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Effects of selective allosteric activation of M_1 and M_4 muscarinic receptors on object recognition memory performance in rats

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An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Master of Arts

In Psychology

2013

Abstract

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By Claire Rebecca Galloway

Rationale Acetylcholine (ACh) signaling through muscarinic receptors (mAChRs) has been shown to benefit memory performance in some conditions, but pan-mAChR activation also frequently leads to peripheral side effects. Drug therapies that selectively target M_1 or M_4 muscarinic receptors may alleviate disease-related memory impairments while minimizing side effects mediated by the other muscarinic receptor subtypes. In healthy individuals, the beneficial range of M_1 or M_4 activation may be narrow and variable, which highlights the importance of considering individual differences in baseline performance when evaluating the efficacy of cholinergic drugs for improving memory performance.
Objectives We tested the ability of three recently developed drugs that selectively activate M_1 or M_4 receptors at allosteric sites to improve performance of rats on an object recognition memory task above baseline.
Methods Long-Evans rats were given subcutaneous (*s.c.*) injections of three different doses of each the M_1 allosteric agonist VU0364572, the M_1 positive allosteric modulator (PAM) BQCA, or the M_4 PAM VU0152100 before performing an object recognition memory task. Each rat also completed three different sessions with 0.9% saline *s.c.* injections to establish baseline performance.
Results The lowest dose (3.0 mg/kg) of VU0152100 markedly improved memory performance of rats who performed poorly at baseline. Rats' general tendency to explore novel objects was not altered in any drug condition.
Conclusions Drug therapies that selectively target M_4 receptors may improve memory performance in individuals with impaired memory.

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Acetylcholine (ACh) signaling through muscarinic receptors (mAChRs) in the central nervous system (CNS) has been associated with memory processes since it was reported that pharmacological blockade of mAChRs with the pan-muscarinic antagonist scopolamine impaired performance on several memory tasks in humans and experimental animals (Aigner and Mishkin 1967; Deutsch and Rocklin 1967; Drachman and Leavitt 1974; Ghoneim and Mewaldt 1975; Meyers et al. 1964; Pazzagli and Pepeu 1965) and that activating mAChRs with pan-muscarinic agonists improved performance or attenuated deficits on several memory tasks in monkeys and rats (Matsuoka et al. 1991; Murray and Fibiger 1986; Prediger et al. 2006; Rupniak et al. 1989; Smith et al. 1996). The five mAChR subtypes (M_1-M_5) are each expressed in both the CNS and the peripheral nervous system (PNS), but the functional significance, prevalence, and regional distribution within the CNS and PNS differ markedly across the receptor subtypes. In particular, M_1 and M_4 receptors have lower expression levels in the PNS relative to the CNS (Levey 1993), and compared to M_2 and M_3 receptors, are less implicated in PNS effects elicited by pan-mAChR agonism (Bymaster et al. 2003). In the CNS, M_1 and M_4 receptors are both prominently expressed in brain regions that are key to several memory systems, including the hippocampus, striatum and amygdala (Levey et al. 1991). M_1 and M_4 receptors are also expressed in areas such as the nucleus basalis magnocellularis (nucleus basalis of Meynert in primates) and prefrontal cortex (Levey et al. 1991), which are important for aspects of cognition that intersect with memory performance, including attention, goal pursuit, and decision making. Thus, M_1 and M_4 receptors likely play a major role in mediating memory performance yet show a decreased potential for eliciting mAChR-mediated peripheral side effects. As a result,

drug therapies involving selective activation of M_1 or M_4 receptors hold considerable promise for the treatment of various diseases that involve memory impairments linked to cholinergic dysfunction, such as Alzheimer's disease and schizophrenia (Bridges et al. 2010; Levey 1996).

A challenge in assessing the potential role of specific mAChR activators in memory is the possibility that the performance-enhancing range of any mAChR subtype activity is narrow and variable across individuals. Several previous studies with non-selective cholinergic drugs have observed a narrow range of beneficial efficacy, a range that varies across individuals, or a strong link between performance during control conditions and the degree of cognitive benefit from cholinergic enhancement (Arauojo et al. 2011; Chuah et al. 2009; Dumery et al. 1988; Flood et al. 1985; Haroutunian et al. 1985; Kukolja et al. 2009; Malkova et al. 2011; Ogura and Aigner 1993; Raffaele et al. 1991; Ye et al. 1999). Accordingly, identifying M_1 or M_4 selective drugs that improve memory performance will likely depend on the use of several doses and accounting for difference in the baseline memory performance level of subjects.

