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Loss of Lever Press-Related Firing of Rat Striatal Forelimb Neurons after Repeated Sessions in a Lever Pressing Task

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Lateral striatal neurons that fire phasically in relation to active movement of the contralateral forelimb (determined via daily sensorimotor examination) were studied during acquisition of cued lever pressing. Rats were trained to lift the contralateral forepaw from the floor to press a lever in the presence of a tone. The tone was presented 70 times per day (session) for 18 consecutive days. All animals acquired the task, evidenced by gradual improvements across sessions and eventual asymptotic levels in tone discrimination, reaction time, and efficiency of the lever press. Forelimb neurons fired in relation to the lever press during early sessions of acquisition but not after repeated sessions on the task. This difference in firing could not be attributed to differences in forelimb movements during lever pressing or to sampling from different populations of neurons in early versus late sessions. In view of evidence that striatal damage impairs acquisition of motor skills, the change in firing suggests that the striatal activity present in early sessions may be necessary for the acquisition of, but not the automatic performance of, learned motor responses.

Key words: striatum; electrophysiology; S–R habit; chronic recording; dopamine; movement

The lateral striatum contains a population of neurons that discharge spontaneously at low rates and phasically in relation to sensorimotor activity of individual body parts. Their functional organization is in register with convergent, patchy somatotopic projections from primary somatosensory and motor cortices (Kunzle, 1975, 1977; Liles, 1979; Crutcher and DeLong, 1984; Alexander and DeLong, 1985; Liles and Updyke, 1985; McGeorge and Faull, 1989; Kimura, 1990; Carelli and West, 1991; Flaherty and Graybiel, 1993). They are projection neurons (Kimura et al., 1990), the main targets of striatal output being the globus pallidus and substantia nigra pars reticulata. The organization of corticostriatal–pallidal connections has led to the suggestion that the distributed representation of body parts of the striatum may allow associative processing involved in sensorimotor learning (Flaherty and Graybiel, 1994).

Neurons related specifically to the forelimb are the best characterized subpopulation of phasic neurons. Although clearly "movement-related," their properties indicate certain dissociations from movement. Firing of most neurons (type IIb of Kimura, 1990) lags behind the onset of electromyogram (EMG) activity of arm muscles (Crutcher and DeLong, 1984b; Liles, 1985; Kimura, 1990), so that any major role in movement initiation is unlikely. The parameter most frequently correlated with firing is the direction of movement, but in half the cases, firing shows no relation to load (Crutcher and DeLong, 1984b; Liles, 1985; Alexander, 1987). Firing also occurs during passive movement and cutaneous stimulation (Crutcher and DeLong, 1984a; Liles, 1985; West et al., 1990; Carelli and West, 1991). These properties suggest a role in integrating signals involved in ongoing movement, such as somatosensory feedback and/or efference copy, transmitted via the corticostriatal system (Kato and Kimura, 1992).

Most of this information has been obtained from trained animals, but training itself influences the responsiveness of neurons in the striatum and substantia nigra. Responses of dopamine (DA) neurons to reward were transferred to a conditioned stimulus (CS) predictive of reward in a simple stimulus–response (S–R) task (Romo and Schultz, 1990; Ljungberg et al., 1992; Mirenowicz and Schultz, 1994), but declined with overtraining as the behavior became automatic (Ljungberg et al., 1992; Schultz, 1993). Tonically active neurons (TANs), a striatal category separate from the slowly discharging, phasic category, developed responsiveness to a CS as a function of acquisition of a sensorimotor association. After overtraining, TANs maintained this responsiveness, in contrast to the decline with overtraining expected of DA neurons, suggesting a possible transfer of information to TANs for storage and use in conditioned motor behavior (Aosaki et al., 1994).

Further suggesting a striatal role in learning, the corticostriatal system is implicated in motor learning and the formation of habits (Marsden, 1982; Mishkin and Petri, 1984; Squire et al., 1993). Acquisition of these forms of nondeclarative memory is impaired after damage to the striatum but characteristically is spared after limbic lesions (Olmstead et al., 1976; Siegfried and Bures, 1980; Martone et al., 1984; Sabol et al., 1985; Whishaw et al., 1986; Heindel et al., 1988, 1991; Phillips et al., 1988; Pisa, 1988; Saint-Cyr et al., 1988; Knopman and Nissen, 1991; McDonald and White, 1993). These data and reports of nigrostriatal changes as behavior becomes automatic encourage further study of striatal activity during motor learning. In an initial study (Carelli and West, 1991b), firing of striatal forelimb neurons during cued lever pressing was time-locked to the lever press during early sessions but not after repeated sessions, suggesting a change as a function of learning. Our objective here was to examine more rigorously

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this change in relation to variables that might also change across sessions, such as cue discrimination, timing and form of the movement, and the ability to sample reliably from the same population.

MATERIALS AND METHODS

Subjects

Long–Evans male rats (Charles River Laboratories, Wilmington, MA), 90- to 120-d-old, were used as subjects. Animals were maintained on a reversed light/dark cycle (on 20:00, off 08:00) so that experiments were conducted during their active period.

Electrophysiological recording

Before they were trained, animals were surgically prepared for chronic single-unit recording by implantation of a base for attaching a miniature microelectrode drive (microdrive) assembly (Josef Biela Engineering, Anaheim, CA) over the left striatum (medial-lateral 3.6 or 4.0 mm, anterior-posterior +0.80 mm relative to bregma, level skull). Details of the microdrive and surgical procedure have been reported elsewhere (Deadwyler et al., 1979; West and Woodward, 1984). Animals were also prepared for chronic EMG recording in the biceps or triceps muscles of the right forelimb and/or the deltoid muscle of the right shoulder. Two flexible, stainless steel wires (7-stranded, Teflon-coated, 125 μm diameter) (A-M Systems, Inc.) were twisted together for differential recording. The wires were intertwined around a 5 × 5 mm piece of Teflon mesh (USCI, a Division of C. R. Bard, Inc.) with the tips extending 4 mm beyond the end of the mesh. The wire tips were stripped of their insulation (0.5 mm length) and arranged into a "V" formation to enable easy insertion into the muscle as well as to provide resistance from being pulled out of the muscle. The wires were routed subcutaneously until the Teflon mesh overlay the muscle, which was gently pushed apart by blunt dissection. The recording wires were inserted into the muscle and secured by suturing both ends of the mesh into muscle. The other end of the bipolar wire was led subcutaneously and finally led through syringe elasticon (Kerr, Inc.) to a connector in the recording headstage attached to the skull. Animals were housed individually and had free access to food (Purina lab chow) and water. After they reattained presurgical body weight, animals were deprived of water and maintained at 82% of that weight.

