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Operant conditioning of cortical cell and muscle response patterns

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Abstract

Operant conditioning of cortical cell
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Part I: In primates, corticomotoneuronal (CM) cells have sufficiently strong synaptic linkages to motoneurons to mediate post-spike facilitation in spike-triggered averages of muscle activity. We investigated the degree to which activity of CM cells and their target muscles could be independently controlled by operantly conditioning their relative activation levels. In two *Macaca nemestrina* monkeys, single cortical neurons were recorded with moveable microwires chronically implanted in the caudal bank of the pre-central gyrus. Rectified EMG of 12 distal forelimb muscles was recorded with sub-cutaneously implanted pairs of wires in each muscle in one monkey from which 35 unique cell-muscle pairs were operantly conditioned. In the other animal, surface electrodes were placed over wrist flexor and extensor muscles (9 unique cell-muscle pairs). Spike-triggered averages of rectified EMG were compiled while the monkeys performed a force target-tracking task about the wrist. Twenty-four CM cells, with post-spike effects at latencies between 6 and 16 ms in one or more forelimb muscles, were selected for activity dissociation conditioning. Monkeys performed an operant conditioning task through which relative activation of the CM cell and a target muscle could be explored (44 unique cell-muscle pairs). Cell and muscle activity controlled the position of a cursor on a screen, with cursor position determined by concurrent cell spike rates (C) and EMG activity of a target muscle (M); one activity assigned the horizontal direction the other, vertical. Monkeys received fruit sauce rewards for holding the cursor

in target positions requiring at least four combinations of increased (+) and suppressed (-) activation relative to levels observed during force generation, namely C+M+; C+M-; C-M+ and C-M-. The monkeys learned to reciprocally activate cells and target muscles exhibiting post-spike facilitation (42 out of 44 cell-muscle pairs), in both directions. For muscles with post-spike suppression (4 out of 4 pairs), monkeys learned to co-activate these cell-muscle pairs. These results indicate that cortical cells with direct synaptic linkages to motoneurons can be flexibly activated relative to their target muscles. Further, CM cell-muscle activity dissociations can be rapid, robust, reversible and are subject to volitional control. Calculation of mutual information between CM cell and muscle activation patterns during reciprocal dissociation events, compared to force target-tracking, implicates involvement of other upstream sources. Activity independence between correlationally-linked components within a neural circuit favors strategies for brain computer interface (BCI) control in which individual neurons are each assigned an individual degree-of-freedom of device output.

Part II: Operant conditioning of neural activity has typically been achieved under controlled behavioral conditions using food reinforcement. To reward cell activity during unconstrained behavior, we sought midbrain sites whose stimulation would support operant responding. Three nemestrina monkeys learned to perform a manual step-tracking task rewarded by fruit sauce. We found sites in nucleus accumbens and surrounding striatum whose stimulation could maintain task performance and verified that response rates increased monotonically with increasing pulse frequency and amplitude. We recorded activity of single neurons with moveable microwires chronically implanted in the precentral gyrus and documented neural modulation with a force-guided target-tracking task. We attempted to condition increased firing rates first with the monkey in the training booth and then during free behavior in the cage using the Neurochip—a head-fixed, autonomous recording and stimulating system. Spikes occurring above baseline rates triggered single or multiple electrical pulses to the reinforcement site (1 mA, 0.2 ms biphasic current pulses). This rate-contingent, unit-triggered stimulation was made available for periods of 1 to 3

minutes separated by 3 to 10 minute time-out periods without stimulation, regardless of cell activity. During in-booth sessions feedback was presented as vertical cursor movement and auditory clicks. During in-cage conditioning, barely audible clicks occurred during each spike-triggered stimulation event. In-booth conditioning produced increases in single neuron firing probability after transition to intracranial reinforcement in 48 of 58 cells. Reinforced cell activity could rise > 5 times that of non-reinforced activity, doubling in most sessions. Activity peaks typically occurred during the first 10 seconds of each time-in period and, for many cells, activity remained elevated above baseline for the full period. In-cage conditioning produced significant increases in post-transition activity in 21 out of 33 sessions. In-cage effects peaked later and lasted longer than in-booth effects but were often comparatively smaller, between 13 and 18 percent above non-reinforced activity. The difference in responding in the two conditioning environments could be due to the dynamic range of candidate cell firing rate, robustness of the reinforcement site and differing levels of attention and competing behaviors in the booth and cage. Controls indicate that stimulation of the reinforcement site did not directly evoke increased cell activity. In several sessions, neighboring, synaptically-linked motor cortex neurons were recorded simultaneously with the trigger cell, revealing network involvement in eliciting conditioned rate changes in the stimulation-triggering neuron.

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GLOSSARY

APL: abductor pollicis longus.

BCI: brain computer interface.

BSR: brain stimulation reward.

C: cortical neuron, cell.

CM: corticomotoneuronal [*cell*].

DBS: deep brain stimulation.

DI: dissociation index.

ECR: extensor carpi radialis.

ECU: extensor carpi ulnaris.

ED4,5: extensor digiti quarti et quinti.

EDC: extensor digitorum communis.

EMG: electromyography.

EPSP: excitatory post-synaptic potential.

F-E: flexion-extension [*axis*].

FCR: flexor carpi radialis.

FCU: flexor carpi ulnaris.

FDI: first dorsal interossei.

FDP: flexor digitorum profundus.

FDS: flexor digitorum superficialis.

FPB: flexor pollicis brevis.

FTT: force target-tracking [*task*].

ICSS: intracranial self-stimulation.

ISI: inter-spike interval.

K-L: Kullback-Leibler [*divergence*].

K-W: Kruskal-Wallis [*test*].

M: muscle, target muscle.

MPI: mean percent increase.

MRI: magnetic resonance image.

MSE: mean squared error.

NAC: nucleus accumbens.

NC: noise correlation [*coefficient*].

NR: non-reinforced [*interval*].

PL: palmaris longus.

PSPE: post-spike effect.

PSPF: post-spike facilitation.

PSPS: post-spike suppression.

R: reinforced [*interval*].

R-U: radial-ulnar [*axis*].

RCST: rate-contingent, spike-triggered [*stimulation*].

REMG: rectified EMG.

SC: signal correlation [*coefficient*].

S.D.: standard deviation.

SPTA: spike-triggered average.

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DEDICATION

To my parents, sister and recovery family.

“Question everything. Learn something. Answer nothing.”

–Euripides

“A fool thinks himself to be wise, but a wise man knows himself to be a fool.”

–William Shakespeare

“Failure is not fatal, but failure to change might be.”

–John Wooden

“Truth can be stated in a thousand different ways, yet each one can be true.”

–Swami Vivekananda

Part I

**INDEPENDENT ACTIVATION OF PRIMATE
CORTICOMOTONEURONAL CELLS AND TARGET MUSCLES
DEMONSTRATED BY OPERANT CONDITIONING**

Chapter 1

INTRODUCTION

In primates, descending commands from primary motor cortex exert a dominant influence in generating voluntary movement [94]. Multiple cortical neurons are involved in activating a single motor unit, and many motor units make up the muscles that drive movements. While motor unit recruitment follows a strict order, from small to large, the convergent influence from cortical neurons could permit flexibility in the manner by which their downstream motor units are depolarized to threshold. A previous study of volitional control of motor cortex cells and related muscles showed that cortical neurons consistently co-activated with particular muscles could be activated in the absence of muscle activity when the monkey was rewarded for that pattern [33]. The reverse dissociation was less successful; the reasons may have involved constraints in relative recruitment of cortical cells and muscles, or simply behavioral fatigue or reinforcement satiation. That investigation rewarded bursts of activity in cortical neurons whose causal role in activating the related muscles remained largely unknown. Here, we investigated flexibility in cortical cell-muscle co-activation for those motor cortical cells that have correlational linkages to forearm muscles. Specifically, we operantly conditioned co-activation and dissociation of corticomotoneuronal (CM) cells and their target muscles—activities that are usually tightly correlated during manual force target-tracking [18], [70]. This represents a stringent test of constraints in relative recruitment of synaptically-linked elements and the proposal that volitionally controlled patterns are determined by “natural” relationships [48].

If peri-contractile CM cell recruitment is indeed flexible, cortico-motoneuronal network architecture and relatively small CM cell synaptic potency should allow the discharge rate of a single CM cell to change without appreciably affecting activation of its target motoneurons. Convergent input from other sources, including other CM cells, could compensate for the missing influence. Flexible CM cell recruitment would allow muscle-activating CM

cell discharge to be suppressed over repeated contractions of the target muscles. Flexible recruitment would also permit elevated CM cell activity while the concurrent target-muscle activity remained low. To probe the flexibility of the relative activation of CM cells and their target muscle we operantly conditioned all combinations of CM cell (C) and isometric target muscle (M) activation, including co-activation (C+M+), reciprocal activation (C+M-, C-M+) and co-inactivation (C-M-). This investigation was motivated to test previous assumptions of fixed CM cell synchronicity with target muscle activation during execution of voluntary movements [34], [70].

1.1 Overview of previous work

Cheney et al. (1991) define “dissociation” of CM cell and target muscle activity as co-activation under some conditions but absence of co-activation or reciprocal activation under other conditions [20]. We have adopted this terminology for this investigation. Here, we define “forward-dissociation” as suppression of muscle activity while the associated cortical neuron remains active (C+M-) and “reverse-dissociation” as the converse: suppression of unit activity during contraction of the associated musculature (C-M+). In this investigation, correlated muscle-CM cell co-activation was initially established during wrist force target-tracking performance. We then explored this relationship under controlled experimental conditions using a specifically-tailored operant conditioning task. Both forward (C+M-) and reverse (C-M+) dissociations were conditioned, as well as co-activation (C+M+) and inactivation (C-M-) of a CM cell and its target muscles.

There is much evidence indicating a variable relationship in the relative activities of CM cells and their downstream target muscles. Davidson et al. (2007) discovered corticomotoneuronal throughput to motoneurons can be rapidly altered as a function of task performance [22]. They found that an individual motor cortex neuron, which is ineffective in eliciting discharge during certain motor behaviors, does facilitate discharge of the same motoneurons during other behaviors. Examples of reverse-dissociation appear in studies monitoring discharge of CM cells during task-associated muscle contraction. Cheney & Fetz (1980) found that during ramp-and-hold wrist movements some CM cells began firing after their target muscles were activated [18]. They also observed that muscle activation

without CM cell activity occurs with rapid, forceful and ballistic movements in comparison to those associated with well-controlled ramp-and-hold movements of their wrist flexion-extension task. Muir & Lemon (1983) and Buys & Lemon (1986) examined discharge in CM cells that facilitated muscle activity in at least one intrinsic hand muscle. They noticed that the CM cells discharged at higher rate during a precision grip task, when the cells' target muscles were less active, in comparison to the rates observed during a power grip task when the muscles were more active [78], [13].

Forward-dissociation of motor-related cortical neurons has been observed in several studies that simultaneously monitored neuronal and muscle discharge. Fetz & Finocchio (1975) conditioned monkeys to alter the relative intensities of muscle activity and co-modulated cortical cells by demonstrating robust forward-dissociation activation patterns [33]. They had limited success, however, demonstrating reverse dissociation—likely due to end-of-session satiation when the C-M+ task component was attempted. In spite of this, slight relative reduction of cortical neuron activity during contraction of proximal limb muscles was demonstrated. Carmena et al. (2003) observed that after shifting control of a robotic arm from manual joystick manipulation to signals derived from the arm-correlated activity of neuronal ensembles in frontoparietal cortex, the original movements used to move the joystick ceased entirely [15]. Similar forward dissociation was observed in other brain-computer interface studies [116], [17]. While cortical neurons recorded in these studies were motor-related, their correlational-linkage to task-relevant muscles remained untested.

1.2 Project significance

In contrast to studies mentioned above, this investigation 1) specifically sought to modify the correlation of concurrent cell and muscle activities directly, using an activity-driven operant conditioning task, 2) sought to demonstrate both forward (C+M-) and reverse (C-M+) dissociations and 3) selected for conditioning only cell-muscle pairs for which correlational linkage was established prior using the method of spike-triggered averaging. It is important to note that demonstrating co-variation of cortical cell discharge with muscle contraction does not prove that the cell is causally involved in activating those muscles. Only by establishing the synaptic connectivity of the cell within the command-generating cortical

network can its causal role be known [31], [28]. For this project, spike-triggered averaging technique was used to determine whether or not a candidate unit is a CM cell with a correlational linkage to muscle.

We demonstrated that co-activation patterns of two, correlationally-linked, components of a neural pathway are dissociable. Secondly, the nature of activity dissociations between CM cells and their target muscles can be rapid, robust, reversible and subject to volitional control. These findings imply that individual neurons, or individual components of a neural circuit (e.g. CM cells and linked motor units), can be assigned to distinct degrees-of-freedom to control brain computer interfaces (BCIs) despite their connection proximity within that circuit. Our results challenge recent interpretations, based on co-activity patterns recorded from parietal reach region, that activity patterns recruited during movements from a monkey's natural repertoire are more favorable for control of BCIs [48].

Chapter 2

GENERAL METHODS

2.1 Subjects

Two male macaca nemestrina monkeys (4-6 years old, weight 4.0 and 5.6 kg) were used in this investigation. All surgical, training and handling procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

2.2 Training

Prior to surgeries, monkeys were trained to perform a center-out force-target-tracking (FTT) task in which isometric wrist torque controlled the 2D position of a computer cursor on a screen. When the cursor entered a target and remained inside for a finite amount of time (1 second or less) a fruit sauce reward signaled completion of that trial. Between moves to peripheral targets, the monkey was required to return to rest position at the screen center. Target placement on the screen determined the direction and magnitude of torque production about flexion-extension (F-E) and radial-ulnar (R-U) axes required to complete each trial. The task used eight peripheral targets (F, FR, R, ER, E, EU, U and FU) plus a central rest target (C) for successive trials. Peripheral targets were presented in random order at equal frequencies. Training was complete when monkeys moved directly from center to each of the eight targets, and held it inside for at least 1 second. During experiments, the FTT task was performed daily to elicit task-related cortical cell firing and muscle EMG for detection of CM cells via spike-triggered averaging.

2.3 Surgery

Chronically implanted microwire arrays enabled stable, long-term recording of corticomotoneuronal cells [50]. Microwires were positioned to advance along the caudal bank of the precentral gyrus, layer V where somata of many CM cells have been identified [97], [112].

In monkey J, EMG wire pairs were implanted subcutaneously in twelve distal forelimb muscles that move the right wrist and hand: flexor digitorum profundus (FDP), palmaris longus (PL), extensor digiti quarti et quinti (ED4,5), extensor carpi ulnaris (ECU), flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), extensor carpi radialis (ECR), flexor carpi radialis (FCR), first dorsal interossei (FDI), abductor pollicis longus (APL), flexor carpi ulnaris (FCU), flexor pollicis brevis (FPB).

2.4 Recording

We recorded activity of single motor cortex cells using chronically implanted, depth-adjustable tungsten microwire electrodes (diameter 50 μm ; impedance 0.5 M Ω ; inter-electrode spacing 500 μm) [50]. To record EMG, we implanted wire pairs subcutaneously in each of 12 wrist muscles in the proximal forearm in animal (Monkey J) and used surface electrodes over flexor and extensor muscles in Monkey D. At the beginning of each session, cortical cell spike events and EMG of forearm wrist muscles were recorded while the monkeys performed the isometric wrist FTT task. Action potentials of cells were identified using time-amplitude window discrimination or a template matching algorithm (MSD, Alpha Omega Engineering). Baseline and task-elevated spike rates were determined for subsequent cell-muscle dissociation conditioning and compiling spike-triggered averages of rectified EMG.

Chapter 3

A FORCE-DRIVEN TARGET-TRACKING TASK REVEALS NON-CONDITIONED CO-ACTIVATION PATTERNS OF CM CELLS AND THEIR TARGET MUSCLES

3.1 *Introduction*

We had monkeys perform the FTT task prior to dissociation conditioning for two reasons. First, to detect possible correlational linkages between task-modulatory cortical neurons and forelimb wrist muscles recruited to control the torque-driven computer cursor. Corticomotoneuronal linkages produce post-spike features in cortical spike-triggered averages of rectified EMG. Following identification of a CM cell and selection of one of its target muscles for dissociation conditioning, cell-muscle co-activation levels produced during the FTT task target holds were used as a basis for comparison when measuring the extent of reciprocal activity dissociations elicited during the subsequent dissociation task.

We plotted concurrent CM cell and target muscle activities as points in a two-dimensional coordinate space. In addition, we also calculated tuning correlation values, using each of the peripheral targets of the FTT task, as an alternative means to gauge cell-muscle activity correlation prior to dissociation conditioning.

3.2 *Methods*

3.2.1 *Spike-triggered averaging*

The output effects of CM cells on muscles were identified by post-spike features in spike-triggered averages (SpTAs) of rectified EMG (rEMG). Spike-triggered averages were compiled by extracting EMG sweeps spanning -30 ms prior, to 50 ms after each spike event produced from a cortical neuron. The collection of rectified EMG snippets were aligned relative to spike occurrence and then averaged. Post spike effect features occurring between 6 and 16 ms, the onsets of which were marked by deviations greater than 20% of pre-spike

baseline activity, were used as evidence of correlational linkage between the cortical neuron and forelimb wrist muscle. In this study, SpTAs were compiled from cortical spike events, and concomitant EMG signals, during performance of the force target tracking task; the performance of which usually elicited simultaneous activation of both the candidate cortical neuron and forelimb wrist muscles for at least one (or more) direction-specific target.

3.2.2 Tuning correlation between cell and muscle task activities

For each CM cell-muscle pair, we measured the signal cross-correlation [125], [6] between their tuning curves generated during force target-tracking. The signal correlation between each CM cell-muscle pair i , is given by

$$SC_i = \frac{\langle (\bar{c}_{i,t} - \langle \bar{c}_{i,t} \rangle_t) (\bar{m}_{i,t} - \langle \bar{m}_{i,t} \rangle_t) \rangle_t}{\sqrt{\langle (\bar{c}_{i,t} - \langle \bar{c}_{i,t} \rangle_t)^2 \rangle_t \langle (\bar{m}_{i,t} - \langle \bar{m}_{i,t} \rangle_t)^2 \rangle_t}} \quad (3.1)$$

where $\langle \rangle_t$ is the expectation operator across target locations, t , and symbols $c_{i,t}$ and $m_{i,t}$ indicate the mean activities from respective cortical and muscle sources during in-target force holds across all trials of a given target. Specifically, cortical activities $\bar{c}_t$, are mean spike counts during force holds inside each target while muscle activities $\bar{m}_t$, represent traces of rectified EMG integrated over target hold times which were then averaged. Monkey J performed the FTT task using 8 peripheral targets, the cursor for which was guided by combined F-E and R-U torque components. Monkey D generated flexion-extension torque to complete trials in a two-dimensional peripheral target task.

The noise correlation between each cell-muscle pair is a measure of the correlation between in-target activities after the signal component, or mean response, has been subtracted. Noise correlation is calculated

$$NC_i = \left\langle \frac{\langle c_{i,t}^n m_{i,t}^n \rangle_N - \bar{c}_{i,t} \bar{m}_{i,t}}{\left(\langle (c_{i,t}^n - \bar{c}_{i,t})^2 \rangle_N \langle (m_{i,t}^n - \bar{m}_{i,t})^2 \rangle_N \right)^{1/2}} \right\rangle_t \quad (3.2)$$

where superscript n indexes the in-target activity average for a single trial and $\langle \rangle_N$ denotes expectation operation (mean) across all N trials of the given target t .

3.3 Results

3.3.1 Spike-triggered averaging detects correlational linkages between CM cells and target muscles

After isolating cell spike activity in motor cortex, we compiled spike-triggered averages of forelimb rEMG to identify post spike effects. Cells whose spike activity affected downstream target muscles were selected for further dissociation conditioning.

As shown in figure 3.1a, sweeps of rectified EMG activity surrounding motor cortex spike events were extracted, aligned and averaged. Corticospinal neurons affect an excitatory influence on neurons onto which they project. Features in these spike-triggered averages occurring between 6 and 16ms after the spike event, that peak above two standard deviations of pre-spike baseline activity, indicate the trigger cell exerts an excitatory, probably monosynaptic, influence on motor units within the recorded muscle [32]. Figure 3.1b illustrates the effect of an example CM cell firing on six concurrently recorded forelimb muscles involved in the target-tracking task. The muscles depicted comprise this cell’s set of target muscles or “muscle field”. Spike-triggered averages of muscles FCU, FDP and ECU reveal “pure” post-spike effects while SpTAs of their antagonists, ECR and EDC, indicate the cell’s suppressive influence, likely mediated through a secondary spinal inhibitory interneurons [91]. The early onset of effect in the ED4,5 average indicates firing synchrony between neurons in motor cortex: the triggering cell fired in tight correlation with other CM cells, not recorded, that also projected onto motoneurons of the muscle. Because spike events of the two cells are usually not perfectly synchronized, early onset of an effect can be observed in spike-triggered averages [7, 112]. In this investigation, PSpF effects were assumed to be superimposed on synchrony effects and were measured between inflection points of appropriate latency (6 to 16 ms).