The orthosteric binding site is highly conserved across mAChRs and has presented a major challenge to the development of subtype-selective drugs (Conn et al. 2010). The recent shift in focus to developing drugs that target allosteric binding sites of mAChRs has produced several promising drugs that are highly specific for the M_1 or M_4 subtypes (Conn et al. 2009; Conn et al. 2010; Farlow 2002; Heinrich et al. 2009). In particular, systemic administration of the M_1-selective allosteric agonist VU0364572 was found to improve memory performance in Morris Water Maze and contextual fear conditioning tasks in rats (Digby et al. 2012). Similarly, the M_1-selective positive

allosteric modulator (PAM) benzyl quinolone carboxylic acid (BQCA) reversed scopolamine-induced deficits in contextual fear conditioning and improved performance on a one-trial object recognition memory task with a 72 hour retention interval that controls performed at chance (Chambon et al. 2011; Ma et al. 2009).

Although the effects of facilitating M_4 activity with a selective M_4 PAM such as VU0152100 on memory performance have never been investigated, several studies indicate that signaling through the M_4 receptor contributes to plasticity in brain regions that are crucial to certain memory systems. For example, i*n vitro* studies with M_4 genetic knock-out (M_4 KO) mice or the M_4-preferring antagonist MT3 suggest that M_4 receptors mediate normal synaptic responses to the application of the cholinomimetic carbachol in the hippocampus and glutamate stimulation in the striatum (Bonsi et al. 2008; Dasari and Gulledge, 2011; Sanchez et al. 2009). Further, MT3 was also shown to impair memory performance of rats (Ferreira et al. 2003; Jerusalinsky et al. 1998), and the M_1/M_4 preferring-agonist Xanomeline showed beneficial effects on memory in psychiatric patient populations and experimental animals (Bartolomeo et al. 2000, Bodick et al. 1997; Cui et al. 2008; Mirza et al. 2003; Shekhar et al. 2008; Si et al. 2010). Thus, there is good evidence that selective M_1 and M_4 activators could be useful tools to improve memory performance.

The goal of the present study was to assess the influence on memory performance of an M_1-selective allosteric agonist (VU0364572), an M_1-selective PAM (BQCA), and an M_4-selective PAM (VU0152100) in the same memory task in the same rats. More specifically, we aimed to find at least one dose of VU0364572, BQCA, or VU0152100 that would improve object recognition memory performance of healthy rats above

baseline performance. The task was based on rats' spontaneous preference for novel objects, involved little in terms of rule learning, and permitted each rat to be tested on all drug conditions. Further, there is substantial evidence that enhancing mAChR signaling can benefit recognition memory (Bradley et al. 2010; Christie et al. 1981; Cui et al. 2008; Ridley et al. 1986; Ridley et al. 1988; Tariot et al. 1988; Uslaner et al. 2013), but the relative efficacy of VU0364572, BQCA, and VU0152100 to improve recognition memory performance in healthy rats has yet to be demonstrated. Three different doses of each drug were administered to increase the likelihood that at least one dose of the drugs administered would benefit memory performance. Furthermore, our analyses took into account individual differences in baseline performance as we assessed the ability of individual drug conditions to produce robust improvements in memory performance.

Method

Subjects

Twelve male Long-Evans rats between 4-7 months of age were tested. Rats were kept on a 12 hour light/dark cycle (testing occurred during light period), individually housed with free access to water, and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All experimental procedures were approved by Emory University's Institutional Animal Care and Use Committee.

Drugs

Three activators of muscarinic acetylcholine receptors were tested, VU0364572, an M_1-specific allosteric agonist (Lebois et al. 2011), BQCA, an M_1-specific positive allosteric modulator (Ma et al. 2009), and VU0152100, an M_4-specific positive allosteric modulator (Shirey et al. 2008). Three doses were used for each drug to maximize the

