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M_1-selective muscarinic allosteric modulation enhances cognitive flexibility and effective salience in nonhuman primates

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Acetylcholine (ACh) in cortical neural circuits mediates how selective attention is sustained in the presence of distractors and how flexible cognition adjusts to changing task demands. The cognitive domains of attention and cognitive flexibility might be differentially supported by the M_1 muscarinic acetylcholine receptor (mAChR) subtype. Understanding how M_1 mAChR mechanisms support these cognitive subdomains is of highest importance for advancing novel drug treatments for conditions with altered attention and reduced cognitive control including Alzheimer's disease or schizophrenia. Here, we tested this question by assessing how the subtype-selective M_1 mAChR positive allosteric modulator (PAM) VU0453595 affects visual search and flexible reward learning in nonhuman primates. We found that allosteric potentiation of M_1 mAChRs enhanced flexible learning performance by improving extradimensional set shifting, reducing latent inhibition from previously experienced distractors and reducing response perseveration in the absence of adverse side effects. These procognitive effects occurred in the absence of apparent changes of attentional performance during visual search. In contrast, nonselective ACh modulation using the acetylcholinesterase inhibitor (AChEI) donepezil improved attention during visual search at doses that did not alter cognitive flexibility and that already triggered gastrointestinal cholinergic side effects. These findings illustrate that M_1 mAChR positive allosteric modulation enhances cognitive flexibility without affecting attentional filtering of distraction, consistent with M_1 activity boosting the effective salience of relevant over irrelevant objects specifically during learning. These results suggest that M_1 PAMs are versatile compounds for enhancing cognitive flexibility in disorders spanning schizophrenia and Alzheimer's diseases.

acetylcholine | attention | donepezil | cognitive control | learning

significance

Muscarinic receptors mediate the procognitive effects of acetylcholine, but it has remained unclear whether they differentially affect the cognitive subfunctions of attentional filtering, set shifting, and learning. To clarify the functional specificity of M_1 mAChRs, we assessed these diverse functions using a recently developed, highly selective M_1 PAM. This M_1 PAM caused domain-specific cognitive improvement of flexible learning and extradimensional set shifting, reduced perseverations and enhanced target recognition during learning without altering attentional filtering functions. These domain-specific improvements contrasted to effects of a nonselective acetylcholinesterase inhibitor that primarily enhanced attention and caused dose-limiting adverse side effects. These results demonstrate domain-specific improvements in cognitive flexibility suggesting M_1 PAMs are versatile compounds for treating cognitive deficits in schizophrenia and Alzheimer's disease.

Cholinergic activity has far reaching consequences on attention and attentional control functions (1, 2) with long-standing suggestions that cholinergic modulation is involved in faster updating of expectations during learning (3–5). Depleting cholinergic innervation to the prefrontal cortex compromises while stimulation of cholinergic activity can enhance attentional control functions (6–10). These cholinergic effects have been suggested to be supported differently by nicotinic versus muscarinic receptors (11, 12). Antagonizing muscarinic cholinergic action with scopolamine in healthy humans and nonhuman primates (NHPs) increases false alarm rates and impairs sustained attention by slowing response times and impairing signal detection in two-alternative choice tasks (13–17). Consistent with these behavioral effects, neuronal recordings in the prefrontal cortex of NHPs have shown that attentional signaling depends on muscarinic receptor activation (18). One key open question from these insights is to what extent are attentional subcomponent processes supported by muscarinic signaling and whether there are subreceptors of the muscarinic receptor family that differentially support separable subcomponent processes underlying attention, such as filtering of distracting information, enhancing the flexible updating and shifting of attention sets, or supporting robust goal representations during goal-directed behavior. Each of these subcomponent processes has been associated in prior studies with the M_1 mAChR, which is widely expressed in the cortex, striatum, and hippocampus (19, 20) and may thus mediate some of these muscarinic procognitive effects (2, 21).

One set of prior studies has implicated the M_1 mAChR in memory processes because M_1-selective ligands can restore deficits in novel object recognition (22, 23) and can partially reverse scopolamine-induced deficits in contextual fear conditioning consistent with M_1-selective compounds enhancing the salience of the (aversive) outcomes during learning (22, 24, 25). But it has remained unclear whether the effects described in these studies are best accounted for by an improvement of memory, or whether enhanced cognitive control processes contribute to more effective encoding of stimuli as opposed to enhancing learning processes. A similar question about the specific cognitive process that is modulated arises

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when considering the M_1 mAChR effects on different forms of attentional performance. While some studies have shown that M_1 mAChR modulation is important for attentional modulation of neural firing (18, 26), behavioral studies using M_1-selective PAMs in NHPs (27) and rodents (28) have not found primary improvements of sustained attention performance. Rather than modulating attention, the M_1 mAChR actions improved behavior only in more demanding task conditions in which M_1 modulation enhanced the adjustment of performance when task requirements changed (28). These results are consistent with findings showing that selective M_1 mAChR ligands can facilitate the use of complex sensorimotor transformations to reach a goal (as in object-retrieval detour tasks) (29), and improve odor-based reversal learning of objects (30). These cognitive enhancing effects suggest that M_1 mAChRs may be particularly important for higher cognitive control processes that go beyond attentional focusing or the filtering of distraction (2). However, it is not apparent which particular control processes might be supported by M_1 mAChRs as the existing studies used widely varying tasks and a study using one of the classical cognitive control task (the antisaccade task) was not successful in identifying neural correlates of M_1 mAChR–specific effects in the prefrontal cortex of NHPs (31).

The current study set out to address these questions about the specific procognitive role of the M_1 mAChR in supporting attention and learning functions. Firstly, to distinguish cognitive subcomponent processes we devised two tasks. A visual search task allowed for distinguishing attentional subcomponent processes by varying distractor load and perceptual interference. And a intra-/extradimensional set-shifting learning task distinguishing cognitive control processes and cognitive flexibility. Secondly, we assessed NHP performance in these tasks using VU0453595, which is a recently developed subtype selective PAM for the M_1 mAChR that does not activate the receptor directly but substantially potentiates the M_1 mAChR response to endogenous ACh (22, 32, 33). This selective M_1 PAM mechanism does not produce the dose limiting side effects which are prevalent with existing orthosteric mAChR agonists and AChEIs (34, 35), and which has the potential to treat deficits in attention control and cognitive rigidity prevalent in schizophrenia, Alzheimer's disease, and substance use disorders (36–40). Assessing the procognitive profile of VU0453595 for these higher cognitive functions is therefore pivotal to advance therapeutic solutions for these widespread neuropsychiatric conditions (22, 41, 42).

We found that the M_1 PAM VU0453595 exerts an inverted-U–shaped improvement of cognitive flexibility with increasing dose, causing faster learning, extradimensional set shifting, and reduced perseverations (i.e., enhanced flexibility), while leaving attentional filtering during visual search unaffected. These results are contrasted to the nonselective AChEI donepezil which improved attentional filtering with only marginal effects on cognitive flexibility (43).

Results

We used four male rhesus macaques, with ages ranging from 7 to 11 y old, as subjects. Each of the four monkeys completed 60 sessions composed of 40 vehicle days and 7 d for each of the three tested doses of VU0453595 (0.3, 1, and 3 mg/kg). No dose-limiting adverse effects were observed in any of the 21 VU0453595 dosing days in any of the monkeys.

M_1 PAM VU0453595 Enhances Learning. Animals performed and consistently completed all 21 blocks of the feature learning task per session in all experimental conditions and expectedly showed faster learning in the easier low distractor load condition versus the more difficult high distractor load condition (Fig. 1 *C* and *D*). Administration of VU0453595 improved multiple measures of the learning performance compared to the vehicle control condition. In order to capture changes in learning speed, we calculated the trials-to-criterion as the first trial in a block that led to >70% accurate performance in the subsequent 10 trials. To reveal any temporally specific effects on learning, we implemented a linear mixed effects model on the median trials-to-criterion for the feature-reward learning (FRL) task (*SI Appendix*, *Supplemental Methods*). We found faster learning with 1 mg/kg dosing as evident in the early, middle, and last thirds of the 21 learning blocks per session with the first third of blocks showing the strongest effects (1 mg/kg, fixed effect: $t(3674) = -2.67$, $P = 0.008$; first third Cohen's $d = -0.228$; overall Cohen's $d = -0.061$) (*SI Appendix*, Fig. S1*A*). For this reason, all future analyses of the FRL task use the first third of blocks. Faster learning was particularly evident at low distractor load for which animals reached the trials-to-criterion at 7.93 (SE: 0.81) trials after a block switch with 1 mg/kg, compared to 11.03 (SE: 0.38) trials with vehicle [$F(3,691) = 3.54$, $P = 0.01$; $\eta^2 = .015$; Tukey's, $P = 0.028$; Cohen's $d = -0.352$] (Fig. 1*E*). After the performance criterion was reached, VU0453595 also enhanced plateau performance (*SI Appendix*, Fig. S1*B*) and increased the proportion of blocks in which the animals reached the learning criterion at the 1 mg/kg dose (*SI Appendix*, Fig. S1*C*).

Faster learning and improved performance accuracy in the 1 mg/kg dose condition were accompanied by faster response times (RTs). Over the course of a learning block, subjects showed a characteristic change of RTs with fast RTs early in the block that slowed down until an inflection point around the trial within the block when animals began to more consistently choose the rewarded target feature (Fig. 2 *A* and *B*). Notably, administering the middle (1 mg/kg) dose of VU0453595 led to significantly faster RTs of 870 ms (SE: 23 ms; low load) and 960 ms (SE: 23 ms; high load) in the low- and high-load conditions relative to vehicle RTs of 960 ms (SE: 11 ms; low load) and 984 ms (SE: 11 ms; high load) [$F(3,1672) = 2.97$, $P = 0.03$; $\eta^2 = 0.005$; Tukey's, $P = 0.04$; Cohen's $d = -0.350$] (Fig. 2*C*). Moreover, the number of trials needed for the RTs to reach this inflection point was significantly fewer with the 1 mg/kg dose taking until trial 6.5 (SE: 0.5) relative to vehicle taking until trial 8.7 (SE: 0.3) [$F(3,193) = 2.67$, $P < 0.05$; $\eta^2 = 0.040$; Tukey's, $P = 0.03$; Cohen's $d = -0.674$) (Fig. 2*D*).

Improved Cognitive Control with M_1 PAM VU0453595. Learning a new feature-reward rule following a block switch entailed either identifying a target feature that was new or from a different feature dimension as in the previous block (extradimensional switches, ED), or from the same feature dimension as the previous target (intradimensional switches, ID). We found that the 1 mg/kg dose with VU0453595 significantly improved learning for both, ED and ID switches (Fig. 2 *E* and *F*) but not switches where the current target was from a novel feature dimension (data not shown). A large improvement was evident for ED switches with the average trials-to-criterion of 4.0 (SE: 0.7) after 1 mg/kg dose administration being significantly lower than the average 12.2 (SE: 1.0) trials-to-criterion of the vehicle condition [$F(3,122) = 3.15$, $P = 0.03$; $\eta^2 = .072$; Tukey's, $P = 0.02$; Cohen's $d = -0.868$] (Fig. 2*E*). Please note that ED switches reported in our task were to a target of the previous distractor feature dimension and thus required disengaging from that dimension in addition to identifying the newly rewarded dimension. ID switches had a more moderate but still significant advantage after administration

