Temporal filtering by prefrontal neurons in duration discrimination

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Keywords: non-human primate, prefrontal cortex, time perception, unit recording

Abstract
Neural imaging studies have revealed that the prefrontal cortex (PFC) participates in time perception. However, actual functional roles remain unclear. We trained two monkeys to perform a duration-discrimination task, in which two visual cues were presented consecutively for different durations ranging from 0.2 to 2.0 s. The subjects were required to choose the longer cue. We recorded single-neuron activity from the PFC while the subjects were performing the task. Responsive neurons for the first cue period were extracted and classified through a cluster analysis of firing rate curves. The neuronal activity was categorized as phasic, ramping and sustained patterns. Among them, the phasic activity was the most prevailing. Peak time of the phasic activity was broadly distributed about 0.8 s after cue onset, leading to a natural assumption that the phasic activity was related to cognitive processes. The phasic activity with constant delay after cue onset might function to filter current cue duration with the peak time. The broad distribution of the peak time would indicate that various filtering durations had been prepared for estimating \( \tau \) duration. The most frequent peak time was close to the time separating cue durations into long and short. The activity with this peak time might have had a role of filtering in attempted duration discrimination. Our results suggest that the PFC contributes to duration discrimination with temporal filtering in the cue period.

Introduction
Intense research efforts have been devoted to psychological aspects of time perception, but the neuronal mechanisms remain unclear (Allan, 1979; Gibbon et al., 1997; Staddon & Higa, 1999; Hellstrom, 2003; Ivry & Spencer, 2004; Mauk & Buonomano, 2004; Buhusi & Meck, 2005). Imaging studies with functional magnetic resonance imaging and positron emission tomography have uncovered that various cortical and subcortical areas are involved in time perception; including the prefrontal cortex (PFC; Onoe et al., 2001; Rao et al., 2001; Macar et al., 2002; Basso et al., 2003; Smith et al., 2003; Livesey et al., 2007), supplementary motor area, premotor cortex, parietal cortex, cingulate cortex, temporal cortex, basal ganglia, thalamus, cerebellum and hippocampus (Onoe et al., 2001; Rao et al., 2001; Nenadic et al., 2003; Coull, 2004; Harrington et al., 2004). Duration activation is identical irrespective of assignment to visual, auditory or tactile stimuli. PFC is a region for the convergence of inputs that originate in primary somatic, auditory, visual, olfactory and gustatory areas, and is considered an area of cross-modal association (Fuster, 1997, 2001). Assuming that the PFC participates in some processes for time perception thus appears reasonable.

Single-unit recording is a powerful method to elucidate functional roles of an area. However, few attempts have been made to apply this approach to the PFC during temporal tasks (Niki & Watanabe, 1979; Sakurai et al., 2004; Genovesio et al., 2006; Oshio et al., 2006). In our previous study, monkeys were instructed to perform a duration-discrimination task in which two visual cues were presented consecutively for different durations, with subjects then requested to choose the cue presented for longer (Oshio et al., 2006). We recorded neuronal activity from the PFC, and found that activity after long first cues differed from that after short first cues, as if a duration category (long or short) of the first cue was encoded before presentation of second cues. A recent study found phasic activity in PFC neurons after cue offset, depending on cue duration, while monkeys responded differently in relation to duration of preceding visual cues by saccadic eye movement (Genovesio et al., 2006). Neuronal processing in the preceding cue period should have brought about these after-cue activities.

The present study addresses cue period activity of monkey PFC neurons during the duration-discrimination task to understand functional roles in estimating duration. We analysed neuronal activity of the first cue period in detail, because the second cue period activity may carry duration information not only of the second cue, but also of the preceding first cue. We demonstrate a variety of neuronal activities of PFC neurons, and then analysed and discussed the most frequent phasic activity.

Materials and methods

Subjects and apparatus

All surgical and experimental protocols were performed in compliance with the science council of Japan’s Guidelines for Proper Conduct of
In our previous study (Oshio et al., 2006), and were approved by the Animal Care and Use Committee of Kinki University. Two male macaque monkeys (Macaca fuscata; weight, 6.4–7.0 kg) participated in the experiment. Subjects were seated in a primate chair with the head fixed to face a flat 30 × 40-cm panel, on which a 6.5-inch computer display and three buttons with a diameter of 3 cm were mounted. The centre of the display was at eye level, 30 cm away from the subject. At 5 cm below the bottom of the display, two buttons were horizontally positioned at a distance of 8 cm from each other as targets for the subject press in response. At 5 cm below these two target buttons, another button was positioned in the centre as a hold button. The subject pressed the hold button to initiate a task trial and kept pressing until making a response. The flat panel was tilted upwards at 24° so that the subject could easily push all buttons. Presentation of visual stimuli and the acquisition, display and storage of data were controlled by personal computers running the tempo system (Reflective Computing, St Louis, MO, USA).

