Original Investigations

Nicotine promotes the utility of short-term memory during visual search in macaque monkeys

Author names and affiliations:
Ryo Sawagashira[1,2] and Masaki Tanaka[1]
Departments of [1]Physiology and [2]Psychiatry, Hokkaido University School of Medicine, Sapporo 060-8638, Japan.

Author contributions:
RS and MT designed research; RS performed experiments; RS analyzed data; RS and MT wrote and revised the paper

Correspondence should be addressed to:
R.S. <rsawa6133@med.hokudai.ac.jp > or M.T. <masaki@med.hokudai.ac.jp>

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Abstract

Rationale: The central cholinergic system is a major therapeutic target for restoring cognitive functions. Although manipulation of cholinergic signaling is known to alter working memory (WM), the underlying mechanism remains unclear. It is widely accepted that WM consists of multiple functional modules, one storing short-term memory and the other manipulating and utilizing it. A recently developed visual search task and a relevant model can be used to assess multiple components of WM during administration of acetylcholine receptor (AChR)-related substances.

Objectives: The effects of systemic administration of AChR-related agents on WM and eye movements were examined during the oculomotor foraging task.

Methods: Three monkeys performing the task received an intramuscular injection of saline or the following AChR-related agents: nicotine (24 or 56 μg/kg), mecamylamine (nicotinic AChR antagonist, 1.0 mg/kg), oxotremorine (muscarinic AChR agonist, 3.0 μg/kg), and scopolamine (muscarinic AChR antagonist, 20 μg/kg). The task was to find a target among 15 identical objects by making eye movements within 6 seconds. The data were analyzed according to the foraging model that incorporated three parameters.

Results: Nicotine and mecamylamine significantly increased the utility but not the capacity of short-term memory, while muscarinic AChR-related agents did not alter any WM parameters. Further regression analysis with a mixed effect model showed that the beneficial effect of nicotine on memory utility remained after considering eye movement variability, but the beneficial effect of mecamylamine disappeared.

Conclusions: Nicotine improves visual search, mainly by increasing the utility of short-term memory, with minimal changes in oculomotor parameters.

Keywords: foraging behavior; visual search; working memory; central executive function; attentional inhibition; acetylcholine receptor; nicotine; mecamylamine; nonhuman primate

Introduction

The central cholinergic system is essential for a variety of cognitive functions, such as working memory (WM), attention, and learning (Azimi et al. 2020; Cools and Arnsten 2021; Disney 2021; Sarter and Lustig 2019; Thiele and Bellgrove 2018). To restore these functions in patients with dementia, acetylcholine receptor (AChR)-related drugs are often administered (Foster et al. 2014; Sun et al. 2012). AChRs are largely classified into nicotinic and muscarinic receptors, both of which are widely expressed in the brain and mediate cognitive functions (Dineley et al. 2015; Kruse et al. 2014).

Previous studies have used nonhuman primates as animal models and have demonstrated that stimulation of AChRs improves WM and blockade of them worsens it (Buccafusco and Terry 2004; Cools and Arnsten 2021; Liu et al. 2017). It has been widely accepted that WM contains multiple functional modules (Baddeley and Della Sala 1996; Baddeley and Hitch 1974). The "slave system" is responsible for storing short-term memory and consists of several subsystems, while the "central executive system" retrieves, integrates, and manipulates the contents of short-term memory according to the context. However, most of the previous studies did not identify which components of WM were primarily affected by the manipulation of cholinergic signaling.

Recently, we developed a method for simultaneously assessing multiple components of WM using a simple visual search task and a relevant model (Sawagashira and Tanaka 2021). In this task, monkeys are urged to find a target among many identical objects within a time limit. An efficient search requires minimizing recursive behavior to the visited items. The recursive behavior of each animal was well explained by a model that incorporated three parameters: memory capacity, memory decay, and utility rate. Memory capacity and memory decay reflected the performance of the slave system, while utility rate determined the use of short-term memory for visual search and was thought to be related to the central executive function

and attentional suppression. In our previous study, administration of low doses of ketamine (N-methyl D-aspartate receptor antagonist) during the task resulted in a slight decrease in memory capacity and a large decrease in utility rate (Sawagashira and Tanaka 2021). Using this framework, it is possible to evaluate the impacts of different psychotropic drugs on each of the multiple components of WM.

In the present study, we assessed the effects of AChR-related agents on WM. The results showed that stimulation of nicotinic AChRs increased the utility of short-term memory with slightly altering saccade parameters, while muscarinic AChRs only mediated changes in eye movements.

Materials and Methods

Animal preparation and surgery

Three adult male monkeys (*Macaca fuscata*, 7–9 kg, monkeys T, N, and O) were used. All experimental protocols were approved in advance by the Hokkaido University Animal Care and Use Committee and were in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006). The animals were sterilely implanted with head holders and an eye coil in separate surgical procedures under general isoflurane and nitrous oxide anesthesia. Analgesics (pentazocine and ketoprofen) were administered during and for a few days following each procedure. The animals were trained on oculomotor tasks after complete recovery from the surgeries. During the training and experimental sessions, animals sat in a custom-made primate chair with their heads fixed in a dark experimental booth. Horizontal and vertical eye positions were recorded using the search coil technique (Enzanshi-Kogyo).

Visual stimuli and behavioral paradigm

The experiments were controlled by a Windows-based real-time data acquisition system (TEMPO, Reflective Computing). Visual stimuli were presented on a 27-inch liquid crystal display (XL2720Z, BenQ; refresh rate, 144 Hz) located 40 cm from the eyes (visual angle, 77° × 62°). The animals were trained on the oculomotor foraging task for several months. In this task, each trial began with the appearance of a red fixation point (FP) at either the center of the display or at one of the four diagonal locations (10° eccentricity, Fig. 1*A*). After a random fixation period (500–1000 ms, < 2° accuracy), the FP was extinguished, and 15 white squares (1°) were simultaneously presented for 6 s. The locations of the stimuli were randomly chosen for each trial from 76 possible sites covering the 40° × 24° area (11 × 7 grid, ≥ 4° apart each other). Only one of the visual stimuli was associated with a liquid reward, and the animals were trained to make sequential saccades until they received a reward within the 6-s time constraint. During the experiments, targeting saccades were detected when eye position remained within 2° of each stimulus for > 100 ms. Once the animals looked at the object associated with a reward, the target turned red, and a liquid reward was delivered after a 200-ms delay period. After the reward delivery, the trial was terminated with a brief high-frequency sound. If monkeys failed to find the target by the end of the time limit, the trial was aborted with a pair of beep sounds. The intertrial interval was always 800 ms.

Procedures of pharmacological experiments

Two monkeys (T and N) received drugs for the first time during the foraging task, while one monkey (O) had received ketamine in a previous study (Sawagashira and Tanaka 2021). In each session, after 100 baseline (pre-injection) trials, a single injection of saline (control) or AChR-related substance was administered to the right quadriceps muscle, followed by the behavioral task for at least 60 min. We tested the effects of the following drugs (all purchased from Sigma-Aldrich, St. Louis, MO, USA): nicotine hydrogen tartrate (nicotinic AChR agonist, 24 or 56

μg/kg), mecamylamine hydrochloride (nicotinic AChR antagonist, 1.0 mg/kg), oxotremorine sesquifumarate salt (muscarinic AChR agonist, 3.0 μg/kg), and scopolamine hydrobromide (muscarinic AChR antagonist, 20 μg/kg). One monkey (N) was tested for the agonist and antagonist of nicotinic AChR only. The dosage of each drug was determined based on previous pharmacological experiments in monkeys (Katner et al. 2004; Vardigan et al. 2015; Witkin 1989). The injection volume was 1.0 mL for all experiments. Pharmacological experiments were spaced at least three days apart to avoid the effects of cumulative administration. We initially tested the drugs associated with nicotinic AChRs and then examined the effects of drugs associated with muscarinic AChRs. Since it took a long period of time to collect all the data (5.3 ± 4.4 months, $n = 3$), the pharmacological effects were evaluated by comparing them with the data after saline injection obtained within 10 days before and after each drug experiment.