Recording sessions began at least 1 week after surgery. Each day, the microdrive was equipped with a tungsten microelectrode (10 MΩ, Haer Corp., Brunswick, ME) and attached to the base on the skull of the animal. As the recording electrode was lowered into the striatum, identification of forelimb-related firing was accomplished by a sensorimotor examination of firing during active movement, passive manipulation, and cutaneous stimulation of the forelimb, as detailed previously (West et al., 1990; Carelli and West, 1992). The exam was conducted before the start of each experimental session in the same experimental chamber in which the task was subsequently conducted, with a black plexiglas wall blocking access to the lever and water trough. Only right (contralateral) forelimb-related neurons were studied during lever pressing in the task.

Neuronal signals were amplified and led through a bandpass filter (500–10,000 Hz). EMG signals were led through a Grass Dual P9 AC Differential Preamplifier and bandpass filter (350–5000 Hz). An AST Premium 386 computer using the Datawave Systems Discovery neurophysiology package (DataWave Systems, Inc., Boulder, CO) was used to simultaneously record single-unit activity from the striatum and EMG activity, and control behavioral aspects of the experiment, as well as for off-line waveform discrimination and construction of perievent histograms (PEHs). Multiunit muscle potentials were analyzed for the purpose of identifying the onset of movements (Ghez and Vicario, 1978; Kimura, 1990) using visual inspection of PEHs. EMG amplitude was not quantified and varied from session to session, most likely a result of slight spontaneous shifts in the location of the subcutaneous wire relative to the muscle.

Experimental chamber

Sessions were conducted in a clear plexiglas chamber (length, 32 cm; width, 17 cm; height, 40 cm) mounted above a treadmill (Sears belt sander model 113.22590). The treadmill belt served as the floor of the chamber and was coated with a thin layer of silicone gasket material (General Electric model 343). One wall of the chamber contained a separate, moveable, clear plexiglas wall with a rectangular opening (3.5 × 2.5 cm), which exposed an operant lever mounted 4 cm from the floor. The wall was hinged to the ceiling of the chamber, enabling it to be moved backward or forward, thereby resulting in complete exposure or retraction of the lever, respectively.

The lever was calibrated to function as a force lever, as follows. The amount of lever depression (downward movement) determined the extent to which the lever interrupted a photocell beam behind it. The extent of interruption was converted using a transistor circuit into five successive "bins" corresponding to five increments in force applied to the lever. Separate inputs to the computer corresponded to initiation of the press, 1–5 gm (bin 1); 6–10 gm (bin 2); 11–15 gm (bin 3); 16–20 gm (bin 4); 21–25 gm (bin 5). Depression to any numbered bin necessarily was preceded by transition through lower numbered bins. Lever depression was accompanied by analog changes in distance and applied force, but transitions between bins were transparent to the subject. Force for each bin was calibrated daily by adjusting the tension in a thin metal band contacted by the far side of the lever. Force, rather than distance, was the programmed variable (described below). Distance was not quantified, but approximated 1 cm vertically in a maximal depression, averaging ~2 mm per bin. Water (0.05 ml) was delivered by activation of a solenoid device (General Valve Co.) into a water trough located 6 cm to the left of the lever and 3 cm from the floor.

Behavioral task

Animals initially were trained to press the lever on a continuous reinforcement schedule . The lever was gradually retracted and animals were required to reach through the small hole in the wall of the chamber with the right forepaw to lever press. The reach covered ~4 cm vertically and 1.5 cm anteriorly. The lever then remained retracted (5 mm behind the wall), and both neural recording and the tone were introduced in session 1. As illustrated in Figure 1, rats were trained to stand facing the lever, before the beginning of each trial, with the right forepaw on a piece of white tape (2.5 × 4.0 cm) situated flat on the floor 1.5 cm in front of the lever. Placement of the forepaw of the animal on (or within 5 mm of) the tape for 0.5–1.0 sec was required before the experimenter activated the tone (1 kHz, 65 dB). The tone was initiated by the experimenter to ensure a consistent starting position that approximated a resting posture of the forelimb on the floor. This was preferred over an automated initiation of the tone, e.g., by requiring depression of a second lever, because that would have confounded the present design. Water delivery was contingent on lifting the forepaw from the tape and pressing the lever within 7 sec of tone onset, to a minimum force of 11 gm (entry into bin 3), which terminated the tone. Lever presses in the absence of the tone were not reinforced. The next trial began with the next placement of the right forepaw of the animal on the white tape. An experimental session consisted of 70 presentations of the tone (70 trials; one session per day for at least 18 consecutive days).

Behavioral measures

"Response to tone" was defined as the percentage of total trials per session in which the animal lever pressed during the tone. "Intertrial interval (ITI) lever press" was defined as the percentage of total ITIs per session during which the animal lever-pressed (without reinforcement) one or more times. "Reaction time" (RT), the time from tone onset to the reinforced lever press, was divided into three nonoverlapping components (Fig. 1). The first component, "tone onset to biceps/deltoid EMG onset" (termed RT_1[tone–EMG]), was interpreted as a reflection of learning to respond during the tone. The second component, "biceps/deltoid EMG onset to lever press" (termed RT_2[EMG–press]), was the time from EMG onset to the onset of the reinforced lever press (i.e., the time period during which the animal lifted the paw, reached toward the lever, and entered bin 1 thereby initiating the lever press). The third component, "lever press onset to reinforcement" (termed RT_3[press–reinforcement]), was the time from the initiation of lever depression (bin 1 entry) to entry into bin 3. RT_2 and RT_3 were interpreted as reflecting motoric performance of the response.

