

# Activation of Monkey Locus Coeruleus Neurons Varies With Difficulty and Performance in a Target Detection Task

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**Rajkowski, Janusz, Henryk Majczynski, Edwin Clayton, and Gary Aston-Jones.** Activation of monkey locus coeruleus neurons varies with difficulty and performance in a target detection task. *J Neurophysiol* 92: 361–371, 2004. First published March 17, 2004; 10.1152/jn.00673.2003. We previously reported that noradrenergic neurons in the monkey locus coeruleus (LC) are activated selectively by target stimuli in a target detection task. Here, we varied the discrimination difficulty in this task and recorded impulse activity of LC neurons to analyze LC responses on error trials and in relation to behavioral response times (RTs). In easy and difficult discrimination conditions, LC neurons responded preferentially to target stimuli with phasic activation. These responses consistently preceded behavioral responses regardless of task difficulty. Latencies for LC and behavioral responses increased similarly for difficult compared with easy discrimination trials. LC response latencies were also shorter for fast RT trials compared with slow RT trials regardless of difficulty, indicating a close temporal relationship between LC and behavioral responses. This relationship was confirmed with response-locked histograms of LC activity, which yielded more temporally synchronized LC responses than stimulus-locked histograms. Population histograms of LC activity revealed that nontarget stimuli resulting in false alarm responses produced phasic LC activation (although smaller than for target-hit trials), and nontarget stimuli resulting in correct rejection responses yielded a small inhibition in LC activity. Population analyses also revealed that LC responses included an early, small excitatory component that was not previously detected. This early response was nondiscriminative because it was similar for target and nontarget stimulus trials. These results indicate that LC neurons exhibit early small magnitude responses that are closely linked to sensory stimuli. In addition, these cells show a later, larger magnitude response that is temporally linked to behavioral responses. These and other results lead us to hypothesize that LC responses are driven by decision processes and help facilitate subsequent behavioral responses.

## INTRODUCTION

The locus coeruleus (LC) is the largest nucleus of norepinephrine (NE) neurons in the brain (Dahlstrom and Fuxe 1964; Moore and Bloom 1979). This set of neurons located in the dorsorostral pons has a diverse set of efferent projections that innervate all levels of the central neuraxis and provides the sole NE innervation of the cerebral, limbic, and cerebellar cortices (Freedman et al. 1975; Garver and Sladek 1976; Ungerstedt 1971). These unique anatomical and neurochemical properties indicate a possible role for this system in behavioral and cognitive functions. Indeed, previous studies based on lesion and pharmacologic manipulations have indicated roles for the

LC-NE system in the sleep-waking cycle (Jouvet 1969), learning and memory (Crow and Wendlandt 1976; Everitt et al. 1983; Harris and Fitzgerald 1991; Mohammed et al. 1986), certain autonomic functions (Miyawaki et al. 1991, 1993; Ward and Gunn 1976), affective states (Siever and Davis 1985; Valentino and Curtis 1991), and vigilance (Aston-Jones 1985; Aston-Jones and Bloom 1981a).

We extended these ideas by studying LC neuronal activity in behaving monkeys during performance of an instrumental target detection task. This task allowed us to measure the link between LC neuronal activity and behavioral responsiveness to unpredictable, conditioned low-level sensory events (as in similar studies in humans; Davies and Parasuraman 1982; Warm 1984). We found that LC neurons responded phasically and selectively to target stimuli and not to other task events or behavioral responses (Aston-Jones et al. 1994). In addition, these neurons exhibited two modes of activity that corresponded to different levels of task performance. The phasic mode was associated with near perfect behavioral performance and included phasic activation of LC neurons preferentially by target (CS+) cues in this task. In contrast, the tonic mode corresponded with elevated tonic LC activity and poor task performance (frequent false alarm errors), but little or no LC response to target stimuli or other task events (Aston-Jones et al. 2000). Neural network modeling studies found that these two modes of LC activity can promote either focused attention (phasic mode) or “scanning” attentiveness/high behavioral flexibility (tonic mode) (Usher et al. 1999).

In additional studies, we found that the phasic responses of LC neurons reversed with the task contingency in a reversal paradigm, confirming that LC phasic responses to targets were not linked to the physical attributes of the stimulus but rather to stimulus meaning (Aston-Jones et al. 1997). Notably, the latencies of LC responses in the reversed (unfamiliar) contingency increased in parallel with behavioral response times (RTs). These results indicated that the extended processing required during the reversed contingency resulted in prolonged LC and behavioral response latencies and led us to propose that the LC response was directly linked to stimulus discrimination. However, the reversal study was not an examination of LC response variability based on cognitive difficulty but rather required the animal to extinguish the association of a previous contingency and establish a new one. The effect observed was transitory. Shortly after reversal, animals quickly assumed performance close to the normal level for the particular con-

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tingency. In the present task-difficulty study, test conditions could be studied for prolonged periods of time, allowing in-depth analysis of the relationship between the LC latency and the behavioral RT. We hypothesized that LC responses to target stimuli should increase in latency, in parallel with RTs, when additional processing is required because of more difficult stimulus discrimination. In this study, we tested this hypothesis by examining whether changes in target detection performance with increased discrimination difficulty are associated with altered LC responsiveness to target stimuli. In addition, our previous studies found that phasic LC responses were reduced in magnitude during the tonic mode of LC activity when high FA error rates occur. In this study, we investigated whether LC responses would occur on FA or miss errors with a more difficult discrimination during the phasic LC mode and focused task performance.

## METHODS

Experimental procedures in this study are similar to those presented in our previous papers (Aston-Jones et al. 1994, 1997).

### Target detection task

Two male cynomolgus monkeys (*Macaca fascicularis*), weighing 5.5 (monkey B) and 8.1 kg (monkey R), were used. Training and recording were performed inside sound- and light-insulating chambers. The monkeys were placed in custom-sized restraining cubicles

that allowed liberal limb and torso movement, restrained by a head-mounted fixation post and a neck yoke. The task is shown in Fig. 1. Animals were trained to continuously depress a pedal and visually fixate a centrally located spot on a computer monitor for  $\geq 200$  (monkey R) or 500 ms (monkey B). After successful fixation, target (infrequent, 20% of trials) or nontarget cues (frequent, 80% of trials) were displayed at this same location in random order across trials, and with randomly varying intertrial intervals (ITIs). Release of the bar within 650 ms after target cue onset was rewarded by juice (Tang); no other responses or trials were rewarded. FA errors were punished by a 3-s time-out during training, but this was found to be unnecessary for later performance and was eliminated. Targets and nontargets were vertical and horizontal light rectangles, respectively (Fig. 1B). Discrimination difficulty was varied by making target and nontarget stimuli more or less similar in shape. For the "easy" task, the length:width ratio of target and nontarget rectangles was 4:1. The length of these stimuli subtended a visual angle of  $7^\circ$  during training and decreased to  $2.4^\circ$  for easy blocks in the mixed easy and difficult task. In the "difficult" discrimination task, the length:width ratio was 1.1:1-1.3:1, and the angular length of stimuli was  $1.4^\circ$ . The speed of trial presentation was also varied. In the "slow" task, the ITI was 1.7 s (0.6 stimuli/s) on average, whereas in the "fast" task, the ITI was reduced to an average of 1.0 s. Some variants were tested in separate sessions, while in other sessions, two or more variants were presented in sequential blocks of trials for the same neural recording. Performance resulted in four categories of possible responses: correct detection (hit), correct rejection, incorrect detection (false alarm), and incorrect omission (miss). The frequency of each response type was used to calculate the signal detection measures  $\beta$  (decision criterion) and  $d'$