likelihood of finding an optimal dose. The doses were selected based on previous work with these drugs (Brady et al. 2008; Lebois et al. 2010; Ma et al. 2009; Shirey et al. 2009). Three control sessions for each were also conducted in which 0.9% saline was administered. Drug administration was counterbalanced between rats using a 12 (subjects) x 12 (sessions) balanced Latin-square design, such that each rat was assigned a unique order of drug administration. Rats were not tested on consecutive days, and testing occurred over a period of 11 weeks. VU0364572 was formulated as a HCl salt in 0.9% saline and administered at doses of 0.1, 10.0, and 30.0 mg/kg, respectively. The pH was titrated to 6.5-8.5 using 1 N NaOH. BQCA was formulated as a sodium salt in a solution of 2-hydroxypropyl-β-cyclodextrin [20% (v/v) in water] at doses of 1.0, 10.0 and 30.0 mg/kg, respectively. The pH was titrated to 6.5-8.5 using 1 M HCl. VU0152100 was formulated as a HCl salt in a solution of 2-hydroxypropyl-β-cyclodextrin [20% (v/v) in water] and administered at doses of 3.0, 30.0, and 56.6 mg/kg, respectively. The pH was titrated to 6.5-8.5 using 1 N NaOH.

Procedure

Figure 1 shows the procedure for a test session. Rats were injected subcutaneously (*s.c.*) with either an experimental drug or 0.9% saline 30 min before being tested on a novel object recognition task that typically lasted about 30 min (mean = 28.56 min), with a range of 17 - 63 min. Previous studies have established that the half-life in brain drug concentration is 46 min for VU0364572, 2.04 hours for BQCA, and 1.12 hours for VU0152100 (Brady et al. 2008; Lebois et al. 2011; Shirey et al. 2009). As drugs remain active for several half-lives, the timing of the procedure ensured each drug would have a high brain concentration until the end of the session. Experimenters were

blind to the drug condition being tested. The object recognition memory task involved rats spontaneously exploring new and repeated objects as they completed clockwise laps on a circular track (outside diameter = 91.5 cm; track width = 7 cm) for a small chocolate reward placed on a central runway upon the completion of each lap. On each trial, one object, ranging in size from 10-2000 cm^3 and made from ceramic, wood, plastic, or metal material, was attached to a small platform adjacent to the track in one of two locations (either 10 or 2 o'clock). The task included a study phase consisting of 12 laps, a 5-min delay in which rats were returned to their home cage, and a test phase consisting of 24 laps, half of which included repeated objects (using duplicates) from the study phase and half of which included new objects. During the initial round of testing, novel objects were used for each session. Prior to testing, rats were trained to complete at least 120 laps on the circular track in 45 min and were given injections of saline on several different days to habituate them to the injection.

Digital video of each session was scored by blind observers to record bouts of object exploration. A rat was considered to be exploring an object only if the rat was within 1 cm of the object and was showing evidence of active investigation (e.g. sniffing and directed attention). Based on past results from similar tasks (e.g. Bass et al. 2012; Ennaceur and Delacour, 1988), recognition memory performance during the test phase was measured by the extent to which rats explored new objects more so than repeated objects. Specifically, for each session a discrimination index was calculated for the test phase by using the mean exploration times of new and repeated objects: new/(new + repeated). A discrimination index of 0.50 indicates chance performance and higher numbers reflect better memory for repeated objects. A discrimination index of 0.66

represents a 2:1 ratio of new:repeated exploration and is typically taken to reflect good performance (Clark and Squire, 2010). Objects that the rat did not explore during the study phase or for which the rat displayed excessive chewing (>5 s) at any point were excluded from analyses. Sessions that included valid data for less than half of the objects in either the study phase or the test phase were deemed unreliable and were retested on a different day.

Analyses focused on the extent to which performance during each drug condition differed from performance averaged across the three control (saline) sessions. Specifically, a linear model (fixed effect = drug condition; random effect = rat) was fitted to the data (dependent variable = DI or exploration time) using Restricted Maximum Likelihood Estimation, and preplanned contrasts were calculated between each drug condition and the control condition. Additionally, to address the possibility that multiple comparisons might lead to type I errors, a Dunnett's correction was also calculated for each contrast. Further, we considered the possibility that different drug conditions might impact performance in a non-uniform manner with respect to a rat's control performance. In particular, the mixed effect approach to the analyses permitted us to compare directly the goodness of fit of linear models in which a rat's performance across conditions was assumed to be correlated (i.e. a compound symmetry covariance structure for the random effect) versus uncorrelated (i.e. a scaled identity covariance structure for the random effect). Additionally, we plotted and analyzed the data separately for 1) all rats, 2) rats who performed well at baseline, and 3) rats who performed poorly a baseline. Data processing and analyses were conducted with SPSS 19 (IBM), R 2.15 (R Foundation), and MATLAB R2011 (Mathworks).

