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Negative Allosteric Modulators Selective for The NR2B Subtype of The NMDA Receptor Impair Cognition in Multiple Domains

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Antidepressant activity of *N*-methyl-D-aspartate (NMDA) receptor antagonists and negative allosteric modulators (NAMs) has led to increased investigation of their behavioral pharmacology. NMDA antagonists, such as ketamine, impair cognition in multiple species and in multiple cognitive domains. However, studies with NR2B subtype-selective NAMs have reported mixed results in rodents including increased impulsivity, no effect on cognition, impairment or even improvement of some cognitive tasks. To date, the effects of NR2B-selective NAMs on cognitive tests have not been reported in nonhuman primates. The current study evaluated two selective NR2B NAMs, CP101,606 and BMT-108908, along with the nonselective NMDA antagonists, ketamine and AZD6765, in the nonhuman primate Cambridge Neuropsychological Test Automated Battery (CANTAB) list-based delayed match to sample (list-DMS) task. Ketamine and the two NMDA NR2B NAMs produced selective impairments in memory in the list-DMS task. AZD6765 impaired performance in a non-specific manner. In a separate cohort, CP101,606 impaired performance of the nonhuman primate CANTAB visuo-spatial Paired Associates Learning (vsPAL) task with a selective impairment at more difficult conditions. The results of these studies clearly show that systemic administration of a selective NR2B NAM can cause transient cognitive impairment in multiple cognitive domains.
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INTRODUCTION

The potential for *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine, to function as antidepressants with rapid clinical onset has led to increased research on their behavioral pharmacology. Clinical studies in small numbers of patients have repeatedly shown that a single, intravenous dose of ketamine can produce an antidepressant effect that lasts for several days in treatment-resistant, depressed patients (Abdallah *et al*, 2015; Coyle and Laws, 2015). However, like most nonselective NMDA receptor channel blockers, ketamine has a number of adverse effects including dissociative effects and cognitive impairment, leading to the search for novel agents that are better tolerated (Morgan and Curran, 2006; Newcomer and Krystal, 2001). One approach is the development of NMDA receptor channel blockers with low potential to become trapped in the channel pore following repetitive channel activation as exemplified by AZD6765 (lanicemine). Although this agent is still a nonselective NMDA receptor antagonist, AZD6765 shows 'low trapping' in electrophysiology studies and appears to be well tolerated in humans (Sanacora *et al*, 2014; Zarate *et al*, 2013). Unfortunately, the antidepressant efficacy of this agent is now uncertain given that the beneficial effects seen in early clinical testing were not observed in a larger clinical trial (Sanacora and Schatzberg, 2015). A second approach is the development of subtype-selective NMDA receptor antagonists with attention focused on the NR2B subtype. CP101,606 (traxoprodil) is a negative allosteric modulator (NAM), which binds to a site present on the N-terminal domain of the NR2B receptor and prevents channel activation (Menniti *et al*, 1998). In a small, proof of concept, clinical trial a single IV infusion of CP101,606 produced an antidepressant effect at a dose with low potential to produce dissociative effects in patients (Preskorn *et al*, 2008).

Despite renewed interest in the antidepressant potential of NMDA receptor antagonists, the clinical development of these agents has historically been challenging, and the ability

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to understand efficacy and tolerability in preclinical species is important. With respect to cognitive impairment, numerous preclinical studies have shown that ketamine impairs cognition in rodents and nonhuman primates, effects similar to those reported in humans (Buccafusco and Terry, 2009; Neill *et al*, 2010; Roberts *et al*, 2010; Taffe *et al*, 2002a). In contrast, the effects of NR2B NAMs have been less well studied and have produced mixed results. Unlike NMDA channel blockers such as MK-801 or phencyclidine systemic administration of NR2B NAMs in rats does not adversely affect spatial memory in operant-delayed match to position tasks (Higgins *et al*, 2003; Smith *et al*, 2011). Similar results were also seen in the Morris watermaze task where systemic NR2B NAM treatment had no effect on memory acquisition or retention for the location of the hidden platform (Guscott *et al*, 2003; Higgins *et al*, 2005; Higgins *et al*, 2003; Okiyama *et al*, 1997). Importantly, evaluation of NMDA receptor occupancy in tissues collected from experimental subjects showed that ~100% NR2B occupancy was achieved in hippocampus and cortex after CP101,606 dosing, suggesting that inadequate dosing or lack of receptor occupancy does not explain the lack of memory impairment (Guscott *et al*, 2003). On the other hand, direct infusion of the NR2B NAM Ro25-6981 into the rat hippocampus impaired spatial working memory assessed using a watermaze or T maze delayed alternation paradigm, suggesting that local versus systemic dosing can lead to different effects (Zhang *et al*, 2013). Mixed results have also been reported in mice with systemic dosing of the NR2B NAM Ro25-6981 producing both an impairment of spatial working memory (Y maze spontaneous alternation task) and an improvement of executive function (attentional set shifting) (Hanson *et al*, 2013; Kos *et al*, 2011). Thus, NR2B NAMs produce very diverse effects on cognition in rodents depending more on factors such as route of delivery, the cognitive domain being assessed and the various tasks explored than on levels of NR2B receptor occupancy.