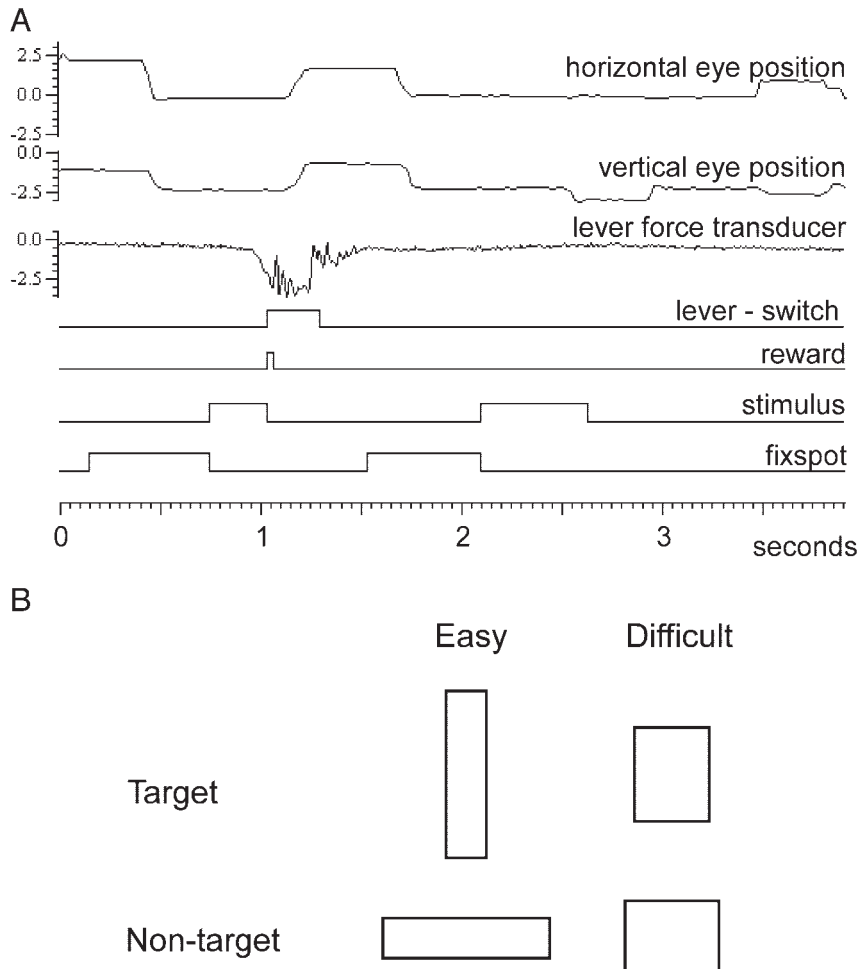


FIG. 1. Illustration of the target detection task used in these experiments. *A*: monkeys were required to depress a lever and foveate a centrally located fixspot on a computer monitor 60 cm away. After successful fixation, a target (CS+; 20% of trials) or nontarget cue (CS-; 80% of trials) was displayed in random order and with randomly varying intertrial intervals. Release of the lever within 650 ms after target cue onset was immediately rewarded with a drop of juice. *B*: targets were vertical light rectangles on a video screen, and nontargets were horizontal rectangles. Task was varied in discrimination difficulty by varying the length/width ratios of target and nontarget stimuli.

(discriminability).  $\beta$  and  $d'$  were computed using algorithms described by Macmillan and Creelman (1991). All animals were highly trained on the task prior to the commencement of neuronal recordings and exhibited performance levels of >90% correct responses.

### Unit recording techniques

Extracellular recordings from LC neurons were obtained using 30- $\mu\text{m}$ -diam, preinsulated tungsten wire electrodes positioned within the brain with the aid of a stereotaxically implanted guide cannula ending 5–7 mm above the LC. The electrode was moved through the brain in short (several micrometers long) steps, driven by a small, remotely controlled, head-mounted microdrive. Different electrode penetrations were achieved by minute alterations in the angle of the guide cannula requiring a movement of <1 mm in the tissue at the distal end of the guide cannula. There were no indications that such manipulations were perceived by the animal, nor did they produce tissue damage that was detectable in histological analyses. The activity patterns recorded from several electrophysiological landmarks aided with electrode placements, with the inferior colliculus, and trochlearis and mesencephalic trigeminal nerves as the most prominent markers. LC neurons were tentatively identified during recording sessions by previously described electrophysiological criteria (Aston-Jones et al. 1994), including broad impulses (>2 ms), a steady low-frequency discharge that decreased with drowsiness, and phasic activation/pause profiles in response to salient stimuli such as tapping on the chamber door. Multi-neuronal recordings from the LC were also identified and included separately for analysis.

### Histology

At the end of all recording sessions, the microwire electrode was repositioned at a location where a putative LC neuron was recorded (identified by its activity profile and the properties of neurons in surrounding structures, as described above), and current (10  $\mu\text{A}$  for 30 s) was passed through a microwire tip. Forty-eight hours later, the animal was deeply anesthetized and perfused with paraformaldehyde, and the brain was sectioned on a cryostat for localization of recording sites. Alternate sections (50  $\mu\text{m}$  thick) through the LC were stained for Nissl using neutral red or for the catecholamine enzyme tyrosine hydroxylase to identify noradrenergic neurons in the LC. The LC in monkey, like that in rat, is composed nearly exclusively of noradrenergic neurons (Freedman et al. 1975; Garver and Sladek 1976). Thus we conclude that recordings from neurons localized to the nucleus LC in our study were derived from noradrenergic neurons.

### Data acquisition and analysis

Continuous monitoring of eye position and pupil diameter was performed by an infrared video-based pupil tracking system (ISCAN, Cambridge, MA). Eye movements during task performance were monitored by a video camera focused on one eye, positioned so as to not obstruct stimuli displayed on the monitor. Data pertaining to horizontal and vertical eye positions as well as pupil diameter were continuously fed to a computer every 16 ms to be used on-line by a program controlling stimulus presentation (CORTEX, courtesy of R. Desimone, National Institutes of Health, Bethesda, MD). Additionally, digitized neural impulses and timing of sensory stimuli and behavioral responses were continuously monitored and stored on-line via a computerized data acquisition system (Cambridge Electronic Design, Cambridge, UK).

Peri-event time histograms (PETHs), raster displays, analog averages, and other display formats for recorded unit data were produced using Spike2 software (Cambridge Electronic Design). Baseline activity of a neuron was calculated as an average discharge rate during the 500-ms epoch immediately preceding the stimuli. PETHs were generated for activity recorded throughout an entire session or a

selected portion of a session. The onset of excitatory responses in PETHs was defined as the first bin of the PETH (10 ms binwidth) whose value exceeded the mean value of baseline bins by 2 SD and which was the first of five consecutive bins whose average was larger than the baseline mean + 2 SD. The offset of such responses was calculated by finding the first subsequent bin smaller than baseline mean + 2 SD, and which was the first of five consecutive bins whose average was smaller than the baseline mean + 2 SD. Similar corresponding criteria were used to detect inhibitory responses (bins < baseline mean - 2 SD).

Response magnitude ( $R_{\text{mag}}$ ) was calculated for every PETH according to the following equation:  $R_{\text{mag}} = (c - x) \times 100/\text{no. trials}$ , where  $c$  = counts in response interval, and  $x$  = counts expected in this interval with no stimulation (based on calculation for 500 ms before the stimulus onset).  $R_{\text{mag}}$  values calculated from individual session PETHs, or from composite cumulative multi-session PETHs, were compared with a  $t$ -test, except in cases with nonnormal distributions, when it was replaced by a Mann-Whitney rank sum test.

The LC mean activation time (MAT) was determined by calculating the time interval between the stimulus and each single neuronal discharge in the response interval of the PETH. Due to the relatively unskewed distribution of response intervals of PETHs, the average was chosen overall as a good representation of the central tendency. This value was close to the median of the LC activation time in PETHs. These LC MATs, and mean behavioral RTs, pertain to response distribution average values and should not be confused with the LC response onset latencies.

In addition to PETHs for individual recordings, population PETHs were generated by including activity across multiple sessions. For such population PETHs, several selected single-session histograms were compiled into a composite histogram by adding corresponding bins together and averaging the cumulative count with the total sweep count. Histograms were also smoothed by averaging across three consecutive PETH bins or by using smoothing algorithms in Sigma-Plot (Deneba). Smoothing parameters were adjusted to facilitate the superimposition of cumulative PETHs in a single figure while reliably preserving the pattern and timing of the responses.

Similar calculations were done for the behavioral RT distributions, with onset and offset values of these distributions established to reject spurious extreme small and large values.

The lever used by the animal to respond to stimulus presentation was typically equipped with a micro-switch. A force transducer (strain gauge) was also installed onto the lever to estimate RT onset.