Results

In general, rats explored most of the objects presented in the study phase of each session (mean number of objects explored out of 12 = 11.09), indicating a good level of voluntary task compliance. Nevertheless, for 7 of the 144 total sessions (involving 4 rats), the rat explored less than 6 out of 12 objects during the study session and the session was marked as unusable. An additional 7 sessions (involving 5 rats) were unusable due to experimenter error (i.e. failure to video-record session or inject rats with full amount of drug). Thirteen of the 14 unusable sessions were retested with objects randomly selected from the entire pool of previously used objects. The final unusable session was a saline session and was not retested. For that rat, control performance was averaged across 2 instead of 3 saline sessions. For another rat, 6 of 12 sessions for one rat were unusable, and attempts to retest that rat did not yield usable data. Consequently, the data from all 12 sessions for this rat were excluded from analyses. Thus, the final data set came from 131 sessions with 11 rats. Of these, 30 sessions were randomly selected to be re-scored by a second observer to check inter-rater reliability. For 3.3% of these trials, scorers disagreed about whether the trial should be included in the final data set. For the remaining trials, the inter-rater reliability scores were high (mean = 0.97; range = 0.89 – 1.00).

Figure 2 shows recognition memory performance for each drug condition calculated as a discrimination index. A mixed effect linear model (see Method) in which a rat's performance was assumed to be correlated across conditions fit the overall data no better than a model that assumed no correlation (-2 times log likelihood of the MLE = -137.78 [12 parameters] and -136.02 [11 parameters], respectively, p=0.18). Thus, the

simpler model was used throughout the analyses. In general, rats performed well, but there was no evidence of an overall effect of drug condition ($F[9,100] = 0.685$, $p = 0.721$), and no preplanned contrasts between control and drug conditions were statistically significant (all uncorrected and Dunnett's-corrected ps > .1). In contrast, when the data were considered separately for the 5 rats who performed relatively poorly (<.60) versus the 6 rats who performed well (>.60) in the control condition, several statistically significant findings emerged. First, for the 6 rats who performed well in the control condition, preplanned contrasts between each drug condition and the control condition suggested memory performance was somewhat decreased by the 30.0 mg/kg dose of VU0364572 ($p = 0.043$), the 3.0 mg/kg dose of VU0152100 ($p = .049$) and the 56.6 mg/kg dose of VU0152100 ($p = 0.039$; all other ps > 0.10). However, a Dunnett's correction indicated that these results might reflect a type I error, as all corrected p values were greater than 0.05. For the 5 rats who performed poorly in the control condition, preplanned contrasts between each drug condition and the control condition suggested memory performance was increased by the 0.1 mg/kg dose of VU0364572 ($p = 0.046$), by the 1.0 mg/kg dose of BQCA ($p = 0.023$), by the 3.0 mg/kg dose of VU0152100 ($p = 0.010$), and by the 30 mg/kg dose of VU0152100 ($p = 0.016$). The increase was particularly marked for the 3.0 mg/kg dose of VU0152100, for which the contrast remained significant following a Dunnett's correction (corrected $p = 0.049$; corrected ps for other conditions >.05).

To address the possibility that the experimental drugs might have altered rats' disposition towards inspecting objects generally rather than their memory performance specifically, we asked if exploration times during the study phase differed by drug

condition. Since all objects were novel in this phase of the task, rats would be expected to show similar exploration times across conditions if the drugs did not influence a rat's overall likelihood of exploring an object. Figure 3 shows the mean exploration times during the study phase from each drug condition and plots the results separately for all rats, for rats who performed well during the baseline, and for rats who performed poorly during the baseline. Exploration times were similar between drug conditions and across groups of rats (all *p*-values for omnibus tests and corrected and uncorrected planned contrasts between drug conditions and control conditions > 0.10).

Discussion

The current study used three recently developed mAChR subtype-selective drugs to investigate whether selectively increasing the activity of the M_1 or M_4 receptor subtypes would improve performance of rats on an object recognition memory task. Our results indicated that low levels of M_4 potentiation significantly improved memory performance in some rats. Specifically, systemic administration of the M_4 PAM VU0152100 at 3 mg/kg resulted in significantly improved memory performance relative to baseline for the five rats who performed poorly across three control sessions. The improvement in memory performance did not appear to result from altered exploration of objects during the study phase but instead seemed to reflect better discrimination between novel and repeated objects during the test phase of the object recognition memory task. The results also suggested that M_1 receptor activation modestly benefited memory performance using either VU0364572 (at 0.1 mg/kg) or BQCA (at 1 mg/kg), yet these results did not maintain statistical significance when corrected for multiple comparisons.

