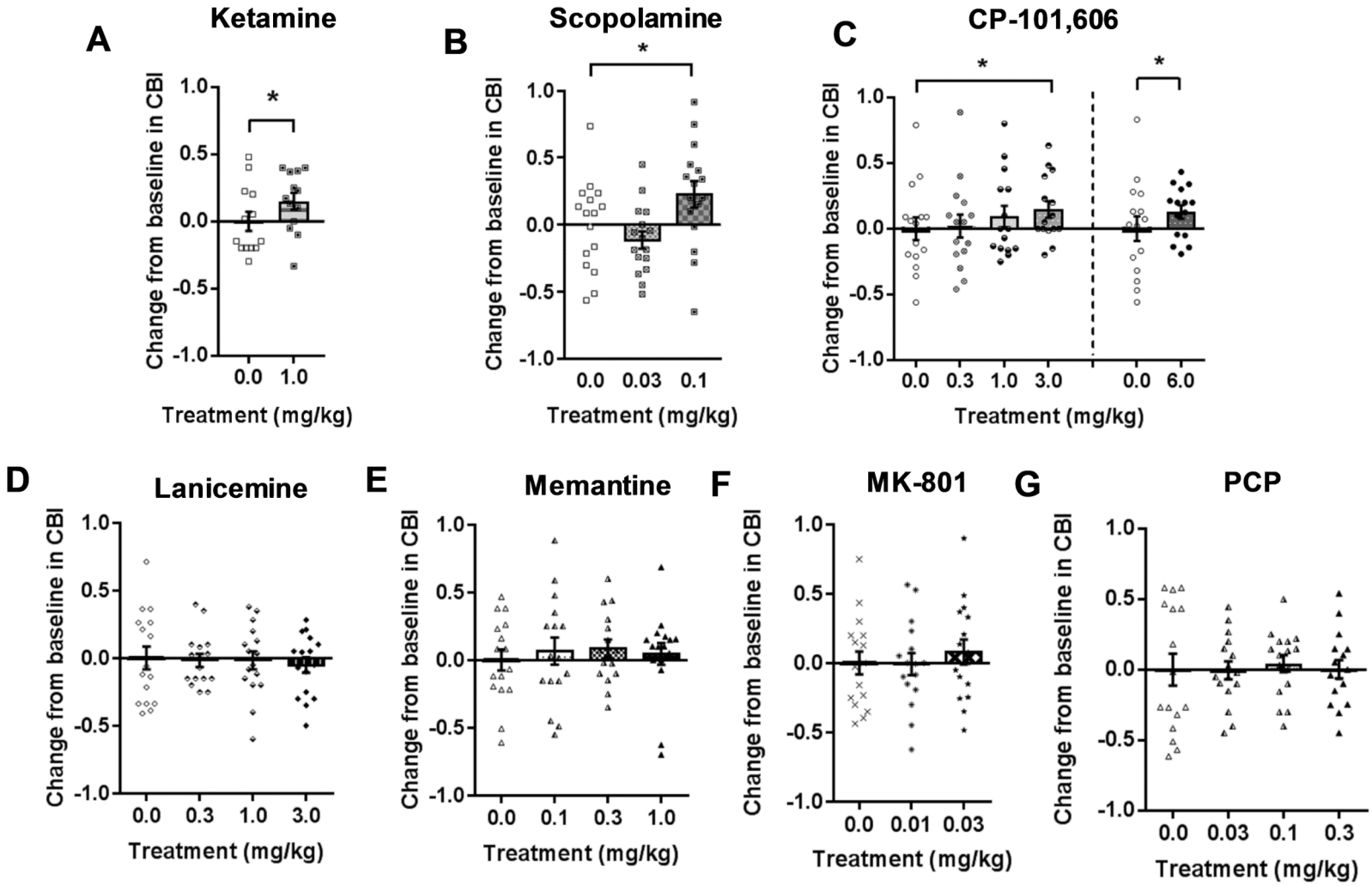
decreased premature responding. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Data represent mean ± SEM (panels B-E) with individual data points overlaid for each rat (panel A). Dashed lines indicate separate, counterbalanced experiments. 60 min pre-treatment. HT - high reward tone; MT - midpoint tone; LT - low reward tone.