Data acquisition and analysis

Signals proportional to eye position were digitized at 16-bit resolution and sampled at 1 kHz along with the event timestamps. Data were saved to files during the experiments and analyzed offline using MATLAB (MathWorks). During offline analysis, saccades were detected when angular eye velocity exceeded 70°/s, eye acceleration exceeded 1000°/s^2, and eye displacement was > 2°.

For each saline and pharmacological experiment, eye velocity, saccade frequency, and inter-saccadic interval (ISI) were measured. To evaluate saccade velocity, we fitted an exponential curve to the relationship between saccade amplitude and peak velocity (main sequence) and estimated the velocity of 20° saccade. Data from sessions in which the animals were clearly unmotivated and did not perform sufficient number of trials were excluded from the further quantitative analysis (4.3%).

The pharmacological effects on WM performance were assessed using the previously

developed foraging model (Sawagashira and Tanaka 2021). To evaluate WM performance, we calculated 1) the proportion of revisiting behavior for each saccade sequence, 2) the number of intervening saccades between initial and recursive choices, and 3) the number of saccades in each trial. The distributions of these three parameters were normalized in area when comparing with data obtained from simulations based on the foraging model (Fig. 2*A–C*, see below).

The foraging model was defined by the following three parameters: memory capacity, memory decay, and utility rate (Fig. 1*B*). We assumed that each saccade target was selected in one of two behavioral strategies. In the exploration mode, animals randomly select an object regardless of the previous search (no reference to short-term memory). In the exploitation mode, animals select a new object based on the memory of the previous search and their performance depends on the memory capacity and memory decay. These two modes are determined according to the utility rate (0 to 1) for each saccade. For example, when the utility rate is unity, monkeys only select from the items that are not registered in short-term memory. When the utility rate is zero, they randomly explore the items for every saccade. The memory capacity (1 to 15) limits the number of items in short-term memory, and the memory decay determines the order in which items are removed from memory when the number of items visited exceeds capacity. When memory decay is unity, the oldest item is deleted. When memory decay equals to capacity, any of the visited items is deleted from short-term memory with equal probability.

A Monte Carlo simulation (5000 iterations) was performed to predict the behavior of the animals for each set of the model parameters (6120 combinations, 0.02 increments for utility rate). We obtained the three distributions of behavioral parameters mentioned above, and then searched for the optimal set of parameters that best explained the actual data in each experiment. The three distributions of the behavioral parameters were normalized in their area to equalize the contribution of each distribution. The goodness-of-fit of the model was evaluated by calculating the coefficient of determination using the formula, $1 - \Sigma(D - S)^2 / \Sigma(D - \bar{S})^2$, where

D and S indicated the combined three normalized distributions of the actual data and the results of the simulation, respectively. To examine whether the three model parameters were stable and specific for each animal, we performed decoding analysis with a support vector machine for all saline experiments (n = 42). A classifier was trained to identify the animal using half of the datasets (three parameter values), and its performance was cross-validated using the remaining datasets. The same procedure was repeated 1000 times to estimate the discriminability.

To evaluate the effect of drugs, the data of each monkey were z-scored based on the data obtained from the saline control. Then, a two-way ANOVA (drug condition × monkeys) was performed for each parameter to assess statistical significance. To quantify the drug effect, the effect size (Hedges' g) was calculated for each WM parameter. The drug effects on each behavioral parameter were also assessed using a linear mixed effect model with a random effect of monkeys as follows,

$$Data \sim \beta_{\mathrm{fix}} \times Condition + \beta_{\mathrm{rand}} \times Monkey$$

Data indicates each of z-scored WM parameters (capacity, utility rate, memory decay) or saccade parameters (eye velocity, saccade frequency, ISI). *Condition* and *Monkey* represent fixed effect variables and random effect variables, respectively. Fixed effect coefficient for the drug condition (β_{drug}) was also calculated with a model incorporating eye velocity, saccade frequency, and ISI as fixed variables to examine whether changes in WM parameter were partially attributable to changes in eye movement parameters. Other details of statistical tests are provided in the relevant text.

Results

Figure 2*A–C* plots the data obtained from a single control experiment in monkey T (gray bars). The WM performance was assessed by the three distributions of behavioral data: 1) the proportion of revisiting behavior for each saccade sequence (Fig. 2*A*), 2) the number of

intervening saccades between initial and recursive choices (*B*), and 3) the number of saccades in each trial (*C*). Using these distributions, we searched for the optimal parameters of the foraging model that best accounted for the actual data (orange lines). The goodness-of-fit of the model was evaluated by the coefficient of determination (CD), which was 0.91 for the representative experiment shown in Figure 2*A–C* and averaged to 0.90 ± 0.02 (SD, $n = 42$) and 0.90 ± 0.02 ($n = 38$) for the experiments with saline and drug injection, respectively (Fig. 2*D*). These values did not differ between drug conditions (unpaired t-test, $t_{78} = -0.85$, $p = 0.40$).

To test the reliability and usefulness of the foraging model, we compared the variability of model parameters between sessions and between monkeys. Figure 2*E* summarizes optimal parameters in control (saline) sessions for each animal. Despite some variability across sessions, the variation in each monkey appeared to be smaller than that between animals, especially in memory capacity and utility rate (Figure 2*E*, left and middle panels). To quantify this, we compared the mean squared error of each model parameter for each monkey (variance in each animal) with that calculated for the global mean (variance in all sessions). These values were significantly different in two of three parameters and the variance in each monkey was smaller than that across all sessions (two-tailed t-test, memory capacity, $t_{82} = -6.01$, $p < 10^{-7}$; utility rate, $t_{82} = -2.91$, $p < 0.01$; decay, $t_{82} = -1.49$, $p = 0.14$), indicating that the foraging model reliably captured the characteristics of each animal. In addition, we also trained a classifier to identify animals based on the three WM parameters (Methods), and its discrimination performance in cross-validation was well above chance (0.86 ± 0.06 versus 0.33). These results further support the reliability and usefulness of the foraging model.

Figure 3*A* displays the distributions derived from the optimal models for three nicotine (24 μg/kg, red lines) and three saline (black lines) experiments in two monkeys. Despite slight inter-session variability, a clear difference between drug conditions was observed in both animals (red versus black lines). For example, the data in the left column shows the decrease in

the proportion of recursive behavior during early saccade sequence following nicotine injection compared to that following saline injection (black arrows). For comparison, Figure 3*B* illustrates the data from the previous experiments with ketamine administration (Sawagashira and Tanaka 2021), in which the proportion of recursive saccades during early sequence increased (arrow in the left panel). The data in the right column show that the distribution of saccade frequency in each trial remained unchanged after nicotine administration in both animals (Fig. 3*A*), whereas the number of saccades was distributed widely following ketamine injection (Fig. 3*B*) and the proportion of trials with intermediate saccade frequency decreased slightly (arrow in the right panel).

The changes in the WM parameters of the optimal model following administration of the agonist (nicotine) and antagonist (mecamylamine) of nicotinic AChR are summarized in Figure 4*A*. To allow for direct comparison across monkeys, the data of each animal were z-scored using control data. The utility rate significantly increased following administration of both the agonist and antagonist of nicotinic AChR (two-way ANOVA, 24 μg/kg nicotine, $F_{1,20} = 26.0$, $p < 10^{-3}$; 56 μg/kg nicotine, $F_{1,19} = 28.5$, $p < 10^{-3}$; mecamylamine, $F_{1,23} = 7.3$, $p = 0.02$), whereas no significant changes in the memory capacity and memory decay were found (24 μg/kg nicotine, $F_{1,20} = 3.4$, $p = 0.09$ and $F_{1,20} = 0.7$, $p = 0.41$; 56 μg/kg nicotine, $F_{1,19} = 0.5$, $p = 0.48$ and $F_{1,19} = 3.2$, $p = 0.09$; mecamylamine, $F_{1,23} = 0.01$, $p = 0.92$ and $F_{1,23} = 3.8$, $p = 0.07$ for memory capacity and memory decay, respectively). Two additional indices were computed to further quantify the drug effects (Table 1). First, we measured the effect size for each WM parameter. Second, we assessed the drug effects with a linear mixed model (Methods), and compared the resulting fixed effect coefficients. Both indices show that nicotine and mecamylamine had a significant effect on utility rate, but not on other WM parameters.

