

Interval Timing and the Encoding of Signal Duration by Ensembles of Cortical and Striatal Neurons

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This study investigated the firing patterns of striatal and cortical neurons in rats in a temporal generalization task. Striatal and cortical ensembles were recorded in rats trained to lever press at 2 possible criterion durations (10 s or 40 s from tone onset). Twenty-two percent of striatal and 15% of cortical cells had temporally specific modulations in their firing rate, firing at a significantly different rate around 10 s compared with 40 s. On 80% of trials, a post hoc analysis of the trial-by-trial consistency of the firing rates of an ensemble of neurons predicted whether a spike train came from a time window around 10 s versus around 40 s. Results suggest that striatal and cortical neurons encode specific durations in their firing rate and thereby serve as components of a neural circuit used to represent duration.

Interval timing, defined as the perception of durations in the seconds-to-minutes range, is necessary for behavioral organization. Although a large body of theoretical work (e.g., Church, 1984; Killeen & Fetterman, 1988; Staddon & Higa, 1999) has produced an understanding of the psychological processes underlying interval timing (Gibbon, 1977), the neural mechanisms involved remain largely unknown. As the properties of the neural structures used in interval timing may dramatically constrain the feasibility of the mechanisms proposed by the various interval-timing models, determining the relationship between the general information-processing components of the interval-timing system and their underlying neural structures is central to understanding temporal control.

All interval-timing models can be conceived of in terms of *clock*, *memory*, and *decision* stages (Church, 1997). Briefly, the clock stage incorporates a clock signal (e.g., a pacemaker), integration mechanisms (e.g., accumulator), and start/stop/reset mechanisms. Output from the clock stage is, by definition, isochronic, as there must be a one-to-one mapping of clock stage output to the

amount of time passed since the clock started. Upon occurrence of a biologically meaningful signal, the clock stage output value is stored in memory. In future opportunities to time, this memorized value is compared with the current output of the clock by means of a decision stage until a similarity threshold is crossed, at which point a “time’s up” response—the presumed final output of the interval timer—is made.

Recent research indicates that corticostriatal circuits, as well as dopaminergic afferents from the substantia nigra pars compacta (SNPC), play a central role in interval timing (for reviews, see Gibbon, Malapani, Dale, & Gallistel, 1997; Harrington & Haaland, 1999). Rats with lesions of the dorsal striatum or SNPC behave as though they have a severely impaired perception of time (Clarke & Ivry, 1997; Dallal & Meck, 1993; Matell, Chelius, Meck, & Sakata, 2000). Dopaminergic drug administration, either systemically (Meck, 1983, 1996) or intrastrially (Neil & Herndon, 1978), alters the speed of clock-stage processing. Parkinson’s patients, who have decreased SNPC functioning, show deficits in reproducing durations when not taking their dopaminergic medications (Malapani, Deweer, & Gibbon, 2002; Malapani et al., 1998). Furthermore, brain imaging studies in humans show that both the cortex and striatum are activated during timing tasks (Harrington, Haaland, & Hermanowicz, 1998; Hinton, Meck, & MacFall, 1996; Lejeune et al., 1997; Meck, Hinton, & Matell, 1998; Rao et al., 1997; Rao, Mayer, & Harrington, 2001).

Electrophysiological recordings also implicate the cortex, SNPC, and striatum in interval timing. The firing rates of agranular cortex neurons in rats have been found to correlate with the attentional demands of timing two stimuli simultaneously (Pang, Yoder, & Olton, 2001). Primate SNPC dopamine neurons fire at the time of an expected, but undelivered, reward (Hollerman & Schultz, 1998), suggesting that they may play a role in timing a specific interval. Data from primates showing ramplike activations in both striatal (Hikosaka, Sakamoto, & Usui, 1989; Niki & Watanabe, 1979) and cortical (Niki & Watanabe, 1979) neurons during the delay period before an anticipated reward or movement

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suggest that these neural areas may also play a role in stimulus expectation or motor preparation.

In light of the above findings, we recently proposed an anatomical substrate for the interval-timing system, in which striatal spiny neurons serve as the temporal integrators of a cortical and thalamic oscillatory clock signal (Matell & Meck, 2000). Much of the memory- and decision-stage processes were also hypothesized to occur through striatal mechanisms, such that striatal firing represented the output of the interval timer. Specifically, the striatal beat frequency (SBF) model (Matell & Meck, 2000), proposed that temporal memories are stored as synaptic weights of corticostriatal synapses, which are modified and updated through a dopaminergic reinforcement signal from the substantia nigra (Wickens, Begg, & Arbuthnott, 1996). Comparisons of the current clock value with previous clock values stored in memory (i.e., decision-stage processing) are based on coincidence detection of these cortical inputs (Beiser & Houk, 1998). In SBF, the temporally isochronic signal of the clock stage is the ever-changing array of membrane potentials across the spiny neuron synapses, and peak-shaped striatal firing is the final "time's up" decision stage output of the internal clock.

Unlike the majority of interval-timing models, SBF makes specific predictions regarding the patterns of neural activity occurring during a timing task. The primary prediction is that striatal activity should peak at the criterion time. As it has not yet been examined whether the striatal and cortical delay-period preparatory activity described above encodes the specific time at which an event is expected to take place, or only readies the subject for an event to occur sometime in the future, it is unclear whether their firing rates continue to increase linearly, reach asymptote, or peak (i.e., symmetrically return to baseline) if the event does not occur at the expected time.

The current experiment was designed to evaluate whether striatal and cortical "preparatory" neurons encode detailed temporal information. To investigate the role that these neural areas play in interval timing, we recorded from ensembles of neurons in the dorsolateral striatum and anterior cingulate cortex in rats trained in a temporal generalization procedure (see Matell & Meck, 1999; Meck, Church, Wenk, & Olton, 1987). By using an interval-timing procedure with two possible times of reinforcement, we were able to observe the neural activity profiles both before and after the expected time of a reward. The use of this multivalued, fixed-interval (FI) timing procedure allowed us to dissociate neural activity from the rats' movements and thereby evaluate whether the firing rates in these structures contain information regarding the specific amount of time that had passed in a trial.

Method

Subjects and Apparatus

Six male Sprague-Dawley rats were initially housed in pairs under a 12-hr light-dark cycle with lights on from 7 a.m. to 7 p.m. They were given continuous access to water and maintained at 85% free-feeding weight by a daily ration of Lab Diet rodent chow (PMI Feeds, St. Louis, MO) given shortly after the daily session. Following electrode implantation, rats were housed individually.

All experimental data were obtained in a standard operant-conditioning chamber. The front and back walls and the roof were aluminum; the sides were transparent acrylic. The floor was composed of 16 parallel stainless

steel bars. A pellet dispenser located 13 cm above the grid floor on the center of the front wall delivered 45-mg food pellets (Formula A; Noyes Precision, Lancaster, NH) to a food cup. One 1 cm wide \times 4 cm long retractable response lever was located 2.0 cm from the side wall and 2.5 cm above the grid floor on the front wall. A 6-W houselight was located on the front wall, 4.0 cm from the ceiling. An 80-dB white noise signal was delivered through a speaker located outside the chamber. Each operant chamber was housed inside a wooden sound- and light-attenuating box, and was equipped with a 10-cm ventilation fan and an eyepiece viewer for observation. The box was equipped with a CCD infrared video camera mounted on the back wall, and had a 5-cm hole in the ceiling allowing the headstage connectors to pass through and attach to a commutator mounted above the sound-attenuating box. An IBM-PC-compatible computer attached to an electronic interface was used to control the experimental equipment and record the behavioral data (MED-PC, MED Associates, Georgia, VT). Neural data was discriminated online with a 32-channel multichannel acquisition processor system (Plexon, Dallas, TX).

Behavior

Rats were trained in a two-interval variant of a discrete trials temporal generalization procedure. In a typical discrete trials FI procedure, a stimulus (e.g., noise) is turned on, indicating the onset of the trial, and the first response after the criterion duration (e.g., 10 s) earns reinforcement and terminates the trial. Early responses have no consequence, and typical data from this procedure show the classic "scallop" pattern of responding as the signal duration approaches the criterion duration. In the current procedure, one of two different FI durations (short = 10 s or long = 40 s) was randomly chosen on each trial, and no cues were provided to instruct the rat which duration would lead to reinforcement. As such, this would cause the rat to press at a high rate at both 10 s and 40 s on the long 40-s FI trials, thereby allowing a comparison between early and late lever pressing. By having differential probabilities for the two trial types, the response rate during the two periods of responding was relatively equal. Only one response lever was available for the two different criterion durations. The response lever was positioned against the right wall of the chamber, and a barrier was constructed around the lever, so that the rat was only able to press the lever with its right forepaw, while its body was directed toward the rear of the chamber. Thus, the current procedure enabled the detection of neural activity differences that are related to the time of responding, largely independent of varying motor responses, as the motor activity (lever pressing) was essentially identical for responses at each duration (see Results).

