Supporting Online Material

Materials and Methods

*Electrophysiological recordings and stimulation* We recorded the activity from single neurons that were located primarily in the CM nucleus, but also in surrounding thalamic nuclei in three adult Japanese monkeys (*Macaca fuscata*, Monkeys SJ, MA, and HD). Under pentobarbital sodium anaesthesia, surgery was carried out to implant head-restraining bolts, a stainless-steel unit-recording chamber, and electromyogram (EMG)-wires. The recording chamber was positioned vertically over the thalamus. Action potentials from single neurons were recorded using tungsten microelectrodes (2 - 5 MΩ at 1kHz) that were inserted through the implanted recording chamber and advanced by means of an oil-drive micromanipulator. The action potentials were amplified, filtered (50 Hz to 3 kHz) and isolated using a spike sorter. We selected neurons that showed burst discharges after unexpectedly presented auditory and/or visual stimuli of long latency (visual; 250 - 350 ms, auditory; 170 - 300 ms), such as a knock on the laboratory door. The neurons were of the long-latency-facilitation (LLF) type, most of which were concentrated in the CM nucleus but some of which were located in surrounding thalamic nuclei ([1, 2](#)) (see section below). We recorded the activity of the flexor carpi ulnaris and biceps brachii arm muscles through chronically implanted wire electrodes in monkey SJ. To electrically activate neurons in the focal CM area while the monkeys were performing the tasks, current pulses (20 biphasic pulses with a 0.2 ms duration at 200 Hz, 20 - 120 µA, median 60 µA) were delivered through the stimulus microelectrode (200 - 400 kΩ at 1kHz) which, at other times, was used to record the activity in single neurons. Stimulation was randomly applied in 12.5 - 50 % of the GO trials. In order to evaluate the
effectiveness of electrical activation on task performance, effective stimulation rate (ESR) was defined as the percentage of stimulated trials in which either reaction time or movement time was longer than the one-sided upper 95% confidence limit of trials carried out without stimulation in the same block of trials. An arrest of action was an extreme effect of stimulation, in which monkey did not initiate or stopped in the midst of an initiated GO action. The Animal Care and Use Committee of Kyoto Prefectural University of Medicine approved all surgical and experimental procedures that were carried out in this study.

**Identification of the recording and stimulation sites** After all studies were completed, monkeys were deeply anesthetized by overdose of sodium pentobarbital (Nembutal, 80 mg/kg, i.p.), and perfused with 10 % formaldehyde (monkey HD) or with 4 % paraformaldehyde (monkey SJ). Coronal 50-μm-thick sections were stained with cresyl violet (Nissl) or by the thiocholine method to demonstrate acetylcholinesterase (AChE) activity. The anatomical limits of the CM were assessed on histological sections (Fig. S1) by referencing the histological criteria of the monkey thalamus (3-5) in conjunction with the assessment of their AChE immunoreactivity (1, 6). In addition, we identified the dorsolateral PF (PFdl) area of neurons, the latter of which were characterized by their densely packed, fusiform perikarya oriented in the mediolateral plane (3, 4). Histological reconstruction of the microelectrode tracks in relation to the electrolytic lesion marks (a total of eight marks made by passing a positive DC current of 20 μA for 30 sec; Fig. S1) allowed us to verify the location of the neuronal recordings and stimulations in monkey SJ (Fig. 4D).
Behavioral task: Monkeys sat in a primate chair in a sound-attenuated and electrically shielded room. They faced a panel in which a rectangular hold button and two instruction buttons were embedded. When the monkeys depressed the hold button for 200 - 600 ms with their hand that was contralateral to the thalamus recording, one of two instruction buttons was illuminated yellow as a cue stimulus. After another 1.2 - 2.2 s holding period, its color turned from yellow to either green or red. With the color green (GO), the monkeys released the hold button and depressed the illuminated target button within 3 s. The color red (NOGO) instructed the monkeys to continue depressing the hold button for another 700 - 800 ms. Combinations of either a large water reward (0.3 ml, +R) after the GO trials and small water reward (0.1 ml, −R) after the NOGO trials or vice versa were run in single blocks of 60 to 120 correct trials. The occurrence of large-reward and small-reward trials was not predictable (the average probability was around 0.5 in monkeys SJ and MA). However, in monkey HD, the single block of GO(+R) and NOGO(−R) trials occurred at a probability ratio of 1:2. A low (300 Hz) or high (1 kHz) tone was sounded after a correct behavioral reaction, which was followed by a large reward or small reward, respectively. The action-reward association was altered in the subsequent block. Error trials included those in which the monkeys either failed to keep the hold button depressed, released the hold button before instructed to do so, or performed an incorrect action (GO or NOGO). When a monkey made an error, all LEDs flashed and the trial was aborted.