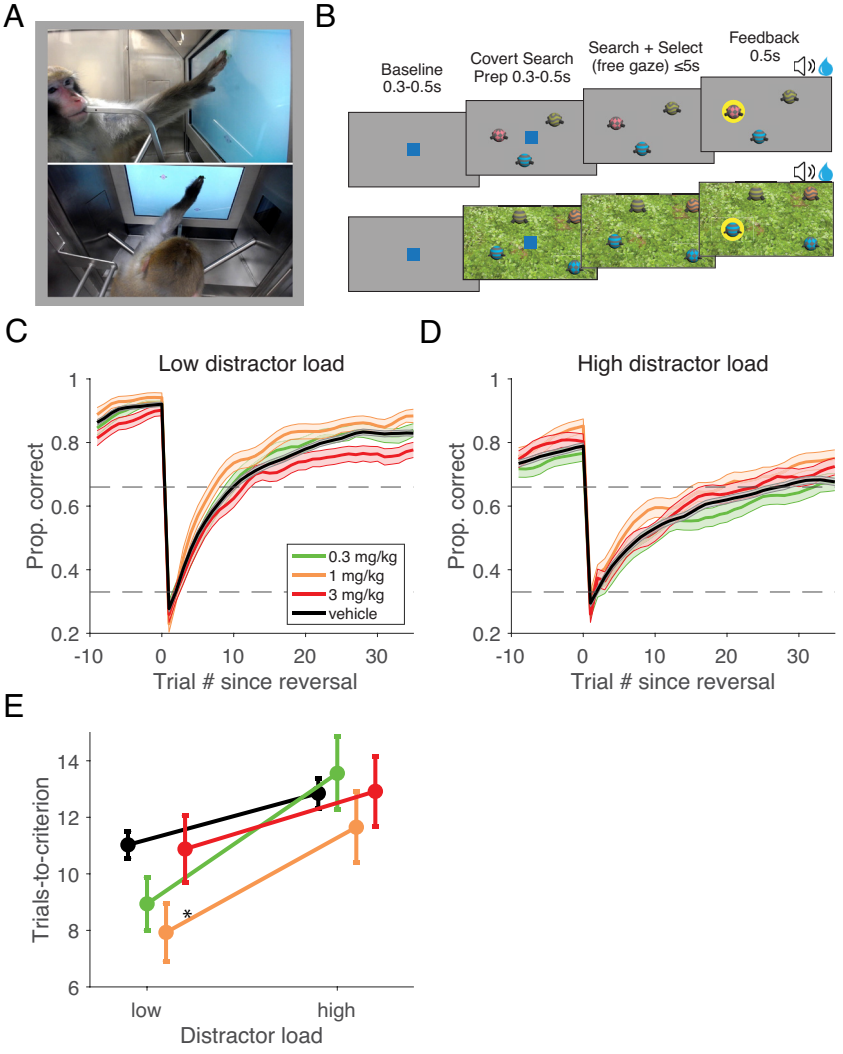

Fig. 1. Task Design and Feature-Reward Learning Task Performance Enhancement by VU0453595 (*A*) Images of cage-mounted kiosk and monkey Ba utilizing the touch screen to perform the feature-reward learning (FRL) task taken via the video monitoring system. Both images are taken from the same time point from different angles. (*B*) Trial progression of the FRL task (*Top*) and the VS task (*Bottom*). The example FRL task here is a block with "high distractor load" where objects vary in both color and pattern. Each object contains only 1 feature from each feature dimension. Although the red checkered object was correctly chosen in this trial, the animal would need to learn through trial and error if the red or checkered feature was correct in order to optimally acquire reward from future objects in this block. The example VS block here shows a trial with three distractors and a target object that is defined by three features: blue, striped, and straight conical arms. The red distractor has zero features in common with the target, the yellow distractor has one feature in common with the target (striped pattern) and the blue distractor has two features in common with the target (blue color and straight conical arms). Trials in either task were initiated by a 0.3 to 0.5 s touch and hold of a central blue square (3° visual radius wide) after which the square disappears (for 0.3 to 0.5 s) and task objects (2.5° visual radius wide) are presented on screen. For the VS task, a structured background scene (here: a lawn) was used to distinguish the VS from the FRL task (which had a grey uniform background). For each visual search block, we drew a different random background scene from a set of five backgrounds independent of the target search feature. In either task, subjects have 5 s to select one of the objects with a 0.2 s touch and hold. Failure to choose an object resulted in an aborted trial which was ignored. Feedback for choice selection was provided 0.2 s after object selection for 0.5 s via both a visual halo around the chosen object as well as a auditory cue alongside any earned fluid. Both the frequency of the audio feedback and color of the feedback halo differed based on outcome. (*C*) Block-wise average learning curves for the low distractor load blocks of the FRL task aligned to block start for vehicle, 0.3, 1, and 3 mg/kg VU0453595, smoothed after the first three trials with a sliding window (shaded area: SE). Dotted horizontal lines signify 0.33 and 0.66 probabilities. (*D*) The same as *C* but for the high distractor load blocks. (*E*) Median trials-to-criterion, calculated as the first trial in a block that led to >70% performance over 10 subsequent trials, for the low and high distractor load blocks of the FRL task. For the low distractor load blocks, trials-to-criterion were 11.03 (SE: 0.38), 8.94 (SE: 0.75), 7.93 (SE: 0.81) and 10.88 (SE: 0.94) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. Only the 1 mg/kg dose was significantly different from vehicle [$F(3,691) = 3.54$, $P = 0.01$; $\eta^2 = .015$; Tukey's, $P = 0.028$; Cohen's d = −0.352). For the high distractor load blocks, trials-to-criterion were 12.85 (SE: 0.43), 13.56 (SE: 1.03), 11.65 (SE: 1.00), and 12.92 (SE: 0.98) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively, with no significant effect [$F(3,565) = .40$, n.s.].

of 1 mg/kg dose of VU0453595 with a trials-to-criterion of 9.3 (SE: 0.7) relative to 12.6 (SE: 0.5) with vehicle [$F(3,518) = 3.26$, $P = 0.02$; $\eta^2 = 0.019$; Tukey's, $P = 0.04$; Cohen's d = −0.349] (Fig. 2*F*).

The learning advantage after ED and ID switches indicates that VU0453595 at the 1 mg/kg dose enhanced cognitive control. Cognitive control also entails the ability to avoid erroneous perseverative responding. We quantified the perseverative responses as the proportion of repeated unrewarded choices to a feature in the target-feature dimension or in distractor-feature dimensions. We found that VU0453595 reduced perseverative responding to other features in the target feature dimension at 1 mg/kg from the 10.7% (SE: 0.2) of vehicle down to 8.5% (SE: 0.6) [$F(3,1679) = 3.74$, $P = 0.01$; $\eta^2 = 0.007$; post hoc analysis of 1 mg/kg condition Tukey's, $P = 0.01$; Cohen's d = −0.243] (Fig. 2*G*). Perseverative responding to objects with features of the distractor dimension was moderately, but nonsignificantly reduced with VU0453595 [$F(3,844) = 2.36$, $P = 0.07$; $\eta^2 = 0.008$] (Fig. 2*H*).

M_1 PAM VU0453595 Has No Consistent Effect on Interference Control. Cholinergic compounds modulate attention and interference control (12, 43, 44). We evaluated these functions using a visual search (VS) task that varied the requirements to control interference from increasing numbers of distractor objects during search, and from increasing the number of features that were shared between target and distractors (target-distractor similarity, see *Methods and Materials*).

Animals showed prominent slowing of target detection times with increasing number of distractors from 3, 6, 9 to 12. VU0453595 did not consistently modulate this slowing with increasing distractor set size as evident by the similar slope of the linear fit across increasing numbers of distractor (Fig. 3 *A* and *B*). Similarly, the accuracy of target detection was not consistently affected by VU0453595 with no change of set size effects. For both, the raw values of target detection times and accuracy, some significant changes were observed (see Supplemental Results) but no systematic pattern could be extracted (Fig. 3 *C* and *D*). Similarly, VU0453595 did not consistently alter perceptual interference operationalized as changes in performance with increasing similarity between the target and distractors (Fig. 3 *E* and *F*). There were no changes in the set size effect for target detection times [first block: $F(3,236) = 0.54$, n.s.; second block: $F(3,236) = 1.81$, n.s.; Fig. 3*E*] or accuracy [first block: $F(3,236) = 0.53$, n.s.; second block: $F(3,236) = 0.51$, n.s.; Fig. 3*F*]. Similar to the

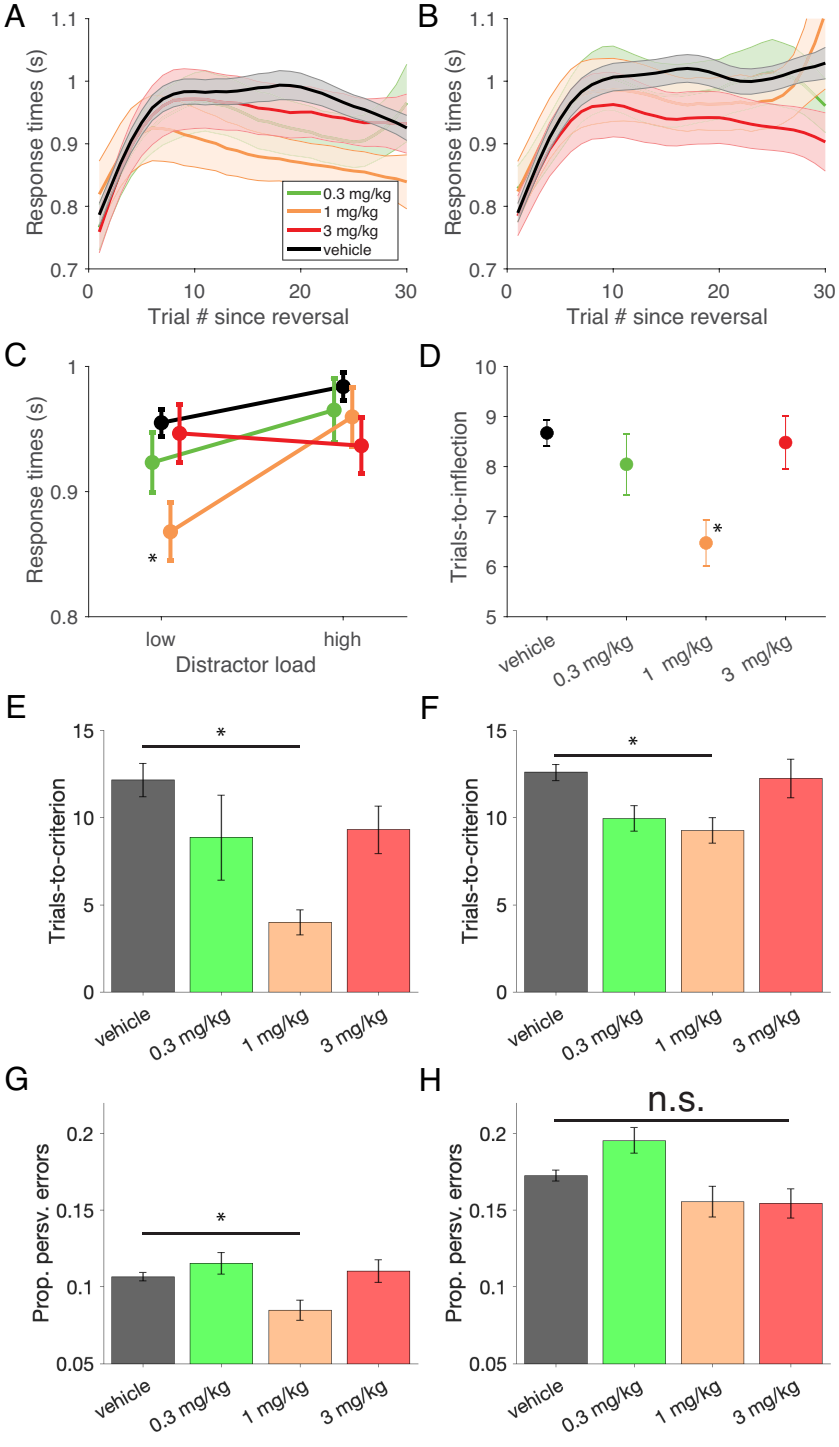

Fig. 2. Feature-Reward Learning Task Efficiency and cognitive flexibility improvements with VU0453595 (*A*) The average RT curve of each session (correct trials only) aligned to block start for the low distractor load blocks of the FRL task for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 (shaded area: SE) (*B*) The same as A but for high distractor load blocks of the FRL task. (*C*) Block-wise averages of the traces plotted in *A* and *B* visualized to compare RTs between distractor load conditions. Low distractor load blocks had RTs of 960 ms (SE: 11), 923 ms (SE: 24), 870 ms (SE: 23) and 974 ms (SE: 23) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. High distractor load blocks had RTs of 984 ms (SE: 11), 965 ms (SE: 26), 960 ms (SE: 23), and 937 ms (SE: 22). Only the 1 mg/kg dose of VU0453595 was significantly different from vehicle [F(3,1672) = 2.97, P = 0.03; η^2 = 0.005; Tukey's, P = 0.04; Cohen's d = −0.350] (*D*) Trials-to-inflection for RTs in the low distractor load blocks defined as the first trial per block (excluding trial 2) that RTs become faster (error bars: SE). Trials-to-inflection was 8.7 (SE: 0.3), 8.0 (SE: 0.6), 6.5 (SE: 0.5) and 8.5 (SE: 0.5) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. Only the 1 mg/kg dose was significantly different from vehicle [F(3,193) = 2.68, P < 0.05; η^2 = .040; Tukey's, P = 0.017; Cohen's d = −0.674]. (*E*) Block-wise average trials-to-criterion after extradimensional shifts were 12.2 (SE:1.0), 8.9 (SE: 2.4), 4.0 (SE: 0.7) and 9.3 (SE: 1.4) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. Only the 1 mg/kg dose showed a significant difference from vehicle [F(3,122) = 3.15, P = 0.03; η^2 = 0.072; Tukey's, P = 0.02; Cohen's d = −0.868]. (*F*) Block-wise average trials-to-criterion after intradimensional shifts were 12.6 (SE: 0.5), 10.0 (SE: 0.7), 9.3 (SE: 0.7) and 12.3 (SE: 1.1) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. Only the 1 mg/kg dose showed a significant difference from vehicle [F(3,518) = 3.26, P = 0.02; η^2 = .019; Tukey's, P = 0.04; Cohen's d = −0.349]. (*G*) The proportion of errors that were perseverative in nature with the feature that was perseverated being from the same feature dimension as the target feature. The proportion of perseverative errors from the target feature dimension were 10.7% (SE: 0.2), 11.5% (SE: 0.7), 8.5% (SE: 0.6) and 11.0% (SE: 0.7) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively, with only the 1 mg/kg dose being significantly different from vehicle [F(3,1679) = 3.74, P = 0.01; η^2 = 0.007; Tukey's, P = 0.01; Cohen's d = −0.243]. (*H*) The same as *G* but with the feature that was perseverated being from the distracting feature dimension (different from the target feature dimension). Proportions of perseverative errors from the distracting feature dimension were 17.3% (SE: 0.3), 19.6% (SE: 0.8), 15.6% (SE: 1.0) and 15.6% (SE: 1.0) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595, respectively. There was a nonsignificant trend for a main effect of experimental condition [F(3,844) = 2.36, P = 0.07; η^2 = 0.008].

distractor effect, the comparisons of how perceptual interference impacted raw target detection times and performance showed no systematic improvements (*SI Appendix*, *Supplemental Results*). We also looked at the relationship between search times and performance in both VS blocks independently and found no significant change in relationship at any of the tested doses (n.s.; fisher r to z transformation; *SI Appendix*, Fig. S2). No changes to speed of processing, operationalized as the time to response during familiarization trials (*Methods and Materials*) were observed with VU0453595 at any dose for neither the first VS block [F(3,236) = 0.56, n.s.] nor the second block [F(3,236) = 0.35, n.s.] (*SI Appendix*, Fig. S3).

Comparison of VU0453595 Effects with the Literature and Consistency of Effects across Monkeys. To evaluate how our study compared to previous studies, we surveyed 20 studies in NHPs (six using M_1-selective PAMs) and thirty-two studies in rodents ((28) using M_1-selective PAMs) and summarize them in Table 1 (study details are described in *SI Appendix*, Tables S2 and S3 for NHP and rodent studies, respectively). This survey highlighted two main ways the current study is distinct from existing studies beyond the use of the compound VU0453595.