Figure 3 – D*ata from mPFC cannulated rats on the judgement bias task.*

Probe tests with no experimental manipulation were conducted before and after mPFC cannulation surgery to ensure that the surgery itself did not effect performance in the judgement bias task. (A) Cognitive bias index became more negative in the probe tests conducted after surgery. (B) The location of the injector placement was confirmed post-mortem and black dots represent the location of the cannula tip as assessed from Cresyl violet-stained brain sections. Coronal sections are +3.7 mm to +2.5mm relative to bregma (Paxinos and Watson, 1998). (C-G) 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. (C) Ketamine, muscimol and scopolamine all caused a positive change in cognitive bias index (CBI) for the midpoint tone. (D) Muscimol decreased accuracy for both reference tones. (E) Muscimol increased response latencies for the high and midpoint tones. (F) For the high and low tones, muscimol increased omissions. (G) Muscimol also increased premature responding. Data represent mean ± SEM (panels A, C-F) with individual data points overlaid for each rat (panel A,C). Black dashed line (panel f) represents 50% accuracy depicting performance at chance. 5 min pre-treatment. ***$p < 0.001$, *$p < 0.05$. HT - high reward tone; MT - midpoint tone; LT - low reward tone.

Figure 4 – *Behavioural data from the judgement bias task following mPFC infusions of CP-101,606.*

541 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
542 by intracerebral infusion in the mPFC to measure the effect on judgement bias. (A) The
543 higher dose of CP-101,606 (3.0 μg/μl) caused a positive change from baseline in CBI. (B)
544 Accuracy was not altered by either dose of CP-101,606. (C) In experiment 1, CP-101,606
545 (1.0 μg/μl) increased response latency for the midpoint tone. (D/E) There was no effect of
546 either dose on omissions or premature responding, *$p < 0.05$. Data represent mean ± SEM
547 (panels B-E) with individual data points overlaid for each rat (panel A). Dashed lines indicate
548 separate, counterbalanced experiments. 5 min pre-treatment. HT - high reward tone; MT -
549 midpoint tone; LT - low reward tone.



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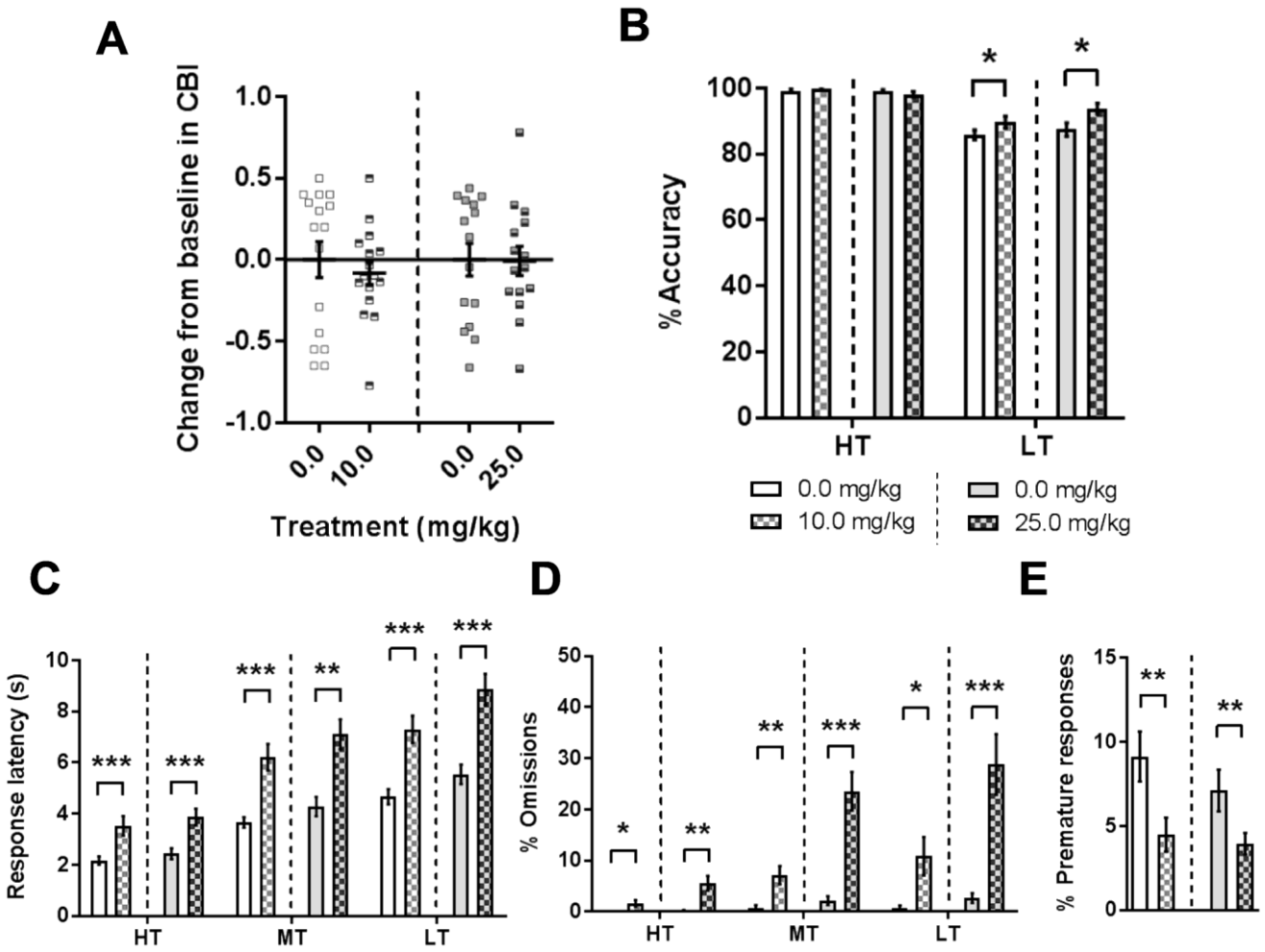