3.3.2 CM cell activation correlates with directional tuning of its facilitated target muscles

We measured correlation between the activities of CM cells and their target muscles by calculating the tuning curves of these activities during performance of the FTT task. The tuning curves of a CM cell and four muscles during performance of a two-dimensional

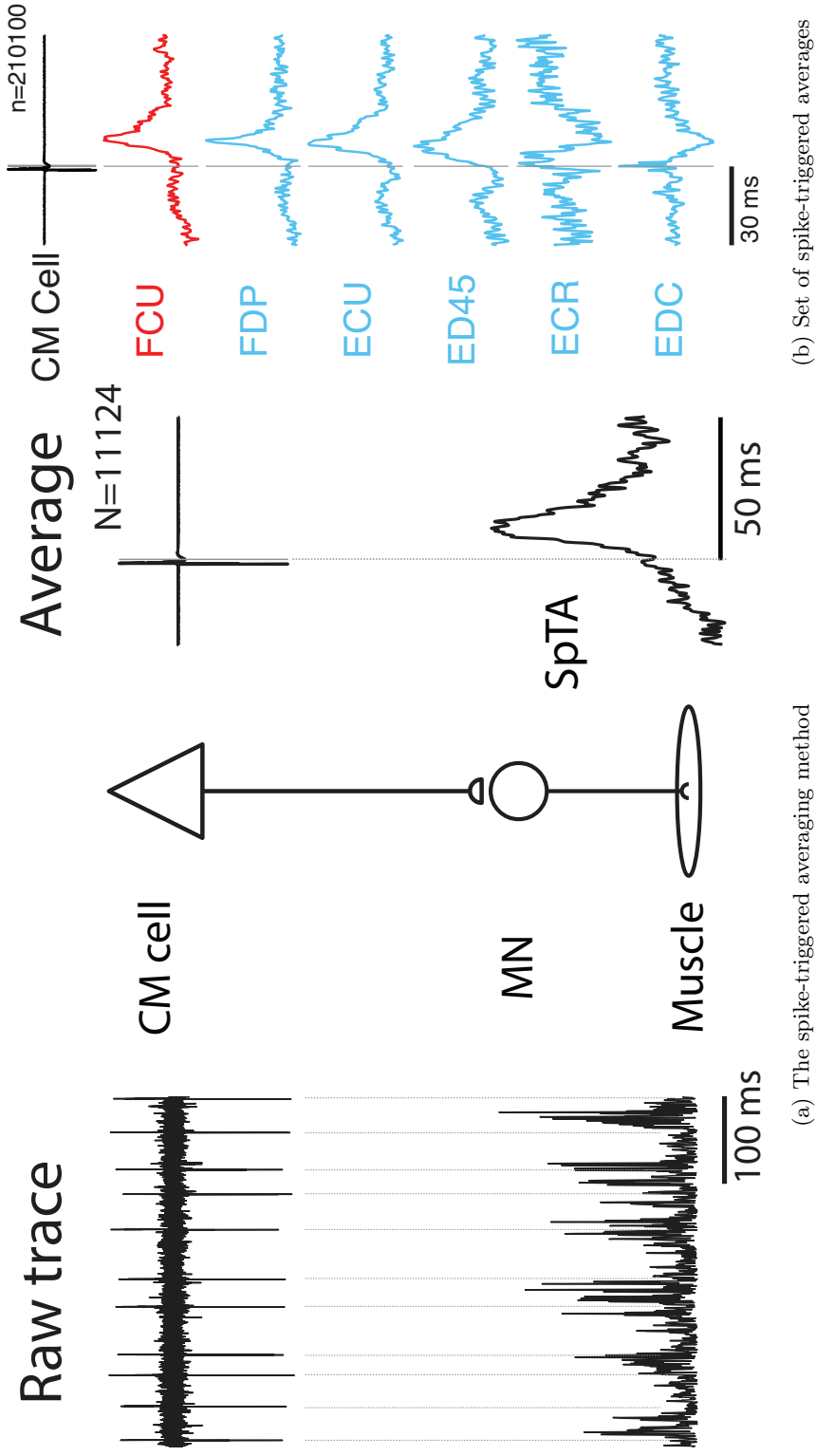


Figure 3.1: Identifying correlationally-linked cell-muscle pairs. (a): In spike triggered averaging, sweeps of rectified EMG activity are aligned around concurrent spike events. Both the cortical cell and muscles must be active to detect cell-muscle correlational linkage. (b): Correlated single unit cortical activity and EMG reveals cortico-motoneuronal linkage between motor cortex neuron and six forelimb wrist muscles.

Target muscle	PSpE type	PSpE MPI	Tuning SC coef.	Tuning NC coef.
PL	facilitation	4.84	0.66	0.20
FCU	facilitation	10.25	0.86	0.23
FDS	facilitation	2.20	0.71	0.29
ECR	suppression	-2.02	-0.63	0.15

Table 3.1: Tuning correlation coefficients and post-spike effect magnitudes between CM cell spike activity and target muscle EMG during an example FTT session. SC=signal correlation. NC=noise correlation. PSpE=post-spike effect. MPI=mean percent increase.

(flexion-extension, radial-ulnar directions) eight-target FTT is depicted in figure 3.2a. The three muscles displaying post-spike facilitation PL, FCU and FDS (figure 3.2b) were “tuned” in the flexion-ulnar direction, roughly the same as that of the CM cell from which they were facilitated. Antagonist muscle ECR is tuned in the opposite extension-radial direction; spike-triggered averaging reveals that this muscle was suppressed by the cell’s spike activity. The diagram in figure 3.2c depicts schematically monosynaptic and disynaptic linkages through which these observed effects were likely mediated. This example also illustrates divergence of projections from a single CM cell onto different motor unit pools. Table 3.1 lists tuning correlation coefficients between cell and muscle co-activity patterns for the same dataset depicted in figure 3.2. Greatest correlation occurred in target muscles with preferred directions approaching that of the upstream CM cell.

3.3.3 Muscle fields of CM cells

Each of the CM cells isolated in this study influenced the set of recorded forelimb wrist muscles in a unique manner, as revealed in SpTAs of their coactivated EMG activity. In this investigation, activity dissociations were demonstrated using a population of CM cells that had a diverse collection of muscle fields from which particular cell-muscle pairs were selected for conditioning. As revealed in subsequent figures, corticomotoneuronal dissociability appears to be a universal quality of the corticospinal system, and not selective for certain CM cells or certain forelimb muscles.

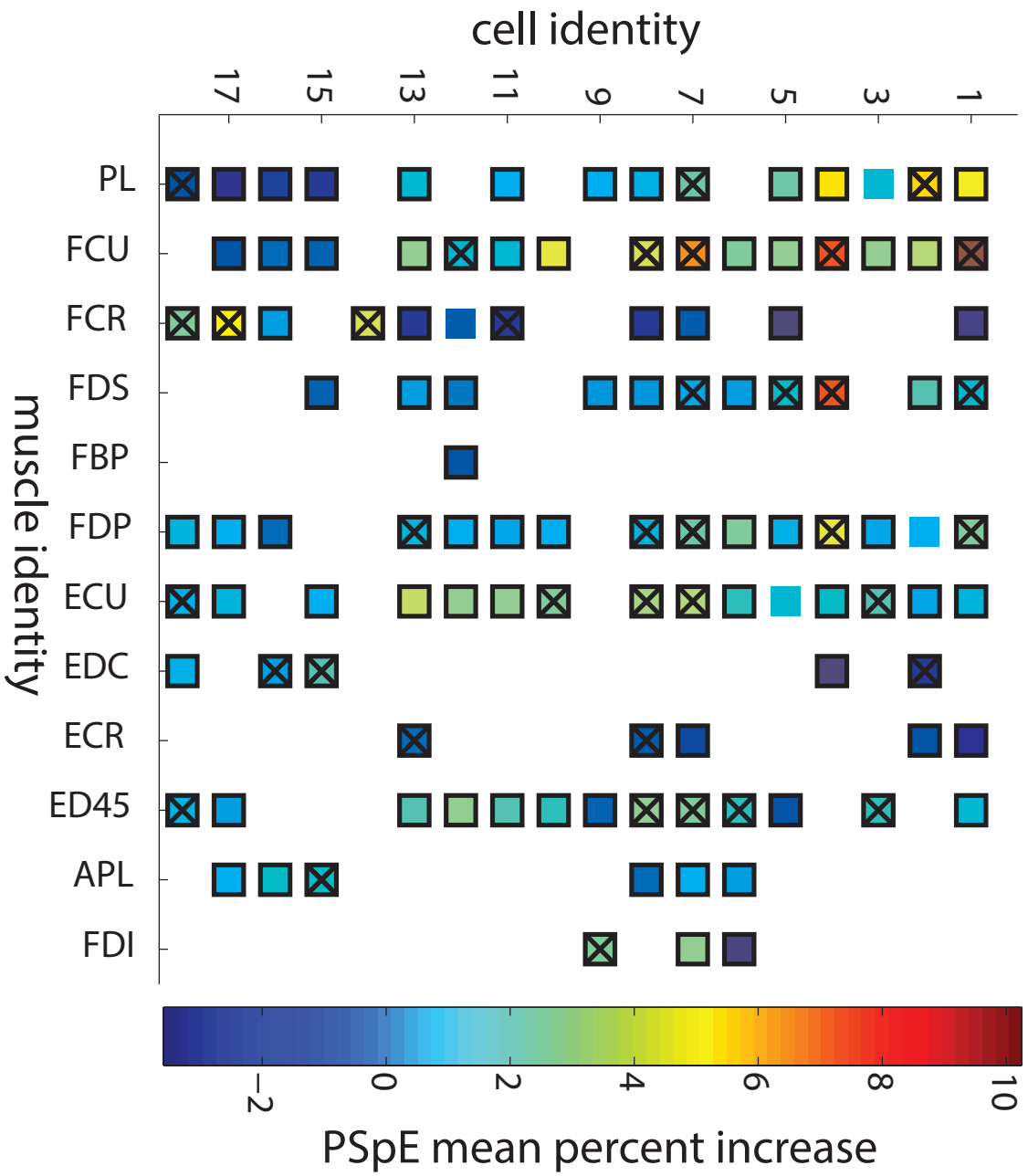


Figure 3.3: Muscle fields of CM cell population in monkey J as revealed by mean percent increases (MPIs) of post-spike effects. Magnitude of increases are coded by color and mapped by cell identity (vertical axis) and muscle type (horizontal axis). Post-spike suppressions are negative. Squares bounded by black outline mark statistically significant ($p < 0.05$) MPIs. Cell-muscle pairs selected for dissociation conditioning are indicated by 'X's overlying squares at their coordinate locations on the map. PSpE=post-spike effect.

Muscle fields of CM cells recorded from monkey J are depicted in figure 3.3 showing mean percent increases (MPIs) in SpTAs from 12 forelimb muscles compiled from 18 CM cells. This matrix shows that wrist flexor muscles FCU, FDS and FCR showed strongest correlational linkages with these cortical neurons. Flexors also showed the greatest diversity in PSpE type: FCU MPIs ranged between 10 to -2% over the population of CM cells. Among wrist extensor muscles, varied effects occur in ECU and ED4,5. Across cell differences in PL, APL and FDI PSpEs are also worth noting. Post spike facilitation predominated in wrist flexors for CM cells isolated in early experiments (cells 1 through 9). In later sessions, CM cells were more positively correlated with wrist extensors (ED4,5, ECU and EDC) and anti-correlated with FCU and FDS muscles.

Cell-muscle combinations that underwent dissociation conditioning are marked with overlaying 'X's in the plot. All muscles except FPB underwent dissociation conditioning with at least one CM cell. The same dissociation task was employed on cell-muscle combinations showing post-spike suppression (2-EDC, 8-ECR, 12-ECR, 11-FCR and 18-PL). In these pairs, the interesting task components are those where cells and muscles co-activated (C+M+) or co-inactivated (C-M-) since the two sources usually reciprocally-activated during force holds in opposite targets of the FTT task. We checked EMG signals for cross-talk between all of the twelve muscles in monkey J and the two muscle groups tested in monkey D. Significant cross-talk was detected between ECR and EDC signals in monkey J. Though signal cross-talk was observed, it did not appear to cause false detection of PSpEs which would have been indicated by frequent and similar PSpEs across ECR and EDC rows in figure 3.3. Crosstalk did not appear to compromise dissociation efficacy in any sessions for which either of the two muscles were tested.

3.3.4 Strong tuning correlation between activities of CM cells and their target muscles

In addition to detecting correlational linkages between cortical cells and forelimb muscles, we measured the correlative relationship between their force hold activities by calculating signal and noise correlation coefficients of their tuning curves. Signal correlation provides a qualitative basis for comparing the extent to which cell and muscle activities were dissociated

during performance of the activity-driven task that followed.

In the above figure, the peak in the distribution of signal coefficients approaching 1.0 indicates near-optimal tuning correlation between CM cells and their target muscles in 11 of the 44 pairs tested. The bulk of signal coefficients fell between 0.28 (25% quantile) and 0.93 (75% quantile). The distribution median is located at 0.76. Several subsets of the population of unique cell-muscle pairs shared a common CM cell—namely, cells that facilitated multiple target muscles, each of which underwent dissociation conditioning. In these cases, tuning correlation was strongest with one muscle in the field over others. The large contribution from surface EMG of extensor muscles, and also the large contribution of strongly anti-correlated values from flexor muscles, is a limitation of tuning resolution of the two-target F-E task; tuning correlation values can take values of only 1 and -1 for these instances.

Noise correlation coefficients report the amount of activity correlation that remains after the signal component, the mean response, has been removed. The comparatively narrow distribution of noise correlation coefficients is centered at 0.18 (mean and median), with standard deviation 0.15, indicates the majority of correlation between activity tuning curves is found in mean response patterns. We believe these results agree with previous work that investigated activity correlation between CM spike activity and power in rEMG of muscles comprising their muscle fields [70]: while strong correlations were detected in many pairs, considerable incongruities were observed as well. In our results, such paradoxical relationships are found on the left of the signal correlation plots in figure 3.4; strong anti-correlation was measured between CM cell-muscle pairs though statistically significant PSpF was observed in the SpTAs of corresponding target muscles. Anti-correlated tuning was observed in 10 of the 44 unique cell-muscle pairs dissociated.

3.4 Discussion

3.4.1 Relevant anatomical and physiological considerations

A small but significant proportion of pyramidal cells in the primate pre-central gyrus project monosynaptically onto spinal α -motoneuron—especially those of finger and wrist muscles

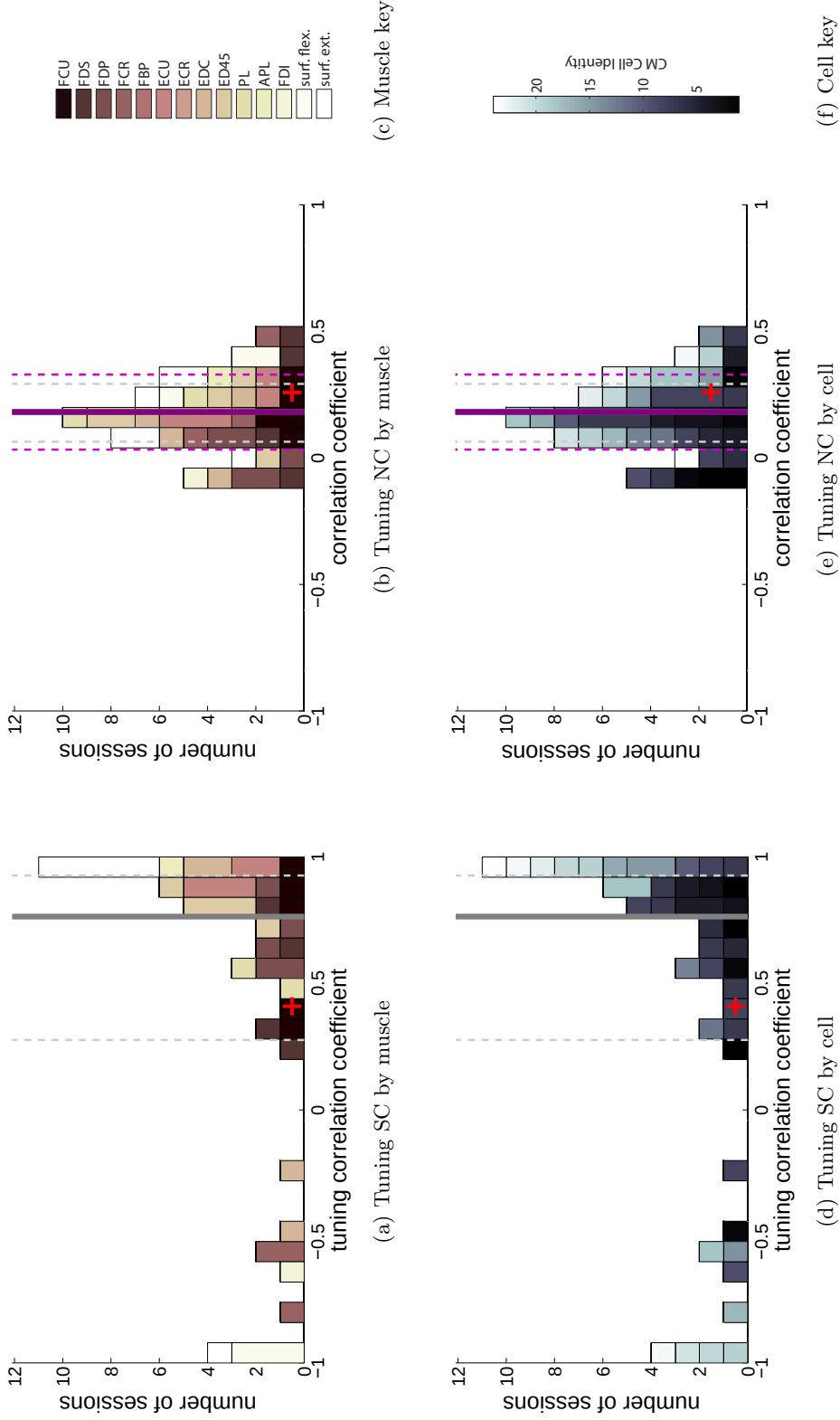


Figure 3.4: Distributions of signal (*left column*) and noise (*right column*) correlation coefficients between cell and muscle activity tuning curves generated from 44 unique pairs. The left-shifted distribution of signal correlation coefficients indicates strongly positive tuning correlation between mean activities of CM cells and their target muscles. Noise correlation contributed little as the its distribution lies near zero. Elements comprising each column are identical distributions; rows depict contribution from each of the two sources. Breakdown by muscle (*top row*) and cell identity (*bottom row*) are coded by color of boxes stacked into bars of each coefficient bin domain. Distribution median (solid grey line), 25 and 75% quantiles (dashed grey lines) characterize the heavily skewed signal correlation distribution while mean (solid purple) and standard deviation (dashed purple) are more appropriate for the symmetric, low-valued, distribution of noise correlation coefficients. Red '+'s mark coefficient values calculated from example session FTT hold activities. SC=signal correlation. NC=noise correlation.

[94, 97]. These CM cells excite motor units that drive motor output and can be identified by post-spike effects in spike-triggered averages of rectified EMG [32]. Many spike-centered sweeps of EMG must comprise a SpTA for a correlated PSpE to be detected amidst competing influence from numerous other synaptic inputs and non-correlated muscle activity. While individual CM cell spike events produce no consistent observable effect in the EMG activity of any target muscle, the arrival of a corticomotoneuronal EPSP in the motoneuronal pool can elicit the next action potential from motoneurons that are near threshold [36, 105]. An individual CM cell lacks sufficiently strong synaptic influence for its firing to depolarize to threshold, the motor units of a target muscle without additional contribution from other sources. This small synaptic contribution should permit a CM cell to spike without concurrent activation of its target muscles. It is important to note however, though the monosynaptic cortico-motoneuronal connection is relatively weak, CM cells often exhibit tight co-variation of activity with their target muscles during movement and force generation tasks (figure 3.4), [70]. Prior to this investigation, there were reports of CM cells dissociating their activity from that of their target muscles under different conditions of a force task [18, 78]. In these instances, though changes in co-activation patterns of CM cells and target muscles were observed, their activities did not directly affect reinforcement. Until this investigation, to our knowledge it was unknown if monkeys could be *operantly conditioned* to activate a CM cell independently of its facilitated muscles and thus, if deviation from their observed correlative activity patterns is subject to volitional control.

3.4.2 CM cells project onto multiple, often synergistic, target muscles

In addition to CM cell synaptic considerations stated above, morphological [109, 61] and electrophysiological [19, 66, 112] evidence suggests divergence of axon collaterals from individual CM cells onto multiple target motoneurons, often in multiple synergistic muscles. It has been argued that CM cell divergence restricts the specificity of influence of single motor cortex neurons on motor output [19]. A more recent study by Smith & Fetz (2009) detected correlational linkages between several CM cells and each of their sets of downstream target muscles, including those showing post-spike suppression in SpTAs of their rEMG

activities [112]. From these data, the authors deduced the simplest corticospinal networks that could have mediated the observed activity correlates whereby CM projections diverged onto several muscles sharing functional synergies while their antagonists received input from inhibitory spinal interneurons onto which the CM cells projected.

3.4.3 Correlated activity patterns between CM cells and their target muscles

High tuning correlation between CM cell and target muscle activities during force target-tracking provides further evidence that CM cell output encodes muscle activation parameters as reflected in EMG [43, 70]. CM cell response patterns during highly-stereotyped force holds showed synergy with the muscles to which they were linked [34]. Recent work by Davidson et. al. (2007) examining post-spike effects during different epochs of a relevant CM cell recruiting task revealed that throughput from these cells could be changed rapidly and dramatically [22]. Novel findings from this investigation—presented in the subsequent chapter (section 4)—demonstrate that, through operant conditioning of CM cell-muscle pairs, the functional role of CM cells does not necessarily remain fixed. Further, success in conditioning changes in CM cell/target muscle co-activation patterns support the hypothesis that neural representations of familiar tasks are unstable [102] and are subject to volitional control.

Chapter 4

DISSOCIATING CM CELLS AND THEIR TARGET MUSCLES

4.1 *Introduction*

In this chapter we present results demonstrating flexibility in relative activation levels of CM cells and their target muscles. Monkeys generated different modes of reciprocal activation—specifically, C+M- forward dissociations and C-M+ reverse dissociations—through operant performance of the activity-driven dissociation task. The extent of dissociation between CM cell firing rates, and concomitant power in target muscle EMG signals, were evaluated by measuring the separation between the C+M- and C-M+ task component co-activity distributions. In addition, we measured their locations with respect to their co-activation levels produced during the C+M+ dissociation task component as well as with respect to their co-activities during force holds inside a chosen target of the FTT task. Dissociation task reciprocal activity patterns that differed significantly from co-activation levels support our claim that CM cells can be operantly conditioned to activate independent of muscles to which they are correlationally linked. The extent of conditioned dissociations was measured by comparing domains of dissociation task components in cell-muscle activity scatter plots, calculating the Kullback-Leibler divergence between activity distributions from the dissociation and FTT tasks as well as calculation of a “dissociation index” designed to quantify relative activity dissociation between the CM cell and target muscle sources.

4.2 *Methods*

4.2.1 *Dissociation conditioning setup*

During each recording session, following wrist force target-tracking during which SpTAs were compiled and PSpEs identified, a spike-correlated muscle was chosen to undergo dissociation conditioning. As depicted in figure 4.1, cursor control was switched so that cell and muscle activity controlled its position on the screen, rather than wrist torque, and a different

target display served as a discriminative cue for the dissociation task. Spike trains and rectified EMG were transformed into suitable control signals by routing them through low-pass filters. Target position on the computer screen indicated relative cell-muscle activities to be reinforced: co-active (C+M+), reciprocal activation (C+M-, C-M+) and co-inactive (C-M-) targets were presented in random sequence. Initially, the four targets appeared at equal frequency, but during some sessions, we increased presentation frequency of more difficult targets. Cursor travel on the screen was adjusted to match the dynamic range of cell spike rate and baseline-to-contraction EMG amplitude. As with FTT, monkeys received fruit sauce rewards for holding the cursor in targets for some minimum hold time. Hold times were initially set at 400 ms and gradually titrated up to a maximum of 1 second over the course of the conditioning session.