First, the type and number of tasks used here differ from typical behavioral assays. None of the surveyed nonhuman primate studies used a set-shifting task or varied the number and

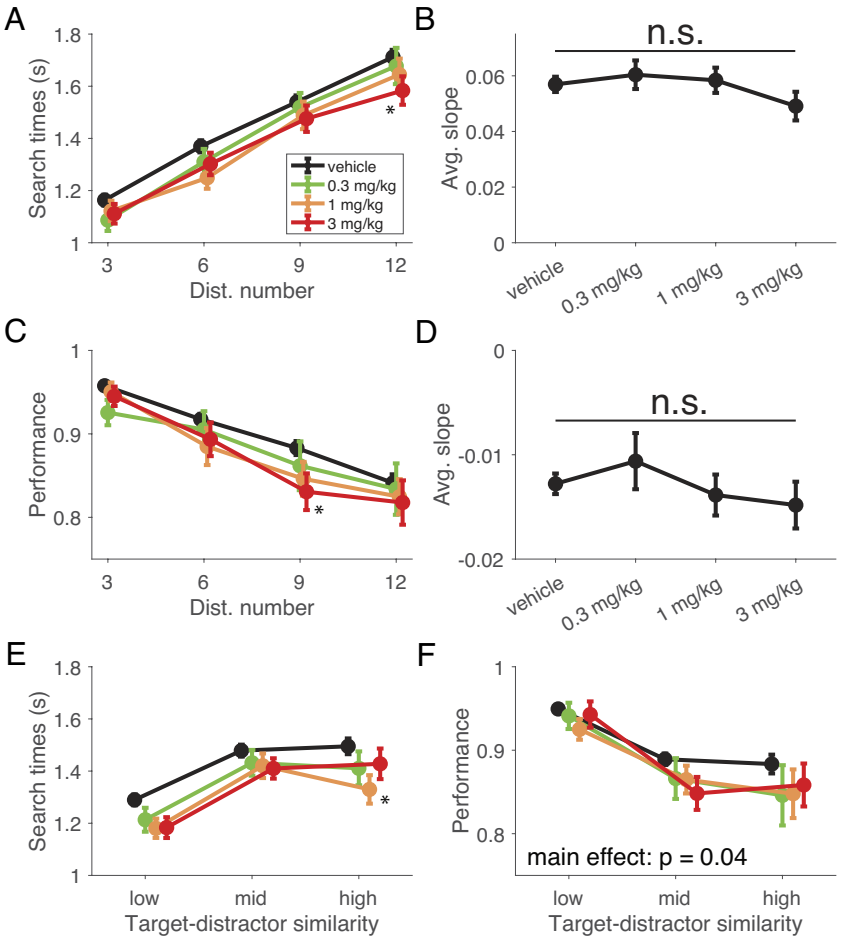

Fig. 3 Distractor Effect and Interference Control Are Not Consistently Impacted by VU0453595 (*A*) Target detection durations (reaction times) as a function of distractor number for the second VS block. There was a significant main effect of experimental condition with a significant different between the 3 mg/kg dose of VU0453595 compared with vehicle [$F(3,944) = 3.67$, $P = 0.01$; $\eta^2 = .008$; Tukey's, $P < 0.05$]. The 3 mg/kg dose improved search times from 1.16 s (SE: 0.02), 1.37 s (SE: 0.02), 1.54 s (SE: 0.03) and 1.72 (SE: 0.03) with vehicle to 1.11 s (SE: 0.04), 1.30 s (SE: 0.04), 1.48 s (SE: 0.05) and 1.58 s (SE: 0.05) for 3, 6, 9, and 12 distractors, respectively. There was no significant change in the first VS block (data not shown). (*B*) The set size effect, operationalized as the slope of the linear fit of search times as a function of distractor numbers for the second VS block (0.057 (SE: 0.003), 0.060 (SE: 0.005), 0.058 (SE: 0.005) and 0.049 (SE: 0.005) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595) was not significant [$F(3,236) = 0.67$, n.s.]. There was also no significant set size effect in the first VS block (data not shown). (*C*) VS task performance as a function of distractor number for the first VS block. There was a significant main effect of experimental condition with a significant different between the 3 mg/kg dose of VU0453595 compared with vehicle [$F(3,944) = 3.80$, $P = 0.01$; $\eta^2 = .010$; Tukey's, $P = 0.04$]. The 3 mg/kg dose reduced performance from 95.8% (SE: 0.4), 91.8% (SE: 0.7), 88.3% (SE: 0.8) and 84.1% (SE: 1.0) with vehicle to 94.5% (SE: 1.2), 89.4% (SE: 2.0), 83.1% (SE: 2.2) and 81.8% (SE: 2.7) for 3, 6, 9 and 12 distractors, respectively. There was no significant change in the second VS block (data not shown). (*D*) The set size effect, operationalized as the slope of the linear fit of performance as a function of distractor numbers for the first VS block [−0.013 (SE: 0.001), −0.011 (SE: 0.003), −0.014 (SE: 0.002) and −0.015 (SE: 0.002) for vehicle, 0.3, 1, and 3 mg/kg doses of VU0453595] was not significant [$F(3,236) = 0.60$, n.s.]. There was also no significant set size effect in the second VS block (data not shown). (*E*) VS task search times as a function of target-distractor similarity for the second VS block. There was a significant main effect of experimental condition with a significant different between the 1 mg/kg dose of VU0453595 compared with vehicle [$F(3,708) = 4.67$, $P = 0.003$; $\eta^2 = 0.018$; Tukey's, $P = 0.02$] but no significant set size effect [$F(3,236) = 1.81$, n.s.]. Search times were faster from 1.29 s (SE: 0.02), 1.48 s (SE: 0.02), and 1.49 (SE: 0.03) with vehicle to 1.18 s (SE: 0.04), 1.42 s (SE: 0.05) and 1.33 s (0.05) for low, medium, and high average target-distractor similarity, respectively. (*F*) VS task performance as a function of target-distractor similarity for the second VS block. There was a significant main effect of experimental condition but no significant post hoc comparison was found [$F(3,708) = 2.84$, $P = 0.04$; $\eta^2 = 0.011$; Tukey's, n.s.]. We also failed to find a significant set size effect [$F(3,236) = 0.53$, n.s.].

similarity of distractors to a target stimulus in an attention task. Of the six studies using M_1 PAM compounds (PQCA, TAK-071, MK-7622, or VU0453595), two contained no behavior, four tested cognitive effects including problem solving (3/4), working memory (2/4), and vigilance/attention (1/4). Critically, this study shows cognitive enhancement relative to vehicle and does not involve reversing pharmacological challenge-mediated deficits in cognition unlike the other M_1 PAM NHP studies surveyed here. Similarly, of the 28 rodent studies using M_1 PAMs (VU compounds: 9/28; BQCA/PQCA: 9/28 studies; PF compounds: 6/28 studies; TAK-071: 4/28; MK-7622: 3/28; Other: 5/28) in diverse pharmacological challenge paradigms and disease models (Table 1 and *SI Appendix*, Table S3). Across these rodent M_1 PAM studies, none quantified ED/ID set shifting, while one reported reversal learning performance (30), alongside behavioral assays testing learning and memory (19/28), locomotion/ motor control (9/28), working memory (6/28), attention/vigilance (3/28), social behavior (3/28), misc. exec. function (2/28), or satiety/drug abuse (1/28).

Second, our approach to test individual monkeys seven times per dose differs from all six previous NHP M_1 PAM studies, which used one determination per dose per monkey. These previous studies focused analyses on the group level with on average eight NHPs used per dose (*SI Appendix*, Table S2), compared to four monkeys per dose in our study. This difference raises the question whether our study design succeeded to find consistent effects not only in individual subjects (which we aimed for by repeating each dose seven times), but also across subjects. We address this question by summarizing in Fig. 4 the average effect of 1 mg/kg VU0453595 relative to the vehicle condition for each subject (marked in different colors) and across all major performance metrics of the set-shifting task and the visual search task. The figure illustrates the consistency of the effects across monkeys and performance metrics, supporting the main conclusions of the previous sections that the M_1 PAM enhanced metrics indexing cognitive flexibility (reduced switch costs, reduced perseverative errors and led to faster learning) with less consistent effects on metrics indexing distractor filtering.

Double Dissociation of VU0453595 and Donepezil for Cognitive Flexibility and Interference Control

VU0453595 improved learning and reduced perseveration, but without reducing interference from distracting objects and features. This pattern of results contrasts to the effects of nonselective AChEI donepezil for which a prior study using the same tasks as in the current study found that an optimal dose range improved VS performance but without affecting reward learning and perseveration (43). To quantify this difference, we re-analyzed the performance of reward learning and visual search with donepezil in the prior study using the best dose for VS improvements (0.3 mg/kg) (43). This comparative approach revealed a

Table 1. Summary of studies in NHPs and rodents assessing cognitive and behavioral effects using M_1 PAMs specifically. For details about the individual NHP studies, see *SI Appendix*, Table S2 and for individual rodent studies, see *SI Appendix*, Table S3

	Working Memory	Learning & Memory	Executive Functioning (Cognitive Flexibility/Reasoning/Problem Solving)	Attention/Vigilance	Social	Motor Control/Locomotion
NHP studies	(↺) (↺)		(↺) (↺) (↺)	(↺)		
Rodent studies	↑ ↺/↺ (↺) (↺) (↺) -	↑ ↑ ↑ ↑ ↑/(↺) ↺ ↺ ↺ ↺/(↺) ↺ ↺ ↺ ↺ ↺ ↺ ↺ (↺) (↺) (↺) (↺) (↺) (↺) (↺) (↺) -	↑/↺	↺ ↺ (↺) -	↺ ↺ ↺	↺ ↺/(↺) (↺) (↺) (↺) (↺) - -
Current study	-	↑	↑	-		

Symbol key. (↺): partial rescue from pharmacological challenge. (↺): partial rescue from genetic/lesion/other challenge. ↺: rescue from pharmacological challenge. ↺: rescue from genetic/lesion/other challenge. ↑: enhancement without any external challenge. ↑: enhancement beyond vehicle after any challenge. -: none of the tested PAMs resulted in significant change.

double dissociation (Table 2). VU0453595 enhanced metrics of learning efficiency and cognitive flexibility but not metrics of interference control during VS, while donepezil made the animals more robust against distraction (improved interference control) during visual search but did so without improving feature-reward learning performance. Furthermore, at this dose, donepezil slowed down response times in the FRL task as well as search times in the VS task and even slowed the speed of processing early, partially as a consequence of dose-limiting side effects that accompanied donepezil. In contrast, VU0453595 at 1 mg/kg sped up response times in the FRL task without slowing down VS search times or the speed of processing and without any observable side effects (Table 2).

Discussion

Here, we found that healthy adult rhesus monkeys show M_1 mAChR–specific improvements of cognitive flexibility in a feature-reward learning task, while leaving attentional filtering unaffected. In particular, the middle of three doses of the M_1 PAM VU0453595 increased the speed of learning a new feature-reward rule, particularly with extradimensional rule changes. At the same dose animals showed less perseveration on unrewarded features. These procognitive effects contrasted to the absence of consistent distractor-dependent changes in accuracy or search times during VS. Although we did find significantly faster search times in the second VS block only and a significant reduction in performance in the first VS block only (both at 3 mg/kg), no attentional effect, i.e., shift in the slope of performance or search times dependent on the number of distractors or on target-distractor similarity was found. At the dose range tested no adverse side effects were noted. This result pattern contrasts with the effects of donepezil which improved attentional filtering during VS at a dose at which it did not affect cognitive flexibility, but already resulted in dose-limiting side effects. Taken together, these findings document a functional dissociation of the role of M_1 mAChR modulation with highly selective M_1 PAMs, suggesting it is a versatile treatment target for disorders suffering from inflexible, rigid cognition and behavior including schizophrenia, Alzheimer's disease, and addiction.

M_1 PAM Enhances Learning and Extradimensional shifts. We found that the medium dose of VU0453595 improved learning of feature values. Compared to the vehicle control, the medium dose allowed subjects to reach the performance criterion 3.10 trials earlier at the low distractor load condition (Fig. 1*E*) and the number of trials to reach RT inflection decreased by 2.20 trials for low distractor load blocks (Fig. 2*D*) (see *SI Appendix*, *Supplemental Discussion* with regard to dose specificity of the effects). The learning improvement was particularly apparent with extradimensional (ED) switches, i.e., when the target feature in a block was from a different feature dimension as the target in the preceding block (Fig. 2*E*). Typically, ED switches take longer and are more difficult than intradimensional switches by requiring the recognition of a new dimension and integrating it in a new attentional set (45), suggesting that VU0453595 particularly benefits the flexible updating and switching of attention sets. This finding in NHPs extends the insights that the M_1-selective ago-PAM BQCA can restore odor-based reversal learning of objects in transgenic mice (30).