**Behavioural task**

Subjects were trained for more than 6 months to sit the primate chair and to discriminate between temporal durations of visual cues. Figure 1A shows event sequence of the duration-discrimination task. All visual stimuli were presented on the computer display against a black background. Each trial was initiated when the subject pressed the hold button with the right hand, making a small white square (2° × 2°) appear for 1.0 s at the centre of the display. This period is referred to as a precue period. After the precue period, two visual cues (a blue or red square, 8° × 8°) of differing duration were displayed successively at the centre of the display. Each of the two cues was followed by a delay period of 1.0 s, in which the small white square (2° × 2°) was presented. The first cue, first delay, second cue and second delay are referred to as C1, D1, C2 and D2, respectively. Visual cues C1 and C2 used two duration sequences [long–short (LS) and short–long (SL)] and two colour sequences (blue–red and red–blue). The sequences were changed randomly between trials. After the D2 period, blue and red squares were presented simultaneously (a choice period). Laterality of blue and red squares was also randomized. If the subjects pressed the target button below the square of the same colour as the longer-
displayed cue on the computer display, a dispenser squirted a drop of juice into their mouth 200 ms after correct response. No feedback signal was given for incorrect responses. Subjects were required to keep pressing the hold button from the start of a trial until the onset of the choice period. If the subjects released the hold button before this or took longer than 1.5 s to respond during the choice period, visual stimuli were turned off and the trial was aborted. Subjects spontaneously started each task after an intertrial interval of 2.0 s.

**Duration parameters**

In our previous study (Oshio et al., 2006), duration parameters for long and short cues did not overlap, and subjects, in principle, could judge whether C1 duration was eventually longer or not before presentation of C2 cues. The present study used duration parameters that overlapped between long and short duration sets. For monkey M11, cue duration varied from 0.2 to 1.6 s in 0.2-s increments, with short-duration cues of 0.2–1.2 s and long-duration cues of 0.6–1.6 s. The minimum difference between long and short duration was 0.4 s. Twenty-one duration pairs were tested in total. Each of these corresponds to a cell in Fig. 1B (top), in which percentages of correct responses are plotted as a greyscale map. Although long and short durations overlapped, the subjects could have obtained good marks only by comparing C1 duration with single criterion duration and ignoring C2. We assessed behavioural performance if responses were made only by comparing C1 duration with a filtering of 0.9 s, as the middle duration of averages of long and short durations. For example, subjects would respond correctly in trials with a duration pair of 1.2 and 0.6 s (a case in which the filtering duration is between C1 and C2 durations), whichever comes first, even though C2 was set aside. Conversely, for a duration pair of 0.6 and 0.2 s (a case in which both C1 and C2 durations are shorter than the filtering duration), subjects would estimate C1 as short and fail in the LS trials (C1 = 0.6 s, C2 = 0.2 s).
C2 = 0.2 s). Responses would be correct in the SL trials (C1 = 0.2 s, C2 = 0.6 s). Considering both LS and SL trials, the percentage of correct responses is assessed as 50% for the duration pair of 0.2 and 0.6 s. This strategy is calculated to provide a percentage of correct responses of 85.7% for all duration pairs. To provide lower correct percentages with such simple strategy, we prepared slightly different duration pairs for the other subject (M16). For monkey M16, 0.6, 0.9 and 1.2 s were taken as standard durations, and then variant durations (s/3, 2s/3, 4s/3, 5s/3) were assigned for each standard duration s. Each of the tested duration pairs corresponds to a cell in Fig. 1B (bottom). The middle duration of the averages of long and short durations was again 0.9 s, as in monkey M11. Similarly to that for M11, the percentage of correct responses for all duration pairs was estimated as 70.8%, if the subject responded by comparing C1 duration with the filtering duration of 0.9 s and ignoring C2. In this estimation, for trials including 0.9 s as duration parameters, percentages of correct responses were assessed as 75% because the subject could not filter out the C1 duration of 0.9 s.

Surgery and recording of neuronal activity

After demonstrating asymptotic performance in the task, the subjects underwent aseptic surgery to enable head fixation, under general anaesthesia initially induced by ketamine hydrochloride (Ketalar; 5 mg/kg, intramuscular) and maintained by sodium pentobarbital (Nembutal; 20 mg/kg, intraperitoneal) with atropine sulphate. Supplemental Ketalar (2 mg/kg, intramuscular) was given as needed. Each monkey was positioned in a stereotaxic apparatus, the skull was widely exposed, small stainless steel screws were implanted in the skull for anchors, the exposed skull and screws were completely covered with transparent acrylic resin and two stainless-steel pipes were mounted with the resin in parallel over the frontal and occipital regions for head fixation. Postoperatively, the subjects were given antibiotics intramuscularly for a period of 1 week to minimize the possibility of infection.

