



Title	Nicotine promotes the utility of short-term memory during visual search in macaque monkeys
Author(s)	Sawagashira, Ryo; Tanaka, Masaki
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1 Original Investigations

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

4

5 Author names and affiliations:

6 Ryo Sawagashira^{1,2} and Masaki Tanaka¹

7 Departments of ¹Physiology and ²Psychiatry, Hokkaido University School of Medicine,
8 Sapporo 060-8638, Japan.

9

10 Author contributions:

11 RS and MT designed research; RS performed experiments; RS analyzed data; RS and MT
12 wrote and revised the paper

13

14 Correspondence should be addressed to:

15 R.S. <rsawa6133@med.hokudai.ac.jp > or M.T. <masaki@med.hokudai.ac.jp>

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37 **Abstract**

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

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

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

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

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

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

62 **Introduction**

63 The central cholinergic system is essential for a variety of cognitive functions, such as working
64 memory (WM), attention, and learning (Azimi et al. 2020; Cools and Arnsten 2021; Disney
65 2021; Sarter and Lustig 2019; Thiele and Bellgrove 2018). To restore these functions in patients
66 with dementia, acetylcholine receptor (AChR)-related drugs are often administered (Foster et
67 al. 2014; Sun et al. 2012). AChRs are largely classified into nicotinic and muscarinic receptors,
68 both of which are widely expressed in the brain and mediate cognitive functions (Dineley et al.
69 2015; Kruse et al. 2014).

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

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

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

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

96

97 **Materials and Methods**

98 *Animal preparation and surgery*

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

110

111 *Visual stimuli and behavioral paradigm*

112 The experiments were controlled by a Windows-based real-time data acquisition system
113 (TEMPO, Reflective Computing). Visual stimuli were presented on a 27-inch liquid crystal
114 display (XL2720Z, BenQ; refresh rate, 144 Hz) located 40 cm from the eyes (visual angle, 77°
115 $\times 62^\circ$). The animals were trained on the oculomotor foraging task for several months. In this
116 task, each trial began with the appearance of a red fixation point (FP) at either the center of the
117 display or at one of the four diagonal locations (10° eccentricity, Fig. 1A). After a random
118 fixation period (500–1000 ms, $< 2^\circ$ accuracy), the FP was extinguished, and 15 white squares
119 (1°) were simultaneously presented for 6 s. The locations of the stimuli were randomly chosen
120 for each trial from 76 possible sites covering the $40^\circ \times 24^\circ$ area (11×7 grid, $\geq 4^\circ$ apart each
121 other). Only one of the visual stimuli was associated with a liquid reward, and the animals were
122 trained to make sequential saccades until they received a reward within the 6-s time constraint.
123 During the experiments, targeting saccades were detected when eye position remained within
124 2° of each stimulus for > 100 ms. Once the animals looked at the object associated with a reward,
125 the target turned red, and a liquid reward was delivered after a 200-ms delay period. After the
126 reward delivery, the trial was terminated with a brief high-frequency sound. If monkeys failed
127 to find the target by the end of the time limit, the trial was aborted with a pair of beep sounds.
128 The intertrial interval was always 800 ms.

129

130 *Procedures of pharmacological experiments*

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

137 $\mu\text{g}/\text{kg}$), mecamylamine hydrochloride (nicotinic AChR antagonist, 1.0 mg/kg), oxotremorine
138 sesquifumarate salt (muscarinic AChR agonist, 3.0 $\mu\text{g}/\text{kg}$), and scopolamine hydrobromide
139 (muscarinic AChR antagonist, 20 $\mu\text{g}/\text{kg}$). One monkey (N) was tested for the agonist and
140 antagonist of nicotinic AChR only. The dosage of each drug was determined based on previous
141 pharmacological experiments in monkeys (Katner et al. 2004; Vardigan et al. 2015; Witkin
142 1989). The injection volume was 1.0 mL for all experiments. Pharmacological experiments
143 were spaced at least three days apart to avoid the effects of cumulative administration. We
144 initially tested the drugs associated with nicotinic AChRs and then examined the effects of drugs
145 associated with muscarinic AChRs. Since it took a long period of time to collect all the data
146 (5.3 ± 4.4 months, $n = 3$), the pharmacological effects were evaluated by comparing them with
147 the data after saline injection obtained within 10 days before and after each drug experiment.

148

149 *Data acquisition and analysis*

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

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

161 The pharmacological effects on WM performance were assessed using the previously

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

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

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

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

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

$$199 \quad \text{Data} \sim \beta_{\text{fix}} \times \text{Condition} + \beta_{\text{rand}} \times \text{Monkey}$$

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

207

208 **Results**

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

212 intervening saccades between initial and recursive choices (*B*), and 3) the number of saccades
213 in each trial (*C*). Using these distributions, we searched for the optimal parameters of the
214 foraging model that best accounted for the actual data (orange lines). The goodness-of-fit of the
215 model was evaluated by the coefficient of determination (*CD*), which was 0.91 for the
216 representative experiment shown in Figure 2*A–C* and averaged to 0.90 ± 0.02 (SD, $n = 42$) and
217 0.90 ± 0.02 ($n = 38$) for the experiments with saline and drug injection, respectively (Fig. 2*D*).
218 These values did not differ between drug conditions (unpaired t-test, $t_{78} = -0.85$, $p = 0.40$).