We next examined the pharmacological effects on oculomotor parameters. Although administration of nicotine sometimes changed saccade velocity and frequency, these changes

were only slight (Fig. 5*A* and Table 2). By contrast, the nicotinic AChR antagonist (mecamylamine) had greater effects, significantly decreasing saccade velocity (two-way ANOVA, $F_{1,23} = 5.3$, $p = 0.03$) and frequency ($F_{1,23} = 7.6$, $p = 0.01$) and increasing the ISI ($F_{1,23} = 29.6$, $p < 10^{-4}$). The increase in ISI was particularly marked, which correlated with utility rate ($r = 0.71$, $p = 0.03$), in contrast to nicotine (low dose, $r = 0.34$, $p = 0.38$; high dose, $r = 0.32$, $p = 0.44$). These results suggest that the mecamylamine effect on utility rate may be partially due to changes in oculomotor parameters. To test this, a regression analysis of utility rate was conducted with a linear mixed effect model incorporating both drug condition and eye movement parameters as fixed effect variables (Methods). The results showed that the fixed effect coefficients for drug condition decreased greatly in the mecamylamine experiment but not in the experiment with high dosage of nicotine (mecamylamine, $\beta_{drug} = 0.10$, $p = 0.89$, $\beta_{ISI} = 0.35$, $p < 0.01$, $\beta_{velocity} = -0.12$, $p = 0.70$, $\beta_{frequency} = -0.16$, $p = 0.51$; low dose nicotine, $\beta_{drug} = 2.01$, $p = 0.08$, $\beta_{ISI} = 0.55$, $p = 0.12$, $\beta_{velocity} = 0.74$, $p = 0.27$, $\beta_{frequency} = -0.89$, $p = 0.10$; high dose nicotine, $\beta_{drug} = 1.73$, $p = 0.03$, $\beta_{ISI} = -0.05$, $p = 0.79$, $\beta_{velocity} = -1.37$, $p < 0.01$, $\beta_{frequency} = -0.31$, $p = 0.39$), suggesting that the significant increase in utility rate after mecamylamine administration was partially attributable to changes in ISI.

When an agonist (oxotremorine) and antagonist (scopolamine) of muscarinic AChR were administered in two monkeys, no significant change in the WM parameters was found (Fig. 4*B* and Table 1). The only significant change was a decrease of saccade velocity following injection of scopolamine (two-way ANOVA, $F_{1,11} = 8.9$, $p = 0.02$, Fig. 5*B*; linear mixed effect model, $\beta_{fix} = -2.03$, $p = 0.006$, Table 2). We also failed to find any significant drug effect on WM parameters even when incorporating oculomotor parameters as fixed effect variables (oxotremorine, $\beta_{drug} = 0.45$, 0.25, 0.39, $p = 0.13$, 0.62, and 0.38 for memory capacity, utility rate, and memory decay, respectively; scopolamine, $\beta_{drug} = -0.14$, −5.48, and −0.93, $p = 0.93$, 0.09, and 0.69, respectively).

Discussion

In this study, we examined the effects of systemic administration of AChR-related agents on the performance of WM and eye movements. A significant increase in utility rate with a slight change in saccade parameter was found after administration of nicotine. Mecamylamine, a nicotinic AChR antagonist, also significantly increased the utility rate, but these changes correlated with changes in saccade parameters. Since changes in utility rate after mecamylamine injection were well explained by changes in ISI rather than drug conditions, the beneficial effect of mecamylamine on WM parameters might be secondary to the slowing of eye movements.

Effects of nicotinic AChR agonist and antagonist

Nicotine administration is known to improve a variety of cognitive functions (Rezvani and Levin 2001). In particular, the effect on WM has been reported in rodents (Rushforth et al. 2011), pigeons (Kangas and Branch 2012), monkeys (Buccafusco et al. 1999; Buccafusco and Terry 2004), and humans (Heishman et al. 2010; Kangas and Branch 2012; Levin et al. 1996; Spinelli et al. 2006; Sultana et al. 2013; Upright and Baxter 2021). Katner et al. (2004) examined the effects of nicotine in monkeys trained on a variety of WM tasks and found that performance improved regardless of memory domain, with more difficult conditions showing a greater effect. However, it is not clear whether the improved performance in these tasks was simply due to better retention of short-term memory or reflected changes in central executive functions. Using the foraging task and a related model, we found in this study that nicotine improved task performance primarily by increasing the use of short-term memory, rather than by increasing the memory capacity. Contrary to nicotine, our previous study showed that low doses of ketamine (an NMDA receptor antagonist) reduced utility rate along with a mild

decrease in memory capacity (Sawagashira and Tanaka 2021). Thus, the performance of visual search depends not only on short-term memory itself, but also on the executive functions that determine whether the stored information is used to control goal-directed behavior.

The use of short-term memory for visual search is likely to be related to visuospatial attention. The "inhibitory tagging" is a mechanism that suppresses recursive behavior during visual search (Klein 1988). The basis for this mechanism is the inhibition of return, which suppresses attentional allocation to an object once attention has been directed to it (Klein 2000; Müller and von Mühlenen 2000; Snyder and Kingstone 2007). Although inhibition of return appears to be an automatic process that persists up to several seconds (Posner and Cohen 1984; Samuel and Kat 2003; Wang and Klein 2010), our monkeys often made recursive saccades to objects they had seen only a few times earlier (Fig. 2*B*). This suggests that inhibitory tagging is not just an automatic passive process, but an active process that intentionally suppresses the allocation of attention based on the memory of previous search. The underlying mechanism for improving the utility rate in this study might be the efficiency of attentional suppression based on short-term memory. During visual search, the priority map for target selection is thought to be dynamically updated by top-down signals (Bisley and Goldberg 2010; Itti and Koch 2001; Veale et al. 2017), which may be regulated by nicotinic AChR.

Since this study was conducted with systemic administration, it is not possible to identify the site of nicotine's action in improving executive function. It is known that α4β2 and α7 subunits of nicotinic AChRs are widely expressed in the brain, and the former is known to regulate cognition, attention, and emotion, while the latter is known to promote memory and learning and protect the brain (Azimi et al. 2020; Paterson and Nordberg 2000; Sarter and Lustig 2019). In particular, the α4β2 subunit shows high affinity for nicotine and is expressed in the frontal cortex and striatum (Gotti et al. 2006), which might contribute to the increased utility rate. In the future, ligands specific for this subtype could be administered systemically or locally

in the brain to further investigate the underlying mechanism.

Previous studies have reported that nicotine shortens saccade latency (Larrison et al. 2004; Reilly et al. 2008; Rycroft et al. 2006), but does not change saccade velocity which reflects brainstem function (Aizawa et al. 1999; Sherr et al. 2002). In the present study, there was no consistent change in saccade parameters following nicotine administration. The exception was a slight decrease in the number of saccades in each trial with a low dose of nicotine (Fig. 5*A* and Table 2), which may be related to an increased utility rate that helps detect targets earlier. We also found a decrease in saccade velocity with a high dose of nicotine, which might reflect an untoward effect by acute administration of nicotine (Withey et al. 2018).

Unexpectedly, mecamylamine, a nicotinic AChR antagonist, slightly increased the utility rate in the present study, although the overall effect size was approximately 30% smaller than that of nicotine (Table 1). On the other hand, the changes in oculomotor parameters after mecamylamine administration were greater than those of nicotine, and the effects were statistically significant for all parameters examined in this study (Fig. 5*A* and Table 2). Furthermore, our regression analysis with a mixed effect model incorporating oculomotor parameters showed that changes in utility rate after mecamylamine administration (but not nicotine administration) were accounted for by the increased ISI rather than drug conditions. These results suggest that the increased utility rate after mecamylamine administration may be secondary to prolonged saccade latency or target selection time during visual search. Taken together, nicotinic AChR stimulation seemed to directly improve central executive function, while inhibition delayed eye movements, which might have resulted in a slight improvement in performance.