Although several studies have explored NR2B NAM treatment in rodents, the effect of NR2B NAM treatment on cognition in humans and nonhuman primates has not been adequately explored. In humans, the effects reported are largely descriptive and include anecdotal reports of impaired delayed word recall memory in healthy humans and abnormal thinking (including dissociation and amnesia) in Parkinson's Disease patients following IV infusion of CP101,606 (Merchant *et al*, 1999; Nutt *et al*, 2008). Unfortunately, the effect of CP101,606 infusion on cognition in patients with treatment-resistant depression was not reported. The therapeutic margin between antidepressant effect and cognitive impairment is therefore not well established. In nonhuman primates there are no published reports describing the impact of systemic NR2B NAM treatment on cognition. Local injection of Ro25-6981 into the dorsolateral prefrontal cortex (dlPFC) of macaques attenuates firing of neurons active during the delay period of a working memory task; however, the impact on task accuracy was not reported and systemic dosing of an NR2B NAM was not examined (Wang *et al*, 2013). Thus, although these electrophysiological results suggest that NR2B NAMs may impair working memory in nonhuman primates, direct demonstration of cognitive impairment has not yet been shown.

The present studies assessed the acute effects of NMDA antagonist treatment on cognitive performance in cynomolgus monkeys. To optimize the translational value of these studies, two tests from the nonhuman primate Cambridge Neuropsychological Test Automated Battery (CANTAB) battery were used: a list-based variant of the delayed match to sample (DMS) task and the visuo-spatial paired associates learning (vsPAL) test. Ketamine and the 'low trapping' nonselective NMDA channel blocker, AZD6765, were chosen for comparison with the selective NR2B NAMs CP101,606 and BMT-108908. BMT-108908 is a novel agent, which potently displaces [^{3}H]Ro25-6981 binding to native human, cynomoglus monkey, and rat brain NR2B receptors (Ki = 1.6, 0.71, and 1.4 nM, respectively). In xenopus oocytes expressing recombinant human NMDA receptors, BMT-108908 selectively inhibits hNR1A/2B receptor function (IC50 = 4.2 nM) and is inactive at the NR2A, NR2C, and NR2D receptor subtypes. In addition, BMT-108908 has no significant activity at 43 relevant CNS receptors and/or enzymes (unpublished data). The detailed pharmacological profile of BMT-108908 will be presented elsewhere.

MATERIALS AND METHODS

Subjects

The cognitive studies used 18 male cynomolgus monkeys (*Macaca Fasicularis*). Each had been used in prior pharmacological studies, and at least a month separated studies. The monkeys were 5–7 years of age and weighed 5.0–8.5 kg. Monkeys were typically pair-housed and fed standard monkey chow (Harlan Teklad Global 20% protein Primate Diet 2050) in sufficient quantities to ensure normal growth while maintaining motivation to perform the tasks (Weed *et al*, 1999).

The pharmacokinetic studies used six male cynomolgus monkeys that had been previously implanted with vascular access ports in a femoral artery and a femoral vein. Housing and care were as described above; however, these animals were not food restricted.

For all animals, water was continuously available except during studies, and fresh fruit or dietary enrichment was provided twice weekly. Toys and foraging devices were routinely provided and television programs were available in the monkey colony rooms. Subjects were fitted with plastic or metal neck collars (Primate Products, Immokalee, FL). Laboratory animal care was according to Public Health Service Policy on the Humane Care and Use of Laboratory Animals, and the Guide for the Care and use of Laboratory Animals (NRC, 2011). The protocol was approved by the Wallingford Animal Care and Use Committee of the Bristol-Myers Squibb Company.

Apparatus

For behavioral testing, monkeys were seated comfortably in nonhuman primate chairs (Primate Products), and placed in a sound-attenuated chamber (Med-Associates, St Albans, VT) with a touch-sensitive computer monitor within easy reach. Session events were controlled by the nonhuman primate CANTAB software; (Lafayette Instruments, Lafayette, IN) running in Whisker Control (Cardinal and Aitken,

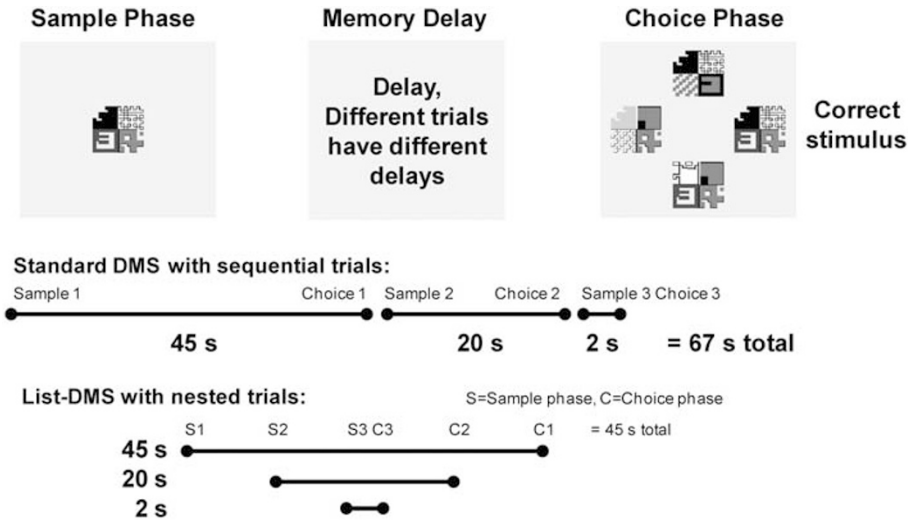

Figure 1 Schematic of the DMS procedure. Actual stimuli are in color and each quadrant of the stimulus is a uniform color. Distracters in the choice phase may mimic the shape of any quadrant of the sample stimulus and use the same or different color. The list-DMS protocol shortens the time required to perform the test, which can be important for compounds with shorter half lives.

2010). Following correct responses, a dispenser delivered 190 mg banana-flavored pellets (Bioserv, Frenchtown, PA).

