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Striatal dopamine modulates timing of self-initiated saccades

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Keywords

self-timing, saccade, dopamine, acetylcholine, basal ganglia, primate

Abbreviations

ACh, acetylcholine; ANOVA, analysis of variance; CNV, contingent negative variation; DA, dopamine; FP, fixation point; mAChR, muscarinic acetylcholine receptor; nAChR, nicotinic acetylcholine receptor; MR, magnetic resonance; MS, memory-guided saccade; MI, modulation index.

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Abstract

The ability to adjust movement timing is essential in daily life. Explorations of the underlying neural mechanisms have reported a gradual increase or decrease in neuronal activity prior to self-timed movements within the cortico-basal ganglia loop. Previous studies in both humans and animals have shown that endogenous dopamine (DA) plays a modulatory role in self-timing. However, the specific site of dopaminergic regulation remains elusive because the systemic application of DA-related substances can directly alter both cortical and subcortical neuronal activity. To investigate the role of striatal DA in self-timing, we locally injected DA receptor agonists or antagonists into the striatum of two female monkeys (*Macaca fuscata*) while they performed two versions of the memory-guided saccade (MS) task. In the conventional, triggered MS task, animals made a saccade to the location of a previously flashed visual cue in response to the fixation point offset. In the self-timed MS task, monkeys were rewarded for making a self-initiated saccade within a predetermined time interval following the cue. Infusion of a small amount of a D_1 or D_2 antagonist led to early saccades in the self-timed, but not the triggered MS tasks, while infusion of DA agonists produced no consistent effect. We also found that local administration of nicotinic but not muscarinic acetylcholine receptor agonists and antagonists altered the timing of self-initiated saccades. Our data suggest that the timing of self-initiated movements may be regulated by the balance of signals in the direct and indirect basal ganglia pathways, as well as that between both hemispheres of the brain.

Introduction

The ability to perform precisely timed actions is crucial in daily life. In many situations, we must make self-initiated movements in anticipation of forthcoming events. In laboratory conditions, scalp potential that is generated prior to an expected event (contingent negative variation; CNV) is known to reflect the prediction of timing for behavioral decisions (Pfeuty et al., 2005; Macar and Vidal, 2003; van Rijn et al., 2011). In experimental animals, a similar gradual ramp-up of neuronal activity before self-timed movements has been reported in the basal ganglia (Romo and Schultz, 1992; Lee and Assad, 2003; Turner and Anderson, 2005), thalamus (Tanaka, 2007), and the cortex (Okano and Tanji, 1987; Kurata and Wise, 1988; Murakami et al., 2014), suggesting that the basal ganglia-thalamocortical pathways are responsible for the generation of the climbing neuronal activity. These activities appear to have a causal role in the adjustment of movement timing (Tanaka, 2006; Kunimatsu and Tanaka, 2012; van Donkelaar et al., 2000), and may partly reflect the subjective passage of time during motor preparation (Macar and Vidal, 2009; Wittmann, 2013).

To report or produce a certain time interval by making a self-initiated movement, the subjects need to suppress the movement until the time comes. Therefore, the impairment of self-timing could also reflect the level of patience, or alternatively, impulsivity could result from overestimation of time intervals (Wittmann and Paulus 2008). Endogenous neuromodulators such as dopamine (DA) and acetylcholine (ACh) regulate signals in the basal ganglia (Calabresi et al., 2000; Surmeier et al., 2007), and have been shown to play a role in self-timing. For instance, patients with Parkinson's disease show reduced CNV (Ikeda et al., 1997), exhibit a difficulty in the self-initiation of actions (Kelly et al., 2002; Jones and Jahanshahi, 2009), and present impairments in the ability to report time interval in the range of seconds (Malapani et al., 1998; Jones et al., 2008; for review, see Allman and Meck, 2012). Pharmacological experiments have also shown that both DA and ACh are crucial for movement timing (for review, see Coull et al., 2011). For example, the systemic administration of DA-related and ACh-related substances altered the peak timing of temporal production in trained rats (Matell et al., 2004; MacDonald and Meck, 2005; Hinton and Meck, 1997), while in humans, the oral administration of DA antagonists disturbed time perception in the range of seconds (Rammsayer et al., 1993). However, because both DA and ACh are known to modulate neuronal processes within the frontoparietal cortices that also participate in temporal processing and decision making (Williams and Goldman-Rakic, 1995; Vijayraghavan et al., 2007; for reviews, see Arnsten, 2009; Noudoost and Moore, 2011; Thiele, 2013; Sarter et al., 2014), the specific roles of neuronal modulators in the basal ganglia remain elusive.

To address this issue, we manipulated DA signals in the striatum of monkeys by locally injecting DA receptor agonists and antagonists while they performed the oculomotor version of the self-timing task. We found that DA receptor antagonists altered the timing of self-initiated saccades, while DA receptor agonists did not produce any consistent effect. Importantly, the effects on timing were only slight for reactive saccades that were triggered immediately by visual stimuli. Furthermore, we found that agonists and antagonists of nicotinic but not muscarinic ACh receptors altered self-timing. These results suggest that the timing of self-initiated movements might be regulated according to the balance of signals between direct and indirect basal ganglia pathways, as well as that between both hemispheres.

Experimental procedures

We used two Japanese monkeys (*Macaca fuscata*, female, 7 and 8 kg, monkeys *B* and *G*) in the present experiments. All experimental protocols were evaluated and approved by the Hokkaido University Animal Care and Use Committee. Much of the experimental procedures were similar to those described previously (Tanaka, 2005; Kunimatsu and Tanaka, 2010).

Animal preparation.

Using sterile procedures and general isoflurane anesthesia, the animals were subjected to two separate surgeries in which we implanted a pair of head holders and an eye coil. Analgesics were administered during each surgery and for several days afterwards. Upon full recovery from the surgery, the monkeys were trained to perform oculomotor tasks. During training and the subsequent experimental sessions, the head of each monkey was secured to a primate chair located in a darkened booth, and horizontal and vertical eye position signals were recorded using the search coil technique (MEL-25; Enzanshi Kogyo). After completion of the eye movement training, we conducted a third surgery in which we placed a recording cylinder over the head-body junction of the caudate nucleus that was verified by means of magnetic resonance (MR) images taken before and after the surgery. Daily recording sessions began after full recovery from the surgery. Water intake was monitored regularly so that monkeys were motivated to perform the behavioral tasks.

Visual stimuli and behavioral tasks.