Cursor rest position and directions of deflection were selected based on the FTT tuning of EMG from the particular muscle selected for conditioning. For example, if FCU was chosen and its preferred direction pointed between FU and U targets, we set the muscle activity axis to the downward vertical direction—parallel to the larger of the two components comprising the tuning vector—and the cell activity axis to the left-going horizontal direction. We did this to preserve, at least partially, the muscle activation-to-cursor deflection mapping to which monkeys were accustomed during the FTT task. When both cell and muscle activities were low (C-M-), the cursor would occupy the upper-right portion of the screen in this example while C+M-, C-M+ and C+M+ targets would be positioned in the top-left, bottom-right and bottom-left quadrants of the screen respectively. We set up all dissociation sessions in this manner, using the specific EMG tuning direction of the selected muscle, for all cell-muscle pairs conditioned.

4.2.2 *Quantitative analysis*

For each CM cell-target muscle pair, we used the cell and muscle activity averages during the dissociation task co-activation (C+M+) holds to normalize corresponding single-trial activities during dissociation task and FTT in-target holds. Specifically, activity sweeps during holds in C+M+ targets were first integrated over -500 to 0 ms relative to each trial

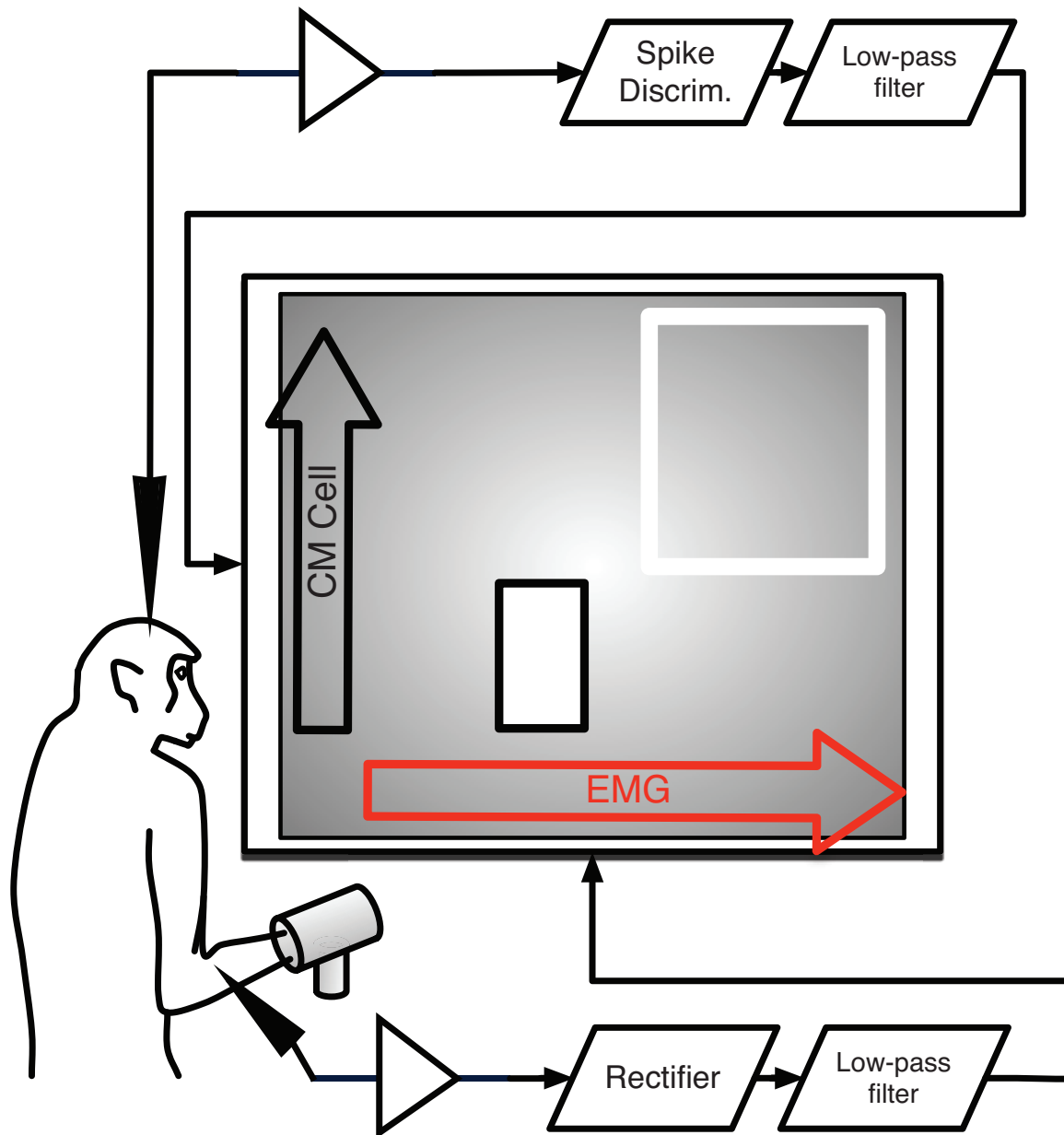


Figure 4.1: During the dissociation task, cursor position was driven by concurrent cell and muscles activities. Spike trains from a CM cell and rectified EMG from a chosen target muscle were low-pass filtered into suitable control signals. Target position on the computer screen indicated the dissociation task component to be reinforced—specifically C+M+ for the target pictured above. The C-M- target would be placed in the lower left corner of the screen, while the upper-left and lower-right corners would contain reciprocal activation targets C+M- and C-M+ targets respectively.

completion event at $t = 0$ ms. The resulting two sets of activity scalars—one of single trial cell activities, the other single trial muscle activities—were then averaged across trials to form the particular pair of normalization factors specific to that cell-muscle pair. Normalizing both target-tracking and dissociation activities in this fashion permits: 1) single-trial activity coordinates to be plotted in a homogeneous two-dimensional space in which relative distance can be measured, and 2) dissociations from different cell-muscle pairs to be compared statistically. Two-dimensional coactivity distributions from the four dissociation task components were plotted on these normalized muscle and cell activity axes for statistical analysis and visual interpretation.

4.2.3 Dissociation index

We devised a simple scalar dissociation index (DI) to measure relative activity dissociation between CM cells and their target muscles. The DI is the logarithm of the ratio of concurrent cell and muscle activation levels expressed as

$$DI_i = \log \left(\frac{c_i}{m_i} \right) \quad (4.1)$$

where c_i and m_i denote cell and muscle activities on a given trial i . The ratio of cell to muscle activity is placed inside a logarithm so that resulting DI values scale symmetrically over a linear axis. Though simple to calculate and easy to interpret, the index is a relative measure only; it conveys no information about the absolute magnitude of activities examined. For this reason we delegated the DI measure to be secondary to scatter plot analyses in which information about activity magnitudes is retained.

4.2.4 Kullback-Leibler divergence

The Kullback-Leibler divergence is an information theoretic measure of relative entropy between two probability distributions that measures information lost when one distribution Q , is used to approximate another, P . The measure can be interpreted as the inefficiency of assuming the distribution is Q when the true distribution is P [21]. For our case, test distribution Q is taken to be the FTT task co-active distribution (expected prior) while

each of the other dissociation task distributions (C+M-, C-M+, C+M+ and C-M-) are the actual P distributions from which dissociation values are drawn. In addition, we also calculated the Kullback-Leibler divergence (in bits) between C+M- and C-M+ reciprocal activation groups. Unlike other dissociation metrics used in this study, the Kullback-Leibler divergence does not depend on distribution means.

4.2.5 Significance testing

Fisher-Pitman permutation tests approximated using Monte Carlo methods were selected to test for statistically significant differences in co-activity patterns between dissociation task and FTT groups. The approximate approach has been shown to produce reliable estimates of exact permutation test values in most instances provided the number of reorganizations is sufficiently large, $\sim 10,000$ [65]. We employed this test rather than the more familiar Student's paired-t because: 1) the distributions being tested are two-dimensional, so a multivariate significance test must be used and 2) the co-activity distributions being evaluated are not necessarily normal, and for some sessions, not sufficiently sampled to pass tests to verify normality. The permutation test has no distribution shape requirement, can be coded to accommodate multivariate distributions, and their test distributions need not have equivalent numbers of samples. As a test of means, the Fisher-Pitman permutation test is most robust when homogeneity of variances is observed [79]—a condition that is not necessarily met for activity groups tested in this investigation. Our use of the permutation test is still valid, however, since the sample sizes of distributions being tested are sufficiently large and roughly equivalent [11].

4.3 Results

4.3.1 Monkeys can alter relative activation levels of CM cells and their target muscles

Having evaluated correlational linkages between a CM cell and forelimb wrist muscles, a particular muscle showing post spike facilitation was then selected for dissociation conditioning. Successful performance of the dual-activity driven task required dissociation of CM cell spike activity from simultaneous EMG recorded from the selected target muscle.

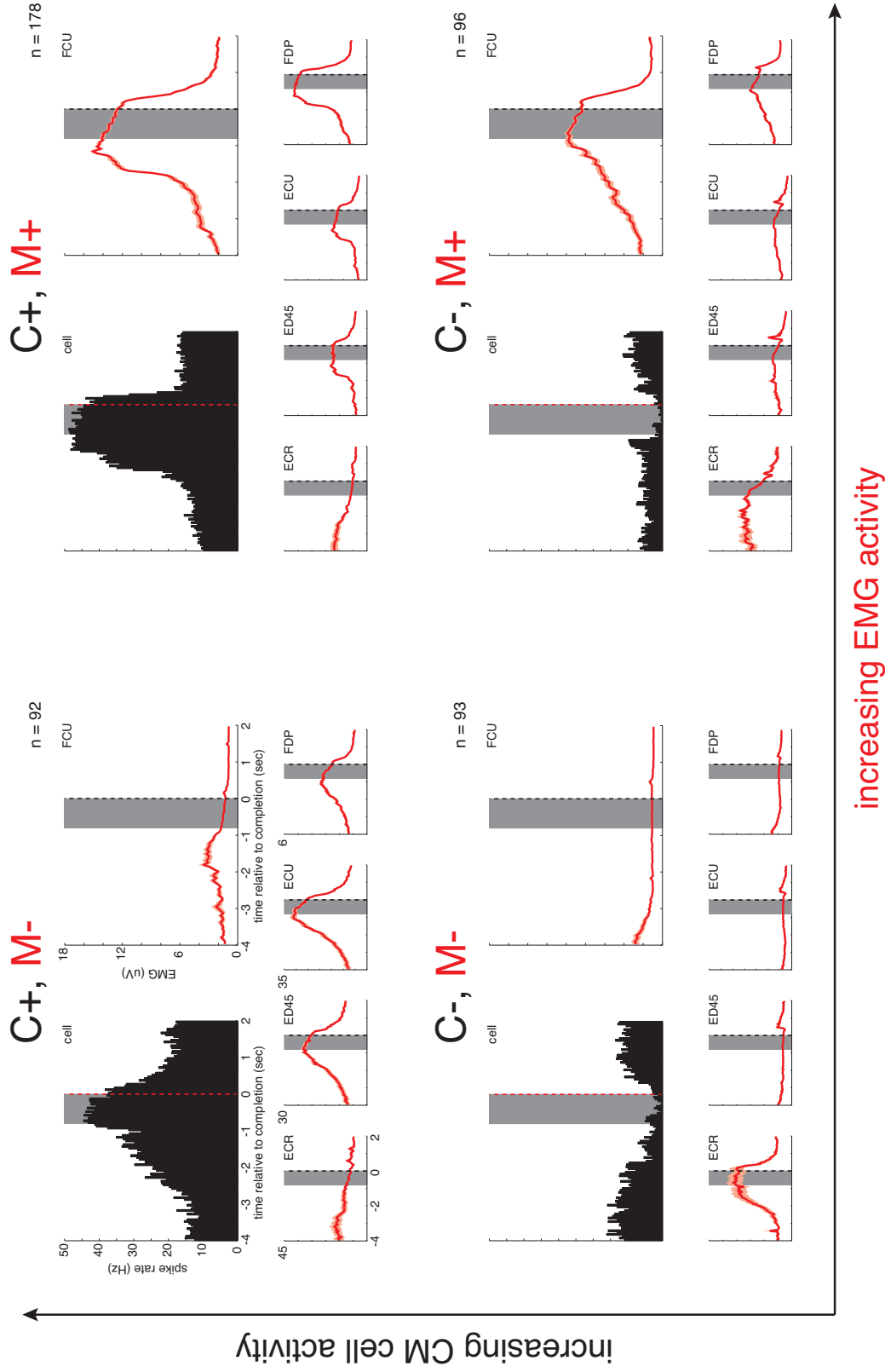


Figure 4.2: Peri-event spike histograms (black, upper left in each group) and wrist muscle EMG averages (red, upper right and bottom row in each group) and wrist muscle EMG sweeps (pink shading) from an example dissociation conditioning session (monkey J). Spike trains and EMG sweeps were aligned at target completions during the activity-driven step-tracking task. For this session, vertical gray bars show the minimum required hold times of 800 ms prior to target completion. In-target activity averages illustrate: co-activation, C+M+ (top right), forward-dissociation C+M- (top left), reverse-dissociation, C-M+ (bottom right) and co-inactive, C-M- (bottom left) patterns between the CM cell and the conditioned target muscle FCU. Activities of other muscles comprising the CM cells muscle field are shown in the bottom row of each task component group.

Figure 4.2 shows averages of CM cell spike trains and target muscle rEMG during performance of the activity-driven dissociation task. In this typical session, peri-event spike histograms and event-triggered averages of rectified EMG are aligned by target completion. These task events required the cursor to be held in the on-screen target domain for at least 400 ms. In this figure, peri-event activity averages are positioned to reflect corresponding target location on the screen during task performance, horizontal axis from left to right codes increasing muscle activity while the increasing vertical axis maps increasing cell activity. These target positions reflect the concurrent cell and muscle activity levels required for trial completion and subsequent reward: co-activation (C+M+), forward-dissociation (C+M-), reverse dissociation (C-M+), and co-inactive (C-M-). Though statistics are reported below, initial inspection of these averages reveal markedly different relative activation levels for the CM cell and its target muscle during the four components of the dissociation task. In this example, monkey J performed more than 90 trial completions of each of the four parts of the cell-muscle activation task.

Target muscle	PSpE type	PSpE MPI	Tuning SC coef.	Tuning NC coef.
FCU	facilitation	4.23	0.41	0.26
FDP	facilitation	1.94	0.64	-0.02
ECU	facilitation	3.55	0.93	0.28
ED45	facilitation	3.39	0.77	0.22
ECR	suppression	-0.82	-0.35	-0.23

Table 4.1: PSpE and tuning correlation values for the cell-muscle configuration in figure 4.2.

This cell was maximally active during force holds in the ulnar direction. Table 4.1 lists the type of post spike effect observed and tuning correlation values computed from FTT task recordings of the same cell-muscle configuration depicted in figure 4.2. As shown in additional EMG averages, non-conditioned target muscles also varied their levels of activation during the dissociation task. Muscle ECU, ulnar agonist of FCU, as well as flexion-agonist FDP, were active during C+M+ and C-M+ alongside the conditioned muscle. Interestingly,

they were also recruited during C+M- components when FCU activity was suppressed. Radial antagonist ECR appreciably activated during C-M- and C+M- trials, when FCU activity was suppressed, and remained quiet during co-active and forward dissociation trials when FCU was active. These dissociation task recruitment patterns of non-conditioned muscles were also observed in a subsequent dissociation session using this same cell and muscle ECU (see section 4.4.1).

4.3.2 Quantifying activity dissociations in CM cell-muscle pairs

The primary aim of this investigation is to explore the extent to which relative activation of CM cells and their target muscles is flexible both absolutely and as compared to non-conditioned, “natural” co-activation patterns observed during force generation. We employed several measures to characterize the relationship between task co-activity patterns.

Groups measured	Kullback-Leibler divergence (bits)	p-value
C+M-, C-M+	48.48	<0.005
C+M-, FTT(U)	15.60	<0.005
C-M+, FTT(U)	22.19	<0.005
C+M+, FTT(U)	10.89	<0.005
C-M-, FTT(U)	12.90	<0.005

Table 4.2: Kullback-Leibler divergence between normalized cell-muscle activity distributions of the example session plotted in figure 4.3.

We plotted single-trial target-tracking and dissociation task co-activities (figure 4.3a) in two-dimensional space in units of normalized muscle activity along the x-axis and normalized cell activity along the y-axis. These coordinate distributions, and their means, are coded by color, indicating the component of the dissociation task to which they belong. Single-trial distributions plotted here are from the same conditioning session as the averages portrayed in figure 4.2. Correlational linkage for this particular cell-muscle pair is shown in the SpTA for FCU in figure 3.1b. The four dissociation task distributions (C-M-: cyan, C+M-: yellow, C-M+: red and C+M+: blue) all differed significantly from the co-activation levels

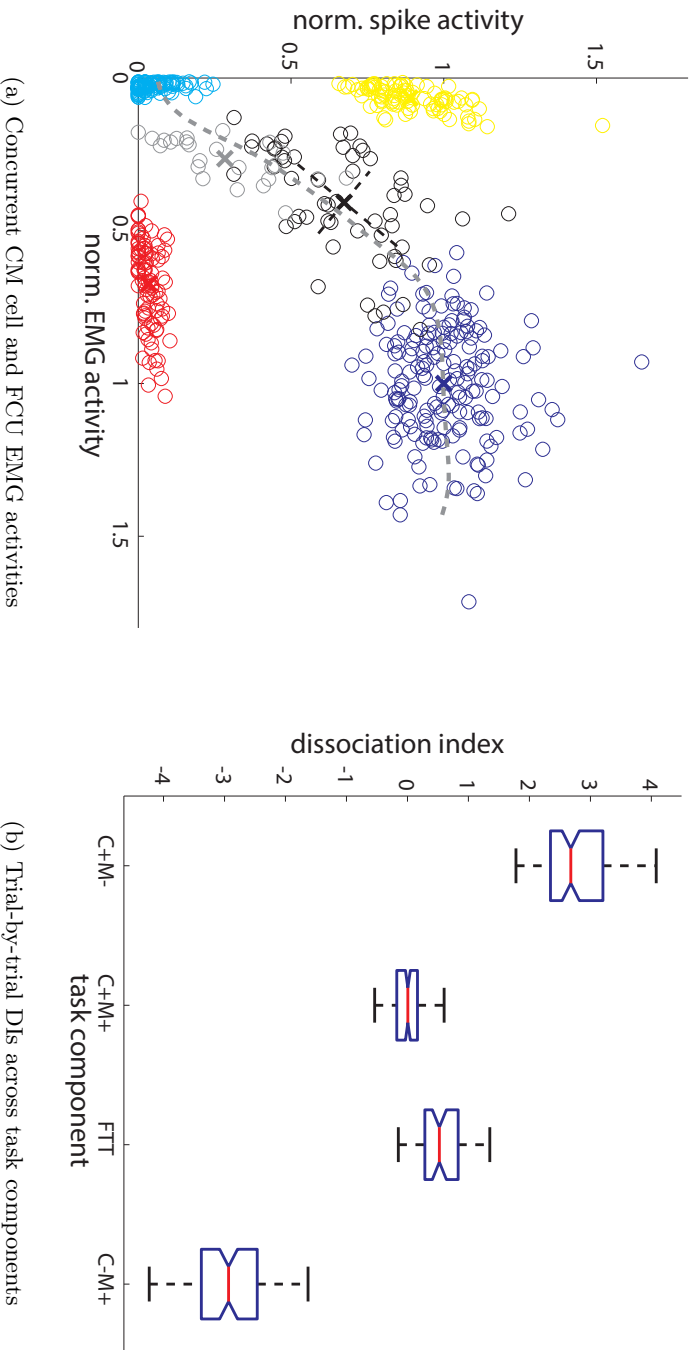


Figure 4.3: (a): Distribution of concurrent CM cell and FCU EMG activities during both force and activity-driven step-tracking trials (monkey J). Activity means (circles) were calculated by integrating over the target hold time prior to trial completion. X's mark distribution means and the lengths of arms depict standard deviation in each dimension. Dark blue circles and crosses depict concurrent cell and muscle activation when the cursor was inside the C+M+ target and cyan codes for the co-inactive (C-M-) task epochs. Reciprocal cell-muscle activations are shown during forward-dissociation (C+M-, yellow) and reverse dissociations (C-M+, red). The black and gray distributions show concurrent cell and muscle activities during the force holds inside respective U and FU targets—the two targets for which the cell and muscle were observed to be maximally co-active in the FTT task. Note that some trials revealed greater dissociation than their distribution averages. The muscle and cell activities are normalized by respective means calculated from the C+M+ dissociation task target holds as indicated by unit-less axes.

(b): Trial-by-trial dissociation indices across FTT and dissociation task components. Dissociation indices calculated for each trial have been grouped by task component. Box plots characterize the four distributions shown (C+M-, C+M+ FTT and C-M+): red lines mark distribution medians and vertical extremities of surrounding blue boxes plot 25% and 75% quantiles. Vertical whiskers indicate maximal values within 1.5 times the interquartile range from the edge of the box. Notches display the confidence interval of the median between samples.

during ulnar (U) target force holds (black) as measured by distance-separating-means two-dimensional permutation tests (see section 4.2.5), for which all p-values $<10^{-4}$. Distances separating the FTT group mean from the C+M- (0.41) and C-M+ (0.69) means reveal that the monkey could alter the relative activation levels of this CM cell and its correlationally-linked target muscle well outside of the variability of co-activation patterns observed during force generation in the U target, whose standard deviation is depicted as crossed dashed lines in figure 4.3a. Cell-muscle co-activation patterns for all FTT task targets are plotted in a subsequent section 5.3.1. For many C+M- trials, EMG of the target muscle remained at rest while the correlated CM cell activated at levels exceeding its average during force target-tracking. Conversely, during the C-M+ task epochs, the same CM cell could remain completely inactive during the target hold period while EMG of the correlated muscle surpassed its target-tracking mean. These findings support the hypothesis that relative activation levels of CM cells and their monosynaptically-linked motor units are flexible and subject to modification through behavioral conditioning.