After a few days to allow recovery from the initial surgery, a 14 × 24-mm area of skull overlying the principal sulcus of the PFC was removed under the above-mentioned anaesthesia. A rectangular stainless-steel recording chamber (20 × 30 mm) with a frame width of 3 mm was then implanted under stereotaxic guidance at this location to record neuronal activity. A week after the initial surgery, the subjects were trained for a few days to perform the task with their head fixed. Single-unit recordings were conducted in a dimly lit, electrically shielded room. Neuronal activity was recorded using epoxy-insulated tungsten microelectrodes (1–2.5 Ω at 1 kHz; FHC, Bowdoinham, ME, USA) that were advanced in the cortex obliquely (45° from vertical in the frontal plane) through the dura mater with a hydraulic microdrive manipulator (MO-951; Narishige, Tokyo, Japan) while monitoring the signal waveform from the electrode. Neuronal activity was therefore evaluated from the left hemisphere contralateral to the responding arm. Behaviour of the subjects during recording was monitored through a CCD camera placed on the ceiling in the shield room. After filtering (band-pass 150–3000 Hz) and amplification, spikes from a single neuron were isolated using a multi-spike detector (Alpha Omega Engineering, Nazareth, Israel) that isolated spiking activity of up to 3 neurons per electrode based on an eight-point template-matching algorithm. Spike and event data were saved with millisecond resolution by personal computers using the tempo system. The electrode was advanced in small steps (approximately 5 μm) while monitoring the signal waveform from the electrode on an oscilloscope and transmitting sound on a speaker. When the multi-spike detector accepted a spike, a signal noting acceptance was also displayed with the corresponding spike on the oscilloscope for confirmation. Visual stimuli and accepted spike timings were also displayed on the tempo display. Intracortical micro-stimulation (ICMS) was applied in the caudal part of the recording chamber. Areas in which ICMS (a train of 12 cathodal pulses of 30 μA, 0.2 ms duration at 333 Hz) evoked saccadic eye movements were recognized as the frontal eye field, and were avoided for further unit recordings. Neuronal activity was stored within a depth of 3 mm from the first spike activity after penetration of the electrode through the dura mater. Judging online whether neurons were related to the task events was difficult, as durations of C1 and C2 periods differed from trial to trial. Spike data for almost all well-isolated neurons were therefore stored for offline analysis. We accumulated neuronal activity data (time of action potentials) and event data (onset and offset times of cues, sequence of cue colours, pattern of choice stimuli [blue (right)/red (left) or red (right)/blue (left)], reaction time, choice time, right/left of the target button that subjects pressed and reward time) for each well-isolated neuron. For almost all analyses, trials with correct responses were submitted for analysis. Error trials in which the subjects responded wrongly were also analysed in a limited case. Throughout the present paper, data analyses were performed using MATLAB (MathWorks, Natick, MA, USA) and custom-made FORTRAN programs running on a Linux machine.

Definition of C1-responsive neurons

Firing rates in the C1 and precue periods were estimated on a trial-by-trial basis. We selected PFC neurons as C1 responsive if firing rates were significantly larger in the C1 period than in the precue period. A simple average obtained by dividing the number of spikes by C1 duration is inappropriate to describe C1 activity in the present task, as cue duration changed between trials, and PFC neurons exhibited various firing patterns, such as phasic (transient) or tonic (sustained) activity. Sliding time windows are also inappropriate for describing C1 activity, because cue duration was variable between trials, and firing rates would be more over- or underestimated, as part of the cue period included in the time window would be getting shorter. We therefore evaluated C1 activity as follows. First, the C1 period was divided into time windows of 200 ms. Before comparing firing rates between C1 and precue periods, the average firing rate was calculated over trials for each time window in the C1 period, and the time window with the highest firing rate was selected as the most-active time window. C1 activity was represented by a set of firing rates on a trial-by-trial basis in the most-active time window. Precue activity was evaluated in a similar way to C1 activity, except that the 200-ms time window was moved by 20 ms. This procedure was allowable as the precue period was uniformly 1 s throughout all trials. The first 300 ms of the precue period was excluded from analysis, as neuronal activity was obviously unstable just after pressing the hold button. Neurons were then defined as C1 responsive if C1 activity was significantly larger than precue activity (two-sample t-test, P < 0.05).

Hierarchical cluster analysis

To investigate a variety of temporal patterns of firing rate during the C1 period for each neuron, and to determine dominant temporal patterns, C1-responsive neurons were classified with a hierarchical cluster analysis, in which the degree of similarity between temporal patterns was accessed. Firing rate data of C1-responsive neurons were pooled together from monkeys M11 and M16. Firing rate was evaluated over trials in 200-ms windows at every 20-ms step for 1.6 s from the onset of C1 in both subjects (71 data points for each neuron). Prior to cluster analysis, firing rates were normalized by the maximum firing rate in each neuron for pattern-based neuron classification. This
normalization is reasonable, as we applied it to the activity of C1-responsive neurons, which met the criterion of providing excitatory responses. The clustering process is depicted as a dendrogram in which horizontal branches of two clusters are linked with a vertical line when the clusters are joined (Fig. 3). The degree of dissimilarity is thus proportional to the length of horizontal branches. Among a variety of methods of hierarchical cluster analysis we applied Ward’s method (Ward, 1963), which is regarded as relatively efficient and has frequently been used over a wide range of studies. Cluster analysis was performed using MATLAB.