Computationally, human and animal studies support the suggestion that an M_1 PAM mechanism might enhance the updating of attention sets. Enhanced learning following ED switches in our task paradigm suggests that the M_1 PAM VU0453595 allowed the animals to more effectively recognize that previously unrewarded, distracting, features became rewarded. The effect of VU0453595 is therefore akin to increasing the effective salience of those targets that were "learned distractors" from the previous block while suppressing the salience of current distractor features (Fig. 1). Recent modeling suggests that increasing effective salience is achieved with an attention–augmentation mechanism that enhances learning from attended features by actively unlearning (forgetting) values of unattended features (46). Various studies have documented that such an attention–augmentation mechanism is important for fast learning in complex tasks like the one used here (46–51). The effect of VU0453595 may thus enhance the effective salience of target features, consistent with neuronal recordings that show M_1 mAChR activation in the prefrontal cortex is necessary during the early processing of targets (52). Support for this suggestion comes from an elegant multitask study in NHPs that found compromising muscarinic activity with scopolamine

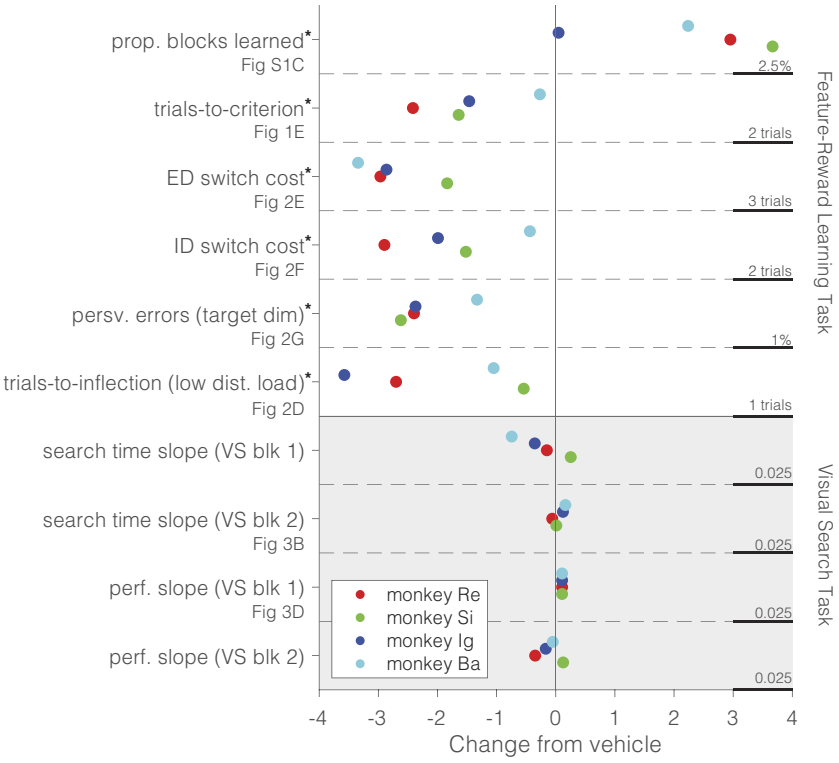

Fig. 4. Results Summary and Consistency across Monkeys. Key results from the feature-reward learning task (*Top*) and the visual search task (*Bottom*). Measures and the respective figures showing each result (if applicable) are stated on the *y*-axis; asterisks indicate a significant effect for all monkeys combined. Values for each monkey represent the average change at the 1 mg/kg VU0453595 dose relative to vehicle scaled arbitrarily for each measure. The scaling for each measure is indicated on the right for 1 arbitrary unit along the *x*-axis. At the 1 mg/kg dose, VU0453595 enhances virtually all measures of the feature-reward learning task to some degree while no reliable changes in the visual search task were observed.

increased the proactive interference of prior spatial information onto current performance in a self-ordered search task (53). The current findings support the interpretation that potentiation of M_1 mAChR activity reduces proactive interference with the net effect of enhanced effective salience.

Recent human studies found that the learning of stimulus-response reward probabilities is enhanced with the AChE inhibitor galantamine (5) and impaired when antagonizing muscarinic receptors with biperiden (54). In a Bayesian framework, these performance improvements were linked specifically to enhanced versus reduced weighting of top-down expectancies and prediction errors during learning (5, 54). In this framework, muscarinic receptor activity determined how fast prediction errors led to belief updating about how stimuli are linked to reward. The results of the current study is consistent with that framework by suggesting that enhanced belief updating and effective salience is mediated specifically through the potentiation of the M_1 mAChR. Supporting this conclusion, in rodents, the M_1-selective ago-PAM BQCA reverses scopolamine-induced deficits in a contextual fear conditioning consistent with M_1 mAChR enhancing the salience of the (aversive) outcomes during learning (22, 24, 25).

These functions of the M_1 mAChR may be realized in the prefrontal cortex. In primates, reversal learning and the extradimensional updating of attentional sets depend on dissociable subareas of the prefrontal cortex with ED shifting and the recognition of attention sets depending particularly on the ventrolateral prefrontal cortex (55, 56). Support for such a prefrontal mechanism comes from a rodent study that found the M_1-selective PAM

Table 2. Comparison of performance metrics with the best doses of VU0453595 and Donepezil

	Extracted Measure	Donepezil (0.3 mg/kg)	VU0453595 (1 mg/kg)
Learning Task	Learning efficiency and performance	↓	↑↑↑
	Cognitive control and flexibility		↑↑↑
Attention Task	Speed of processing	↓	
	Distractor interference	↑↑↓	↑*
	Perceptual interference	↑↓	

From the FRL task and VS task, we extracted five different performance metrics. Learning efficiency and performance entails the number of trials-to-criterion, plateau performance, proportion of learned blocks, response times, and trials-to-inflection for response times. Cognitive control and flexibility entails perseverative error measures and the role of block switches (e.g., ED and ID) on learning efficiency (trials-to-criterion). Speed of processing is a single measure extracted from familiarization trials. Distractor interference entails search time and performance changes as a function of the number of distractors. Perceptual interference entails search time and performance changes as a function of target-distractor similarity.
*no systematic effect of 1 mg/kg of VU0453595 was found in the VS attention task.

TAK-701 can partially reverse a deficit of target detection selectively on signal trials that followed no-signal trials when the deficit was induced by partially (~60%) depleting ACh afferents to the prefrontal cortex (28). These considerations support the notion that M_1 mAChRs in prefrontal cortex are pivotal for the improved updating of attentional sets (2).

In previous work, faster learners of feature-reward associations were shown to have improved working memory capacity (46), which raises the possibility that M_1 mAChR allosteric modulation may have affected learning through enhanced short-term memory of targets. We believe this is unlikely. While the nonselective muscarinic antagonist scopolamine impairs short term memory retention and nonselective AChE inhibitors partially reverse the deficit (57–61), the short-term deficits can be independent of the delay and more prominent for short or intermediate delays, making it unlikely that muscarinic receptors have primary effects on recurrent persistent delay representations (53, 59, 62, 63).

M_1 PAM Reduces Perseverative Responding. A second main result of the current study is VU0453595 reduced response perseveration, allowing animals to avoid repeating erroneous responses to objects with the same nonrewarded features (Fig. 2*E*). This finding supports early insights into the effects of the muscarinic antagonist scopolamine in the prefrontal cortex to increase omissions (64), suggesting that it is the M_1 mAChR that is particularly important for minimizing error rates. Support for the M_1 specificity of these effects also comes from a study treating transgenic mice with an M_1-selective ago-PAM which resulted in reduced erroneous choices of compound object discrimination in the trials after reversing object-reward associations (30).

Perseverative responding is the key characteristic of inflexible, habitual responding because it reflects that performance feedback is not utilized for adjusting behavior. It has been shown that performance feedback triggers transient activation of cholinergic neurons in the basal forebrain in mice (65) and activates the basal forebrain in humans (66). In the prefrontal cortex, cholinergic transients trigger gamma activity (67) that depends specifically on local M_1 mAChRs (52), and thus could be a mechanism underlying improved recognizing feedback that leads to avoiding perseveration in our study.

Taken together, the reduction of perseverative responding with VU0453595 implicates the M_1 mAChRs in the effective processing of feedback to adjust future performance. Perseverative, habitual responding is a hallmark of multiple psychiatric disorders including schizophrenia, obsessive compulsive disorder and substance use disorders (68, 69). The current result therefore bears particular relevance by suggesting that potentiating the M_1 mAChR critically reduces perseverative response tendencies (70).

M_1 PAM Has No Consistent Effect on Interference Control over Distractors. We found that VU0453595 did not affect VS performance differently with few or many distractors. Target detection response times were moderately faster and accuracy was moderately lower to a similar extent for 3, 6, 9, or 12 distractors (Fig. 3 *A*–*D*). This finding shows that the VU0453595 dose range that improved cognitive flexibility did not alter attentional filtering of distracting information. This finding adds clarity to diverse results in previous studies. Firstly, the absence of M_1-specific distractor effects resonates with a recent finding in rodents that the M_1-selective PAM, TAK-071, did not modulate the distracting effects of light on/off switches during a sustained attention task, but started to improve performance in the second half of testing when distraction ended, and the animals adjusted to a no-distractor regime (28). This result pattern is congruent with our result pattern. Allosteric modulation of the M_1 mAChR improved adjusting behavior to challenges, but without improving interference control from distraction. A similar lack of effects of muscarinic modulation on distractor interference control were found in other task contexts. Scopolamine-induced deficits of continuous recognition performance can be partially reversed with an M_1-selective agonist (71) or the nonselective muscarinic agonist milameline (72, 73), but this deficit reversal is independent of the similarity between distracting and target objects (27). Similarly, scopolamine does not alter distractor effects in an attentional flanker task, but rather causes an overall slowing and selective impairment of learning reminiscent of the reward learning effect we found (74).

The observed result pattern with the M_1 PAM VU0453595 contrasts to apparent effects to reduce distraction with nicotinic modulation (12, 44, 75, 76), with nonselective cholinergic increases using donepezil (43) (Table 2), or with the improvement of target detection accuracy and visuospatial attentional orienting when enhancing cholinergic transmission from the basal forebrain (1, 77–79). Particularly relevant in this context is a prior NHP study that found the nicotinic alpha-4/beta-2 receptor agonist selectively enhanced distractor filtering when two stimuli underwent salient changes but had no effect on reversal learning speed (44).

One caveat when interpreting the absence of an effect with the M_1 PAM VU0453595 is that it is unclear whether a higher dose of this ligand would have affected distractor filtering during VS performance. The highest dose used in this study (3 mg/kg, oral) is a magnitude lower than the maximum 30 mg/kg doses that previous studies found to have only mild adverse side effects (80), suggesting that future studies will need to identify possible dose-specific effects on attention functions.

Limitations. While our study already tested multiple markers of cognitive flexibility and attention, it was not yet incorporating tests of other domains that M_1 mAChR modulating ligands might affect and which are compromised in psychiatric patient populations such as long-term memory and motivation (13, 81–83). Further tasks, where we can extract measures for these domains would be important additions for a more comprehensive characterization of possible M_1 mAChR dependent behaviors (*SI Appendix, Supplemental Discussion*). Such an expansion of extracted measures would align well with efforts to develop multi-task batteries for NHPs covering a wide range of cognitive domains (53, 84–87).

Conclusion. In summary, the M_1 PAM VU0453595 produced selective improvements in cognitive flexibility in the absence of adverse side effects. The results were obtained with cognitive tasks that tap into real-world cognitive demands for adjusting to the changing relevance of visual objects. This result pattern suggests that M_1 PAMs will be powerful targets for drug discovery efforts to augment cognitive flexibility.

Methods and Materials

Subjects. Four adult male rhesus macaques (*Macaca mulatta*) were separately given access to a touchscreen Kiosk Station attached to their housing unit where they performed a visual search attention task and a feature-reward learning task (85) (Fig. 1*A*) (*SI Appendix, Supplemental Materials*).

Compounds and Procedures. The scale up of the M_1 PAM VU0453595 used in the present study was synthesized at the Molecular Design and Synthesis Center within the Vanderbilt Institute of Chemical Biology, Vanderbilt University, School of Medicine (30, 33) and mixed with a vehicle of 18 g of

strawberry yogurt and 2 g of honey provided to the monkeys in a small paper cup (oral administration). All monkeys received vehicle or VU0453595 2 h prior to the start of behavioral performance and were observed to ensure full consumption of vehicle or VU0453595. VU0453595 was administered once per week to allow appropriate washout. Based on the weight of each animal, its volume was calculated for 0.3, 1, and 3 mg/kg doses. Side effects were assessed 15 min following VU0453595 administration and after completion of the behavioral performance with a modified Irwin Scale for rating autonomic nervous system functioning (e.g., salivation) and somatomotor system functioning (e.g., posture and unrest) (43, 88–90). Furthermore, monkeys' behavioral status was video-monitored throughout task performance.

The pK for VU0453595 has previously been reported in cynomolgus macaques where peak concentrations at 3 mg/kg dosing occurred ~2 h after oral administration (80). The same study found changes to qEEG spectral power 0 to 4 h after VU0453595 administration in macaques, consistent with the time window for behavioral performance in our study. While changes in qEEG spectral power were also observed in mice, M_1-KO mice did not exhibit these changes. Furthermore, the agonist and PAM activity of VU0453595, through calcium mobilization assays have been previously reported in M_1-expressing CHO cells (22).

Behavioral Paradigms. Monkeys performed a sequence of two tasks in a single behavioral session including an initial VS task block, 21 reward learning task blocks and finally, a second visual task block. Rewarded and unrewarded objects in the VS task and FRL task were multidimensional, 3D rendered objects (named "Quaddles") (91) that shared a variable number of different feature dimensions (colors, shapes, arms and/or body patterns). The VS task varied the perceptual target-distractor similarity by changing the average number of common features between distractors and the target object. The FRL task varied the complexity of the feature space by varying features of objects in only one or two feature dimensions from trial to trial.

At the start of each session, animals performed a VS task block and again at the end of each session, they performed a second VS task block. Each VS block contained an initial ten "familiarization trials" followed by 100 search trials. During the familiarization trials, only the rewarded object was presented on screen, without any distracting objects. The rewarded object was made up of three features of three different feature dimensions. The 10 familiarization trials were followed by a set of 100 search trials, where the rewarded object (learned during the familiarization trials) was always presented amongst 3, 6, 9, or 12 (counter-balanced and randomly selected) distracting objects (Fig. 1*B*, *Bottom*). These distracting objects could each share 0, 1, or 2 of the three features with the target. Animals received fluid reward for touching the learned target object presented during that block's familiarization trials. Each of the two VS blocks in a daily session was accompanied by one of five patterned background images, selected without replacement daily. These images bore no relationship to the target objects and served to cue the animal to the task rule in contrast to the FRL task which contained a neutral gray background.

Between the two VS blocks, the animals performed 21 blocks of the FRL task where they had to identify the single feature value associated with high reward probability (85%). In each block (35 to 60 trials) of the FRL task, animals were required to learn, by trial-and-error, which single feature was associated with the reward. The FRL task indexes cognitive flexibility by testing how fast subjects learn which feature is rewarded when the feature-reward rule switched between blocks. The newly rewarded feature after the uncued block switches could be from the same or from a different feature dimension than the previously rewarded feature. This makes the task similar to attentional set-shifting tasks, but different by using a larger set of features that varied within and across sessions in order to control task difficulty. In each trial, three objects were shown that varied either in the features of one feature dimension (e.g., each object having different colors or body shapes), or that varied in features of two feature dimensions (e.g., each object having different colors and body shapes). Thus, in a single trial, no two objects contained any overlap in the presented features (Fig. 1*B*, *Top*). Choosing the object with the correct feature was rewarded with a probability of 0.85. Blocks where only 1 feature dimension varied (low distractor load) were easier as there were less distracting features than in blocks with two varying feature dimensions (high distractor load). Blocks switched after the completion of a minimum of 35, 40, or 45 trials (random jittering) if a performance threshold of $\geq$ 80% in the previous 10 trials was reached. Otherwise, blocks would switch after the completion of 60 trials.

