Can Delay-Period Activity Explain Working Memory?

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Submitted 17 October 2003; accepted in final form 7 August 2004

Sobotka, Stanislaw, Mark D. Diltz, and James L. Ringo. Can delay-period activity explain working memory? J Neurophysiol 93: 128–136, 2005. First published August 18, 2004; doi:10.1152/jn.01002.2003. Working-memory tasks often lead to elevated delay-period discharge rates in cortical neurons. When this altered neuronal discharge rate, called delay activity, shows stimulus specificity, it is a good candidate for a neuronal mechanism of working memory. If the delay activity is indeed the carrier of memory, then experimental manipulation during the delay period that disrupts delay activity should also disrupt behavioral performance. We tested this hypothesis in two macaque monkeys with a delayed matching-to-sample task (delay time: 8 or 10 s) in which only two visual images were used. In each trial, one of the images was randomly chosen as the sample. In control trials (without disruptive stimulation), the monkeys performed at the level of 74.3% correct recognition. Three electrical stimulation levels (mild: a 0.25-s train of electrical pulses; medium: 1-s train; strong: 4 s), delivered to the hippocampal formation or to the orbitofrontal and inferotemporal cortices during delay period, decreased the performance to 71.4, 66.8, and 58.0% respectively (all are significantly less than control performance, P < 0.05 for mild stimulation and P < 0.0001 for other stimulation levels). Three hundred and thirty-four cells were recorded from inferotemporal (211 cells) and prefrontal (123 cells) cortices. Significant (P < 0.05) stimulus-specific delay activity was found in about one-third of recorded cells. For these cells in control trials, the mean difference in delay-period spike rates between preferred and nonpreferred images was 26%. The electrical stimulation reduced this difference to 20% (not a statistically significant reduction) in trials with mild stimulation, to 14% (P < 0.05) with medium stimulation, and just to 4% (P < 0.0005) with strong stimulation. These results, that increasing electrical stimulation reduced neuronal selectivity and at the same time reduced behavioral performance, directly support the hypothesis that delay activity is the carrier of memory through the delay period.

INTRODUCTION

In monkeys engaged in the delayed matching-to-sample task using visual images, the sample presentation induces a delay-period activity. This is an altered discharge rate in neurons of medial temporal lobe and prefrontal cortex usually lasting throughout the delay period. Sometimes different neurons are differentially active for different stimuli. It seems plausible that this activity across the population of neurons encodes memory of that which is to-be-remembered. In fact, this function has indeed been suggested by essentially every study that finds the delay activity to be a neuronal correlate of working memory.

Stimulus-selective activity during the delay period in visual image-recognition tasks has been reproducibly found in neurons recorded in inferotemporal cortex (Colombo and Gross 1994; Fuster 1990; Fuster and Jevey 1981, 1982; Miyashita and Chang 1988) and in the inferior convexity of prefrontal cortex (Fuster et al. 1982; Rosenkilde et al. 1981; Wilson et al. 1993). Ablative lesions, cooling, and electrical stimulation of the same regions (inferotemporal cortex: Fuster 1981; Gaffan and Murray 1992; Horel et al. 1987; Kovner and Stamm 1972; inferior convexity of prefrontal cortex: Kowalska et al. 1991; Passingham 1975; Stamm 1973) produce performance deficits in the same tasks.

A crucial factor in creating robust delay activity appears to be the repeated use of just a few images (Miyashita 1988). Electrical stimulation of the brain during the delay period disrupts monkey performance (Ringo 1993, 1995). This disruption is particularly severe if only a few images are repeatedly used throughout the whole study, whereas the disruption is only mild if fresh images are used in each trial (Ringo 1993, 1995; Ringo and Diltz 1994).