Behavioral Procedures: List-DMS

Animals were first trained to proficiency on the standard nonhuman primate CANTAB DMS where an abstract stimulus was presented in the center of the screen for the monkey to touch ('sample phase') and, after a memory delay, the sample stimulus and three distracter stimuli were presented in the 'choice phase' (total of four stimuli in each corner of screen).

List-DMS differs from standard DMS in that three sample stimuli were presented sequentially, prior to any choices (Figure 1). The choice phases for the respective sample stimuli also occurred sequentially, after their respective memory delays. The three trials are thus 'nested' within the longest memory delay, reducing the session length, which may be important for testing short acting compounds.

For this study the memory delays were 2, 20, and 45 s. The sequence of events was: sample stimulus A (which would have a 45-s memory delay) was presented and touched, resulting in a screen blank. Next, sample stimulus B (which would have a 20-s memory delay), was presented and touched, resulting in another screen blank. Sample stimulus C (which would have a 2-s delay) was touched, resulting in a screen blank for the 2-s memory delay, after which the choice stimuli for stimulus C were presented. After a response, pellets were delivered (for a correct response) or not (for an error), and the screen was blanked until the 20-s memory delay for stimulus B expired. The choice phase for stimulus B proceeded as for stimulus C, followed by a screen blank until the 45-s memory delay from stimulus A expired. Following the end of the choice phase for stimulus A there was a 10-s screen blank before the process repeated with all new stimuli.

In either the sample or choice phase, the monkey had 5 s to respond to the stimulus, or the trial was considered an 'omission'. If the omission was in the sample phase, the choice phase for that sample stimulus was not presented, but the timing of sample and choice phases for other stimuli remain unchanged. Three distracter stimuli were used for all delays, and therefore, chance responding would yield 25% correct in this procedure. A session consisted of 20 lists, allowing for 60 total trials.

Behavioral Procedures: vsPAL

In brief, the monkey was required to learn and remember the association of a visual stimulus and a location on the touch screen (Supplementary Figure 1; Taffe *et al*, 2002b). For a given trial there were either two, three, or four stimulus/location pairs to remember. Sample stimuli were presented in a given location in a list sequence, with 1 s between stimuli. After all stimuli were sampled in their appropriate position, there was a 15-s memory delay. In the choice phase, a stimulus was presented in the correct location as well as three incorrect locations. Positions were randomly selected from nine possible locations in a 3×3 grid. Each touch in the correct location resulted in food pellet delivery. If the animal failed to respond within 30 s, or if the animal responded in an incorrect location, the trial stopped there. After a 10-s time out, the same sequence of stimuli in the same locations repeated, starting with the sample phase. The animal had the initial attempt plus up to five repetitions to learn the correct stimulus-location pairs. If the animal responded to all stimuli correctly on the initial presentation, the trial was scored as 'initial correct'. If a trial was completed correctly after an initial error, the trial was scored as correct eventually ('eventual correct'). Stimuli changed after correct trials or after six failed attempts. As with previous studies, a session consisted of 10 trials at each difficulty level presented in an ascending order of difficulty (Taffe *et al*, 2002b).

Drugs

Racemic ketamine HCl was purchased as a 10 mg/kg solution (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA). CP101,606 (traxoprodil, methanesulfonic acid salt, vehicle: 5% DMSO/10% propylene glycol/15% methylcellulose/70% sterile water), AZD6765 (lanicemine, HCl, vehicle: 5%

Table 1 Summary of Details from RM ANOVAs on List-DMS Performance

	% Correct, dose–response function				
	Difficulty	**Treatment**	**Interaction**	**Response latency**	**% Task completion**
Ketamine	$F_{2,16} = 42.3$; $p < 0.0001$	$F_{4,32} = 12.4$; $p < 0.0001$	$F_{8,64} = 1.4$; $p > 0.05$	$F_{4,32} = 5.9$; $p = 0.001$	$F_{4,32} = 6.2$; $p = 0.0008$
AZD6765	$F_{2,14} = 35.9$; $p < 0.0001$	$F_{3,21} = 12.4$; $p < 0.0001$	$F_{6,42} = 12.5$; $p < 0.0001$	$F_{3,21} = 0.2$; $p > 0.05$	$F_{3,21} = 4.9$; $p = 0.01$
CP101,606	$F_{2,16} = 219.3$; $p < 0.0001$	$F_{5,40} = 32.6$; $p < 0.0001$	$F_{10,80} = 5.1$; $p < 0.0001$	$F_{5,40} = 2.1$; $p = 0.09$	$F_{5,40} = 3.2$; $p = 0.02$
BMT-108908	$F_{2,16} = 211.6$; $p < 0.0001$	$F_{4,32} = 39.5$; $p < 0.0001$	$F_{8.64} = 12.8$; $p < 0.0001$	$F_{4,32} = 4.4$; $p = 0.006$	$F_{4,32} = 0.9$; $p > 0.05$

% correct after 30 min PT data were analyzed with a two-way RM ANOVA. Response latency, % task completion, and pretreatment time were analyzed with one-way RM ANOVAs.

DMSO/95% sterile water), and BMT-108908 (free base, vehicle: 30% hydroxypropyl beta cyclodextrin/70% citrate buffer, pH 4.0) were synthesized at Bristol-Myers Squibb. Compounds were administered intramuscularly at 0.1–0.2 ml/kg. Doses refer to the free base.