Data analysis: Special attention was paid to the error trials in which wrong actions were made and in which neuronal activity was examined. Except for this analysis, we did not analyze the data collected during every error trial, nor during retrials after the error trial or the initial 8 correct trials during the transitional phase between blocks of trials with
different action-reward associations. The statistical significance of increases in discharge was evaluated by a two-sample Wilcoxon test ($p < 0.05$) in sliding by 10 ms with 30 ms windows which were compared to the 500 - 750 ms window that occurred before the start of the trials. Based on this evaluation, we determined the latency and duration of the neuronal responses. To estimate which factors significantly influenced neuronal activity, we used a two-way ANOVA ($p < 0.01$) with reward (+R/−R) and action (GO/NOGO) as the factors. This analysis was carried out by examining the activity of each neuron for 250 - 500 ms after the GO and NOGO request.

Supporting Online Text

**Relationship between LLF neuronal activity and performance reaction time**

Neuronal activity was high during the performance of the GO(−R) task, which occurred with a longer reaction time than performance of the GO(+R) trial. This observation raised the question as to whether our results could simply be explained by the ‘suppression’ hypothesis which posits that LLF neurons simply inhibit action and delay reaction. However, this hypothesis is not consistent with the following two of our findings. First, the magnitude of neuronal activation was not monotonically related to reaction times in the GO(−R) trials. To examine the relationship between neuronal activity and task performance reaction time, we divided all GO(−R) trials into 4 groups for each neuron based on reaction times. We then averaged the activity of 33 small-reward-action-selective LLF neurons from two monkeys in the 4 groups of trials (Fig. S3). What we found was that the magnitude of neuronal activity was similar between groups, though there was a slight tendency towards enhanced neuronal activity in the longer reaction time group except for the smallest activation in the longest reaction
time group (purple in Fig. S3). Results from a representative CM neuron are described in
the main text (Fig. 2B). A second inconsistency with the ‘suppression’ theory was that the
onset times of neuronal activity correlated with the reaction times of the GO(−R) action.
Although LLF neurons stopped discharging when the lever was released (GO reaction) in
all 4 reaction time groups of trials (Fig. S3B), the earlier the onset of activation the shorter
the reaction times (Fig. S3A). These data suggested that the discharge of LLF neurons
induced rather than suppressed GO(−R) actions. Thus, our results suggest that longer
reaction times might not be caused by suppression of the GO(-R) action by LLF neurons.

Relationship between LLF neuronal activity and location in the thalamus

We studied the characteristics of particular neurons in the intralaminar nuclei of the
thalamus which were previously described as LLF type neurons (1, 2). We examined the
relationship between the properties of neuronal activity and cellular location in the
thalamus through the histological reconstruction of the electrode penetration and
electrolytic lesion marks (Fig. S1). In monkey SJ, 40 LLF neurons were recorded of
which 31, 5, and 4 were located in the CM, PFdl, and PF, respectively. Thus, most of LLF
neurons were located in the CM nucleus while only a small number were found outside of
the nucleus. Furthermore, all of the neurons that were selectively activated after a
small-reward-action request in monkey SJ (n = 23) were located in the CM, except for
one neuron that was located in the PFdl. Therefore, the CM was the source of the neuronal
signals that were selectively activated when a small-reward-action was required at a time
when a large-reward-option was anticipated. We further examined the distribution pattern
of neurons within the CM nucleus itself. Specifically, we compared the medial two-thirds
of the CM that contained neurons that projected their axons to the striatum, with the
neurons located in the lateral one-third, whose axons projected mainly to the primary motor cortex (7). Our results showed that there were no significant differences vis-à-vis the small-action selectivity of these neurons within the CM (medial CM, 13/19; lateral CM, 9/12, $\chi^2 = 0.15$, df = 1, $p = 0.69$).