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

233 Figure 3*A* displays the distributions derived from the optimal models for three nicotine
234 ($24 \mu\text{g}/\text{kg}$, red lines) and three saline (black lines) experiments in two monkeys. Despite slight
235 inter-session variability, a clear difference between drug conditions was observed in both
236 animals (red versus black lines). For example, the data in the left column shows the decrease in

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

246 The changes in the WM parameters of the optimal model following administration of the
247 agonist (nicotine) and antagonist (mecamylamine) of nicotinic AChR are summarized in Figure
248 4A. To allow for direct comparison across monkeys, the data of each animal were z-scored using
249 control data. The utility rate significantly increased following administration of both the agonist
250 and antagonist of nicotinic AChR (two-way ANOVA, 24 $\mu\text{g}/\text{kg}$ nicotine, $F_{1,20} = 26.0$, $p < 10^{-3}$;
251 56 $\mu\text{g}/\text{kg}$ nicotine, $F_{1,19} = 28.5$, $p < 10^{-3}$; mecamylamine, $F_{1,23} = 7.3$, $p = 0.02$), whereas no
252 significant changes in the memory capacity and memory decay were found (24 $\mu\text{g}/\text{kg}$ nicotine,
253 $F_{1,20} = 3.4$, $p = 0.09$ and $F_{1,20} = 0.7$, $p = 0.41$; 56 $\mu\text{g}/\text{kg}$ nicotine, $F_{1,19} = 0.5$, $p = 0.48$ and $F_{1,19}$
254 $= 3.2$, $p = 0.09$; mecamylamine, $F_{1,23} = 0.01$, $p = 0.92$ and $F_{1,23} = 3.8$, $p = 0.07$ for memory
255 capacity and memory decay, respectively). Two additional indices were computed to further
256 quantify the drug effects (Table 1). First, we measured the effect size for each WM parameter.
257 Second, we assessed the drug effects with a linear mixed model (Methods), and compared the
258 resulting fixed effect coefficients. Both indices show that nicotine and mecamylamine had a
259 significant effect on utility rate, but not on other WM parameters.

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

262 were only slight (Fig. 5A and Table 2). By contrast, the nicotinic AChR antagonist
263 (mecamylamine) had greater effects, significantly decreasing saccade velocity (two-way
264 ANOVA, $F_{1,23} = 5.3, p = 0.03$) and frequency ($F_{1,23} = 7.6, p = 0.01$) and increasing the ISI ($F_{1,23}$
265 $= 29.6, p < 10^{-4}$). The increase in ISI was particularly marked, which correlated with utility rate
266 ($r = 0.71, p = 0.03$), in contrast to nicotine (low dose, $r = 0.34, p = 0.38$; high dose, $r = 0.32, p$
267 $= 0.44$). These results suggest that the mecamylamine effect on utility rate may be partially due
268 to changes in oculomotor parameters. To test this, a regression analysis of utility rate was
269 conducted with a linear mixed effect model incorporating both drug condition and eye
270 movement parameters as fixed effect variables (Methods). The results showed that the fixed
271 effect coefficients for drug condition decreased greatly in the mecamylamine experiment but
272 not in the experiment with high dosage of nicotine (mecamylamine, $\beta_{\text{drug}} = 0.10, p = 0.89, \beta_{\text{ISI}}$
273 $= 0.35, p < 0.01, \beta_{\text{velocity}} = -0.12, p = 0.70, \beta_{\text{frequency}} = -0.16, p = 0.51$; low dose nicotine, β_{drug}
274 $= 2.01, p = 0.08, \beta_{\text{ISI}} = 0.55, p = 0.12, \beta_{\text{velocity}} = 0.74, p = 0.27, \beta_{\text{frequency}} = -0.89, p = 0.10$; high
275 dose nicotine, $\beta_{\text{drug}} = 1.73, p = 0.03, \beta_{\text{ISI}} = -0.05, p = 0.79, \beta_{\text{velocity}} = -1.37, p < 0.01, \beta_{\text{frequency}} =$
276 $-0.31, p = 0.39$), suggesting that the significant increase in utility rate after mecamylamine
277 administration was partially attributable to changes in ISI.

278 When an agonist (oxotremorine) and antagonist (scopolamine) of muscarinic AChR
279 were administered in two monkeys, no significant change in the WM parameters was found
280 (Fig. 4B and Table 1). The only significant change was a decrease of saccade velocity following
281 injection of scopolamine (two-way ANOVA, $F_{1,11} = 8.9, p = 0.02$, Fig. 5B; linear mixed effect
282 model, $\beta_{\text{fix}} = -2.03, p = 0.006$, Table 2). We also failed to find any significant drug effect on
283 WM parameters even when incorporating oculomotor parameters as fixed effect variables
284 (oxotremorine, $\beta_{\text{drug}} = 0.45, 0.25, 0.39, p = 0.13, 0.62, \text{ and } 0.38$ for memory capacity, utility
285 rate, and memory decay, respectively; scopolamine, $\beta_{\text{drug}} = -0.14, -5.48, \text{ and } -0.93, p = 0.93,$
286 $0.09, \text{ and } 0.69$, respectively).