The experiments were controlled by the TEMPO system (Reflective Computing). Visual stimuli were presented on a 24-inch cathode-ray tube monitor (refresh rate: 60 Hz) positioned 38 cm from the eyes, and subtended 64 × 44° of the visual angle. We used three saccade paradigms (Fig. 1). In the visually-guided saccade task, the saccade target appeared at the time of the fixation point (FP) offset, and monkeys made an immediate saccade. In the conventional,

triggered memory-guided saccade task (triggered MS task; Hikosaka and Wurtz, 1983), a visual cue was presented briefly (100 ms) during central fixation. Monkeys were required to remember the cue location, and maintain fixation for an additional delay interval which was random 1500 ± 900 ms and 1000 ± 200 ms for monkeys *B* and *G* respectively. The animals made a saccade to the cue location within 400 ms of the FP offset. In the self-timed memory-guided saccade task (self-timed MS task; Tanaka, 2006, 2007; Ashmore and Sommer, 2013), the animals were required to make a saccade to the cue location within a predetermined time interval following the cue offset (1100 ± 300 ms and 1200 ± 300 ms for monkeys *B* and *G*, respectively). The FP disappeared only after the animals generated a self-initiated saccade, i.e., when eye position deviated > 3° from the FP. For all three tasks, the color of the FP was initially gray for 400 ms and then changed to red (triggered MS task and visually-guided saccade task) or blue (self-timed MS task) to signal the trial type. The saccade target and visual cue were presented either 16° left or right of the FP. The size of the eye position window was 2° for initial fixation and 4° for the saccade target. Correct performance was reinforced with a drop of liquid reward at the end of each trial. Each trial was presented in a pseudo-random order within a block that consisted of six different trials (three different tasks in opposite directions). Detailed behavioral analyses for these saccade tasks have been previously performed (Kunimatsu and Tanaka, 2012).

Neuronal recording.

To record from single neurons, a glass-coated tungsten electrode (Alpha Omega Engineering) was lowered into the striatum through a 23-gauge guide tube using a micromanipulator (MO-97S; Narishige). The location of each penetration was adjusted using a grid system attached to the recording cylinder. Signals obtained from the electrodes were amplified (Model 1800; A-M Systems), filtered (Model 3625; NF Co.), and monitored online using oscilloscopes and an audio device. We isolated the waveforms of single neurons using a real-time spike sorter with template-matching algorithms (MSD or ASD; Alpha Omega Engineering). During the experiments, occurrences of action potentials were time stamped and saved in files along with the data of eye movements and visual stimuli.

Pharmacological experiments.

For the local application of dopamine- (DA) or acetylcholine- (ACh) related agents, we used a custom-made injectrode consisting of an epoxy-coated tungsten microelectrode (FHC Inc.) and a silica tube (Polymicro Technologies Inc.). Injections were conducted at or near the sites where saccade-related neuronal activities were previously recorded. To locate the injection sites, multiunit activities were recorded through the electrode in each experiment. The silica tube was

connected to a 10-μL Hamilton microsyringe, and a small amount of drugs (1 μL for each of 2–3 sites along the penetration, > 500 μm apart) was pressure-injected using a micropump (NanoJet, Chemyx Inc.). We used the following agents at the specified concentrations: D_1 agonist (SKF38393, 3.0 μg/μL), D_1 antagonist (SCH23390, 8.0 μg/μL), D_2 agonist (quinpirole, 5.0 μg/μL), D_2 antagonist (eticlopride, 6.0 μg/μL), nicotinic ACh (nACh) agonist (nicotine, 8.0 μg/μL), nACh antagonist (mecamylamine, 6.0 μg/μL), muscarinic ACh (mACh) agonist (oxotremorine, 6.0 μg/μL), and mACh antagonist (scopolamine, 10.0 μg/μL; AF-DX, 5.0 μg/μL). The concentrations of these drugs were chosen based on the previous experiments in monkeys and rodents (Hernandez-Lopez et al., 1996; Floresco et al., 2003; Pratt and Kelley, 2004; Hiranita et al., 2006; Mark et al., 2006; Nakamura and Hikosaka, 2006). For each site, the effects of the drugs were tested in the following order; eticlopride, quinpirole, SCH23390, SKF38393, mecamylamine, nicotine, oxotremorine and AF-DX. For monkey G, the effects of DA-related agents were examined in both hemispheres while those of ACh-related agents were examined only in the left hemisphere. For monkey B, experiments were conducted only in the right hemisphere. To compare the effects of agents, different drugs were administered repeatedly at single sites on different days. We assessed the resulting effects by comparing eye movements before and 15–90 min after drug infusion. In separate control experiments, we injected saline (1 μL for each of 2–3 sites along the penetration, > 500 μm apart) into the same locations to ensure that the changes in behavior could not be attributed to the non-specific volume effects. The injection sites were reconstructed based on MR images (Siemens, Prisma 3T, 0.5-mm-thick slices).

Data acquisition and analyses.

Spike timing and eye movement data were sampled at 1 kHz and saved in files during the experiments. Further off-line analyses were performed using Matlab (MathWorks). Saccades were detected when angular eye velocity exceeded 40°/s and eye displacement was > 3°. Trials were excluded from the analysis when saccades landed > 5° from the cue location. Saccade latency was defined as the time from either the cue offset (self-timed MS task) or the FP offset (triggered MS task and visually-guided task) to the time of saccade initiation. In the self-timed MS trials, the monkeys sometimes generated saccades that were too early or too late. These trials were aborted as error trials during the experiment, but were included in the subsequent analyses. The rate of successful trials in the self-timed task before drug infusion were 85% and 91% for monkeys *B* and *G*, respectively.

We considered neurons to be task-related when they showed differential activity between the baseline period (250 ms before the change in FP color) and the delay period (200–

600 ms before saccades) in the self-timed MS trials in either direction (paired *t*-test, $p < 0.05$). We quantified the directionality of the delay period activity by computing the modulation index (MI), defined as (*Contra* − *Ipsi*)/(*Contra* + *Ipsi*), where *Contra* was neuronal activity during the delay period for contraversive self-timed saccades and *Ipsi* was that measured for ipsiversive saccades. We also assessed directionality for individual neurons by performing a statistical test (unpaired *t*-test) between saccades in the opposite directions.

To assess changes in saccade latencies following local drug infusion, we used Kolmogorov-Smirnoff test for the comparison in each experiment. For multiple sites in each or both monkeys, we measured the difference in median saccade latencies in each condition, and applied Wilcoxon signed-rank test and the analysis of variance (ANOVA). We chose the difference rather than the ratio of saccade latencies for quantification because self-timed saccade latency must reflect neural processes for both temporal monitoring and saccade production while that in the other two tasks reflected only the latter.

Results

Properties of preparatory activity

To determine the sites of drug infusion, we initially searched for neurons in the caudate nucleus that discharged before self-timed saccades. We recorded from a total of 104 neurons that exhibited preparatory activity before saccades. Most of these neurons (98/104, 95%) were found in the anterior part of the caudate nucleus, while the remaining neurons (5%) were recorded from the more posterior body part of the caudate nucleus (Fig. 2A). Figure 2B shows the data for a representative neuron responding to ipsiversive saccades during the self-timed and the triggered MS tasks. Although neuronal activity started just after the cue offset and increased gradually until saccade initiation in both tasks, the activity was much greater in the self-timed task. For 59 of the 104 recorded neurons (57%), neuronal activity during the delay period in the self-timed saccade task was directional (unpaired *t*-test, $p < 0.05$, Fig. 2C, filled bars). Of these, 51% (30/59) showed a preference for contraversive saccades, while the remaining neurons (29/59, 49%) showed a preference for ipsiversive saccades. We assessed the directionality of the population of neurons by computing the modulation index (Methods), which averaged -0.03 ± 0.45 (SD) and was not different from zero (one sample *t*-test, $p = 0.96$, Fig. 2C). These results were consistent with the previous study showing that neuronal activity in the striatum during saccade preparation exhibited no significant directional bias (Lau and Glimcher, 2007).