The evidence that delay activity is the holder of the memory is, however, indirect and has never been adequately tested. There have been a few previous experimental attempts to show that sustained delay activity indeed forms the basis for working memory. Funahashi et al. (1989) compared delay-related activity on correct and incorrect trials. They found that robust delay activity present in correct trials vanished during incorrect trials. Pessoa et al. (2002) showed that delay activity could predict performance. The correlational character of both of these studies suggests a need for experimentation that directly manipulates the important variables. The goal of the present experiment was to test the hypothesis that delay activity is the holder of the memory by such manipulation. We wish to know what unit activity embodies the behavior not just what unit activity is associated with it. Our experimental manipulation was done through disruption of delay activity by electrical stimulation. If delay activity in a particular region mediates memory, then reduction or elimination of the delay activity should disrupt the behavioral memory in predictable ways. This prediction was tested.

In the present experiment, we measured the stimulus-selective delay activity of single units in inferotemporal cortex and the inferior convexity of the prefrontal cortex when electrical stimulation was simultaneously applied to these structures during the delay period. A range of electrical stimulation magnitudes was applied, causing the units’ delay activity to range from being almost unaffected to completely eliminated.

Single-unit recording combined with electrical stimulation, and its precise temporal control, provides a unique opportunity to test rigorously the hypothesis that the stimulus-selective delay-period activity is the carrier of the memory in the delayed matching-to-sample task. If this hypothesis is true,
then neuronal activity in the investigated regions should predict the behavior. Behavioral performance should fall in a monotonic way with experimentally controlled reductions in the stimulus selectivity of the units’ delay activity.

**METHODS**

**Subjects**

Two young, adult female macaque monkeys, *Macaca nemestrina* and *M. mulatta* (∼4 kg each) were used in this study. All experimental procedures were approved by the University of Rochester’s University Committee on Animal Resources and adhere to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Stimulating electrodes**

Two bipolar electrodes for electrical brain stimulation were implanted in the left hemisphere and two in corresponding brain regions in the right hemisphere (see Fig. 1 for electrode placements). The electrodes, made from Teflon (PTFE) insulated, 5-mil platinum-iridium wire, were soldered into a 25-pin Winchester connector, which was cemented to the skull via dental acrylic and miniature titanium (0.5 mm) and stainless steel (0.86 mm) screws.

In one monkey, the electrodes were placed on the outer surface of inferotemporal and orbital cortex, between cortex and dura mater. A 10-mm end portion of the wire was stripped of its Teflon insulation and shaped into a 3-mm-diam circle. Two such circles separated by 1.5-mm distance, sewn on a small rectangular piece of Teflon, formed a bipolar electrode. In each hemisphere, one such bipolar electrode was implanted (Teflon outside, wire circles inside) under visual guidance, on the ventral surface of the prefrontal cortex and one pair on the surface of the inferotemporal cortex (the location of stimulation electrodes is shown in Fig. 1, top right).

In the other monkey, electrodes were implanted with a stereotactic approach in the rostral and caudal hippocampal region (Ringo 1993). Bipolar electrodes were made from two segments of the wire. The segments were sharpened at one end (which stripped the insulation from the tip) and glued together with a 2-mm vertical separation between tips. In each hemisphere, one electrode pair was stereotactically aimed at the anterior hippocampal formation (A11) and one pair at the posterior hippocampal formation (A8, see Fig. 1, bottom right).

![FIG. 1. The location of stimulating and recording electrodes in our monkeys (M1 and M2) is presented on drawings of coronal brain sections taken from the atlas of Winters et al. (1969). The number given after the letter A indicates how many millimeters anterior to the external auditory meatus is the particular brain section. Left: the position of recording electrodes. Electrode traces were recovered from histology and projected from the left and right hemispheres on single sections. Several electrode penetrations were made at each position indicated with each vertical line. Right: the position of stimulation electrodes. In the monkey M1, 4 surface electrodes were used. Electrodes (pairs of 3-mm diam uninsulated wire rings, see description in METHODS) were placed under visual control at the surface of ventral-prefrontal cortex and the inferotemporal cortex. Electrode positions are shown in the figure with small pairs of circles on ventral and lateral views of brain surface. In the monkey M2, 4 deep electrodes were implanted into the brain. They were placed stereotactically with electrophysiological guidance in the frontal and posterior hippocampus. Electrode positions were confirmed by histology and shown in the figure on coronal brain sections.](http://jn.physiology.org/content/93/1/129/F1)

*J Neurophysiol* • VOL 93 • JANUARY 2005 • www.jn.org
To secure accurate location of the electrode tips in the hippocampus, electrical activity was continuously monitored from the stimulation electrodes during surgical implantation. After each 1-mm step of lowering electrodes into the brain, electrical activity was checked for existence of very characteristic afterdischarges (lasting ~60 s) indicating hippocampus.