## RESULTS

### Task performance varied with discrimination difficulty and trial speed

Increased discrimination difficulty in the slow task ( $n = 14$  sessions) resulted in a significant increase in the number of incorrect omissions ( $P < 0.005$ ) and a nearly significant increase in FAs ( $P = 0.057$ ). In the fast task ( $n = 4$  sessions), both the number of incorrect omissions and number of FAs increased significantly with increased difficulty ( $P < 0.02$  and  $P < 0.005$ , respectively). With increased difficulty in the slow task, the signal detection discriminability measure  $d'$  decreased from 4.97 to 4.19 ( $P < 0.02$ ), while changes in the  $\beta$  parameter were found to be nonsignificant. Unlike our previous studies (Aston-Jones et al. 1994; Usher et al. 1999), we did not observe alternating epochs of near perfect versus error-prone performance. The increase in the FA frequency with the difficult task observed here was distributed throughout sessions and did not vary appreciably to produce alternating periods of good and poor performance. Thus behavior here was characterized by apparently consistent attentiveness to the tasks.

### LC neurons exhibit discriminative responses to target stimuli

One hundred thirty-five recordings from LC neurons were obtained during these tasks; 60% of this number were classified as single-unit recordings, and the remainder were multi-unit. Task difficulty was changed within a single session as well as between sessions. Most recordings ( $n = 96$ ) were performed during the easy task condition, and a smaller number ( $n = 23$ ) during the difficult task only. In addition, some neurons were studied during alternating epochs of easy versus difficult discrimination, with fast ( $n = 5$ ) or slow ( $n = 11$ ) task conditions.

LC neurons preferentially responded to target stimuli but produced no response, or a small activation only, to nontarget cues in both easy and difficult tasks (Figs. 2–4). The typical response to target presentation in the “easy/slow” condition (similar to our previously reported task; Aston-Jones et al. 1994) consisted of a brief activation followed by a short-lasting depression of activity. For monkey B, the mean onset latency for these excitatory responses was  $114.8 \pm 5.3$  (SE) ms and duration was  $118.5 \pm 18.5$  ms ( $n = 41$ ), and in monkey R, these values were  $136.7 \pm 3.7$  and  $148.0 \pm 10.3$  ms ( $n = 45$ ), respectively. It is noteworthy that latencies for LC phasic activation were significantly shorter than behavioral RTs, which were  $260.6 \pm 2.6$  ms in monkey B and  $320.2 \pm 5.6$  ms in monkey R.

To test whether the response to targets was due to their low probability of occurrence (20% of trials), we introduced an infrequent nontarget stimulus (square of the same area as the rectangular target and nontarget cues; 20% of trials). As shown in Fig. 3, in these sessions, LC neurons continued to be selectively activated by target stimuli and did not respond significantly (by our 2 SD criterion) to either the infrequent or frequent nontarget cues (20 and 60% of trials, respectively). In

addition, phasic LC activation in response to target cues was significantly larger than LC response to either infrequent ( $P = 0.023$ ,  $n = 5$ ) or frequent ( $P = 0.012$ ,  $n = 5$ ) nontarget cues. This analysis was done by comparing response  $R_{\text{mag}}$  for target versus nontarget cues within the response time-window for target stimuli.

LC neurons were not activated by other task-related events, such as fixspot presentation, reward delivery, or task related movements (Fig. 2). In addition, as seen in Fig. 4, LC neurons were not significantly activated by target stimuli that failed to produce behavioral responses (omission error trials).

### Responses to nontarget stimuli

Nontarget stimuli typically produced only an insignificant modulation in LC activity compared with target stimuli (Fig. 2). Nevertheless, because nontarget stimuli were presented four times more frequently than target stimuli, when all nontarget trials were included, some recordings yielded enough data to produce significant responses. In both monkeys and in both easy and difficult tasks, nontarget stimuli that produced correct rejections yielded a small activation that appeared to correspond to an early nondiscriminative response that followed all stimuli. The mean latency for such activation was  $89.7 \pm 3.2$  ms in monkey R and  $75.5 \pm 9.7$  ms in monkey B. This early response was followed by a weak inhibition with an onset (by the 2 SD criterion) at 252 ms in monkey R and 168 ms in monkey B.

Nontargets yielded FA responses more frequently in the difficult than in the easy task (see *Task performance*). Weak LC responses in the latency range of discriminative responses to target cues were detected for nontarget stimuli that produced FA errors in the difficult task (monkey R; onset latency = 184

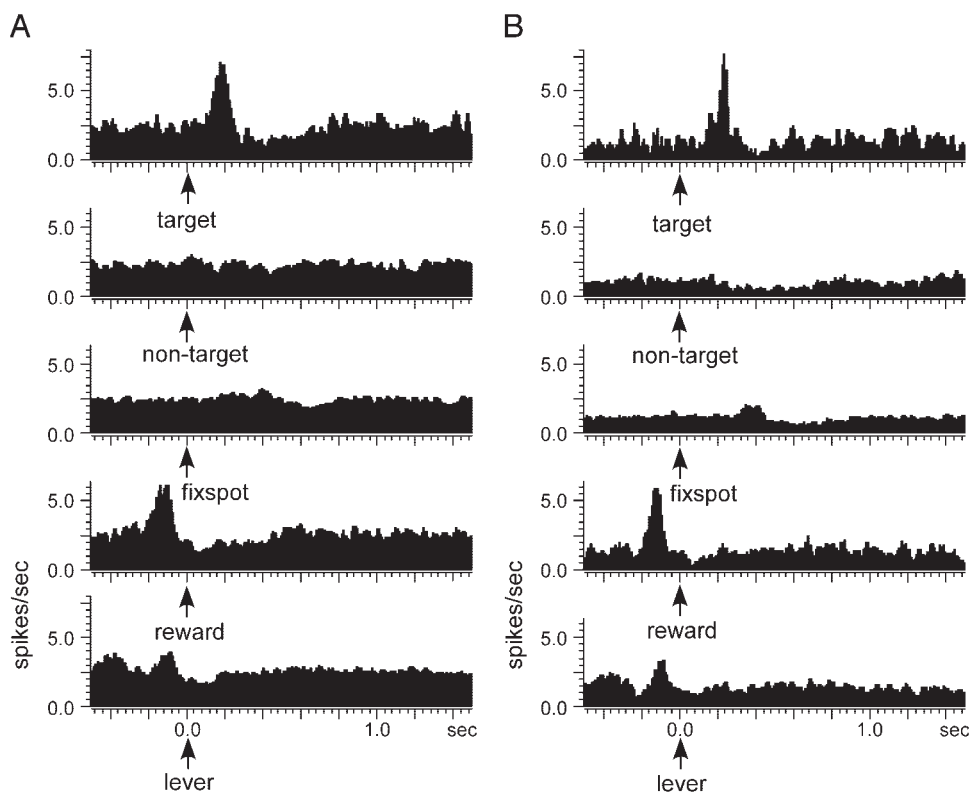


FIG. 2. Peri-event time histograms (PETHs) showing activity of 2 locus coeruleus (LC) neurons (A and B) recorded simultaneously during 1 task session. *Top row*: target stimuli elicited a short latency phasic activation. *Second row*: nontarget stimuli elicited no significant LC response in A but a small decrease in activity in B. *Third to bottom rows*: neither neuron was activated by the fixspot, presentation of the juice reward, or with lever motion (hit, FA, and intertrial responses included). Increased activity before juice or lever reflects responses to target stimuli. Numbers of trials from *top to bottom*: A—573, 2317, 5054, 995, 2177; B—200, 769, 1949, 384, 870.

