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A dual-task paradigm for behavioral and neurobiological studies
in nonhuman primates

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Highlights

We introduce a novel dual-task paradigm for neurobiological experiments in monkeys.

The present paradigm is analogous to that used in human studies.

Monkeys exhibited dual-task interference effect on this paradigm.

We describe the method to efficiently teach monkeys to learn the dual task.
Abstract

Background: The dual-task paradigm is a procedure in which subjects are asked to perform two behavioral tasks concurrently, each of which involves a distinct goal with a unique stimulus-response association. Due to the heavy demand on subject's cognitive abilities, human studies using this paradigm have provided detailed insights regarding how the components of cognitive systems are functionally organized and implemented. Although dual-task paradigms are widely used in human studies, they are seldom used in nonhuman animal studies.

New Method: We propose a novel dual-task paradigm for monkeys that requires the simultaneous performance of two cognitively demanding component tasks, each of which uses an independent effector for behavioral responses (hand and eyes). We provide a detailed description of an optimal training protocol for this paradigm, which has been lacking in the existing literature.

Results: An analysis of behavioral performance showed that the proposed dual-task paradigm (1) was quickly learned by monkeys (less than 40 sessions) with step-by-step training protocols, (2) produced specific behavioral effects, known as dual-task interference in human studies, and (3) achieved rigid and independent control of the effectors for behavioral responses throughout the trial.

Comparison with Existing Methods: The proposed dual-task paradigm has a scalable task structure, in that each of the two component tasks can be easily replaced by other tasks, while preserving the overall structure of the paradigm.

Conclusions: This paradigm should be useful for investigating executive control that underlies dual-task performance at both the behavioral and neuronal levels.
Keywords

● Monkey
● Behavioral neurophysiology
● Dual-task paradigm
● Training protocol
1. Introduction

1.1. Dual-task paradigm in human and nonhuman animal studies

The study of human dual-task performance has received considerable interest in the fields of experimental psychology and cognitive neuroscience because it provides detailed insights into the functional architecture of human cognitive systems. The general principle of the dual-task paradigm is to present, either simultaneously or in rapid succession, two component tasks that involve their own distinct goals and stimulus-response associations. In its basic procedure, a trial of one component task intervenes in a trial of the other component task, such that, for example, a series of noun/verb judgments is inserted during the memory maintenance period of a verbal short-term memory (STM) task with word memoranda (Wager et al., 2014). Alternatively, the two component tasks can be initiated simultaneously by the presentation of two cue stimuli after an inter-trial interval (e.g., Kuo et al., 2008), or can be performed along an independent, continuous stream of cue presentation (e.g., D’Esposito et al., 1995). Despite the remarkable flexibility of cognitive abilities, human subjects often exhibit decreased performance in the component tasks under dual-task conditions (Pashler, 1994). Often, the insertion of a more cognitively-demanding secondary task produces stronger disruption in the performance of the primary task. This effect, known as dual-task interference, has been extensively studied to examine the functional organization of hierarchical, multi-component working memory systems (Baddeley and Hitch, 1974; Just and Carpenter, 1992), and to make inferences about the dynamics of cognitive resource allocation (Kahneman, 1973; Wickens, 1980). Theories based on human dual-task performance emphasize the importance of executive control which coordinates the information-processing streams of multiple tasks (Meyer and
Kieras, 1997; Sigman and Dehaene, 2006).

The dual-task paradigm has rarely been adopted in studies of nonhuman animals. In the existing literature, dual-task experiments in monkeys have typically used a delayed matching-to-sample (DMTS) paradigm as a primary task, with a variety of tasks inserted during the retention period of the DMTS task as a secondary task. These experiments have primarily relied on manual responses using response buttons attached to a screen (Moise, 1970), joystick (Washburn and Aster, 1998; Smith et al., 2013), or touch screen (Basile and Hampton, 2013). Most of these studies did not focus on the psychological mechanisms that are specifically related to the information processing required for dual-task performance. Rather, these studies used dual-task paradigms as a tool for evaluating the functional similarity of STM between humans and nonhuman animals, such as the involvement of active rehearsal in STM.

In human studies, neuroscientific approaches involving functional magnetic resonance imaging (fMRI) have identified the key brain regions, including the lateral and medial prefrontal cortices, in the information-processing required for dual-task performance (D’Esposito et al., 1995; Klingberg, 1998). However, the precise functional role and mechanisms of these prefrontal regions remain largely unknown due to the lack of appropriate dual-task paradigms that are directly amenable to various invasive neurobiological techniques, including single-neuron recording, histology and lesion/inactivation approaches. Thus, the development of appropriate tasks for studies in nonhuman primates would be highly desirable for better understanding the mechanisms underlying dual-task processing.