We used low-level electrical stimulation of the hippocampus because previous work in human (Halgren et al. 1985), monkey (Ringo 1993), and rabbit (Salafia and Allan 1980) has suggested that such stimulation could disrupt memory processes in the memory task. There is strong evidence that the inferotemporal cortex receives massive projections from the medial temporal lobe especially entorhinal and perirhinal cortex (Van Housen 1982; Webster et al. 1991).

Recording electrodes

Stainless steel guide tubes with outer diameter of 1.07 mm (7 tubes in monkey M2 and 8 in monkey M1) were implanted in the frontal and temporal cortex of each hemisphere. They were stereotactically positioned to guide electrodes at the prefrontal cortex and the inferotemporal cortex during recording sessions (see Fig. 1). At each position indicated with a vertical line in Fig. 1, several electrode penetrations were made. The use of permanent spaced guide tubes restricted regions of recordings to small-diameter cylindrical regions and made the identification of area where units were located relatively certain. The tubes were fixed in an acrylic mass, which was in turn secured to the skull via titanium and stainless steel screws. Two platinum wires inserted into occipital cortex were used as a ground. When not in use, each guide tube was filled with a stainless steel obturator and covered with a screw-top cap.

Eye coil

A scleral magnetic search coil (to record eye position) was implanted under the conjunctiva around the eyeball of one eye (Judge et al. 1980).

Before each recording session the system for monitoring eye gaze was calibrated. A small white dot (~0.5" diam) was presented at the screen in different locations. The monkey was trained to fixate her gaze on the dot to be rewarded with a small (~1 ml) squirt of fruit juice.

Unit recording

Single-unit activity was recorded with parylene-coated tungsten microelectrodes (Microprobe). The impedance of the electrodes was ~700 kΩ measured at 1 kHz). An electrode with 0.48-mm stainless steel guard tube was inserted into the brain through the permanently implanted guide tube. The end of the guard tube was positioned ~10 mm above the recording region, and then the electrode was advanced to the recording destination with a hydraulic microdriver. The frame of the microdriver was screwed into a sleeve embedded in the acrylic mass secured to the skull. The depth of penetration was determined by measuring distance between the tip of the electrode and the top of permanently implanted guide tube. The measurement of the distance from the top of the guide tube to the bottom of the brain was made before the first recording from each guide tube. The stainless steel wire (0.1-mm diam) was manually gently advanced until clear resistance, indicating the bottom of the brain, was felt.

When cell activity was encountered, the signal was amplified, filtered (0.25–10 kHz bandwidth), displayed on an oscilloscope, and sent to a PC computer with DataWave software for data acquisition. The signal was sampled at 32 kHz, and all candidate spikes (signals crossing user chosen triggering level) were stored for off-line analysis. The analysis allowed the authenticity of a candidate spike to be determined, by extracting and examining the distribution of up to eight parameters from the stored waveform (typically, the height and width of the spike, the first two principal components and a template match).

Histology

After experimentation, electrolytic lesions were made to facilitate histological verification of electrode positions. Bipolar electrodes (tips ~2 mm apart) were inserted into those guide tubes through which cells with significant delay activity were recorded. The lower tips of the bipolar electrodes were positioned 10 mm above the bottom of the brain. DC current of 1 mA was provided for 60 s for each polarity. Lesions were added to the four permanent stimulation electrodes aimed at the anterior and posterior hippocampal formation with the same current parameters as in the preceding text. One week later monkeys were killed with an overdose of barbiturate and perfused transcardially with saline followed by 10% formalin. The brains were blocked in situ, extracted, and immersed in 10% formalin. Later the brains were frozen, sectioned, and stained. Figure 1 shows the positions of recording electrodes as well as the electrodes used for electrical stimulation, presented on standard brain drawings from the atlas of Winters et al. (1969).