Data analysis

PEHs were constructed to determine relationships among forelimb-related neuronal activity, EMG activity, and behavioral events. Events included the reinforced lever press (bin 3 entry) and tone onset during the task, and cutaneous stimulation or passive manipulation of the limb during the exam. Simultaneously with each cutaneous stimulus or passive manipulation, the experimenter pressed a computer key as an approxi-

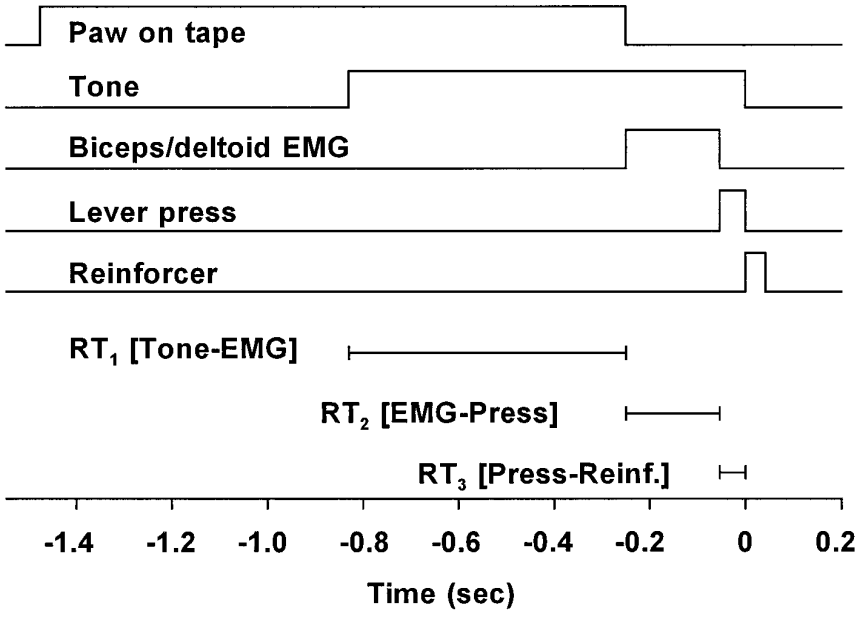

Figure 1. Schematic diagram of behavioral task and RT measures. Animals were trained to place the contralateral forepaw on a piece of tape on the floor, situated directly in front of the lever. Correct forepaw placement for 0.50–1.0 sec activated a tone, during which the animal lifted its forepaw from the tape (activation of biceps/deltoid EMG), and pressed the lever activating water delivery. See text for description of RT measures. Times are approximate means across animals and represent behavior of a trained animal. Time 0 = entry of lever into bin 3 (11 *g* force).

mate synch pulse, with no intention of determining the latency to onset of neuronal discharges.

Signal-to-baseline ratio. Firing of forelimb neurons as depicted in PEHs relative to the reinforced lever press was converted into a numerical expression termed "signal-to-baseline ratio" (S:B) for statistical analysis. S:B was defined as follows. "Baseline" for each neuron was the firing rate immediately before reaching toward the lever, during which time the animal was stationary in front of the lever with the forepaw positioned on the white tape. The duration of the baseline period ranged from 150 to 500 msec across all animals and sessions. EMG activity was examined to verify that no forelimb movement occurred during the baseline period. The "signal" was designated as the higher of two firing rates (depending on the neuron), observed during either (1) the reach toward the lever (beginning with onset of biceps or deltoid EMG activity) or (2) lever depression. Each responsive neuron exhibited a clear relationship to one period but not the other. The duration of the period in which the signal was determined averaged 200 msec, with bin 3 entry as either the onset or offset in nearly all cases. S:B was calculated by dividing signal by baseline. This ratio expressed the magnitude of change in neuronal firing correlated with the change from static limb position on the floor to completion of the lever press.

Tone-evoked discharges. PEHs (70 trials) were analyzed for the presence of short-latency tone-evoked responses. For each neuron, mean firing rate during the 100 msec immediately after tone onset was compared with that during the 100 msec before tone onset, with a difference of 50% defined as a minimum response.

Statistical analysis

Behavioral and neural data for each animal were analyzed as a function of session number using the Change-Point test (Siegel and Castellan, 1988), a form of the Mann–Whitney–Wilcoxon test. One-tailed tests were used, predicting either a change in the direction corresponding to improvement in the task (decline in errors or RTs), or a decline in S:B, as predicted by the initial study (see introductory text). Only significant changes ($p < 0.01$) are reported as changes.

Histology

After the last experiment, each animal was anesthetized (sodium pentobarbital, 150 mg/kg), and a small lesion was placed, using the microdrive, in a location at which a particular striatal neuron had been recorded. After intracardial perfusion and staining of coronal sections, the location of the lesion was used to reconstruct the three-dimensional positions within the striatum of all forelimb neurons recorded from the animal (Carelli and West, 1991). The location of EMG wires was determined for each animal at the time of perfusion. The right (contralateral) forelimb was dissected to determine (1) location of the Teflon mesh and (2) EMG wire placement within the biceps, triceps, or deltoid muscles. Muscle anatomy was verified according to Greene (1963).

RESULTS

Preliminary examination

Seven hundred fourteen striatal neurons were recorded in the dorsolateral striatum of three rats. Of these, 210 cells (29%) were related to whole-body movement, 197 cells (28%) were unresponsive, and 307 cells (43%) were related to sensorimotor activity of individual body parts. Of the latter category, 86 neurons were related specifically to the right (contralateral) forelimb, and 53 of these were recorded during the lever-pressing task. During the exam, all 53 forelimb neurons increased firing during active movement. Of the 53 neurons, 31 increased firing during cutaneous stimulation of the contralateral forelimb, 32 increased firing during passive manipulation of the forelimb, and 20 responded to both.

Behavior during the task

General description

Each session began with a click of the solenoid, removal of the black blocking wall, and exposure of the lever and water trough. Animals immediately approached the trough and drank the water. After the animal drank, the body of the animal was positioned between the trough and the lever, oriented and positioned to touch the floor tape, press the lever, and drink the water. Once in this position, the animal either lever-pressed (i.e., ITI error) or placed the forepaw on or near the floor tape. The latter resulted in presentation of the tone, during which a lever press was reinforced. After the click of the solenoid, the forepaw was removed from the lever, and the upper body was maneuvered to drink the water, as described above. Minimal postural adjustments were involved, consisting mainly of maneuvering the upper body between the lever and the trough.