1.2. Oculomotor- and hand-movement-based dual-task paradigm
While the dual-task paradigms in previous behavioral studies are suitable for assessing the behavioral performance of monkeys, they are not directly applicable to neurobiological investigations, primarily due to the lack of adequate control of the effectors for behavioral responses throughout the task phases. For example, while pressing a button (Moise, 1970) or touching a screen (Basile and Hampton, 2013) are intuitive behaviors for monkeys to learn, unconstrained movement of the effector prior to the response period (i.e., during the delay period) would make it difficult for the researcher to interpret neural activities during that time period. To achieve a long-term goal of elucidating the neural mechanisms that underlie dual-task performance and the interference effect, we developed a novel dual-task paradigm by combining two cognitive tasks that are frequently used in neurophysiological experiments, i.e., the visuospatial working memory task (Funahashi et al., 1989; Constantinidis et al., 2001) and the visuospatial attention task (Chelazzi et al., 1993; Buschman and Miller, 2007). The visuospatial working memory task was performed using memory-guided saccadic eye movements to the location where a visual cue had been presented. The monkeys were required to keep gazing at the fixation spot presented at the center of a screen before the response period. In the response period, a behavioral report of memory content was expressed by making a memory-guided saccade toward a correct target. The visuospatial attention task was performed using simple manual responses. The monkeys were required to hold down a lever placed in front of them while they attended to a location where a small circle was presented on the monitor. They were then required to perform a quick lever-release when they detected a change in the color of that circle. The combination of these two tasks is particularly useful because each component task is performed using a quick, standardized behavioral response of an
independent effector. This would eliminate spontaneous and inappropriate behavior of the animals and warrant the adequate, independent control of task-related effectors throughout a trial both before and during the response period, which is critical in experiments involving neurophysiological techniques. The use of independent effectors would also enable independent analyses of dual-task effects on recorded neuronal activities throughout the trial (i.e., from cue presentation to response execution) for each of the two component tasks, by minimizing the confounding of response-preparatory and response-executant activities related to the performance of the other component task.

The present paradigm offers several potential advantages for exploring the neural mechanism of dual-task performance. First, the two component tasks in this paradigm, the visuospatial working memory and visuospatial attention tasks, have been extensively studied in neuroscience. The neural mechanisms that underlie the performance of these tasks are well characterized in humans and nonhuman animals. Both tasks are known to require intact function of the lateral prefrontal cortex (LPFC) and recruit the activation of many LPFC neurons. In particular, spatially selective neuronal activities in the LPFC are known to play a key role in the performance of these tasks, such as the deployment of covert spatial attention in the attention task, and the encoding and maintenance of mnemonic contents in the memory task. Second, as we will discuss later, the present paradigm has a highly scalable task structure, in that each of the two component tasks can be replaced by other tasks, while preserving the overall structure of the paradigm and the amenability to various neurobiological approaches.

In the following sections, we show that the present dual-task paradigm (1) was efficiently learned by monkeys with a step-by-step training regimen; (2) produced a
significant dual-task interference effect; and (3) successfully minimized the influence of
the effect of behavioral responses in the other component task and achieved a clear
separation of processes dedicated to response preparation for the two concurrent tasks.
We emphasize the training method of dual-task paradigms in monkeys because it is
often presumed that dual-task performance requires sustained, complex cognitive
processing and that nonhuman animals are not suitable for performing such complex
tasks (Wager et al., 2014). Apart from a brief description of the training regimen by
Moise (1970), previous reports on animal studies have lacked detailed descriptions of
the training protocols for a dual-task. Therefore, in this report we hope to promote the
use of the dual-task methodology in nonhuman animals by presenting a step-by-step
training protocol for our dual-task paradigm that can serve as a benchmark example for
future experiments.

2. Materials and Methods

2.1. Subjects and apparatus

The subjects were two adult Japanese monkeys (Macaca fuscata; monkey S, male, 9.1
kg; monkey A, female, 5.5 kg). Prior to the present experiment, a stainless-steel
head-restraint device (Narishige, Tokyo, Japan) and scleral eye coil (Cooner Wire
Company, Chatsworth, CA) were implanted in aseptic surgeries that have been
described elsewhere in detail (Watanabe et al., 2006; Watanabe and Funahashi, 2007).
The training of the present dual-tasks was conducted on weekdays (i.e., 5–6 sessions per
week, 1 session per day). Access to water was restricted in their home cages except for
weekends. The monkeys sat in a custom-made primate chair in a dark, sound-attenuated
cubicle and their head movements were restricted. Visual stimuli were presented on a
21-inch CRT monitor (RD21GZ, Mitsubishi Electric Corporation, Tokyo, Japan) placed 45 cm from the monkey’s face. A lever (customized microswitch) was attached to the front wall of the chair. Eye movements were monitored using a magnetic scleral search coil technique (Robinson, 1963; Judge et al., 1980). A TEMPO system (Reflective Computing, St. Louis, MO) was used for the control of task events and the acquisition of behavioral data. All experimental protocols were approved by the Animal Research Committee at the Graduate School of Human and Environmental Studies, Kyoto University, and were in full compliance with the guidelines of the Primate Research Institute, Kyoto University.

2.2. Task procedure

The present dual-task paradigm required the simultaneous performance of a visuospatial attention task and a visuospatial memory task. Each task engaged an independent effector for the behavioral response (hand and eyes, respectively) (Fig. 1).

Trials began with the attention task component, in which monkeys were required to attend to a target ring and perform a rapid lever-release when its color changed to red (Fig. 1A). To initiate a trial, the monkeys were required to press the lever to turn on a central fixation ring (FR) and to fixate within 6 degrees (visual angle) imaginary square window centered at the FR throughout the trial. At 1.0 s after the start of fixation, two peripheral (Up and Down) rings were presented in the left (monkey S) or right (monkey A) visual field (Fig. 1D). After a further 1.0-s interval, an attention cue (red filled circle) was presented on one of the three rings for 0.4 s to indicate the target ring for the current trial. The different locations of the attention cue indicated different attention conditions: the (attend) Up, Down and FR conditions. The location of
the attention cue was randomized across trials. After a random-length waiting period from attention cue offset (wait1 period, 2.0-5.0 s), in 60% of the trials (short trials) the color of the target ring changed to red ($T_{col}$ change) for 0.4 s, and the monkeys were required to release the lever within 0.6 s (lever-release). In monkey S only, lever-release RTs of up to 0.8 s were allowed in some sessions. In the remaining 40% of the trials (long trials), termination of the wait1 period was followed by a change of the color for one of the two non-target rings (catch change). The monkeys were prohibited from responding to catch changes. The monkeys waited for another 0.4-3.4 s from the offset of the catch change (wait2 period) before $T_{col}$ change.