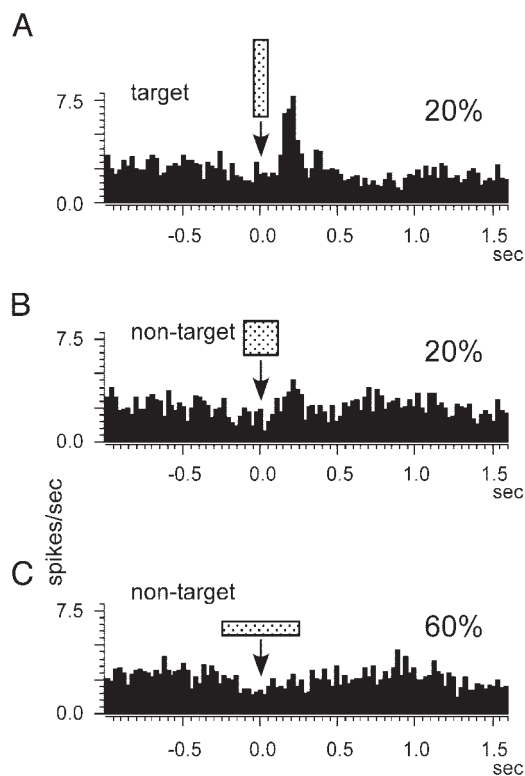


FIG. 3. Responses of LC neurons are not due to infrequent stimulus presentation. *A*: infrequent target stimuli (20% of trials) produced robust activation of this LC neuron. *B*: nontarget stimuli presented with the same frequency (20%) resulted in no significant LC response. *C*: nontarget stimuli presented on 60% of trials also elicited no LC response.

ms, MAT = 234 ms; Fig. 4). This compared with a behavioral RT of  $343.5 \pm 7.2$  ms, on FA trials, which was not significantly longer than RTs for hit responses ( $329.9 \pm 2.6$  ms;  $P = 0.076$ ). Unlike correct rejection trials, inhibition of LC activity was not apparent. Additional analyses were performed on FAs that had RTs within the range of RTs for hit trials; these were compared with the hit trials that followed each of these FAs. In this analysis, the  $R_{\text{mag}} = 42$  for FA trials versus 82 for the

paired hit trials in stimulus-locked population histograms and the  $R_{\text{mag}} = 53$  versus 83 in response-locked population PETHs, respectively.

#### Early nondiscriminative activation

LC neurons are preferentially activated phasically by target cues in this task (Aston-Jones et al. 1994). However, construction of population PETHs revealed additional response features not previously detected, presumably reflecting enhanced sensitivity due to greatly increased numbers of trials in population histograms. In particular, these population PETHs revealed a small, early LC excitatory response component that appeared to be present for all trial types. This response corresponds to the early response detected on nontarget trials in single-session PETHs. Because it was present for all trial types, we denote this as a nondiscriminative response, to be distinguished from the longer-latency excitatory response that occurs preferentially for target stimuli (discriminative response). The early nondiscriminative response on target trials was especially evident during the difficult task because the larger discriminative LC response component was delayed, producing a clear separation between the early nondiscriminative and later discriminative response components. As shown in Fig. 4, responses for all trial types diverge from baseline slightly before the onset latency of the more prominent excitatory responses seen in hit and FA trials. This early activation was found to exceed baseline activity by the 2 SD criterion (see METHODS) in 11 of 23 neurons in the difficult task, where the early response could be analyzed separately. The mean onset latencies of this early activation on target and nontarget trials for these 11 PETHs were  $82.0 \pm 2.3$  and  $89.7 \pm 3.2$  ms, respectively, and the average duration was  $60.0 \pm 3.3$  ms in monkey R. Because only the easy task was used in monkey B, separate analysis of the early activation on target trials was not possible, but the mean value for early response onset on nontarget trials was  $75.5 \pm 9.7$  ms in monkey B. Although apparent on FA trials, the early nondiscriminative response was not significant by the 2 SD criterion, presumably due to the small number of trials. Comparisons between correct rejection trials in easy versus

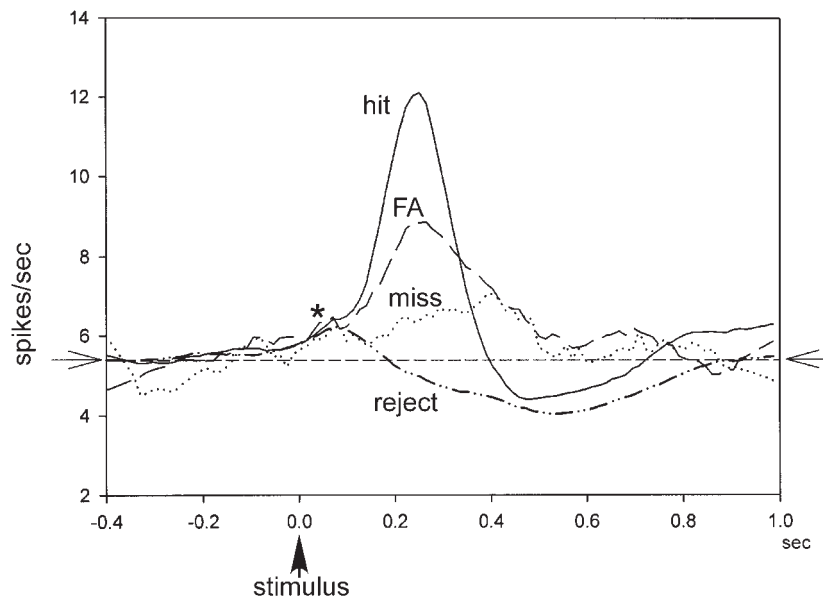


FIG. 4. Population PETHs for responses of LC neurons during the difficult/slow discrimination task in monkey R in 4 categories of task responses: hits (solid line, 8,116 trials), false alarms (FA; dashed line, 598 trials), incorrect omissions (miss; dotted line, 212 trials), and correct rejections (reject; hashed-double dotted line, 32,528 trials). Horizontal dashed line shows mean level of prestimulus activity. \*Early, nondiscriminative activation common to all 4 traces.

difficult task population PETHs, where the lack of the larger discriminative LC response left the early activation period exposed for analysis, revealed significant activations by our 2 SD criteria; there was no significant difference in the  $R_{\text{mag}}$  of these responses in easy versus difficult trials ( $P > 0.05$ ).

#### *Effects of changing task difficulty: delayed discriminative response in difficult trials*

Sessions in which the same recording was maintained for alternating epochs of 500-1,000 trials of both difficult and easy conditions ( $n = 16$  neurons) allowed direct comparisons of the effects of task difficulty on LC responses. Changing task difficulty had parallel effects on performance and neuronal responses. In general, the more difficult task resulted in an increased number of errors, increased behavioral RTs, and increased LC response latencies, as shown for a typical single cell in Fig. 5.

The differences in responses of LC neurons between easy and difficult discriminations were most apparent with superimposed population PETHs (Fig. 6). Eleven neurons were recorded in the slow task with sequentially switching blocks of difficult and easy tasks. Responses to target stimuli in easy versus difficult tasks had the same general pattern, with a similar onset time of about 88.5 ms (in numerous cases starting with a distinct nondiscriminative early activation) and postactivation depression. The most apparent difference between easy and difficult trials was that the activation peak for the discriminative response on target trials in stimulus-locked PETHs was significantly delayed in the difficult task. The LC MAT in the easy task was  $205.7 \pm 2.7$  ms, and for the difficult task, it was  $245.5 \pm 2.9$  ms ( $P < 0.001$ ). The durations of these responses were 155 and 173 ms, respectively.

In contrast to these differences in LC responses to easy versus difficult discrimination trials observed in stimulus-locked PETHs, LC activity for these different trials was almost identical when viewed in response-locked PETHs. As seen in Fig. 6C, the LC responses for easy and difficult discrimination trials were closely overlapping in response-locked populations PETHs. This indicates that LC responses are more closely associated with behavioral responses than with the presentation of the sensory cues.

The responses of LC neurons to nontarget stimuli that elicited correct rejections did not differ significantly between easy and difficult task conditions (Fig. 6B). FAs were too few in number in the easy task to compare with those in the difficult task. LC neurons responded to nontargets on FA trials in the difficult task with a weak activation.

The RT also changed significantly and prominently between easy and difficult trials (Fig. 6). Compiled across sessions, the mean RT for the easy task was  $323.3 \pm 2.3$  ms, but was  $345.5 \pm 2.3$  ms for the difficult task ( $P < 0.001$ ). The similar SE for these mean values indicates that the variances in the RT distributions did not differ appreciably between easy and difficult conditions. As noted above, the LC MAT was delayed in the difficult task compared with the easy task by  $\sim 40$  ms. Thus the mean latency of LC neuronal activity was affected by the difficult task requirement somewhat more than was the RT. This is primarily due to a somewhat longer LC response duration in the difficult versus easy task (described below).