Behavioral measures

Task requirements restricted variation in the forelimb movement involved in the lever press, and experimenter observation confirmed that reaching from the floor to press the lever remained similar in form throughout all sessions. Figure 2 (*middle* and *bottom right*) shows that no significant changes as a function of session number were observed for any animal in RT_2[EMG–press] or in RT_3[press–reinforcement]. Because the

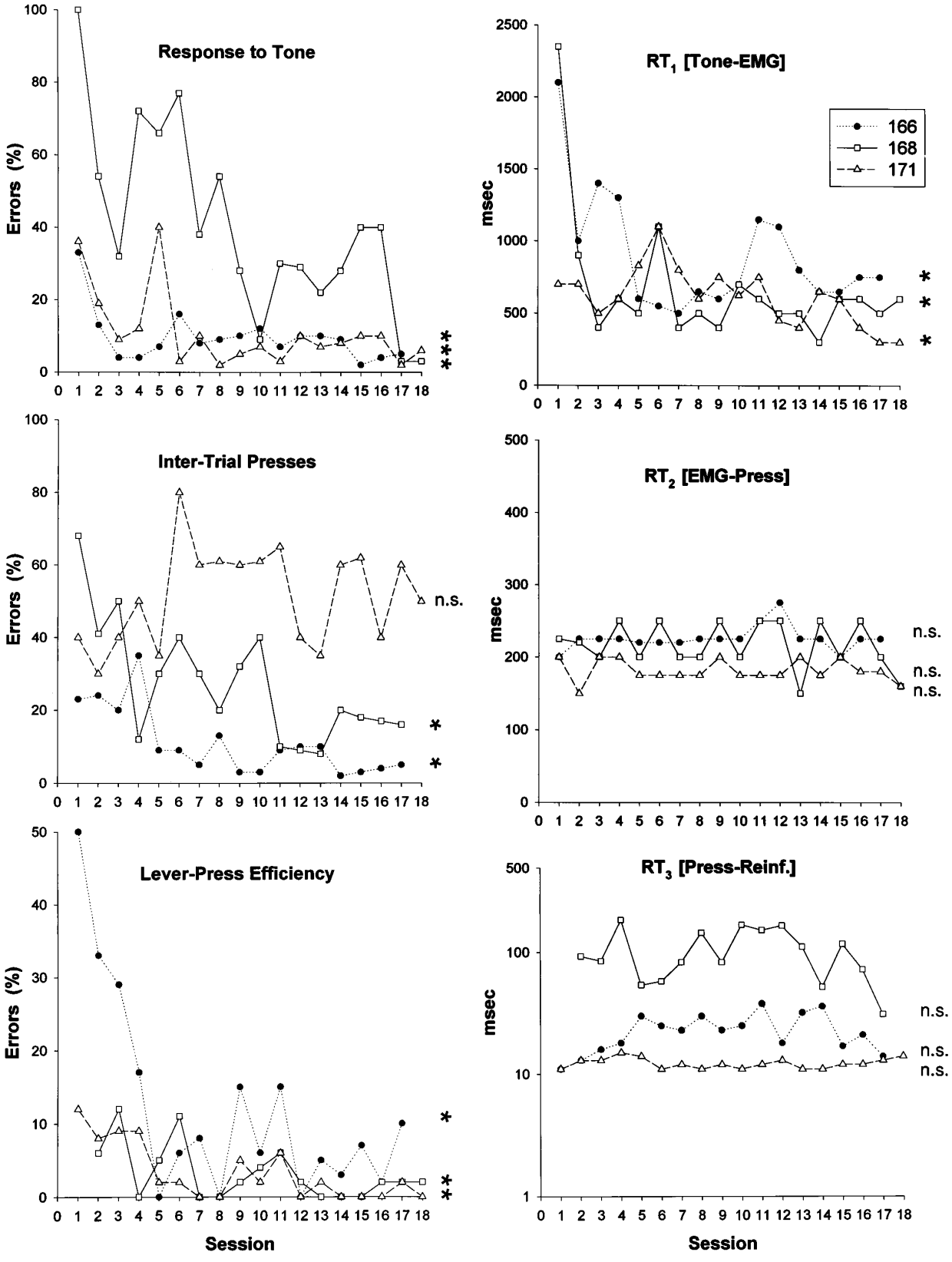

Figure 2. *Left column*, Percentage of trials in each session in which the animal responded during the tone, i.e., completed a reinforced lever press (*top*); completed one or more (unreinforced) lever presses during the ITI (*middle*); or pressed the lever beyond the force required for water delivery (*bottom*). *Right column*, RT measures (msec). RT_1 = time from onset of tone until onset of biceps or deltoid EMG activity. RT_2 = time from onset of biceps or deltoid EMG activity until onset of lever press. RT_3 = time from onset of lever press until lever depression to the force required for water delivery. *Asterisk* indicates significant ($p < 0.01$) change as a function of session number (*n.s.*, not significant).