As a key feature of this dual-task, a memory task component was added to the attention task component (Fig. 1B,C) by the presentation of a memory cue (small white square) in one of five peripheral locations for 0.4 s (Fig. 1E, memory cue is shown as a small black square for visualization purpose only) at random timing between attention cue offset and $T_{col}$ change onset. The monkeys were required to memorize this location while performing the attention task component. At the end of the attention task component, after lever-release and the subsequent follow-up fixation period (0.4 s), all of the rings disappeared and small place-holders were presented at the five possible memory cue locations. The follow-up fixation period was implemented to ensure temporal separation of the two behavioral responses, so that the response-related behavioral and neuronal events would become tractable separately for each task in separate time windows. The monkeys were required to make a saccade within 0.6 s to the location where the memory cue had been presented and to keep gazing at it for 0.6 s. Saccade and gaze-keeping had to fall within a 6 degree imaginary square window centered at the location of the correct place-holder. The location and timing of the
Memory cue presentation were selected randomly and independently of the event of the attention task component (timing: 1.6-5.1 s after attention cue offset). Importantly, presentation of the memory cue that was scheduled after the timing of $T_{col}$ change onset never occurred, leaving this trial as a simple attention task trial. This timing rule led to presentation of the memory cue in two thirds of attention task trials, which made these trials a dual-task. The remaining one-third of trials were performed as single attention task trials. Correct behavioral responses (lever-release in the case of single attention task trials, and lever-release followed by a correct saccade in the case of dual-task trials) were rewarded with a drop of juice (approximately 0.5 ml) delivered to a drinking spout placed in front of the monkeys’ mouth, and the screen went blank for an inter-trial interval (4 - 7 s). Incorrect lever-release response, fixation break from the FR, erroneous saccadic response toward a wrong place-holder, and failure to hold gaze at a correct place-holder after a saccade resulted in abortion of the trial and started an inter-trial interval. We expected that the requirement for concurrent attention task performance would cause an interference effect in the performance of the memory task component during memory task trials under the dual-task condition (DMT). Since the attention task component had three conditions (Up, Down, and FR), hereafter, the memory tasks performed under these three attention task conditions are referred to as DMT-Up, DMT-Down, and DMT-FR, respectively (Fig. 1F).

After the completion of dual-task training, to examine whether the monkeys exhibited dual-task interference effect in the DMT conditions, we compared the performance in the DMT conditions with the performance in the memory task alone (SMT, single memory task) (Fig. 1E). The time course of the event sequence in the SMT condition was the same as that in the DMT condition except that all attention task
events were scheduled but executed as empty-events without occurrence of any physical stimulus change. The SMT trial was initiated automatically by the presentation of the FR. The Up and Down rings were presented to match the ring stimuli layout in the DMT condition. The memory cue was presented in every trial except the trials in which the memory cue presentation was scheduled after the termination of the wait1 or the wait2 period. Since the SMT condition was to serve as a task to determine the spatial characteristics of single-neuron activity in future neurophysiological experiments, eight memory cue locations that covered the entire visual field were used in this condition.

With regard to the nature of processing requirements in the attention task, we had previously addressed the question regarding whether this task required continuous deployment of covert visuospatial attention by introducing a control condition (Supplementary Figure 3 in Watanabe and Funahashi, 2014). In the control condition, non-cued trial was randomly inserted (50% of trials) among three attention conditions (cued trial) (the Up, Down and FR conditions). In non-cued trial, since red filled circles were simultaneously presented at all three rings during the attention cue period, the monkeys did not receive any information regarding the to-be-attended ring. The monkeys were required to release the lever by responding to the color change of any one of the three rings. The comparison of behavioral performance between the cued and non-cued trials showed the typical cueing effect, such as shorter lever-release RTs and higher percent correct rates in cued trials than in non-cued trials. Therefore, we concluded that the current “attention” task indeed required the deployment and maintenance of covert attention to the cued target ring, which supports our labeling of the present task as the attention task. However, we admit that the presence of the cueing effect does not negate the memory requirement in the present attention task, because the
to-be-attended location was marked only briefly by the presentation of a visual cue and the monkeys needed to keep in mind this cued location for a few seconds in order to perform correct lever-release response.

Fig. 1  Behavioral tasks. (A) Event sequence of the attention task component in the present dual-task. The monkey was asked to attend to a target ring and perform a rapid lever-release when its color changed to red. Eye fixation on the fixation ring (FR) was required throughout the trial. (B) Addition of the memory task component to the attention task component (short trial). A memory cue was presented during the wait1 period. After completion of the attention task component, all of the three rings
disappeared and small place-holders were presented at five possible memory cue locations. (C) Same as in (B), but the trial type of the attention task was long trial. (D) Location of visual stimuli for the attention task (monkey S). The Up and Down rings were presented in the left visual field. For monkey A, the configuration was inverted relative to the vertical meridian, and the Up and Down rings were presented in the right visual field. Because we intended to minimize the monkeys’ confusion about where to fixate among three rings on the screen, and because the FR also served as a fixation point for the memory task, the size of the FR was set to be smaller than two peripheral rings. (E) Location of memory cue presentation for the memory task (monkey S). Black filled squares indicate the five possible memory cue locations in the present dual-task. Open squares depict the three additional locations used in the memory task alone (SMT) (Fig. 3). For monkey A, the spatial configuration was symmetrically inverted relative to the vertical meridian. (F) Combinations of the attention and memory tasks in three DMT and SMT conditions.