Visual images

Eight complex visual images (~2° wide and 2° high, see Fig. 2) were used. Each image was produced by a random (with constrains) combination of three geometric elements with each element in one solid color (chosen randomly among 5 colors). Multimedia Grasp (Paul Mace Software), and user-written programs in C language were used to generate and display the images. Images were presented on the computer display (100-Hz frame refresh rate), which was located in front of and 26 cm away from the monkey.

Procedure

Each monkey performed a delayed matching-to-sample task. In each particular experimental session, only two images (a pair indicated by A, B, C, or D in Fig. 2, randomly selected before the start of the session) were used. A single trial consisted of “sample,” “delay,” and “test” periods (see Fig. 3). First, a sample (an image randomly chosen from the 2 images used in the current session) was presented. To improve performance, the sample was presented three times in a row (each sample presentation lasted 1 s and was separated from neighboring samples by a 0.8-s interval of darkness). The place on the display screen of each sample presentation was randomly selected from five locations: the center of the monitor or 7.5° to the left, right, up or down from the center. Correct fixation on the sample (contin-

![FIG. 2. The entire set of abstract, complex images used in the study. In a single experimental session, only 2 of these images were presented (randomly chosen from the set of 4 pairs marked with A–D).](http://jn.physiology.org/lookup/doi/10.1152/jn.00713.2004)
uous gaze during ≥300 ms within a fixation window of 3° radius) was rewarded with a squirt of fruit juice (~1 ml) delivered to the monkey’s mouth through a tube. After the last presentation of the sample, there was a delay period of complete darkness. To get overall similar performance levels for the two monkeys, the delay period lasted 10 s in one monkey and 8 s in the other monkey. After the delay period (with no requirements for eye fixation), both images (1 image matching, the other image not-matching the sample image) were presented simultaneously 7.5° to the left and right of the center (left and right locations randomized across trials). The monkey’s task was to respond by directing its gaze at the image that was identical to the sample. A correct response, defined as a noninterrupted 800-ms gaze on the image matching the sample, was rewarded with juice. Both images disappeared immediately after any response (correct or incorrect), defined as a noninterrupted fixation for 800 ms on any of the test images or after 1.5 s (if no valid response was given).

In a pseudorandomly chosen 30% of all trials, 1 s after offset of the last sample presentation, a series of bipolar electrical current pulses was simultaneously delivered to all stimulating electrodes in the left hemisphere (ventral prefrontal and inferotemporal cortex in monkey M1; both the anterior and posterior hippocampus in the monkey M2, see the location of stimulating electrodes in Fig. 1). The stimulation consisted of a train of 0.2-ms pulses separated by 10-ms interpulse intervals. Eight-milliampere current was used for surface electrodes in monkey M1 and 1 mA for deep electrodes in monkey M2. The length of time during which the train of pulses was delivered determined the strength of stimulation. Three stimulation levels were used: weak (in 10% of all trials), medium (in 10% of all trials), and strong (in 5% of all trials). After initial exploratory work with various stimulation levels the following lengths of stimulation were chosen for the monkey with stimulation electrodes placed at the brain surface: weak stimulation lasted for 0.25 s, medium stimulation lasted 1 s, and strong stimulation lasted 4 s. In the other monkey (with stimulation electrodes placed in the hippocampal formation), there were fluctuations in effectiveness of stimulation throughout the experiment. Therefore the stimulation periods were periodically adjusted to produce constant behavioral effects of stimulation. Weak stimulation lasted between 0.125 and 0.25 s, medium stimulation lasted between 0.5 and 1 s, and strong stimulation lasted between 2 and 4 s. In 70% of the trials (control trials), no electrical stimulation was delivered. Before the first experimental session, each monkey was initially run in dim light and observed for possible behavioral reaction to stimulation. This procedure was periodically repeated later. Also during the initial experimental sessions the monkeys were continuously monitored (by a researcher who was well adapted to darkness) for any effects of stimulation. No stimulation effects (body or limb movements, eye movements, changes in alertness) were noticed.