Alternate measures for quantifying cell-muscle dissociation were also employed in this study. Parenthesized values listed in this paragraph are those calculated for the session depicted in figures 4.2 and 4.3 with p-values from corresponding significance testing listed after where appropriate. These included: 1) Measuring distances separating the C+M+ dissociation task mean from the C+M- mean (0.94, $p < 10^{-4}$) and C-M+ mean (1.02, $p < 10^{-4}$). 2) Smoothing force target-tracking data into an approximate cell-muscle co-activation function (gray dashed line) and then measuring how far the C+M- group deviated for comparable cell activation (difference 0.57, 90% deviation) and how far the C-M+ group deviated for comparable muscle activation levels (difference 0.89, 96% deviation). 3) Calculating the Kullback-Leibler divergence (in bits) between C+M- and C-M+ reciprocal activation groups as well as relative to the FTT co-activity distribution (table 4.2). 4) Calculating the mutual information (in bits) of all co-activity distributions (table 4.3) to measure the mutual dependence of concurrent cell and muscle activity patterns during the dissociation task as compared to activities from the same pairs during force holds. The value calculated for the C+M- component is greater than that for the FTT group; this instance departs from the C+M- session population trend in which mutual information declines compared to FTT

activities (figure 5.3). The p-values listed here arise from bias-correction (see section 5.2.1); those less than 0.05 indicate the calculated mutual information value was distinguishable from noise and could be corrected.

Task comp.	Mutual Information (bits)	p-value
C+M+	0.010	p = 0.78
C-M-	0.089	p = 0.10
C+M-	0.489	p <0.001
C-M+	0.269	p = 0.005
FTT(U)	0.322	p <0.001

Table 4.3: Mutual information between concurrent cell and muscle activities for each task component of the example session plotted in figure 4.3a.

4.3.3 *Rapidity and reversibility of CM cell-muscle dissociations*

During dissociation conditioning, targets eliciting concurrent C-M-, C+M-, C+M+ and C-M+ activity patterns were presented in random order following each return to co-inactivity event that separated successive trials. Session time was not divided into distinct blocks devoted to each task component. This format required monkeys to (usually) alter the correlative relationship between activities of CM cells and target muscles to acquire the next target presented. Despite this added difficulty compared to blocked trials, monkeys were able to rapidly switch between all possible concurrent activation levels required by the four-target dissociation task (figure 4.4). In all sequences of the example session, the monkey was observed to transition within 4 seconds and often less than 2. It is important to note that a between-trial minimum of 0.5 second duration imposed a lower bound on these transition times. Because shifts in correlative relationship were observed to change on the order of seconds, we attribute observed activity dissociations to arise from relative changes in activation of existing pathways that converged onto motoneuron pools.

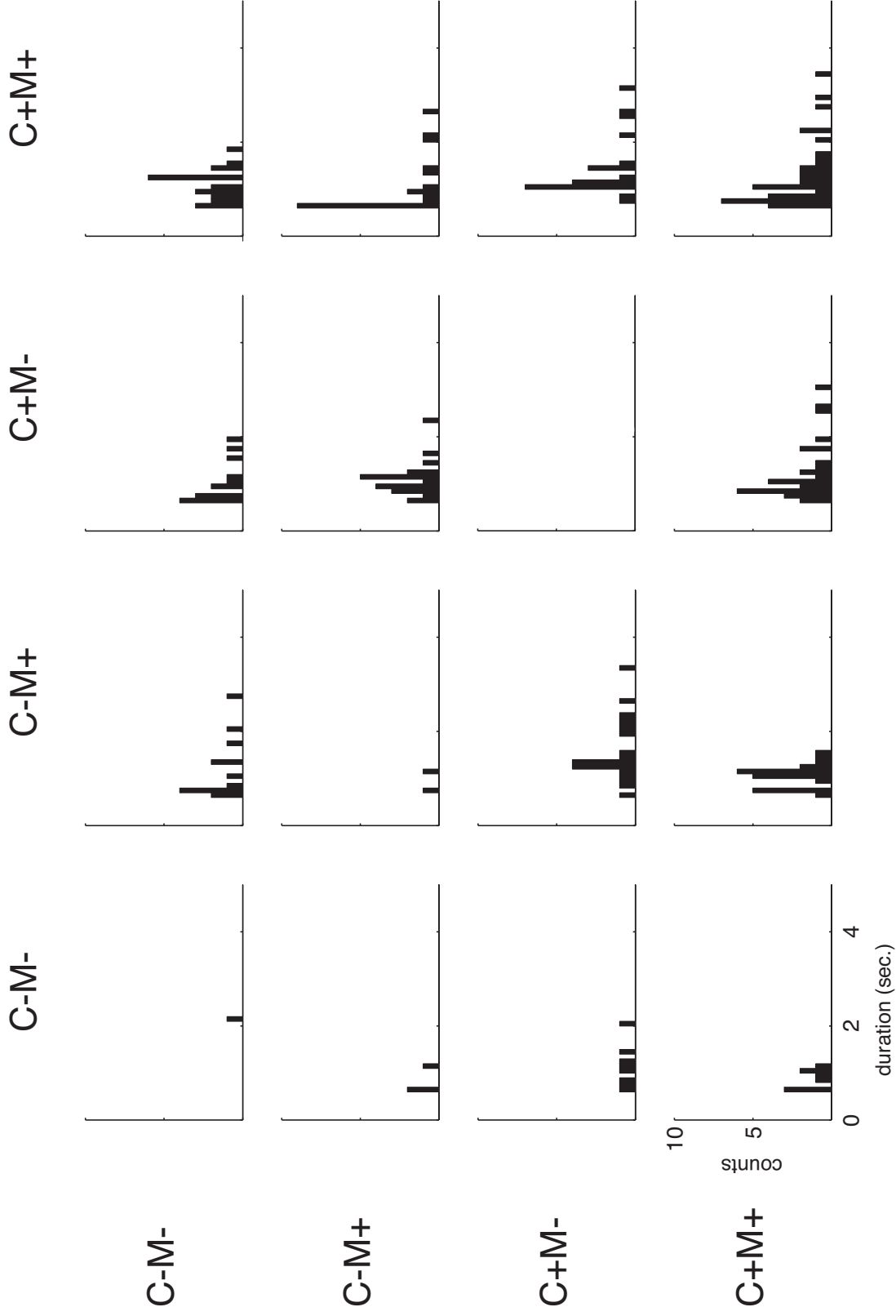


Figure 4.4: Monkeys can rapidly shift between all possible concurrent activation states of CM cells and target muscles. We measured durations spanning each completed trial event to the time at which the cursor first entered the peripheral target of the next trial. Since target presentation was randomized, monkeys were required to transition between 16 possible two-component sequences comprised of the four task components (C-M-, C-M+, C+M- and C+M+) over the course of each conditioning session. Histograms of transition duration are ordered in matrix form above: rows code identity of the completed target while destination targets are ordered by column.

4.3.4 *Activity dissociations for CM cell-muscle combinations in both monkeys*

Dissociation conditioning, force target-tracking and their analyses described in the above example (figure 4.3) were repeated for 57 total sessions across two monkeys. Conditioning sessions from 44 unique cell-muscle pairs were selected for population analysis. Selected pairs were linked by statistically significant post-spike facilitation effects (35 in monkey J and 9 in monkey D). Twenty-four CM cells ($n = 18$, monkey J and $n = 6$, monkey D) were each identified through spike-triggered averaging of rectified EMG during performance of the wrist-force target-tracking task. Out of the 44 dissociation conditioning attempts on unique cell-muscle combinations, only two failed to yield significant reciprocal activation patterns in either direction—one unsuccessful session per monkey. During three recording sessions, target muscles did not co-activate with their CM cell during any target holds of the force target-tracking task. These occurred for FDI, a muscle probably not mandatory for successful performance of the FTT, and for two muscles that maximally activated in directions opposite to the preferred direction of their CM cell in spite of their positive correlational linkage established via spike-triggered averaging. Again, these instances were rare; occurring in only 2 out of 44 cell-muscle combinations tested.

We repeated dissociation conditioning for many CM cell-muscle pairs to assess the ubiquity of the activity dissociation phenomenon. Though the extent of dissociations varied across the population, statistically significant deviations in cell-muscle co-activity patterns were observed in the 95% of conditioned pairs.

For the population of unique cell-muscle pairs exhibiting post-spike facilitation, we plotted session activation means along coordinate axes of normalized cell and muscle activities for each of the five task components. Means from each component occupied mostly distinct domains of the cell-muscle activation space depicted in figure 4.5a. The distribution of means from the C+M- task component (yellow) lies substantially displaced from the domain of FTT co-activation levels (black). Their difference in location is statistically significant, as indicated by distance-separating-means permutation test (table 4.4). Similarly for reverse dissociations (C-M+), the distribution of session means also differed significantly from the force target-tracking group using the same test for significance. Non-significant dissocia-

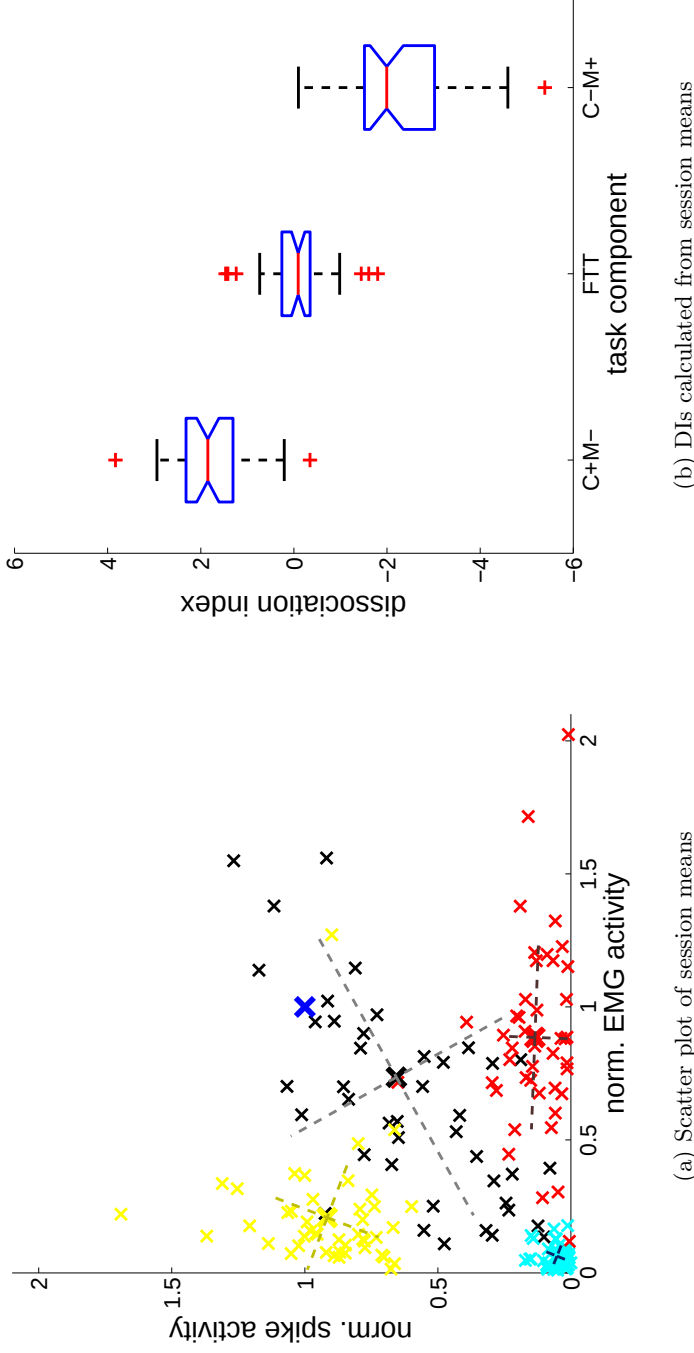


Figure 4.5: (a): Scatter plot of session means. Dissociation task and force target-tracking task session means color-coded by task component: FTT (black), C-M- (cyan), C+M- (yellow) and C-M+ (red). All activities were normalized by C+M+ dissociation task means (blue)—hence the lack of variability in the C+M+ across-session group located at (1,1). Averaged co-activity coordinates of all sessions are depicted as large 'X's for each task component and dashed lines illustrate standard deviation of each distribution along its primary and secondary principal axes. Data from 44 unique cell-muscle combinations from two monkeys comprise the above scatter plot. All pairs showed post-spike facilitation in spike-triggered averages of EMG with statistically significant mean percent increases between 6 and 16 ms following spike occurrence. Two outliers of the FTT set, located at (2.05, 2.69) and (3.51, 0.580), lie outside the boundaries of the above plot.

(b): Dissociation indices calculated from the session population of activity means. Index values calculated from session means are pooled into distributions categorized by FTT and reciprocal dissociation task components. As in figure 4.3b, box plots characterize the three DI distributions depicted: C+M-, FTT and C-M+. Red '+' mark values outside of 1.5 times the inter-quartile range.

tions from the two sessions are evident as yellow and red ‘x’s in the midst of FTT and C-M- coordinate clouds (see section 4.3.6). Session time constraints and lapses in subject motivation could have contributed to these rare failures to elicit reciprocal activation patterns (see section 4.4.2).

The distribution of session activity means during the C+M- task component (yellow) lies substantially displaced from the domain of FTT co-activation levels (black). Their difference in location is statistically significant, as indicated by distance-separating-means permutation test (table 4.4). Similarly for reverse dissociations (C-M+), the distribution of session means also differed significantly from the force target-tracking group using the same test for significance.

Task comp.	$\vec{\mu}$	σ^{1°	θ^{1° (deg.)	σ^{2°	θ^{2° (deg.)	p-value
FTT	(0.736, 0.656)	0.595	29.3	0.455	119.2	-
C+M+	(1.00, 1.00)	-	-	-	-	$<10^{-4}$
C+M-	(0.021, 0.916)	0.210	-20.7	0.208	69.6	$<10^{-4}$
C-M+	(0.885, 0.135)	0.346	-2.4	0.123	87.7	$<10^{-4}$
C-M-	(0.006, 0.005)	0.004	67.7	0.004	157.8	$<10^{-4}$

Table 4.4: Mean and variability of session activity means grouped by task component.

Among the five task components tested, concurrent cell-muscle activities during FTT trials varied most across sessions compared to C+M- and C-M+ dissociation task components (table 4.4). Values denote standard deviations (σ) along distribution primary (1°) and secondary (2°) principal axes, at angles (θ) with respect to the x-axis, as depicted as dashed lines in figure 4.5a. Greater intersession variability of the FTT task component reflects that, within a given session, absolute activity levels of C+M+ and FTT groups often differed substantially as no attempt was made to match them. Because the C+M+ component mean was used to normalize all activity values in a given session, all C+M+ session means are located at (1, 1) in the above scatter plot.

4.3.5 *Dissociation efficacy is unrelated to muscle group and CM cell identity*

To summarize observed activity dissociations across the population of conditioned CM cell-muscle pairs, we calculated single-valued measures, each characterizing a particular quality of the co-activity scatter plot compiled for each dissociation session. These resulting values from all unique cell-muscle pairs were pooled and binned into histograms (figure 4.6). Comparing population statistics to optimal idealized values permits the extent of cell-muscle dissociation to be quantitatively analyzed. We reported the identities of cells and muscles comprising parameter distributions to examine if any overly contributed to any value range—that is, if robust distributions were more likely for some cells or muscles than others.

To evaluate activity dissociation across the population of 44 unique CM cell-muscle combinations, we calculated three measures to summarize dissociation efficacy for each session, then pooled these values into distributions (figure 4.6). An idealized model dissociation session would have an C+M- activity coordinate cloud centered near (0, 1) and the opposite reciprocal activation distribution, C-M+, located near (1, 0). Variability along the inactive coordinate axes (muscle axis for C+M- and cell axis for C-M+) would be minimal. In this ideal case, the distance separating the two distributions should approach $\sqrt{2} \approx 1.414$ and the angle of separation with respect to the positive-going x-axis is $-\frac{\pi}{4} \approx -0.785$. The angle of separation measures dissociation symmetry. Calculating the Kullback-Leibler divergence on simulated distributions with these parameters yielded values between 35 and 45, depending on the variability of the simulated distributions. Strong examples of reciprocal cell-muscle activation comprise the right tails of distance separating means and K-L divergence distributions and values greater than $\sqrt{2}$ resulted from sessions where cell activation in C+M- groups and/or muscle activation in C-M+ activities surpassed corresponding C+M+ levels.

A frequency histogram illustrates the distribution of distances separating reciprocal activation groups (C+M- and C-M+) across sessions (figure 4.6a and 4.6e). Distribution mean and standard deviation are listed in table 4.5, as well as median, 25% and 75% quantile parameters that more accurately characterize this non-symmetric distribution. The angles of vectors separating these reciprocal activation groups were more symmetrically distributed (middle column) with nearly overlapping mean and median values that fell very close to the

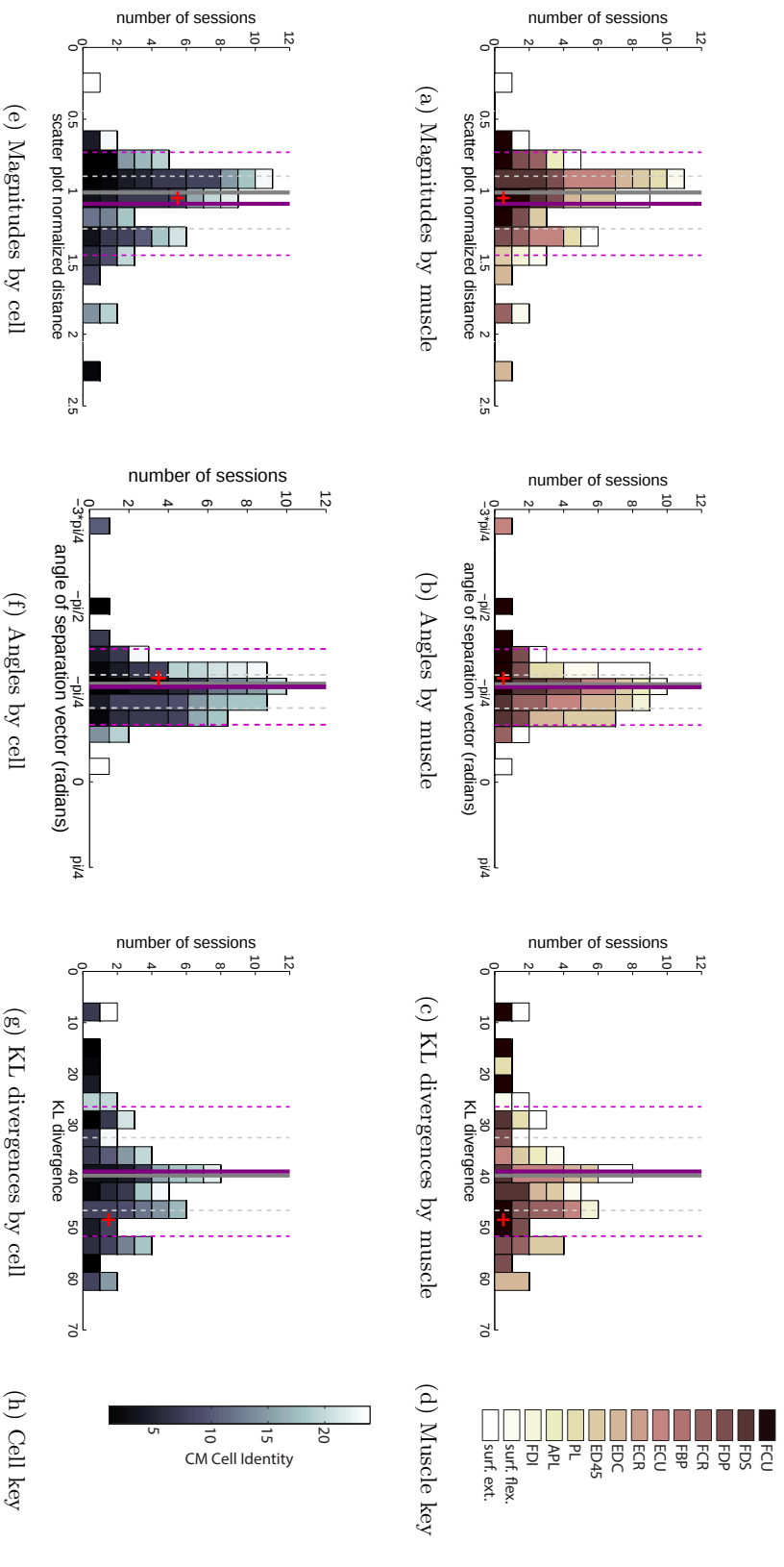


Figure 4.6: Dissociation measures across sessions. Task outcomes from 44 dissociation sessions binned into frequency histograms measuring: the magnitude of vectors spanning between C+M- and C-M+ group means (*left column*), the angle of these vectors with respect to the x-axis (*middle column*) and the Kullback-Leibler divergence separating these same C+M- and C-M+ activity distributions (*right column*). Elements comprising each column are identical distributions; rows depict contribution from each of the two sources. Contributions by muscle (*top row*) and cell identity (*bottom row*) are illustrated using color-coded regions stacked within bars. Color guides for these are located at the far right of each row. Standard deviations (purple dashed) about means (purple solid) as well as medians (gray solid) and 25% and 75% quantiles (gray dashed) depict parameters characterizing these distributions measured from the population of unique cell-muscle combinations conditioned. Red crosses mark values measured from the example session depicted in figures 4.2 and 4.3.

idealized angle of $-\frac{\pi}{4} \approx -0.785$ considering distribution variability (S.D. = 0.347). Distant outliers located at the far-left and far-right of the histogram will be addressed in the next section. Kullback-Leibler divergences were also symmetrically distributed as indicated by nearly overlapping mean and median values and nearly equidistant 25% and 75% quantile values about the distribution median (table 4.5).

Measure	mean $\pm$ S.D.	median	25% quant.	75% quant.
Sep. vector magnitude	1.090 $\pm$ 0.359	1.012	0.898	1.265
Sep. vector angle (rad.)	-0.871 $\pm$ 0.347	-0.897	-0.982	-0.671
K-L Divergence	39.06 $\pm$	39.85	32.47	46.67

Table 4.5: Parameters characterizing separation between reciprocal activation components (C+M- and C-M+) from 44 sessions.

The top row of histograms depicted in figure 4.6 contain color-coded regions within bars illustrating the contribution each muscle made to the overall sum within each binned domain. For bars contained within one standard deviation of the mean, no single muscle group contributed more than half to its total sum. This was the case for all three dissociation measures examined. Muscles that were conditioned in multiple sessions (each session with a unique CM cell) took different values throughout each of the three distributions. Examples of this can be seen for FCU, FDP and ED4,5 muscle types. The above statements regarding non-specificity of dissociation efficacy across muscle type similarly hold across the population of conditioned CM cells as illustrated in the bottom row of figure 4.6.