2.3. Data acquisition and analysis

Data collected for each trial included the attention condition (Up, Down or FR condition), trial type (short or long trial), length of the wait1 and wait2 periods, memory cue positions, and latencies of lever-release and saccadic responses. Eye-position data were recorded throughout the trial. Statistical analyses were conducted using MATLAB (MathWorks, Natick, MA). For the analysis of attention task performance, raw percent correct rates and median lever-release response times (RTs) were calculated for each session. In the calculation of percent correct rates, only two types of errors were considered: premature lever-release before $T_{col}$ change and failure to initiate lever-release within 0.6 s after $T_{col}$ change. The remaining category of error, fixation break (FB) before $T_{col}$ change, was excluded from the analysis. For the analysis of behavioral performance in the memory task component, session-by-session percent correct rates were calculated as the number of correct trials divided by the number of trials in which monkeys successfully completed the attention task component.
(i.e., trials in which lever-release was made within 0.6 s of $T_{col}$ change onset). Correct trials in the memory task component were defined as those that resulted in both (1) successful saccadic target acquisition within 0.6 s after place-holder onset, and (2) successful gaze-keeping at the correct place-holder for 0.6 s. All other types of eye movements that occurred after lever-release in the attention task were considered as errors. To compensate for a chance-level difference between the SMT (12.5%) and DMT conditions (20%), we also examined memory task performance after transforming the raw percent correct rates of each session into correction-for-guessing scores. This transformation was made by using a conventional formula, $S = (C - E/(n - 1))/(C + E)$, where $S$ is the corrected score, $C$ is the number of correct responses, $E$ is the number of error responses, and $n$ is the number of alternatives (i.e., eight and five in SMT and DMT, respectively). The results obtained by this procedure were qualitatively identical to the raw percentage correct rates.

2.4. Preliminary training
Before the dual-task training, the monkeys were originally trained to perform the attention task and the memory task separately over a 4-month period. The results reported here began after the completion of training for each task. The monkeys were first trained in the attention task, and then in the memory task. For both tasks, training of the task was completed when the monkeys achieved a correct response rate of over 90% in three consecutive sessions. Between the completion of the attention task training and the commencement of the memory task training, the monkeys performed a modified attention task. In this task, during the wait1 and/or wait2 period in every trial, small white squares that were identical to the memory cues in the memory task were presented
1–3 times for 0.4 s each as a distractor. The aim of the modified attention task was to acclimatize the monkeys to the basic sequence of stimulus presentation in the dual-task paradigm. Monkey A adapted quickly to the modified attention task. From the first training session of this task, it maintained stable fixation despite distractor presentation, and showed a quick, reliable lever-release response after \( T_{\text{col}} \) change. Monkey S initially showed poor adaptation to the modified attention task. As a remedial action, the duration and frequency of distractor presentation were reduced as much as possible (one video frame and one distractor per trial), and then gradually increased to the same level as in monkey A. The training of the memory task was begun after adaptation to the modified attention task. After the monkeys learned the memory task, they entered into the final stage of the preliminary training. In this stage, both the modified attention task block and the memory task block were introduced in a repetitively alternating manner within a single session. This training was continued until the monkeys learned to quickly adapt to the task switch. In general, neither monkey confused the behavioral requirements for the modified attention task with those for the memory task; during the performance in the modified attention task, they did not make a saccade to the location where a small white square was presented, since the reward was given immediately after lever-release and the follow-up fixation period, and because small place-holders were not presented, which served as a go-signal for saccadic eye movements in the memory task.

3. Results

3.1. Dual-task training regimen
Training of the dual-task was conducted in a step-by-step manner, since we reasoned that step-by-step instructions would be necessary for the monkeys to acquire complex structures of stimulus presentation, task structure and response execution in the present dual-task. The two monkeys underwent a similar training regimen.

Monkey A had a total of five training phases. The objective of phase 1 was for the monkeys to adapt to the complex structure of response execution in the dual-task condition. In this phase, the monkeys needed to learn to produce two sequential responses using two different effectors. To this end, the complexity of stimulus presentation and the task structure was reduced as much as possible. The attention task component was simplified; the Up and Down rings were not presented and the attention cue was always presented on the FR. The memory task component was also simplified; the number of possible cue locations was reduced to three (0, 90, and 270° locations relative to the FR; see the schematic illustration of the memory task in “phase 1” of the training stage in Fig. 2A) and the memory cue was presented repetitively at the same location for up to consecutive 5 trials. In addition, the duration of a trial was shortened so that only short trials were presented. The timing of memory cue presentation was adjusted so that, as in the planned dual-task design (Fig. 1), the memory cue was presented in approximately two thirds of attention task trials. In preliminary training sessions, the monkey had already been exposed to the modified attention task, in which a small white square identical to the memory cue was presented as a distractor. Therefore, the objective of this phase was simply for the monkey to regard this white square as task-relevant (i.e., cue for the memory task component), and to make a saccadic eye movement after completion of the attention task component. We expected that this learning would be facilitated by the presentation of the place-holders that mark
the possible saccade targets, since presentation of the place-holders had been associated with the execution of saccades during training of the memory task alone in the preliminary training sessions. To further facilitate learning, when the monkey made saccade errors repeatedly in the memory task component, a “hint” was provided after completion of the attention task, by presenting a single place-holder at the correct target location. In addition, the requirement of post-saccadic gaze-keeping (0.6 s) at the correct target was shortened (0.2-0.4 s). The maximum value of the RT for the memory task was increased up to 2 s in the first two sessions.