Drug Testing

All compounds were tested in the list-DMS procedure. Monkeys were included in dose–response analyses if they achieved stable baseline performance (±15% over the week prior to testing). If an animal's percent trials completed dropped below 20% for a given test session, their accuracy and latency data were discarded and interpolated with the group mean for the repeated-measures analysis of variance (RM-ANOVA). Ketamine was administered 15 min before the session and all other compounds were administered 30 min prior to the session. To determine whether cognitive impairment of CP101,606 was specific to the list-DMS task, CP101,606 was also tested in the vsPAL procedure. Determinations of performance after vehicle occurred prior to, during, and at the end of the dose–response function for each drug (minimum of three vehicle determinations for a given drug). Drugs were tested twice weekly with baseline performance sessions on days in between. On test days the cohort was divided roughly in half, and one-half received one dose of the drug and the other half received another dose of the drug. Drug doses were thus administered in a mixed order.

To determine exposure-effect relationships for cognitive impairment of CP101,606 and BMT-108908, the effects of both compounds were also studied after 3 and 5 h pretreatment (in addition to the 0.5 h pretreatment used in the dose–response function). Blood samples were obtained immediately following the behavioral session at these times. Vehicle pretreatments at 3 and 5 h were determined for each compound, and comparisons were made between the mean of the respective drug and vehicles. In addition, for the long-delay condition, the difference between performance after vehicle and drug was calculated and related to the exposure.

Statistical Analysis

Performance accuracy (% correct (list-DMS); % initially and eventually correct (vsPAL)) was analyzed with RM-ANOVA with the two within-subject factors of difficulty (memory delay or number of stimuli per trial) and treatment. Omissions were not included in the percent correct measure, but contributed to percent task completed. Percent task completed was the proportion of trials on which the animal responded (in both sample and choice phase) divided by the total number of trials (ie, # responded/(# responded+# omitted)). Latencies to respond in the choice phase (averaged across delays) and percent task completed for the entire session were analyzed with a one-way RM-ANOVA. Holm–Sidak's method was used for *post hoc* comparisons against vehicle. Statistical tests were performed in Prism v6.05 (Graphpad, San Diego, CA) and presented in detail in Tables 1 and 4.

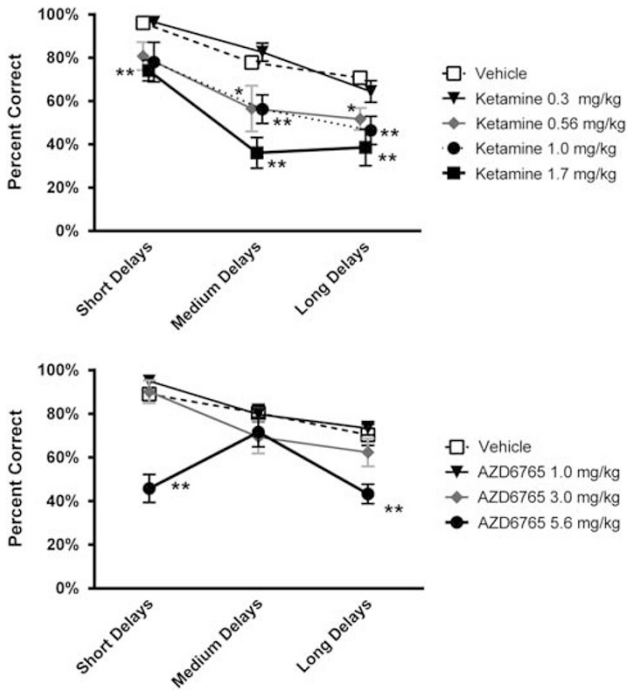

Figure 2 Effect of acute treatment with the NMDA channel blockers RS-ketamine (top, $N = 9$) and AZD6765 (bottom, $N = 8$) on list-DMS performance in cynomolgus monkeys. Results are presented as the group mean ± SEM; * and ** indicate that performance differs from vehicle treatment at the $p < 0.05$ and $p < 0.01$ levels, respectively.

RESULTS

List-DMS Performance

Acute treatment with ketamine impaired performance on list-DMS in a dose- and difficulty-dependent fashion

Table 2 Group Means for Overall Response Latency in List-DMS in Milliseconds

	Ketamine (ms)	AZD6765 (ms)	CP101,606 (ms)	BMT-108908 (ms)
Vehicle (SEM)	2405 (82.4)	2459 (72.0)	2526 (70.7)	2443 (80.3)
0.10 mg/kg (SEM)	n.t.	n.t.	n.t.	2381 (171.6)
0.30 mg/kg (SEM)	2501(137.2)	n.t.	2382 (135.7)	2574 (82.8)
0.56 mg/kg (SEM)	2521(192.7)	n.t.	n.t.	n.t.
1.00 mg/kg (SEM)	2386 (215.1)	2460 (107.3)	2604 (107.5)	2798 (65.42)*
1.70 mg/kg (SEM)	2994 (49.2)**	n.t.	n.t.	n.t.
3.00 mg/kg (SEM)	n.t.	2563 (140.2)	2557 (122.8)	2744 (94.78)
5.60 mg/kg (SEM)	n.t.	2456 (215.3)	2431 (142.7)	n.t.
10.00 mg/kg (SEM)	n.t.	n.t.	2802 (98.7)	n.t.

Abbreviation: n.t., not tested. * and ** indicate performance differed from vehicle at $p<0.05$ and $p<0.01$ levels, respectively, using Holm–Sidak test.

Table 3 Group Means for List-DMS Percent Completion

	Ketamine 15 min PT	AZD6765 30 min PT	CP101,606 30 min PT	BMT-108908 30 min PT
Vehicle (SEM)	98.4% (0.5%)	97.0% (0.7%)	96.9% (1.1%)	96.4% (1.7%)
0.10 mg/kg (SEM)	n.t.	n.t.	n.t.	94.8% (4.7%)
0.30 mg/kg (SEM)	75.4% (12.8%)	n.t.	95.8% (1.8%)	96.2% (2.4%)
0.56 mg/kg (SEM)	74.0% (8.2%)	n.t.	n.t.	n.t.
1.00 mg/kg (SEM)	65.6% (9.8%)*	84.5% (11.8%)	76.2% (8.8%)	95.1% (1.8%)
1.70 mg/kg (SEM)	43.2% (12.5%)**	n.t.	n.t.	n.t.
3.00 mg/kg (SEM)	n.t.	94.1% (3.3%)	83.7% (7.8%)	90.9% (6.8%)
5.60 mg/kg (SEM)	n.t.	55.8% (14.2%)**	66.7% (12.3%)*	n.t.
10.00 mg/kg (SEM)	n.t.	n.t.	70.4% (9.2%)	n.t.