4.3.6 *Unsuccessful conditioning attempts are outliers in session population*

The smallest separation value in the distance-separating-means distribution (figures 4.6a and 4.6e, leftmost value: 0.179), the near zero angle of separation in the histogram (figures 4.6b and 4.6f rightmost value: -0.06 radians) and one of the three values comprising the left-hand tail of the K-L divergence distribution (figures 4.6c and 4.6g, 6.250) were each calculated from a session whereby neither C+M- nor C-M+ activities deviated significantly from FTT task co-activation levels (cell 24, surface EMG extensors, monkey D). The C+M-

and C-M+ activity means from this failed session reside inside the black FTT task cloud in figure 4.5a, closest to the distributions center of mass. In the opposite extreme, the leftmost value of the angle of separation distribution (figures 4.6b and 4.6f, leftmost value: -2.420 radians) marks the result of the other session in which reciprocal activation patterns were not demonstrated (cell 10, ECU, monkey J). In this case, the C+M- group did not substantially differ from the FTT distribution while attempted C-M+ activities fell close to the inactive C-M- group. The resulting angle far-negative of $-\frac{\pi}{2}$ indicates that the monkey failed to activate muscles in during the C-M+ task component, and failed to calm them during C+M- trials.

4.4 Discussion

4.4.1 Possible mechanisms mediating activity dissociations in CM cell-muscle pairs

Though not conclusive, inspection of non-conditioned target muscle activity during performance of the dissociation task suggests monkeys employed alternating muscle recruitment strategies to achieve activity dissociation of conditioned CM cell-muscle pairs (examples: figures 4.2 and 4.7). Target muscles showing post-spike suppression from the CM cell, ECR and FCR (figure 4.7), generated force in the opposite direction to that of the conditioned target muscle ECU that exhibited PSpF and opposite the preferred, ulnar, direction of the CM cell. The muscle antagonists were active during C-M+ trials along with the conditioned target muscle ECU, while upstream CM cell activity was suppressed. These results suggest the monkey adopted a strategy of contracting muscle antagonists that effectively limited recruitment of the conditioned CM cell. In this model, upstream cortical sources suppressed CM cell activation during recruitment of muscle antagonists. An ulnar agonist to ECU, FCU, remained inactive during C-M+ trials as well. For the case of forward dissociation (C+M-), both the conditioned target muscle and its muscle antagonists remained quiet, while the CM cell and non-conditioned agonist FCU, co-activated. Interestingly, though both ECU and FCU were facilitated muscles in the CM cell's muscle field, the monkey selectively activated FCU, without ECU; possibly as a means to recruit the conditioned cell during C+M- trials.

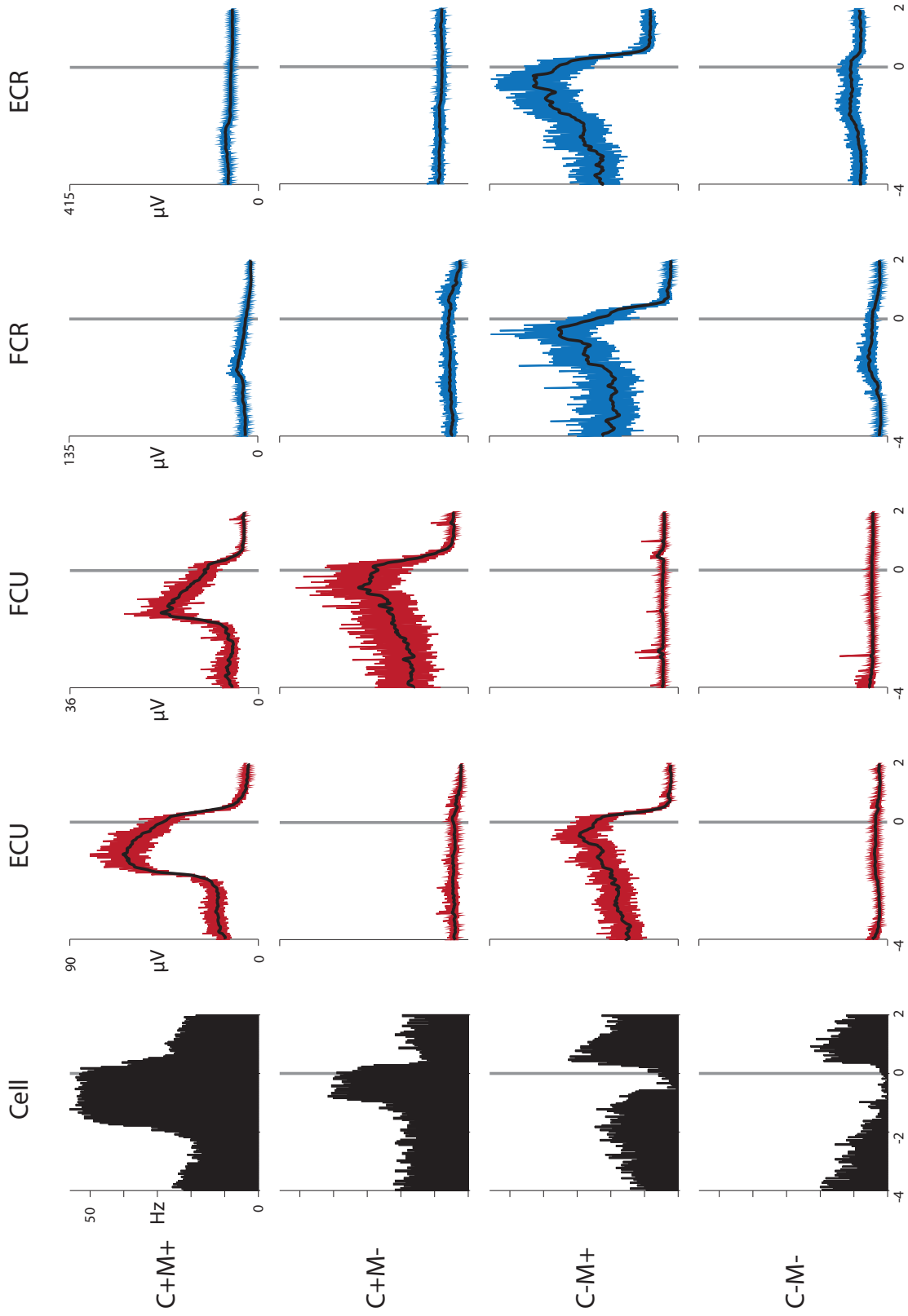


Figure 4.7: Another example of the alternating muscle recruitment strategy. Peri-event spike histograms (black, left column) and wrist muscle EMG averages (red and blue, columns) and standard error about mean (background shading) from a subsequent dissociation conditioning session using same CM cell as depicted in figure 4.2 and ulnar synergist muscle ECU. Red EMG traces indicate the muscles exhibited post spike facilitation in FTT task spike-triggered averages, while blue traces code for muscles that showed post-spike suppression. Peri-target completion activities of the CM cell and target muscles (columns) for each dissociation task component (rows) are arranged in matrix form.

The motoneuron pools of a given a CM target muscle can receive inhibitory input from spinal interneurons when antagonist muscles are activated during performance of an auxotonic force target-tracking task [91, 34]. In this reciprocal inhibition model, inhibitory input from these interneurons could oppose direct excitatory input from an active CM cell, and thus prevent activation of the target muscle during forward dissociations (C+M-). Evidence supporting involvement of inhibitory interneurons would be activation of muscle antagonists during the C+M- component of the dissociation task. Indeed, non-conditioned partial antagonist ECU was highly active during the C+M- task component in figure 4.2. Conversely, additional support for this mechanism could be observed in cases of reverse dissociation: suppressed activation of muscle antagonists during C-M+ trials (ECU, C-M+ group, figure 4.2) could suggest involvement of inhibitory interneurons that project onto motoneuron pools of antagonist muscle groups. In this model, these interneurons would be recruited by activation of the conditioned target muscle.

The rapidity and reversibility of CM cell-muscle dissociations strongly favor reciprocal inhibition as a mechanism over modified synaptic strength of input projections (e.g. Hebbian plasticity) however “implausible” the suggestion of synaptic alteration might be. In a recent investigation, changes in post-spike effects were observed after continuous conditioning using spike-triggered spinal stimulation over several hours. Induced changes also persisted for minutes to hours after conditioning ceased [84]. These timescales are incompatible with reversibility of dissociation task activity patterns that occurred within 4 seconds among all possible sequences of task components (figure 4.4).

Monoaminergic drive, subject to volitional control, could also be a mechanism permitting activity dissociations in cell-muscles pairs. Persistent inward currents at motoneuron dendrites can determine magnitude of pre-synaptic input from corticospinal pathways [45], and possibly the synaptic efficacy of descending input from a corticomotoneuronal cell. Modifying the effective impact of CM cell input could enable forward dissociation during C+M-task components by reducing the amplitude of CM cell-evoked EPSPs, thus suppressing motoneuron depolarization. It could also enable reverse dissociations by amplifying suppressed CM input and/or input from other parallel sources to effectively activate the target muscle.

Finally, pre-synaptic inhibition might also play a role in mediating CM cell-muscle activity dissociations. In a model suggested by Yanai et. al. (2007), descending command supplies both cortical input to motoneurons, and mediates presynaptic inhibition of afferent information to motor units [125]. Though prior work demonstrates that corticospinal terminals are not involved [81, 49], pre-synaptic inhibition of afferents that terminate on motoneurons of the conditioned target muscle could effectively reduce membrane potential of a motoneuron below threshold while the conditioned upstream CM cell is active. Such a mechanism could also permit reverse dissociation via release of pre-synaptic inhibition of convergent afferent terminals. Emergence of excitatory afferent input would combine with other non-CM cell input with the net result of keeping motoneurons of the target muscle depolarized while CM cell input is reduced or absent.

4.4.2 Contributing factors affecting dissociation task performance

The extent of CM cell-muscle activity dissociation was subject to the monkeys' motivation to perform the activity dissociation task. Monkeys were observed to improve their performance on each of the task components as sessions progressed. Usually, one of the two reciprocal activation task components was the most difficult for the monkey to acquire—often requiring multiple attempts during early target presentations. Waning motivation, reward satiation and “frustration” over task difficulty could have detracted from dissociation performance, leading to sub-optimal dissociation examples from some sessions. Because we employed operant conditioning to elicit dissociations in this investigation, we cannot draw conclusions regarding the maximum extent to which CM cells and their target muscles could be further dissociable. Also, we cannot conclude that attempted dissociations of any cell-muscle pair were impossible for the monkeys to demonstrate. There were two conditioning attempts for which cell-muscle co-activation levels of C+M- and C-M+ dissociation task components did not significantly deviate from those observed during target-tracking—one session for each of the two monkeys tested. These sessions lasted the usual amount of time (~ 2 hours) and positions of reciprocal activation targets were gradually displaced away from the cell-muscle co-activation domain as was typical for the majority of successful sessions. We suspect that

the monkeys did not develop a successful strategy for dissociating co-activation patterns within the time constraints of the conditioning session. An alternative explanation, that activity dissociations of the two particular cell-muscle pairs were beyond their volitional capacity to demonstrate, is also possible. The tuning correlation coefficients for these cell-muscle pairs were quite strong (monkey J: 0.979, monkey D: 0.995) but not the highest among all pairs attempted.

Chapter 5

COMPARING CM CELL-MUSCLE ACTIVATION PATTERNS ACROSS TASKS

5.1 *Introduction*

Having demonstrated independent activation in multiple cell-muscle pairs, we next examined if “natural” co-activation patterns—that is, concomitant CM cell and target muscle activation levels that occurred during force holds for the FTT task—were predictive of their dissociability. In the first section, we verify that the dissociation task elicited relative co-activation levels beyond those observed during FTT task performance. In the subsequent section, dissociation indices are plotted over corresponding FTT task tuning signal correlation values for each of the unique 44 conditioned cell-muscle pairs. These analyses characterize trends between the efficacy of cell-muscle reciprocal activation conditioning and the strength of correlation between their activities during force generation whereby their activation did not directly affect operant reinforcement. Finally, we present changes in the mutual information between CM cell activities and those of their target muscles induced during instances of dissociation task reciprocal activation as compared to FTT task co-activation levels.

5.2 *Methods*

5.2.1 *Mutual information calculation*

Mutual information measures the amount of information one random variable contains about the other—in our case: given a level of cell activity how well can one predict the level of muscle activity (and vice versa). We intend to see if this mutual information between concurrent cell and muscle activities changes depending on task component (i.e. C+M- and C-M+ co-activity groups) as compared to FTT hold activities.

To calculate mutual information, two dimensional coordinate distributions of concurrent

cell and muscle activities were binned into frequency histograms. Coordinates were assigned to a 10×10 bin array, bin widths along each dimension were scaled by standard deviation of the distribution along that dimension. Histogram bin space was centered about the distribution mean. The resulting joint histogram $p(c_i, m_j)$ was then normalized and summed along each dimension to generate marginal probability distributions $p(c_i)$ and $p(m_j)$. Using these, mutual information $I(c; m)$ was calculated for discrete distributions with

$$I(c; m) = \sum_{i=1}^{10} \sum_{j=1}^{10} p(c_i, m_j) * \log_2 \left(\frac{p(c_i, m_j)}{p(c_i)p(m_j)} \right) \quad (5.1)$$

where i and j index bin locations along respective cell and muscle dimensions of the distributions. The resulting positive scalar sum indicates mutual information in bits between concurrent cell and muscle activities. Using this method to calculate mutual information, bias in the statistic arose from three contributing factors: approximation of the joint probability distribution through discretization (binning procedure), noise in the finitely sampled dataset compared to the actual underlying population, and small sample sizes. These three sources of bias were corrected using the bootstrap method of Optican et al. [89] whereby mutual information bias contribution from these sources is ascertained by shuffling sample distribution pairing, then recalculating $I(c_{sh}; m_{sh})$ on each bootstrap iteration. These sources are additive, so the bias measured from the resulting bootstrap I_b were removed using the following relation:

$$\hat{I} = \left[1 - \left(\frac{I}{I_b} \right)^2 \right] I. \quad (5.2)$$

We calculated the bias-corrected mutual information of the five co-activity distributions (FTT, C+M+, C+M-, C-M+ and C-M-) on each conditioning session.

5.2.2 Statistical analysis: Box-plots and the Kruskal-Wallis test

We grouped mutual information statistics and dissociation indices by task component and calculated box-plot parameters that characterize the resulting distributions. Boxplots depict median values (middle horizontal lines), 25% to 75% quantiles (vertical box extremities) and more outlying values within the 5-95% quantile range (whiskers) of each distribution. Distribution values that lie outside of whisker boundaries are plotted as individual points.

The Kruskal-Wallis (K-W) test appraises statistical significance, without bias from repeated measures, that would otherwise arise by running a series of pair-wise rank sum tests. We applied the K-W test to evaluate significance of changes between above-bias FTT mutual information distribution and their corresponding reciprocal activation counterparts. Wilcoxon rank-sum tests were also applied to statistically assess differences between C-M+ and FTT mutual information values, as well as C+M- and FTT values.

5.3 Results

5.3.1 *Dissociation conditioning elicits reciprocal activation patterns beyond FTT task domains*

We sought to examine variance of CM cell-muscle co-activation patterns during repeatable, highly-stereotyped movement behaviors. Single-target peri-completion activities during FTT task performance serve as a basis for comparison when measuring the extent of conditioned activity dissociations since:

1. CM cell throughput to muscles has been previously documented to change during different movements [22]. In the context of this investigation, different targets of the FTT task require different wrist torque angles for their successful completion, so only the elicited activities during hold in one of the eight peripheral targets would contain co-activity patterns minimally compromised by changes in cortico-spinal throughput.
2. CM cell-muscle co-activation patterns during moves into a single target, compared to multiple FTT targets, provide a more representative sampling of co-activation variability when the CM cell and target muscle are recruited during more “natural” – or at least, non-dissociation-eliciting – behaviors.

We feel it strengthens our claim of *operantly conditioned* CM cell-muscle dissociations if dissociation task co-activation patterns are compared to peri-completion co-activation levels from *all* targets of the preceding FTT task (figure 5.1). Reciprocal component distributions that occupy domains distinct from all FTT task co-activities reveal that activity-guided dissociation task could extend cell-muscle dissociations beyond those demonstrable through

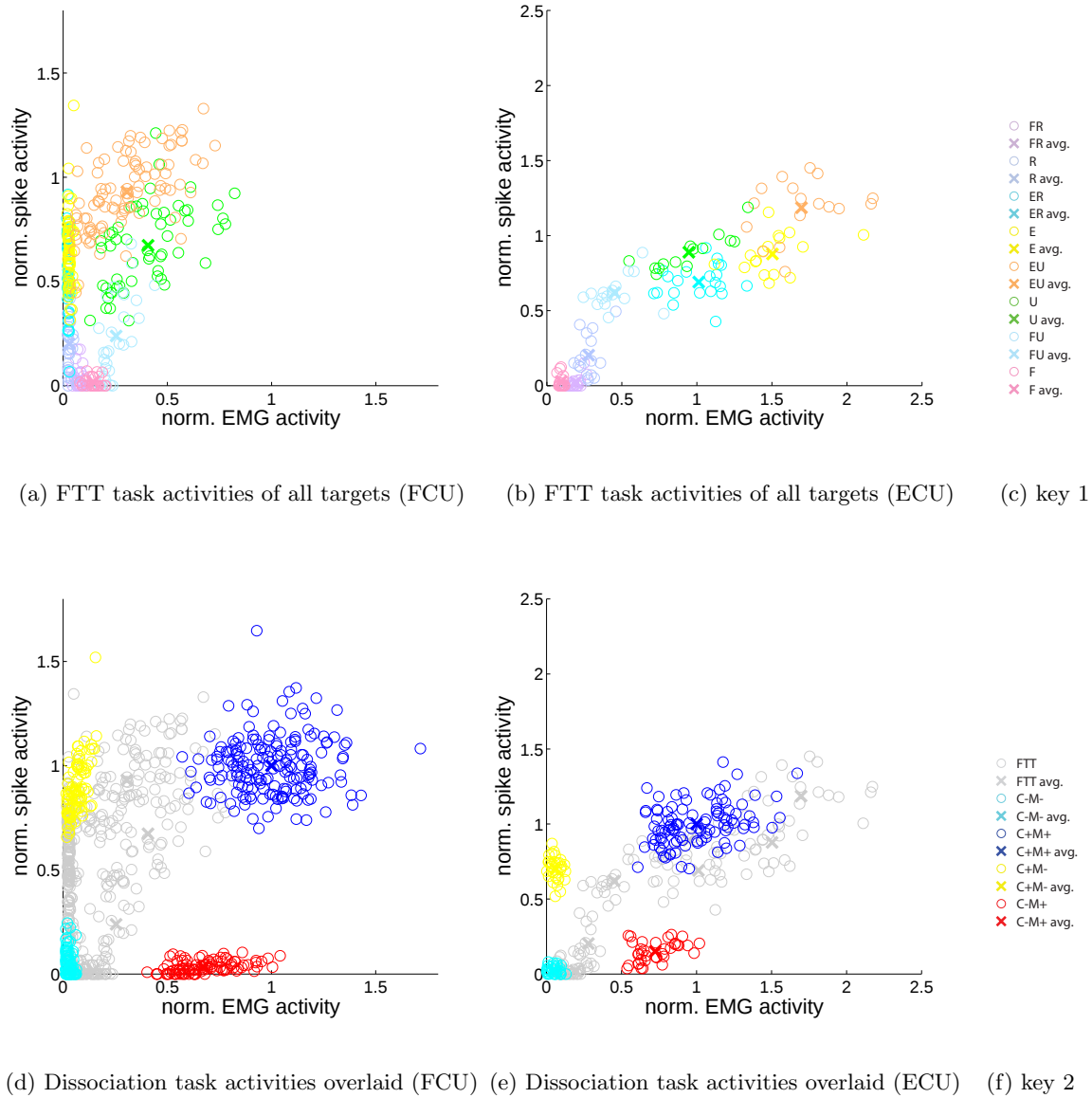


Figure 5.1: The activity-driven dissociation task elicits concurrent CM cell-muscle response patterns different from co-activation levels generated during the torque-guided FTT task. Normalized activities (circles) from a CM cell and two of its target muscles (FCU: *left column* and ECU: *right column*) plotted for all FTT task target completion events (*top row*) and with dissociation task component activity patterns overlaid (*bottom row*). Colors code FTT task target identities (key 1) and dissociation modes (key 2) with the same FTT data grayed out. As in previous figures, bold face 'x's are located at mean levels of muscle (horizontal axes) and cell (vertical axes) activation for each target group.

FTT task performance. Co-activation levels from the dissociation task epochs as well as from all FTT targets are shown from two example CM-cell/target muscle combinations in figure 5.1. In both examples, the monkey generated co-activation levels during the activity-guided dissociation task well outside of the aggregate domain of all FTT target co-activation levels: specifically, the red reverse-dissociation cloud in figure 5.1d and both the forward- (yellow) and reverse-dissociation clouds in figure 5.1e.

In previous sections (4.3.2 and 4.3.4), we selected the FTT task target that recruited the greatest peri-target-completion activation levels from *both* the CM cell and the chosen target muscle as the representative FTT co-activities group plotted in co-activity scatterplots (coded in black) and used subsequent analyses (e.g. section 4.3.2, figure 4.3a) for the two reasons listed above.

5.3.2 *Reverse dissociation strength varies inversely with cell-muscle tuning correlation*

In figure 5.2 we show dissociation indices (DIs) plotted over tuning signal correlation values calculated for each of the 44 unique CM cell-muscle pairs that underwent activity dissociation conditioning. Cells and target muscles with stronger tuning correlation showed relatively weaker dissociation during reciprocal activation task components (C+M- and C-M+) compared to CM cell-muscle pairs with weaker tuning correlation; whose activities were more robustly dissociated. Similar trends held for cell-muscle pairs showing strong negative tuning correlation in both reciprocal activation cases as well. The distribution of dissociation indices from FTT-task maximally co-active targets centered about zero across varied tuning correlation values from those 44 sessions. This indicates that the population of DIs from this group was unbiased toward reverse dissociation in either direction. We observed stronger correlative trends between dissociation index and tuning in the reverse dissociation (C-M+) task component than for the forward dissociation (C+M-) component (table 5.1), reaching statistical significance in the domain of positive tuning signal correlation values.