In phase 2, the complexity of the task structure was increased from that in phase 1. In this phase, the length of the attention task trials was randomly selected from the short and long trials at a prescribed ratio (6 : 4). For the memory task component, the number of locations for memory cue presentation was increased to five, and the location of memory cue presentation was randomized across trials. Since only the FR ring was presented in the attention task component and the attention cue was always presented on the FR, the catch change was scheduled, but executed as an ‘empty event’ without any actual change of items on the display. In addition, a single place-holder that guided a correct memory-guided saccade (the “hint” in phase 1) was not presented. All other conditions stayed the same as in phase 1.

In phases 3 and 4, the complexity of stimulus presentation was increased from that in phase 2. In these phases, two attention conditions (Up and Down conditions) were added to the dual-task condition. However, in phase 3, only the short trials of the attention task component were presented. In phase 4, although the length of the attention task trials was selected from the short and long trials at a prescribed ratio (6 : 4), the catch change scheduled in the long trial was executed as an ‘empty event’
without any actual change of the items on the display. The duration of the gaze at the correct target after a memory-guided saccade was gradually increased to 0.6 s during these two phases.

The dual-task condition in phase 5 was identical to that in phase 4, except that the catch change scheduled in the long trial was executed as an actual event. Thus, the dual-task performed in this phase was identical to the planned dual-task design (Fig. 1).

Monkey S needed only four training phases. The training phases for monkey S consisted of the same training regimen as that for monkey A until phase 3. Phase 4 in monkey S corresponded to phase 5 in monkey A. In phase 4, monkey S performed a dual-task identical to the final version of the dual-task. Learning in the final training phase was judged to be complete when the monkey showed a correct response rate above 80% for three consecutive sessions. The correct rate was obtained as a product of the raw percent correct rates in the attention and memory tasks.
Fig. 2  Step-by-step training regimen and performance in individual sessions in dual-task training. (A) The performance of individual sessions in the five training phases (1 – 5) in monkey A. The monkey was exposed to the dual-task condition for the first time in session 1 of phase 1. Gray filled squares and gray filled triangles depict the percent correct rates in the attention and memory task components, respectively. Black filled circles indicate the overall percent correct rates, which were obtained by multiplying the percent correct rates in the attention and memory task components. Inset plots indicate the performance of the attention and memory task components during the first four sessions in phase 1. Schematic diagrams beneath the graph illustrate the number and possible locations of the attention cue and the memory cue. (B) Within-session changes in behavioral performance in the attention and memory task components in monkey A. For each of the four sessions in phase 1 (inset), percent correct rates in the attention and memory task components are plotted separately for the first and second halves of the session. (C) Same as in panel (A), but for monkey S. (D)
3.2. Progress of dual-task training

Each monkey performed a different number of trials in each session, depending on its motivational level. However, both monkeys performed an adequate number of trials per session throughout the entire training phases (monkey A, 197 ± 98 trials; monkey S, 237 ± 95 trials, mean ± s.d.). In the final training phase, both monkeys consistently performed a large number of trials per session (monkey A, 231 ± 76 trials; monkey S, 262 ± 82 trials). Five and three sessions in monkeys S and A, respectively, were excluded from the analysis because the monkey stopped task performance soon after the start of the session (< 30 trials).

The progression of each monkey’s performance during training is shown in Fig. 2. Both monkeys steadily progressed in the training regimen, and met the criteria for learning of the present dual-task after a total of 36 sessions. Throughout the training sessions, the performance of the attention task component in dual-task trials (filled gray squares) remained consistently high (mean ± s.d. and range across all sessions: monkey A, 94.5 ± 3.8%, 80.5 to 100%; monkey S, 92.2 ± 4.6%, 79.7 to 100%). On the other hand, the performance of the memory task component in dual-task trials (filled gray triangles) showed an abrupt decrease when the dual-task became more difficult by moving one phase further along in the training regimen. This result indicates that the attention task component was generally performed as a primary task whereas the memory task component was performed as a secondary task, and that this additional, secondary requirement for memory component task processing was gradually accommodated in the primary process for the attention task component to accomplish the goal of the two tasks together.
At the beginning of phase 1, the monkeys were exposed to the dual-task condition for the first time. In session 1 of phase 1, the performance of the attention task component in dual-task trials was lower than that of the memory task component in both monkeys (monkey A, Fig. 2A inset: 80.5% in the attention task, 88.4% in the memory task; monkey S, Fig. 2C inset: 86.9% in the attention task, 91.8% in the memory task). However, this pattern was reversed in subsequent sessions, such that performance of the attention task component consistently gave higher percent correct rates than that of the memory task component (session 3 for monkey A, Fig. 2A inset: 94.7% in the attention task, 75.9% in the memory task; session 3 for monkey S, Fig. 2C inset: 89.1% in the attention task, 70.0% in the memory task). Furthermore, we examined whether or not this trend was observed at the within-session level, by comparing the performance of the attention and memory tasks between the first and second halves of a session. The result showed that, even within each session, this initial attenuation and subsequent recovery of performance of the attention task component was also observed within each of the first two sessions in phase 1 in monkey A (Fig. 2B), but not in monkey S (Fig. 2D). This indicates that the switching task preference occurred more rapidly in monkey A than in monkey S. These results suggest that, during the first few sessions of dual-task training, the monkeys learned, through trial-and-error, which of the two component tasks should be performed as the primary task, and began to optimize the weighting of processing commitment for the two component tasks. These results also suggest that learning of dual-task performance consists not only of learning of the complex structure of stimulus presentation, but also of learning to coordinate the concurrent performance of two component tasks via prioritization of processing between the two tasks. The present study showed that the
latter aspect of learning occurred earlier than the former and was accomplished rather quickly.