Magazine training. Rats were given one session of food magazine training. A session consisted of delivery of a 45-mg food pellet once per minute for 60 min.

Autoshaping. Rats were given three sessions of lever-press training. During these sessions, a food pellet was delivered, independent of responding, once per minute for 60 min. Lever training was accomplished by autoshaping, in which the lever was retracted and extended 1 s before delivery of the food pellet. In addition, all responses made on the lever were reinforced with a food pellet. When rats pressed the lever 60 times on 2 consecutive days, they met the lever-pressing criterion and were switched to the next phase of training.

Lever barrier training. Once the rats reached criterion levels of lever pressing, an aluminum barrier was mounted around the lever so that it could only be pressed while the rat was positioned directly in front of the lever, rather than from an angle. The aluminum barrier was gradually extended over days until only a small (1-cm) window was available through which the rat could extend its right forepaw and press the lever. Criterion performance for each stage of the lever extension was assessed by 100 responses on a fixed-ratio 1 schedule. Training time varied between four and eight daily sessions for individual rats.

Temporal generalization training. A multiple-duration, FI procedure was used. Trials began with the onset of an 80-dB white noise signal. On

short trials, the first lever press after 10 s had elapsed was reinforced and terminated the signal, ending the trial. On long trials, the first lever press after 40 s was reinforced and terminated the signal. Responses made before the criterion duration of each trial had no consequence. The subject was not given any cues as to which duration would be primed for reinforcement on each trial. The proportion of trials was 25% short and 75% long in order to equate reinforcement density at the two criterion durations, and trials were chosen pseudorandomly. A random, uniformly distributed, 30–50-s inter-trial interval separated the trials. Training continued until the response distribution showed both a peak at 10 s and a scallop pattern up to 40 s on the long trials, and roughly equivalent peak response rates at the two criterion durations. Once performance reached this behavioral criterion, rats were implanted with electrodes, given 1 week to recover, and retrained on the interval-timing procedure until they returned to similar response rates at both criterion durations, at which point electrophysiological recording commenced.

Surgery

Electrodes. Chronic microwire assemblies (NBL Labs, Denison, TX) were used in our experiments. Arrays composed of 50- μ m Teflon-coated, stainless steel wires, assembled into 2×8 matrices held with epoxy, were used in this study. The arrays measured approximately 1 mm in width \times 2 mm in length.

Procedure. Rats were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). One of the electrode arrays was lowered into the anterior dorsolateral striatum (center at AP +1.0, ML 3.0 [left hemisphere], and DV 4.0). A second 2×8 array was placed around the boundary of premotor cortex 2 and cingulate cortex 1 (center at AP +3.00, ML 0.75 [left hemisphere], and DV 2.00). This area of the cortex was chosen because it was shown to provide afferents to the striatal region (Sesack, Deutch, Roth, & Bunney, 1989). Furthermore, previous recordings from anterior cingulate and dorsolateral prefrontal cortex showed preparatory activation prior to both movements and cues during a differential reinforcement of low rates (DRL) timing task in monkeys (Niki & Watanabe, 1979). In order to fit both arrays onto the skull and get viable recordings from these two proximal areas, the striatal array was implanted at a 15° angle. The striatal array was lowered until it was approximately 1 mm from the target location, and then the prefrontal array was lowered straight down. Once the prefrontal array was in place, the striatal array was lowered the remaining amount. In this manner, movement of the brain from the insertion of the electrode arrays would have a minimal impact on the viability of the recording sites. Rats were given 5 days of recovery, with free access to food and water, before being returned to food deprivation.

Recording Procedure

Once 85% body weight was reobtained, rats were retrained on the full procedure until they reached presurgical performance levels. Following task reacquisition, neural recordings were begun. The rat was briefly anesthetized with halothane, headstage cables were plugged into the implants, and the rat was placed in the operant-conditioning chamber, with the door left open so as to maximize the contextual differences of the chamber during spike sorting. The anesthesia was used to allow the connection of the cables to the headstage, without any excessive torque to the skull that could conceivably occur if the rat moved its head while the connection was being made. All observable effects of halothane on behavior disappear within 5 min, and any effects on the neural activity are undetectable within 20 min (E. Fanselow, personal communication, April, 2000). Therefore, the behavioral procedure was not begun for at least 30 min after administration of anesthesia.

The electrical activity recorded from each microwire was monitored with the multichannel acquisition processor and subjected to the procedures for discrimination of single units. Specifically, multiple voltage and

time-voltage windows combined into a real-time principal component algorithm were used for spike discrimination (Nicolelis & Chapin, 1994; Nicolelis, Ghazanfar, Faggin, Votaw, & Oliveira, 1997). This method separates spike waveforms on the basis of the amplitudes of the waveform peaks, the time between peaks, and computed parameters based on the waveform shape. Each of the channels is processed separately. After the units were discriminated, the recordings from each wire were amplified on an audio monitor. Ensembles of single neurons, distributed across the striatum and cortex, were then recorded simultaneously (Nicolelis et al., 1997). After the discrimination of single units, the door to the operant-conditioning chamber was closed and the interval-timing procedure was initiated. The discriminated spike times were transferred, along with the onset and offset times of behavioral events (e.g., reward delivery, lever press, stimulus onset), to a database for subsequent analysis.

Analysis

Determination of single units. Single- and multiunit clusters were initially judged on the basis of visual inspection of waveform shape, size, and discriminability in reference to other single units and the baseline noise on each channel. Units that were visually identified as discriminable were then verified as single units by evaluating the interspike interval histogram. A single unit is defined as any discriminated unit in which less than 1% of its interspike interval histogram falls below 2 ms and the histogram shows a characteristic Poisson shape. Discriminated units that did not pass these criteria were not analyzed further.

Although data were collected for more than one session, we found that the neural waveforms frequently differed across days. As such, we could not reliably determine whether we were recording the same cells from one session to the next. To alleviate analysis problems associated with possible changes in cell numbers/identity, we analyzed data from only a single session. The data used for analysis were selected by choosing the session with the greatest number of single cells.

Histological analysis. Rats were perfused intracardially, first with saline, and then with 10% Formalin. Brains were frozen, sliced at 80 μ m, and stained with a Nissl stain. Electrode tracks were identified, and placement of the electrodes was verified.

Data analysis. Analyses were based on changes in the firing rate of single neurons, as assessed by construction of peristimulus time histograms (PSTH) and rasters around behavioral events.

Comparison periods. To determine whether a neuron was significantly modulated across a trial in a manner that could conceivably encode signal duration, we defined two time windows (times of interest [TOIs]). These TOIs correspond to the periods of high lever-press activity occurring just prior to the two criterion durations (10 s and 40 s), and they reflect the times at which the rat was beginning to expect that reward might be delivered. Previous research has shown that the peaks of the short and long criterion durations are composed of single-step functions that vary from a low operant rate to a constant high rate of lever pressing, and vary from trial to trial only in terms of the start and stop times for responding (Church, Meck, & Gibbon, 1994; Schneider, 1969). As such, these periods of high lever pressing for both the short and long criterion durations are roughly identical on a trial-by-trial basis and can be logically compared.

However, because the rats stopped pressing after reward delivery at 40 s, only the left half of the short peak was used in comparisons with activity from the long scallop. The peak times of each rat's mean lever-press function were used to determine these comparison periods. The short peak, centered around 10 s, was defined by fitting the mean lever-press data with a three-parameter Gaussian curve, using the data that made up the first peak (e.g., the data were cut off at the time bin that had the fewest responses separating the two peaks of the mean function). The standard deviation of this Gaussian function was then used to define the width of the TOI for the two durations. The short TOI was defined as extending backward in time from the mean of the Gaussian function by 1 *SD*. The long TOI was defined

as extending from 40 s backward in time by the same 1 *SD* taken from the fit of the short peak.

These TOI periods are associated with an increasing, temporally specific, behaviorally expressed expectation of potential reward, and were used in order to allow us to compare neural firing rates during periods of similar motor output. These asymmetric periods, rather than a symmetric period around the short criterion duration, were selected to make the comparison periods as psychologically equivalent as possible (although there was a difference in reinforcement rate), given the observation that separate thresholds may be used to start and stop responding around a criterion time (Rakitin et al., 1998). In addition to these quantitative comparisons across the TOIs, visual observation of the shapes of the histograms over the entire trial provided us with the ability to qualitatively evaluate whether the neurons in question broadly peak at or around the criterion time, or whether they display variations in firing rate that would fail to be noted by this technique. Thus, despite the fact that our TOIs preceded the criterion times, we were still able to evaluate the possibility that neurons fired with precise temporal activations only at or after the criterion times.