Gaussian fitting of peak activities
To characterize peak activities of phasic neurons, firing rate curves were fitted with the Gaussian function: \( f(t) = a \exp\left(-\frac{(t - b)^2}{2c^2}\right) \), where \( f(t) \) is the firing rate as a function of time \( t \), parameter \( a \) is peak height, \( b \) is the centre time of the peak and \( c \) is a parameter related to peak width. The parameter \( 2c \) is often used as an index characterizing width of the Gaussian function. The peak width of \( 2c \) corresponds to a height of \( 0.6065a \). An example of the fitting is shown in Fig. 2A (bottom). The fitting was performed on a main peak for each phasic neuron, as non-phasic portions might have adversely affected the fitting. Gaussian fitting was performed using an interactive plotting program, GNUPLOT ver. 4.0 (Williams & Kelly, 1998) on the Linux machine.

Unimodality test
The unimodality of the peak-width and peak-time distribution was assessed statistically using Hartigan’s dip test (Hartigan & Hartigan, 1985). The dip statistic is the maximum difference between the empirical distribution function and the unimodal distribution function that minimizes that maximum difference. The dip measures departure of the sample from unimodality. This test was performed using a FORTRAN algorithm (Hartigan, 1985) on the Linux machine.

Histology
After collecting neuronal data, small marking lesions were placed at known electrode coordinates by passing negative DC currents through the tips of microelectrodes (30 μA for 30 s). Monkeys were anaesthetized deeply with an overdose of sodium pentobarbital (Nembutal; 50 mg/kg, intraperitoneal), and perfused transcardially with 0.1 m phosphate-buffered saline (PBS), pH 7.3, followed by 10% formalin dissolved in 0.1 m PBS, pH 7.3. The brain was removed immediately from the skull, saturated with 10% then 30% sucrose in 0.1 m PBS, pH 7.3, at 4°C, and cut serially into 50-μm-thick coronal sections (parallel to recording electrode penetrations) on a freezing microtome. Every fourth section was histochemically stained using Cresyl violet. Recording sites were reconstructed, referring the marking lesions.

Results

Differences between trial types in performance
We collected 25 769 trials from monkey M11 (12 853 LS trials and 12 916 SL trials) and 23 418 trials from M16 (11 780 LS trials and 11 638 SL trials) for performance analysis. Percentages of correct responses were calculated separately in LS and SL trials for each of 21...
and 12 duration pairs tested in monkeys M11 and M16, respectively, then plotted as greyscale maps (Fig. 1B). In monkey M11 (Fig. 1B, top), the horizontal axis represents long duration (0.6–1.6 s), and the vertical axis represents short duration (0.2–1.2 s). In monkey M16 (Fig. 1B, bottom), the horizontal axis represents three standard durations x (0.6, 0.9 and 1.2 s), and the vertical axis represents four variant durations (s/3, 2s/3, 4s/3, 5s/3) for each standard duration x. The percentage of correct responses is scaled so that 100% correct is shown as white, and 50% and 70% correct are shown as black for monkeys M11 and M16, respectively. Percentages of correct responses were 76.1% for monkey M11 (LS trials, 71.1%; SL trials, 81.1%; range, 48.1–94.4%) and 88.5% for M16 (LS trials, 88.0%; SL trials, 89.0%; range, 72.5–98.8%).

Monkey M11 performed well in easy trials with large differences in duration (top right cells in Fig. 1B, top) and did worse in difficult trials with small differences (diagonal cells) in both trial types (LS and SL trials). However, the subject exhibited different performance between trial types. Grey values in corresponding cells between trial types (Fig. 1B, top) were different from each other, even though a duration parameter for the cells was identical (paired t-test; t = 3.603, df = 20, P = 0.0018). Percentages of correct responses were more variable along the vertical axis than along the horizontal axis in SL trials (Fig. 1B, top right). The vertical axis corresponds to C1 duration in SL trials. The vertically variable tendency of correct percentages in SL trials thus means that the correct percentages were more sensitive to C1 than C2 duration, indicating a C1-weighted performance. In LS trials (Fig. 1B, top left), we observed a horizontally variable tendency, supporting the C1-weighted performance. We evaluated dependence of the correct percentages on duration parameters by linear regression analysis that was performed to estimate an effect of short duration on the correct percentages for each long duration [regression coefficient (mean ± SE), –0.139 ± 0.0303 for LS, –0.403 ± 0.0562 for SL]. Comparison of the regression coefficients between trial types demonstrated that the subject exhibited different parameter dependence between trial types (paired t-test; t = 3.898, df = 4, P = 0.0176). In the previous experiment, in which long and short cue duration did not overlap, we reported that subjects responded differently between trial types (Oshio et al., 2006). Here, we observed different performance between trial types again, even if long and short duration overlapped. These results suggest not that C1 and C2 durations were estimated independently and then compared, but that encoding of C1 duration affected encoding of C2 duration and/or comparison between C1 and C2 durations.