How then, in phase 1, could the monkeys learn to prioritize between information processing in the two component tasks so quickly? An analysis of errors in the attention task component in dual-task trials indicated that, in early sessions of phase 1, the great majority of errors consisted of the failure to perform a lever-release after $T_{col}$ change (i.e., miss). This suggests that the monkeys’ attention was drawn by the presentation of a memory cue, and they forgot about performing the attention task. However, due to the present task design (see Materials and Methods), a substantial number of single attention task trials were performed between dual-task trials, and both monkeys achieved high percent correct rates in the performance of these single attention task trials (monkey A, > 87%; monkey S, 100%, for the first two sessions in phase 1). This trend continued throughout all of the training phases. This result demonstrates that the inclusion of single attention task trials facilitated learning of the coordination between two component tasks in dual-task trials, by reminding the monkeys about the attention task component. In addition, the inclusion of single attention task trials could help to maintain the animals’ motivation. These observations suggest that, when training animals in dual-tasks, the trials of one of the two component tasks should be included as an independent task among trials of the dual-task condition.

3.3. Analysis of behavioral effects of dual-task performance

After the completion of dual-task training, we examined whether or not the behavioral performance of the monkeys exhibited a dual-task interference effect. This analysis was performed using behavioral data obtained in 16 consecutive sessions that were collected
after the completion of phases 5 and 4 in monkeys A and S, respectively. To compare the performance in the memory task between the SMT and DMT conditions, we introduced a block of SMT trials before or after a block of DMT trials in a daily session. The order of the SMT and DMT blocks was counterbalanced across sessions. For the analysis of attention task performance, the performance in dual-task trials was compared to that in single attention task trials in the same dual-task block.

Analyses of memory task performance showed that both monkeys exhibited lower percent correct rates in the DMT conditions than in the SMT condition (Fig. 3A,B). A one-way repeated-measures ANOVA for percent correct rates revealed a significant main effect of memory task condition in both monkeys (monkey A, $F_{3,45} = 6.75, P = 0.0007$; monkey S, $F_{3,45} = 4.01, P = 0.01$). Post-hoc multiple comparison (Ryan’s method) confirmed the presence of a significant dual-task performance decrement in the DMT-Up condition ($t = 3.96; P = 0.0002$) and a marginally significant dual-task performance decrement in the DMT-Down condition ($t = 1.86; P = 0.07$) in monkey A, and the presence of a significant dual-task performance decrement in the DMT-Down condition ($t = 3.44, P = 0.001$) and a marginally significant dual-task performance decrement in the DMT-FR condition ($t = 1.75, P = 0.09$) in monkey S. After a correction-for-guessing transformation (dashed black lines in Fig. 3A,B; see *Materials and Methods*), there was a significant decrease in percent correct rates in the DMT conditions relative to that in the SMT condition in both monkeys (for both monkeys, $F_{3,45} > 4.89, P < 0.005$, one-way repeated-measures ANOVA).

Analyses of attention task performance showed that both monkeys exhibited lower percent correct rates and longer RTs in the dual-task trials than in the single attention task trials (Fig. 3C,D). For the percent correct rates, a two-way
repeated-measures ANOVA with factors of trial type (single-task trial or dual-task trial) and attention task condition (Up, Down, or FR condition) only showed a significant main effect of trial type in monkey A ($F_{1,15} = 6.22, P = 0.02$) (Fig. 3C, left). In monkey S (Fig. 3D, left), the analysis revealed a marginally significant main effect of trial type ($F_{1,15} = 4.24, P = 0.06$) and a significant interaction effect ($F_{2,30} = 5.04, P = 0.01$), with dual-task trials exhibiting significantly lower percent correct rates than single-task trials in the Down condition ($F_{1,45} = 11.57, P = 0.001$; simple effect analysis of trial type on percent correct rates). For lever-release RT data in monkey A (Fig. 3C, right), a two-way repeated-measures ANOVA revealed only a significant interaction effect ($F_{2,30} = 4.65, P = 0.02$), with dual-task trials exhibiting significantly longer RTs than single-task trials in the Up condition ($F_{1,45} = 5.37, P = 0.03$). In monkey S (Fig. 3D, right), the analysis of RT data showed only a significant interaction effect ($F_{2,30} = 7.71, P = 0.002$), with dual-task trials exhibiting significantly longer RTs than single-task trials in the Up and Down conditions (Up, $F_{1,45} = 7.27, P = 0.01$; Down, $F_{1,45} = 4.19, P = 0.046$).

These results indicate that a significant cost of dual-task performance was manifest among the performance of each of the two component tasks in the present dual-task. These results also demonstrate that, although the monkeys were able to perform this dual-task, their performance exhibited a typical dual-task interference effect similar to that observed in humans. Thus, we concluded that the present dual-task paradigm for nonhuman primates is an appropriate analogue of the dual-task paradigms that are used in human studies, and that this paradigm is useful for the study of behavioral effects and neural mechanisms of dual-task performance in nonhuman primate subjects.
Fig. 3 Behavioral performance in 16 consecutive sessions after the completion of dual-task training. (A) Box plots show median and distribution of session-by-session percent correct rates in the single memory task (SMT) and the memory task under three dual-task conditions (DMT-Up, DMT-Down, and DMT-FR) in monkey A. Throughout the figures, whiskers extend from the box to the lowest and highest data points that are still within a 1.5 interquartile range of the lower and upper quartiles, respectively. Small gray crosses indicate values beyond whisker ends. Open black circles indicate mean values. The dashed line shows the mean percentage correct rate after a 'correction-for-guessing' transformation (chance-level correction; see Materials and Methods). (B) Same as in panel (A), but for monkey S. (C) Left: median and distribution of session-by-session percent correct rates in the attention task under the three dual-task (D) conditions (Up, Down and FR conditions) and the single-task (S)
condition in monkey A. Right: median and distribution of session-by-session median RTs in the attention task under the three dual-task (D) conditions and the single-task (S) condition in monkey A. (D) Same as in panel (C), but for monkey S.