Effects of dopamine signals on saccade timing

To explore the causal role of striatal dopamine (DA) in self-timing, we injected a small amount of DA receptor agonist or antagonist into three caudate nuclei of two monkeys. Figure 3A plots the data from a single experiment. When a D_2 antagonist (eticlopride, 2.0 μL in volume, concentration 6.0 μg/μL) was injected into the right caudate nucleus, the latency of ipsiversive self-timed saccades slightly decreased (left column, Kolmogorov-Smirnoff test, $p < 0.01$), whereas those of triggered memory-guided saccades and visually-guided saccades remained unchanged ($p > 0.05$).

To quantify the effects of D_2 antagonist, we compared median saccade latencies across the tasks (Fig. 3B). Among 11 injection sites (5 and 6 sites in monkeys *B* and *G*, respectively), a significant change in self-timed saccade latency was found in 9 sites (Kolmogorov-Smirnoff test, $p < 0.05$, Fig. 3B, left panel and Table 1). Of these, 3 sites (33%) altered the saccade latency in both directions, while the remaining sites altered the latency of either ipsiversive ($n = 3$) or contraversive ($n = 3$) self-timed saccades. When the animals made memory-guided saccades in response to the FP offset (triggered MS task), a D_2 antagonist shortened the latency of ipsiversive saccades in 4 out of 11 experiments and prolonged the latency of contraversive saccades in 2 experiments (Fig. 3B, middle panel). In contrast, when the animals generated immediate saccades in response to the onset of visual stimuli (visually-guided saccade task), a D_2 antagonist *increased* the latency of contraversive saccades in more than half of the experiments (64%, 7/11, Fig. 3B, right panel).

Overall, ipsiversive self-timed saccades were significantly facilitated following D_2 antagonist injection (Wilcoxon signed-rank test, $p < 0.01$, $n = 11$, Fig. 3B), while the effects on contraversive self-timed saccades varied from site to site and did not reach a statistically significant level ($p = 0.32$). Coefficient of variation for self-timed saccade latency remained unchanged for saccades in both directions (paired *t*-test, $p > 0.05$). In addition, contraversive visually-guided saccades were delayed following D_2 antagonist infusion (Wilcoxon signed-rank test, $p < 0.01$, Fig. 3B), whereas ipsiversive saccades remained unchanged ($p = 0.24$). A two-way analysis of variance (ANOVA) showed that the amount of changes in latency significantly depended both on the task and saccade direction ($p < 0.05$) without interaction between them ($p = 0.90$). Post hoc multiple comparisons indicated that changes in latency were greater for the self-timed task than the other two tasks (Scheffe's methods, $p < 0.05$). When the changes in ipsiversive saccade latencies were normalized for the standard deviation in the control block, the values averaged –1.01 ± 1.80 (SD, $n = 11$), 0.29 ± 0.37 and 0.03 ± 0.66 for the self-timed, triggered MS, and visually-guided saccade tasks, respectively. These values did not differ significantly between the tasks (one-way repeated measures ANOVA, $p = 0.10$).

We repeated similar experiments with various DA receptor agonists and antagonists. For individual experiments, Table 1 summarizes the effects on the self-timed saccade latency in each experiment. Each panel in Figure 4 compares the changes in median latency in the three saccade tasks following the drug infusion. Again, a D_2 antagonist shortened the latency of ipsiversive saccades in the self-timed task and the triggered MS task (Fig. 4A, Wilcoxon signed-rank test, $p < 0.05$, $n = 11$), while the same drug delayed contraversive visually-guided saccades ($p < 0.05$). In contrast, a D_2 agonist (quinpirole) failed to alter saccade latency in any task (Fig. 4B, Wilcoxon signed-rank test, $p > 0.05$, $n = 11$). For a D_1 antagonist (SCH23390), the latency of ipsiversive self-timed saccades significantly decreased (Fig. 4C, $p < 0.01$, $n = 11$), while that in the triggered MS task remained unchanged. The same drug also shortened the latency of ipsiversive visually-guided saccades ($p < 0.05$) and delayed contraversive saccades ($p < 0.05$). Conversely, a D_1 agonist (SKF38393) showed no effect in the population ($p > 0.05$, $n = 9$), while slight but significant changes in latency were observed in a few experiments (Fig. 4D, filled symbols). These results were consistent between the two animals (three-way ANOVA, $p > 0.05$ for the factor of animals). When the changes in ipsiversive saccade latencies were normalized for the SD in the control block, the values for D_2 antagonist averaged $–0.19 \pm 0.25$, $–0.16 \pm 0.47$ and $–0.29 \pm 0.41$ for the self-timed, triggered MS, and visually-guided saccade tasks, respectively. These values did not differ significantly between the tasks (one-way repeated measures ANOVA, $p = 0.73$). When a similar amount of saline (2.0–3.0 μL) was injected into 9 effective sites in three caudate nuclei, saccade latency remained unchanged in the population (Wilcoxon signed-rank test, $p > 0.05$, $n = 9$), while contraversive self-timed saccades in a few experiments exhibited a significant change in latency (Table 1). Because saline infusion did not alter ipsiversive saccade latency in all nine experiments, the effects of DA antagonists mostly found in ipsiversive saccades (Figs. 4A and C) were unlikely to be attributed to the volume effects. Although the previous study demonstrated that the different effects of DA antagonist injection showed clear topographic organization (Nakamura and Hikosaka, 2006), we failed to find any such trend, possibly because our injection sites clustered in a narrow range and extended more anteriorly than the previous study.

Role of acetylcholine in saccade timing

Because endogenous acetylcholine is known to regulate neuronal activity in the striatum (Zhou et al., 2002), we next examined the effects of ACh-related substances in both hemispheres of two monkeys. We initially tested the effects of a non-selective mAChR antagonist (scopolamine), but failed to find any significant changes in saccade latency ($n = 6$ sites,

Wilcoxon signed-rank test, $p > 0.05$). This might be because different subtypes of mAChRs regulate neuronal activity in the striatum in opposing directions, and the multiple effects of a non-selective antagonist might be canceled out. We therefore examined the effect of a partially selective (M_2/M_4) mAChR antagonist (AF-DX) and agonist (oxotremorine sesquifumarate), as well as that of a non-selective nAChR antagonist (mecamylamine) and agonist (nicotine) on eye movements.