In Fig. 1B (bottom), the vertical axis represents SL ratios to the standard duration denoted on the top row. Monkey M16 performed well in easy trials with large discrepancies in duration (top row with SL ratio of 1/3, and bottom row with SL ratio of 5/3), and did worse in difficult trials with small discrepancies (middle rows with SL ratios of 2/3 and 4/3). Grey values in corresponding cells between trial types were not different significantly from each other (paired t-test; t = 0.416, df = 11, P > 0.05). However, the subject also performed differently between trial types, as seen with monkey M11. In each row (constant SL ratio) for SL trials (Fig. 1B, bottom right), the longer the standard duration, the higher the percentage of correct responses. For LS trials (Fig. 1B, bottom left), on the other hand, performance pattern was roughly opposite to that for SL trials. Similarly to the case in monkey M11, linear regression analysis was used to estimate dependence of the correct percentages in duration parameters [regression coefficient (mean ± SE), –0.0635 ± 0.0400 for LS, 0.275 ± 0.0593 for SL]. In this case, regression coefficients were estimated for each C1/C2 ratio (each row in Fig. 1B, bottom). The subject also performed differently (paired t-test; t = 7.667, df = 3, P = 0.0042). Weber’s law, one of the psychological laws quantifying distinguishability of sensory stimuli, states that a just noticeable difference represents a constant ratio of the absolute strength of the stimuli. This law has been observed in psychophysical studies of time perception (Gibbon et al., 1997). If Weber’s law held in data from monkey M16, percentages of correct responses in each row of Fig. 1B (bottom) would have been constant, as ratios between two durations of each pair in each trial were constant. However, percentages of correct responses in each row actually differed (χ² = 11.1–57.8, df = 2, P < 0.01). Only for parameters of the top row in LS trials (Fig. 1B, bottom left) were significant differences not seen across columns (χ² = 1.33, df = 2, P > 0.01).
C1-responsive neurons (neural activity)

Our previous paper (Oshio et al., 2006) noted that D1 activity of PFC neurons after long C1s differed from that after short C1s, as if a duration category (long or short) of C1 was encoded as early as the D1 period before presentation of C2. The present study explored whether PFC neurons show C1 activities related to this D1 response to clarify functional roles of the PFC in duration discrimination. We recorded single-unit activity from 847 neurons (511 in monkey M11, 336 in M16) in the left dorsolateral PFC as monkeys were performing the duration-discrimination task. Both subjects responded with the right hand. Penetration sites were distributed around the principal sulcus in the PFC (Fig. 1A, inset).

At first, neurons showing excitatory responses in the C1 period were picked out. If C1 activity was significantly larger than precue activity (two-sample t-test, \( P < 0.05 \)), neurons were defined as ‘C1 responsive’ (see Materials and methods). We identified 160 neurons as C1 responsive (118 from monkey M11, 42 from M16). Typical neuronal activities are shown in Fig. 2. We frequently found phasic activity after a constant delay from C1 onset in each trial as shown in Fig. 2A (neuron #1102251). Ramping activity was also observed, which increased firing rate gradually toward the offset of C1 (Fig. 2B, neuron #1103041). Some PFC neurons exhibited sustained activity, maintaining a higher firing rate from just after C1 onset until C1 offset (Fig. 2C; neuron #1602691).

Cluster analysis of C1-responsive neurons

We analysed C1 activity of the 160 C1-responsive neurons. Various temporal patterns were found in neuronal activity (Fig. 2). As a first step of analysis, the C1-responsive neurons were tentatively classified with a hierarchical cluster analysis of the firing rate curve so that relatively homogeneous groups could be formed to determine prevailing temporal patterns (see Materials and methods). The resulting dendrogram is shown in Fig. 3. The C1-responsive neurons were tentatively sorted into 10 groups. Branches of the dendrogram for each group were drawn in the same colour, and each group was named alphabetically. To identify the prevailing temporal patterns, average firing rates from each group are illustrated in Fig. 4. Temporal patterns with a single peak were frequently seen (e.g. groups A, B and G). Sustained activity was also observed (e.g. groups C and F), which can be characterized by the high and stable firing rate throughout the C1 period. To assess the sustained activity for each group in Fig. 4, we calculated the average firing rate and the maximum deviation (a sum of absolute values of the positive and negative maximum deviation from a line defined between the first and last data points of the firing rate curve). As shown in Fig. 5, groups C and F showed the high firing rate and low deviation, and were isolated from others as ‘sustained’ neurons (29 neurons; 22 from M11, 7 from M16). We tried Gaussian fitting to peaks of the C1-responsive neurons, except for the sustained neurons. If Gaussian fitting was completed, and a peak was contained within the C1 period [a sum of fitting parameters \( b \) (peak time from C1 onset) and \( c \) (half of peak width) < 1.6 s], such neurons were defined as ‘phasic’ neurons (83 neurons; 65 from monkey M11, 18 from M16). Otherwise, if Gaussian fitting was completed, but a peak was later than the C1 offset, such neurons were defined as ‘ramping’ neurons, in the sense that firing rate kept increasing toward the C1 offset (34 neurons; 23 from M11, 11 from M16). Note that a decreasing type of ramping was also possible, but had been already excluded in the processes by which C1-responsive neurons were selected, as neuronal activity during the precue period was larger than that during the C1 period in such cases. Fourteen neurons were not successful in fitting, and referred to as ‘other’ (8 from M11, 6 from M16). The ratio of the numbers of neurons with each temporal pattern (phasic, ramping, sustained or other) is illustrated in Fig. 6. More than half of the C1-responsive neurons were phasic. For each group in Figs 3 and 4, the numbers of neurons displaying each temporal pattern are listed in Table 1.