Future studies with VU0364572 and BQCA would be useful to ask if different dosages of these drugs might benefit object recognition memory performance.

The current results with VU0152100 are consistent with previous studies that found that increasing synaptic ACh levels may provide cognitive benefits for humans and experimental animals only with naturally pre-existing or experimentally-induced (sleep deprivation) impairments in baseline memory performance (Chuah et al. 2009; Kukolja et al. 2009; Malkova et al. 2011). These previous findings are consistent with the results from the present study that indicate the benefit of M_4 potentiation on memory performance is dependent on baseline levels of performance. Whether the underlying cause of differences in baseline memory performance is due to generally low levels of endogenous ACh, M_4 receptor activity in particular, or other factors, remains to be determined.

One important possibility to consider with respect to our results is that, by partitioning the groups by control performance, statistically significant differences between the control condition and drug conditions may reflect a type of regression to the mean (Nesselroade et al. 1980; Barnett et al. 2005). For example, a statistically significant increase in performance for a drug condition might emerge because performance in the control condition is spuriously low rather than because performance in the drug condition is actually high. Several points argue against this interpretation, especially for the result from the 3 mg/kg dose of VU0152100. First, the rats were categorized according to their mean performance on three separate saline sessions that were evenly distributed throughout the course of testing. This approach of using multiple baselines at different points in testing has been recommended as a method for reducing

the possibility of spurious results related to regression to the mean (Barnett et al. 2005). Second, the performance for the 3mg/kg dose of VU0152100 by the five rats who performed poorly in the control condition (mean ± S.D. = 0.725 ± 0.095) was more than one standard deviation higher than the overall mean (mean performance of all rats in the saline condition = 0.623). Third, for the 3 mg/kg dose of VU0152100, performance for the five rats who performed poorly at baseline was significantly better than the performance of the six rats who performed well at baseline (independent samples t-test; $t[9] = 2.256$, $p = .050$). That is, there was no indication that the poor baseline performance by the five rats represented a spurious observation or that their very good performance for the 3 mg/kg dose of VU0152100 simply represented regression to the mean.

The observation from the current results that none of the drugs significantly altered exploration times of novel objects during the study phase of the task suggests that the drugs did not impact rats' overt attention or general inclination to explore novel objects. However, the drugs may have influenced memory performance indirectly by modulating perception or covert attention. Indeed, mAChR activity has been found to influence synaptic plasticity and excitation in the visual (e.g., Choi et al. 2005; Kimura and Baughman, 1997; Kirkwood et al. 1999; Silver et al. 2008), olfactory (e.g., Saar et al. 2007; Wilson 2001), and somatosensory cortices (e.g., Eggerman and Feldmeyer 2009; Levy et al. 2008; Rahman and Berger 2011). Many past studies have also highlighted the role of mAChRs in attention (e.g., Chen et al. 2004; Dunne and Hartley 1986; Ellis et al. 2006; Gulledge et al. 2009), and in particular BQCA was shown to increase firing activity of medial prefrontal cortex neurons of rats as they performed an auditory detection task

(Shirey et al. 2009). Thus, the drugs in the present study may have impacted memory indirectly through altered perception or covert attention.

An additional indirect influence on memory performance may have occurred via modulation of mAChRs in the striatum (Hersch et al. 1994; Zhang et al. 2002), which in turn may have altered reward-related dopaminergic signaling (Bernard et al. 1992; Dencker et al. 2012; Weiner et al. 1990). Indeed, several studies with M_4 KO mice indicate that M_4 modulates dopamine release in the nucleus accumbens (Jeon et al. 2010; Tzavara et al. 2004), and both the M_1 and M_4 subtypes influence the response of experimental animals to drugs of abuse (Carrigan and Dykstra 2007; Schmidt et al. 2011; Thomsen et al. 2012). Nevertheless, in the present study the only incentive provided was a small food reward that was consistently provided to rats after the completion of each lap on the circular track irrespective of exploration times. Further, it is unclear how the drugs might have impacted a rat's motivation to explore novel objects during the test phase without impacting the rat's inclination to explore novel objects during the study phase.