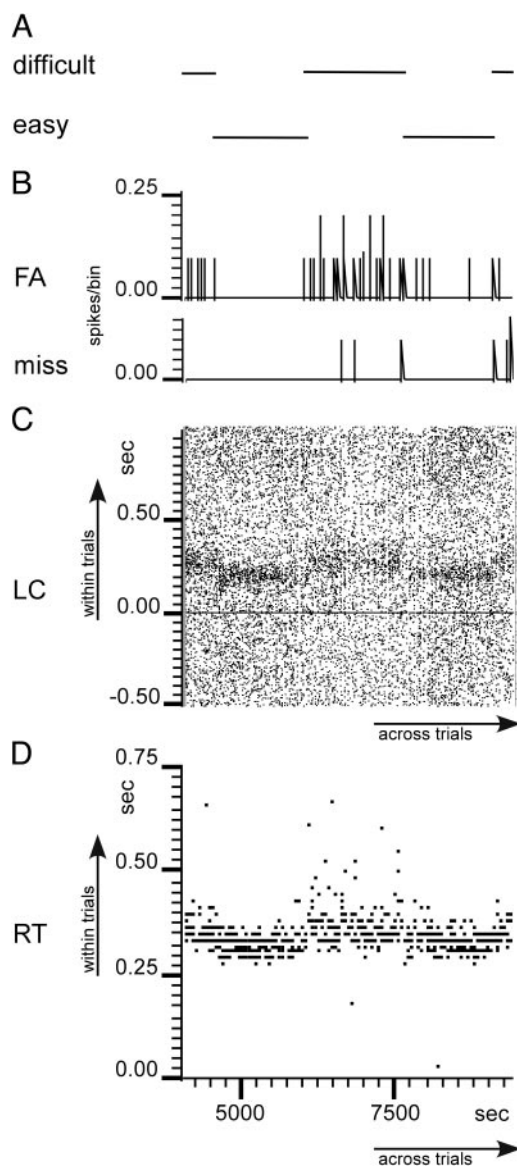


FIG. 5. Effects of switching task difficulty in a single recording session. *A*: horizontal line segments mark time epochs when either difficult task (*top*) or easy task (*bottom*) is presented. *B*: FA and miss errors are denoted. Most errors occurred during difficult task performance. *C*: raster plot of LC activity during task performance showing altered LC response latency with different discrimination difficulties. Each vertical line of dots (corresponding to the vertical time scale, with stimuli marked by the horizontal line at *time 0*) represents discharge of an LC neuron during a single trial. Consecutive trials are organized *left to right* along the horizontal time scale. Note that altered LC response corresponds with change in task difficulty. *D*: similar raster display as above, except that events marked by dots denote the time of the lever release. Vertical shift in raster-dot concentration shows difficulty-related variation in response time (RT).

To validate comparisons of population data, an average was calculated for onset latencies in individual sessions. Overall, this analysis produced results similar to the population analysis. For individual sessions, the mean latency of onset for LC responses in the easy task occurred at  $160.8 \pm 4.6$  versus  $191.5 \pm 4.2$  ms for the difficult task ( $n = 11$  single neurons,  $P = 0.002$ ). Note that this single-session analysis is less effective in revealing the early nondiscriminative response described above for population PETHs. Also, the onset latencies are somewhat longer with this analysis than with popula-

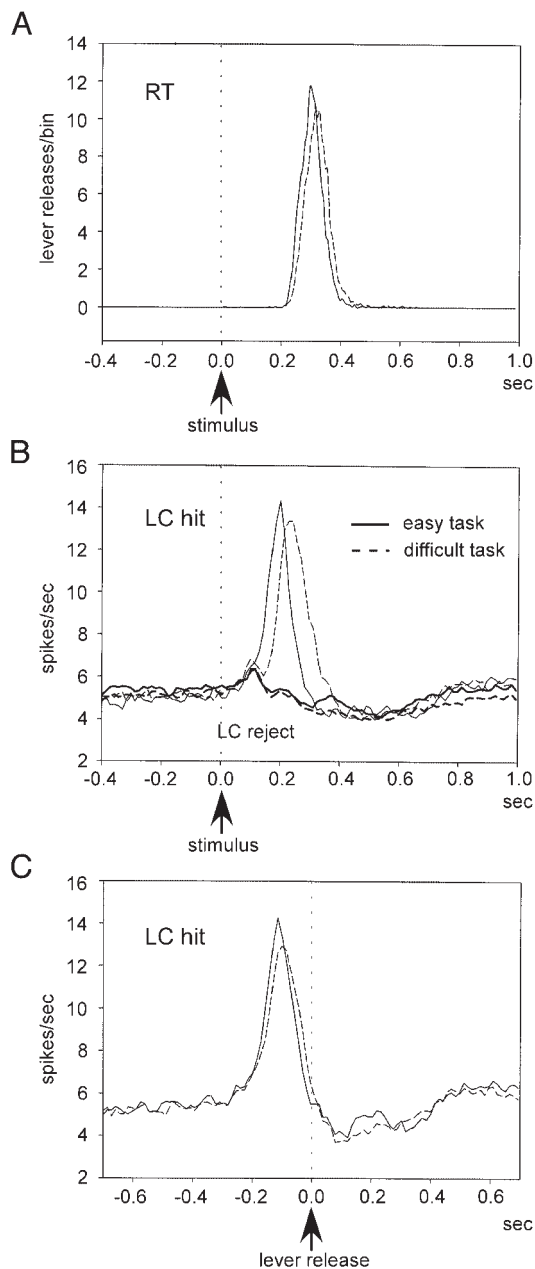


FIG. 6. RT distribution and LC responses for easy vs. difficult tasks. *A*: RTs were longer overall for difficult (dashed line) than easy trials (solid line). *B*: stimulus-locked population PETHs of LC responses to task stimuli for the easy (solid lines) vs. difficult discrimination (dashed lines). Hits, thin lines; correct rejections, thick lines. Note that LC responses are longer in latency for the difficult compared with easy discrimination. *C*: LC responses for easy (solid line) and difficult discrimination trials (dashed lines) in response-locked PETHs. These LC responses are more closely aligned compared with stimulus-locked PETHs in *B*, indicating that LC responses are more closely related to behavioral responding than stimulus presentation. Stimulus presentation is at time 0 in *A* and *B*, and behavioral responses are at time 0 in *C* (indicated by arrows).

tion PETHs or with analyses including multiunit recordings (as above), which effectively yield a larger sample. The MAT for LC responses on target trials was  $201.5 \pm 5.8$  ms in the easy task and  $244.7 \pm 4.1$  ms in the difficult task ( $P < 0.001$ ); note that this is very similar to LC MATs in population PETHs above. Although the easy task condition produced LC activation distributed mostly before movement onset, the difficult

condition resulted in a broader LC activation distribution (response duration =  $97.1 \pm 7.8$  ms in the easy vs.  $129.3 \pm 13.7$  ms in the difficult task,  $P = 0.05$ ).  $R_{\text{mag}}$  was also calculated and compared for the above data. These analyses revealed that the  $R_{\text{mag}}$  for LC responses on hit trials did not significantly differ for difficult versus easy tasks ( $P > 0.05$ ).

#### LC response corresponds with RT

As noted above, the latencies of LC activation and behavioral RTs increased with task difficulty. This indicates that LC responses may be temporally linked to behavioral responses, as would be expected if the LC activation facilitates corresponding lever responses. To analyze this possibility, correlation analyses between the latencies of spikes within the discriminative response period and the corresponding RT were performed on a trial-by-trial basis for 16 single LC neurons recorded during alternating periods of easy and difficult discrimination. Results revealed significant correlations, with correlation coefficients of  $r = 0.33$  and  $0.18$  for easy and difficult discriminations, respectively ( $P < 0.001$  for each).

The relationship between the RT and LC response latencies was also analyzed by computing LC response latencies for short versus long RT trials. In each of 11 recording sessions with single LC neurons recorded during easy and difficult tasks, two populations of LC responses were produced by separating trials whose RTs were shorter versus longer than the mean RT for the session (population PETHs for 11 neurons). These results are summarized in Fig. 7 and Table 1. As seen there, LC response latencies were longer for long compared with short RT trials. Comparison of response-locked versus stimulus-locked PETHs for short- and long RT trials in Fig. 7 revealed once again that LC activation is more closely associated with behavioral responding than with the sensory stimulus. Quantitative analysis of the response-locked population PETHs in the difficult task also revealed that LC responses begin as early as 250 or 255 ms before the lever response (long and short RTs, respectively). This time interval between LC and behavioral responses is considerably longer than what is calculated by comparing LC versus RT latencies in single session stimulus-locked histograms (Table 1). This discrepancy is primarily due to the larger number of trials in the population PETHs (which produces a better estimate of results) compared with the single session PETHs used for Table 1. Indeed, extrapolation of onset latency for the discriminative LC response in the stimulus-locked population PETH for hit trials in Fig. 4 (omitting the early, nondiscriminative response) indicates a value of about 100 ms. The difference between this and the mean RT of the difficult task (Table 1; Fig. 4) is 254 ms, close to the value obtained for population response-locked PETHs.