Because the two criterion durations were equated for reinforcement density, response rates between the two TOIs were highly similar. To facilitate a meaningful comparison of short versus long neural activity, we dissociated possible motor confounds by comparing short versus long neural activity during trials in which the number of lever presses during the short and long portions of the trial were identical (on average). Because the early and late TOIs were the same width, the response rates across these comparison periods were also identical. By these criteria, approximately 90% of the trials in each rat (~ 45/50 trials) were used for neural analysis.

Neuronal activity during the TOI. For the evaluation of neural responsiveness as a function of time, 99% confidence intervals were computed from the session's firing statistics. Neural firing was evaluated for crossing the 99% confidence interval over the number of time bins that made up 40% of the width of the TOI, when divided into 1-s bins. This procedure enabled a determination of neurons with near maximal or minimal firing rates at or around the times of expected reward.

Differential firing over time. A multivariate analysis of variance (MANOVA; Duration \times Neuron) was used to compare the number of spikes from each neuron on each trial during the short versus long TOI, using each trial as a separate case. The *p* level for these tests was set at .05.

Video analysis of behavior. In order to evaluate whether there were variations in the rats' behavior over time, we analyzed videotapes of the behavioral sessions frame by frame. The rats' overall behaviors could be clearly characterized as bouts of lever pressing interspersed with locomotion to and from the magazine to check for food. Other behaviors (e.g., rearing and grooming) occurred very infrequently. As such, three behaviors (the onset of locomotion from response lever to the magazine, crossing the food cup with the head, and onset of locomotion from the magazine to the response lever) were scored, and the times of their occurrence were recorded.

Motor versus time analysis. It was necessary to evaluate and compare the neural activity during epochs of highly similar motor behavior across the TOI. To this end, we defined a bout of pressing as a series of presses preceded and followed by a 1-s pause in pressing. We then compared the neural activity that fell within press bouts that occurred during short and long TOIs. Specifically, the mean spike rate as a function of a within-bout press was determined for each trial at each duration. In addition, to more adequately match the comparison epochs, we computed press statistics (e.g., press duration, interpress interval, and release-to-press interval) for each trial and used them as covariates in a multivariate analysis of covariance (MANCOVA; Duration \times Neuron). The *p* level for these tests was set at .05.

Dynamic motor coding. Alterations in press-related firing over time were evaluated by construction of PSTHs around short and long TOI presses (± 2 s, 100-ms bins). Changes in the firing patterns were assessed

with a chi-square test for distribution similarity. Changes in background spike rate were assessed by a paired sign test. The *p* level for these tests was .05.

Single-trial analyses. Behavior on individual trials in an FI procedure has been described as switching from a low response rate to a high response rate (Schneider, 1969). This analysis has been expanded for use with the peak-interval procedure (Church et al., 1994). We applied this analysis to the present data (both behavioral response patterns and neural activity of temporally specific neurons). Briefly, the data were fit with low-high-low-high step functions by minimizing squared deviations, thus allowing each step to have an independent rate, with the exception that both high states must be higher than both low states. Important statistics from these analyses are the times at which the states switch (e.g., from low to high).

Ensemble analysis. The role of multiple neuronal coding of intervals was evaluated by means of ensemble analysis techniques. We were interested in determining whether the information provided by multiple neurons would be considerably better than information from the best single neuron in the ability to characterize whether a spike train came from one or the other of the two TOIs. To this end, linear discriminant analyses were used to evaluate whether additional information is contained in the correlations between neuronal firing rates among the ensembles of neurons (for details regarding these analyses, see Laubach, Wessberg, & Nicolelis, 2000; Nicolelis, Lin, & Chapin, 1997). Briefly, we compared spike counts during the short versus long TOI on each trial, either in a single neuron or across varying size ensembles, in order to determine whether the number of spikes in each of these TOIs could be used to discriminate whether the spike train occurred early or late in the trial. In other words, we measured the degree of trial-by-trial consistency in the PSTH plots and evaluated whether this consistency could be improved by looking at multiple neurons simultaneously. The relationship between trial type (i.e., short or long) and spike rate/correlation statistics was computed by using all but one trial (i.e., the algorithm was trained). The remaining trial was then classified as short or long by computing the similarity of the firing statistics on the current trial with the session-based statistics. All trials were reassessed in turn, and the percent correct classification was determined. We investigated the predictability of each single neuron alone, the ensemble as a whole, and the ensemble after the omission of individual neurons so that the performance of the remaining neurons was maximized.

Timing function. In order to assess a possible underlying timing function contributing to the mean neural activity patterns, the distribution of neural activity for those neurons that showed an increasing peak-shaped activation was fit by using a least-squares minimization, with a function composed of the sum of the scaled distribution of presses, a baseline firing rate, and either a linear decay function or a Gaussian peak function. These functions represent the basic predicted clock readouts of two timing models (e.g., accumulator models [Gibbon, 1977] and oscillator models [Matell & Meck, 2000]).

Results

The mean lever-press distribution from the long trials, averaged across the 6 rats, is shown in Figure 1. As expected, response rates on the lever increased to form a peak near the first criterion duration of 10 s, decreased in a near-symmetrical manner, and then increased again to approximately the same peak rate in a scallop pattern at 40 s.

The average peak time (mean of the Gaussian function) for the short peak was 12.5 s (± 1.7 s), and the width of the peak (standard deviation of the Gaussian function) was 7.1 s (± 1.9 s), resulting in a coefficient of variation of 0.56 (± 0.09). Because Gaussian fits could not be performed on the second scallop, the standard deviation of the long function, and therefore superimposition, could not

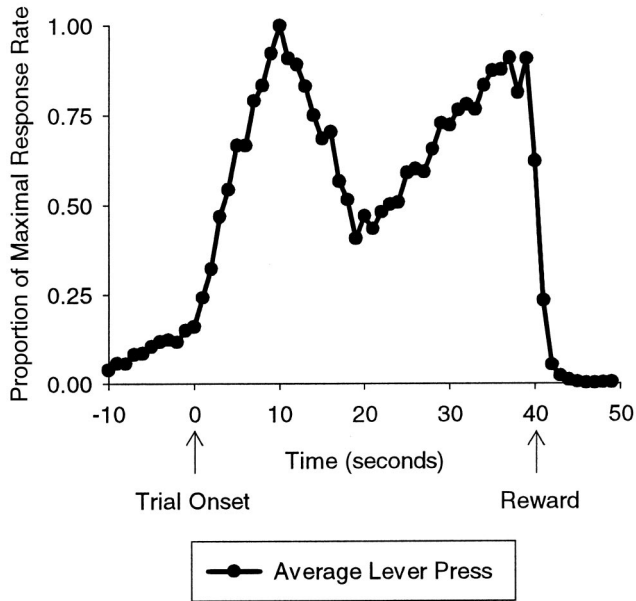


Figure 1. Mean distribution of lever presses across the long (40-s) trials. These 40-s trials make up 75% of the total trials. The remaining 25% are made up of 10-s fixed-interval trials. This differential probability of reinforcement produces roughly equal rates of responding at both the 10-s and 40-s peaks.

be determined in this manner. However, an alternative method of assessing scalar timing is to compare the widths of the functions at half-maximal responding. With a scalar timing process, these widths should be directly proportional to the duration being timed (i.e., the width of the 40-s function should be 4 times the width of the 10-s function). Surprisingly, if we assume that the “long” response function would peak at 40 s, these data do not show precise scalar timing by this measure, in that the width of 40-s function (29.3 ± 7.5 s) was only 2.4 times the width of the 10-s function (12.5 ± 2.3 s). In addition, a single-trials analysis was performed on these data by fitting the lever-press responses on each trial with a multiple step function (low-high-low-high). The average onset time for the first high response state was 9.3 s (± 1.9 s), whereas the average offset time was 17.9 s (± 1.2 s), thereby leading to a width of 8.6 s (± 1.5 s) with a midpoint time of 13.6 s (± 1.4 s). The average onset time of the second high state was 29.7 s (± 1.5 s), which, with a 40-s peak time, would give a width of 20.5 s (± 3.0 s), which is 2.4 times greater than the width of the first high state. Thus, the data from the single trials analyses were similar to the mean function data, in that exact scalar responding was not seen. A correlation analysis between the offset time of the first high state and the onset of the second high state failed to show significant correlations in the majority of the subjects, suggesting that these two durations were timed independently (note: 2 subjects did have very small, but significant, positive correlations [$R^2 \leq .10$] between the offset of the first high state and the onset of the second high state).