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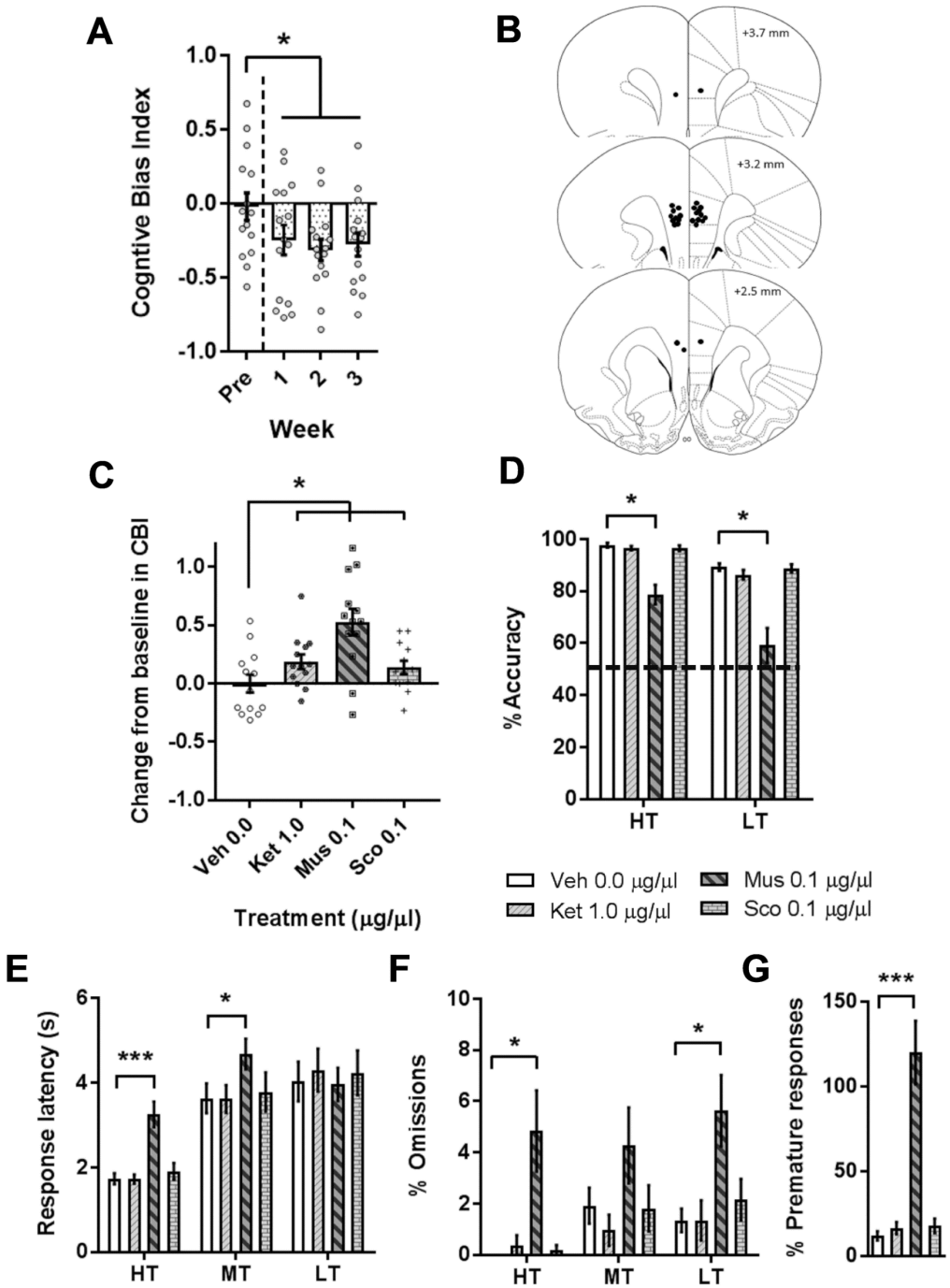