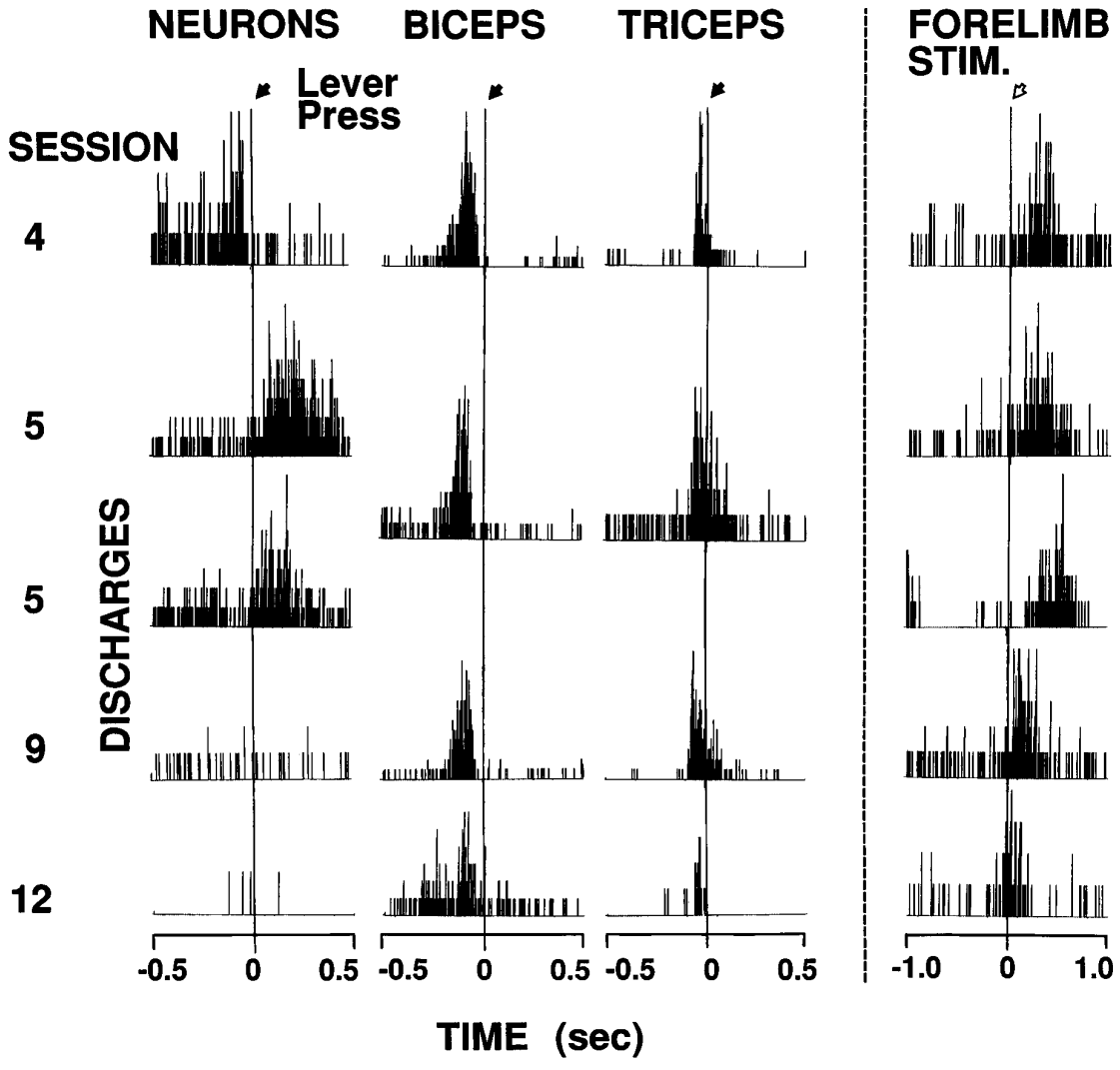

Figure 3. Decline in lever press-related firing of striatal forelimb neurons after repeated sessions. *Three left vertical columns*, PEHs display activity of forelimb neurons and simultaneously recorded biceps and triceps EMG activity across representative sessions for one animal (166). Reinforced lever press is "node" (bin 3 entry, time 0), indicated by *solid vertical lines* and *filled arrows* (70 trials in each). Neuronal activity was time-locked to lever press during acquisition (e.g., sessions 4 and 5, the latter yielding neurons) but not after repeated sessions (e.g., 9 and 12). Timing of onset of biceps/triceps EMG activity remained similar across sessions (amplitude was not quantified; 2 msec/bin). *Vertical column* to *right* of *dashed line*, PEHs show responsiveness of each neuron at *far left* to cutaneous stimulation during exam before session (60 repetitions of tapping or rubbing limb/paw, indicated by *solid vertical line* and *open arrow*; 4 msec/bin).

distances associated with RT_2 (floor to lever) or RT_3 (distance the lever travelled to enter bin 3) did not change, it can be concluded that no change as a function of session number occurred in the average velocity of either the reach or the press.

PEHs showing activity recorded from prime movers of the forelimb were constructed using the reinforced lever press (bin 3 entry) as the node (time 0, Figs. 3 and 4). Biceps and deltoid EMG activity increased as the animal lifted the paw to reach for the lever, then decreased to baseline during lever depression. Triceps EMG activity remained at baseline until it increased during lever depression, beginning ~50 msec before completion of the reinforced lever press, and returned to baseline when the animal withdrew the forepaw from the lever. Only slight variations in the timing of EMG activity relative to the reinforced lever press were observed across all sessions and animals: biceps mean onset = -177 ± 5 msec, mean duration = 156 ± 7 msec; deltoid mean onset = -155 ± 5 msec, mean duration = 125 ± 5 msec; triceps mean onset = -43 ± 9 msec, mean duration = 49 ± 13 msec. This low variance and the lack of change in RT_2 or RT_3 (Fig. 2) indicate that animals did not alter the timing of the reach and the press across sessions.

All three animals showed significant improvement in measures of tone discrimination as a function of repeated sessions on the task (Fig. 2). As session number increased, animals made fewer errors of omission (failure to respond to the tone) and commission (ITI presses; with one exception: rat 171), and responded to the tone with shorter RTs (RT_1, tone onset to EMG onset).

All three animals also showed significant improvement in the efficiency of the lever press as session number increased. Figure 2 (*lower left*) shows a reduction in the percentage of trials containing "errors," which consisted of superfluous fluctuations of the lever beyond the force requirement, after bin 3 entry/water delivery.