#### Varying the ITI

The frequency of stimulus presentation was increased from 0.6/s (as in the preceding experiments) to 1/s in 5 recording sessions with intermixed blocks of difficult and easy task contingencies, as well as in 18 sessions with the difficult task alone. Table 2 combines data for 11 neurons in the slow task and 5 neurons in the fast task, all examined across easy/difficult task conditions. The data show that increasing task

difficulty slowed LC response onset in both the slow and fast tasks. Increasing task difficulty also slowed the RT significantly. In contrast, increasing task frequency resulted in substantially faster performance and somewhat (although not significantly) shorter LC onset latencies. (Note that these analyses

TABLE 1. LC latencies in easy and difficult tasks for short vs. long RT trials

	Easy			Difficult		
	Short RT	Long RT	<i>P</i>	Short RT	Long RT	<i>P</i>
LC	137.5 ± 6.7	162.5 ± 9.2	0.041	164.4 ± 9.5	188.0 ± 8.5	0.086
RT	292.3 ± 4.5	332.2 ± 5.3	0.04	308.3 ± 4.7	354.1 ± 4.8	0.001

Values are mean ± SE. Each session's RT distribution was split along the average RT into short RT trials and long RT trials. Data were computed separately for each RT group. LC data gives mean onset time of LC activation. RT data shows mean RT. *P* values are from *t*-tests. No data were included from the fast task. *n* = 16 single and multiunit recordings.

were taken from individual PETHs rather than population PETHs to avoid including the early nondiscriminative response and to provide more accurate mean latency estimates.) LC MAT was shorter for fast compared with slow trial presentation in the difficult task (Table 2). Other measures did not change with increased frequency of stimulus presentation (e.g., the duration of LC responses or  $R_{mag}$ ;  $P > 0.05$  for each).

#### Spontaneous impulse rates

The mean discharge rate of LC neurons was determined from the 0.5 s preceding stimulus presentation. Rates were compared to ascertain whether changing the task difficulty or ITI affected the tonic level of impulse activity of LC neurons. As shown in Table 3, the average discharge rate for single LC neurons was not significantly different in the easy versus difficult tasks. Across all recordings, multi-unit included, the average discharge rate was  $5.4 \pm 1.1$  spikes/s for the easy task and  $5.0 \pm 1.0$  spikes/s for the difficult task ( $P = 0.6$ , paired *t*-test). However, spontaneous activity was significantly higher on the fast compared with slow task (Table 3). As seen in Fig. 4 for population PETHs, spontaneous rate for LC neurons did not differ for hit versus FA trials.

#### DISCUSSION

These findings revealed that responses of LC neurons to target stimuli vary with the level of difficulty in a target detection task. The LC discriminative activation following targets exhibited longer latencies for more difficult discriminations, as did behavioral RTs, and consistently preceded behavioral responses. Population PETH analyses in these results also revealed a novel early activation of LC neurons following task stimuli. This small magnitude response occurred similarly for target and nontarget stimuli and was distinct from the longer-latency response elicited selectively by target cues. We propose that this early response reflects primarily sensory-related information reaching the LC.

The values obtained here for LC and RT latencies in the easy task are very similar to those we previously reported for LC recordings in this task in other monkeys (Aston-Jones et al. 1994, 1997; Usher et al. 1999). The present results are consistent with, and extend, our prior findings during reversal of the target detection task (Aston-Jones et al. 1997). There, we found increased latencies of LC responses to the new target in parallel with increased behavioral RTs shortly following reversal of stimulus meaning. Additional processing was required for the reversed contingency, as reflected by the increased RTs. The

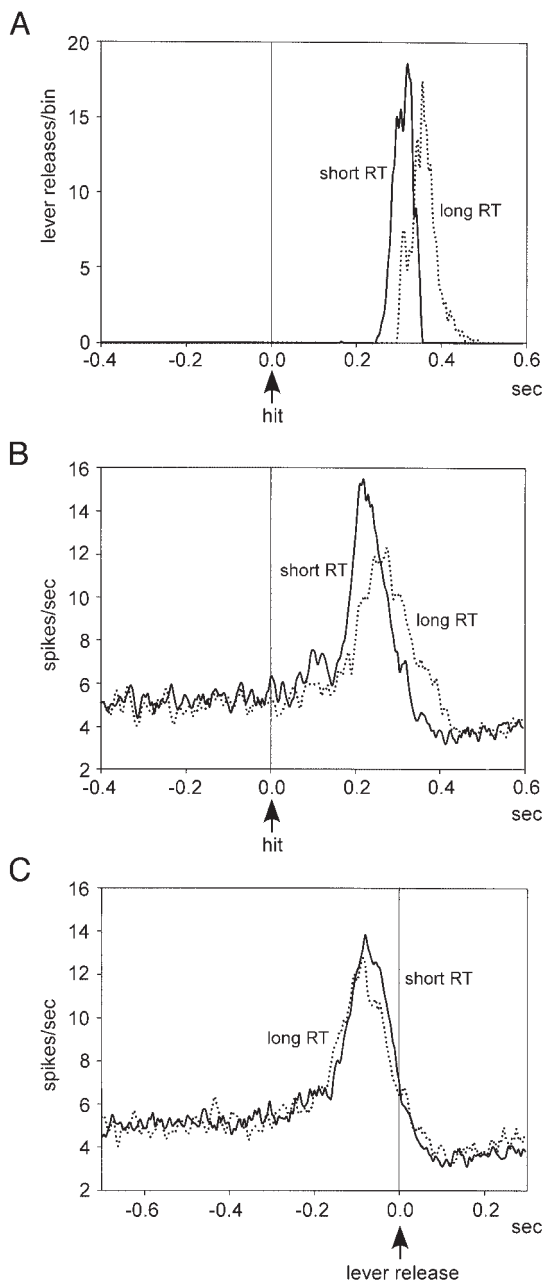


FIG. 7. RT distributions and LC responses for short vs. long RT trials. *A*: RT distribution for each of 11 recording sessions was split to produce RTs shorter or longer than the mean RT. Population RT distributions for short (solid line) vs. long RTs (dotted line) compiled across all 11 sessions are displayed. *B*: stimulus-locked population PETHs of LC responses to target stimuli that produced hit responses for short (solid line) vs. long RT trials (dotted line). LC responses are longer in latency for the long compared with short RT trials. *C*: LC responses for short (solid line) and long RT trials (dotted line) in response-locked population PETHs. These LC responses are more temporally aligned compared with stimulus-locked PETHs in *B*, indicating that LC responses are more closely related to behavioral responding than stimulus presentation. This result is comparable to that for easy vs. difficult discrimination in Fig. 6. Stimulus presentation is at time 0 in *A* and *B*, and behavioral responses are at time 0 in *C* (indicated by arrows).

TABLE 2. LC latencies and RTs in tasks that differed in difficulty and speed

	LC Onset			LC MAT			RT		
	Easy	Difficult	<i>P</i>	Easy	Difficult	<i>P</i>	Easy	Difficult	<i>P</i>
Slow	160.8 ± 4.8	191.5 ± 4.4	<0.01	201.5 ± 5.8	244.7 ± 4.1	<0.05	320.2 ± 5.6	340.2 ± 3.6	<0.001
Fast	151.8 ± 14.1	175.6 ± 13.1	>0.05	195.2 ± 15.0	224.2 ± 9.6	<0.05	275.4 ± 7.3	299.6 ± 5.4	<0.01
<i>P</i>	>0.05	>0.05		>0.05	<0.05		<0.001	<0.001	

Values are mean ± SE. LC onset time, mean LC activation time (MAT), and mean RT were computed for  $n = 11$  single units in the slow task and  $n = 5$  single units in the fast task. *P* values from *t*-tests.

parallel increases in RTs and LC response latencies during reversal, and during increased discrimination difficulty in this study, therefore support the idea that LC responses reflect a high level of stimulus processing, and that LC responses may play a role in behavioral responses to the corresponding stimuli (Aston-Jones et al. 1994; Usher et al. 1999).