An experimental session was composed of 400 trials and lasted ~2 h. Single-unit activity was recorded from inferotemporal and prefrontal cortices. The monkey’s head movement was restrained during experimental sessions. In front of her head, there was a transparent plate fitted snugly across the snout that did not obstruct vision. Behind the head there was a piece of plastic, shaped to the back of the head, which prevented withdrawal from the frontal plate. This restraint was sufficient to allow stable single unit recording. One experimental session was run for each monkey each day, 6 days/wk. The monkeys had restricted access to water. A portion of their daily fluids (50 ml · kg⁻¹ · day⁻¹ per monkey) consisted of rewards received in the behavioral task, the rest was added shortly after finishing the experimental session. Hydration levels were carefully monitored, and free water access was allowed at least once each week.

**Data analysis**

**INITIAL ANALYSIS.** All recordings of single units, which were clearly separated from noise and activity of other units, were checked for consistency of firing frequency throughout the whole experimental session. Only cell recordings with consistent firing rates during the entire session or most of the session were selected. When firing rates changed near the end of a session the recording was truncated to eliminate the period of changed activity.

Additional selection eliminated units recorded during sessions in which the monkey’s performance was poor or electrical stimulation during delay activity was not effective. To be included in further analysis, unit recording had to be done while the monkey’s performance was better than 70% correct in trials without electrical stimu-
lation and reduced by $\geq 10\%$ in trials with the strongest stimulation. All decisions about whether the quality of recording was sufficiently high to add it into the analyzed database were made blind to the single-unit results.

NORMALIZED DELAY ACTIVITY. The averaged frequency of spikes from the last 2 s of the delay period (just prior to the moment when the pair of test images was displayed on the screen) was used to calculate delay activity. This time bin was chosen to provide substantial time for spike data collection but also to allow $\geq 1$ s for recovery of neuronal activity after the end of electrical stimulation. In monkey M2 (with 8-s delay period), the longest 4-s stimulation train (which started 1 s after offset of the last sample) ended just 1 s before the start of data acquisition for computation of delay period.

To decrease the variability of data caused by slow fluctuations in unit activity a normalized delay activity measure was computed for each trial and used in further data analysis. Normalized delay activity was calculated as a difference between delay activity in the current trial and the baseline delay activity (averaged delay activity from neighboring trials with the same electrical stimulation but the other visual image) divided by the sum of the two measures:

$V = (V_c - V_b)/(V_c + V_b)$.

$V_c$ is normalized delay activity, $V_b$ is activity in a current trial, and $V_b$ is averaged delay activity from neighboring trials (5 before and 5 after) in identical electrical stimulation condition, but to the visual image other than that used in the current trial.

SELECTION OF CELLS WITH VISUAL SPECIFICITY OF DELAY ACTIVITY. From all recorded units, which passed initial data screening, we selected cells that had statistically different delay activity in response to different visual images. The selection was based on only a portion of control trials to leave aside the rest of the control trials for a measure of delay activity in further analyses. This procedure provided a measure of delay activity, which was unbiased by the cell selection. In the selection process, all control trials (without electrical stimulation during the delay period) were divided into two groups. The first group of control trials (consisted of every 3rd control trial) was used to select units with statistically significant visual specificity of delay activity—i.e., those units in which the two visual images generated statistically different delay activity ($t$-test, $P < 0.05$) and, in the case of significant difference to indicate, which of the two visual images produced stronger delay activity. Data from this group of control trials were not included in further analysis. The second group of control trials was used to evaluate the degree in which electrical stimulation of different intensities influenced both behavioral recognition and delay activity.