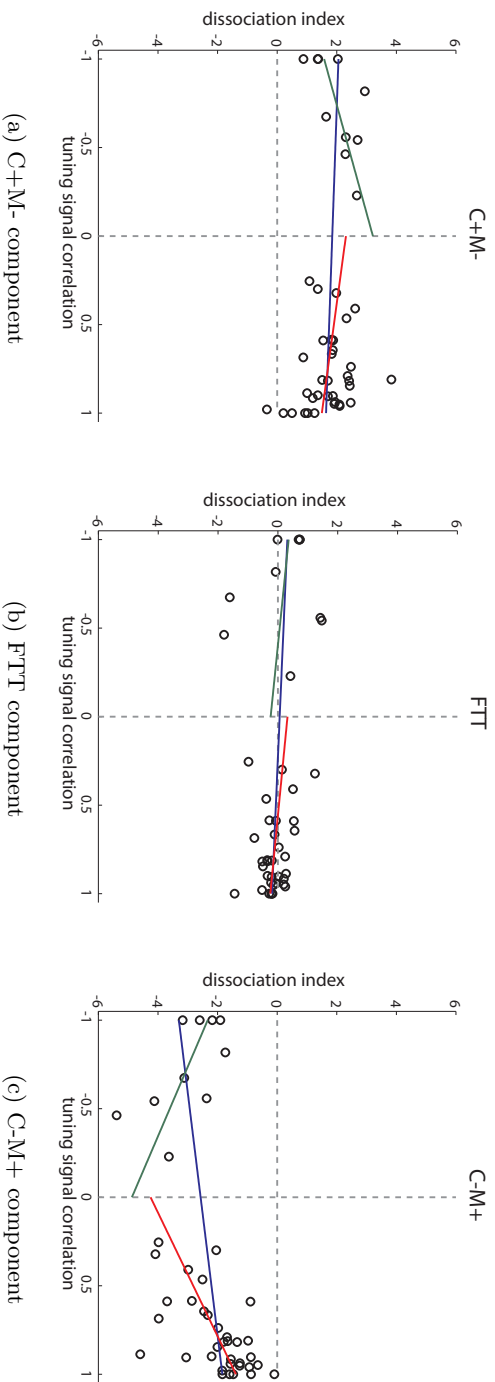


Figure 5.2: Testing dependence of cell-muscle co-activation patterns on FTT task tuning. In the above scatter plots, each point (black circle) plots the dissociation index of a given cell muscle pair (y-axis) over their tuning signal coefficient (x-axis) computed from the FTT task for that session. Values from the 44 unique cell-muscle pairs are plotted here. Each plot illustrates values from reciprocal dissociation task components (C+M-: (a), C-M+: (c)) and co-activation patterns observed during force target-tracking (FTT: (b)). Regression lines fitted across the entire domain of correlation values $[-1, 1]$: blue), as well as positive (red) and negative (green) domains, illustrate trends between the two measures. Refer to table 5.1 below for the R^2 and corresponding p-values for regression lines.

Task comp.	ρ	p-value	Slope	$ R $
C+M-, SC(all)	-0.18	0.2316	-0.21	4.95
C+M-, SC(+)	-0.23	0.1949	-0.81	4.38
C+M-, SC(-)	0.66	0.0360	1.64	1.53
FTT, SC(all)	-0.26	0.0926	-0.26	4.29
FTT, SC(+)	-0.26	0.1308	-0.57	2.66
FTT, SC(-)	-0.15	0.6819	-0.60	3.33
C-M+, SC(all)	0.43	0.0040	0.73	6.91
C-M+, SC(+)	0.58	0.0003	2.87	5.10
C-M+, SC(-)	-0.63	0.0533	-2.54	2.64

Table 5.1: Linear dependence and goodness-of-fit parameters for dissociation-tuning scatter plots. SC=tuning signal correlation coefficient.

5.3.3 *Cell-muscle mutual information declined during periods of reciprocal activation compared to co-activation during force holds*

We calculated the bias-corrected mutual information values of concurrent cell-muscle activity distributions from five task components (FTT, C+M+, C+M-, C-M+ and C-M-) for each dissociation experiment. Twelve sessions in which mutual information during the FTT task epoch reached statistical significance above noise ($p < 0.05$, see methods) were selected for further examination. From these sessions, mutual information values of reciprocal activation groups (C-M+ and C+M-) were gathered for comparison (figure 5.3). Mutual information in at least one of the three task components differed statistically from the other two ($p = 0.013$, Kruskal-Wallis test, table 5.2); this test appraises statistical significance without bias from repeated measures that would otherwise arise by running a series of pairwise rank sum tests. Posthoc Wilcoxon rank-sum tests for significant difference between C-M+ and FTT mutual information values, as well as C+M- and FTT values, revealed that mutual information during periods of forward dissociation (C-M+) and reverse dissociation (C+M-) declined significantly compared to co-activation during force generation

Task comp.	25% quantile	50% quantile	75% quantile	KW p-value	WRS p-value
C-M+	0.007	0.100	0.218	-	p = 0.005
FTT	0.150	0.296	0.467	p = 0.013	-
C+M-	0.020	0.133	0.277	-	p = 0.035

Table 5.2: Parameters characterizing across-session mutual information, in bits, by task component.

(table 5.2). As one might expect, cell and muscle activities had the highest interdependence during force holds and least during modes of reciprocal activation.

5.4 Discussion

5.4.1 Decline in CM cell-mediated information suggests input from parallel sources

Mutual information in cell-muscle co-activity distributions was significantly greater during normal force generation (FTT) than during each of the two dissociation task components eliciting reciprocal activation patterns (C+M- and C-M+). These results (figure 5.3, table 5.2) indicate that during both forward and reverse dissociations, the influence of synaptic input from the upstream CM cell was markedly reduced. The example session (figure 4.3a and table 4.3) shows increased mutual information during the C+M- component, when muscle activity is reduced, as compared to target tracking. This means that for the small amount of muscle activity present, it was actually more predictive of concurrent spike activity than the non-conditioned FTT activities during force holds. Though this instance was atypical compared to the group trend, it demonstrates that reduced information content cannot be assumed to be due simply to reduced activity of one of the two sources.

Reduction in cell influence suggests input from other convergent sources onto motor units of the conditioned target muscle, likely contributed suppressive input to effectively hinder muscle activation during forward dissociations. The overall reduction in cell activity during high target muscle activation requires that other sources or alternate mechanisms made up for the CM cells missing excitatory contribution—as indicated by comparatively reduced mutual information values for the C-M+ co-activation distribution. Unfortunately, statisti-

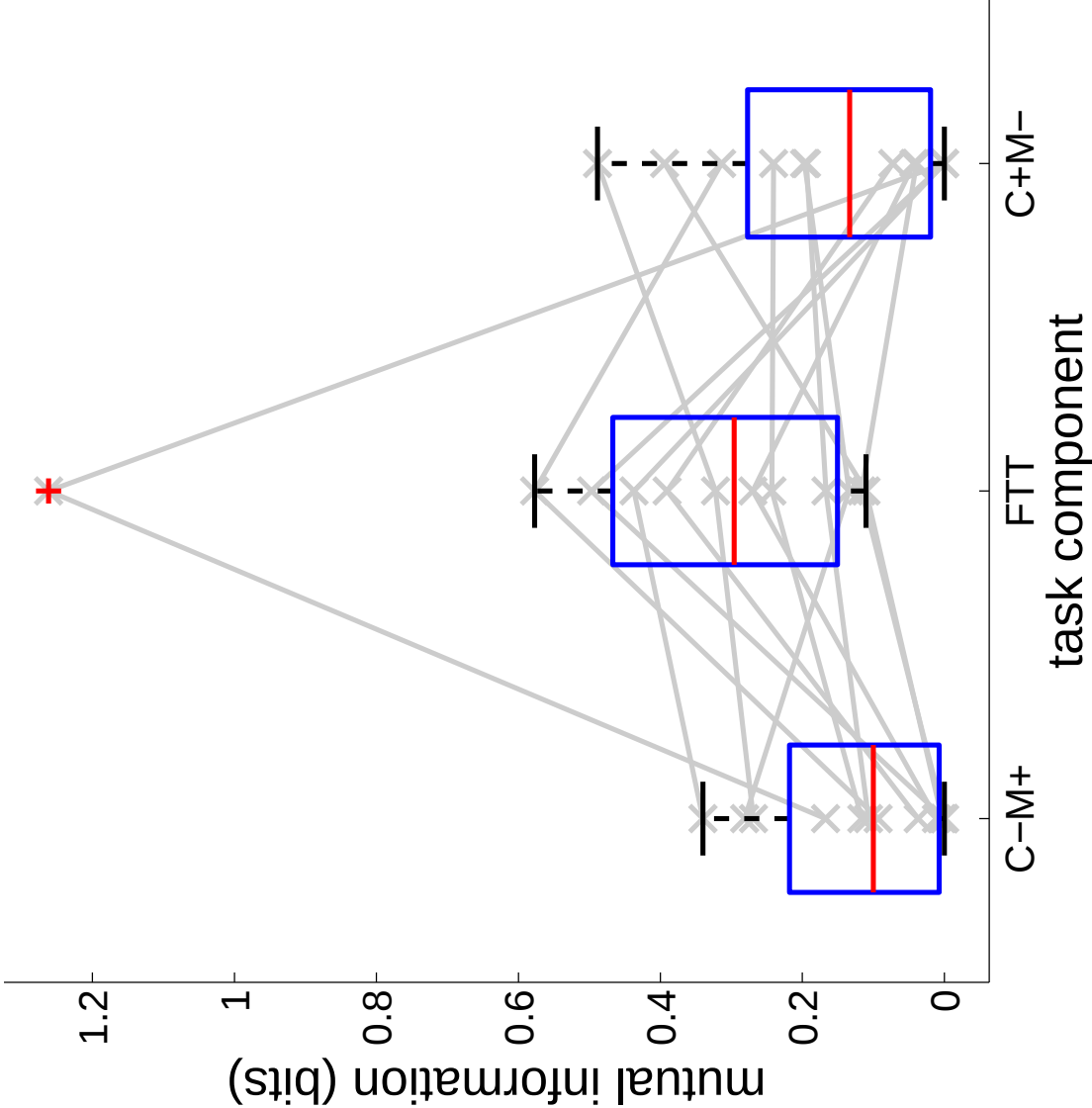


Figure 5.3: Mutual information shifts across task component: Mutual information values (grey ‘X’s) plotted for 12 selected sessions across FTT and reciprocal activation (C-M+ and C+M-) task components. Grey lines link values from a given session across groups. Boxplots depict median values (red), 25 to 75% quantiles (box floor, box ceiling) and most extreme values within the 5-95% quantile range (black whiskers) of each distribution. The 12 sessions plotted are those whose mutual information values calculated for their FTT task groups were significantly greater than noise levels (monkey J: $n = 8$, monkey D: $n = 4$).

cally significant mutual information values were detectable in only 12 of the 42 cell-muscle pairs that were successfully dissociated. This small sample population was the result of undersampling response activities (for the purpose of mutual information characterization) during FTT of most recording sessions.

Chapter 6

ACTIVITY DISSOCIATION OF TWO CM-CELL TARGET MUSCLES

6.1 Introduction

Previous work reported dissociations in concurrent activity patterns between CM cells and a single target muscle [78] or multiple target muscles [22, 105] without systematic examination of CM cell activity patterns when individual muscles activated. In this project, we investigated the relative levels of CM cell activation when target muscles are activated both individually and concomitantly.

We used the same dual activity-driven task to condition reciprocal activation levels between CM cells and target muscles, but this time routing the EMG activities of two target muscles as input sources. Thus, we operantly conditioned co-activation (M_1+M_2+), co-inactivation (M_1-M_2-), and reciprocal-activation of the two muscles in both combinations, M_1+M_2- and M_1-M_2+ , while recording concomitant firing of the cortical CM cell. Knowledge of CM cell firing levels during each of these dissociation task components enabled us to document CM cells' activation with individual or coactivation of their target muscles.

6.2 Methods

We implanted monkey J with pairs of stainless steel electrodes in six to twelve muscles of the right forearm, first subcutaneously, and later transcutaneously. Muscles driving wrist flexion, extension, abduction and adduction movements were selected for their involvement in the two dimensional wrist force target tracking task (see methods sections 2.3 and 3.2.1). During performance of the task, we recorded single unit cortical activity to detect correlational linkages between the candidate cortical neurons and the implanted wrist muscles by averaging peri-spike sweeps of rectified EMG activity. When at least two of the recorded muscles showed significant facilitatory post spike effects, we continued with the dual-muscle activity-driven dissociation task. For two sessions, we also conditioned reciprocal- and co-

activation of a muscle pair in which one muscle showed post spike facilitation with the CM cell while the other muscle exhibited post-spike suppression.

In a manner similar to the CM cell/muscle activity dissociation task (section 4.2.1), we used EMG signals from two target muscles to control the computer cursor. We first rectified, then low-pass filtered each EMG signal; the resulting pair of DC output signals controlled the cursor’s 2-D position on the screen—one muscle for each axis.

As in cell/muscle activity dissociation conditioning, target position on the screen conveyed dissociation task components: co-inactivation (M_1-M_2-) and co-activation (M_1+M_2+) located along one diagonal, while the two reciprocal activation boxes, M_1+M_2- and M_1-M_2+ , occupied the two off-diagonal quadrants. We selected associated screen dimension for each muscle using the same rationale as for CM cell-muscle dissociation conditioning. The tuning vector of the target muscle showing greatest post-spike facilitation (muscle 1) determined which screen dimension would be associated with each muscle. Specifically, we selected the greatest component of muscle 1’s tuning vector, either horizontal or vertical, as the screen dimension for muscle 1. The screen dimension that remained was assigned to muscle 2. During performance of the dual-muscle activity dissociation task, we recorded spike activity of the muscles’ CM cell for subsequent analysis.

6.3 Results

In figure 6.1, we show spike-triggered averages of cortical activity and EMG from two forelimb muscles selected for subsequent dual-muscle dissociation conditioning. Statistically significant deflections from pre-spike baseline levels that occur between 6 to 16 ms after cortical spikes indicate correlational linkage consistent with monosynaptic projections from motor cortex onto motor units of the activated muscle. Thus, the facilitatory post-spike effects in the two averages of figure 6.1 indicate that ECU and FCU are both muscles within the CM cell’s muscle field.

In figure 6.2 we show peri-event averages of instantaneous spike rate and low-pass filtered EMG activity from conditioned target muscles ECU and FCU during each component of the dual-muscle dissociation task. The animal co-activated and reciprocally activated the two target muscles through successful performance of muscle-driven operant conditioning task.

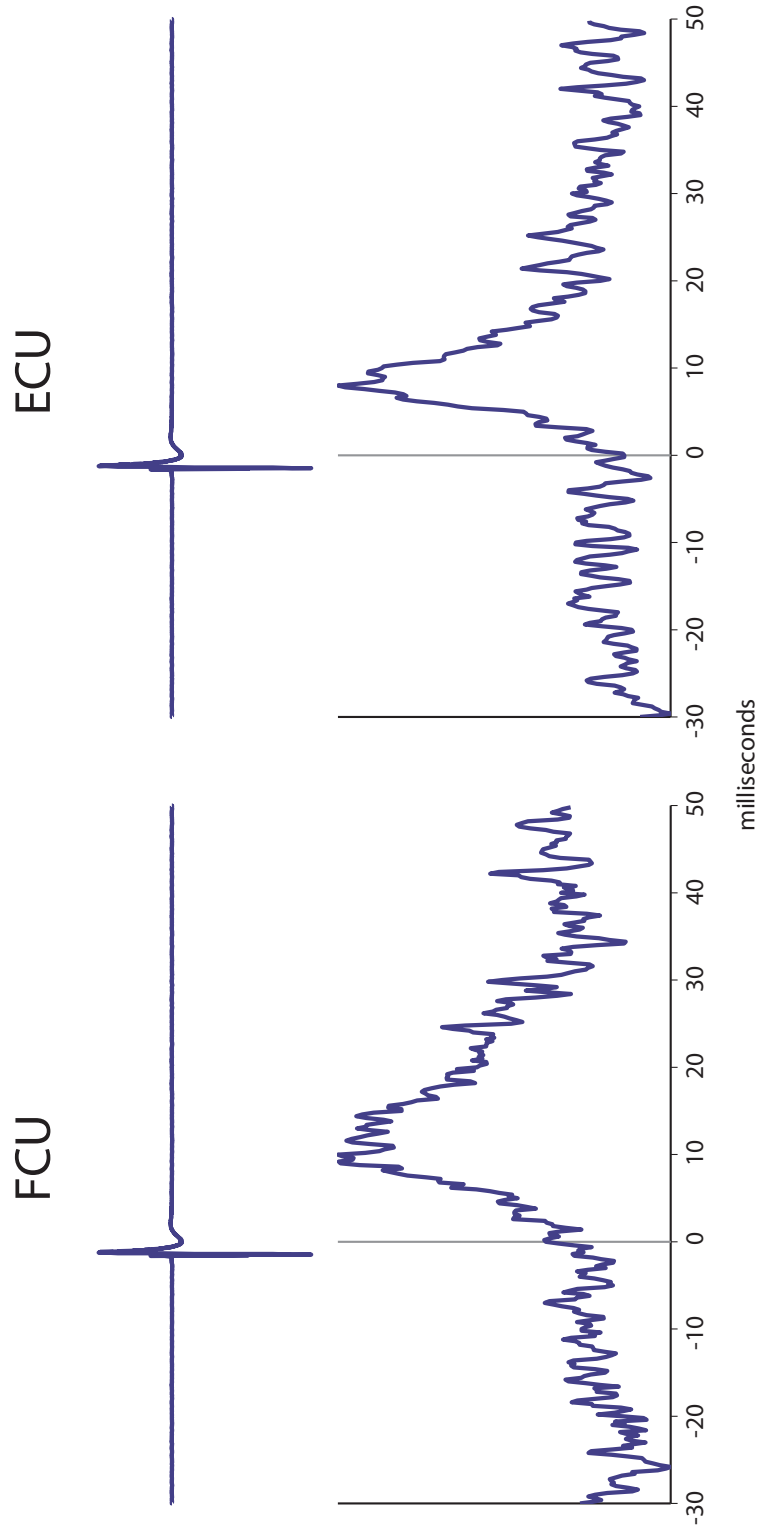


Figure 6.1: Spike-triggered averaging reveals the “muscle-field” of a CM cell. During performance of force target tracking, sweeps of rectified EMG from forelimb wrist muscles were extracted about cortical cell spike events and then averaged. These spike-triggered averages show correlational linkages between CM cell spikes (*top row*) and two target muscles selected for subsequent activity dissociation conditioning: FCU (*bottom left*) and ECU (*bottom right*). Averages are displayed in a range from -30 to 50 milliseconds with reference to spike occurrence. Both rEMG averages show statistically significant post spike effects at latencies (6-16 ms) consistent with corticomotoneuronal synaptic transmission times across a single synapse. Session: J20100318.

In this representative example, concomitant firing rates of the CM cell were greatest during co-activation of the two muscles and significantly reduced during muscle activity holds in the two targets that required reciprocal activation of the two muscles. Corticomotoneuronal cell firing rates during the two reciprocal task components were roughly equivalent as differences between rate distributions of these two groups did not reach statistical significance ($p = 0.121$, permutation test).

Plotted in figure 6.3¹ are color-coded distributions of concomitant activity patterns from a CM cell and two of its downstream target muscles during the four components of the muscle-driven dissociation task (cyan, blue, red, green). Also plotted are data-points from the target recruiting maximal muscle co-activation during the force target tracking task (black). Three-dimensional coordinates of each data point have been normalized by their corresponding average values calculated from M_1+M_2+ component levels. Each data-point in the scatterplot was normalized as follows: its absolute cell activity (i.e. number of spike counts that occurred in the 1 second preceding target completion) was divided by the average spike count of all M_1+M_2+ task component trials to give its normalized value along the z-axis. The data-point's location along the x-axis (normalized EMG activity from muscle 1) was calculated by dividing the time-integrated rEMG from muscle 1 by the group average of that measure during co-active trials (M_1+M_2+). Similarly, the data-point's y-coordinate (normalized EMG activity from muscle 2) was calculated by dividing integrated rEMG from muscle 2 by muscle 2's average activity during co-active trials.

We plotted activities in this fashion to investigate how CM cells were activated as more of their target muscles become active. If CM cell activation exhibits scaling, the activation level of the CM cell would be greater when the two target muscles are co-active compared to

¹Guidelines for viewing stereographs: The scatterplots that follow we show as stereographs should readers choose to enhance their perception of data-points in three-space. The two figures in each stereograph are the same images rotated 8° with respect to one another. One must cross one's eyes to view the 3-D effect. To aid convergence of the two images, hold your finger halfway between your eyes and the page and focus on your finger. Be conscious of the two images behind your finger that are out of focus. Move your finger slightly forward or back until the two images converge into one. Hold that convergence while changing your focus from your finger to the page. You can now remove your finger from the field of view. Moving the page closer or farther from you may facilitate focusing on the converged image. If this method of viewing proves to be too difficult or causes pain or headaches, one of the two images may be viewed normally, without the 3-D effect.