Effects of muscarinic AChR agonist and antagonist

Since previous studies have demonstrated that stimulating muscarinic AChRs improves and

362 blocking them impairs WM performance (Cools and Arnsten 2021; Liu et al. 2017; Sarter and
363 Lustig 2019; Thiele and Bellgrove 2018), we expected changes in foraging behavior after
364 administration of muscarinic AChR agonist and antagonist. However, we failed to find any
365 significant effect on WM parameters, even though the same dosages of drugs significantly
366 altered WM performance in previous studies (Vardigan et al. 2015; Witkin 1989). This negative
367 result may simply be due to differences in behavioral tasks, as nicotine has a greater effect in
368 more difficult WM tasks (Katner et al. 2004). In addition, since different subtypes of muscarinic
369 AChR are known to mediate different functions (Eglen 2006), the multiple effects of systemic
370 administration of nonspecific agonist (oxotremorine) or antagonist (scopolamine) in the present
371 study might be canceled out and were not detected as behavioral changes. To further investigate
372 the effects of muscarinic AChR on visual search in future studies, it is necessary to consider the
373 use of subtype-specific drugs or local administration, or both.
374
375 In conclusion, our novel finding was that nicotinic AChR agonist significantly
376 increased the utility of short-term memory with a slight change in saccade parameters. Since
377 the use of short-term memory to guide goal-directed behavior is one of the key elements of
378 executive function, nicotine may promote cognitive functions in general. Consistent with this,
379 nicotine has also been shown to facilitate other forms of executive functioning, such as attention
380 (Spinelli et al. 2006; Witte et al. 1997), associative learning (Hahn et al. 2018), and deliberate
381 control in difficult tasks (Ettinger et al. 2017; Petrovsky et al. 2013). On the other hand, the
382 current results also indicate that nicotine does not improve short-term memory itself during
383 visual search, although previous studies have shown beneficial effects (Castner et al. 2011;
384 Hironaka et al. 1992; Katner et al. 2004). These differences may be due to the fact that most of
385 the previous studies have not separately assessed the multiple components of WM. Other drugs,
386 such as dopamine and adrenergic receptor agonists, are known to improve short-term memory

387 at the cellular level (Arnsten and Goldman-Rakic 1985; Ott and Nieder 2017; Vijayraghavan et
388 al. 2016; Wang et al. 2004; Williams and Goldman-Rakic 1995); therefore it seems important
389 to investigate and compare the effects of these drugs in our paradigm.

References

Aizawa H, Kobayashi Y, Yamamoto M, Isa T (1999) Injection of nicotine into the superior colliculus facilitates occurrence of express saccades in monkeys. J Neurophysiol 82: 1642-6.

Arnsten AF, Goldman-Rakic PS (1985) Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science 230: 1273-6.

Azimi M, Oemisch M, Womelsdorf T (2020) Dissociation of nicotinic α7 and α4/β2 sub-receptor agonists for enhancing learning and attentional filtering in nonhuman primates. Psychopharmacology (Berl) 237: 997-1010.

Baddeley A, Della Sala S (1996) Working memory and executive control. Philos Trans R Soc Lond B Biol Sci 351: 1397-403; discussion 1403-4.

Baddeley AD, Hitch G (1974) Working Memory. In: Bower GH (ed) Psychology of Learning and Motivation. Academic Press, pp 47-89

Bisley JW, Goldberg ME (2010) Attention, intention, and priority in the parietal lobe. Annu Rev Neurosci 33: 1-21.

Buccafusco JJ, Jackson WJ, Jonnala RR, Terry AV, Jr. (1999) Differential improvement in memory-related task performance with nicotine by aged male and female rhesus monkeys. Behav Pharmacol 10: 681-90.

Buccafusco JJ, Terry AV (2004) Donepezil-induced improvement in delayed matching accuracy by young and old rhesus monkeys. J Mol Neurosci 24: 85-91.

Castner SA, Smagin GN, Piser TM, Wang Y, Smith JS, Christian EP, Mrzljak L, Williams GV (2011) Immediate and sustained improvements in working memory after selective stimulation of α7 nicotinic acetylcholine receptors. Biol Psychiatry 69: 12-8.

Cools R, Arnsten AFT (2021) Neuromodulation of prefrontal cortex cognitive function in primates: the powerful roles of monoamines and acetylcholine. Neuropsychopharmacology.

Dineley KT, Pandya AA, Yakel JL (2015) Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol Sci 36: 96-108.

Disney AA (2021) Neuromodulatory Control of Early Visual Processing in Macaque. Annu Rev Vis Sci 7: 181-199.

Eglen RM (2006) Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. Auton Autacoid Pharmacol 26: 219-33.

Ettinger U, Faiola E, Kasparbauer AM, Petrovsky N, Chan RC, Liepelt R, Kumari V (2017) Effects of nicotine on response inhibition and interference control. Psychopharmacology (Berl) 234: 1093-1111.

Foster DJ, Choi DL, Conn PJ, Rook JM (2014) Activation of M1 and M4 muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. Neuropsychiatr Dis Treat 10: 183-91.

Gotti C, Zoli M, Clementi F (2006) Brain nicotinic acetylcholine receptors: native subtypes

and their relevance. Trends Pharmacol Sci 27: 482-91.

Hahn B, Wells AK, Lenartowicz A, Yuille MB (2018) Nicotine effects on associative learning in human non-smokers. Neuropsychopharmacology 43: 2190-2196.

Heishman SJ, Kleykamp BA, Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology (Berl) 210: 453-69.

Hironaka N, Miyata H, Ando K (1992) Effects of psychoactive drugs on short-term memory in rats and rhesus monkeys. Jpn J Pharmacol 59: 113-20.

Itti L, Koch C (2001) Computational modelling of visual attention. Nat Rev Neurosci 2: 194-203.

Kangas BD, Branch MN (2012) Relations among acute and chronic nicotine administration, short-term memory, and tactics of data analysis. J Exp Anal Behav 98: 155-67.

Katner SN, Davis SA, Kirsten AJ, Taffe MA (2004) Effects of nicotine and mecamylamine on cognition in rhesus monkeys. Psychopharmacology (Berl) 175: 225-40.

Klein R (1988) Inhibitory tagging system facilitates visual search. Nature 334: 430-1.

Klein RM (2000) Inhibition of return. Trends Cogn Sci 4: 138-147.

Kruse AC, Kobilka BK, Gautam D, Sexton PM, Christopoulos A, Wess J (2014) Muscarinic acetylcholine receptors: novel opportunities for drug development. Nat Rev Drug Discov 13: 549-60.

Larrison AL, Briand KA, Sereno AB (2004) Nicotine improves antisaccade task performance without affecting prosaccades. Hum Psychopharmacol 19: 409-19.

Levin ED, Kim P, Meray R (1996) Chronic nicotine working and reference memory effects in the 16-arm radial maze: interactions with D1 agonist and antagonist drugs. Psychopharmacology (Berl) 127: 25-30.

Liu R, Crawford J, Callahan PM, Terry AV, Jr., Constantinidis C, Blake DT (2017) Intermittent Stimulation of the Nucleus Basalis of Meynert Improves Working Memory in Adult Monkeys. Curr Biol 27: 2640-2646.e4.

Müller HJ, von Mühlenen A (2000) Probing distractor inhibition in visual search: inhibition of return. J Exp Psychol Hum Percept Perform 26: 1591-605.

Ott T, Nieder A (2017) Dopamine D2 Receptors Enhance Population Dynamics in Primate Prefrontal Working Memory Circuits. Cereb Cortex 27: 4423-4435.

Paterson D, Nordberg A (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 61: 75-111.

Petrovsky N, Ettinger U, Quednow BB, Landsberg MW, Drees J, Lennertz L, Frommann I, Heilmann K, Sträter B, Kessler H, Dahmen N, Mössner R, Maier W, Wagner M (2013) Nicotine enhances antisaccade performance in schizophrenia patients and healthy controls. Int J Neuropsychopharmacol 16: 1473-81.