Figures 5A and B summarize the observed changes in median saccade latency following the local application of nAChR-related agents. For individual experiments, a blockade of nAChR (mecamylamine) in the caudate nucleus facilitated contraversive self-timed saccades in 3 out of 8 experiments (Fig. 5A and Table 2, Kolmogorov-Smirnoff test, $p < 0.05$) and ipsiversive self-timed saccades in 4 experiments ($p < 0.05$). Overall, the saccade latency before and after the mecamylamine injection differed only during the contraversive self-timing task (Wilcoxon signed-rank test, $p < 0.05$, $n = 8$). For nAChR agonist (nicotine), a significant change in contraversive self-timed saccade latency was observed in only one out of eight experiments (Fig. 5B and Table 2, Kolmogorov-Smirnoff test, $p < 0.05$), while the delay in saccade latency was statistically significant in the population (Wilcoxon signed-rank test, $p < 0.01$). When the changes in contraversive saccade latency were normalized for the SD of control trials, the values for nAChR antagonist averaged –0.44 ± 0.40, 0.13 ± 0.46 and 0.02 ± 0.17 for the self-timed, triggered MS and visually-guided saccade tasks, respectively. The values for the self-timed saccades significantly differed from those for the other two tasks (one-way repeated measures ANOVA followed by post hoc multiple comparisons with Scheffe's method, $p < 0.05$). However, the normalized values for nAChR agonist did not differ significantly between the tasks (0.26 ± 0.60, 0.15 ± 0.34 and –0.10 ± 0.32 for the self-timed, triggered MS and visually-guided saccade tasks, respectively, $p = 0.27$).

Figures 5C and D plot the data for mAChR-related substances. In the population, the mAChR antagonist did not produce any significant effect across conditions (Fig. 5C, Wilcoxon signed-rank test, $p > 0.05$, $n = 8$). During individual experiments, local application of the mAChR antagonist (AF-DX) occasionally facilitated ($n = 2$) or delayed ($n = 2$) contraversive self-timed saccades (Fig. 5C and Table 2, Kolmogorov-Smirnoff test, $p < 0.05$). Furthermore, a partially selective mAChR agonist (oxotremorine) also failed to alter saccade latency in all conditions except for contraversive triggered MS trials (Fig. 5D and Table 2). Thus, nAChR in the striatum appears to be involved in self-timing, while the roles of mAChRs in this context are uncertain.

Discussion

We initially searched for neurons in the caudate nucleus that exhibited a gradual ramp-up of activity prior to self-timed saccades. Although many individual neurons exhibited directional firing modulation during saccade preparation, the population as a whole showed no response preference for ipsiversive or contraversive saccades (Fig. 2C), consistent with the previous study (Lau and Glimcher, 2007). This is sharp contrast to the significant contralateral bias of visual response for neurons in the caudate nucleus reported previously (Kawagoe et al., 1998; Lau and Glimcher, 2007), suggesting that the origin of signals for the visual response in individual neurons might differ from that for saccade preparation. Bilateral representation of saccade preparation in the self-timing task has also been reported in the oculomotor thalamus (Tanaka, 2007) and the supplementary eye field (Kunimatsu and Tanaka, 2012). The basal ganglia-thalamocortical loop consisted of these regions might generate the climbing activity that appears to regulate the timing of self-initiated saccades.

Roles of striatal dopamine in saccade timing

We found that the timing of self-initiated movements was regulated by DA signals in the striatum. The reversible blockage of D_1 and D_2 receptors in the striatum facilitated self-timed saccades, while the effects on externally triggered saccades were, if any, only small (Figs. 4A and C). Conversely, the local application of DA agonists sometimes delayed self-timing but the overall effects did not reach a significant level (Figs. 4B and D), suggesting that endogenous dopamine might have been functioning in our experimental conditions such that additional DA had only a minor effect on self-timed behavior.

Striatal DA modulates signals in the direct and indirect basal ganglia pathways through D_1 and D_2 receptors, respectively (Gerfen et al., 1990). Although the D_2 antagonist often had a stronger effect compared with the D_1 antagonist (Fig. 4), suggesting a dominant role for the indirect pathway, the different concentrations and affinities of the drugs precludes a definitive conclusion. According to the known anatomical connections, D_1 and D_2 antagonists are expected to elevate activity in the output nodes of the basal ganglia, thereby reducing the level of activity in the thalamocortical pathways (Albin et al., 1989; Mink, 1996). Because the previous study showed that inactivation of the oculomotor thalamus delayed self-timed saccades (Tanaka, 2006), the promotion of self-timed saccades observed in the present study appeared to be paradoxical to the existing theory. Furthermore, the modulation of *ipsiversive* saccades definitely requires additional consideration on the interaction between both hemispheres via the crossed connections in the basal ganglia-thalamocortical pathways (see below).

In addition to the facilitation of self-timed saccades, we also found a slight but significant delay of *contraversive* visually-guided saccades following injection of DA antagonists (Figs. 4A and C). This was consistent with the previous findings by Nakamura and Hikosaka (2006) showing that application of D_1 and D_2 antagonists prolonged contraversive saccade latency in trials with large and small rewards, respectively, resulting in the alteration of the magnitude of contextual modulation of saccade latencies. Such diverse effects of DA antagonists imply that striatal DA may play multiple roles in saccade generation depending on the behavioral context. Recent studies have shown that DA neurons in the dorsolateral part of the substantia nigra respond to behaviorally-salient stimuli (Matsumoto and Hikosaka, 2009), while those in the ventromedial region represent 'reward prediction error' signals (Schultz et al., 1997). Through the projection from the dorsolateral substantia nigra to the dorsal striatum (Haber et al., 2000), the DA system may amplify saliency signals in the striatum that may consequently regulate value-based spatial attention (Krauzlis et al., 2013). Indeed, the aforementioned study by Nakamura and Hikosaka (2006) showed that DA antagonists altered the effects of a reward schedule on saccade latency, possibly through the reduction of saliency signals. Similarly, the changes in contraversive visually-guided saccade latency following the application of DA antagonists observed in this study might be attributed to a decrease of value-based saliency signals that likely promote reactive saccades.

Besides the temporal monitoring during the delay period, animals were required to actively suppress early saccades in the self-timed MS task. Therefore, the reduction of self-timed saccade latency following the local infusion of DA antagonists could also be viewed as a consequence of increased impulsivity. Increased impulsivity might be linked with impaired time perception, such that the promotion of the subjective passage of time may facilitate early impulsive responses (Wittmann and Paulus, 2008; Pine et al., 2010). Recently, correlation between impulsivity and eye movements has been shown in humans (Munoz et al., 2003; Cirilli et al., 2011; Hakvoort Schwerdtfeger et al., 2012) and monkeys (Rajala et al., 2012). In particular, Rajala et al. (2014) found that the availability of DA transporter in the basal ganglia correlated with the rate of early saccades in the triggered MS task with a long delay (up to 6 s), suggesting that reduced DA might cause impulsive behavior. Considering that striatal DA improves the signal-to-noise ratio in medium spiny neurons (Rolls et al., 1984; Kiyatkin and Rebec, 1996; for review, see Nicola et al., 2000), increased baseline activity following the application of a DA antagonist might render the system more responsive to distracting stimuli.