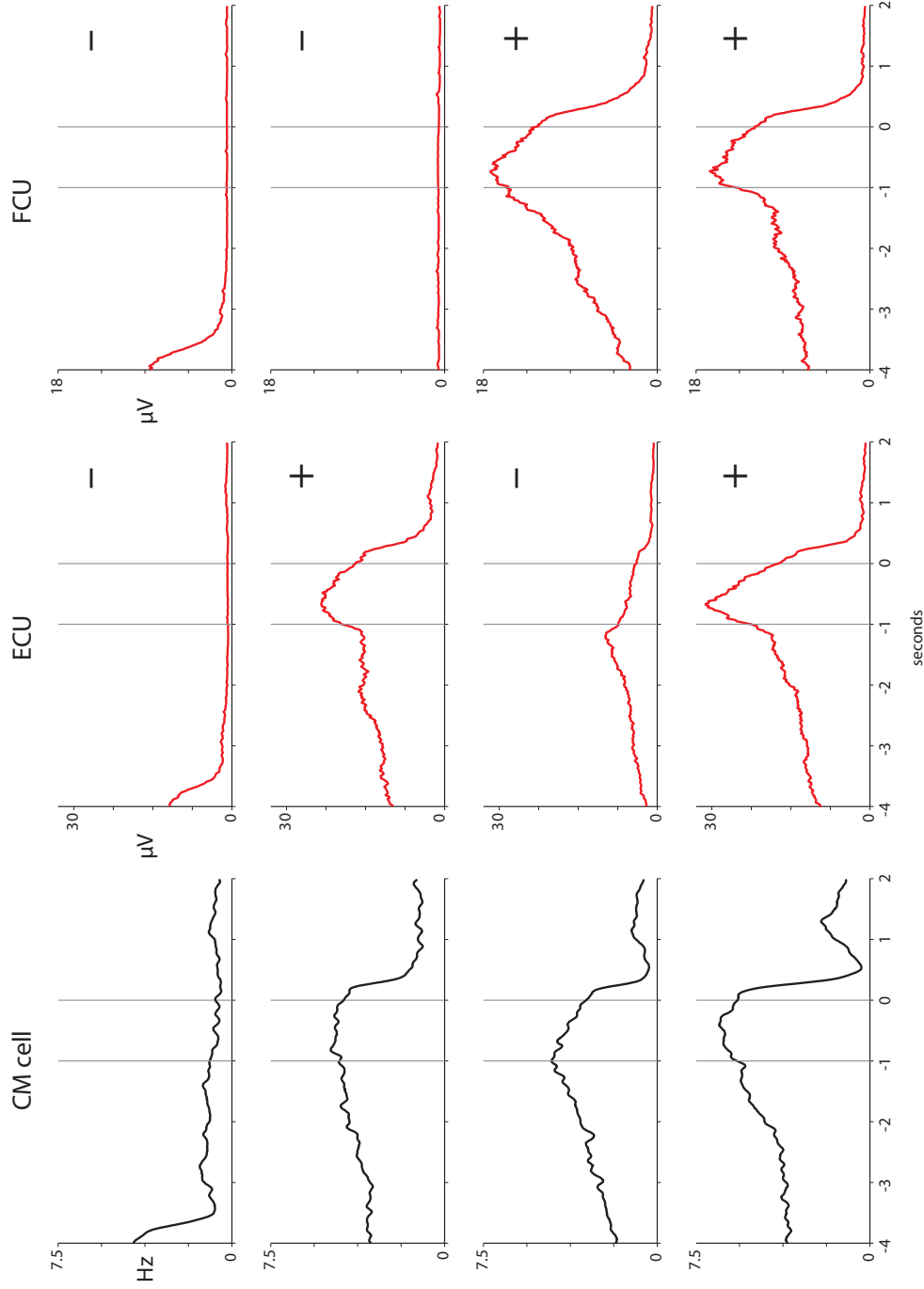


Figure 6.2: Concomitant CM cell activity varies between paired and reciprocal activation of two target muscles. Peri-event averages of instantaneous spike rate (*left column*) and low-pass filtered EMG activity (*middle and right columns*) during each component of the dual-muscle dissociation task. Activity sweeps are aligned by target completion of co-inactivation (ECU-FCU-, *top row*), reciprocal activation (ECU+FCU-, *second row* and ECU-FCU+, *third row*) and co-activation (ECU+FCU+, *bottom row*). The cell and muscle averages are displayed in a range from -4 to 2 seconds surrounding time $t = 0$, which marks trial completions when the EMG-guided cursor remained within the target box for the required hold time of 1 second. Gray lines at -1 second mark the time when the cursor first entered the corresponding target box. Session J20100318

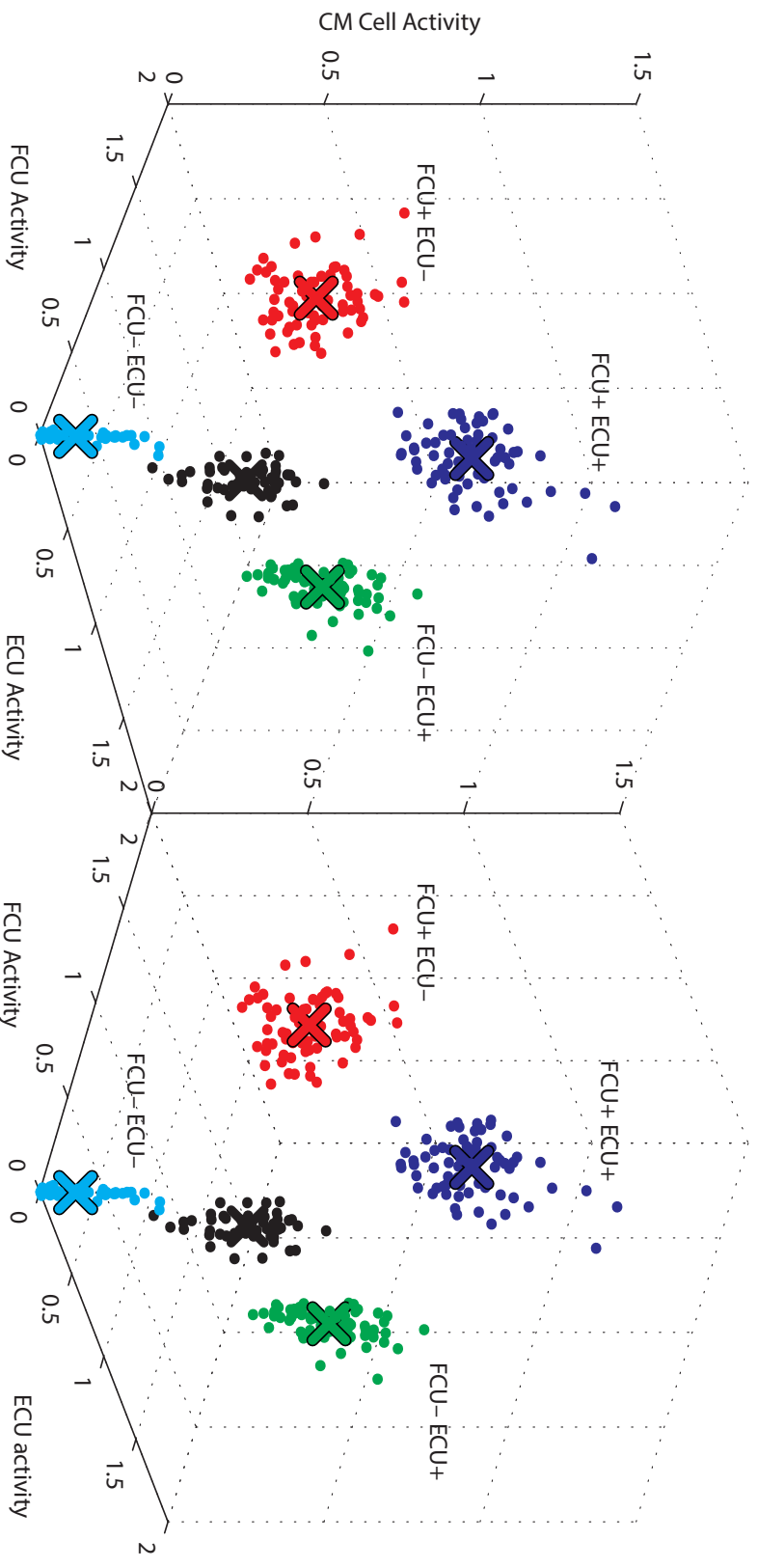


Figure 6.3: CM cell activity that scales positively with target muscle activation. Concomitant activities from the CM cell and two of its selected target muscles are plotted in three dimensional space. The location of each circle illustrates the relative levels of the three activities preceding each target completion during the muscle-driven dissociation task. Individual data-points have been normalized with respect to the average of co-active task component trials. Cyan, blue, red and yellow colors code one of the 4 possible target boxes of the dissociation task. Co-inactive target box levels (FCU-ECU⁻, $n = 68$) are cyan, ECU-reciprocal patterns (FCU-ECU⁻, $n = 69$) are green, FCU-reciprocal patterns (FCU+ECU⁻, $n = 69$) are red and co-active levels (FCU+ECU⁺, $n = 67$) are dark blue. Crosses mark average concomitant activation levels of the three sources for the corresponding color-coded group. Notice that CM cell activity during co-activation of the two muscles (green and red) are equally reduced compared to its average activity during co-activation of the two muscles. Black points represent the cell and muscle activities during the wrist force step tracking prior to the dissociation experiment for a single target box that recruited maximal co-activation the of the CM cell and target muscle activities: the ECU target in this example ($n = 54$).

when each of the target muscles are individually active. In addition to examples of positive additivity (figure 6.3), scaling could also manifest through dissociation of a heterogeneous muscle pair whereby one muscle revealed post spike facilitation and the other, post-spike suppression. In this case, scaling would manifest as a reduction in CM cell firing during co-activation of the two antagonist muscles compared to CM cell rates during isolated activation of the positively correlated muscle. This second phenomenon we call “negative additivity” in the results that follow (figure 6.4).

Cases in which the concurrent activity of the CM cell during co-activation of both target muscles is equal to levels during single activation of one target muscle, and substantially less during single activation of the other we categorize as “muscle-selective” or “muscle-preferred” (figure 6.5). Finally, “counter-intuitive” cases are those in which CM cell activity levels are less during dual-muscle activation than during activation of one of the target muscles despite positive correlational linkages demonstrated for both. In these cases, the additional activation of the second target muscle effectively reduced activation of the cortical neuron.

Figure 6.3 shows an example of target muscle positive additivity in concurrent CM cell activation. Specifically, CM cell activation during M_1+M_2- and M_1-M_2+ trials were significantly less than the distribution of activities of that cell during the co-active M_1+M_2+ component of the task. Distribution averages and permutation test p-values are listed in table 6.1. Corticomotoneuronal cell muscle-selectivity can be interpreted from the scatterplot in figure 6.5. In this example, while monkey J produced statistically significant reciprocal activation levels of the two conditioned muscles, concomitant firing patterns of the CM cell did not significantly differ between the M_1+M_2- reciprocal activation task component and the co-active M_1+M_2+ component while levels during the other reciprocal component, M_1-M_2+ , did (separation values and statistical test p-values listed in table 6.1). For completeness, we also show a counter-intuitive example in which inclusion of one of the PSpF-linked muscles served to suppress CM cell activity (figure 6.6); the corresponding p-values and distribution separation distances are also listed in table 6.1.

Of the 16 valid dissociation sessions, we observed co-activation patterns indicative of CM cell activation scaling in 9 of them: positive additivity in 7 sessions in which the

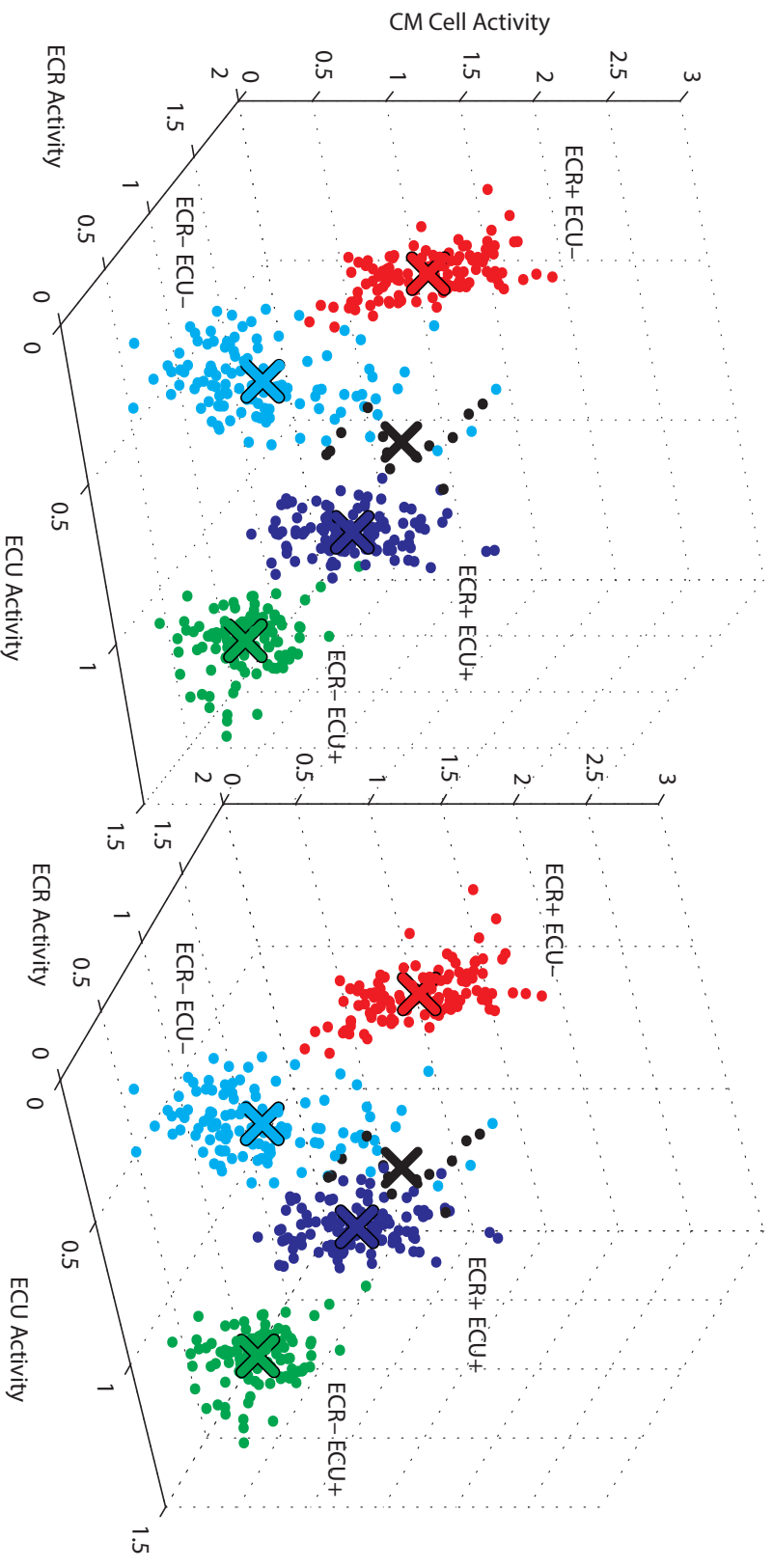


Figure 6.4: CM cell activity that scales negatively with activation of PSpS-linked muscle. Same format as figure 6.3. Co-inactive target box levels (ECR-ECU-, $n = 115$) are cyan, ECU-reciprocal patterns (ECR-ECU+, $n = 115$) are green, ECR-reciprocal patterns (ECR+ECU-, $n = 116$) are red, and co-active levels (ECR+ECU+, $n = 115$) are dark blue. Notice that CM cell activity is greatest during isolated activation of the positively-correlated target muscle (red), and is comparatively reduced during co-contraction with the negatively-correlated target muscle (dark blue). Black points represent the cell and muscle activities during the wrist force step tracking prior to the dissociation experiment for a single target box that recruited maximal co-activation the of the CM cell and target muscle activities: the FU target in this example ($n = 13$). Session J20110302.

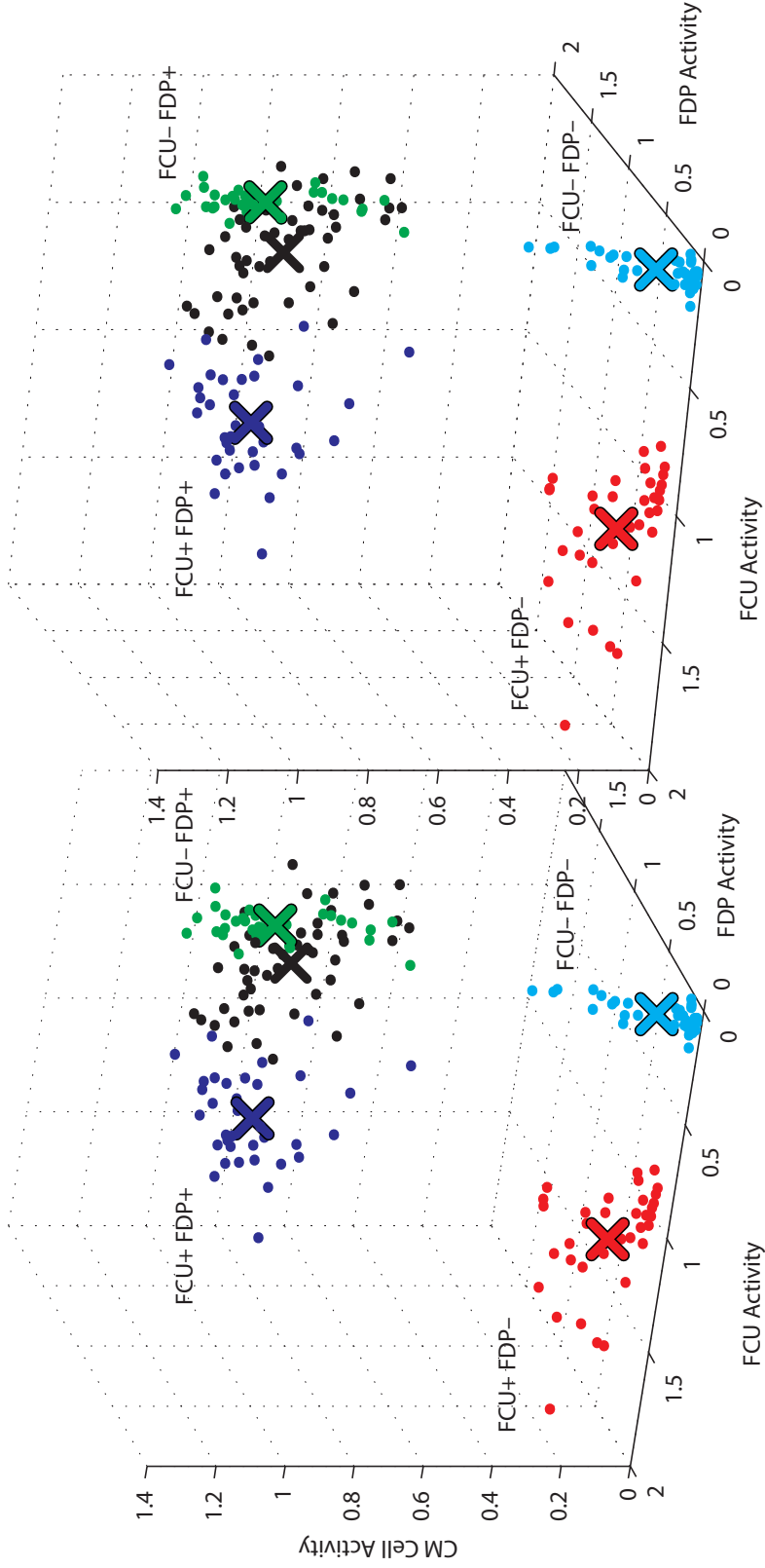


Figure 6.5: A CM cell with target muscle preference. Same format as figure 6.3. Co-inactive target box levels (FCU-FDP-, $n = 37$) are cyan, FDP-reciprocal patterns (FCU-FDP+, $n = 36$) are green, FCU-reciprocal patterns (FCU+FDP-, $n = 35$) are red, co-active levels (FCU+FDP+, $n = 35$) are dark blue. Notice that CM cell levels during isolated activation of FDP are nearly equal to cell activities during target muscle co-activation (dark blue) though both FCU and FDP showed post-spike facilitation in their SpTAs. Black points represent the cell and muscle activities during the wrist force step tracking prior to the dissociation experiment for a single target box that recruited maximal co-activation the of the CM cell and target muscle activities: the EU target in this example ($n = 49$). Session J20100326.

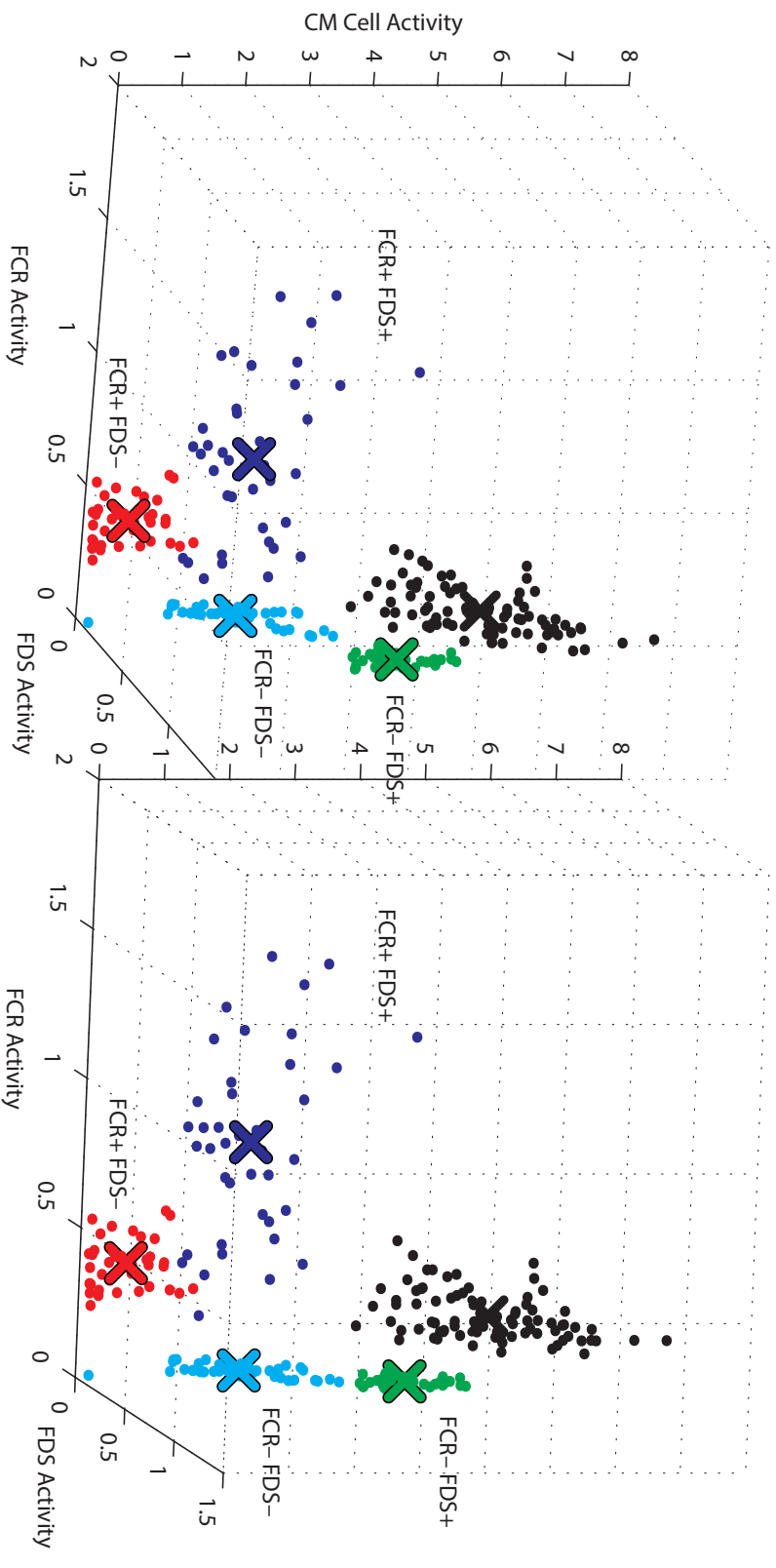


Figure 6.6: “Counter-intuitive” CM cell/target muscle co-activation. Same format as figure 6.3. Co-inactive target box levels (FCR-FDS-, $n = 40$) are cyan, FDS-reciprocal patterns (FCR-FDS+, $n = 40$) are green, FCR-reciprocal patterns (FCR+FDS-, $n = 40$) are red, and co-active levels (FCR+FDS+, $n = 40$) are blue. Notice CM cell activities during isolated contraction of FDS (green) surpassed levels during when both positively-correlated target muscles co-activated (dark blue). Black points represent the cell and muscle activities during the wrist force step tracking prior to the dissociation experiment for a single target box that recruited maximal co-activation the of the CM cell and target muscle activities: the U target in this example ($n = 86$). Session J20101020.

dissociated muscles both showed facilitatory PSpEs and negative additivity in 2 sessions guided by EMG from one facilitation-linked target muscle and another muscle that showed post-spike suppression. Six of the seven examples of positive additivity involved dissociation of FCU. We found examples of non-scaling firing patterns in a total of 7 sessions. More specifically, we observed muscle-selective firing patterns in CM cells in 4 sessions while 3 sessions supported counter-intuitive CM cell activation patterns in facilitated muscle pairs. We should note that all cases of counter-intuitive CM cell activation patterns occurred when the same pair of target muscles were conditioned: FCR and FDS. Table 6.2 elucidates activation patterns generated by the 12 CM cells used in this study, as well as their target muscles dissociated for each session. We checked for cross-talk in EMG signals between all pairs of target muscles that guided muscle-muscle dissociation attempts. Only pairs for which non-significant cross-talk was observed are included in session counts above.