Temporal characteristics of phasic activity during the C1 period

A single, dominant peak (Fig. 2A) was frequently observed for each phasic neuron. We characterized phasic activity during the C1 period with three fitting parameters for the Gaussian fitting: \( a \) (peak height); \( b \)
(peak time); and $c$ (peak width) (see Materials and methods). The phasic activity in Fig. 2A, for instance, had a peak time of 792 ms from C1 onset and peak width of 306 ms according to the Gaussian fitting analysis. Distributions of the peak width and peak time for the 83 phasic neurons are summarized in Fig. 7A and B, respectively. Peak width was estimated as $2c$, a typical index characterizing peak width and close to the full width at half maximum, where $c$ is the standard deviation of the Gaussian distribution. The histogram of peak width exhibited an approximately unimodal distribution with the most frequent range of 0.4–0.6 s (Fig. 7A; Hartigan’s dip test; $P = 0.982$; dip value, 0.0265). Peak time was given by the fitting parameter $b$ (peak centre), and was distributed about 0.8 s from C1 onset as the main distribution, and additionally at 0.4–0.5 s as a satellite distribution (Fig. 7B). The peak time distribution was unimodal (Hartigan’s dip test; $P = 0.418$; dip value, 0.0407). The peak time of 0.8 s was close to the specific duration of 0.9 s that discriminates between possibly long and short durations in the present study. Phasic activity about such timing would be convenient for sorting current duration into possibly long or short. In this case, the phasic activity at inappropriate timing may have caused wrong responses. For instance, the subjects may have tended to sort C1 as a short cue when phasic neurons with the peak time separating into long and short responded later. To test this speculation, we performed a peak-time analysis in error trials. Twenty-five phasic neurons with peak time from 0.7 to 0.9 s were selected for the analysis. Among them, we analysed 16 neurons with more than 10 error trials, excluding trials of C1 durations shorter than 0.6 s and longer than 1.2 s, which would have been less involved in the phasic activity at about 0.8 s. Peak times in error trials for each neuron were calculated using the Gaussian fitting, separately for LS and SL trials. The fitting analysis was successful in 14 of the 16 neurons for each trial type. The distribution of difference of the peak time between the correct and error trials for each neuron was shown in Fig. 8. As expected, the peak time in error trials was later in LS trials and earlier in SL trials than the correct trials (mean ± SE; 96 ± 76 ms in LS trials; −99 ± 43 ms in SL trials). Wrong responses would have related to other processes in cases when the peak time came earlier in LS trials and later in SL trials. We thus averaged the difference of the peak time for LS and SL trials in cases when the peak time came earlier in LS trials and later in SL trials. The peak time was shifted a few hundred milliseconds in error trials (mean ± SE; 308 ± 55 ms in LS trials; −161 ± 28 ms in SL trials). This error analysis therefore supports the speculation that phasic neurons would have contributed to sort C1 duration into possibly long or short. Additionally, no correlation was observed between peak width and peak time (Fig. 7C; $r = −0.082$, df = 82, $P = 0.461$). The phasic neurons were distributed uniformly within the recorded area.

### Ramping and sustained neurons

A subpopulation of the C1-responsive neurons [34 neurons (21%)] exhibited ramping activity, that is, a gradual increase with time until
offset of C1 [23 neurons (20%) in monkey M11, 11 (26%) in M16]. These ramping neurons were sorted with hierarchical cluster analysis into groups D, I and J in Figs 3 and 4 (Table 1). The average firing rate started ramping 0.5 s after C1 onset, and kept on until C1 offset (Fig. 9A). Ramping activity might have represented temporal integration. Another subpopulation was sustained neurons [29 neurons (18%); 22 (19%) in monkey M11, 7 (17%) in M16], which exhibited high firing rate throughout the C1 period with no clear peak (Fig. 9B). The sustained neurons were sorted into groups C and F in Figs 3 and 4 (Table 1). These sustained neurons may have monitored whether visual stimuli were ongoing. Ramping and sustained neurons were also distributed uniformly within the recorded area.