TOIs were defined as the periods of time preceding both the first peak time and the trial end (40 s) by the width of the standard deviation of the first peak. Thus, the average short TOI extended from 5.4 s to 12.5 s, and the average long TOI extended

from 32.9 s to 40.0 s. These TOIs represent the signal durations at which the rats expected food reward, as they lever pressed at roughly maximal rates during these periods.

A total of 108 single units were recorded, with 54 of these from anterior dorsolateral striatum, and 54 from dorsal medial prefrontal cortex, specifically the region of anterior cingulate cortex 1 (Paxinos & Watson, 1986). Representative electrode track marks and the placements across subjects are shown in Figure 2. There were an average of 9.0 ± 2.9 striatal units and 9.0 ± 5.5 cortical units per rat.

Trial-Based Firing Modulations

Of the 54 striatal neurons recorded, 39 (72%) were responsive during the periods preceding the short, long, or both expected reward times. Similarly, 65% (36/54) of 54 cortical neurons crossed the 99% confidence interval during the periods prior to expected reward delivery. In all cases, these neurons fired (or had inhibitions in activity) throughout one or both of the press periods, thereby peaking at or very close to the time of the peak in the lever-press function. These results suggest that a large proportion of cortical and striatal neurons are involved in the behavioral expression of a temporal generalization task.

However, if these neurons are directly involved in the representation of specific signal durations, their neural activity must be dissociable from the lever-press activity that occurs during these TOIs, and it must also be different across the two TOIs. Of the 54 striatal cells, 15 (28%) had significantly different activity at one of these criterion durations compared with the other criterion duration. Similarly, of the 54 cortical cells, 11 (20%) had significantly different firing rates across the two TOIs. This differential activity occurred despite the fact that the number of lever presses was identical across both TOIs. Representative examples of these neurons are shown in Figures 3 (striatal) and 4 (cortical).

The differential firing rates across the short and long TOIs in the striatal and cortical neurons suggest that they may play a part in the representation of specific signal durations. However, to be confident that this interpretation is accurate, the rats’ behaviors needed

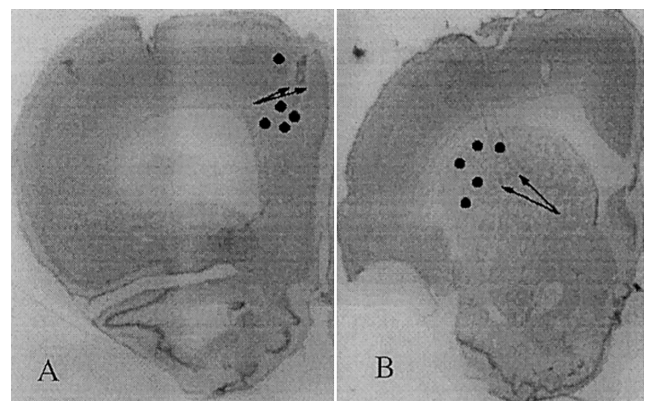


Figure 2. Representative track marks and location of electrodes across subjects. Track marks from the electrode arrays are shown in scanned sections and are indicated by arrows (cortical, Panel A; striatal, Panel B). Filled circles on sections indicate array positions in remaining subjects. Image contrast and brightness were enhanced for presentation.

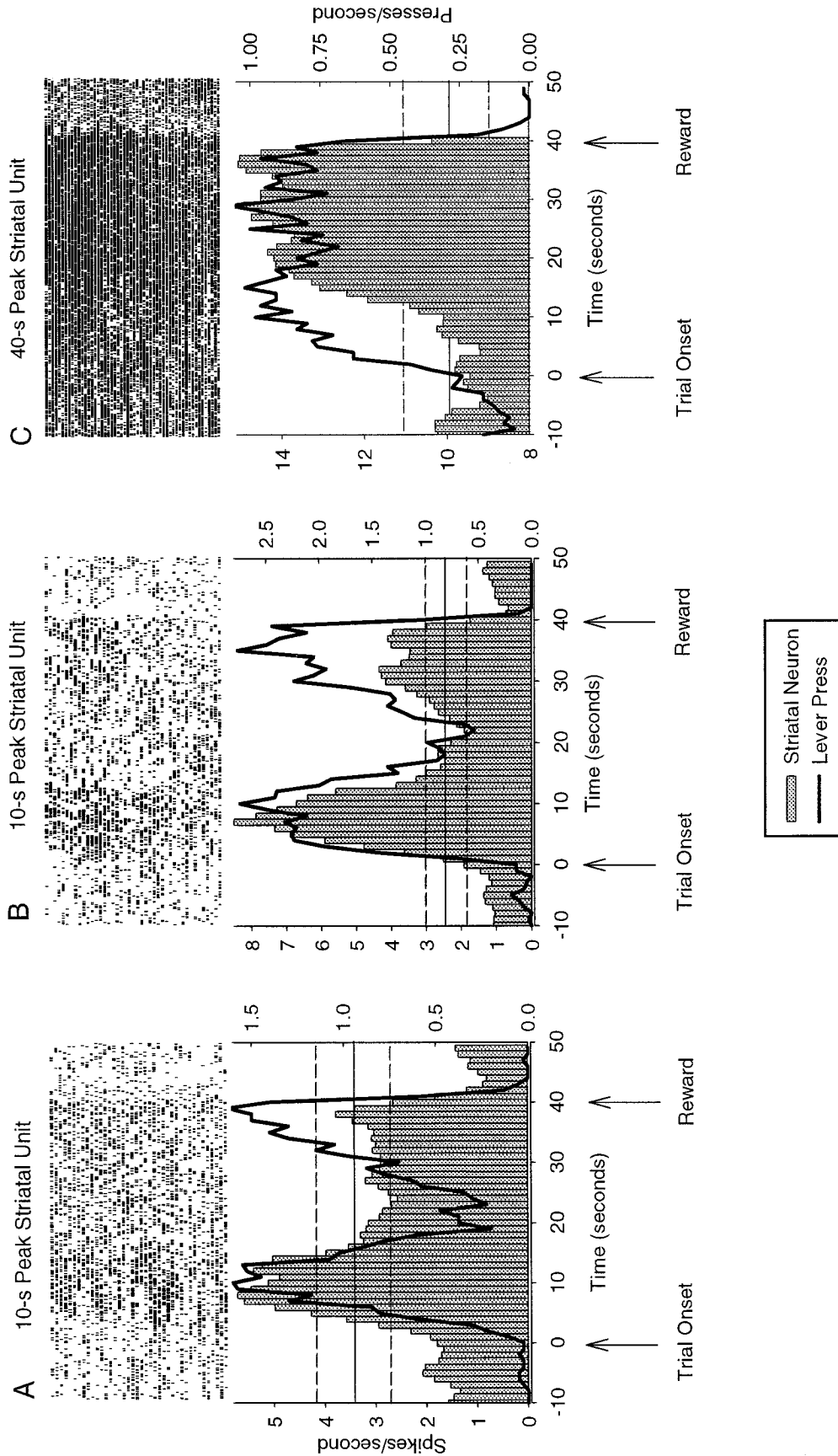


Figure 3. Striatal activity, showing peak firing patterns with maximal firing at only one of the criterion durations. A: Striatal neuron showing a strong increase in firing rate at the first criterion duration of 10 s but failing to show a robust increase at 40 s. B: Another striatal neuron showing a clear peak at 10 s, and a much smaller peak at 40 s. C: Striatal neuron showing a large increase in firing rate at 40 s, with little change around 10 s. This neuron is likely multitasking, as a dramatic drop in firing rate can be seen at the end of the trial. Here and in Figure 4, the distributions of neural activity contrast with the lever-press distributions, which show equivalent peaks at both 10 s and 40 s (shown by the black line). These peak firing patterns were found in seven of eight striatal neurons showing differential activity across the two times of interest in both the mean functions and the press-topography controlled analyses. Although the neural activity was primarily associated with the lever-pressing activity for only one of the two periods of high pressing, the smaller, secondary peak at the other criterion duration was frequently seen. The mean firing rate is denoted by a solid horizontal line, and the 99% confidence intervals of firing rate are denoted by dashed horizontal lines. Because of its high firing rate, the y-axis in Panel C has been only partially shown to emphasize the match between spike rate and 40-s lever pressing. Spike distributions have been smoothed by a 3-s running mean for presentation.