287

288 **Discussion**

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

297

298 *Effects of nicotinic AChR agonist and antagonist*

299 Nicotine administration is known to improve a variety of cognitive functions (Rezvani and
300 Levin 2001). In particular, the effect on WM has been reported in rodents (Rushforth et al.
301 2011), pigeons (Kangas and Branch 2012), monkeys (Buccafusco et al. 1999; Buccafusco and
302 Terry 2004), and humans (Heishman et al. 2010; Kangas and Branch 2012; Levin et al. 1996;
303 Spinelli et al. 2006; Sultana et al. 2013; Upright and Baxter 2021). Katner et al. (2004)
304 examined the effects of nicotine in monkeys trained on a variety of WM tasks and found that
305 performance improved regardless of memory domain, with more difficult conditions showing
306 a greater effect. However, it is not clear whether the improved performance in these tasks was
307 simply due to better retention of short-term memory or reflected changes in central executive
308 functions. Using the foraging task and a related model, we found in this study that nicotine
309 improved task performance primarily by increasing the use of short-term memory, rather than
310 by increasing the memory capacity. Contrary to nicotine, our previous study showed that low
311 doses of ketamine (an NMDA receptor antagonist) reduced utility rate along with a mild

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

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

329 Since this study was conducted with systemic administration, it is not possible to
330 identify the site of nicotine's action in improving executive function. It is known that $\alpha 4\beta 2$ and
331 $\alpha 7$ subunits of nicotinic AChRs are widely expressed in the brain, and the former is known to
332 regulate cognition, attention, and emotion, while the latter is known to promote memory and
333 learning and protect the brain (Azimi et al. 2020; Paterson and Nordberg 2000; Sarter and Lustig
334 2019). In particular, the $\alpha 4\beta 2$ subunit shows high affinity for nicotine and is expressed in the
335 frontal cortex and striatum (Gotti et al. 2006), which might contribute to the increased utility
336 rate. In the future, ligands specific for this subtype could be administered systemically or locally

337 in the brain to further investigate the underlying mechanism.

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

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

359

360 *Effects of muscarinic AChR agonist and antagonist*

361 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.

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524 **Figure legends**

525 **Figure 1. Behavioral paradigm and the foraging model.**

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

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

535

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

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

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

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

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

550

551 **Figure 3. Changes in visual search performance after administration of low-dose nicotine.**

552 A: Comparison of the best-fit data following injection of saline (black lines) and nicotine (24
553 $\mu\text{g}/\text{kg}$, red lines) in two monkeys. Data from three saline and three nicotine experiments are
554 overlaid on each panel.

555 B: Comparison of the best-fit data following injection of saline (black lines) and ketamine (1.5
556 mg/kg , cyan lines) in monkey S, adopted from the previous study (Sawagashira and Tanaka
557 2021). The arrows indicate that the effect of ketamine is different from that of nicotine.

558

559 **Figure 4. Effects of AChR-related substances on the foraging model parameters.**

560 A: Changes in the foraging model parameters after administration of an agonist (nicotine, 24
561 and 56 $\mu\text{g}/\text{kg}$) and antagonist (mecamylamine) of nicotinic AChR. Data were collected during
562 60 min after drug administration and were normalized (z-scored) with data obtained in control
563 (saline) sessions. Each datapoint indicates single experiment and different symbols represent
564 different animals. The gray bar indicates the mean of each condition. The p -values of the main
565 drug effect obtained from two-way ANOVA are shown for statistically significant conditions
566 only. The effect sizes and the results of regression analysis with a mixed effect model are
567 summarized in Table 1.

568 B: Changes in the foraging model parameters after injection of an agonist (oxotremorine) and
569 antagonist (scopolamine) of muscarinic AChR. The graph convention is the same as in A.

570

571 **Figure 5. Effects of AChR-related substances on saccade parameters.**

572 A: Changes in saccade velocity, saccade frequency, and inter-saccadic interval (ISI) after
573 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

580 **Table 1. Pharmacological effects on WM performance**

		Hedges' <i>g</i>	LMM	
			β_{fix}	<i>p</i> -value
Nicotine (24 $\mu\text{g}/\text{kg}$)	Capacity	0.59	0.62	0.16
	Utility	1.31	3.16*	< 0.01
	Decay	0.31	0.29	0.44
Nicotine (56 $\mu\text{g}/\text{kg}$)	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

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

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

		Hedges' <i>g</i>	LMM	
			β_{fix}	<i>p</i> -value
Nicotine (24 $\mu\text{g}/\text{kg}$)	Velocity	0.65	0.55	0.12
	Frequency	0.94	-0.93*	< 0.05
	ISI	0.11	0.18	0.78
Nicotine (56 $\mu\text{g}/\text{kg}$)	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.