Abbreviation: n.t., not tested. * and ** indicate performance differed from vehicle at $p<0.05$ and $p<0.01$ levels, respectively, using Holm–Sidak test.

(Figure 2, top; Table 1 and Supplementary Table S1). RM-ANOVA (Table 1) and *post hoc* tests confirmed that impairment was dose-dependent as 0.56 mg/kg, and higher doses impaired accuracy, whereas 0.3 mg/kg did not differ significantly from vehicle (Figure 2, top; Supplementary Table S1). Ketamine's effects were difficulty dependent for the 0.56 and 1.0 mg/kg doses as accuracy at short delays did not differ from vehicle, whereas accuracy was significantly impaired at medium and long delays. Latency to respond and percent task completion were not impaired following 0.56 mg/kg ketamine, consistent with a specific reduction in memory at this dose (Tables 1, 2, and 3). Ketamine decreased percent task completed at 1.0 and 1.7 mg/kg. After 1.7 mg/kg ketamine list-DMS performance was impaired on all measures, the contribution of memory impairment relative to motivational or psychomotor impairment could not be determined for this dose. Average plasma ketamine concentrations measured 15 min after dosing with 0.56 and 1.0 mg/kg were 595 and 705 nM, respectively (Supplementary Table S2).

Acute treatment with AZD6765 had no effect on performance accuracy at any delay condition at doses of 1 and 3 mg/kg i.m. (Figure 2, bottom; Table 1 and Supplementary Table S1). In addition, latency to respond and % task completed were also not affected at these doses (Tables 2 and 3). In contrast, a significant decrease in accuracy was observed at both the short- and long-delay conditions in subjects treated with 5.6 mg/kg AZD6765. A significant reduction in % task completed was also observed at this dose, suggesting that non-specific effects may account for the apparent memory impairment. Higher doses of AZD6765 could not be examined, as prior studies showed severe emesis in animals treated with 10 mg/kg AZD6765. The average plasma concentration determined 30 min after dosing with 5.6 mg/kg AZD6765 was 7168 nM (Supplementary Table S3).

Acute treatment with CP101,606 produced a dose- and difficulty-dependent impairment in performance accuracy in the list-DMS task. At doses $\geqslant$ 1 mg/kg, a selective impairment at the medium- and long-delay conditions was observed, whereas accuracy at short delays was not affected (Figure 3, top; Table 1 and Supplementary Table S1). CP101,606 did not impair response latency at any dose tested (Table 2); however, a modest, but significant, reduction in % task completed was observed at the 5.6 mg/kg dose (Table 3). Doses of 1.0 and 3.0 mg/kg impaired accuracy without significantly affecting response latency or percent task completed, consistent with a selective disruption of memory at medium and long delays. The average plasma concentration measured 30 min after dosing with 1.0 mg/kg or 3.0 mg/kg CP101,606 was 471 and 1137 nM, respectively (Supplementary Table S4).

Figure 4, top left panel illustrates the time course of impairment of list-DMS performance following 1.0 mg/kg CP101,606 as represented by % correct in the long-delay condition (RM-ANOVA of time course: $F_{3,24} = 15.6$; $p < 0.0001$). Although performance was significantly impaired 30 min after dosing, the effect was no longer significant at 3 h ($p = 0.09$ from Holm–Sidak test) and returned to vehicle-control levels by 5 h post dose. Recovery from impairment was closely related to plasma CP101,606 concentrations and cognitive impairment was consistently observed at plasma concentrations $\geqslant$ 471 nM (Figure 4, top right panel; Supplementary Table S4).

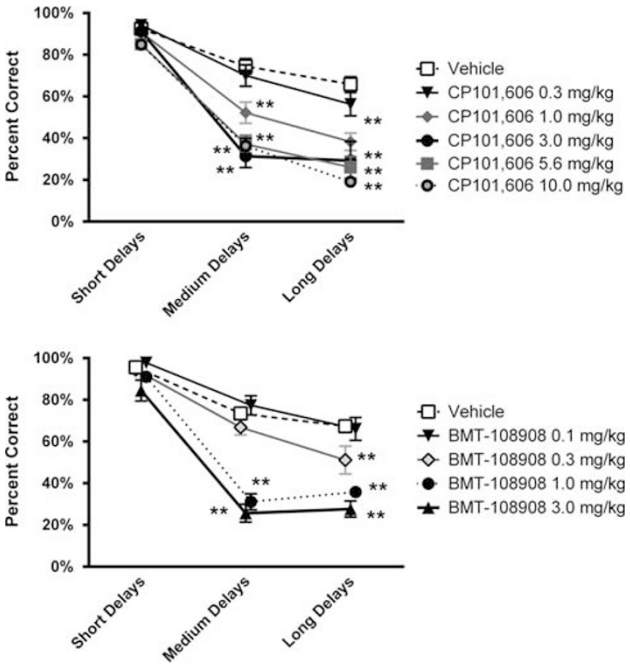

Figure 3 Effect of acute treatment with the NR2B-selective NAMs CP101,606 (top, $N = 9$) and BMT-108908 (bottom, $N = 9$) on list-DMS performance in cynomolgus monkeys. Results are expressed as the group mean ± SEM; * and ** indicate that performance differs from vehicle treatment at the $p < 0.05$ and $p < 0.01$ levels, respectively.