Properties of LC neurons were similar throughout the nucleus in this study, as previously reported for other studies of these neurons in monkey (Aston-Jones et al. 1994; Grant and Redmond 1984; Grant et al. 1988) and other species (Abercrombie and Jacobs 1987; Aston-Jones and Bloom 1981a,b; Morilak et al. 1987; Rasmussen and Jacobs 1986). Thus this study adds to the prior evidence that LC neurons are physiologically similar throughout the nucleus and that the population acts as an ensemble to carry out a uniform function.

#### Effects of increased task difficulty

These results revealed that increased task difficulty resulted in longer RTs and latencies for discriminative LC responses to target cues. This would be expected if LC and behavioral responding reflect the extended processing required to make a discrimination in the more difficult condition. This possibility is consistent with our finding that the latencies of spikes in the LC phasic response were significantly correlated with the RT latencies on corresponding trials and also consistent with our prior findings of a similar nature (Aston-Jones et al. 1994). Thus shorter latency LC responses were associated with shorter latency behavioral responses. We hypothesize that the delayed LC response with increased task difficulty, like the delayed LC response that we reported previously during task reversal (Aston-Jones et al. 1997), indicate that LC responses are not purely stimulus-bound but are driven by processes that are integrally involved with discriminating and identifying the target stimulus.

LC response latencies increased somewhat more with increased difficulty than did the RTs (Fig. 6; Table 2). In contrast, the difference in LC response latencies between short and long RT trials was smaller than the difference between the

TABLE 3. Spontaneous discharge rate across different task conditions

	Slow Task	Fast Task	<i>P</i>
Easy task	2.9 ± 0.3	5.2 ± 0.5	0.002
Difficult task	3.2 ± 0.5	4.9 ± 0.6	0.065
<i>n</i>	10	4	
<i>P</i>	>0.05	>0.05	

Values are mean ± SE of single unit spontaneous rates computed for the different tasks, as indicated. *P* values computed from *t*-tests.

corresponding RTs (Table 1). These results indicate that, although they are related, the mechanisms that delay LC responses and behavioral RTs are not identical. This also indicates that these LC responses are not simply motor or premotor in nature, as also apparent from the lack of LC activation during lever responding between trials.

We estimate that motor cortex activation on this task occurs about 150 ms prior to the bar release [100 ms for motor cortex to activate spinal mechanisms and produce muscle contraction (Evarts 1974), and 50 ms for the mechanics of activating the microswitch on the bar]. The present results show that LC activation begins about 250 ms before the lever response. Because 50-100 ms is needed for LC impulses to reach cortical targets, we conclude that NE release from LC activation is occurring at about the same time as motor cortex activation associated with the lever response is beginning. This indicates that LC discriminative responses occur at the correct time to facilitate behavioral responses on the corresponding trial.

#### Effects of increased task speed

Increasing task cue frequency resulted in less accurate but faster performance (shorter RTs). The difference in RTs in the fast versus slow tasks was substantial and exceeded the difficulty-related difference in RTs by about 1.9-fold. Increased task speed resulted in more rapid responding with more errors, indicating a shift on the speed-accuracy trade-off curve to favor a more risky response strategy. It is notable that the associated increase in the tonic discharge rate did not preclude phasic responses to target stimuli, which were maintained in the fast task; thus this was not a shift to the tonic mode of LC activity as occurs in other situations (Usher et al. 1999). It is possible that the increased tonic discharge rate during the fast task with phasic responding to task stimuli plays a role in the change in response strategy observed during increased time pressure. Further studies are needed to test this possibility.

The LC response tended to also decrease in onset latency in the fast task, but to a smaller degree than the RT. Thus as with changes in discrimination difficulty, although the LC response times and behavioral RTs are related, they do not always change by identical amounts with different task parameters.

#### Responses on nontarget trials

Our previous results in the target detection task revealed either no response or a small inhibition on nontarget trials (Aston-Jones et al. 1994, 1997). These prior studies contained too few FA trials during periods of good performance (when LC neurons elicit phasic responses to task stimuli; phasic LC mode; Usher et al. 1999) to determine whether nontargets that

elicit FAs also evoke LC responses. The present results show that during the difficult discrimination nontargets often elicited FA responses despite a continuing phasic LC mode with responses also to target stimuli. On FA trials the nontarget stimuli evoked a clear phasic activation of LC neurons that was weaker than responses to target stimuli that elicit hits (Fig. 4). The present population PETH analysis also revealed that nontarget trials that produced correct rejection responses were associated with a long-latency weak inhibition of LC activity. Depression of LC activity also typically followed the phasic excitatory LC response on target trials. This is probably caused primarily by postactivation mechanisms such as the strong Ca-dependent K current and autoreceptor inhibition known to exist in LC neurons (Aghajanian et al. 1983; Andrade and Aghajanian 1985; Williams et al. 1984). Such factors do not play a role in the inhibition seen on nontarget trials. It is possible that the inhibition following the target is generated by, in part at least, a different mechanism than the inhibition after nontarget stimuli on omissions.

#### *Nondiscriminative response*

The early, small magnitude response on all task trials was detected here as a result of the increased sensitivity afforded by population PETHs. Because this early response occurred similarly for all trial types, it may reflect sensory-driven influence on LC activity, independent of stimulus meaning, i.e., a nondiscriminative response, distinct from the later and larger magnitude discriminative response for the target stimuli. This early response co-occurred and preceded the LC response that is elicited preferentially by target cues and appears in isolation on other task trials in stimulus-locked PETHs. In contrast, this early response was not detected in response-locked PETHs, further indicating its preferential association with sensory stimuli. The functional significance of this response is unclear, but these results indicate that a prediscriminative sensory signal reaches the LC, in addition to the conditioned signal reflecting the target stimulus meaning.

#### *What is driving the LC response?*

Is LC activation linked to active responding or to expectation of the reward? Our results reveal that for a stimulus to be effective in driving LC activity, it must have motivational value and be a salient event. Our finding that LC neurons do not alter activity during lever release outside of the task (Fig. 2) indicates that LC responses are not purely motor or premotor in nature. However, additional evidence indicates that LC activation is not related simply to anticipation of reward either. In a recent study (Clayton et al. 2002, 2003), we trained a rhesus monkey on a two-alternative forced choice task with frequent cues that led to juice reward (80% of all trials) and infrequent cues (20% of all trials) that signaled the unavailability of reward (noncue trials). The animal responded to the noncue stimuli even after learning that they signaled no reward on these trials (as indicated by longer RTs). We hypothesize that the animal made these responses to terminate the trial and initiate the next trial, which would likely lead to reinforcement. Notably, LC neurons exhibited phasic activation on the noncue trials similar to that for other trial types in response-locked PETHs. These results indicate that stimuli need not signal the immediate availability of reward to elicit LC activation.

#### *Caveats and limitations*

A general problem of monkey neurophysiology studies is that only a small number of subjects can be studied. This problem is somewhat lessened in studies of structures such as the LC where neuronal properties are generally quite similar across the population (as in this study). Thus studies in different species, laboratories, and preparations have consistently found remarkably similar properties for LC noradrenergic neurons (Berridge and Waterhouse 2003; Foote et al. 1983). This allows a reasonable sample of one class of cells to be obtained in a small number of subjects.

Although these results reveal a close relationship between LC phasic responses and behavioral responses to task stimuli, they do not establish a causal role for the LC in performance of this task. For that, additional studies would be necessary employing selective manipulations of LC activity. Preliminary results with local microinjections into the LC are supportive of our hypothesis that the phasic LC responses facilitate behavioral responses to the corresponding target stimuli (Aston-Jones et al. 2000; Ivanova et al. 1997).

#### *Proposed role of LC responses in task performance*

These results reveal that discriminative LC responses to target cues in this task reflect neither purely sensory nor motor/premotor activities. Rather, our results indicate that these phasic LC responses are driven by decision processes involved with identification of motivationally targeted stimuli, consistent with recent neural network modeling studies of the LC (Usher et al. 1999) and our recent results for LC activity during a forced choice task (Clayton et al. 2002, 2003). We propose that the outflow of NE from the LC efferent network increases the synaptic responsiveness of target neurons, as previously described in many physiological studies (Berridge and Waterhouse 2003). This in turn serves to facilitate wide-spread stimulus-response processing that is temporally associated with the decision to respond, and accelerates the associated behavioral response. In this way, we propose that these LC responses serve to support and facilitate task-driven phasic behaviors and thereby promote selective behavioral responding.