There are several mechanisms by which the 3.0 mg/kg dose of VU0152100 may have more directly influenced object recognition memory. First, M_4 receptors are expressed in the perirhinal cortex, a region known to be important for object recognition memory (Brown and Aggleton, 2001; Murray 1996; Murray and Bussey 1999). For example, removal of ACh afferents from the basal forebrain to the perirhinal cortex with 192- IgG-saporin impairs object recognition memory performance in rats (Winters and Bussey 2005), and blocking muscarinic receptors in the perirhinal cortex has been shown to disrupt both LTP and LTD *in vitro* and impair object recognition memory performance

in rats and monkeys (Balderas et al. 2012; Massey et al. 2001; Tang et al. 1997; Warburton et al. 2003). Second, M_4 receptors are also densely expressed in both the hippocampus and entorhinal cortex (Levey et al. 1991; Levey et al. 1995; Mulugeta et al. 2006; Piggott et al. 2002; Rodriguez-Puertas et al. 1997), two areas that are also thought to be important for recognition memory (Broadbent et al. 2009; Clark et al. 2000; Galani et al. 1998; Jutras and Buffalo 2010; Lima et al. 2009; Manns et al. 2003; Parron and Save 2004). In particular, based on both receptor localization and previous in vitro studies, M_4 receptor activation may play an important role in modulating glutamate release in the hippocampus at CA3-CA1 synapses (Amaral and Witter 1989; Dasari and Gullege 2011; Hasselmo and Schnell 1994; Levey et al. 1995; Witter et al. 1988). Thus, VU0152100 may have improved object recognition memory performance by acting in the hippocampus or the adjacent perirhinal and entorhinal cortices to influence synaptic plasticity or network dynamics in the extended hippocampal memory system (Hasselmo and McGaughy 2004).

In previous studies, the M_1/M_4-preferring mAChR agonist Xanomeline modestly improved memory in psychiatric populations (Bodick et al. 1997; Shekhar et al. 2008). However, it was unclear the extent to which activity of M_1 versus M_4 receptors contributed to the improvement. The results of the present study demonstrated for the first time that selectively potentiating M_4 receptors can improve memory and thus that a large part of the efficacy of Xanomeline in patient populations may have been due to its actions at M_4 mAChRs. Nevertheless, further research is needed to replicate the findings of the present study and investigate if memory enhancement from M_4 receptor potentiation will hold across species and extend to other cognitive tasks. A key question

is whether selective M_4 receptor activators like VU0152100 would be beneficial when administered to individuals with compromised memory, such as AD patients or schizophrenics. In pursuing that goal, future studies in experimental animals will be helpful in asking the extent to which VU0152100 influences memory performance directly by modulating activity in the hippocampal memory system or indirectly by modulating activity in other brain areas. Finally, the relevance of individual differences in baseline performance to the cognitive outcome from M_4 potentiation indicate subject variability in cognitive abilities at treatment onset may be an important factor to consider when evaluating the efficacy of cholinergic drugs to improve memory performance.

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Figure Captions

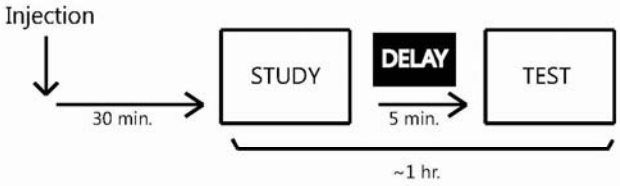
Fig. 1 Schematic of the testing procedure. (a) 30 min. after injection rats completed a testing session consisting of a study phase, a 5-min delay, and a test phase that together lasted about 1 hr. (b) On each trial of the study phase and the test phase, a rat encountered a single object as it completed a clockwise lap around a circular track. During the study phase, rats encountered 12 novel objects. During the test phase, rats encountered duplicates of the 12 objects from the study phase and an additional 12 novel objects. Individual objects are denoted by "O". *indicates duplicates of study objects

Fig. 2 Memory performance for each drug condition. The results are shown as mean Discrimination Index (DI) and are plotted for (a) all rats (n = 11), (b) rats who performed well at baseline (n = 6), and (c) rats who performed poorly at baseline (n = 5). The rats who performed poorly at baseline showed significantly improved memory performance for the 3 mg/kg dose of VU0152100, even after correction for multiple comparisons. Error bars show SEM. The dashed line indicates chance performance. All drug doses are mg/kg. * = $p < 0.05$ with LSD simple comparisons, # = $p < 0.05$ after Dunnett's correction

Fig. 3 Exploration times during the study phase for each drug condition. The results are shown as mean object exploration durations in seconds and are plotted for (a) all rats (n = 11), (b) rats who performed well at baseline (n = 6), and (c) rats who performed poorly at baseline (n = 5). The tendency to explore novel objects did not seem to be influenced by any of the drug conditions. Error bars show SEM. All drug doses are mg/kg

Fig. 1

a.