In a natural environment, the DA signals in both hemispheres likely work cooperatively to control patience and/or temporal processing. Nevertheless, our data regarding

the unilateral perturbation of DA signals may provide insight into the underlying mechanisms. Although previous studies have mainly implicated the basal ganglia in contraversive movements (Kravitz et al., 2010; Tecuapetla et al., 2014), the predominant effects of DA antagonists on *ipsiversive* self-timed saccades observed in this study indicate that this region plays a role in modulating bidirectional movements. Previous anatomical studies have shown that the basal ganglia-thalamocortical pathways contain several commissural connections. For example, intratelencephalic corticostriatal neurons project to the striatum bilaterally (Wilson, 1987; Lei et al., 2004), regulating both the direct and indirect pathways (Kress et al., 2013). In addition, there is also a crossed connection from the substantia nigra pars reticulata to the thalamus and the superior colliculus (Beckstead et al., 1981; Jiang and Stein, 2003). In terms of physiological recordings, a recent study in rodents showed firing modulation of neurons in the contralateral substantia nigra and motor cortex during unilateral optogenetic inhibition of the indirect pathway in the striatum (Tecuapetla et al., 2014). As with the opposing effects of the direct and indirect basal ganglia pathways on movement generation, the crossed connections might provide pathways for mutual inhibition between hemispheres. As suggested recently in a study of the rodent premotor cortex (Li et al., 2015), the unbiased directional preference of commissural projections might complicate the role of the basal ganglia in motor control.

As discussed above, altered saccade latency in different tasks following the application of DA antagonists might be associated with reduced value-based saliency and increased impulsivity possibly resulting from impaired temporal processing. Reduced attention and increased impulsivity are hallmarks of attention deficit hyperactivity disorders, for which application of DA stimulants is a major treatment (Faraone et al., 2015). In the future studies, their symptoms and the underlying neural mechanisms could be assessed by using the behavioral tasks similar to the present study.

Roles of striatal acetylcholine in saccade timing

We found that the application of a nAChR agonist and antagonist into the striatum altered self-timed saccade latency (Figs. 5A and B). Cholinergic interneurons in the striatum are known to modulate local neuronal processes through multiple mechanisms. For example, stimulation of nAChR at the terminal of DA neurons (α3-subunit) promotes DA release (Wonnacott, 1997), while those at GABAergic interneurons (β2-subunit) inhibits medium spiny neurons (Grilli et al., 2009). Because non-selective nAChR substances were used in this study, contribution of each subunit remained unknown. Although the effects of non-selective nAChR agonist and antagonist on self-timing were generally consistent with those of DA receptors, the effects of

nAChR agonists appeared to be stronger than those of DA agonist. Because DA release is regulated by the interplay between DA neuron firing and the activation of nAChR (Threlfell et al., 2012), such nonlinear effect of nAChR on DA release, as well as the other effects on GABAergic interneurons, makes it difficult to interpret the present data. Nevertheless, the significant effects of nAChR signals on self-timing are likely attributable to the alteration of DA signals, at least in part.

We failed to find any consistent effect of mACh-related agents on saccade timing (Figs. 5C and D). This was unexpected because a non-selective mAChR antagonist (scopolamine) has been found to ameliorate Parkinson's disease (Langmead et al., 2008). Furthermore, the previous study showed that systemic administration of mAChR antagonist can impair interval timing (Meck and Church, 1987), suggesting a role for mAChR outside of the striatum in temporal processing. However, considering the complex metabolic effects of mAChR and their multiple expressions in different neurons, it is difficult to exclude the possible involvement of striatal mAChR signals in timing. We examined the roles of M_2/M_4 receptors in this study, but because they are expressed at the terminals of dopaminergic, glutamatergic, and GABAergic neurons in the striatum (Goldberg et al., 2012), the different synapses might regulate temporal information in different ways. Application of subtype selective mAChR-related substances or optogenetic approaches would be helpful to address this issue in future studies.

Conclusions

The present study examined the role of DA and ACh in the striatum on self-timing. We found that local infusion of D_1 or D_2 antagonist facilitated self-timed saccades. Nicotinic but not muscarinic ACh receptor agonists and antagonists also altered the timing of self-initiated saccades. Because DA release in the striatum is known to be regulated by the activation of nAChR, the effects of nAChR signals on self-timing are attributable to the alteration of DA signals. We suggest that striatal DA are essential for self-timing by modulating the balance of signals in the direct and indirect basal ganglia pathways as well as that between both sides of the brain.

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Figure captions

Figure 1. Sequence of events in the three saccade tasks. In the self-timed memory saccade task, a cue in the peripheral visual field flashed briefly (100 ms) during central fixation. Monkeys were required to maintain fixation and remember the cue location. Animals made a memory-guided saccade to the cue location within a predetermined time window (900–1500 ms or 800–1400 ms) following the cue offset. In the conventional, triggered memory-guided saccade task, monkeys made a saccade to the previously presented cue location in response to the fixation point offset. In the visually-guided saccade task, the animals were required to make a saccade toward a visible target within 400 ms. Different trial types were randomly interleaved within a block.

Figure 2. Locations of microinjections and the directional properties of task-related neurons. ***A,*** Injection sites were overlaid on frontal sections drawn from MR images. The levels of the sections are shown in millimeters as anterior or posterior distances from the anterior commissure (AC). Injection sites between successive sections are projected anteriorly. *Cd*: caudate nucleus, *Put*: putamen. ***B,*** Activity of a representative neuron recorded from the injection site. Trials are sorted by saccade latency and are aligned according to the initiation of ipsiversive saccades during the self-timed saccade task (upper panel) and the triggered saccade task (lower panel). The diamond on each raster line represents the cue offset. The delay interval in the triggered task was chosen randomly from 1500 ± 700 ms. The black bar in the upper panel indicates the interval used to quantify the directionality of the preparatory activity. ***C,*** Distribution of the directional index. Data for neurons with significant directional modulation (unpaired *t*-test, $p < 0.05$) are shown as filled bars. The triangle with horizontal lines indicate the mean ± SD.

Figure 3. Effect of D_2 antagonist on saccade latency. ***A,*** Traces indicate horizontal eye position in the three saccade paradigms before (black) and after (red) D_2 antagonist infusion (eticlopride, 6 μg/μL, 2 μL). The animal was rewarded for saccades generated during the time window indicated by the upper horizontal line. The rewarded time window in the self-timed memory saccade task was 800–1400 ms after the cue offset. The number on each panel indicates the median latency. ***B,*** Each panel compares the median latencies in trials before and after D_2 antagonist injection. Blue and red symbols indicate contralateral and ipsilateral saccades, respectively. Filled plots indicate data showing a statistically significant difference (Kolmogorov-Smirnoff test, $p < 0.05$).