Acute treatment with BMT-108908 also produced a dose- and difficulty-dependent impairment in performance accuracy in the list-DMS. At doses $\geqslant$ 0.3 mg/kg a selective impairment in the medium- and/or long-delay condition was observed, whereas accuracy in the short delay condition was not altered (Figure 3, bottom; Table 1 and Supplementary Table S1). BMT-108908 did not impair % task completion at any dose tested; however, a significant increase in the average latency to respond of 355 ms was observed at the 1.0 mg/kg dose (Tables 2 and 3). Performance after 0.3 mg/kg BMT-108908 was consistent with a selective impairment in memory at the long-delay condition. Average plasma concentrations of BMT-108908, 30 min after dosing with 0.3 mg/kg, were 279 nM (Supplementary Table S5).

Figure 4, bottom left panel illustrates the time course of impairment of list-DMS performance following 1.0 mg/kg BMT-108908 as represented by % correct at the long-delay condition. With 30 min and 3 h pretreatment times there was a significant impairment, with full recovery to vehicle-treated control levels by 5 h post dose (RM ANOVA of time course: $F_{3,24} = 8.8$; $p = 0.0004$). Recovery from impairment was closely related to plasma BMT-108908 concentrations and cognitive impairment was consistently observed at plasma concentrations $\geqslant$ 275 nM (Figure 4, bottom right panel; Supplementary Table S5).

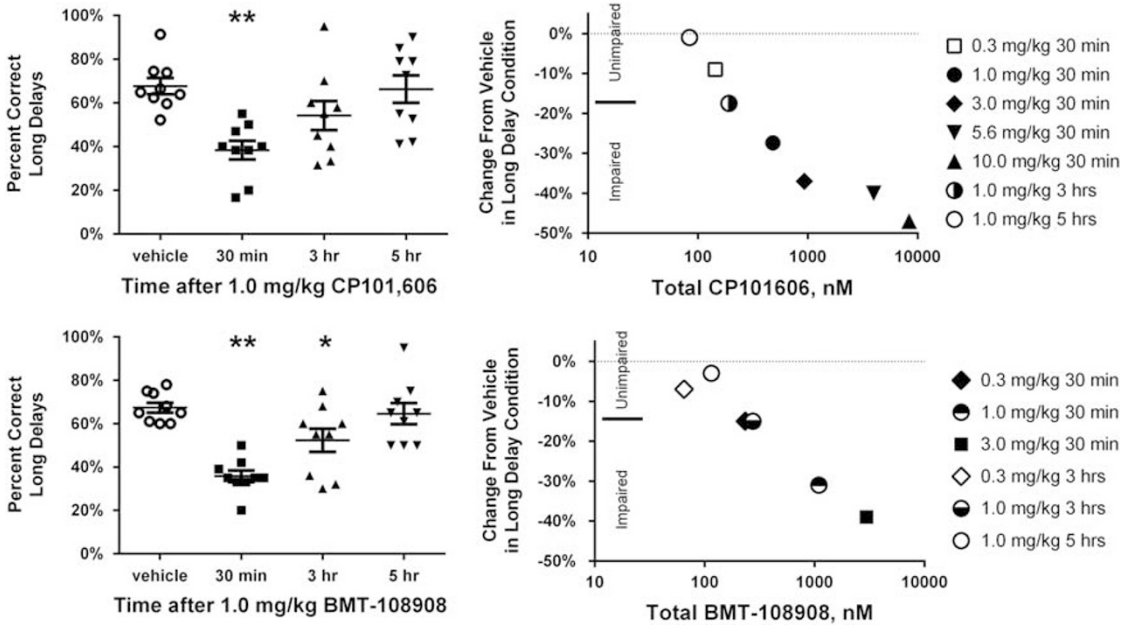

Figure 4 Time course of list-DMS impairment by CP101,606 and BMT-108908 (left panels) and plasma exposure *vs* impairment relationship for CP101,606 and BMT-108908 (right panels). Left panels: *y* axis is percent correct responding for long delays after 1 mg/kg CP101,606 (top left) and 1 mg/kg BMT-108908 (bottom left). Bars are SEM of group and line is group mean. * and ** indicate that performance differs from performance after vehicle at the $p < 0.05$ and $p < 0.01$ levels, respectively. Right panels: mean % change from vehicle at long delay is plotted against average plasma concentration of total compound for CP101,606 (top) and BMT-108908 (bottom). Filled or half-filled symbols indicate significantly impaired list-DMS performance.

vsPAL Performance

Acute treatment with CP101,606 produced a dose- and difficulty-dependent impairment of initial accuracy in the vsPAL task (Figure 5, top; Table 4, Supplementary Table S6). This impairment was only observed at the most difficult four-stimuli condition at 0.3 mg/kg CP101,606 but was apparent on both the three- and four-stimuli conditions after 1.0 mg/kg. After 3.0 mg/kg, performance was impaired at all difficulty levels. In addition, CP101,606 impaired eventual accuracy in the four stimulus condition after the 1.0 mg/kg dose (Figure 5, bottom; Table 4 and Supplementary Table S6). After 3 mg/kg, eventual accuracy was impaired at all difficulty levels and significant impairments in response latency and % trials completed were also observed (Table 5). The results suggest a selective cognitive impairment at the 0.3 and 1.0 mg/kg doses of CP101,606.