The difference between the preferred image (i.e., that which produced larger delay activity in the 1st group of control trials) and the nonpreferred image was compared in different stimulation conditions: without stimulation (calculated from the 2nd group of control trials) and with weak, medium, and strong stimulation. The comparisons were made both separately in the groups of cells from the inferotemporal and prefrontal cortices and jointly, across all recorded cells.

RESULTS

Behavioral performance

Over the whole period of the study, the monkeys worked at a steady performance level. The mean correct recognition level from trials without stimulation was 73% in one monkey and 75% in the other. Electrical stimulation during the delay period significantly disturbed performance. The performance level of each monkey consistently decreased with increased stimulation level. Averaged (from the 2 monkeys) performance levels were: 74% without stimulation, 71% with weak stimulation, 67% with medium stimulation, and 58% with strong stimulation (see Fig. 4). Two-way, repeated-measures ANOVA with stimulation level (none, weak, medium, and strong) and monkey (M1 and M2) as the main factors showed strong statistical significance of the stimulation level ($F = 93.93, df = 3/291, P < 0.0001$ after Greenhouse-Geisser correction for more than 2 levels factor) (see SAS/Stat User’s Guide 1988). The Duncan test showed a statistically significant drop in performance level in each condition with electrical stimulation when compared with the performance obtained without stimulation ($P < 0.02$ for weak, $P < 0.0001$ for medium, and $P < 0.0001$ for strong stimulation).

In addition the analysis showed a small but statistically significant difference in the overall performance of the monkeys (df = 1/97, $F = 9.42, P < 0.01$) but no significant interaction between the two main factors ($F = 2.27, df = 3/291, P > 0.05$). This lack of interaction indicates similar influence of electrical stimulation on behavioral performance in the two monkeys.

Delay activity

CELL NUMBERS AFTER SELECTION. In the two monkeys, we recorded 411 units (273 from inferotemporal cortex, and 138 from prefrontal cortex), which were clearly separated from noise and had invariable firing frequency during the entire or a substantial portion of the experimental session.

For further analysis, from this group of units we selected only stimulus-specific cells recorded from sessions in which monkey’s behavioral performance was good. We defined good behavioral performance as performance in trials without electrical stimulation of $\geq 70\%$ correct recognition level and with $\geq 10\%$ reduction in trials with the strongest electrical stimulation. There were 334 such units (211 from inferotemporal cortex and 123 from prefrontal cortex).

We then selected units in which the two visual images used in the session produced significantly different delay activity in
control trials (see preceding text). Among 211 units from the inferotemporal cortex, 75 cells (40 cells in monkey M1 and 35 in monkey M2) showed statistically significant (P < 0.05) visual specificity in trials without electrical stimulation. Among 123 units from the prefrontal cortex, 38 cells (33 cells in monkey M1 and 5 in monkey M2) showed such visual specificity.

The cells in which we found visual specificity of delay activity in trials without electrical stimulation (75 inferotemporal cells and 38 prefrontal cells) were further analyzed. We tested how the difference in delay activity evoked by the two images (average difference was 0.78 spikes/s in control trials) was affected by electrical stimulation during the delay period.

**VISUAL SPECIFICITY IN TRIALS WITH ELECTRICAL STIMULATION.** Our experiments showed an increasing disruption of both neuronal activity and behavior with increases in the intensity of electrical stimulation (see for comparison Fig. 4 and 6). Figure 5 presents the perievent time histograms of neuronal spike activity in control trials and in trials with different intensity of electrical stimulation from an example unit. The statistically significant difference in delay activity in responses to the two visual images observed in trials without electrical stimulation, disappears with gradually increased level of electrical stimulation.

All the stimulus selective cells were analyzed using a three-way, repeated-measures ANOVA with main factors of location (prefrontal and temporal), stimulation level (none, weak, medium, and strong), and monkey (M1 and M2). This ANOVA showed the statistical significance of stimulation level (F = 5.89, df = 3/327, P < 0.005) after Greenhouse-Geisser corrections for >2 levels of this factor, the Greenhouse-Geisser corrections were also applied in all other ANOVA analyzes presented in the following text). The ANOVA did not show any other significant effects. Location was not a significant factor (F = 0.001, df = 1/109, P > 0.05). There was also no significant interaction between stimulation and location (F = 2.28, df = 3/327, P > 0.05). These results suggest a similar influence of electrical stimulation on the specificity of delay activity in inferotemporal and prefrontal cortices as well as the average from all cells in both regions followed similar pattern.