Posner MI, Cohen Y (1984) Components of visual orienting. In: Houma H, Bouwhuis DG (eds) Attention and performance X: control of language processes. Erlbaum, Hillsdale, pp. 531-556

Reilly JL, Lencer R, Bishop JR, Keedy S, Sweeney JA (2008) Pharmacological treatment effects on eye movement control. Brain Cogn 68: 415-35.

Rezvani AH, Levin ED (2001) Cognitive effects of nicotine. Biol Psychiatry 49: 258-67.

Rushforth SL, Steckler T, Shoaib M (2011) Nicotine improves working memory span capacity in rats following sub-chronic ketamine exposure. Neuropsychopharmacology 36: 2774-81.

Rycroft N, Hutton SB, Rusted JM (2006) The antisaccade task as an index of sustained goal activation in working memory: modulation by nicotine. Psychopharmacology (Berl) 188: 521-9.

Samuel AG, Kat D (2003) Inhibition of return: a graphical meta-analysis of its time course and an empirical test of its temporal and spatial properties. Psychon Bull Rev 10: 897-906.

Sarter M, Lustig C (2019) Cholinergic double duty: cue detection and attentional control. Curr Opin Psychol 29: 102-107.

Sawagashira R, Tanaka M (2021) Ketamine-Induced Alteration of Working Memory Utility during Oculomotor Foraging Task in Monkeys. eNeuro 8:ENEURO.0403-20.2021.

Sherr JD, Myers C, Avila MT, Elliott A, Blaxton TA, Thaker GK (2002) The effects of nicotine on specific eye tracking measures in schizophrenia. Biol Psychiatry 52: 721-8.

Snyder JJ, Kingstone A (2007) Inhibition of return at multiple locations and its impact on visual search. Visual Cognition 15: 238-256.

Spinelli S, Ballard T, Feldon J, Higgins GA, Pryce CR (2006) Enhancing effects of nicotine and impairing effects of scopolamine on distinct aspects of performance in computerized attention and working memory tasks in marmoset monkeys. Neuropharmacology 51: 238-50.

Sultana R, Ameno K, Jamal M, Miki T, Tanaka N, Ono J, Kinoshita H, Nakamura Y (2013) Low-dose nicotine facilitates spatial memory in ApoE-knockout mice in the radial arm maze. Neurol Sci 34: 891-7.

Sun X, Jin L, Ling P (2012) Review of drugs for Alzheimer's disease. Drug Discov Ther 6: 285-90.

Thiele A, Bellgrove MA (2018) Neuromodulation of Attention. Neuron 97: 769-785.

Upright NA, Baxter MG (2021) Effects of nicotinic antagonists on working memory performance in young rhesus monkeys. Neurobiol Learn Mem 184: 107505.

Vardigan JD, Cannon CE, Puri V, Dancho M, Koser A, Wittmann M, Kuduk SD, Renger JJ, Uslaner JM (2015) Improved cognition without adverse effects: novel M1 muscarinic potentiator compares favorably to donepezil and xanomeline in rhesus monkey. Psychopharmacology (Berl) 232: 1859-66.

Veale R, Hafed ZM, Yoshida M (2017) How is visual salience computed in the brain? Insights from behaviour, neurobiology and modelling. Philos Trans R Soc Lond B Biol Sci 372.

Vijayraghavan S, Major AJ, Everling S (2016) Dopamine D1 and D2 receptors make

dissociable contributions to dorsolateral prefrontal cortical regulation of rule-guided oculomotor behavior. Cell Rep 16: 805-16.

Wang M, Vijayraghavan S, Goldman-Rakic PS (2004) Selective D2 receptor actions on the functional circuitry of working memory. Science 303: 853-6.

Wang Z, Klein RM (2010) Searching for inhibition of return in visual search: a review. Vision Res 50: 220-8.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376: 572-5.

Withey SL, Doyle MR, Bergman J, Desai RI (2018) Involvement of Nicotinic Receptor Subtypes in the Behavioral Effects of Nicotinic Drugs in Squirrel Monkeys. J Pharmacol Exp Ther 366: 397-409.

Witkin JM (1989) Central and peripheral muscarinic actions of physostigmine and oxotremorine on avoidance responding of squirrel monkeys. Psychopharmacology (Berl) 97: 376-82.

Witte EA, Davidson MC, Marrocco RT (1997) Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. Psychopharmacology (Berl) 132: 324-34.

Figure legends

Figure 1. Behavioral paradigm and the foraging model.

A: The oculomotor foraging task. After the initial fixation period, monkeys were presented with fifteen identical objects (white squares). One object was assigned as the target and the other objects were assigned as distractors. As the animals looked at the target for > 100 ms within six seconds, the target turned red and they received a liquid reward after 200 ms.

B: A schematic of the foraging model. In the exploitation mode, the animals select among unseen objects based on the memory of previous search, and their performance depends on both the capacity and decay of short-term memory. In the exploration mode, the animals randomly select an object without referring to their memory. These modes are switched according to the utility rate for each saccade.

Figure 2. An example of model-based analysis and the validity of model fitting.

A–C: Data in a control session in monkey T (gray bars) were compared with the data from a Monte Carlo simulation of the foraging model (5000 iterations, orange lines). The model parameters were selected from 6120 combinations to optimize the following three distributions of behavioral data (Methods): the relationship between saccade sequence and the proportion of recursive behavior (A), the relative frequency of revisiting behavior as a function of the number of intervening saccades (distance) from the previous same choice (B), and the distribution of saccade number in each trial (C). In this example, the coefficient of determination (CD) for model fitting was 0.91.

D: Distribution of the CD in all sessions. White and green histograms represent saline (control) and drug experiments, respectively.

E: Optimal WM parameters of the foraging model for each monkey control session. Boxes and whiskers indicate the first and third quartiles and the range of the data, respectively. The red

horizontal line indicates the median and the blue X indicates the mean.

Figure 3. Changes in visual search performance after administration of low-dose nicotine.
A: Comparison of the best-fit data following injection of saline (black lines) and nicotine (24 μg/kg, red lines) in two monkeys. Data from three saline and three nicotine experiments are overlaid on each panel.
B: Comparison of the best-fit data following injection of saline (black lines) and ketamine (1.5 mg/kg, cyan lines) in monkey S, adopted from the previous study (Sawagashira and Tanaka 2021). The arrows indicate that the effect of ketamine is different from that of nicotine.

Figure 4. Effects of AChR-related substances on the foraging model parameters.
A: Changes in the foraging model parameters after administration of an agonist (nicotine, 24 and 56 μg/kg) and antagonist (mecamylamine) of nicotinic AChR. Data were collected during 60 min after drug administration and were normalized (z-scored) with data obtained in control (saline) sessions. Each datapoint indicates single experiment and different symbols represent different animals. The gray bar indicates the mean of each condition. The *p*-values of the main drug effect obtained from two-way ANOVA are shown for statistically significant conditions only. The effect sizes and the results of regression analysis with a mixed effect model are summarized in Table 1.
B: Changes in the foraging model parameters after injection of an agonist (oxotremorine) and antagonist (scopolamine) of muscarinic AChR. The graph convention is the same as in A.