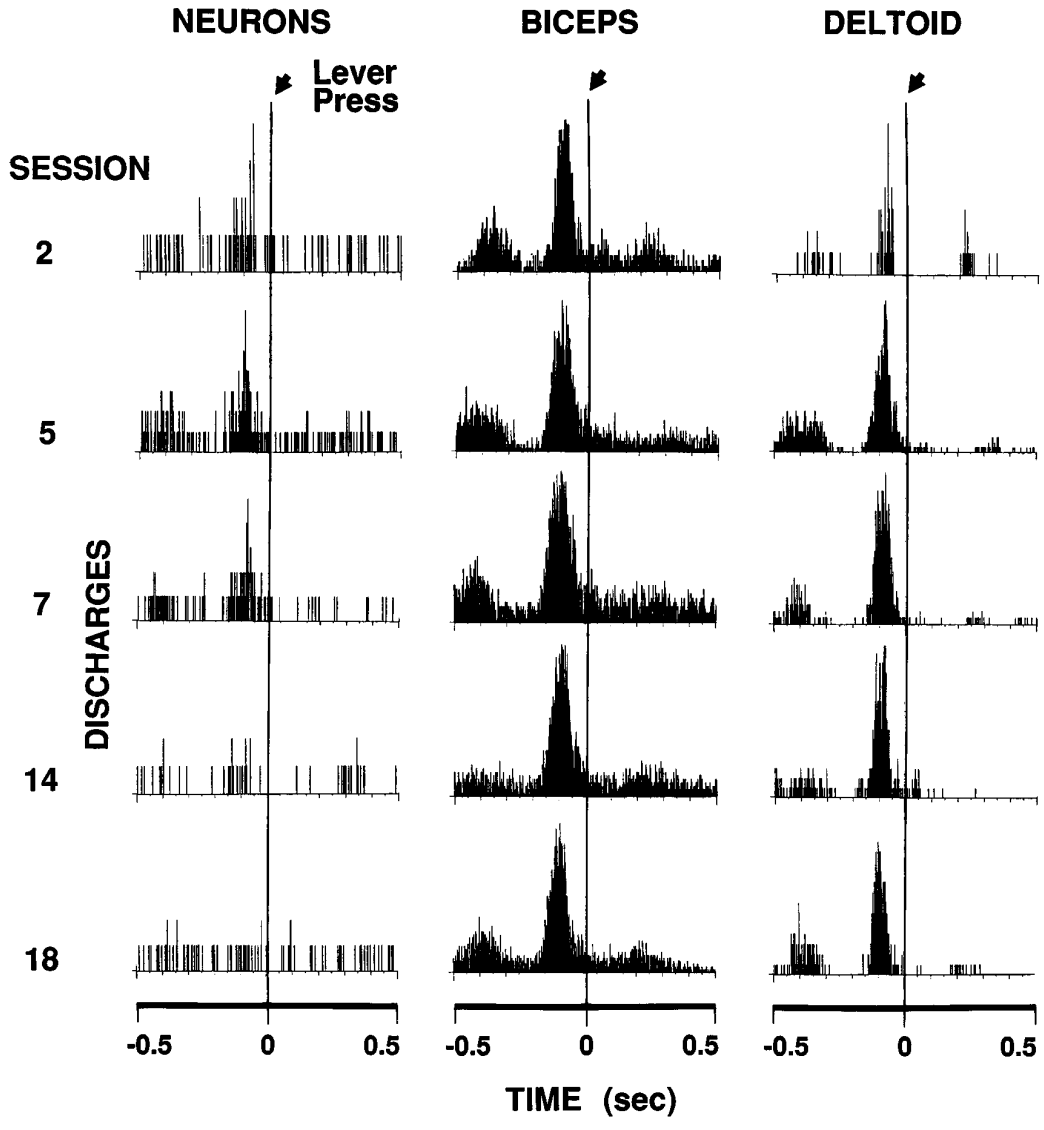

Figure 4. Decline in lever press-related firing of forelimb neurons after repeated sessions in a different animal (171). Firing was time-locked to the reach toward lever during acquisition (e.g., sessions 2, 5, and 7) but not after repeated sessions (e.g., 14 and 18). Onset of biceps/deltoid EMG activity was similar across sessions. Secondary peaks in neuronal activity and EMG activity at −0.50 sec reflect residual from ITI lever presses, which persisted at three to four per ITI for this animal. Details as in Figure 3, *left*.

Disappearance of firing related to the lever press after repeated sessions on the task

The main finding of this study was that forelimb neurons fired in relation to the lever press during early sessions, but did not do so after repeated sessions on the task. This was true even though all neurons were verified by the exam to fire in relation to active movement specifically of the forelimb, as well as to passive manipulation and/or cutaneous stimulation for some neurons (e.g., Fig. 3, *right column*). Examples of activity recorded from single forelimb neurons and simultaneously recorded EMG activity are illustrated in PEHs for representative sessions of two animals in Figures 3 and 4. One forelimb neuron (Fig. 3, session 4) exhibited an increase in firing rate during the reach toward the lever, whereas two others (session 5) recorded simultaneously increased firing after the onset of lever depression; however, after repeated sessions on the task (e.g., sessions 9 and 12), no such activity related to the lever press was observed. A similar pattern is shown for another animal in Figure 4. Increased firing rate related to reaching toward the lever was observed in early sessions (e.g., sessions 2, 5, and 7), and an absence of firing related to the lever press was observed in late sessions (e.g., sessions 14 and 18).

A graphic summary of these changes is presented in Figure 5, in which S:B for each of the 53 forelimb neurons is plotted as a function of session number for each animal. Although some values <1.5 were observed in early sessions, most forelimb neurons recorded in the first few sessions showed severalfold increases in firing rate as the forelimb moved from its position on the floor to reach for and press the lever. Such increases were not observed for any forelimb neuron recorded in the later sessions. This pattern showed a striking consistency in every animal tested; in all three cases, S:B showed a significant ($p < 0.01$) decrease with increasing session number.

Of 53 forelimb neurons recorded from all three animals during the task, 21 showed firing time-locked to the lever press (S:B $>$ 1.5). For these 21 neurons, the firing rate associated with reaching toward (14 neurons) or pressing (7 neurons) the lever, i.e., signal,