The analysis also did not show any significant influence of the monkey factor on the specificity of delay activity in inferotemporal and prefrontal cortices (see Fig. 6). The contrast in spike rates between responses to preferred and nonpreferred images (from prefrontal and temporal recordings of both monkeys) was defined as the difference in the spike rates divided by the sum of those rates. The contrast between the preferred and nonpreferred images was 0.13 without stimulation, 0.10 with weak stimulation, 0.07 with medium stimulation, and 0.02 with strong stimulation (see Fig. 6). A
Duncan test was used to compare these values in trials without stimulation and those with different levels of stimulation. The test showed a statistically significant drop in specificity from medium ($P < 0.05$) and strong stimulation ($P < 0.0005$). In the case of weak stimulation, the drop in specificity did not reach the level of statistical significance ($P > 0.05$).

In addition, we analyzed separately the cells from the frontal and temporal cortex with two two-way, repeated-measures ANOVAs (with monkey and stimulation level as the main factors). Both analyses showed statistically significant influence of stimulation ($F = 3.80, df = 3/108, P < 0.05$ and $F = 3.77, df = 3/219, P < 0.02$ in the frontal and temporal cortices, respectively). Both the factor of monkey and interaction between monkey and stimulation were not statistically significant in any of the analyses. Duncan tests showed that the differences between trials without stimulation and with the strongest stimulation were statistically significant both in frontal ($P < 0.001$) and temporal ($P < 0.005$) cortices.

**Correspondence between behavioral performance and neural delay activity**

Behavioral performance for each stimulation level matched, almost perfectly, the relationship between delay activity and stimulation level (see Figs. 4 and 6). The Pearson correlation coefficient between behavioral performance (percent of correct recognition, averaged from all sessions in which visually specific cells were recorded) and delay activity (averaged specificity measure of delay activity in all visually specific cells) was 0.982 ($df = 3; P < 0.02$). However, it should be underlined that this correlation coefficient is so high only with the assumption that the correlations are identical in both monkeys.

**DISCUSSION**

There is wide interest in the hypothesis that the delay activity is the principal neuronal mechanism of working memory (for reviews, see Fuster 2001; Goldman-Rakic 1995). Part of the attractiveness of this idea stems from the scarcity of alternative single-unit mechanisms of memory, which could hold memory traces in working memory (Ringo 1996; Sobotka 2000; Sobotka and Ringo 1996). However, such a hypothesis has never been directly tested. In the present experiment, we tested this hypothesis directly through analysis of monkey performance when the delay activity was gradually eliminated through electrical stimulation.

**Fulfillment of two necessary conditions**

The experimental conditions of the present study, in which two monkeys were involved in the delay-matching-to-sample task, created two circumstances required to test the hypothesis that delay activity is indeed the holder of the memory.

The first requirement is that the units showed stimulus specific delay activity. In the current study, about one-third of units showed such specificity. These values are comparable, although somewhat larger than those of Colombo and Gross (1994) recording in inferotemporal cortex and Rosenkilde et al. (1981) recording in inferior convexity. Both these research groups found that 18% of their samples showed stimulus-selective delay period activity in a visual delayed matching-to-sample task. The somewhat higher percentage of units with stimulus specific delay activity found in the current study may be a result of the method of “normalized delay activity,” which we used to measure the significance of delay activity. This method increases sensitivity to significant differences by removing from statistical analysis the portion of additional variability caused only by slow drifts of cell activity.