Discussion

Roles of phasic neurons in duration discrimination

We found that more than half of the C1-responsive neurons in the PFC showed the phasic activity during the C1 period in the duration-discrimination task. Most of the peak times of the phasic activity were later than 0.4 s after the C1 onset and were distributed unimodally at about 0.8 s. This delay from C1 onset is substantially late for simply visual responses, leading to a natural assumption that the phasic activity was related to cognitive processes in the present task. The phasic activity after a specific delay could be used to filter the current duration with the delay time. The broad distribution of the peak time would indicate that a variety of filtering duration had been prepared for estimating C1 duration. Durstewitz has explicitly postulated the existence of phasic neurons that signal the end of some duration as ‘response units’ in a simulational study (Durstewitz, 2003). In the study, climbing activity (the ramping activity in this article) was produced using a spiking leaky-integrate-and-fire neuron model, and the response unit was introduced to represent the readout of the climbing activity by signalling an expected time of occurrence. The present phasic neurons might correspond to the response units.

The unimodal peak-time distribution centred at 0.8 s suggests that the subjects might have responded by comparing C1 duration with a single filtering duration. The middle duration of averages of long and short cues was 0.9 s for both monkeys, close to the peak time of 0.8 s. The phasic activity with the peak time of about 0.8 s seems to represent the endpoint of the filtering duration for a temporal filter that sorts C1 durations into possibly long or short cues (Fig. 10). The subjects would have filtered C1 durations by testing whether the phasic activity was included in the C1 period or not. This speculation was supported by the fact that the peak times of phasic activity were shifted in error trials (Fig. 8). Also, this strategy might have been related to the C1-weighted performance shown in Fig. 1B. Durations of 0.6 and 0.2 s cannot be discriminated using the single filtering duration, yet the subjects (particularly in monkey M16) discriminated between these durations much better than at chance level. Phasic activity of the satellite peak at about 0.4–0.5 s could have contributed to filter out the shortest C1 durations of 0.2 and 0.4 s. In the previous paper (Oshio et al., 2006), we reported that PFC neurons exhibited categorical responses during the D1 period, meaning neuronal activity

Fig. 7. Distributions of the peak width (A) and peak time (B) for phasic activity during the C1 period for the phasic neurons. The peak width was estimated as ‘2c’, where c is the standard deviation of the Gaussian distribution (see Materials and methods). The peak time was given using fitting parameter ‘b’ (peak centre). (C) Correlation between the peak width and peak time. The linear regression line is also plotted. No correlation was observed.
differed depending on whether the preceding C1 duration was long or short. Results of temporal filtering by the phasic neurons with the peak time of about 0.8 s would have encoded as the categorical responses in the D1 period. The strategy may have been simpler for subjects than comparison of C1 and C2 durations after independent encoding.

**Phasic activity in other temporal tasks**

Phasic activity for representation of time interval is familiar in the auditory brain systems of a wide range of animals, such as cats, rats, mice, bats and frogs (Potter, 1965; Casseday et al., 1994; He et al., 1997; Brand et al., 2000; Ma & Suga, 2001). Neurons showing such phasic activity after a specific time has elapsed are referred to as duration-tuned or duration-selective neurons. Duration-tuned neurons have been found in the inferior colliculus, and responded to specific duration from several to several tens of milliseconds as a long-pass, short-pass or band-pass filter for analysis of vocalization or echolocation in rats, mice and bats (Casseday et al., 1994; Brand et al., 2000; Ma & Suga, 2001; Fremouw et al., 2005; Perez-Gonzalez et al., 2006). Duration-tuned neurons have been also recorded from the cat auditory cortex (He et al., 1997), and responded to a wider range of durations from several tens to several hundreds of milliseconds. In this manner, phasic activity is ubiquitous for representing specific duration.

**Ramping activity in PFC**

Of the 160 C1-responsive neurons, 34 (21.3%) exhibited ramping (or build-up) activity. Although this type of neural activity was in the minority here, other monkey temporal experiments with unit-recording methods have already observed similar activity from the PFC (Niki & Watanabe, 1979), parietal cortex (Leon & Shadlen, 2003; Janssen & Shadlen, 2005), motor cortex (Roux et al., 2003; Renoult et al., 2006) and inferotemporal cortex (Reutimann et al., 2004). The pacemaker-accumulator (PA) model, a well-known model for integrating duration,
postulates an internal pacemaker that regularly emits clock pulses and an accumulator that counts these pulses (Gibbon, 1977; Meck & Malapani, 2004). The ramping neurons are favourably positioned to work as the accumulator. Ramping activity has also been observed widely in non-temporal experiments, such as preparation for movement in the PFC (Fuster et al., 1982), growing certainty in the parietal cortex (Shadlen & Newsome, 2001), and anticipatory activity in the primary and supplementary motor cortices (Tanji & Evarts, 1976; Tanji & Kurata, 1985). This ramping activity may be also utilized as the accumulator in temporal tasks such as duration discrimination and time reproduction. In this instance, no specialized accumulator for time integration is required, but clock pulses can be accumulated in disparate areas including the PFC. Specific roles of accumulators in each area should be investigated in detail.