Figure 4. Quantitative summary of the effect of dopamine-related substances on saccade latency. Changes in median latency are plotted for different tasks during a local injection of a D_2 antagonist (eticlopride, *A*), D_2 agonist (quinpirole, *B*), D_1 antagonist (SCH23390, *C*), and D_1 agonist (SKF38393, *D*). Blue and red plots indicate contralateral and ipsilateral saccades, respectively. Filled symbols indicate data showing significant difference (Kolmogorov-Smirnoff test, $p < 0.05$). Each horizontal line indicates the mean for each condition. Data showing a statistically significant difference in the population are denoted by asterisk (Wilcoxon signed-rank test, $p < 0.05$). One outlier point (–193) has been omitted from the plot showing the effects of D_2 antagonist on ipsiversive self-timed saccades.

Figure 5. Quantitative summary of the effect of acetylcholine-related agents on saccade latency. Changes in median latency are plotted for different tasks during a local injection of a nicotinic receptor antagonist (mecamylamine, *A*) and agonist (nicotine, *B*), and a muscarinic receptor antagonist (AF-DX, *C*) and agonist (oxotremorine, *D*). Conventions are the same as in Fig. 4. One outlier point (–187) has been omitted from the plot showing the effects of mAChR agonist on contraversive self-timed saccades.