Statistical Analysis. Data were analyzed with standard nonparametric and parametric tests with test statistics, *P* values, and effect sizes reported where appropriate in text. For detailed statistical methods, please see the *SI Appendix*.

Financial Disclosures. The authors declare no competing financial interests.

Data, Materials, and Software Availability. .mat and .m files data have been deposited in Github (https://github.com/att-circ-contrl/PNAS_VU595_code-data) (92).

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Supplemental Information for

M_1 selective muscarinic allosteric modulation enhances cognitive flexibility and effective salience in nonhuman primates

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Content:

Supplemental Materials and Methods

Subjects

All animal related experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the Society for Neuroscience Guidelines and Policies, and approved by Vanderbilt University Institutional Animal Care and Use Committee.

Four pair-housed adult male rhesus macaques (*Macaca mulatta*), 7-11 years old and weighing ~8-15 kg were subjects in this experiment. Monkeys in each pair were separately given access to a cage-mounted Kiosk Station attached to their housing unit uniformly at either 11am (monkeys Ig and Ba) or at 1pm (monkeys Re and Si). Each monkey was overtrained and engaged with and completed a visual search attention task and a flexible feature-reward learning task via a touchscreen interface (1) with the software being controlled by the Unified Suite for Experiments (USE) (2).

Of the four monkeys, two (monkeys Si and Ig) had previously been involved in a similar study utilizing the acetylcholinesterase inhibitor donepezil (3) with over 6 months between experiments for washout. Prior to the donepezil experiments, monkey Ig had also previously been exposed to a

different experimental M_1 PAM. Monkeys Ba and Re were naïve to VU0453595 and other neurological or psychiatric medications.

Comparison table

The comparison table (Table 1) between the best dose of donepezil and VU0453595 was based on the data collected in a previous study (3) but for all measures, identical methods were applied to both datasets consistent to what is described here.

Statistics

Within the feature-reward learning (FRL) task, we use trials-to-criterion to quantify learning efficiency with the criterion being defined as the first trial after at least 1 error which preceded a string of 10 trials with 70% or greater performance. Note that 70% trials-to-criterion measure is different from the backward-looking threshold of 80% which determined the switching of blocks during FRL task performance. The 70% performance threshold is different from our previous work (3) which was set to 80% performance, which was and still is the criterion for block switches in the FLR task. We found this new threshold to better reflect the occurrence of learning and led to only a mean 0.37 (0 median) trial difference in baseline trials-to-criterion overall. The comparisons between VU0453595 and donepezil were made using the same definition for each measure.

Block switches in the FRL task were labelled based on the status of the target feature relative to the previous block as extra-dimensional, intra-dimensional or as involving a novel target feature dimension. For novel target blocks, the rewarded feature dimension was not present in the previous block, independent of the present or previous block's dimensionality. Similarly, intra-dimensional shift blocks involved the same rewarded feature dimension but a different rewarded feature (e.g. a different color) as the previous block, independent of their dimensionality. However, for extra-dimensional shift blocks, the previous block must have been a high load (objects varying in 2 feature dimensions) block where the current block's target feature was from the previous one's distracting feature dimension. Extra-dimensional shift blocks themselves could be either low or high distractor load.

In the FRL task, for each session, reaction times (RTs) were averaged and smoothed using a 5 trial shifting window for low and high distractor load blocks separately (**Fig. 2A,B**). We then defined the time to plateau as the first trial per session, excluding trial two, where the RTs began to decrease.

Perseverative errors were quantified based on the features of the erroneously chosen object. The consecutive errors could be made with objects containing the same feature from the distracting or target feature dimensions. The proportion of perseverative errors are reported as a percentage of all errors (**Fig. 2G,H**).

In order to account for temporally specific effects on learning efficiency with VU0453595, as seen with other cholinergic compounds (3), we applied a linear mixed effects model (LMEM) to the trials-to-criterion. The LMEM had three main effects: experimental condition, distractor load and temporal bin (thirds), while individual monkeys were treated as random effects:

$$Trial\ to\ criterion = ExpCond \times DistractorLoad \times TemporalThirds + (1|Monkey) + b + \varepsilon$$

Given the results of the LMEM and the maximal effect size with the first third of blocks in the FRL (**Fig. S1A**), all analyses for the FRL task used only the first third of blocks to capture the period where VU0453595 had its strongest effect on performance.

Effect sizes were reported as either eta squared values when referring to ANOVA results or Cohen's d when appropriate (i.e. when post-hoc analysis showed a significant effect at a single dose). The Cohen's d was computed by directly comparing vehicle to the significant dose using this formula:

$$d = \frac{M_2 - M_1}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}}$$

Supplemental Results

Feature-reward learning task

After reaching performance criterion, VU0453595 also resulted in higher plateau accuracy (compound condition main effect: ($F(3,1672) = 3.22$, $p = .02$; $\eta^2 = .005$); low distractor load accuracy: 90.9% (SE: 1.9%); 95.9% (SE: 0.9%); 90.9% (SE: 1.8%); 91.0% (SE: 0.7%) for 0.3, 1, 3 mg/kg and vehicle respectively; high distractor load accuracy: 74.4% (SE: 3.0%); 80.5% (SE: 2.9%); 84.8% (SE: 2.3%); 77.9% (SE: 1.1%) for 0.3. 1, 3 mg/kg and vehicle respectively) (**Fig. S1B**). The middle dose of VU0453595 (1 mg/kg) also increased the proportion of blocks in which animals reached the learning criterion of 70% over the subsequent 10 trials using a forward looking 10 trial window ($F(3,1369) = 2.93$, $p = .03$; $\eta^2 = .006$; Tukey's, $p = .02$). Animals reached the learning criterion of 70% accuracy over 10 successive trials in 90.5% (SE: 2.4; low load) and 72.1% (SE: 3.7%; high load) of blocks in the vehicle condition. Tukey's HSD multiple comparisons test among proportions revealed that at the 1 mg/kg dose, VU0453595 significantly increased the proportion of learned blocks in the low load condition to 98.7% (SE: 2.6%) ($p = .04$) (**Fig. S1C**).

Visual search task

In the first VS block, target detection times across distractor conditions were not different with VU0453595 relative to vehicle control ($F(3,944) = 1.67$, n.s.; $\eta^2 = .004$), with the exception of faster target detection times in the second VS block at the 3 mg/kg dose (experimental condition main effect: $F(3,944) = 3.67$, $p = .01$; $\eta^2 = .008$; Tukey's, $p < .05$). With regards to performance, in the VS block at the end of the session there were no significant effects, while in the first VS block there was a significant main effect of compound ($F(3,944) = 3.80$, $p = .01$; $\eta^2 = .010$) with reduced accuracy at 3 mg/kg dose (Tukey's, $p = .04$) irrespective of the number of distractors.

Despite the lack of set size effects, the raw target detection times were overall significantly faster with the 1 mg/kg dose in the second block ($F(3,708) = 4.67$, $p = .003$; $\eta^2 = .018$; Tukey's, $p = .02$) with more improvement with high target-distractor similarity (cohen's $d = -.447$) than low target-

distractor similarity (cohen's d = -.427) (**Fig. 3E**). There was also a general reduction in performance during the first block ($F(3,708) = 2.84$, $p = 0.04$; $\eta^2 = 0.011$) (**Fig. 3F**). We also tested if there was a speed-accuracy trade-off at any dose of VU0453595 during either the first or second VS block. We found no significant changes in the speed-accuracy relationship between vehicle and any of the administered doses of VU0453595 (**Figure S2**).

Characterization of adverse cholinergic side effects

Each subject was observed during their consumption of VU0453595 stirred into a strawberry yogurt and honey vehicle (20 g total) placed in a small paper cup. Of the 4 subjects, 1 placed the entire cup immediately in their mouth while the other 3 consumed all of the paper cup's content before either ripping it and licking it clean or eating parts of the paper cup alongside the yogurt. None of the subjects had any day where they spilled an observable volume of yogurt. A modified Irwin test, which measures cholinergic effects on the autonomic and somatomotor systems, was applied to all subjects twice daily (**Table S1**). Subjects were observed throughout the experiment through a video monitoring system (**Figure 1A**) and formally evaluated for the modified Irwin test once, ~110 minutes after administration (immediately before start of task), and a second time, immediately after subjects finished all behavioral tasks for their session (< 2 hours after the first assessment). Ratings of 0, 1 or 2 were assigned to each item on the test reflecting no change, a slight change, or a significant change respectively. During the first 1 mg/kg dose of VU0453595, 4/4 monkeys experienced slight changes in arousal while 2/4 monkeys experienced slight unrest and 1/4 experienced a slight increase in yawning. No other symptoms were observed beyond the first 1 mg/kg dosing. At the 3 mg/kg dose, 4/4 monkeys experienced slight unrest, 3/4 monkeys experienced slight changes in arousal and a single monkey experienced vasodilation (redness of the face). Most of the symptoms observed at the 3 mg/kg dose also occurred during the first 3 mg/kg dosing event. Only a single monkey had any symptoms post-task completion, once at the 1 mg/kg dose and once at the 3 mg/kg dose, both instances involved a slight change in arousal. No changes were observed at the 0.3 mg/kg dose.

Supplemental Discussion

M_1 PAM literature review

We compiled an exhaustive summary of M_1 PAM NHP (4–9) and rodent papers (4, 7, 10–35) to the best of our knowledge (**Table S2 & S3** respectively) and identified the tasks they utilized and which cognitive domains their extracted measures were informative of. A large majority of all of the identified papers contain some pharmacological challenge (i.e. scopolamine, amphetamine, PCP, haloperidol etc) in order to demonstrate the efficacy of the candidate M_1 PAM (5/6 NHP papers and 16/28 rodent papers; of the remaining rodent papers 7 involved a genetic disease model and another 4 contained some lesion or prion component). In contrast, 0/6 NHP studies and 4/28 rodent studies used varying task parameters in order to manipulate cognitive demand for pharmacological testing. One potential reason for this is the relatively simple, and easy-to-train, design of the commonly used behavioral tasks, where over trained animals have near ceiling performance. Such tasks also suffer from being only informative of a single cognitive domain and require modification for further utility. For example, the Morris water maze was used in 8 rodent M_1 PAM studies to assay learning and memory, however, the addition of a reversal component allowed for measures of cognitive flexibility and a dissociation of the impact of VU0486846 in

one study (36; **see Table S3**). Both **Tables S2** (37–48) and **S3** (41, 49–51) also include a select few non-M_1 PAM cholinergic drug studies. Although focusing on the symptoms of diseases of interest is an attractive approach for identifying the efficacy of a candidate compound, it is important to dissociate its impact on multiple cognitive domains in order to identify differences in optimal dosing and potential cognitive trade-offs (e.g. 3, 19).

Possible M_1 agonism

Although *in vitro* data suggests little to no agonistic properties of VU0453595 (21), we cannot completely rule out the possibility that the inverted-U shaped responses observed with this compound may be due, in part, to ago-PAM activity at the highest dose tested *in vivo*. This would suggest that the endogenous signaling at the M_1 mAChR supporting cognitive flexibility is sensitive to exogenous intervention. The possible agonism of M_1 PAMs such as VU0453595 *in vivo* will be the subject of future studies.

Possible contributions of M_1 potentiation of memory or motivation/effort control

The current study dissociated the relative importance of an M_1 PAM (VU0453595) for cognitive flexibility and attentional filtering and contrasted these effects to those of donepezil (**Table 1**). The functional dissociation of the effects highlights the importance of a multi-task paradigm for understanding ligand actions on behavior (3, 45, 52) and supports efforts to develop multi-task batteries covering a wide range of cognitive domains (1, 45, 52–54). While our study tested already multiple markers of cognitive flexibility and attention, it was not yet incorporating tests of domains that M_1 modulating compounds might also affect. For example, scopolamine challenges have long suggested that M_1 mAChRs in the medial temporal lobe support longer-term memory processes (55–57), making it possible that M_1 mAChR modulation might have positive consequences in this domain.

Motivation and the ability to control effort are other domains that we did not test and which some studies have suggested to be modulated by mAChRs. The task we deployed varied cognitive load which inevitably increases difficulty and the amount of effort subjects needed to exert. Although we did not control for motivational factors explicitly, visual inspection suggested it was not modulated by VU0453595 because the learning improvements were somewhat more pronounced at lower than higher load in the learning task and did not vary with increasing distractor difficulty (target-distractor similarity) in the search task. These findings resonate with the results of a scopolamine challenge study in NHP that found no effects of increasing difficulty in a stimulus-location association learning task (58). However, when testing for a memory load effect with a visuo-spatial paired associate task, Taffe and colleagues (59) found that scopolamine reduced performance particularly when 3 or 4 stimulus-object associations needed to be learned and retrieved but not when 1 or 2 associations were involved. Such a memory load differs from the cognitive load that we imposed by increasing the number of distracting features in the learning task and from the perceptual load that we varied with increasing target distractor similarity. However, it will be important to identify in future studies which motivation or load dependent processes are modulated specifically by M_1 selective mAChR modulation.