The second requirement is that the electrical stimulation during the delay period was effective in reducing the visual specificity of neuronal delay activity both in the inferotemporal cortex and in the prefrontal cortex. Specificity of cell response declined with increases of stimulation level. The specificity during the strongest stimulation was about one-sixth that of the specificity in control trials (without stimulation). It was possible (through selection of appropriate stimulation current intensity) to achieve comparable levels in specificity of cell delay activity in two monkeys with two different methods of stimulation delivery. In one monkey, stimulation electrodes were inserted into the brain, whereas in the other monkey external electrodes were placed between the dura and the brain. The currents used in both monkeys were moderate in that they were well below after-discharge threshold yet clearly sufficient to influence delay activity of recorded cells.

The strength of the present experiment is that manipulation of the delay-period neuronal activity was linked so closely with the subsequent behavior. When the electrical stimulation was so weak as to only lightly affect the stimulus selective delay-period activity, then the behavioral performance was only lightly affected. When the stimulation was strong enough to almost wipe out the delay-period activity, the behavioral performance was almost at chance. When stimulation markedly reduced but still left significant delay-period activity, the behavioral performance was likewise markedly reduced but still not at chance level. Such close linkage suggests, but of course cannot prove, a causal relation.

Sustained activity during the delay on working-memory tasks may reflect other factors than working memory (see review in Funahashi and Takeda 2002; Wise et al. 1996). For example, it can reflect the coding of the task rules in prefrontal cortex. The damage of this brain region may produce difficulty in following rules in humans (Milner 1963). Rushworth et al. (1997) studied learning ability in both simultaneous and delayed matching tasks in monkeys. The lesion of the inferior convexity caused strong deficit in ability to relearn matching task even when there was no delay, suggesting that the lesion impaired the representation of the task rule. Wallis et al. (2001) ran an experiment in which monkeys were trained to switch between delay matching and nonmatching tasks. In each trial, a cue indicated which particular rule (matching or nonmatching) was currently in effect. The authors found rule-selective neurons in prefrontal cortex.

Therefore one could suspect that our stimulation during the delay period might not affect the memory of the sample image but simply prevent the monkey from applying the rule of delay matching to sample task. However, such a possibility does not seem to be a likely explanation of our results because such a possibility would leave as a remarkable and unexplained coincidence that the stimulus selective differential unit activity and the behavior moved together closely in a “dose”-dependent relationship.
fashion. Furthermore, in previous work from this laboratory (Ringo 1993, 1995), it was found that electrical stimulation of the hippocampal region (very similar to monkey M2) during the delay period was not disruptive to a trial-unique version of the delayed matching-to-sample task, a version of the task not usually associated with induction of delay period activity (see INTRODUCTION). These studies suggest that such stimulation does not disrupt the procedural memory required for the delayed matching-to-sample task.

Delay activity as the neuronal mechanism of memory—comparison between prefrontal and inferotemporal cortices

Over all the recorded units, memory performance correlated very well with the neuronal signal. Pearson correlation coefficient between the two studied variables (behavioral performance and neuronal activity) was equal almost 1.0 (but only with the assumption that the correlations are identical in both monkeys), showing that the reduction in behavioral performance very closely resembled the reduction observed in stimulus-selective delay activity in response to the electrical stimulation. When units just in IT or just prefrontal cortex were analyzed, each group showed a statistically significant effect of electrical stimulation.

Similar behavioral and electrophysiological effects of electrical stimulation in two monkeys in spite of profound difference in stimulation method

The present study produced similar results in spite of different locations of stimulating electrodes in both monkeys. In one monkey, electrical stimulation was made through electrodes implanted to the anterior and posterior regions of hippocampal formation, whereas in the other monkey, electrodes were placed at the cortical surface, one on anterior IT and the other on orbital cortex. The levels of stimulation intensity were chosen during preliminary experiments to have similar behavioral effectiveness in both monkeys. It seems that the exact route through which stimulation that interferes with selective delay activity reaches cells carrying delay period activity is not very important to the present results.

ACKNOWLEDGMENTS

Sincere thanks are due to S. O’Neill for expert technical assistance and A. Cecala for comments.

GRANTS

This work was supported by National Institute of Child Health and Human Development Grant HD-36032.

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