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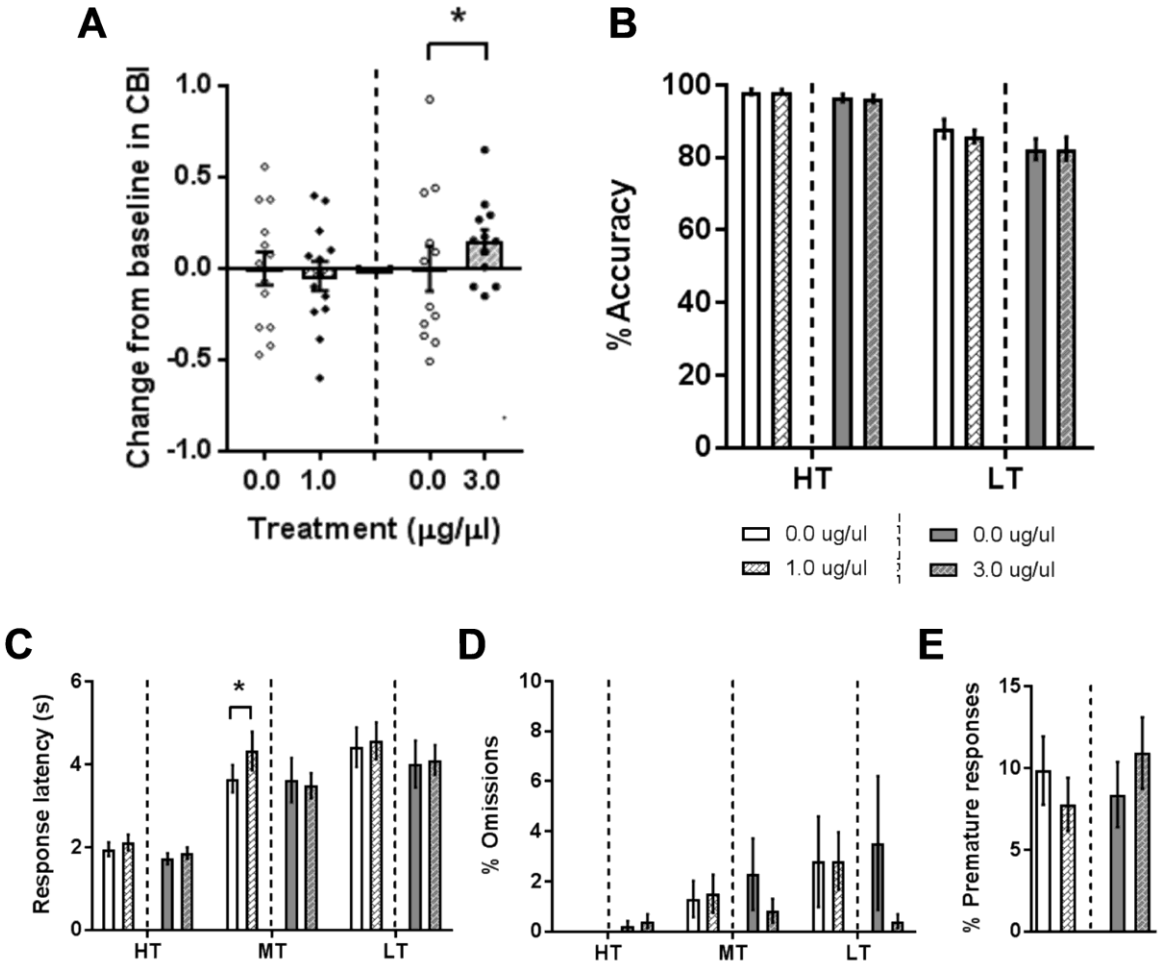

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CP-101,606 (Expt 1: 00.0, 1.0 µg/µl, n = 13; Expt 2: 0.0, 3.0 µg/µl, n = 12) was administered by intracerebral infusion in the mPFC to measure the effect on judgement bias. (A) The higher dose of CP-101,606 (3.0 µg/µl) caused a positive change from baseline in CBI. (B) Accuracy was not altered by either dose of CP-101,606. (C) In experiment 1, CP-101,606 (1.0 µg/µl) increased response latency for the midpoint tone. (D/E) There was no effect of either dose on omissions or premature responding, *$p < 0.05$. Data represent mean ± SEM (panels B-E) with individual data points overlaid for each rat (panel A). Dashed lines indicate separate, counterbalanced experiments. 5 min pre-treatment. HT - high reward tone; MT - midpoint tone; LT - low reward tone.

Table 1 – *Data for behavioural measures from the JBT.*

Drug / Session	Dose (mg/kg) / Week	Behavioural Measure								
		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 significant differences (compared to vehicle 0.0 mg/kg session for that drug). *p<0.05, **p<0.01, ***p<0.001.