Figure 5. Effects of AChR-related substances on saccade parameters.
A: Changes in saccade velocity, saccade frequency, and inter-saccadic interval (ISI) after administration of an agonist (nicotine) and antagonist (mecamylamine) of nicotinic AChR. The

574 data for each animal were normalized (z-scored) with the data in control (saline) sessions. The
575 effect sizes and the results of regression analysis with a mixed effect model are summarized in
576 Table 2.
577 B: Changes in saccade parameters after injection of an agonist (oxotremorine) and antagonist
578 (scopolamine) of muscarinic AChR.
579

Table 1. Pharmacological effects on WM performance

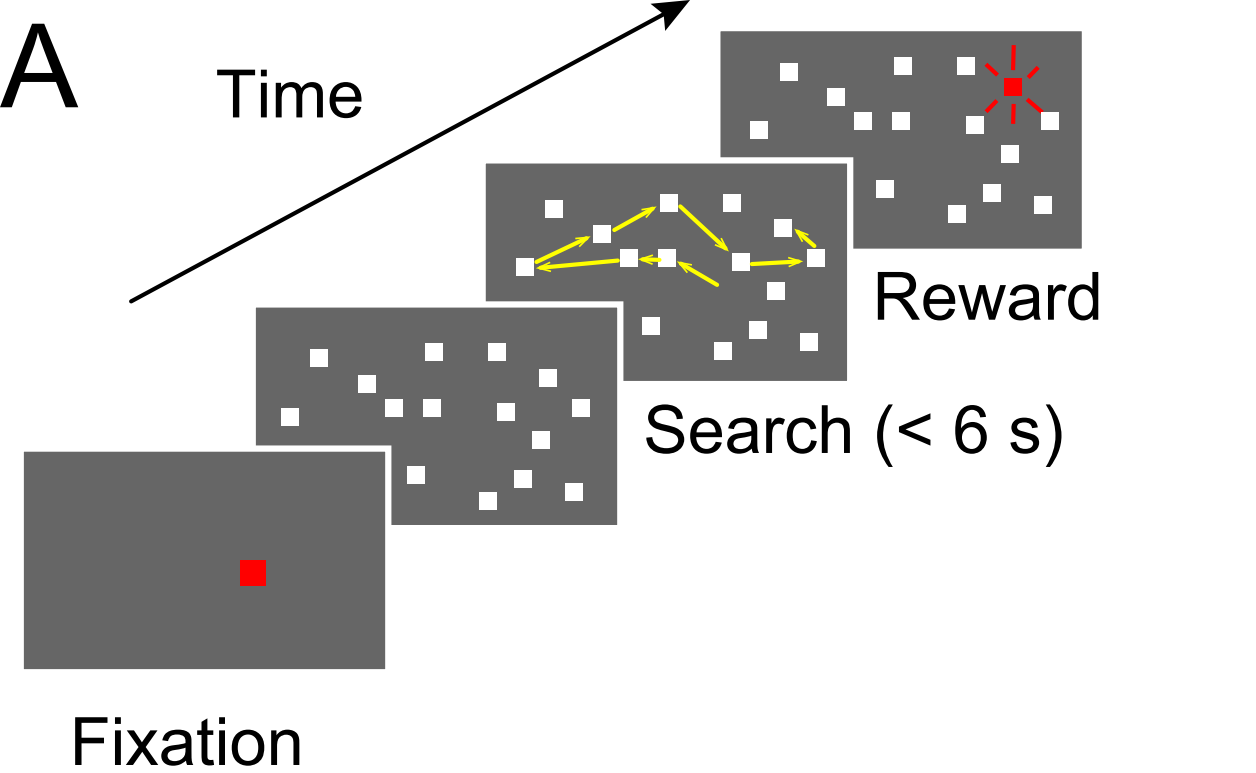
			LMM	
		Hedges' *g*	β_{fix}	*p*-value
Nicotine (24 μg/kg)	Capacity	0.59	0.62	0.16
	Utility	1.31	3.16*	< 0.01
	Decay	0.31	0.29	0.44
Nicotine (56 μg/kg)	Capacity	0.38	0.43	0.36
	Utility	1.41	3.07*	< 0.01
	Decay	0.79	1.15	0.07
Mecamylamine (nAChR antagonist)	Capacity	0.05	0.04	0.90
	Utility	0.98	1.57*	< 0.05
	Decay	0.76	1.07	0.06
Oxotremorine (mAChR agonist)	Capacity	0.38	0.30	0.44
	Utility	0.50	0.54	0.31
	Decay	0.86	0.87	0.12
Scopolamine (mAChR antagonist)	Capacity	0.66	0.87	0.20
	Utility	0.18	0.43	0.71
	Decay	0.42	0.80	0.40

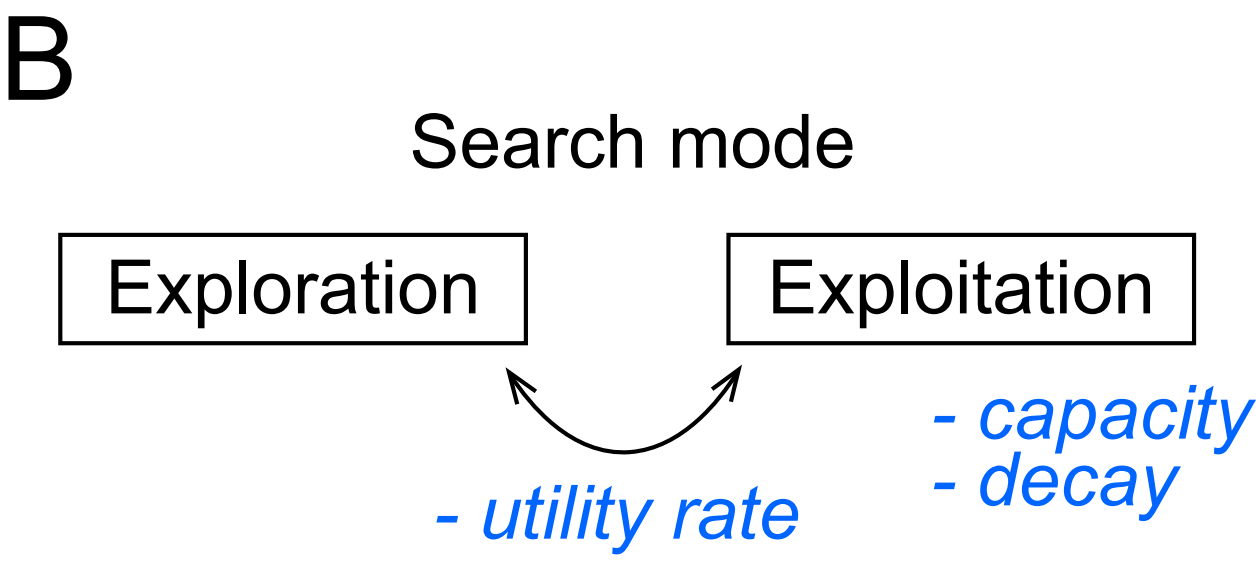
Asterisk indicates significant drug effect in each condition. LMM, linear mixed effect model.

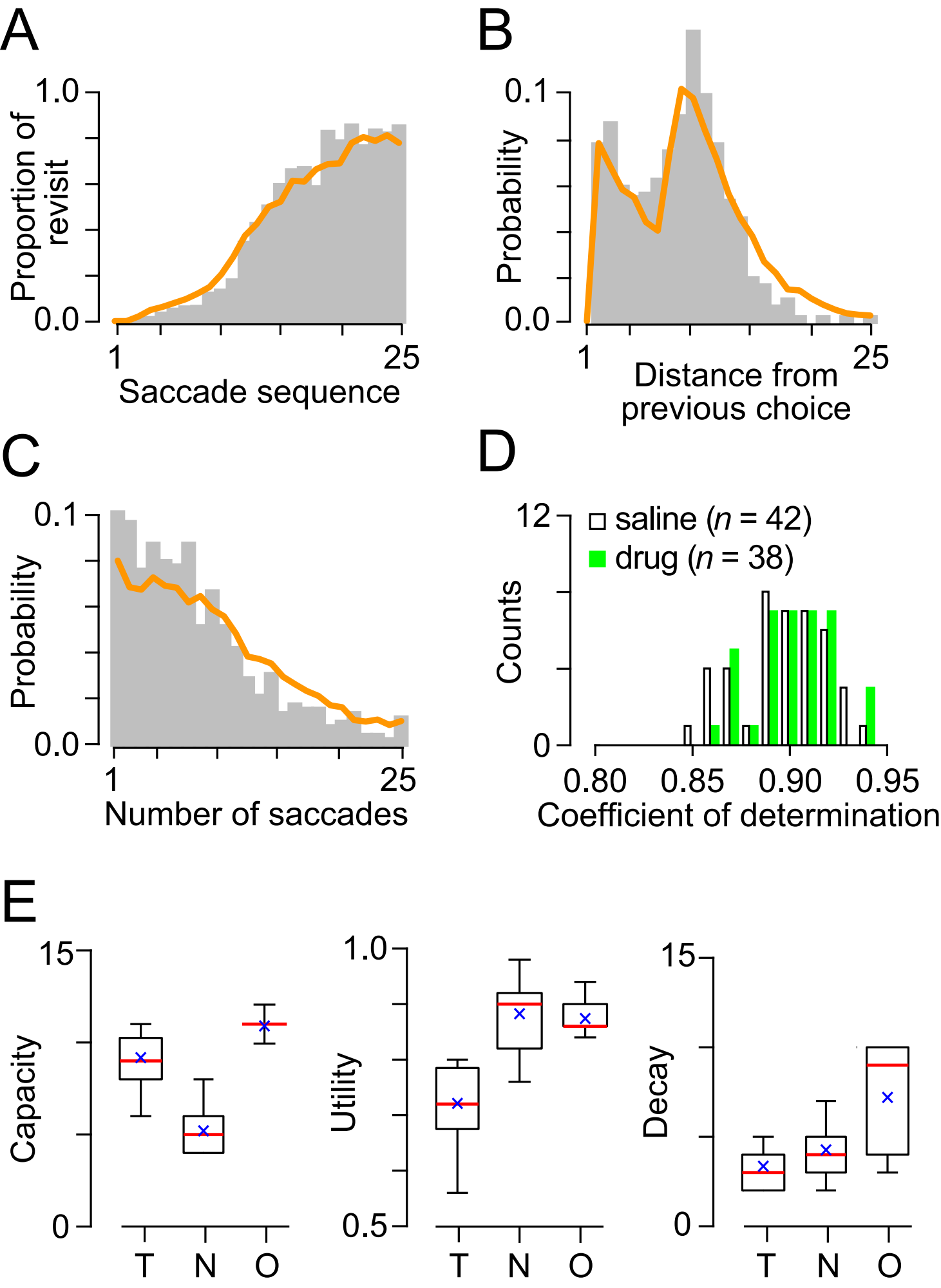
582 **Table 2. Pharmacological effects on eye movements**