3.4. **Analysis of eye movements during fixation between the two behavioral responses.**

In the present dual task, the monkeys were required to separately execute two behavioral responses: lever-release and saccadic eye movement. To examine whether or not the control of saccadic eye movement for the memory task component was affected by the preceding lever-release response for the attention task component, we compared the trajectories of eye movements between before and after the lever-release response.

In the present dual-task, the monkeys were required to keep fixating on the central fixation ring (FR) before as well as after the lever-release response. However, a growing body of behavioral evidence suggests that slight eye movements during the fixation period often reflect the subject’s internal state (Haddad and Steinman, 1973; Hafed and Clark, 2002; Ko et al., 2010). Therefore, if there was crosstalk between movement preparation for the two behavioral responses in the present dual-task, changes in eye movement patterns during fixation should be observed between before and after execution of the lever-release response.

As illustrated in **Fig. 4**, the lever-release response hardly affected the pattern of eye movements during fixation in both monkeys. While eye movements during fixation in monkey S were almost completely unaffected by the lever-release response (**Fig. 4C,D**), there was a small, systematic disturbance in eye movements during fixation after the lever-release response in monkey A (**Fig. 4A,B**). As shown in vertical (blue lines) and horizontal (red lines) eye-position traces for trials in which the memory cue was
presented in the 315° location (Fig. 4A), a small downward drift of eye movement occurred after the lever-release response. However, the magnitude of the drift was negligible compared with the travel distance required for correct memory-guided saccades (horizontal dotted lines). Furthermore, an analysis of gaze-position distribution (Fig. 4B) using the entire data sets obtained in the 16 consecutive sessions used in Fig. 3 showed that, although the small, downward eye movement drift persisted, the drift was confined exclusively within an area subtended by ± 2° relative to the center of the FR, which was much smaller than the fixation window (white dotted square). In addition, when the distribution of gaze position was analyzed separately for trials in which the memory cue was presented in each of the five possible locations, similar drift patterns were observed across the five memory cue conditions (Fig. 4B, bottom panels). These results indicate that in monkey A, the preparation of the subsequent memory-guided saccade for the memory task component was not affected by the lever-release response for the attention task component in a saccade-direction-dependent manner. Thus, we concluded that both monkeys could successfully achieve the independent control of two effectors in the present dual-task paradigm. The clear separation of two behavioral responses would warrant detailed analyses concerning the behavioral performance in the present dual-task, and allow the independent examination of response-related neural activities for each component task in neurophysiological experiments.
Fig. 4  Analysis of eye movements during fixation before and after the lever-release response. (A) Vertical (blue lines) and horizontal (red lines) eye-movement traces for trials in which the memory cue was presented at the 315° location in monkey A. Eye-movement traces are aligned at the onset of the lever-release response. The gray shaded area shows the area of the fixation window. The data were obtained from five consecutive sessions among the 16 sessions used in the analysis shown in Fig. 3. Throughout the figure, data are plotted for all trials in which the attention task was performed correctly, regardless of whether or not the subsequent saccade response for the memory task was performed correctly. (B) Top panels: comparison of the 2-dimensional distribution of gaze positions between immediately before and after the lever-release response in monkey A. The data were obtained from the 16 sessions used in the analysis in Fig. 3. To construct these plots, gaze-position data were re-sampled at
200 Hz, and the relative frequency of the gaze position falling in each spatial bin (a 0.325° square window in visual angle) was calculated. The obtained 2-dimensional matrix is shown as a contour plot, with warmer colors corresponding to higher frequencies. White filled squares depict the five possible locations for memory cue presentation. The white unfilled circle indicates the fixation ring (FR), and the white dotted-square window surrounding the FR indicates the fixation window. The result inside the fixation window was enlarged and re-plotted for visualization purposes. Bottom panels: relative frequency distribution of gaze position (after lever-release) in each of the five memory cue location conditions. Data are shown for the area subtended by the fixation window. (C) Same as in panel (A) but for trials in which the memory cue was presented at the 225° location in monkey S. (D) Same as in (B) but for monkey S.

4. Discussion

The present study demonstrated that, if an adequate step-by-step training regimen is introduced, monkeys can learn the complex structure of a dual-task paradigm quickly. After the preliminary training of two component tasks for four months, both monkeys successfully learned to perform the present dual-task within 40 sessions. The number of sessions required to finish training of the present dual-task is similar to that reported by Moise (1970), who used a dual-task composed of a visual DMTS task and a manual reaction-time task. In this study, the preliminary training of the component tasks lasted about three months, and the subsequent dual-task training required 44 sessions (days). These observations indicate that the training of dual-tasks in monkeys can be accomplished relatively quickly, once the subjects become familiar with the component tasks.

Based on the analysis of behavioral performance during dual-task training, we identified at least three factors that are important for the efficient implementation of dual-tasks in monkeys. First, at the initial stage of dual-task training, the focus should
be on learning to coordinate two sequential responses using two different effectors in one trial. Therefore, each component task should be simplified as much as possible at this stage. In fact, an analysis of behavioral performance at the initial stage of dual-task training (Fig. 2A,C) showed that the monkeys, which had never been exposed to the dual-task condition, first needed to adapt to the novel requirement of concurrent preparation for two behavioral responses through the prioritization of information processing between the two component tasks. Therefore, the general scheme for dual-task processing needs to be learned before the complex structure of stimulus presentation and the order of responses is learned.