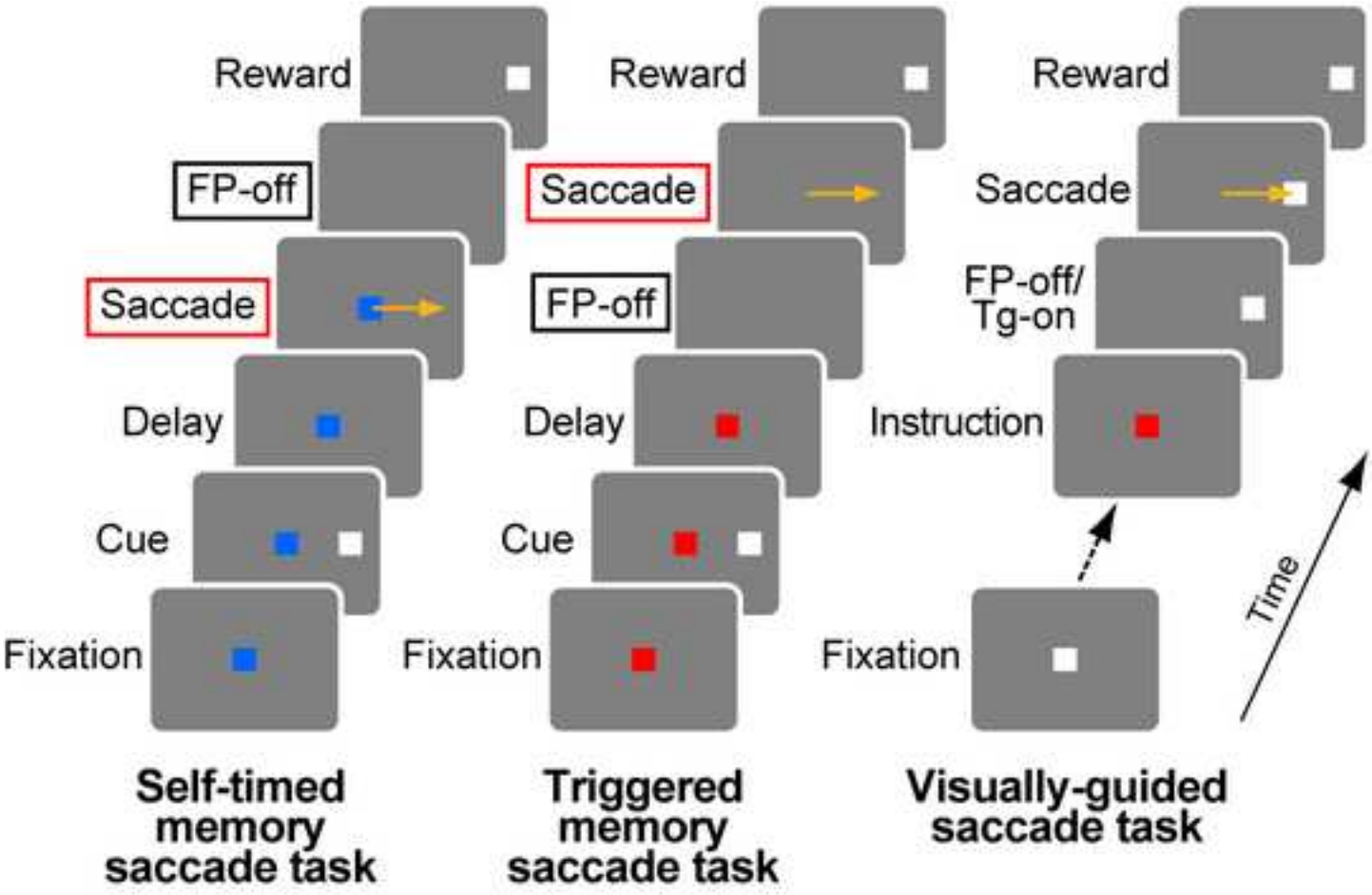


Figure 1, Kunimatsu & Tanaka

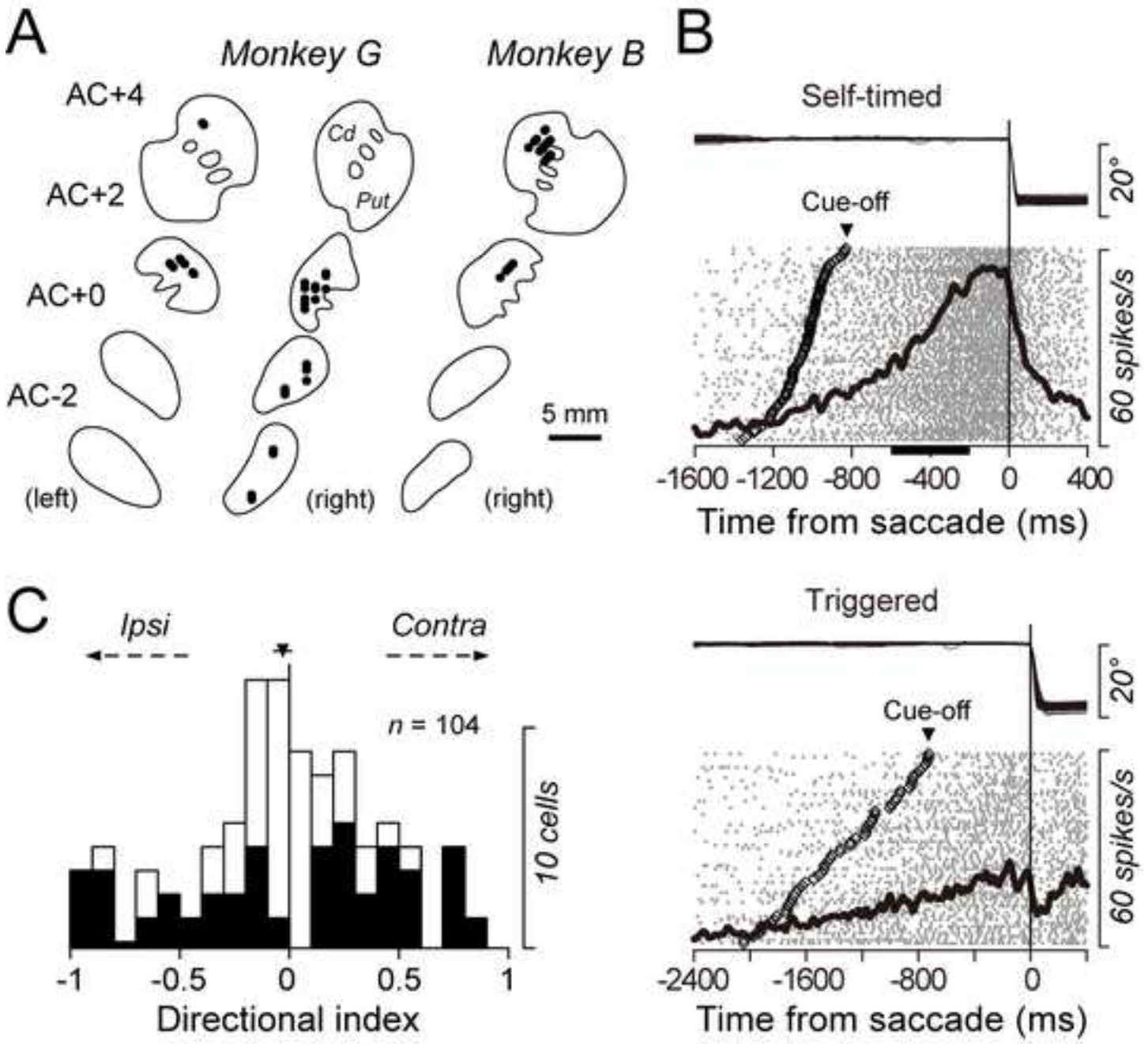


Figure 2, Kunimatsu & Tanaka

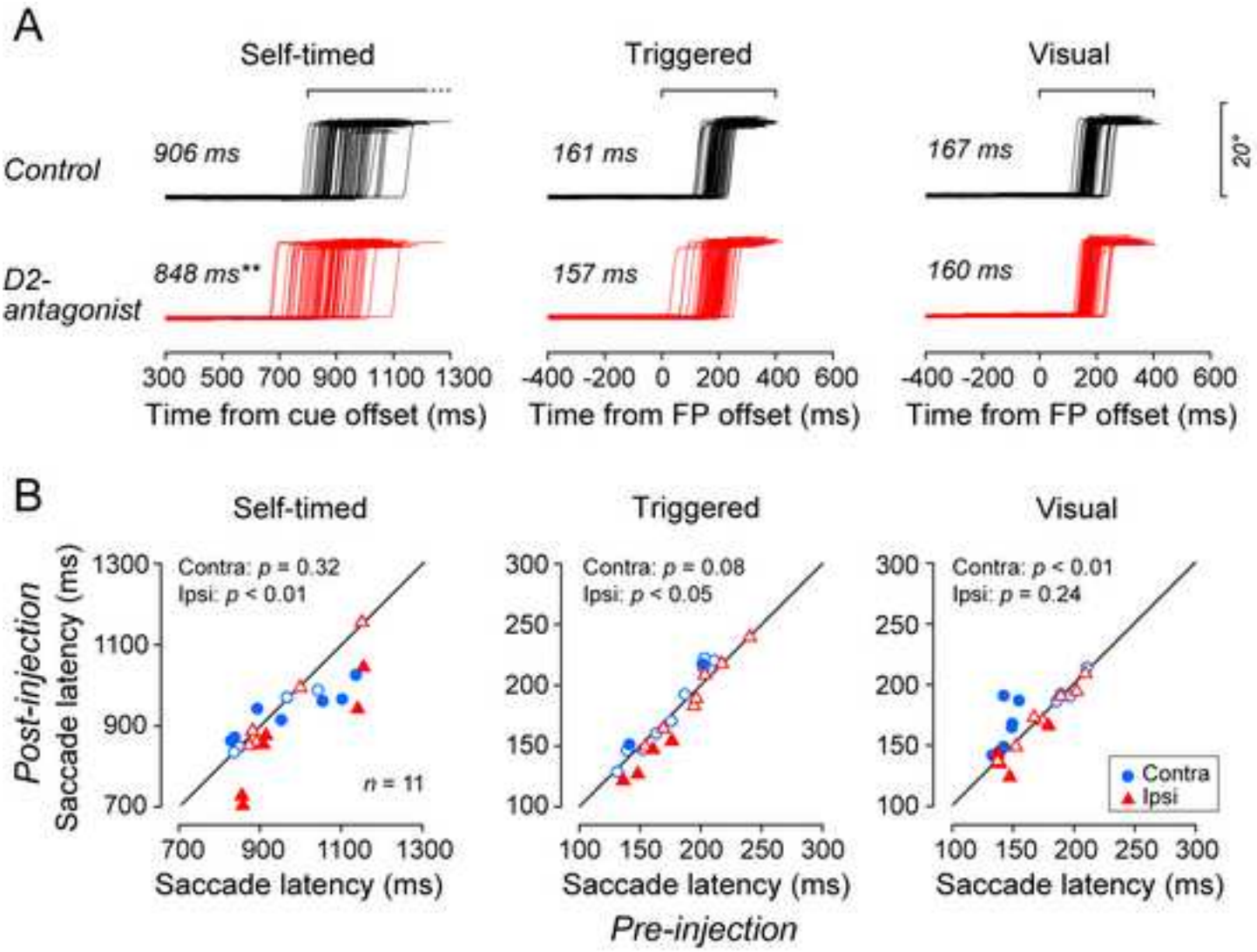