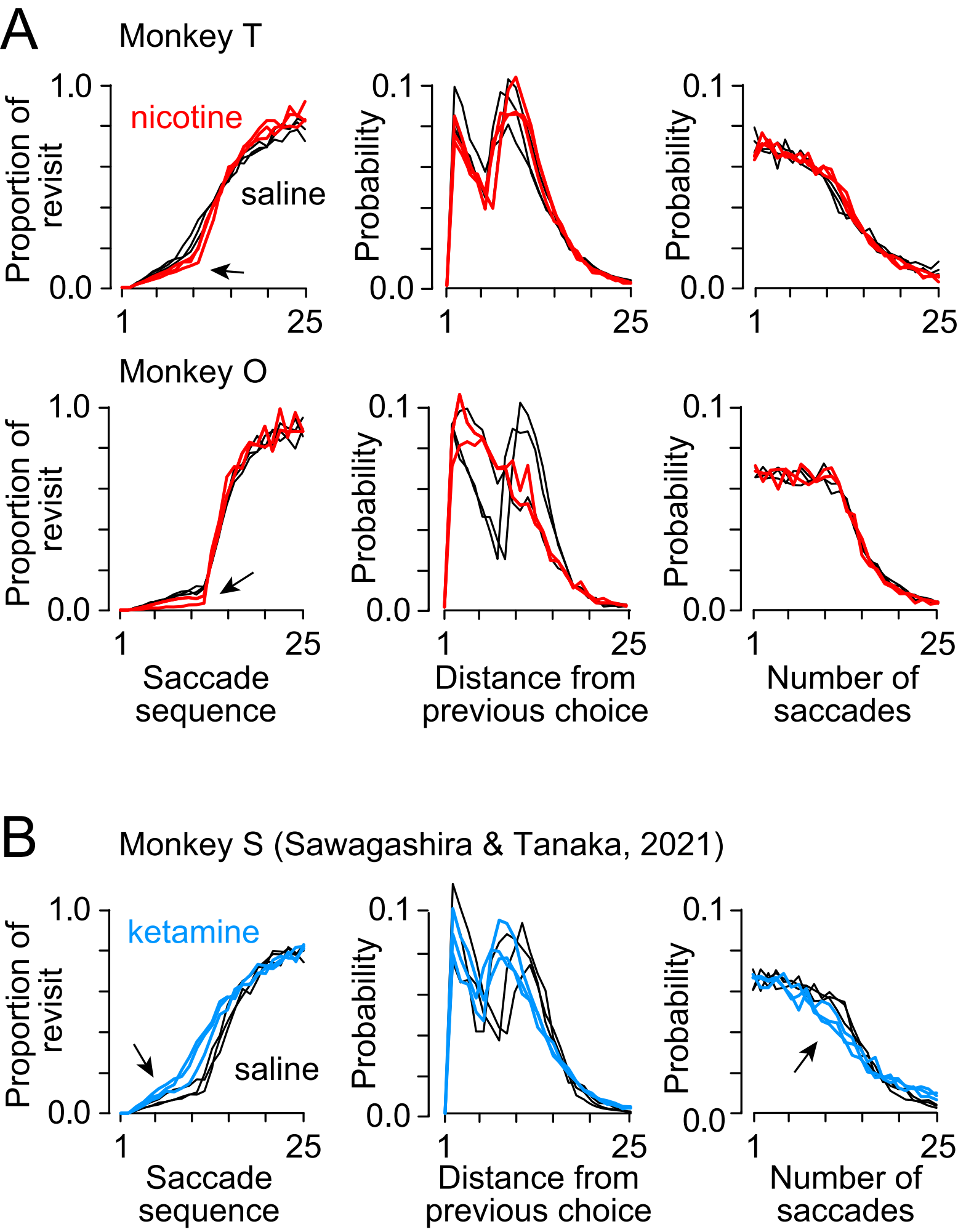
			LMM	
		Hedges' g	β_{fix}	p-value
Nicotine (24 μg/kg)	Velocity	0.65	0.55	0.12
	Frequency	0.94	−0.93*	< 0.05
	ISI	0.11	0.18	0.78
Nicotine (56 μg/kg)	Velocity	0.83	−0.93	0.06
	Frequency	0.26	−0.26	0.53
	ISI	0.09	0.30	0.74
Mecamylamine (nAChR antagonist)	Velocity	1.02	−0.88*	< 0.05
	Frequency	1.02	−1.17*	< 0.05
	ISI	1.31	3.18*	< 0.01
Oxotremorine (mAChR agonist)	Velocity	0.51	0.54	0.38
	Frequency	0.64	0.78	0.29
	ISI	0.13	0.18	0.79
Scopolamine (mAChR antagonist)	Velocity	1.66	−2.03*	< 0.01
	Frequency	0.47	0.52	0.35
	ISI	0.79	−2.14	0.13