Second, the training of dual-tasks should include singly-performed trials of at least one of the two component tasks, and these trials should be embedded within dual-task trials. In the present study, a substantial number of single attention task trials were embedded in dual-task trials. The performance of these single attention task trials was stable at high percent correct rates throughout the dual-task training sessions. In addition, this trend was evident even at the very beginning of dual-task training (i.e., the first few sessions in phase 1). These observations suggest that the inclusion of single attention task trials facilitated learning of the present dual-task paradigm by continuously reminding the monkeys about the requirement of the attention task component.

Third, before introduction of the dual-task condition, the monkeys should be exposed to quasi-dual-task trials, in which the stimuli used in one of the two component tasks appear as task-irrelevant items during the performance of the other component task. The aim of this procedure is to acclimatize monkeys to the complex sequence of stimulus presentations in dual-task trials beforehand. In the present study, we inserted
the presentation of a visual stimulus identical to the memory cue during preliminary training of the attention task alone. The presentation of a salient visual stimulus in the peripheral visual field often induces reflexive eye movements. The occurrence of such eye movements would be judged as a fixation-break error in dual-task trials. Therefore, we introduced quasi-dual-task trials to reduce the proportion of fixation-break errors in dual-task training.

The key feature of the present dual-task paradigm is the assignment of independent effectors to each component task. As shown in Fig. 4, this procedure enabled rigid control of the movements of each effector both before and during the behavioral responses, and the good separation of two successive behavioral responses. These features are essential in neurobiological experiments using nonhuman primates. Use of the present effector combination, eye movement and manual lever response, should enhance the versatility of this dual-task paradigm for nonhuman primates, while preserving its immediate applicability to experiments involving neurobiological techniques (Watanabe and Funahashi, 2014, 2015). For example, the order of cue presentation for the attention and memory tasks can be reversed in order to examine the effect of the task-order in dual-task processing. In the present study, presentation of the attention cue always preceded that of the memory cue. This task-order could have influenced the pattern of dual-task interference in two component tasks. The present design would also make it possible to manipulate the difficulty of each component task independently. The difficulty of the attention task component can be parametrically manipulated by varying the intensity of the target ring change (Reynolds et al., 2000), while the difficulty of the memory task component can be manipulated by presenting distractors during the delay period (Artchakov et al., 2009; Qi et al., 2015). The
manipulation of the difficulty in one component task may produce varying degree of
dual-task interference in the other task, and thus may enable to identify brain areas
critical for dual-task processing. Neuronal activities in such areas are likely to be
strongly correlated with the magnitude of dual-task interference effect. In order to
investigate behavioral and neuronal activities in different task combinations, researchers
can replace the attention task part of the present dual-task with other behavioral tasks
that can be performed by manual lever-release responses, such as a conditional
Go/No-go task (Tanibuchi et al., 2009), a DMTS task (D'Amato, 1973), or a serial probe
recognition task (Wright et al., 1985). The use of a multi-directional joystick
(Heimbauer et al., 2012) could further expand the repertoire of the manually performed
component tasks in the present dual-task by enabling varieties of response modes
beyond a yes-no response indicated by lever deflection.

In neurophysiology, there have been a few experiments that used dual-task-like
paradigms (Bisley and Goldberg, 2003; Lebedev et al., 2004; Messinger et al., 2009;
Miyazaki et al., 2013). For example, in Bisley and Goldberg (2003), monkeys were
required to plan a saccade to a remembered location that had been instructed by a visual
cue, and then required to judge an orientation of a Landolt C that instructed them to
execute the saccade or cancel it. Although these studies demonstrated the ability of
monkeys to perform complex cognitive tasks that involved processing of multiple lines
of task information, the tasks were not clearly divided into two component tasks that
involved their own distinct goals and stimulus-response associations. In addition, the
focus of these studies was not on the dual-task interference effect. Thus, the present
study extends previous literatures by demonstrating that dual-task paradigms analogous
to those used in humans can be implemented in behavioral and neurophysiological
experiments in monkeys.

It could be argued that tasks involving eye-movement responses can require invasive techniques, such as the surgical implantation of a head-restraining device (i.e., head-post) on the skull and of a search coil into the sclera of the eye (Robinson, 1963; Judge et al., 1980), and such invasive procedures might not be suitable for experiments that only aim to collect behavioral data. However, as shown in recent studies (Washburn et al., 2010; Amemori et al., 2015), the restraint of a monkey’s head movement can be achieved using non-invasive head-restraining devices. These non-invasive head-restraining techniques, together with the use of commercially available video-based, non-contact optical eye-movement tracking systems, could enable implementation of the present eye-hand-based dual-task paradigm in non-invasive behavioral experiments.

5. Conclusions
The dual-task paradigm has been used in human studies extensively for many decades, and the data obtained in this paradigm have contributed significantly to the understanding of the functional architecture of human cognition. However, these efforts have been limited due to the absence of dual-task paradigms that are suitable for the investigation of behavior-neuronal correlates of dual-task performance in nonhuman animals under various non-invasive and invasive techniques. To facilitate the use of the dual-task methodology in nonhuman animals, the present study described the scalable design, training protocol, and important reminders during training for the implementation of a dual-task paradigm in nonhuman primates. The present results may form the foundation for future studies to more finely characterize the mechanisms that
underlie information-processing in dual-task performance.
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