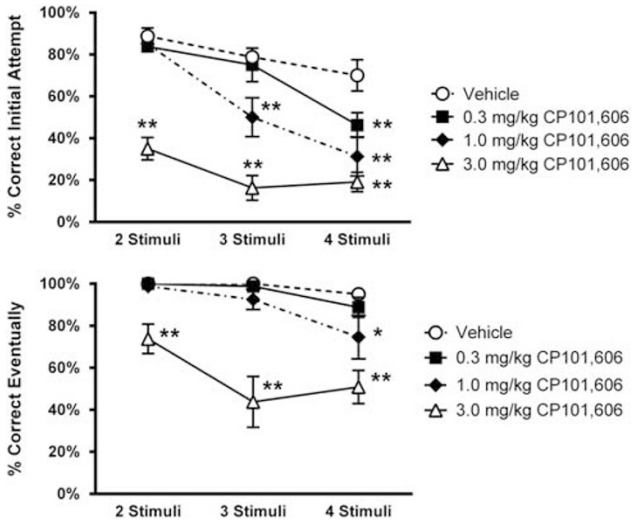

Figure 5 Effect of acute treatment with CP101,606 on accuracy in the vsPAL procedure ($N = 8$). Top panel: accuracy on the initial attempt. Bottom panel: eventual accuracy (initial+ five possible repeats). Both panels: *x* axis is difficulty of trial in terms of number of stumuli. * and ** indicate that performance differs from performance after vehicle at the $p < 0.05$ and $p < 0.01$ levels, respectively.

DISCUSSION

The present studies are the first to demonstrate cognitive impairment in nonhuman primates after administration of NR2B NAMs. The results of these studies clearly show that treatment with a selective NR2B NAM can impair pattern recognition memory (list-DMS) as well as learning and memory of associations between stimuli and location (vsPAL) in cynomolgus monkeys. The use of the least-difficult trials as procedural controls (eg, 2-s delay in list-DMS or two stimuli condition in vsPAL) allows for the separation of impairment in the cognitive domain of the task from other factors affecting performance such as the ability to: (a) remember the rules of the task, (b) perform the motor movements necessary, (c) encode stimuli into working or short-term memory, and (d) accurately recognize the stimuli (or stimulus/location pairing). In addition, factors influencing motivation and psychomotor effects are detected on measures such as percent task completion and response latency. For both CP101,606 and the novel agent, BMT--108908, a delay- or difficulty-dependent impairment was observed at doses not affecting task completion or latency to respond. The results from both tasks are consistent with selective impairment in longer-term memory, and to a lesser extent, learning (vsPAL task) after acute NR2B NAM treatment.

Interestingly, NR2B blockade of the dlPFC neurons studied by Wang *et al* (2013) is not likely to be responsible for the deficits in DMS performance in the current studies. Lesions of medial but not dorsolateral PFC have been shown to impair DMS performance (Bachevalier and Mishkin, 1986; Passingham, 1975). Patients with frontal lobe lesions were not impaired on the CANTAB DMS task, although frontal patients were impaired on the vsPAL (Owen *et al*, 1995). Therefore, dlPFC neurons, such as those studied by Wang *et al*, (2013) may be involved in the spatial memory components of vsPAL; however, the DMS impairment implicates regions outside of the dlPFC. Performance in both vsPAL and DMS tasks is similarly sensitive to function in temporal cortical and hippocampal structures, as well as medial PFC structures, suggesting their involvement in the cognitive impairment observed in the present studies. Additional studies would be needed to provide further information on the relative contribution of different brain areas to the cognitive impairment seen after NR2B NAM administration.

In healthy human subjects, the ability of ketamine to produce cognitive impairment is well documented. To examine the translational utility of the nonhuman primate CANTAB list-DMS task we also examined the effect of acute ketamine treatment and measured the plasma ketamine concentrations that were achieved after dosing. The ketamine-induced memory impairment in the list-DMS procedure is consistent with previous reports in ketamine-treated monkeys performing the standard CANTAB DMS task (Taffe *et al*, 2002a). Furthermore, our results are consistent with the dose-dependent impairment of DMS task performance reported in healthy male subjects after i.v. ketamine infusion (Newcomer *et al*, 1999). In the latter study, the steady state plasma ketamine concentration associated with impaired DMS performance in humans was 90 ng/ml (379 nM) and is within the exposure range measured over the testing period at the minimum effective dose (0.56 mg/kg) in cynomolgus monkeys (average plasma concentration 15–60 min post dose = 595–342 nM). Indeed, the range of plasma ketamine concentrations associated with memory impairment and impairment on frontal cortical tasks (eg, Wisconsin Card Sort Task) in humans is overlapping with the exposures reported in the present studies (543 nM-1102 nM, (Morgan *et al*, 2004); 841 nM (Krystal *et al*, 1998); 513-618 nM, (Hetem *et al*, 2000)). These results suggest that, at least for ketamine, there is a good correlation between exposure/effect relationships in monkeys and humans.