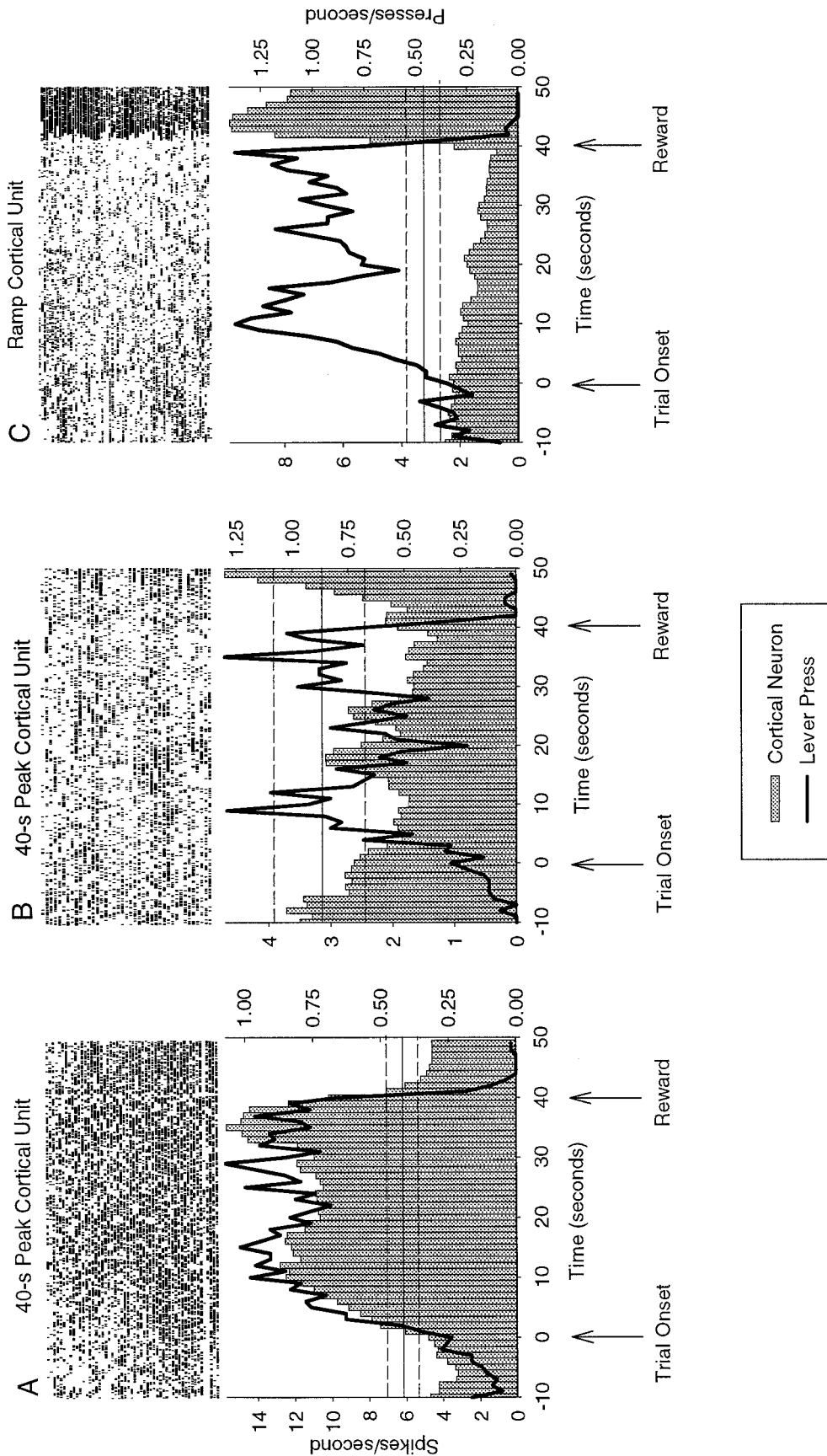


Figure 4. Cortical activity as function of signal duration. A: Cortical neuron showing significantly greater activity during the second criterion duration of 40 s as compared with the first criterion duration of 10 s. B: Cortical neuron showing significantly greater inhibition of activity around 40 s as compared with its inhibition at 10 s. C: Cortical neuron showing a decreasing ramp firing pattern as a function of trial duration. Note that this neuron is multitasking, showing a dramatic increase in firing rate on reward delivery. Peak-shaped firing patterns were found in two of four cortical neurons, and ramp firing patterns were found in two of four cortical neurons showing differential activity across the two times of interest in both the mean functions and the press-topography controlled analyses. Lever-press distribution, trial onset, reward, mean firing rate, confidence intervals, and smoothing are the same as indicated in Figure 3.

to be more closely examined. Although we initially selected trials so that the total numbers of lever presses during the short and long TOIs were identical, close inspection of the behavioral data showed that within the TOI, bouts of lever pressing were separated by short intervals in which the rats were not pressing the lever, and instead were performing collateral behaviors. All of the rats exhibited the same general behavioral pattern: a bout of lever pressing separated by a turn toward the magazine, placement of their nose over the food cup, and return to the lever for another bout of pressing. Although there were no observable differences in posture or position of the rats as they pressed during the short and long TOIs, plots of the collateral behaviors as a function of time in the trial revealed differences in the amount of one or another of these collateral behaviors across the two TOIs in 5 of the 6 rats. Occasionally these plots showed a high degree of visual similarity to the neural firing patterns in the PSTHs, suggesting that a small subset of these neurons may have been involved in coding these collateral behaviors (Figure 5). In order to more adequately address whether these collateral behaviors could be responsible for the firing rate differences found across the TOI, we reevaluated the spike rates of these neurons during periods of time in which the rat was not performing any collateral behaviors (i.e., during the times when the rat was solely performing a bout of pressing).

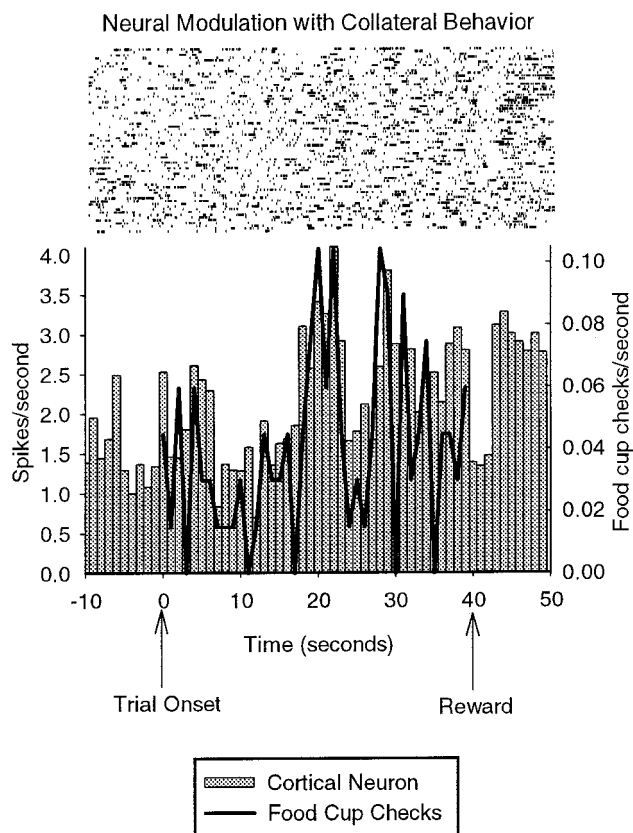


Figure 5. Cortical neuron with a temporal modulation in firing rate caused by collateral behavior. Although this neuron displays a differential spike rate across the two times of interest, this modulation is clearly due to collateral behavior, and it failed to have a significant difference when reanalyzed using only those periods of time during a press bout. Collateral behaviors were scored only during the trial.