The lack of response in LC neurons on correct omission trials (in which the animal correctly withheld responding) indicates that the LC responds only for decisions that produce an active behavioral response; LC responses do not occur for decisions that require no response. This is consistent with our overall view that the decision-driven phasic LC response functions to facilitate the ensuring behavioral response. This function is only needed if an active behavioral response is reinforced by the stimulus-driven decision; on no-go trials, there would be no advantage to facilitating behavioral responding with a phasic LC response. We propose that during task acquisition the circuits associated with the "go" decision increase connectivity with the LC so that they come to more strongly drive LC responses than the no-go decision. This plasticity may involve reinforcement learning or a similar mechanism; further work is needed to delineate the processes involved in this learning.

Our results also reveal that LC responses on FA trials are smaller in magnitude than on hit trials. This could reflect a mixture of genuinely erroneous decisions by the animal (on

which trials we would expect LC responses to be similar to those for hit trials) plus occasional exploratory responses (e.g., to test for a possible change in reward value of nontarget cues) or responses during attentional lapses that were not driven by a similar decision process as on hit trials. Additional work is needed to test these ideas and further specify the role of phasic LC responses in adaptive behavioral activity.

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#### REFERENCES

- Abercrombie E and Jacobs B.** Single unit response of noradrenergic neurons in locus coeruleus of freely moving cats. II. Adaptation to chronically presented stressful stimuli. *J Neurosci* 7: 2844–2848, 1987.
- Aghajanian GK, Vandermaelen CP, and Andrade R.** Intracellular studies on the role of calcium in regulating the activity and reactivity of locus coeruleus neurons in vivo. *Brain Res* 273: 237–243, 1983.
- Andrade R and Aghajanian GK.** Opiate- and  $\alpha_2$ -adrenoceptor-induced hyperpolarizations of locus coeruleus neurons in brain slices: reversal by cyclic adenosine 3':5'-monophosphate analogues. *J Neurosci* 5: 2359–2364, 1985.
- Aston-Jones G.** Behavioral functions of locus coeruleus derived from cellular attributes. *Physiol Psychol* 13: 118–126, 1985.
- Aston-Jones G and Bloom FE.** Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci* 1: 876–886, 1981a.
- Aston-Jones G and Bloom FE.** Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J Neurosci* 1: 887–900, 1981b.
- Aston-Jones G, Rajkowski J, and Cohen J.** Locus coeruleus and regulation of behavioral flexibility and attention. *Prog Brain Res* 126: 165–182, 2000.
- Aston-Jones G, Rajkowski J, and Kubiak P.** Conditioned responses of monkey locus coeruleus neurons anticipate acquisition of discriminative behavior in a vigilance task. *Neuroscience* 80: 697–715, 1997.
- Aston-Jones G, Rajkowski J, Kubiak P, and Alexinsky T.** Locus coeruleus neurons in the monkey are selectively activated by attended stimuli in a vigilance task. *J Neurosci* 14: 4467–4480, 1994.
- Berridge CW and Waterhouse BD.** The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* 42: 33–84, 2003.
- Clayton EC, Rajkowski J, Cohen JD, and Aston-Jones G.** Activation of monkey locus coeruleus with stimulus identification in the Eriksen flanker task. *Soc Neurosci Abstr* 28: 86.88, 2002.
- Clayton E, Rajkowski J, Cohen JD, and Aston-Jones G.** Decision-related activation of monkey locus coeruleus neurons in a forced choice task. *Soc Neurosci Abstr* 29: 722.27, 2003.
- Crow TJ and Wendlandt S.** Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine. *Nature* 259: 42–44, 1976.
- Dahlstrom A and Fuxe K.** Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* 62: 5–55, 1964.
- Davies DR and Parasuraman R.** *The Psychology of Vigilance*. London: Academic Press, 1982.
- Evarts EV.** Precentral and postcentral cortical activity in association with visually triggered movement. *J Neurophysiol* 37: 373–381, 1974.
- Everitt BJ, Robbins TW, Gaskin M, and Fray PJ.** The effects of lesions to ascending noradrenergic neurons on discrimination learning and performance in the rat. *Neuroscience* 10: 397–410, 1983.
- Foote SL, Bloom FE, and Aston-Jones G.** Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. *Physiol Rev* 63: 844–914, 1983.
- Freedman R, Foote SL, and Bloom FE.** Histochemical characterization of a neocortical projection of the nucleus locus coeruleus in the squirrel monkey. *J Comp Neurol* 164: 209–231, 1975.
- Garver DL and Sladek JJR.** Monamine distribution in primate brain. II. Brain stem catecholaminergic pathways in macaca speciosa (arctoides). *Brain Res* 103: 176–182, 1976.
- Grant SJ, Aston-Jones G, and Redmond DEJ.** Responses of primate locus coeruleus neurons to simple and complex sensory stimuli. *Brain Res Bull* 21: 401–410, 1988.
- Grant SJ and Redmond DJ.** Neuronal activity of the locus coeruleus in awake Macaca arctoides. *Exp Neurol* 84: 701–708, 1984.
- Harris GC and Fitzgerald RD.** Locus coeruleus involvement in the learning of classically conditioned bradycardia. *J Neurosci* 11: 2314–2320, 1991.
- Ivanova S, Rajkowski J, Silakov V, Watanabe T, and Aston-Jones G.** Local chemomanipulations of locus coeruleus (LC) activity in monkeys alter cortical event-related potentials (ERPs) and task performance. *Soc Neurosci Abstr* 23: 1587, 1997.
- Jouvet M.** Biogenic amines and the states of sleep. *Science* 163: 32–41, 1969.
- Macmillan NA and Creelman CD.** *Detection Theory: A User's Guide*. Cambridge: Cambridge University Press, 1991.
- Miyawaki T, Kawamura H, Hara K, Suzuki K, Usui W, and Yasugi T.** Differential regional hemodynamic changes produced by L-glutamate stimulation of the locus coeruleus. *Brain Res* 600: 56–62, 1993.
- Miyawaki T, Kawamura H, Komatsu K, and Yasugi T.** Chemical stimulation of the locus coeruleus: inhibitory effects on hemodynamics and renal sympathetic nerve activity. *Brain Res* 568: 101–108, 1991.
- Mohammed AK, Callenholm NE, Jarbe TU, Swedberg MD, Danysz W, Robbins TW, and Archer T.** Role of central noradrenergic neurons in the contextual control of latent inhibition in taste aversion learning. *Behav Brain Res* 21: 109–118, 1986.
- Moore RY and Bloom FE.** Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu Rev Neurosci* 2: 113–168, 1979.
- Morilak DA, Fornal CA, and Jacobs BL.** Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats. III. Glucoregulatory challenge. *Brain Res* 422: 32–39, 1987.
- Rasmussen K and Jacobs BL.** Single unit activity of locus coeruleus neurons in the freely moving cat. II. Conditioning and pharmacologic studies. *Brain Res* 371: 335–344, 1986.
- Siever LJ and Davis KL.** Overview: toward a dysregulation hypothesis of depression. *Am J Psychiatry* 142: 1017–1031, 1985.
- Ungerstedt U.** Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand Suppl* 367: 1–48, 1971.
- Usher M, Cohen JD, Servan-Schreiber D, Rajkowski J, and Aston-Jones G.** The role of locus coeruleus in the regulation of cognitive performance. *Science* 283: 549–554, 1999.
- Valentino RJ and Curtis AL.** Antidepressant interactions with corticotropin-releasing factor in the noradrenergic nucleus locus coeruleus. *Psychopharmacol Bull* 27: 263–269, 1991.
- Ward DG and Gunn CG.** Locus coeruleus complex: elicitation of a pressor response and a brain stem region necessary for its occurrence. *Brain Res* 107: 401–406, 1976.
- Warm JS.** *Sustained Attention in Human Performance*. New York: John Wiley, 1984, p. 352.
- Williams JT, North RA, Shefner SA, Nishi S, and Egan TM.** Membrane properties of rat locus coeruleus neurons. *Neuroscience* 13: 137–156, 1984.