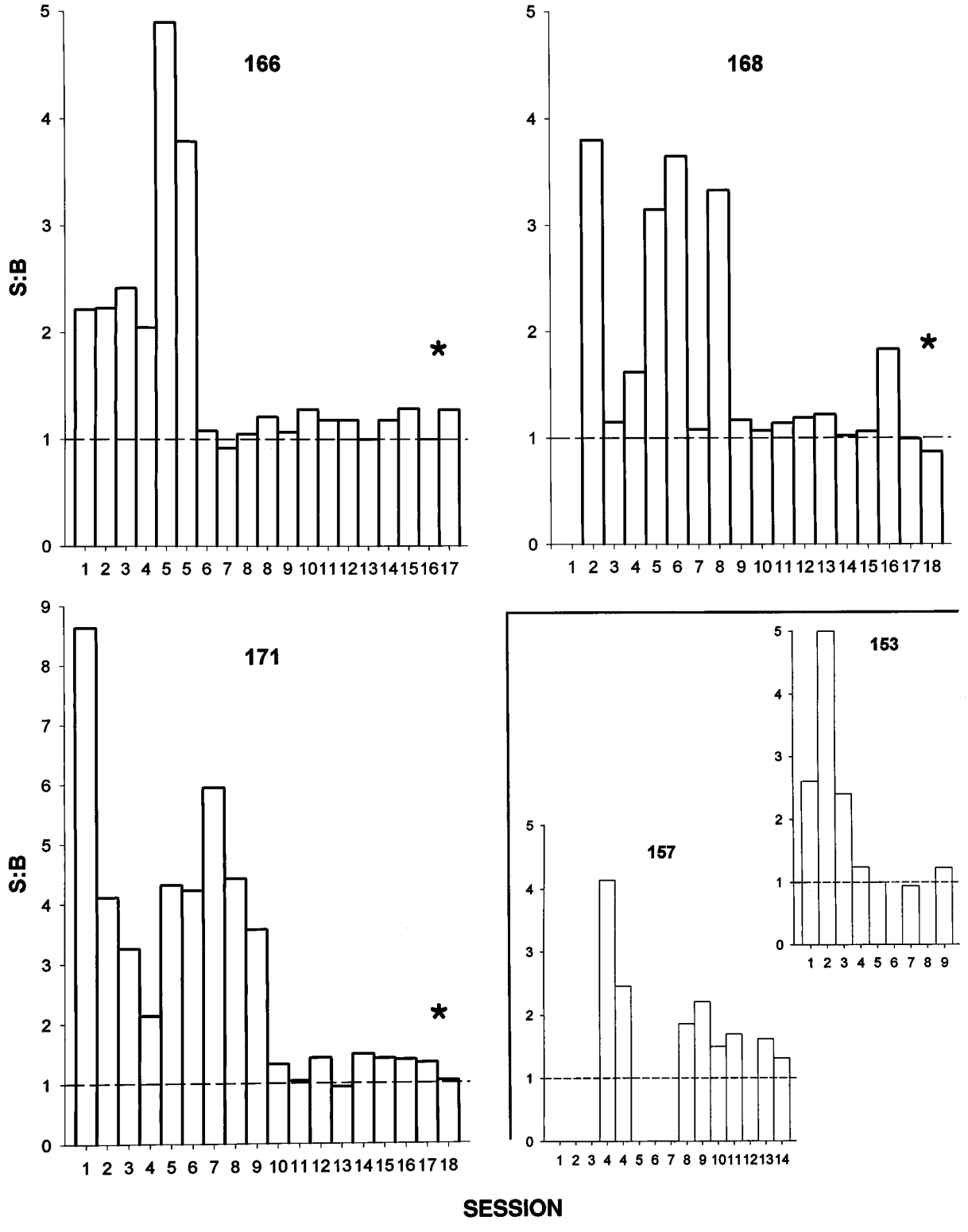

Figure 5. S:B across all sessions for each animal (animal *number* at *top*). Each *vertical bar* represents S:B for one forelimb neuron. Ratio near 1 (*dashed horizontal line*) indicates that firing rate did not change as the paw moved from position on tape to reach and press the lever. *Asterisk* indicates significant ($p < 0.01$) change in S:B as a function of session number. *Inset*, Similar trend in S:B as a function of session number obtained from two animals in the initial study.

was as much as 9 times greater than that during baseline, when the paw was stationary on the floor. Of the 21 neurons showing time-locked firing, all except one were recorded early in training, i.e., sessions 1–9, representing 71% of the 28 neurons recorded in those sessions. Only one neuron that showed time-locked firing (S:B = 1.8) was recorded after session 9, constituting 4% of the 25 neurons recorded in late sessions (10–18).

It was not possible to track the activity of the same neuron for

18 sessions, but the data lead to the conclusion that the activity of the population of forelimb neurons changed as a function of session number. An alternative is that forelimb neurons might belong to separate subpopulations (indistinguishable in the exam): "time-locked" and "non-time-locked" to the lever press, the latter sampled mainly in late sessions. Accordingly, our differential of 20 time-locked neurons (early) to one time-locked neuron (late) occurred by random sampling from the two hypothetical subpopulations. Because 28 total neurons (early) and 25 total neurons (late) were sampled, the probability (relative frequency) of sampling any given neuron early versus late is 28 of 53 (0.528) and 25 of 53 (0.472), respectively. The probability of obtaining the observed differential of 20:1 for time-locked neurons by random sampling is $0.528^{20} \times 0.472^{1} \times 21 = 0.00003$, effectively eliminating this alternative.

Neural data are included for the two animals from the initial study (Carelli and West, 1991b), in which the full complement of neural and behavioral data were not collected. Both animals exhibited a decrease in S:B as session number increased (Fig. 5, *inset*). Available behavioral data (response to tone, and total RT only) showed that both animals also evidenced acquisition of tone discrimination. One showed 43% errors in responding to the tone in session 1, which improved to asymptotic values of 7%, 3%, and 2% in sessions 7, 8, and 9. Total RTs similarly improved from 0.74 sec to 0.43, 0.66, and 0.65 sec in those same sessions. The second animal improved from 35% errors in responding to the tone in session 4 to 22% in session 14; total RT improved from 2.6 to 1.7 sec in those sessions.

Lack of short-latency tone-evoked neuronal responses

Comparison of firing between the periods −100 msec versus +100 msec relative to tone onset was not confounded by forelimb movement because (1) the preceding 100 msec corresponded to stationary forelimb position on the floor and (2) forelimb movement did not begin for at least 300 msec after tone onset (RT_1 in Fig. 2, *top right*). Of the 53 forelimb neurons, only one showed tone-evoked activity (inhibition; rat 168, session 3). No tone-evoked activity was observed for any forelimb neuron during the late sessions, i.e., after acquisition of tone discrimination.

Histology

Reconstruction of three-dimensional locations of electrode tips (Carelli and West, 1991) revealed that all forelimb neurons were located in the forelimb region of the dorsolateral striatum (+0.20 to +1.6 mm anteroposterior) (compare West et al., 1990, and Cho and West, 1997). Dissection of the right forelimb and the right shoulder of each animal revealed that in all instances muscle formed around the Teflon mesh used to secure the EMG wires in place. The EMG wires were located underneath the mesh, within the triceps or biceps muscles of the right forelimb, or the deltoid muscles of the right shoulder.

DISCUSSION

An initial study (Carelli and West, 1991b) had shown that the firing of striatal forelimb neurons during lever pressing declined after animals learned to lever press. Therefore, the most important aspect of the present design was to eliminate variability from the task and from daily protocols that could potentially account for such changes in firing. Animals were required simply to press a lever from a particular starting point in response to a tone cue. This contingency remained the same on every trial and every session. In early sessions, the majority of forelimb neurons fired in relation to the lever press, as expected; however, after repeated daily sessions, striatal neurons related to movement of the forelimb no longer fired in relation to virtually the same movement to which firing had been time-locked during early sessions.

The change in firing could have been related to one or more of the behavioral variables that changed across sessions. No changes were observed in forelimb movement involved in reaching and pressing the lever to the level required for reinforcement. Nonetheless, all three animals improved in the efficiency of lever pressing, measured as fewer extraneous fluctuations beyond that level of force. This effectively reduced force and distance by ~5–10 gm and 2–4 mm in later sessions; however, it is not likely that these reductions explain the elimination of firing time-locked to the lever press. The most compelling reason is that any such contribution could not have applied to the majority of neurons studied. An estimated two-thirds of all neurons were related to the reach toward, not the depression of, the lever (estimated on the basis of the 2:1 ratio of the former to the latter). Therefore, declining force or distance could have contributed to the decline in S:B for only an estimated one-third of the neurons studied. Second, firing rates of load-related or distance-related striatal forelimb neurons are reduced, not eliminated by, reductions in force (Crutcher and DeLong, 1984b; Liles, 1985) or distance (Kimura, 1990) of forelimb movement. Thus, the observed partial reductions in force and distance as a function of session number do not explain the absence of time-locked firing in later sessions. Furthermore, ~50% of striatal forelimb neurons are load-related (Crutcher and DeLong, 1984; Liles, 1985). This leaves only 50% of the estimated one-third of our sample (i.e., the neurons related to depression of the lever) in question, further restricting any explanatory power of this argument. Therefore, it is reasonable to conclude that with increasing session number the gradual disappearance of firing in relation to the lever press (1) occurred despite the similarity of numerous movement parameters across sessions and (2) cannot be accounted for satisfactorily by the partial reduction in force or distance exhibited in later sessions.