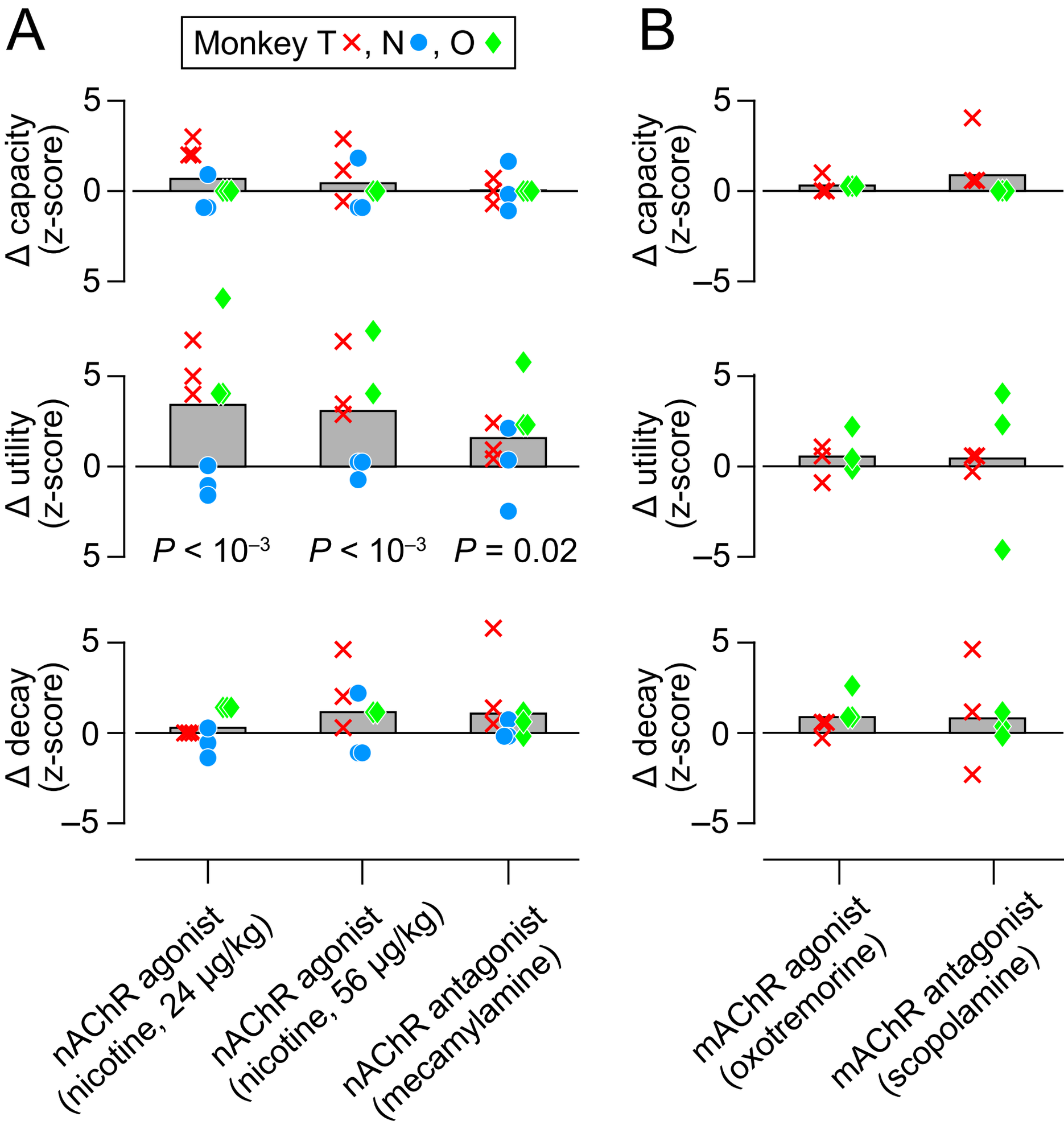
583 Asterisk indicates significant drug effect in each condition. LMM, linear mixed effect model.

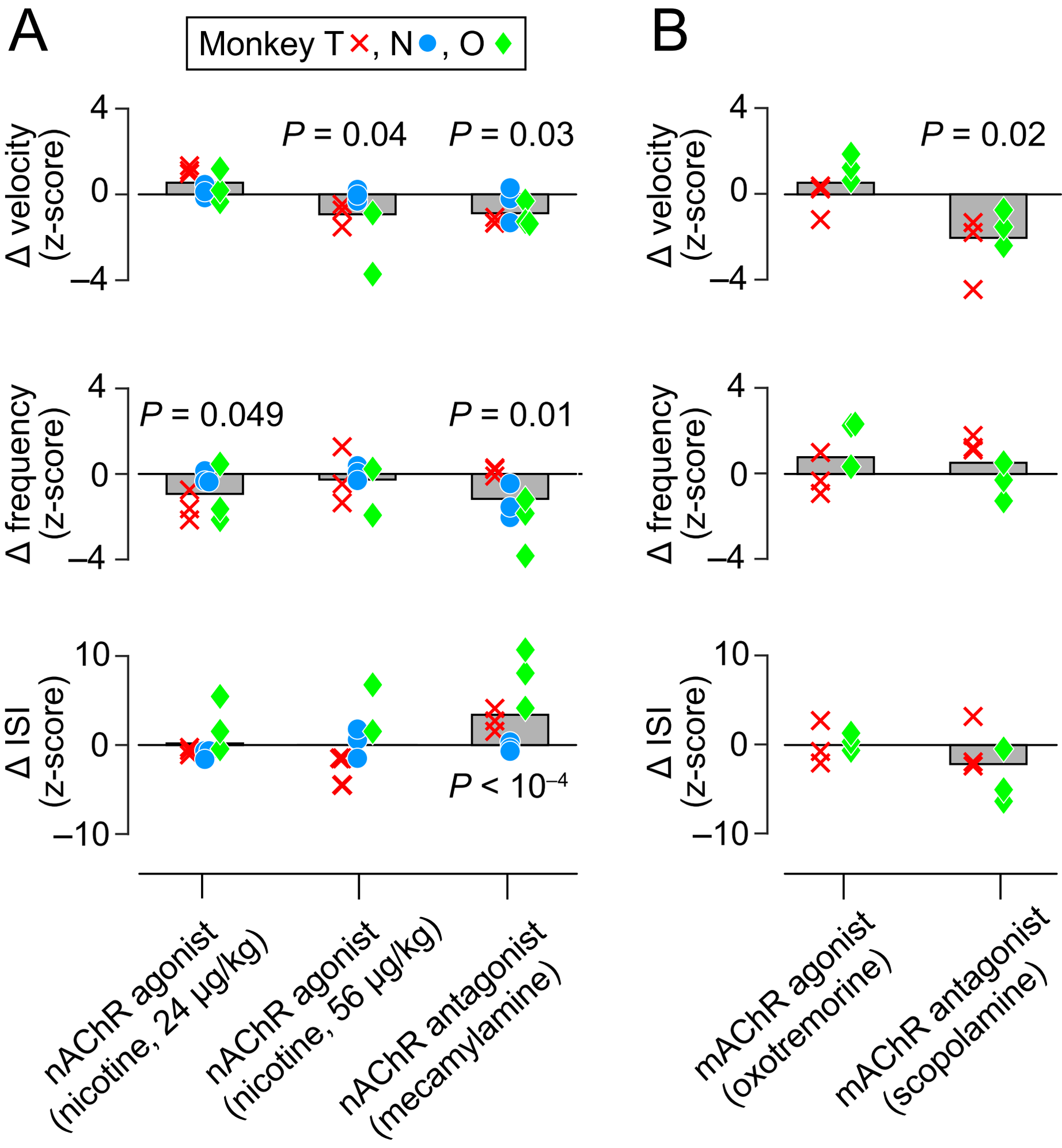

A
Time
Reward
Search (< 6 s)
Fixation


B
Search mode
Exploration
Exploitation
- capacity
- decay
- utility rate


A
1.0
0.0
Proportion of revisit
1
25
Saccade sequence
B
0.1
0.0
Probability
1
25
Distance from previous choice
C
0.1
0.0
Probability
1
25
Number of saccades
D
12
0
Counts
saline (n = 42)
drug (n = 38)
0.80
0.85
0.90
0.95
Coefficient of determination
E
15
0
Capacity
T
N
O
1.0
0.5
Utility
T
N
O
15
0
Decay
T
N
O


A
Monkey T
Proportion of revisit
1.0
0.0
nicotine
saline
1
25
Probability
0.1
0.0
Monkey O
Saccade sequence
Distance from previous choice
Number of saccades
B
Monkey S (Sawagashira & Tanaka, 2021)
ketamine


A
Monkey T ×, N ●, O ♦
Δ capacity (z-score)
Δ utility (z-score)
Δ decay (z-score)
5
0
−5
P < 10−3
P < 10−3
P = 0.02
nAChR agonist (nicotine, 24 μg/kg)
nAChR agonist (nicotine, 56 μg/kg)
nAChR antagonist (mecamylamine)
B
mAChR agonist (oxotremorine)
mAChR antagonist (scopolamine)


A
Monkey T ×, N ●, O ◆
Δ velocity (z-score)
4
0
–4
P = 0.04
P = 0.03
Δ frequency (z-score)
P = 0.049
P = 0.01
Δ ISI (z-score)
10
–10
P < 10–4
nAChR agonist (nicotine, 24 μg/kg)
nAChR agonist (nicotine, 56 μg/kg)
nAChR antagonist (mecamylamine)
B
P = 0.02
mAChR agonist (oxotremorine)
mAChR antagonist (scopolamine)