Specifically, in order to quantitatively determine whether the neural firing differences across the TOI were due to the differential context of signal duration, we compared the spike rates that occurred only during press bouts that coincided with the short or long TOI. These comparison periods were further matched by using lever-press topography statistics, including press duration, inter-press interval, and release-to-press interval as covariates for the comparison. These press statistics were included as covariates in the analysis because they varied slightly as a function of duration (see Figure 6). A MANCOVA showed that signal duration (short vs. long TOI) was a significant factor accounting for the differences in the firing rate of 22% (12/54) of the striatal neurons and 15% (8/54) of the cortical neurons. Of these neurons, 67% (8/12) of the striatal neurons and 50% (4/8) of the cortical neurons could be further conceived as *primarily* coding duration in this experiment, as they also showed significant differences in the analysis of their mean functions (prior to the covariate adjustment) and crossed the 99% confidence interval during one, or both, of the TOIs. These primarily duration-coding neurons were classified as ramp shaped (i.e., they had monotonically increasing or decreasing functions, $n = 3$) or peak shaped (i.e., they had peaks of activity around the criterion times, $n = 9$). By calculating the ratio of the differences between the spike rates at the two TOIs as compared with baseline, we are able to quantify the extent to which a peak-shaped neuron's firing rate is specific for a single duration. In other words, this ratio is a measure of how much larger one peak is as compared with the other. Of the 9 peak-shaped neurons, the average ratio of peak sizes was 2.7 ± 1.5 . There was a significant difference between the two neural regions investigated. The 7 striatal peak-shaped cells had an average ratio of 3.1 ± 1.5 , whereas the 2 cortical peak-shaped cells had an average ratio of 1.4 ± 0.1 , $t(6) = 3.08$, $p < .05$. Only these neurons, which were significant in all three analyses, are presented in Figure 3 (striatal) and 4 (cortical). The remaining 8 neurons (4 striatal, 4 cortical) showing significant differences across TOIs were not given shape classifications, as the differences in their firing rates evolved as a combined function of the stimulus duration and the rats' lever-pressing behaviors, so that their shapes in the mean functions were not indicative of their temporally varying contributions. Nevertheless, these neurons may be construed as functioning to represent stimulus duration through a combinatorial code accounting for both stimulus duration and press topography.

We further evaluated whether these 20 timing neurons had differential anatomical distributions. Blocking the recording arrays into four regions (anterior lateral, anterior medial, posterior lateral, posterior medial), we found that the striatal timing cells were more likely to be found in the medial portion of our arrays, $\chi^2(1, N = 12) = 8.3$, $p < .01$, whereas the cortical timing cells were more likely to be found in the anterior portion of the array, $\chi^2(1, N = 8) = 4.5$, $p < .05$. Given the dimensions of our array (1 mm wide \times 2 mm long), the striatal cells would still reside in the dorsolateral region of the striatum, whereas the cortical cells may be approaching the border between cingulate and prelimbic cortices (Paxinos & Watson, 1986).

In the majority of the neurons that showed a peak-shaped firing pattern at one TOI, a secondary, smaller peak could frequently be seen during the other TOIs. These smaller secondary peaks suggest that there may be a modulation in the strength or relationship of lever press-induced neural activity as a function of signal duration,

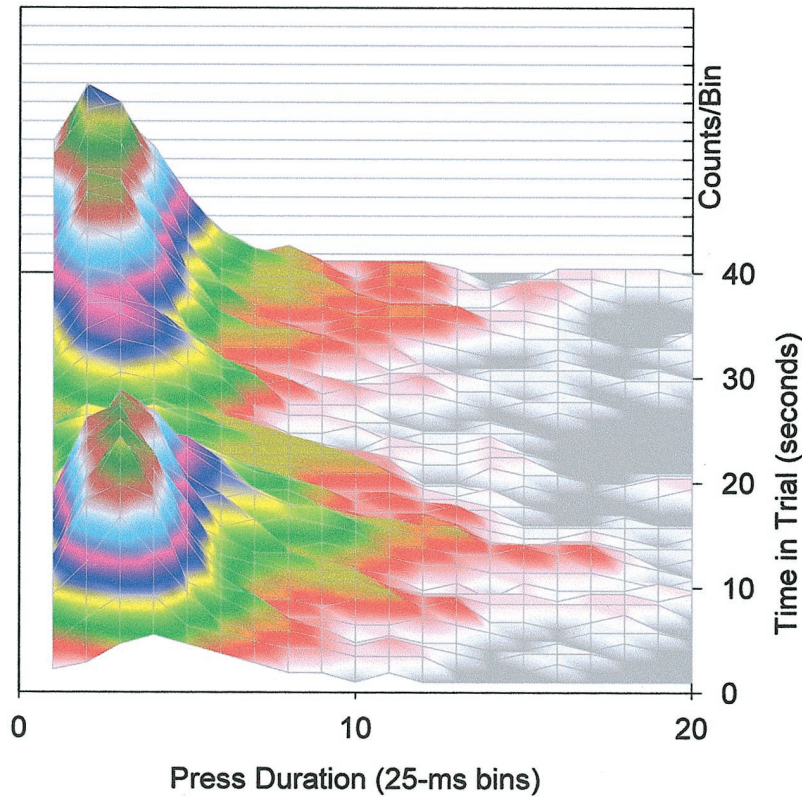


Figure 6. Distribution of lever-press durations (time from lever press to release) as a function of time in the trial. Press duration is slightly longer around the short criterion time than around the long criterion time. Therefore, this variable (along with interpress interval and release-to-press interval) was used as a covariate in order to evaluate whether duration, rather than press topography, was an important factor driving the temporally varying spike rates. Data in this figure have been smoothed with a three-bin running mean along the *x*- and *y*-axes for presentation.

such that these neurons are strongly related to pressing at one TOI, and weakly related at the other TOI. The neural responses produced in relation to lever presses occurring at each of the two TOIs were compared to evaluate whether the neural responses during lever pressing were consistent over time. Representative striatal cell activity patterns surrounding a lever press from both the short TOI and the long TOI are shown in Figure 7 (Panels A and B, respectively).

As can be seen in Figure 7, the neural activity occurring around a lever press varied as a function of signal duration, such that the firing pattern around a short press had two inhibitory valleys of similar magnitude, one before and one after the press, whereas the firing pattern around a long press had a much smaller before-press inhibition, thereby resulting in a more apparent press-related excitatory peak. A chi-square test of distribution similarity confirmed this change in firing pattern from short versus long TOI, and chi-square tests on the remaining neurons (those that were identified as primarily duration coding) indicated that differential firing patterns occurred in all but two of the neurons tested (one cortical, one striatal). However, it is also clearly apparent that the background firing rate is considerably higher around short TOI presses as compared with long TOI presses. A paired sign test comparing the bin-by-bin values confirmed this observation. This difference

in overall firing rate was true for all of the neurons tested. Although such differences in background rate cannot be clearly separated from the concurrent changes in firing pattern, these results suggest that coding of signal duration in these neurons may occur through multiple mechanisms, one a direct duration code via an alteration of background spike rate, and the other through an alteration of the neural-behavioral relationship.

Multiple Neuron Coding

Using discriminant analyses, we evaluated the ability of both a single neuron and an ensemble of neurons to predict the trial period on the basis of spike count. The best single neuron in each rat allowed the algorithm to predict, on average, 65.6% ($\pm 4.0\%$) of the trials correctly. In all rats, the best single neuron from the discriminant analyses also showed significant differences in the tests on the mean functions used above. Use of all the neurons recorded in each rat as an ensemble led to a modest increase in predictability, as the performance improved to 72.4% ($\pm 6.6\%$) correct classification. However, these results are based on equal weightings of all the recorded neurons, independent of their individual contributions. In other words, neurons that performed at chance levels when tested singularly were included in these results,

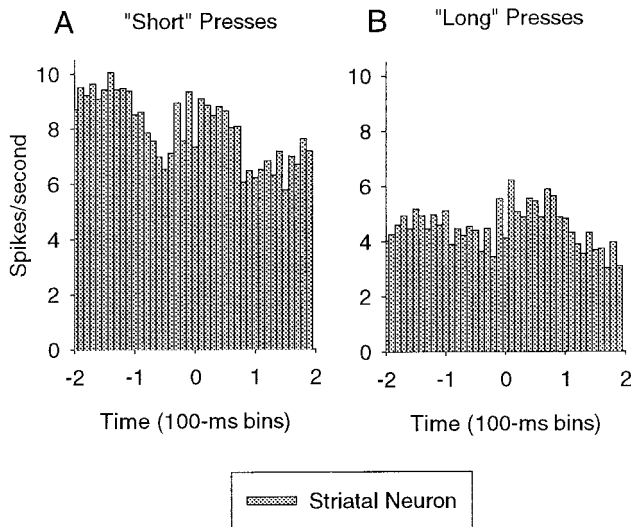


Figure 7. Variation in firing rate in a temporally specific striatal neuron (neuron from Figure 3B) in relation to presses made during the short time of interest (TOI; A) or the long TOI (B). The press-related firing pattern is different for short and long presses, suggesting a modulation in press coding as a function of signal duration. In addition, the background firing rate is higher over the entire peristimulus time histogram in the short TOI as compared with the long TOI, suggesting that the background rate may be separate from press-related firing modulations. These differences are not due to differences in the amount or timing of other lever presses occurring in the histogram range. Such temporally modulated changes in firing rate are hypothesized to represent the duration specificity of the neuron.

and could lower the classification. To assess this possibility, the algorithm was rerun after omitting each neuron in turn, in order to maximize the classification performance. As a result of omitting neurons that poorly predicted the trials (e.g., removing those neurons that were at chance levels when tested individually), the ensembles were able to predict 79.6% ($\pm 7.8\%$) of the trials correctly. These “best” ensembles used only 9.3 (± 4.6) neurons. The classification improvements obtained over single neurons by using all the neurons, or a select combination of neurons, were significant as tested by an ANOVA $F(2, 15) = 7.3, p < .01$. Paired t tests of single neuron versus all neurons and all neurons versus best ensemble showed that each step was a significant improvement over the previous, $t(5) = 5.3, p < .01$; $t(5) = 6.5, p < .01$, respectively.