Instead, the gradual reduction in superfluous fluctuations beyond the required level of lever depression may be viewed more appropriately as an improvement in efficiency or accuracy, concomitant with acquisition of skill in the task. These fluctuations appear to be analogous to final "current control," i.e., small, oscillating corrections made before the end of a movement to a target. Such fluctuations predominate early in learning a movement but tend to be eliminated as a function of experience, as ballistic movements become more acurate and/or predominant (Brooks, 1979) (see below).

Acquisition was demonstrated further by changes in other key behavioral measures that were not measures of the movement per se. All animals exhibited tone discrimination, by (1) reducing the percentage of tone presentations to which animals failed to respond, and (2) reducing RT_1, time to initiate forelimb movement in response to tone onset. Two animals also reduced the number of lever presses made in the absence of the tone, whereas one did not. With that single exception, the topography of each measure (response to tone, RT_1, intertrial presses, and efficiency) as a function of session number was curvilinear, exhibiting a negative acceleration and approximate asymptote. These topographies conform to exponential models of learning and demonstrate acquisition of what has been termed a lever-pressing habit (Hull, 1943; Estes, 1959; Spence, 1960).

The acquisition of a habit involves the gradual development of specific S–R bonds (Mishkin et al., 1984; Squire et al., 1993). A

habit is distinguished by the tendency to be "response-like" in that it is triggered automatically by a particular stimulus or stimulus complex (Dickinson, 1985). Furthermore, acquisition of a skilled movement involves a progression from movements requiring current control and feedback, to ballistic movements that are made correctly with less current control and are uninfluenced by peripheral feedback (Polit and Bizzi, 1978; Moroz and Bures, 1983; Zhuravin and Bures, 1986; Saling et al., 1992; Hocherman, 1993; for reviews, see Keele, 1968; Brooks, 1979; Cooke, 1980). The striatum has been suggested to play a role in the acquisition of habits and certain motor skills (Marsden, 1982; Mishkin et al., 1984). Parkinson's patients have been described as "having lost the advantages of working ballistically, notably those of increased speed and reduced information load in the sensory-motor system" (Flowers, 1975).

If the striatum and its DA input are necessary for the acquisition of motor skills and/or habits, then activity of neurons in the nigrostriatal system ought to show correlations with their acquisition. Such correlations have indeed been found, specifically with respect to neuronal responses to conditioned stimuli after overtraining (Ljungberg et al., 1992; Schultz, 1993; Aosaki et al., 1994). The present report is the first, to our knowledge, to demonstrate a disappearance of *movement*-related firing of striatal neurons as a function of acquisition.

Initially, DA may be necessary for processing by striatal forelimb neurons of lever press-related sensorimotor information projected to them via the corticostriatal system. In turn, their activity may be necessary to their targets in premotor areas for developing computations that will be used subsequently, by neural networks controlling the lever press after it has become (in various terminologies) automatic, preprogrammed, or S–R habit. As the processing of sensorimotor variables is eliminated, striatal forelimb neurons may participate less in the lever press, provided conditions remain constant. This decline in sensorimotor processing is in line with the proposed decline in processing of conditioned stimuli as a function of learning (Pearce and Hall, 1980). Our interpretation is also consistent with the suggestion that computations for executing certain learned movement sequences may be performed outside the striatum (Marsden, 1982). That the striatum may participate, in certain instances, in the acquisition of motor skills whose computations are stored elsewhere is analogous to the conceived role of the medial temporal/diencephalic memory system in the initial stages of forming permanent declarative memories, which are ultimately stored elsewhere (Milner, 1970; Squire, 1993).

Other studies have shown a continued presence of task-related striatal activity after extensive training (e.g., Crutcher and DeLong, 1984b; Liles, 1985; Alexander and Crutcher, 1990a,b; Crutcher and Alexander, 1990; Gardiner and Nelson, 1992; Kimura et al., 1992; Jaeger et al., 1993). Those tasks continued to engage the activity of striatal neurons, presumably because they required processing of complex sensorimotor variables (Alexander et al., 1992) that changed unpredictably from trial to trial. The tasks lacked the simple, repetitive contingency held constant on every trial of every session in our task, in which the single stimulus was programmed to provide no information other than a temporal reference for the single response. Indeed, after primates were overtrained in a task similar to the present task, responses of DA neurons to the CS were reduced considerably (Ljungberg et al., 1992), which was interpreted as a potential influence on striatal processing involved in the acquisition of habits.

Only 1 of 53 forelimb neurons exhibited a short latency response to the tone S^D, consistent with previous studies (Crutcher and DeLong, 1984; Kimura et al., 1984; Alexander, 1987). In this respect, the present sample resembles type IIb neurons (Kimura, 1990). Discharges initiated by a conditioned stimulus (West et al., 1987; Schultz and Romo, 1988) are characteristic of other functionally defined populations of striatal neurons, such as type IIa (Kimura, 1986; 1990; Gardiner and Nelson, 1992; Romo et al., 1992) and TANs (Kimura et al., 1984; Apicella et al., 1991; Aosaki et al., 1994).

Each neuron recorded in each of 18 sessions was verified in the preliminary sensorimotor exam to be responsive specifically during active movement of the forelimb, and in most cases also during passive manipulation and cutaneous stimulation of the forelimb (compare West et al., 1990). Thus, the loss of firing is not attributable to any compromised ability to sample forelimb neurons or to tissue damage. In addition, firing was later reinstated under certain altered conditions (not studied systematically). This is similar to a previous study in which phasic striatal firing during treadmill locomotion was reinstated after having disappeared after exposure for 30 sessions to an unchanging treadmill cycle (West et al., 1987). The gradual disappearance of striatal firing suggest that movement-related activity may cease during certain movements that have become automatic or habitual, but not before that activity may have contributed to the formulation in other areas (e.g., premotor areas) of computations needed to carry out the automatic movement.

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