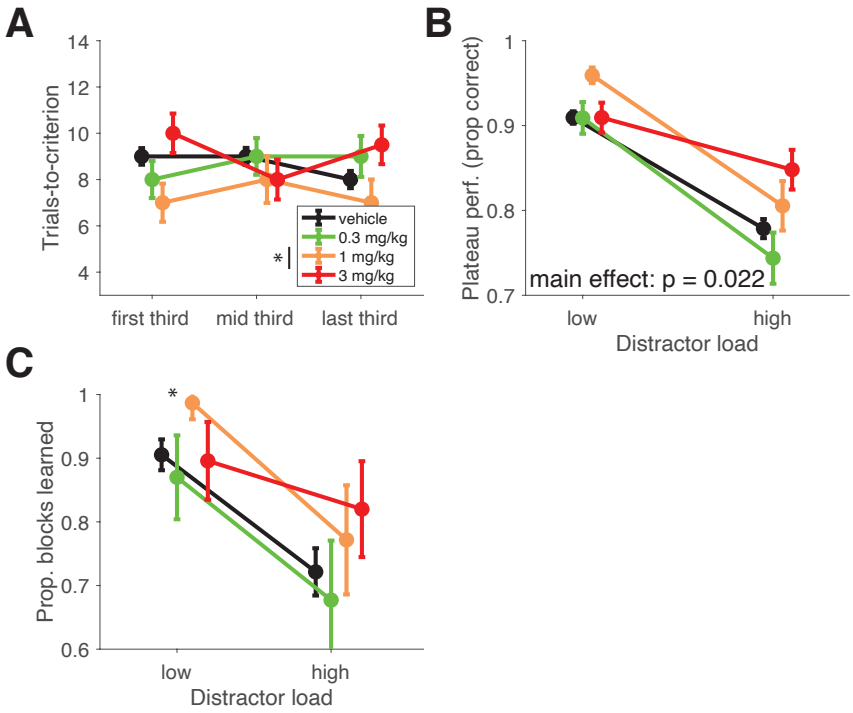

Figure S1. VU0453595 enhances multiple measures of learning performance (**A**) The median trials-to-criterion, visually combined for low and high distractor load conditions temporally split by their presentation within a session (7 blocks in each third) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595. The LMEM used the experimental condition, temporal bin (thirds) and distractor load as fixed effects. There was significantly faster learning with 1 mg/kg which showed the strongest effect size during the first third of FRL blocks (1 mg/kg fixed effect: $t(3674) = -2.67$, $p = .008$; first third Cohen's $d = -.228$; overall Cohen's $d = -.061$). (**B**) Average performance in the final 10 trials of low and high distractor load blocks of the FRL task. For the low distractor load blocks, plateau performance was 90.95% (SE: 0.73), 90.90% (SE: 1.86), 95.92% (SE: 0.92) and 90.94% (SE: 1.76) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. For the high distractor load blocks, plateau performance was 77.86% (SE: 1.12), 74.37% (SE: 3.01), 80.54% (SE: 2.91) and 84.80% (SE: 2.34) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. There was a significant main effect of experimental condition ($F(3,1672) = 3.22$, $p = .022$; $\eta^2 = .005$) but post hoc analysis (Tukey's) showed no single dose as significantly different from vehicle. (**C**) Average proportion of learned blocks (defined as blocks that reached the 70% performance over 10 trials; the same measure as trials-to-criterion) per session in the FRL task. For the low distractor load blocks, the proportion of blocks learned was 90.54% (SE: 2.42), 87.00% (SE: 6.59), 98.68% (SE: 2.56) and 89.58% (SE: 6.11) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. For the high distractor load blocks, the proportion of blocks learned was 72.14% (SE: 3.71), 67.71% (SE: 9.35), 77.17% (SE: 8.58) and 82.00% (SE: 7.53) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. Pair-wise comparisons between the VU0453595 doses and vehicle revealed a significant improvement at the low distractor load with the 1 mg/kg dose (Tukey's multiple comparison test among proportions: $q = 4.082$, $q_{crit} = 3.633$) and no significant changes at the high distractor load (Tukey's multiple comparison test among proportions, n.s.).

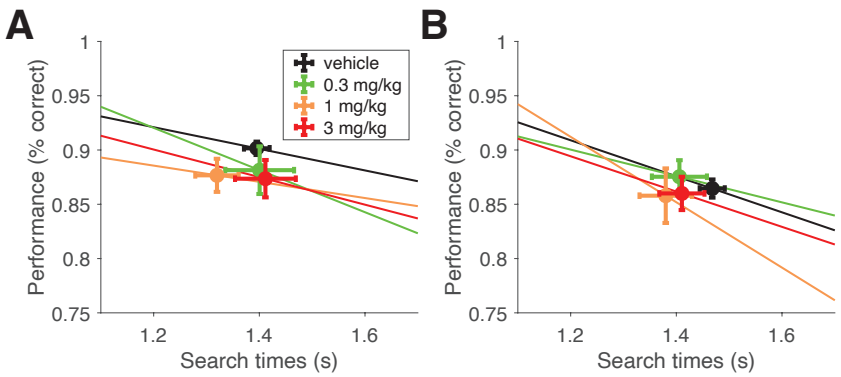

Figure S2. VU0453595 does not change the speed-accuracy trade-off in the visual search task. (**A**) The mean search times and block performance across all of the first VS blocks. The lines represent the linear relationship between the search times and performance for these blocks. No significant difference in the relationship between performance and search times was observed (all n.s.; fisher r to z transformation). (**B**) Same as A but for the second VS block. No significant differences were observed in the relationship between performance and search times (all n.s.; fisher r to z transformation).

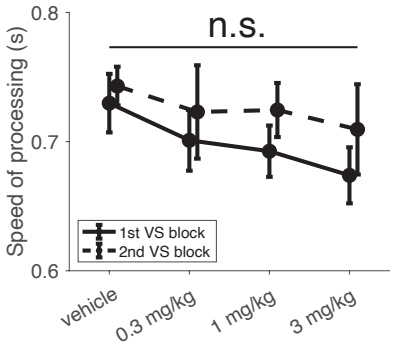

Figure S3. VU0453595 does not impact the speed of processing. The speed of processing for the first and second VS blocks, defined as the time animals took to touch the only object on screen (during familiarization trials). In the first VS block, speed of processing was 0.730 s (SE: 0.023), 0.701 s (SE: 0.024), 0.693 s (SE: 0.020) and 0.674 s (SE: 0.022) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. In the second VS block, speed of processing was 0.743 s (SE: 0.015), 0.723 s (SE: 0.036), 0.725 s (SE: 0.021) and 0.710 s (SE: 0.035) for vehicle, 0.3, 1 and 3 mg/kg doses of VU0453595 respectively. No significant changes were observed for the first ($F(3,236) = .56$, n.s.) or second VS blocks ($F(3,236) = .35$, n.s.).

	VU0453595	0.3 mg/kg		1 mg/kg		3 mg/kg	
	Observation	Pre-task	Post-task	Pre-task	Post-task	Pre-task	Post-task
Autonomic Nervous System	Salivation	-	-	-	-	-	-
	Lacrimation	-	-	-	-	-	-
	Urination	-	-	-	-	-	-
	Defecation (amount)	-	-	-	-	-	-
	Defecation (consistency)	-	-	-	-	-	-
	Emesis	-	-	-	-	-	-
	Miosis	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-
	Exophtalmos	-	-	-	-	-	-
	Piloerection	-	-	-	-	-	-
	Respiratory Rate	-	-	-	-	-	-
	Yawn	-	-	+	-	-	-
	Vasodilation	-	-	-	-	+	-
	Vasoconstriction	-	-	-	-	-	-
	Irritability	-	-	-	-	-	-
	Body Temp.	-	-	-	-	-	-
Somatomotor Systems	Physical Appearance	-	-	-	-	-	-
	Tremor	-	-	-	-	-	-
	Leg Weakness	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-
	Visuo-Motor Coordination	-	-	-	-	-	-
	Posture	-	-	-	-	-	-
	Unrest	-	-	+	-	+	-
	Stereotypies	-	-	-	-	-	-
	Arousal	-	-	+	+	+	+
	Sedation	-	-	-	-	-	-
	Oral Dyskinesia	-	-	-	-	-	-
	Bradykinesia	-	-	-	-	-	-
	Dystonia	-	-	-	-	-	-

Table S1. A summary of observed dose-limiting side effects. The effect of VU0453595 (0.3, 1 and 3 mg/kg PO) on autonomic and somatomotor system function were evaluated. The mean score of 4 monkeys was classified as follows: - no effect; + 0-0.15; ++ 0.16-0.3; +++ 0.31-0.45

Table S2. Summary of NHP studies with compounds acting at the M_1 receptor. Light gray: sample studies without an M_1 PAM; white: studies using M_1 PAMs; dark gray: summary. Background color on tasks defines their cognitive domain they pertain to. White: no task; green: working memory; blue: learning and memory; purple: executive functioning; orange: attention and/or vigilance; cyan: social; yellow: motor control or locomotion; red: motivation.

Reference	Sample/Subject Info	Ligands & Doses	Reported Adverse Effects	Task(s)	Challenge	Number of Determinations	Cognitive Domain(s)	Positive Results
Glick & Jarvik 1970	8 rhesus macaques	Muscarinic antagonist (Scopolamine): 0.025, 0.05, 0.1 mg/kg (im)	None reported	1. Delayed match to sample task	Behavioral (delay)	At least 2 per dose	Working memory	All doses reduced accuracy and response frequency
Bartus & Johnson 1976	8 rhesus macaques (m; adolescent)	Muscarinic antagonist (Scopolamine): 0.03, 0.04, 0.05 mg/kg (im)	Ptosis, pupil dilation	1. Delayed match to sample task	Behavioral (delay)	1 per dose	Working memory	Severe disruption of performance
Buccafusco et al., 2003	12 rhesus macaques (m/f; >20 y.o)	AChE-I (Donepezil): 0.01, 0.025, 0.05, 0.1 mg/kg (im)	None reported	1. Delayed match to sample	Behavioral (delay)	1 per dose	Working memory	Enhanced accuracy @ 0.025mg/kg (m) and @ 0.1mg/kg (f)
Buccafusco & Terry 2004	17 rhesus macaques (m/f; 9-29 y.o.)	AChE-I (Donepezil): 10, 25, 50, 100 ug/kg (im)	None reported	1. Delayed match to sample	Behavioral (delay)	1 per dose	Working memory	Enhanced accuracy during medium & long delays @ 25ug/kg
Buccafusco et al., 2008	32 rhesus macaques (m; avg. 18.8 y.o.)	AChE-I (Donepezil): 5, 10, 25, 50, 100, 125 ug/kg (im & po)	None reported	1. Delayed match to sample	Scop.	1, 4, 4, 2, 1, 1 per dose respectively	Working memory	Partial rescue @ 50ug/kg (po)
Rupniak et al., 1997	9 rhesus macaques (m; young adult)	AChE-I (Donepezil): 0.003, 0.01, 0.03, 0.04, 0.05, 0.06, 0.1, 0.5, 1, 1.75 mg/kg (im)	Tremors, jerking, retching, mouth movements, salivation, pallor & lethargy @ 2 mg/kg	1. Spatial delayed response task	Scop.	1 per dose	Working memory	Partial rescue @ 0.5, 1 & 1.75 mg/kg
				2. Visual recognition task	None	1 per dose	Attention/vigilance	Pre-treatment enhanced performance @ 0.03 (best) & 0.05 mg/kg
Oliveira et al., 2021	25 (5 per treatment group) black tufted-ear	AChE-I (Donepezil): 0.5 mg/kg (sc)	None reported	1. Novel object recognition task	A: Scop. B: MK-801 challenge	1 per dose	Learning and memory	A: rescue of novel object exploration preference. B: no effect

	marmosets (f/m; 4.5-8 y.o.)				(NMDA antagonist)			
Tsukada et al., 2004	10 rhesus macaques (m; half ~5 & half ~20 y.o.)	AChE-I (Donepezil): 50, 250 ug/kg (iv)	None reported	1. Oculomotor delayed response task	Monkey age	1 per dose	Working memory	Partial rescue @ both 50 and 250 ug/kg in old monkeys. Trend in young monkeys (n.s.)
				2. Visually guided saccade task	Monkey age	1 per dose	Motor control; attention/vigilance	No effect
Taffe et al., 1999	6 rhesus macaques (m; ~4 y.o.)	Muscarinic antagonist (Scopolamine): 3, 10, 14, 17, 24 ug/kg (im)	None reported	1. Delayed non-match to sample	Behavior (delay)	Tasks 1-5: 1 per dose for 4 separate doses *all animals were dosed at 3, 10 & 17 ug/kg. 3 of the animals also received 24 ug/kg dose while the other 3 received a 14 ug/kg dose	Working memory	Reduced accuracy (no delay interval interaction)
				2. Self-ordered spatial task	Behavior (number of objects)		Working memory; attention/vigilance	Reduced accuracy (with interaction)
				3. Reaction time	RT		Speed of processing; attention/vigilance	Increased movement time only @ 14 &17ug/kg
				4. Progressive ratio task	Behavior (satiation)		Motivation	Reduced reinforcer acquired @ 14, 17, 24ug/kg
				5. Bimanual motor task	RT		Motor control	Increased latency @ 14, 17 & 24ug/kg
Knakker et al., 2021	6 rhesus macaques (m; ~5 y.o.)	AChE-I (Donepezil): 100, 200 ug/kg (im)	None reported	1. Delayed match to sample task	Scop.	1 per dose	Working memory	Partial reversal during medium delay @ 200 ug/kg
Callahan 1999	6 rhesus macaques (m/f; 12+ y.o.)	AChE-I (Tacrine): 0.03, 0.1, 0.32, 1 mg/kg (im) Muscarinic agonist (Milameline): 0.001, 0.003, 0.01 mg/kg (im)	None reported	1. Continuous performance task	Scop.	1 per dose	Attention/vigilance	Partial to full rescue of response omissions @ 0.32 mg/kg Tacrine. Partial rescue of response omissions @ 0.003 or 0.01 mg/kg milameline. Partial rescue @ tested combinations with stronger effect than the same dose of either ligand alone
Callahan et al., 2013	7 rhesus macaques (m/f; aged)	AChE-I (Donepezil): 0.003, 0.01, 0.025, 0.05, 0.1, 0.2 mg/kg (po)	None reported	1. Delayed match to sample task	Behavioral (delay)	6-7 dosing events total	Working memory	Accuracy enhancement with donepezil @ 0.01, 0.025, 0.05, 0.1 & 0.2 mg/kg. Accuracy enhancement with donepezil (0.003 mg/kg) + PNU-120596 @ 3 & 10 mg/kg (PNU-120596 alone did not show result in significant improvement)