We also evaluated the role of striatal and cortical ensembles on their own. We found no significant difference between the ability of just striatal or just cortical neurons to predict whether a spike train came from the short or long TOI, using either the entire regional or best regional ensemble. However, performance of the best regional ensemble (using either region) was significantly worse than performance of the best ensemble when both regions were combined: striatal, $t(6) = -3.7, p < .05$; cortical, $t(5) = -3.4, p < .05$, suggesting that both regions have the capacity to uniquely contribute to temporal processing.

We also evaluated the correlations between neural activity and lever pressing on a trial-by-trial basis. The spike train from each neuron on each trial was fit with either a single-transition step function (low state–high state) or a three-transition step function

(low state–high state–low state–high state) as appropriate for each neuron. Average transition times determined using these analyses were 10.8 ± 1.8 s for the first transition, 17.9 ± 2.7 s for the second transition, and 28.5 ± 1.5 s for the third transition. Overall, these neural transition points fell near the transition points calculated from single-trials analyses of the presses (average deviation between common points = -0.1 s, negative indicating that neural transitions fell after lever-press transitions). However, despite these similarities in average transition times between neural activity and lever-press behavior, we found only slight correlations in a small subset of neurons between lever-press transition times and neural transition times when calculated on a trial-by-trial basis (average $R^2 = .02$, maximum $R^2 = .18$). Thus, these data, like the discriminant analyses data, suggest that individual neural responses play a relatively minor role in controlling the behavior of the animal.

Underlying Temporal Functions

In an effort to elucidate the timing mechanism contributing to the temporal discrimination ability of these rats, we fit those neural data showing excitatory peak-shaped activity patterns ($n = 7$) with a combination of a baseline rate, the lever-press function itself (with a scaling factor), and either a linear or Gaussian peak function. A representative example of the functions resulting from these fits can be seen in Figure 8.

In all cases, a linear accumulator function provided less accurate fits than the Gaussian-based function (R^2 values for the linear function averaged $.53 \pm .14$, whereas the Gaussian function R^2 value averaged $.75 \pm .08$). As the peak function has a greater number of parameters, it is not wholly surprising that it provided better fits. However, the fits using a peak function were uniformly better at capturing the height of the dominant peak, suggesting that

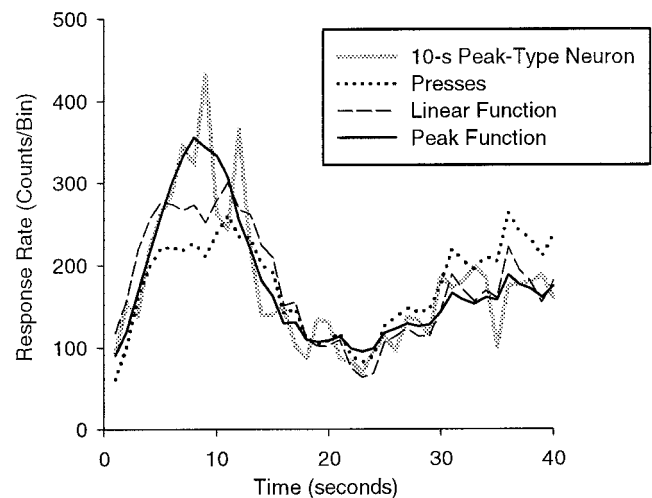


Figure 8. Functions constructed by the summation of a baseline rate, a scaled lever-press function, and a theoretical temporal integration function (i.e., linear accumulator or Gaussian peak function), in an attempt to fit the neural data from neurons with excitatory peak-shaped firing patterns ($n = 7$). In all cases, the peak function fit the neural activity pattern quite well, whereas the linear function frequently failed to capture the height of the dominant peak.

the improved fits were not simply the result of randomly adding parameters to the function.

Discussion

This study examined the role that striatal and cortical neurons play in interval timing. Previous research has demonstrated ramp-like preparatory activity in these brain areas (Hikosaka et al., 1989; Niki & Watanabe, 1979), suggesting that they are involved in the temporal control of behavior. However, it was unclear whether this preparatory activity represented a specific temporal code or only served as a general expectancy signal that readied the subject for a future event, but did not provide information as to when that event would occur. The current findings suggest that a subset of neurons within the dorsolateral anterior striatum and anterior cingulate cortex can encode specific signal durations. One common firing pattern of temporally specific neurons was a gradual increase in firing rate up to the time of an expected reward (i.e., 10 s), followed by a gradual decrease in firing rate back to baseline if the reward did not occur (Figures 3A and 3B). Because the firing rates show a peak at the expected time of reinforcement, these neurons may encode specific signal durations as a direct function of their spike rate.

The rats in this study were trained to press a response lever in order to receive a food reward at one of two possible times (i.e., 10 s and 40 s). As expected, their lever-pressing distribution peaked twice on 40-s trials, once at 10 s, and then again at 40 s (Figure 1). The 10-s peak firing pattern found in the striatal neurons described above roughly coincided with the 10-s peak in the lever-press function, but it did not match the 40-s peak in the lever-press function (Figures 3A and 3B). Because the neural activity pattern diverged from the lever-press distribution as a function of signal duration, the motor behaviors associated with lever pressing cannot account for the neural peak function. Furthermore, the differences in firing rate across time remained significant after temporal variations in press rate and press duration were controlled for. The firing pattern and the lever-press activity were also highly incongruent in those striatal neurons showing maximal firing at 40 s (Figure 3C). Taken together, these results suggest that neurons in the striatum can serve to encode the time of expected reward occurrence in this task.

A subset of cortical neurons also showed peak-shaped firing rate differences as a function of signal duration (Figures 4A and 4B). However, the deviations between their firing patterns and the lever-press distributions were subtler than those seen in the striatal neurons. These cortical neurons, as well as those cortical and striatal neurons that showed significant differences over time only after variations in press topography were controlled, may be understood as being modulated as a function of signal duration, rather than representing signal duration directly. Such modulatory action suggests that these neurons may be involved in coding nontemporal cognitive and/or behavioral variables, and that interval timing processes modulate the strength of these representations. As such, these modulations may indicate that components of the interval timing system are embedded within neural networks primarily involved in other cognitive and behavioral processes.

There were two different sources of firing variation over time in the majority of temporally specific neurons (Figure 7). First, these neurons had a change in their basal press-related spike rate as a

function of time, suggesting that these neurons may directly code specific learned durations as a function of firing rate. Second, variations in the pattern of press-related firing between short and long presses imply that the relationship between neural activity and behavior is contextually dynamic (i.e., the passage of time is an alteration of context). A similar contextually dynamic neural-behavioral relationship has also been shown in the striatum during the processing of sequential events (Aldridge & Berridge, 1998; Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999).

It is also clear from the current results that a relatively small number of duration-coding cells, when working together as an ensemble, could quite accurately represent the amount of time that had passed in the trial. Using the most consistent single neuron in each rat, we were able to predict whether a spike train came from the short versus long TOI on 66% of the trials. In contrast, by evaluating the covariance across neurons, the short versus long classification accuracy of the most informative ensemble in each rat was almost 80% correct. Although these ensembles always included those single neurons that had significant differences across durations in the mean functions, they also included neurons that did not show significant differences when tested individually. These ensemble classification levels are quite striking when compared with the behavioral accuracy of the interval-timing system in rats, which perform at about 90% correct on a comparable duration discrimination procedure (Church & Deluty, 1977). It is worthwhile to note here that we did not systematically remove any neurons from the ensemble analyses, and as such, neurons that may have been primarily involved in representing press topographies were used in the ensemble classification. Such a finding does not negate the importance of these data, as it is clear that animals can, and will, use their behavioral state as a source of temporal information (Killeen & Fetterman, 1988). However, these investigators (Fetterman, Killeen, & Hall, 1998) have also shown that overt behavioral state assessment is not necessary for accurate timing performance, suggesting that a "pure" duration percept may be required for some timing tasks. As such, the fact that press-topography related neurons and duration-coding neurons were both found within the same neural regions suggests that the temporally informative output of the basal ganglia may be composed of multiple "clock" sources.