Neural mechanisms of time integration

As discussed above, the phasic neurons would have represented the endpoints of the filtering durations. The duration must be integrated in some way by other neurons to bring the phasic activity. How is duration integrated in the brain? The PA model has been applied to behavioural and neural data in timing studies (Gibbon, 1977; Meck & Malapani, 2004). If temporal duration was estimated in the frame of the PA model, estimation error would have been proportional to the duration to be estimated. Assuming that the accumulator evokes the phasic activity, the peak width of the phasic activity would be wider as the peak time is later. However, we observed no correlation between the peak time and peak width (Fig. 7C), indicating that the phasic activity in the PFC did not reflect the PA model. Of course, this result does not immediately reject the PA model. The time integration may be executed in other brain areas as denoted in the previous section. Also, whether the PA model is applicable to behavioural and neural data may depend on time ranges. Meta-analysis indicated that behavioural data satisfying Weber’s law were frequently taken from temporal tasks with duration of 1.5–500 s (Gibbon et al., 1997). In the present task, cue durations were changed from 0.2 to 2.0 s, and neural mechanisms suitable for the PA model may not have worked for duration estimation. However, Weber’s law was observed in a delayed eye movement task, in which a time range from 0.1 to 2.5 s was tested (Janssen & Shadlen, 2005). The law may hold better in motor timing tasks than duration-discrimination tasks. Further research should examine the relationship of Weber’s law to time ranges and task types.

Alternatively, pacemaker-free models have also been proposed for temporal integration (Ivry, 1996; Buonomano & Karmarkar, 2002). For instance, a state-dependent networks (SDN) model has been proposed (Karmarkar & Buonomano, 2007). The SDN model postulates that neural circuits are inherently capable of temporal processing as a result of the natural complexity of cortical networks coupled with the presence of time-dependent neuronal properties, such as synaptic plasticity and both fast and slow inhibitory postsynaptic potentials (Buonomano & Merzenich, 1995). A simulational study demonstrated that neural networks based on the SDN model provided burst firings without correlation between peak time and peak width in the sub-second range, similar to the present phasic activity (Buonomano & Merzenich, 1995). Local networks of PFC neurons may have integrated time in the framework of the SDN model and brought about the phasic activity representing the filtering durations. A cascade-timing model also postulates no specialized timing process such as a pacemaker, and hypothesizes that memory strength that decays over time acts as a clock (Staddon & Higa, 1999; Staddon, 2005). Although we failed to identify decreasing firing rate over time in the PFC, other areas may have participated in this type of temporal processing. After all, the neural mechanisms of time integration represent a holy grail for research into time perception, and many further studies will be required to reach this goal.

Roles of the PFC in duration discrimination

The present experiment found that the majority of the C1-responsive neurons exhibited phasic activity during the C1 period. The peak time was broadly distributed at about 0.8 s after the C1 onset. The phasic activity would have encoded endpoints of filtering durations. Phasic neurons with the peak time about 0.8 s would have functioned to sort current duration into possibly long or short. We previously reported that PFC neurons exhibited categorical responses for C1 durations either in the D1 or D2 period (Oshio et al., 2006). The previous result indicates that the PFC would contribute to implementation of strategic processes in temporal processing associated with trial types (LS or SL), such as representation of the trial type, retention of cue information or anticipation of the forthcoming cue. In summary, our results suggest that the PFC would mainly contribute to the temporal filtering (comparison process) during the cue period, and to the strategic processes during the delay periods in duration discrimination, but less to temporal integration. Additionally, the temporal filtering by the phasic neurons conjures up ‘predictive coding’ in the medial frontal cortex (Summerfield et al., 2006). The predictive coding proposes that the brain resolves perceptual ambiguity by anticipating the forthcoming sensory environment, generating templates that should be matched with observed sensory evidence. The phasic activity we observed would be associated with the template in the predictive coding. Further investigations such as parametric analysis of neuronal activity in the D1 and C2 periods, and experiments using different ranges of cue duration should be performed to test whether the phasic activity actually reflects temporal filtering, and to determine the overall roles of the PFC in duration discrimination. Also, studies with inactivations and lesions are needed to establish the function of the PFC.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (17021039, 18500314, 19500230), and the Cooperation Research Program of the Primate Research Institute at Kyoto University. We would like to thank the technical staff of the Life Science Institute of Kinki University for animal care, and Akira Murata, Katsumi Nakajima and Hiroaki Ishida for helpful discussions. We are grateful to anonymous reviewers whose comments led to improvements and clarity of this manuscript.

Abbreviations

CMS, intracortical micro-stimulation; LS, long–short; PA, pacemaker-accumulator; PBS, phosphate-buffered saline; PFC, prefrontal cortex; SDN, state-dependent networks; SL, short–long.

References

Temporal filtering by prefrontal neurons