		Nicotinic (α7) PAM (PNU-120596): 1, 3, 10 mg/kg (po)						
Gould et al., 2020	8 cynomolgus macaques (m; 4-8 y.o.)	M_1 PAM (VU0453595): 3, 10, 30 mg/kg (ig) M_1/M_4 agonist (Xanomeline): 1, 3 mg/kg (sc) AChE-I (Donepezil): 3, 10 mg/kg (po)	Increased urination, reduced respiration, changes in posture, motor coordination, leg weakness @ 30 mg/kg VU0453595	None (qEEG study)	N/A	1 per dose	N/A	VU0453595: Beta power (18-30Hz) increased @ 30mg/kg, gamma power (30-50Hz) increased @ 10 & 30mg/kg
Kurimoto et al., 2019	4 (per group) cynomolgus macaques (m; 3-5 y.o.)	M_1 PAM (TAK-071): 0.3, 1, 3 mg/kg (po) AChE-I (Donepezil): 0.3, 3 mg/kg (po) M_1/M_4 agonist (Xanomeline): 1 mg/kg (sc)	None reported	None (qEEG study)	Scop. (increased delta, theta and alpha power)	1 per dose	N/A	TAK-071: alone it decreased alpha power @ 3 mg/kg and theta + alpha power @ 1 mg/kg. It lead to partial rescue of alpha power @ 1 mg/kg (delta lowered but n.s.). It also lead to partial rescue of alpha and delta power @ 3 mg/kg (delta lowered but n.s.) Donepezil: alone it increased alpha power @ 0.3 mg/kg. It also lead to partial rescue of delta, theta and alpha power. Xanomeline: partial rescue of delta, theta and alpha power (all trends; n.s.).
Uslaner et al., 2013	6 rhesus macaques (m; 4-6 y.o.) 6 cynomolgus macaques (f; ~15 y.o.)	AChE-I (Donepezil): 0.3 mg/kg (po) M_1 PAM (PQCA): 3, 10, 30 mg/kg (po)	None reported	1. Object retrieval detour task	Scop.	1 per dose	Reasoning & problem solving (exec. functioning)	Partial rescue @ 10 & 30mg/kg PQCA
				2. Self-ordered spatial task	Scop.	1 per dose	Working memory	Partial rescue @ 10mg/kg PQCA & 0.3mg/kg donepezil
Uslaner et al., 2018	8 rhesus macaques (m; adult)	M_1 PAM (MK-7622): 0.1, 0.3, 1 mg/kg (po)	None reported	1. Object retrieval detour task	Scop.	1 per dose	Reasoning & problem solving (exec. functioning)	Partial rescue @ 0.3 & 1 mg/kg
Lange et al., 2015	18 rhesus macaques (m; adult)	M_1 PAM (PQCA): 0.3, 1, 3, 10, 30 mg/kg AChE-I (Donepezil): 0.1, 0.25, 0.3, 1, 3 mg/kg	None reported	1. Paired-associates learning task	Scop.	1 per dose (po)	Working memory	Partial rescue @ 10 & 30 mg /kg PQCA (po)
				2. Continuous performance task	Scop.	1 per dose (im)	Attention/vigilance & impulsivity (exec. functioning)	Partial rescue @ 0.3 & 1 mg/kg PQCA (im)

Vardigan et al., 2015	8-12 rhesus macaques (m/f)	M_1 PAM (PQCA): 3, 30, 50 mg/kg (po) M_1/M_4 agonist (Xanomeline): 0.03, 0.1, 0.3 mg/kg (im) AChE-I (Donepezil): 0.3, 0.56, 1, 1.8, 3, 5 mg/kg (po)	Increase in feces (weight) @ 5 mg/kg donepezil & 0.3 mg/kg xanomeline. Increase severity of salivation & emesis @ 0.3 mg/kg xanomeline	1. Object retrieval detour task	Scop.	1 per dose	Reasoning & problem solving (exec. functioning)	Partial rescue @ 1, 1,.8, 3 mg/kg donepezil & 0.1 mg/kg xanomeline; partial rescue also @ 0.3 mg/kg donepezil + 3 mg/kg PQCA
Current study	4 rhesus macaques (m; 7-11 y.o.)	M_1 PAM (VU0453595): 0.03, 0.1, 0.3 mg/kg (po)	None reported	1. Feature-reward learning task	Distractor load	7 per dose	Learning and memory; cognitive flexibility (exec. functioning)	Better performance, faster learning and less perseverations @ 0.1 mg/kg
				2. Visual search	Distractor interference & perceptual interference	7 per dose	Speed of processing; attention/vigilance; working memory	No reliable change
Total studies utilizing PAMs: 6	4-18 monkeys with a median of 8	M_1 PAMs: -VU0453595 (1/6) -TAK-071 (1/6) -MK-7622 (1/6) -PQCA (3/6)	Reported for PAMs (VU0453595) and other cholinergic agents (donepezil, xanomeline)	2/6 tasks had no behavioral component 2/6 had 2 tasks 2/6 had only 1 task	Studies with behavior and even 1 of the 2 studies without behavior used a scopolamine challenge.	Average number of determinations: 1 (6/6 had 1 determination per dose)	Cognitive domains tested: - Reasoning & problem solving (3/4) -Working memory (2/4) -Attention/ Vigilance (2/4)	Rescue of scopolamine challenged behavior in all cases with least 1 dose of the tested PAM.

Table S3. Summary of rodent studies with compounds acting at the M_1 receptor. Light gray: sample studies without an M_1 PAM; white: studies using M_1 PAMs; dark gray: summary. Background color on tasks defines their cognitive domain they pertain to. White: no task; green: working memory; blue: learning and memory; purple: executive functioning; orange: attention and/or vigilance; cyan: social; yellow: motor control or locomotion; red: motivation/drug abuse.

Reference	Sample/Subject Info	Ligands & Doses	Reported Adverse Effects	Task(s)	Challenge	Number of Determinations	Cognitive Domain(s)	Positive Results
Buccafusco et al., 2008	Albino Wistar and Long-Evans rats (m; 2-3 m.o.)	AChE-I (Donepezil): 1, 2 mg/kg (sc)	None reported	1. Morris water maze	Scop.	1 per dose	Learning and memory	Partial rescue @ 2mg/kg
				2. Delayed stimulus discrimination task	Scop.	1 per dose	Working memory	Partial rescue @ 1mg/kg
Lebois et al., 2017	5XFAD mice (6 m.o.)	M_1 agonist (VU0364572): 10 mg/kg (in drinking water)	None reported	1. Morris water maze	Mice with elevated beta amyloid	10 mg/kg daily dosing for 4 months (from 2-6 m.o.)	Learning and memory	Partial rescue after 4 months of chronic dosing
Digby et al., 2012	Sprague-Dawley rats (m)	M_1 allosteric agonist (VU0364572): 0.03, 0.056, 0.1, 0.3, 0.56 mg/kg (ip) M_1 allosteric agonist (VU0357017): 0.03, 0.1, 0.3, 0.56, 1, 3 mg/kg (ip)	None reported	1. Morris water maze	None	5 per dose	Learning and memory	Enhanced performance (swim distance) with VU0364572 @ 0.1 (best) & 10 mg/kg.
				2. Contextual fear conditioning	None	1 per dose	Learning and memory	Better acquisition of contextual fear with VU0364572 @ 0.056, 0.3 & 0.56 mg/kg as well as VU0357017 @ 0.1, 0.3, 0.56, 1 & 3 mg/kg
Xiong et al., 2019	C57BL/6 mice	M_1 agonist (77-LH-28-1): 5 ul of 5 uM via cannula (intra-cerebroventricular)	None reported	1. Morris water maze	GluA2 mutated mice	3 per dose	Learning and memory	Better (faster) reversal performance. No enhancement observed in GluA2 mutated mice.
Gould et al., 2020	Sprague-Dawley rats (4-6 m.o.) 10 C57BL/6NTac mice (m; 22-26 m.o.)	M_1 PAM (VU0453595): 3, 10, 30 mg/kg (ip) M_1 PAM (BQCA): 3, 10, 30 mg/kg (sc) AChE-I (Donepezil): 1, 3, 10 mg/kg (ip)	None reported	None (sleep/wake study)	N/A	N/A	N/A	BQCA increased duration awake and reduced REM and NREM sleep

	6 M_1 KO (m; 4-6 m.o.)							
Uslaner et al., 2013	Wistar Hannover rats (m)	AChE-I (Donepezil): 1.8 mg/kg (ip) M_1 PAM (PQCA): 3, 10, 30 mg/kg (ip)	None reported	1. Novel object recognition	Scop.	1 per dose	Working memory	Partial rescue @ 10mg/kg PQCA & 3mg/kg donepezil
Chambon et al., 2011	Sprague-Dawley rats (m; adult)	M_1 PAM (BQCA): 5, 10 mg/kg (ip)	None reported	1. Novel object recognition task	Behavioral	1 per dose	Learning and memory	Retained familiar object memory @ 72h delay with BQCA 10 mg/kg (but not with vehicle or BQCA 5 mg/kg)
Chambon et al., 2012	Sprague-Dawley rats (m; adult)	M_1 PAM (BQCA): 10 mg/kg (ip) M_1/M_3 agonist (Cevimeline): 1, 3 mg/kg (po)	Cevimeline increased salivation. None reported for BQCA.	1. Spontaneous alternation task	Scop.	1 per dose	Working memory	Partial rescue of alternations & trials completed @ 10 mg/kg BQCA
Bradley et al., 2017	Tg37 hemizygous mice	M_1 PAM (BQCA): 15, 20, 30 mg/kg (ip) M_1 PAM (BQZ-12): 1.5, 5 mg/kg (ip) AChE-I (Donepezil): 0.5, 1, 2.5 mg/kg (ip) M_1/M_4 agonist (Xanomeline): 5, 10, 15, 30 mg/kg (ip)	None reported for BQCA (up to 30 mg/kg) or BQZ-12 (up to 5 mg/kg). Donepezil (1 & 2.5 mg/kg) & Xanomeline (10-30 mg/kg): piloerection, squinting, subdued and hunched posture. Donepezil also resulted in impaired mobility, laboured respiration, ataxia and paralysis.	1. Contextual fear conditioning	Prion disease	1 per dose	Learning and memory	Donepezil (0.5 mg/kg), Xanomeline (5 mg/kg) (controls), BQCA (15 mg/kg) & BQZ-12 (1.5 mg/kg) fully rescued behavior when administered before conditioning. Daily BQCA (15 mg/kg) treatment prolonged survival.
Dwomoh et al., 2022	Tg37 hemizygous mice	M_1 PAM (VU0486846): 10 mg/kg	None reported	1. Contextual fear conditioning	Prion disease	1 per dose	Learning and memory	VU0486846 (10 mg/kg) fully rescued behavior when administered before conditioning.

								Daily VU0486846 treatment resulted in slower disease progression and prolonged survival in some animals
Rook et al., 2018	Sprague-Dawley rats (m) *C57BL/6 mice (m; 7-8 w.o.) used for adverse effects testing	M_1 PAM (VU0486846): 1, 3, 10 mg/kg (ip)	Minor piloerection and pinna reflex loss @ 100 mg/kg (ip)	1. Novel object recognition	Behavior	1 per dose	Working memory	Enhanced recognition memory in rats @ 3 & 10 mg/kg
				2. Contextual fear conditioning	Risperidone challenge	1 per dose	Learning and memory	Rescue @ 10 mg/kg
Ma et al., 2009	12-16 (per group) B6SJL mice (m; 10 w.o.)	M_1 PAM (BQCA): 5, 10, 15, 20 mg/kg (ip) M_1 allosteric agonist (TBPB): 10, 30 mg/kg (ip) M_1 allosteric agonist (AC-42): 3, 10, 30 mg/kg (ip)	None reported	1. Contextual fear conditioning	Scop.	1 per dose	learning and memory	Full rescue @ 15 and 20 mg/kg
Puri et al., 2015	Tg2576 transgenic mice (f; 3-6 & 9-12 m.o.)	M_1 PAM (PQCA): 0.1, 1, 10 mg/kg (ip) AChE-I (Donepezil): 0.1, 0.3, 1, 3 mg/kg (ip)	None reported	1. Novel object recognition task	Aged mice with elevated beta amyloid	1 per dose	Learning and memory	Recognition improved to comparable levels of WT aged mice @ 0.3, 1 mg/kg donepezil & 10 mg/kg PQCA. Combining subthreshold doses (0.03 mg/kg donepezil & 1 mg/kg PQCA) also resulted in a similar enhancement.
Rook et al., 2017	C57BL/6 mice	M_1 PAM (VU6004256): 1, 3, 10 mg/kg (ip) M_1 PAM (PF-06764427): 1, 3, 10 mg/kg (ip) * Other M_1 PAMs were tested for adverse effects (BQCA; VU6004877; VU6006270; VU6006251; VU6005263; all ip)	Using 100 mg/kg of each ligand: BQCA, VU6004877, VU6006270, & PF-06764427 induced convulsions. VU6004256 induced mydriasis, piloerection, loss of some fine motor control.	1. Novel object recognition task	None (single condition)	1 per dose	Learning and memory	Recognition was enhanced with pretreatment of VU6004256 @ 3 & 10 mg/kg. Trend for enhanced recognition (n.s.) with pretreatment of PF-06764427 @ 10 mg/kg