Given the fact that animals will typically vary their behavior as a function of time, and given the historical association between striatal activity and motor activity, one of our primary concerns in designing this experiment was to minimize potential motor confounds that could hinder the interpretation of the neural data. To this end, we chose to use a matched behavior strategy that enabled comparisons of the neural activity across bouts of lever-pressing behaviors that occurred at different times. Given the scalar nature of timing, however, the current experimental design leads us to be comparing a more precise temporal estimate (i.e., the short criterion time) with a more variable temporal estimate (i.e., the long criterion time). Although this fact does not cloud the current results in terms of demonstrating that neural activity in both the striatum and cingulate cortex varies as a function of time, it does present some concerns regarding an interpretation of whether these neurons participate in the timing of one or two durations (i.e., the short and/or long criterion times). As such, it is conceivable that the second smaller peak seen in the neural activity profiles is related to timing a second duration, rather than being directly related to the

motor activity, as we have alluded to above. As the present coincidence-detection formulation of SBF requires that striatal neurons time only a single duration, the current experimental design does not allow us to address violations of this prediction. In other words, cells that time both durations with equal firing rates have been eliminated from our analysis because we would be unable to dissociate this potential timing-related activity from motor-related activity. These issues raise the possibility that the actual proportion of striatal and cortical cells involved in timing may be considerably higher than that reported (e.g., ~70% of our cells showed significant activation during the TOI periods).

Related to this issue is the seemingly low percentage of neurons found to encode specific stimulus durations through variations in their firing rate across the two TOIs in this study. Specifically, 22% of the striatal neurons and 15% of the cortical neurons had significantly different numbers of spikes across the two periods of reward expectation. However, these percentages are fairly similar to those typically reported in the literature for “preparation” or “expectation” type cells, which usually fall between 20% and 40% of the recorded cells (e.g., Hassani, Cromwell, & Schultz, 2001; Hollerman, Tremblay, & Schultz, 1998; Niki & Watanabe, 1979). Furthermore, the present set of cells showed differential activation between the two periods of reward activation, and most could not be construed as general reward expectation cells, like those found in these other studies.

Although it is possible that other variables such as press force or unidentified collateral behaviors may account for some of the observed firing differences, we extensively evaluated the rats’ collateral behaviors and lever-press topography and did not find that these behaviors were responsible for the firing rate differences. Press force is normally highly correlated with press duration ($r = .84$; Notterman & Mintz, 1965), and we controlled for press duration in our analyses. Because we evaluated the spike rates that occurred only within short and long press bouts, any collateral behaviors that could account for the temporally specific firing rate differences would have to have been emitted simultaneously with the lever presses. Because we were unable to detect any postural or positional differences in pressing throughout the trial, we view this possibility as an unlikely explanation for our data. Of course, despite our attempts to minimize potential motor influences on the neural data, nonlinear relationships between the neural activity and the lever-press activity may exist that could have contributed to the present results.

Another possible explanation is the differential probability of reinforcement at each of the criterion durations. However, to achieve a higher peak at 10 s than at 40 s, these neurons would need to have had a large increase in firing rate when the probability of reinforcement was relatively low (at 10 s) and a small increase when probability was high (at 40 s). As the probability of reinforcement was actually lowest at the beginning and middle of the trial, this probability-coding hypothesis would predict that the highest firing rates would have occurred at these unrewarded times (e.g., 0 s and 20 s), an effect which was not seen.

Also associated with this differential probability of reinforcement is the qualitative difference between partial and full reinforcement at 10 s and 40 s, respectively. In retrospect, it may have been beneficial to provide partial reinforcement of the 40-s duration as well, so as to provide more equivalent comparison periods and allow an evaluation of the entire period of high-state respond-

ing. However, one consequence of partially reinforcing the long duration would be to decrease response rates, necessitating a decrease in the short duration reinforcement density, as well as an extension of session duration, which could conceivably hinder our ability to collect sufficient data for a thorough analysis.

How does the existing framework of interval timing models handle these neural firing data? As described in the introduction, interval timing models can be broken down into clock, memory, and decision stages (Church, 1997). Final output from the interval timer may be broadly conceived of as a decision-stage similarity function comparing the current clock signal with past clock values stored in memory (see Gibbon, Church, & Meck, 1984). This final output function is thought to be reflected in the temporally specific behaviors obtained from a variety of interval timing procedures (Church & Deluty, 1977), and it is remarkably similar to the Gaussian-shaped firing patterns of the peak neurons obtained in this experiment (Figures 3A and 3B), suggesting that these neurons may be directly related to the output of an internal clock used to time intervals in the seconds to minutes range.

We have recently proposed that cortical–striatal interactions are at the heart of interval timing (Matell & Meck, 2000). We have further suggested that the mechanisms of interval timing may be realized by striatal spiny neuron-based coincidence detection of oscillatory cortical inputs (SBF). As the current data show cortical and striatal activity varying as a function of time, the present results data provide strong support for the most global predictions of SBF (i.e., cortical and striatal involvement in timing). Further, the firing patterns of the striatal neurons were most frequently peak shaped, with large differences in peak heights across the two TOIs, a finding that is also predicted by SBF (Matell & Meck, 2000). However, finding these broad, peak-shaped striatal firing patterns does not rule out alternative timing models, as the shape of this activity pattern is consistent with the decision stage output function of a generalized timing model (Church, 1997). Nevertheless, finding such temporally specific neural activity does put some constraints on alternative timing models (most of which are not implemented through specific brain mechanisms).

In contrast, SBF also proposes that the cortical inputs producing the clock signal are oscillatory in nature. In the present study, oscillatory patterns were not found in any of the striatal or cortical neurons recorded, a result that severely challenges interval-timing models that rely on cortical oscillatory input for their clock signal. Although oscillatory signals may be coming from brain areas other than those recorded, the current data suggest that future modeling may need to investigate the use of alternative computations for arriving at a temporally meaningful signal.

Instead, the majority of temporally specific firing patterns in the cortex had relatively minor differences in spike rate magnitudes across the two TOIs, suggesting that the cingulate cortex may be involved in modulating cognitive or behavioral output (i.e., alterations in attention as a function of duration), rather than directly encoding specific durations. It is conceivable that the source of this temporal modulation is the more prominent peak-shaped temporally varying activity of the striatum, which may be relayed through the basal ganglia output channels to the thalamus, and from there to the cingulate cortex. Taking this feedback loop one step further suggests that these cortical cells might also serve as input to the striatal cells, leading to a dynamic interval timing system, in which cortical activity leads to striatal firing, which in

turn alters the ongoing cortical activity, thereby altering striatal firing further, et cetera.

Determining the initial (or ongoing) cortical input patterns that are potentially integrated by the striatum to achieve peak-shaped activity is therefore a critical next step to understanding the interval timing system. One potential candidate for these inputs is the linear ramp patterns seen in a couple of neurons in the present study and consistently found in primate prefrontal cortex (Fuster, Bauer, & Jervey, 1982). Given their apparent linearity, these ramp patterns are unlikely to be the left side of a peak-shaped function that has not reached its criterion time (e.g., Figures 3C and 4), and thus these cells may function as a temporal accumulator and/or a general, non-temporally specific expectation signal. In addition, other neural activity patterns, such as decelerating decay functions (Fuster, 1997) and peak functions that peak at times other than the reward period (Kojima & Goldman-Rakic, 1982), may also be used for integration into a temporally meaningful signal. In addition, there is considerable evidence showing that the cerebellum is involved in the timing of short-duration behaviors, such as conditioned eyeblink responses (Raymond, Lisberger, & Mauk, 1996), saccadic eye movements (Thier, Dicke, Haas, & Barash, 2000), and repetitive movements (Ivry & Keele, 1989), as well as duration of discriminations shorter than 1 s (Ivry & Keele, 1989). Nevertheless, it remains unclear to what extent the cerebellum plays a role in timing stimuli and behavior in the seconds-to-minutes range.

In summary, the current results suggest that single neurons within the dorsolateral anterior striatum can encode specific signal durations through slow changes in their firing rate. Striatal firing patterns are peak shaped, suggesting that this activity may be the final output of an internal clock. A small number of striatal and cortical neurons used as an ensemble can discriminate durations with nearly the same accuracy as the behavioral performance of the rat. These data suggest that the striatum and cortex may play an important role in timing and time perception.

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