Injection
30 min.
STUDY
DELAY
5 min.
TEST
~1 hr.

b.

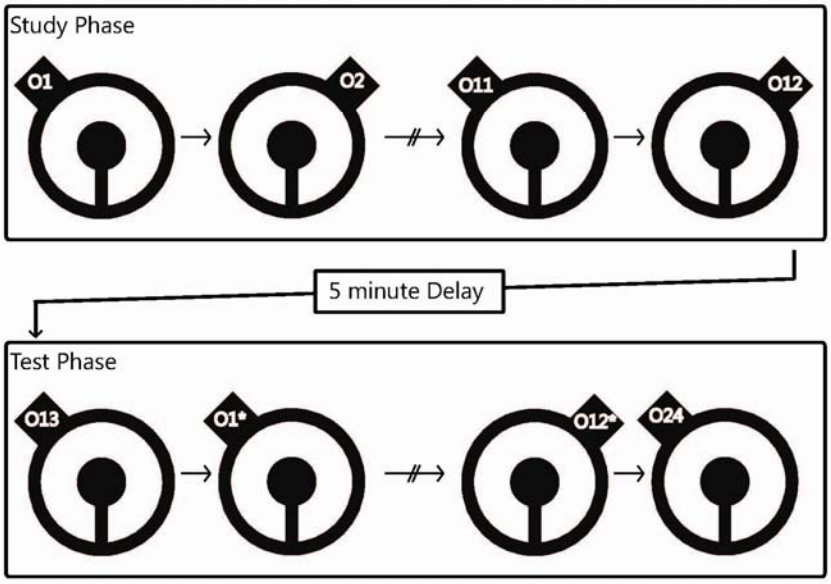

Study Phase
O1
O2
O11
O12
5 minute Delay
Test Phase
O13
O1*
O12*
O24

Fig. 2

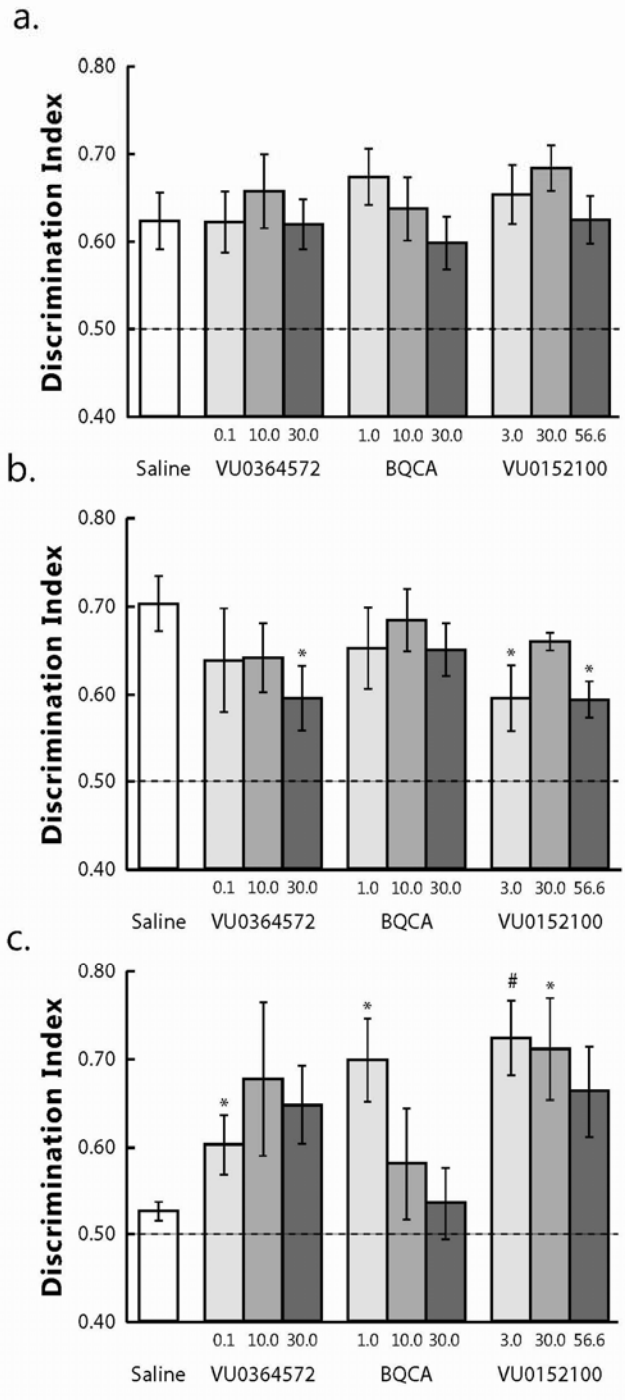
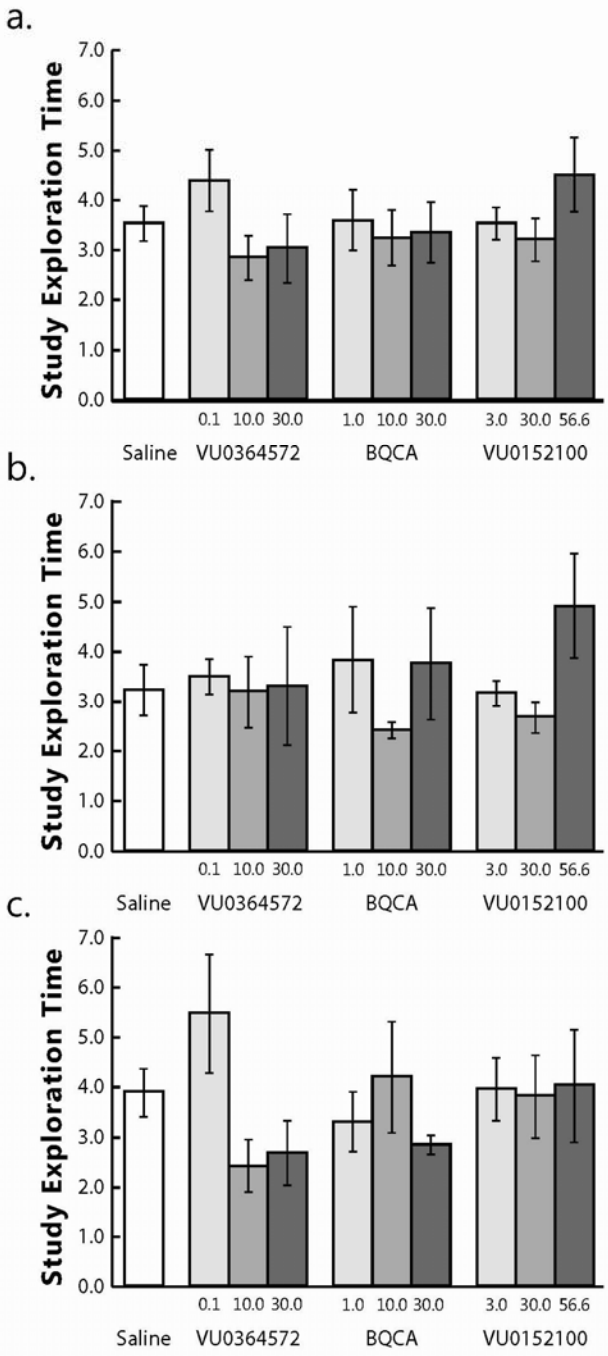

a.
Discrimination Index
0.80
0.70
0.60
0.50
0.40
0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
VU0364572
BQCA
VU0152100
b.
Discrimination Index
0.80
0.70
0.60
0.50
0.40
*
*
*
0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
VU0364572
BQCA
VU0152100
c.
Discrimination Index
0.80
0.70
0.60
0.50
0.40
*
*
#
*
0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
VU0364572
BQCA
VU0152100

Fig. 3


a.
Study Exploration Time
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
VU0364572
BQCA
VU0152100
b.
Study Exploration Time
7.0
6.0
5.0
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0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
VU0364572
BQCA
VU0152100
c.
Study Exploration Time
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.1 10.0 30.0
1.0 10.0 30.0
3.0 30.0 56.6
Saline
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