Figure 3, Kunimatsu & Tanaka

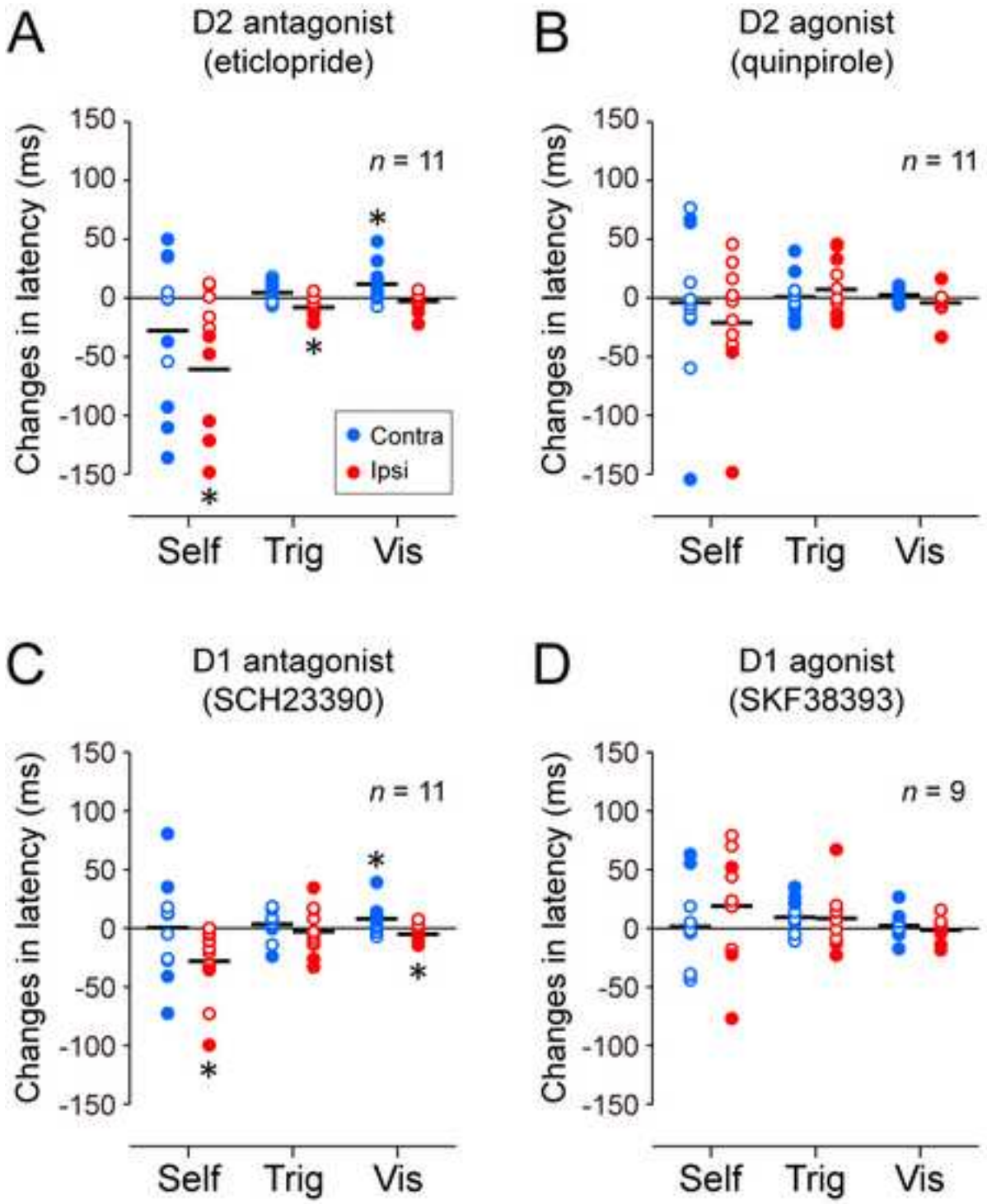


Figure 4, Kunimatsu & Tanaka

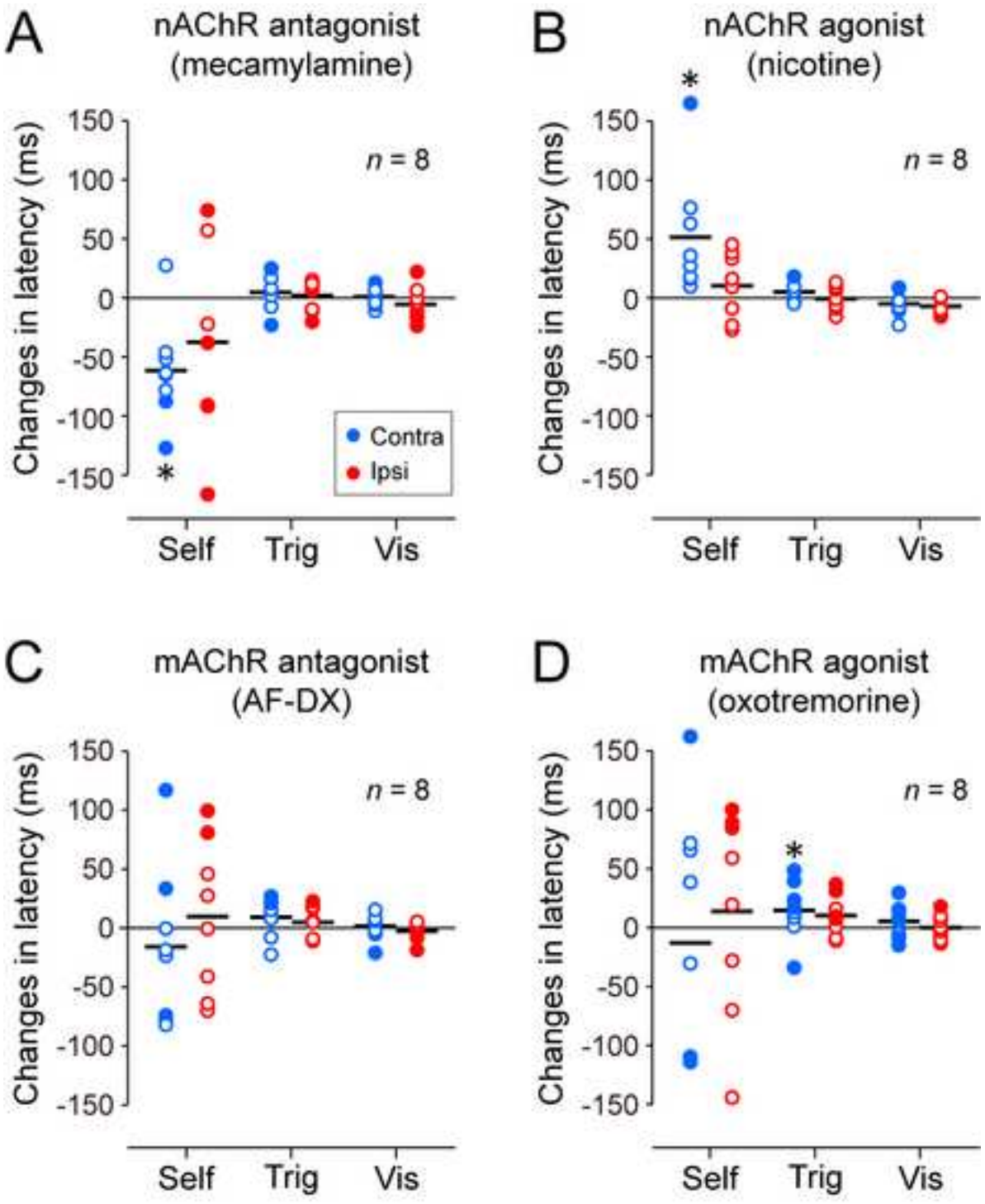


Figure 5, Kunimatsu & Tanaka