			PF-06764427 impacted corneal reflex, pinna reflex, salivation & motor activity					
Davoren et al., 2016a	C57BL/6J mice	M_1 PAM (PF-06764427): 1, 3.2, 10 mg/kg (sc)	None reported	1. Locomotion	Amphetamine-induced hyperactivity	1 per dose	Locomotion	Partial rescue of amphetamine-induced hyperactivity @ 3.2 & 10 mg/kg
Davoren et al., 2016b	Task 1: C57BL/6J mice (m; 6-8 w.o.) Task 2: Wistar rats (m) Task 3: Sprague-Dawley rats *Sprague-Dawley rats (m/f; 7-10 w.o.) & Beagle dogs (m) were used for adverse effects testing	M_1 PAM (PF-06767832): 0.32, 1, 3.2 mg/kg (mice: sc; rats: po)	Rats: increased food intake and body weight gain @ 10, 15 & 30 mg/kg. Dogs: loose stool, emesis & salivation @ 3 mg/kg and higher doses with convulsions @ 45 mg/kg	1. Locomotion	Amphetamine-induced hyperactivity	1 per dose	Locomotion	Partial rescue of amphetamine-induced hyperactivity @ 1 & 3.2 mg/kg.
				2. Morris water maze	Scop.	1 per dose	Learning and memory	Partial rescue @ 0.32 mg/kg
				3. Pre-pulse inhibition	Amphetamine disruption	1 per dose	Sensori-motor/vigilance	Partial rescue @ 1 mg/kg
Moran et al., 2018	C57BL6/J mice (m)	M_1 PAM (VU0453595): 0.3, 1, 3, 10 mg/kg (po) M_1 PAM (MK-7622): 1, 3, 10 mg/kg (po)	Convulsions induced @ 30 & 100 mg/kg MK-7622. None reported for VU0453595	1. Novel object recognition task	None	1 per dose	Learning and memory	Enhanced recognition memory @ 1, 3, 10 mg/kg
Davoren et al., 2017	Task 1: C57BL/6J mice (m; 6-8 w.o.) Task 2: Wistar rats (m)	M_1 PAM (PF-06827443): 0.32, 1, 3.2 mg/kg (sc)	Rats: soft feces @ 15 mg/kg & convulsions @ 45 mg/kg (po).	1. Locomotion	Amphetamine-induced hyperactivity	1 per dose	Locomotion	Partial rescue of amphetamine-induced hyperactivity @ all doses.
				2. Morris water maze	Scop.	5 per dose	Learning and memory	Partial rescue at 0.32 mg/kg and full rescue at 1 and 3.2 mg/kg

Abd-Elrahman et al., 2022	APPswe mice (f; 9 m.o.) *control: B6C3F1/J mice (f; 9 m.o.)	M_1 PAM (VU0486846): 10 mg/kg	None reported	1. Novel object recognition task	Mice with elevated beta amyloid	Daily dosing for 4 or 8 weeks	Learning and memory	Partial rescue with 4 week exposure. Enhancement of scores with 8 week exposure (*worse score for WT mice; WT vehicle mice performed worse @ 8 week relative to 4 week)
				2. Morris water maze + reversal morris water maze	Mice with elevated beta amyloid	Daily dosing for 4 or 8 weeks	Learning and memory; cognitive flexibility (exec. functioning)	Partial rescue for both 4 & 8 week exposure regimes.
				3. Open field test	Mice with elevated beta amyloid	Daily dosing for 4 or 8 weeks	Anxiety-like behavior (locomotion)	Partial to full rescue of anxiety-like behavior for both 4 & 8 week exposure regimes.
Sako et al., 2019	Sprague-Dawley rats (m) & Long Evans rats (m) *C57BL/6J mice were also used for identifying adverse effects	M_1 PAM (TAK-071): 0.03, 0.1, 0.3, 1, 3 mg/kg (po) M_1 PAM (T-662): 0.03, 0.1, 0.3 mg/kg (po); Controls: AChE-I (Donepezil): 0.1, 0.3, 1, 3 mg/kg (po) AChE-I (Rivastigmine): 0.1, 0.3, 1 mg/kg (ip)	Rats: Loose stool, salivation, miosis & fasciculation in rats induced by donepezil @ 10 mg/kg. Lacrimation, salivation, miosis & fasciculation induced by rivastigmine @ ≥ 3 mg/kg. Loose stool induced by TAK-071 @ 3 mg/kg and T-662 @ 0.1 mg/kg Mice: Diarrhea induced with TAK-071 @ 3 mg/kg & T-662 @ 10 mg/kg.	1. Novel object recognition task	Scop.	1 per dose	Learning and memory	Partial rescue by donepezil @ 0.3 & 1 mg/kg. Full rescue by rivastigmine @ 0.3 & 1 mg/kg. Full rescue by TAK-071 @ 0.3, 1 & 3 mg/kg. Full rescue by T-662 @ 0.1 & 0.3 mg/kg.
Ghoshal et al., 2016	C57BL6/J mice (m; 8-9 w.o.)	M_1 PAM (VU0453595): 1, 3, 10 mg/kg (ip)	None reported	1. Novel object recognition task	PCP challenge	1 per dose	Learning and memory	Partial rescue by VU0453595 @ 1, 3 & 10 mg/kg
				2. Social interaction assay	PCP challenge	1 per dose	Social interaction	Full rescue by VU0453595 @ 1, 3 & 10 mg/kg

Shirey et al., 2009	Tg2576 mice (m/f; 10-12 w.o.)	M_1 PAM (BQCA): 30 mg/kg (sc)	None reported	1. Reversal learning digging task	Mice with elevated beta amyloid	2 per dose *received compound twice but only 1 pre-reversal & 1 post-reversal measure	Cognitive flexibility (exec. functioning)	Full rescue of reversal performance by BQCA. Enhanced performance beyond just reversal (relative to even WT mice) for the initial discrimination
Fisher et al., 2016	Task 1: Wistar rats (3 m.o.) Task 2: 3xTg-AD mice (f; 12 m.o.)	M_1 PAM (AF710B): Task 1: 1, 3, 10, 30, 100 ug/kg (po) Task 2: 10 ug/kg (ip)	None reported	1. Passive avoidance	Trihexyphenidyl challenge	1 per dose	Learning and memory	Partial rescue with AF710B @ 1, 3, 10 & 30 ug/kg (po)
				2. Morris water maze	Mice with frontotemporal dementia mutation (tau mutation)	Daily dosing for 2 months (from 10-12 m.o.)	Learning and memory	Partial rescue with AF710B @ 10 ug/kg (ip)
Grannan et al., 2016	C57BL/6J mice (8-10 w.o.)	M_1 PAM (VU6004256): 1, 3, 10 mg/kg (ip)	None reported	1. Novel object recognition task	NMDA receptor subunit (NR1) knock-down mice	1 per dose	Learning and memory	Enhancement in novel object recognition of WT mice @ 10mg/kg. Full rescue @ 3 and 10 mg/kg.
				2. Cue-mediated fear conditioning task	NMDA receptor subunit (NR1) knock-down mice	1 per dose	Learning and memory	Partial rescue @ 10 mg/kg.
				3. Spontaneous locomotor activity	NMDA receptor subunit (NR1) knock-down mice	1 per dose	Locomotion	Full rescue @ 1 & 10 mg/kg
Maksymetz et al., 2019	C57BL/6J mice	M_1 antagonist (VU0255035): 3, 10, 30 mg/kg (ip) M_1 PAM (VU0453595): 10 mg/kg (ip)	None reported	1. Contextual fear conditioning	None	2 per dose (VU0255035)	Learning and memory	Significantly impaired contextual extinction with VU0255035 @ 30 mg/kg
				2. Stress-enhanced fear learning	None	1 per dose (VU0453595)	Learning and memory	Enhanced extinction with VU0453595 @ 10 mg/kg
Smith et al., 2022	Mecp2 hetero- and homozygote mice (f; 20 w.o.)	M_1 PAM (VU0453595): 10 mg/kg (ip)	None reported	1. Open field test	Mecp2 heterozygotes	1 per dose	Anxiety-like behavior (locomotion)	No effect
				2. 3-chamber social preference assay	Mecp2 heterozygotes	1 per dose	Social recognition and memory	Rescue of biased exploration towards new mice

				3. Novel object recognition assay	Mecp2 heterozygotes	1 per dose	Learning and memory	Full rescue of novel object preference
				4. Contextual fear conditioning	Mecp2 heterozygotes	1 per dose	Learning and memory	Full rescue of freezing behavior
Walker et al., 2022	6-8 (per group) Indiana alcohol-preferring rats (m; 8 w.o.)	M_1 PAM (PF-06767832): 1 mg/kg (ip)	None reported	1. Locomotion	None (alcohol preferring rats only)	1 per dose	Locomotion	No effect
				2. Alcohol self-administration	None (alcohol preferring rats only)	2 per dose (1 per measure)	Alcohol self-administration; motivation	Reduced alcohol self-administration without altering motivation for alcohol
				3. Sucrose self-administration	None (alcohol preferring rats only)	2 per dose (1 per measure) *same mice used across assays 2 & 3	Food and water self-administration	Reduced sucrose self-administration, as well as food and water consumption
Kurimoto et al., 2021	C57BL/6 mice (11-18 w.o.)	M_1 PAM (TAK-071): 0.03, 0.1, 0.3 mg/kg (po) M_1 PAM (MK-7622): 1, 3, 10 mg/kg (ip) *NO positive results and only used in task 1	None reported	1. Social approach-avoidance test	A: Schizophrenia mice model (miR-137 Tg) B: maternal exposure to poly I:C (reported to induce Schizophrenia symptoms) C: haloperidol challenge	1 per dose	Sociability	B: Full rescue of social sniffing @ 0.1 & 0.3 mg/kg
				2. Y-maze task		1 per dose	Working memory	A: Full rescue of alternations @ 0.3 mg/kg B: Full rescue of alternations @ 0.1 mg/kg A/C: Full rescue of haloperidol-induced reduction in alternations in miR-137 Tg mice.
				3. Novel object recognition task		1 per dose	Learning and memory	A: full rescue @ 0.1 & 0.3 mg/kg
				4. Pre-pulse inhibition test		1 per dose	Sensori-motor/vigilance	A: full rescue @ 0.3 mg/kg
Kucinski et al., 2021	Sprague-Dawley rats (f; 2-3 m.o.)	M_1 PAM (TAK-071): 0.1, 0.3 mg/kg (ig)	None reported	1. Michigan complex movement control task	Rats with dual cholinergic-dopamine loss	7 per dose (1 daily)	Complex motor control	Partial rescue of complex motor control @ both 0.1 (best) & 0.3 mg/kg
Mandai et al., 2020	Long Evans rats (7 w.o.) *Sprague-Dawley rats were used for	AChE-I (Donepezil): 0.1, 1 mg/kg (po) M_1 PAM (MK-7622): 1, 3, 10 mg/kg (po)	T-495 induced diarrhea and in 1/6 rats, convulsions +	1. Novel object recognition task	Scop.	1 dose per regime (both PAMs alone or combined with donepezil)	Learning and memory	Rescue by T-495 @ 1 & 3 mg/kg. Partial rescue by MK-7622 @ 3 & 10 mg/kg. Rescue by donepezil (0.1 mg/kg) & T-495 (0.3 mg/kg).

	characterization of aversive side effects	M_1 PAM (T-495): 0.3, 1, 3 mg/kg (po)	salivation @ 100 mg/kg. MK-7622 induced diarrhea at 3 mg/kg	2. Contextual fear conditioning	Mouse model of dementia and Parkinson's (CaMKIIα-tTA/A543T α-syn dTg)	2 per doses (donepezil or T-495)	Learning and memory	Partial rescue by donepezil @ 1 mg/kg. Partial rescue by T-495 @ 3 mg/kg.
				3. Y-maze	Mouse model of dementia and Parkinson's (CaMKIIα-tTA/A543T α-syn dTg)	1 per dose (donepezil or T-495)	Working memory	Full rescue by donepezil @ 1 mg/kg. Partial rescue by T-495 @ 3 mg/kg.
Kucinski et al., 2020	3-4 (per group) Sprague-Dawley rats (m/f; 2-3 m.o.)	M_1 PAM (TAK-071): 0.1, 0.3 mg/kg (ig)	None reported	1. Sustained attention task	A: signal duration B: Cholinergic lesioned rodents C: distractor (flashing light)	6 per dose (1 daily)	Attention/vigilance	A/B: no effect A/C: no effect A/Post C (after termination of distractor): enhanced performance for lesioned and non-lesioned mice (strongest effect on lesioned) @ 0.1 mg/kg
Choy et al., 2016	C57Bl/6J (m; 2-4 m.o.)	M_1 PAM (BQCA): 1, 3, 5, 10, 20 mg/kg (sc or ip); combined with anti-psychotic drugs but only considered here when used alone	None reported for BQCA	1. Pre-pulse inhibition	MK-801 challenge	1 session per dose (3 pulses)	Sensori-motor/vigilance	No effect with BQCA alone
				2. Y-maze	MK-801 challenge	1 per dose	Working memory	No effect with BQCA alone
Total studies utilizing PAMs: 28	Rodents used: -Sprague-Dawley rats: 9/28 studies -C57BL mice: 13/28 studies -Long Evans rats/Wistar rats/ other rodents: 13/28 studies	M_1 PAMs: -VU compounds: 9/28 studies -BQCA/PQCA: 9/28 studies -PF compounds: 6/28 studies -TAK-071: 4/28 studies -MK-7622: 3/28 studies	Reported for several PAMs and other cholinergic agents	1/28 tasks had no behavioral component 14/28 had 1 only 1 task 6/28 had 2 tasks 5/28 had 3 tasks 2/28 had 4 tasks	-Scopolamine challenge: 7/28 studies -Other compound challenges (amphetamine, PCP, alcohol etc): 9/28 studies -Aged rodents/ genetic model	Average number of determinations: 1 (vast majority used only 1 determination) -4/28 studies contained some daily dosing regime -5/28 studies contained more	Cognitive domains tested: -Learning and memory (19/28; 5 of those had more than 1 task in this domain) -Locomotion/ motor control (9/28) -Working memory (6/28)	Some behavioral enhancement (usually a rescue) in almost all studies with at least 1 dose of M_1 PAMs

		-Other: 5/27 studies			of disease: 7/28 studies -Behavioral: 3/28 studies -Other (prions, lesions): 4/28 studies -None: 3/28 studies	than 1 determination in at least 1 dose (non-daily dosing)	-Attention/ vigilance (3/28) -Social behavior (3/28) -Cognitive flexibility/exec. function (2/28) -Satiety/drug abuse (1/28)	

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