Although CP101,606 has been administered to humans, the relationship between exposure and cognitive impairment in humans is not well understood, in part because the literature reports of cognitive impairment are largely descriptive. In addition, CP101,606 is metabolized by CYP2D6, a cytochrome P450 enzyme that is highly polymorphic, leading to variable plasma pharmacokinetics

Table 4 Summary of Details from RM ANOVAs for the Effects of CP101,606 on vsPAL Performance

	Difficulty	Treatment	Interaction
Initial accuracy			
Two-way RM-ANOVA CP101,606 PAL	$F_{2,14} = 43.8$; $p < 0.0001$	$F_{3,21} = 41.6$; $p < 0.0001$	$F_{6,42} = 5.1$; $p = 0.0005$
Eventual accuracy			
Two-way RM-ANOVA CP101,606 PAL	F2,14 = 9.6; $p = 0.002$	F3,21 = 33.2; $p < 0.0001$	F6,42 = 2.8; $p = 0.02$

	Response latency	% Task completion
One-way RM-ANOVA CP101,606 PAL	$F_{3,21} = 15.2$; $p < 0.0001$	$F_{3,21} = 5.9$; $p = 0.004$

Table 5 Group Means for vsPAL Overall Latency to Respond and % Task Completion

CP101,606	Overall response latency (ms) (SEM)	% Task completion (SEM)
Vehicle	1664.8 (111.4)	99.5% (0.4%)
0.3 mg/kg	1709.6 (171.8)	97.3% (1.6%)
1.0 mg/kg	1734.7 (161.3)	92.4% (5.2%)
3.0 mg/kg	2322.8 (232.4)[a]	80.0% (6.4%)[a]

[a]Indicates performance differed from vehicle at $p < 0.01$ level using Holm–Sidak test.

in subjects who extensively or poorly metabolize the drug (Johnson *et al*, 2003). Nevertheless, retrograde amnesia and impairment of delayed word recall was reported in healthy humans treated with a 2 h IV infusion of CP101,606 (Merchant *et al*, 1999). Amnesia was also reported in healthy subjects receiving an i.v. bolus/infusion administration lasting 72 h (Merchant *et al*, 1999). Although plasma drug concentrations were not reported for the 2 h infusion, the 72 h infusion paradigm achieved levels of 1466 and 3848 nM in extensive and poor metabolizers, respectively. In the current study, plasma CP101,606 concentrations for impairment of list-DMS performance ranged from 471 to 6423 nM across the effective dose range (1.0–10 mg/kg; 30 min post dose ie, at the initiation of testing). Thus, the effect/exposure relationship of CP101,606 in cynomolgus monkeys appears similar to the limited information available from human testing. Interestingly, performance of the nonhuman primate vsPAL appears to be even more sensitive to CP101,606 with a modest but significant impairment detected at the most difficult condition after treatment with 0.3 mg/kg. These results highlight the potential for NR2B NAMs to impair cognition in humans and suggest that well-standardized testing, using tools such as the CANTAB battery, should be incorporated into early clinical testing of novel agents.

These studies are also the first preclinical studies published comparing the cognitive effects of 'low trapping' *vs* 'high trapping' NMDA antagonists. Preclinical studies have shown that AZD6765 is a modest potency, voltage-dependent, nonselective, NMDA receptor channel blocker (Sanacora *et al*, 2014). Although this profile is similar to ketamine, the 'low trapping' effects seen in electrophysiology assays are hypothesized to better preserve use-dependent block under conditions of normal synaptic transmission, leading to improved tolerability (Mealing *et al*, 1999). Indeed, preliminary studies evaluating the antidepressant potential of i.v. infusions of AZD6765 appear consistent with this hypothesis, as AZD6765 showed low potential for dissociative effects, psychotomimetic effects, or cognitive impairment at doses of 100 and 150 mg in patients (Sanacora *et al*, 2014; Zarate *et al*, 2013). In the present studies acute treatment with AZD6765 did not impair list-DMS performance at doses of 1 and 3 mg/kg, achieving plasma drug concentrations of 1 μM–656 nM and 3.7–4.2 μM, respectively, during the testing period. Although impaired performance accuracy was observed at the highest dose tested, a marked reduction in task completion was also seen, indicating a more general impairment in performance. Indeed we have observed severe emesis in monkeys treated with 10 mg/kg AZD6765, indicating poor tolerability at higher doses. Although limited plasma exposure information is available in humans, i.v. infusion of 150/160 mg AZD6765 is reported to achieve concentrations of ~ 6 μM, after a 1 h infusion (Zarate *et al*, 2013), similar to levels achieved in these studies.

The ability of NR2B NAMs to impair cognition in nonhuman primates raises the important question as to whether antidepressant efficacy can be dissociated from cognitive effects for this approach. Although this remains to be resolved, it is important to note the temporal disconnect between the transient, short lasting cognitive impairment, which is tightly coupled to plasma exposure and antidepressant effects, which emerge slowly and persist beyond the elimination of ketamine or CP101,606 in humans. Furthermore, although deficient synaptic plasticity (LTP) is thought to underlie cognitive impairment, the delayed antidepressant effect is thought to be driven by enhanced synaptic plasticity in key brain regions implicated in MDD (Duman *et al*, 2012). In particular, in rodent models of chronic stress, ketamine and NR2B NAMs have been shown to activate the BDNF-mTOR signaling pathways, leading to increased translation of synaptic proteins, enhanced synaptogenesis, and alleviation of depression-related behaviors (Duman *et al*, 2012; Li *et al*, 2010, 2011). Indeed, using hippocampal LTP as a measure of plasticity it was recently reported that LTP is robustly enhanced in slice preparations taken from animals dosed 24 h previously with i.v. ketamine or NR2B NAMs

(Graef *et al*, 2015). This effect contrasts sharply with the LTP impairment seen following direct application of NMDA antagonists to hippocampal slices (Shipton and Paulsen, 2014). Importantly, although separation of the antidepressant from cognitive effects may ultimately not be achievable, transient cognitive impairment is likely manageable in patients treated with i.v. agents and should not preclude their development. Thus, i.v. infusions will require administration in a clinical setting where patients can be monitored until any transient cognitive issues have resolved. However, the potential for prolonged cognitive impairment following oral NR2B NAMs is of concern especially for agents with long pharmacokinetic half lives. These observations highlight the importance of further studies to investigate tolerance to repeated dosing of NR2B NAMs and the monitoring of cognitive impairment in the clinical development of these agents.

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