**Effects of electrical stimulation of the CM and its surrounding thalamic nuclei**

In the main text, we described how electrical stimulation of the CM after a large-reward-action request resulted in sluggish rather than brisk performance (Fig. 4A, B). The effective sites of the stimulation were concentrated in the CM, and the average effective stimulation rates (ESRs) were significantly higher within than outside of the CM (Fig. 4C). The most effective sites were in the CM except for two sites i.e., the dorsal border of the PFdl and in the MD. Two factors might have been responsible for the high ESRs following stimulation outside of the CM, especially in the region dorsal to the nucleus. One was the fact that only a small number of LLF neurons were observed in the PFdl and PF (as well as in the CM). The other may have been the high current intensity that was used in some cases, which may resulted in the activation of axons of CM neurons that projected antero-dorso-laterally from the CM to the striatum and/or cerebral cortex (8). The highest ESR was obtained following stimulation of the MD (Fig. 4D) with the highest current intensity (120 $\mu$A) used in this study (range was 20 - 120 $\mu$A, median 60 $\mu$A).

**Does LLF neuronal activity reflect long-term reward expectation or a complementary process to response bias?**

LLF neurons increased their response to a small-reward-action request concomitantly with rising expectation of a large-reward in the subsequent trial (Fig. 3). These neurons
may play a role in long-term expectation of a large reward such that their neuronal discharge increases trial-by-trial on the path to a large-reward trial. However, this suggestion does not seem to be well supported by the specificity of the neuronal activity. Strong activation occurred selectively after a small-reward-action request; even when a large-reward was highly expected in the subsequent trial, neuronal activation did not occur after the large-reward request. In addition, the pursuit of the requested action was critical for neuronal activation to occur. Therefore, LLF activity could not reflect the expectation of a long-term reward. The performance of the task by the monkeys did not appear to be influenced by the expectation of a long-term reward. Two monkeys reacted faster and more accurately in the large-reward GO trials than in small-reward trials. This finding was consistent with previously published reports that demonstrated that animals reacted faster and more accurately when they were motivated to obtain a reward (9), a specifically desirable reward (10), or a larger reward (11). While the monkeys could have developed a strategy that allowed them to perform the small-reward trials as fast as the large-reward trials in order to get the total amount of reward faster, this did not seem to be the case. Monkeys might react quickly and correctly in large-reward trials in order that they might secure the expected reward and not because of long-range reward estimation. These results let us to conclude that LLF activation was selective for the small-reward option and that it was complementary to response bias to a large-reward option.
References


**Fig. S1.** Photomicrograph of a Nissl stained frontal section of the recording and stimulation sites in and around the left CM nucleus of monkey SJ. The dots indicate the boundary between different parts of the nuclear group. Two microelectrode tracks passed through the CM in the dorsal to ventral direction. In the lateral track, two electrolytic lesion marks were made (*) - one at the bottom of the CM and the other in the PFdl. PFdl - dorsolateral part of PF (parafascicular nucleus); FR - fasciculus retroflexus; MD - medio-dorsal nucleus; CL - nucleus centralis lateralis. Scale bar: 1 mm
Fig. S2. Relationship between LLF neuronal activation time and activation of the arm muscle (flexor carpi ulnaris) during task performance. Neuronal and muscle activity was centered at the time of the GO and NOGO requests. The dotted line in C indicates the average activity during the (+R) condition.
Fig. S3. Relationship between the magnitude of neuronal activation and reaction times in the GO(−R) trials in a block of NOGO(+R) vs. GO(−R) trials. The curves represent the activity of 33 LLF neurons that showed significant (−R) selectivity in the trials performed at the reaction times, divided into 4 groups from the shortest (0 - 25 %) to longest (76 - 100 %). Activity was aligned at the GO(−R) request (A) and lever release (GO action) (B). Note that the magnitude of neuronal activity was similar between groups except for that which showed the smallest activation in the longest reaction time group.
Table S1. Latencies of LLF neuron activation after GO and NOGO requests. Values are the mean and SD in ms, while the number of responsive neurons in monkeys SJ and MA are indicated in the parentheses.

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<tr>
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<th>GO Onset</th>
<th>GO Offset</th>
<th>NOGO Onset</th>
<th>NOGO Offset</th>
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<td>387 ± 116 (19)</td>
<td>334 ± 54 (27)</td>
<td>421 ± 51 (27)</td>
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<tr>
<td>-R</td>
<td>309 ± 47 (36)</td>
<td>429 ± 76 (36)</td>
<td>313 ± 55 (43)</td>
<td>446 ± 57 (43)</td>
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