6.4 Discussion

Our description of cortico-motoneuronal network architecture (section 3.4.1) cited both anatomical and electrophysiological evidence for divergence of CM cell projections onto multiple motoneuron pools, including those comprised of motor units in different muscles. Given this fact, activation of an upstream CM cell would act to facilitate target muscle activation via an excitatory, monosynaptic correlational linkage. Motoneurons receive convergent input from many sources, permitting flexibility in the manner by which CM cells can be activated with their target muscles, since CM cell facilitatory influence is relatively small compared to the aggregate input from all sources received by motoneurons [36, 105].

In light of their direct linkage with motor units, we endeavored to study CM cell activation patterns as increasing numbers of their target muscles became active. In a grander scheme, we wondered if the activation patterns generated by the cortico-motoneuronal-muscle networks investigated here reflect a general property observed by other physiological neural networks as well, given that cortico-motoneuronal circuits are relatively rare within the primate nervous system [94]. This remains an open question as far as we know. Corticomotoneuronal cell firing during reciprocal- and co-activation of two target muscles fell into two general categories: 1) activity scaling whereby CM cell firing levels increased as

	Muscle co-activity difference		Cell activity difference		Cell activity difference	
	$(M_1 + M_2^-) - (M_1^- M_2 +)$	p-value	$(M_1 + M_2 +) - (M_1 + M_2^-)$	p-value	$(M_1 + M_2 +) - (M_1^- M_2 +)$	p-value
J20100318 (+)	1.208	< 0.002	0.246	< 0.002	0.336	< 0.002
J20110203 (-)	1.231	< 0.002	0.224	< 0.002	-0.780	< 0.002
J20100326 (sel.)	1.207	< 0.002	0.052	0.152	0.905	< 0.002
J20101020 (c.i.)	0.701	< 0.002	-2.890	< 0.002	0.440	0.002

Table 6.1: Distances separating distribution mean coordinate values, and their corresponding permutation test p-values, are tabulated above for each example session depicted in figures 6.3, 6.4, 6.5 and 6.6 that illustrate examples of positive additivity scaling, (+), negative additivity scaling, (-), muscle selectivity, (sel.) and counter-intuitive, (c.i.) relations respectively. The first column lists distances separating the two reciprocal activation distributions projected onto the muscle-muscle co-activity plane—these values measure the extent and significance of dissociations conditioned during the dual-muscle activity-driven dissociation task. Columns of distances, and p-values to the right list differences in normalized cell activity during dual target muscle co-activation $M_1 + M_2 +$ and mean cell activity levels during $M_1 + M_2^-$ (*middle column*) $M_1^- M_2 +$ (*right column*) reciprocal activation dissociation task components. Negative valued normalized distances indicate that average CM cell activity for the associated reciprocal activation distribution was greater than during dual muscle co-activation.

cell identity												
	1	2	3	4	5	6	7	8	9	10	11	12
positive additivity (+)	FCU-	FCU-	FCU-				FCU-		FCU-	FCU-		
	ECU	ECU	ECU				FDS		FCR	FCR		
			FDP-									
			ECU									
negative additivity (-)					FDS-							ECU-
					EDC							ECR
muscle- selective	FDP-			FCU-		FCR-					FCU-	
	ECU			FDP		ECU					FDS	
counter- intuitive							FCR-	FCR-			FCR-	
							FDS	FDS			FDS	

Table 6.2: Summary of all CM cells and target muscle pairs categorized by their activity relation with the dual-muscle dissociation task. All sets tabulated above are from sessions that demonstrated statistically significant dissociation between the two target muscles whose EMG signals were not significantly affected by cross-talk.

more facilitated target muscles became active—or declined when negatively-correlated target muscles were recruited, and 2) activity preference by CM cells for some of its target muscles over others.

In their “simplest model”, Smith & Fetz (2009) proposed a plausible candidate network architecture to account for observed correlations linking cortical neurons and forelimb wrist muscles: reciprocal inhibition between CM cells that exert opposing effects on target muscles [112]. We believe that negative additivity examples from this investigation support this model of reciprocal inhibition, at least partially. The model predicts a decline in CM cell activity when the negatively correlated muscle co-activates with the facilitated one, due to concurrent inhibitory input from sources that are recruited on activation of the antagonist muscle. Such input could be introduced through axon collaterals of CM cells projecting onto inhibitory interneurons, which in turn project onto other CM cells whose muscle fields do not overlap that of the monitored CM cell.

According to previous work reviewed in Fetz et al. (1991), in addition to findings reported by Jackson et al. (2003) and Smith & Fetz (2009), common input is the most frequently observed synaptic interaction between cortical neurons [52, 35, 112]. Further investigation revealed preferential distribution of common synaptic input among CM cells that facilitated common target muscles [112]. Thus, these investigators most frequently detected common-drive correlations between CM cells whose muscle fields overlapped. These findings, as well as the corticomotoneuronal network model of Smith & Fetz can explain cases of positive additivity that we observed: CM cell activity preference for co-activation of target muscles over individual activation of the same muscles. For the purpose of concision in the explanation that follows, we have chosen to call CM cells whose muscle fields encompass the relevant target muscles, muscle-spanning cells. Under the simplest model, common-drive input would serve to activate muscle-spanning CM cells that, in turn, facilitate both of the co-active target muscles. Co-activation selective common-drive would facilitate firing rates of muscle-spanning CM cells during target muscle co-activation, compared to cases of individual muscle activation, when such common-drive would be reduced or absent. During reciprocal activation of target muscles, motoneuron pools of the active target muscle could receive input from other, more-specific, sources to compensate for the lack of con-

tribution from muscle-spanning cells. In figure 6.7, we show a schematic representation of corticomotoneuronal circuits described here.

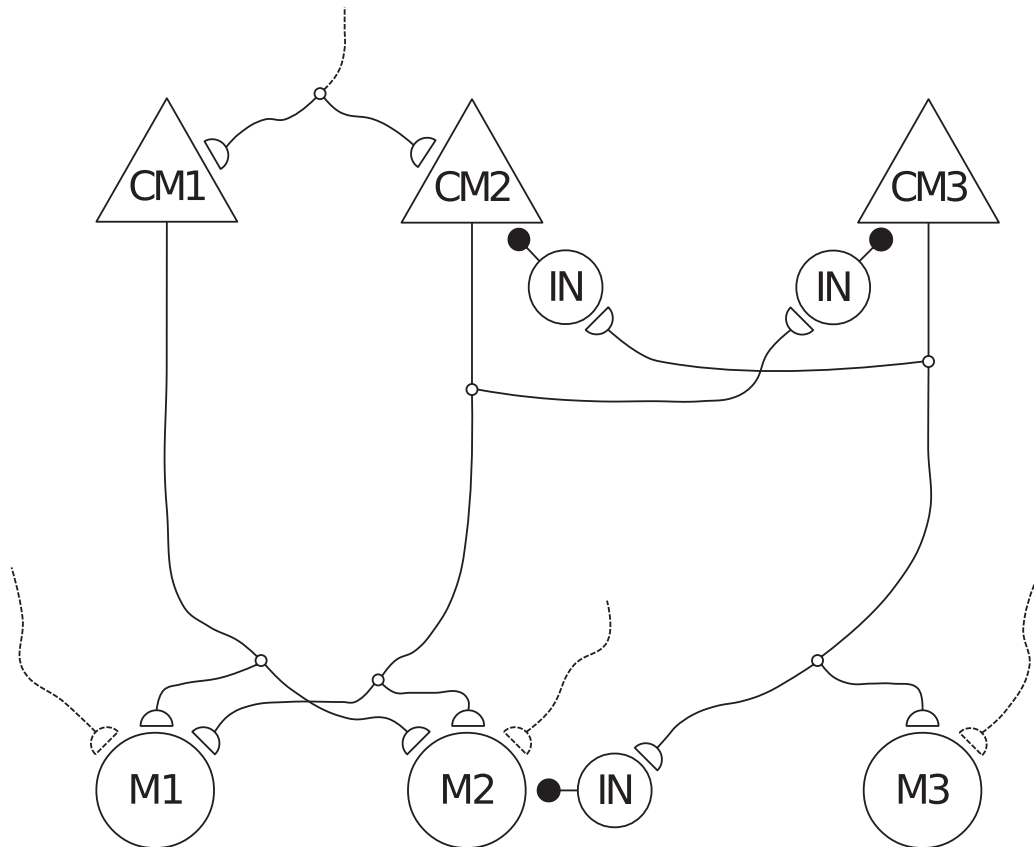


Figure 6.7: Positive and negative additivity can be explained by Smith & Fetz CM circuit model. Generalized schematic representation of synaptic interactions between CM cells projecting to synergistic and antagonistic muscle groups. Large circles at the bottom represent motoneuron pools that innervate the dissociated target muscles in positive additivity examples ($M1$ and $M2$) as well as in cases of negative additivity ($M2$ and $M3$). Large triangles represent somata of corticomotoneuronal (CM) cells and mid-size circles labeled “IN” depict inhibitory interneurons as indicated by their inhibitory synaptic terminals (*solid small circles*). Excitatory synapses we portray as open semi-circles and branch-points from which axon collaterals originate are small open circles. Dashed projections onto motoneuron pools represent other sources of input, not recorded in our sessions or part of the model of Smith & Fetz (2009), that we believe play a role in reciprocal activation of selected target muscles (see discussion section). In this diagram, line crossings not marked by branch-point nodes are unconnected.

Besides compliance with the corticomotoneuronal circuitry model of Smith & Fetz (2009), there are other possible explanations for observed CM cell activity scaling with target muscle dissociations. Activity scaling may arise as the consequence of a sort of “size principle” observed by CM cells, for which concurrent activation of multiple target muscles could involve recruitment of larger motor units with higher activation thresholds, thus requiring increased levels of CM cell input. Another possibility could be that the effective excitatory influence of a CM cell during selective activation of a single target muscle might be greater than that during co-activation of two or more target muscles, due to a possible absence of suppressive parallel input that is otherwise present during co-activation of other muscles.

One possible explanation for target muscle selectivity by CM cells might be that a given CM cell exerts stronger influence over some of its linked target muscles than others. This property could be useful for motor behaviors requiring more cortical, rather than spinal, control of specific target muscles; for example, muscles involved in fine-scale hand movements. In this case, observed muscle selectivity could arise when only some of the existing corticomotoneuronal linkages had been strengthened—through repeated co-activation of the CM cell and selectively-recruited target motor units—via the mechanism of Hebbian plasticity. To put it more concisely, the stronger linkages could have been the product of repetition of motor behaviors that recruited only a subset of the CM cells target muscles. Those that most often co-activated with the CM cell, through repetition eventually developed the strongest input linkages with the cortical neuron.

We observed counter-intuitive CM cell activation patterns most often when one or more of the dissociated target muscles showed relatively weak correlational linkage with the CM cell. That is, when the post-spike effects of the selected muscle(s) were comparatively small in amplitude and more difficult to distinguish from background EMG. In counter-intuitive examples, mean percent increases (MPIs) of post-spike effects relative to pre-spike baseline activity were 2.30%, 2.16% and 1.32% in FCR and 2.24%, 1.45% and 1.71% in FDS. While statistically significant, these MPIs are notably smaller than those measured in target muscles of activity scaling examples, which ranged between 8.27% and 2.83%.

Corticomotoneuronal cells 7 and 8 comprised two of the three cases of counter-intuitive spike patterns during the dual-muscle dissociation task (table 6.2). The two cells were

recorded using the same cortical wire, and each produced a distinct spike profile from which they were individually discriminated during force target tracking and dual-muscle dissociation tasks. Though differences in overall activity levels and disparate autocorrelation features argue against their common identity, the two cells did exhibit similar tuning preferences during target tracking. Cross-correlation spike histograms compiled from their spike trains revealed the two cells received input from a common source as indicated by a central peak. We believe that because CM cells 7 and 8 were correlated during dissociation conditioning, they should not be considered fully independent examples of counter-intuitive activity patterns.

Chapter 7

GENERAL REMARKS**7.1 *Significance for neural representations***

The dissociations between CM cells and target muscles were demonstrable through performance of an activity-driven operant conditioning task—its successful performance revealed the correlative relationship between the activity of CM cells and motor units onto which they project, is subject to volitional control. Forty-four unique cell-muscle pairs were conditioned, and of these, there were only two instances in which monkeys did not generate statistically significant deviations from co-activation levels observed during force target-tracking. The extent of CM cell-muscle activity dissociations were robust: parameters characterizing separation between forward (C+M-) and reverse (C-M+) dissociation groups approached ideal values, and, in several conditioned cell-muscle pairs, surpassed them. Neither cell identity nor muscle type determined the extent of these operantly-conditioned dissociations as most elements of these categories contributed throughout distributions of each dissociation measure. For the subset of twelve sessions in which statistically discernible mutual information values could be detected in force hold activities, mutual information between CM cells and their target muscles significantly decreased during reciprocal activation task components when compared to target-tracking patterns of those same cell-muscle pairs. These results reflect the decreased role of CM cell input during instances of activity dissociation with downstream target muscles.

7.2 *Implications for control of brain-machine interfaces*

Recent findings from parietal reach region have been interpreted to suggest that cortical activity signals recruited for movements of a monkey's natural motor repertoire are more favorable for controlling BCIs than more direct control strategies [48], specifically: each degree-of-freedom of a BCI controlled by an individual neuron, citing work of Moritz et.

al. (2008) as an example [76]. While results from our investigation do not refute the above conclusion, demonstration of robust activity dissociations between correlationally-linked CM cells and their target muscles provides strong evidence that the central nervous system, specifically the corticospinal pathway, is flexible enough to permit independent control at the level of single neurons regardless of their network connectivity. Tuning correlation between CM cells and their target muscles is often quite strong (figure 3.4) but can also exhibit counter-intuitive incongruities [70]. The fact that co-activity patterns of two monosynaptically-linked components within a neural circuit are subject to independent volitional control and can be rapidly and robustly dissociated suggests the gains in dimensionality of control, via direct assignment of degrees-of-freedom to individual neurons, is at least an equally viable alternative. One can also pose the question: what exactly are “natural”, or stable, cortical activity patterns when this study, and several others investigating the activity of CM cells during relevant motor behaviors, have revealed such striking variability in the cortical cell-muscle co-activation relationship under varying conditions [18, 78, 22, 105]. Other investigators posit that multiple configurations of synaptic strengths within the motor cortex network could underlie any single motor behavior [102]. Investigations in primate and cat models suggest the possibility that the corticospinal tract is functionally divided into a dual system: one that acts through phylogenetically old circuits alongside another that acts to circumvent these circuits to produce more fractionated movements [62, 124]. In this model, descending command from corticomotoneuronal cells comprise the evolutionary newer group and successful activity dissociation between CM cell-muscle pairs could be mediated through another parallel system that supplies compensatory input during reverse dissociations and suppressive influence during forward dissociations. Future work will be needed to determine the degree of flexibility between linked components in cortico-motoneuronal and other cortical circuits during learning of a BCI or complex motor task.

Part II

**OPERANT CONDITIONING OF MUSCLE AND CORTICAL
ACTIVITIES DURING CONSTRAINED AND FREE BEHAVIOR
USING ACTIVITY-CONTINGENT INTRACRANIAL STIMULATION**

Chapter 8

GENERAL DESCRIPTION

8.1 Introduction

8.1.1 Project overview

It has been shown that bursts of spike activity of motor cortex neurons can be operantly conditioned using food rewards and bio-feedback, as first demonstrated by Fetz (1969) [27] and Fetz and Baker (1973) [30]. It has remained an open question, however, whether monkeys can learn to control neural activity when intracranial electrical stimulation is employed as the sole source of reinforcement. For this investigation, we sought to operantly condition spike rates of motor cortex neurons, as well as electromyographic (EMG) activity recorded from proximal limb muscles, using activity-contingent, behaviorally-reinforcing, brain stimulation reward (BSR). We sought to condition these activities both in the training booth and as the monkeys moved freely about their home cages. First, we sought midbrain sites whose stimulation would support operant responding of a manual target-tracking task on which the monkeys had already been trained with applesauce reward. We recorded activity of single neurons with microwires chronically implanted in motor cortex and documented their activity modulation with the manual target-tracking task. Inspecting these task-related modulation levels, we set a rate-threshold for each cell candidate for conditioning. To reinforce instances of elevated cell discharge, spikes occurring above our assigned threshold rates triggered electrical pulse stimulation of the reinforcement site. This rate-contingent, spike-triggered (RCST) stimulation was typically made available for periods of 1 to 3 minutes separated by 3 to 10 minute periods of no stimulation. To check for conditioned changes, we compared firing rates between non-reinforced (NR) and reinforced (R) periods of the alternating conditioning schedule and also calculated time-series statistics on the sequence of R and NR period activity averages to detect temporal patterns across session responding.

8.1.2 *Project motivation*

Several questions motivated this project. We wondered if monkeys could gain direct and specific control of their cortical activity, even at the level of single neurons, without need of physical actuation (i.e. motor task and its associated task-related feedback). The non-restrained, free behavior paradigm expands in the means by which monkeys could learn to modulate their cortical activity. In addition, we wondered if monkeys could incorporate an augmented artificial cortico-striatal pathway into their normal brain functioning and if the artificial linkage could affect cortical activity patterns, motor function, operant responding and overall general behavior. Successful demonstration of BSR-mediated conditioning of cortical activity implies individuals have the potential to gain volitional control over their cortical activity without the need of extensive practice via activity-guided tasks. Multiple hour-long conditioning sessions permit investigation of learning, adaptation and habituation that occur over timescales much longer than the typical duration of most in-booth sessions; inspection of in-cage conditioning records could reveal these phenomena. Finally, BSR enables more temporally-precise (within several ms) reinforcement of selected spike patterns than food or water rewards whose delivery typically occurs over several hundreds of milliseconds. Millisecond-order temporal precision of BSR permits reinforcement of inter-spike intervals or autocorrelation values calculated from an ongoing spike train in real-time; a very difficult, if not impossible, feat to achieve using conventional food rewards.

8.1.3 *Background*

Brain computer interfaces permit long-term conditioning under non-restricted conditions

Until recently, traditional techniques for recording action potentials of single neurons in behaving animals have limited the scope of motor neurophysiology to investigation of constrained behavior. Typically, a primate sits in a chair with its head fixed and limbs restrained so that action potentials can be recorded as it performs highly trained, stereotyped movements. While this approach enables movements to be controlled and systematically varied, such constrained task-related movements differ from natural behavior. Repetition of the same task-movements may influence the neural representation of trained actions [85] and

neural-movement correlations established under particular task conditions may not hold under non-task conditions [14, 1, 54].

Jackson et. al (2007) demonstrated that an implanted autonomous recording system allows cortical neurons to be monitored while the animal exhibits a full repertoire of normal behavior, including sleep [54]. While unconstrained movements may be more difficult to quantify and may not sample the movement space systematically, an unconstrained paradigm allows the study of natural synergistic motor control that is not possible under restraint. In recent years, Brain Computer Interfaces (BCIs) have been used to engage learning and adaptation [16], induce plasticity changes [53], modify synaptic strength [84], control prosthetic devices [117, 16], reanimate musculature through device-routed cortical control [77, 93, 26], facilitate sensory and motor transformation [86], and study sensorimotor and cognitive skill formation [58, 40]. BCIs enable investigators to fully define functional circuits for action, an attribute of great investigatory and rehabilitative potential in neuroscience research. To our knowledge, this is the first investigation that employs BCIs to operantly condition changes in cortical spike rates by means of activity-dependent delivery of Brain Stimulation Reward (BSR) in primates. Recently published work in rodents reports BSR-elicited rate increases in prefrontal cortex neurons using a similar paradigm [121]. Employing rack-mounted instrumentation, the Neurochip or the Neurochip2-HV, we delivered rate-contingent, spike-triggered stimulation to an ICSS-sustaining site in the ventral striatum with the aim of rewarding increased spike discharge of the trigger neuron.

In the context of this project, the activity of cortical neurons may very well depend on environment. Differences in the degree or time course of conditioned changes in spike rate during free behavior, as compared to those established in the training booth, would suggest a contextual dependence. Behaviorally induced changes in firing rate during free-behavior could differ from rate changes conditioned in-booth due to several possible reasons; these include: the longer duration over which monkeys can receive reinforcement during in-cage conditioning sessions, lack of cues aimed to help focus monkeys' attention and the broader repertoire of behaviors that monkeys are free to emit in their home cage environments.

8.2 *Methods*

8.2.1 *Subjects*

Three male macaca nemestrina monkeys P, D and J (4-6 years old, weight 6.0, 5.6 and 4.0 kg) were used in this investigation. All surgical, training and handling procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

8.2.2 *Training*

Prior to surgeries, monkeys were trained to perform a center-out force-target-tracking (FTT) task in which isometric wrist torque controlled the position of a computer cursor on a screen. When the cursor entered a target and remained inside for a finite amount of time (1 second or less) a fruit sauce reward signaled completion of that trial. Between moves to peripheral targets, the monkey was required to return to rest position at the screen center. Target placement on the screen determined the direction and magnitude of torque production about the flexion-extension (F-E) axis required to complete each trial. The task used two peripheral targets, flexion (F) and extension (E) plus a central rest target (C) for successive trials. Peripheral targets were presented in random order at equal frequencies. Training was complete when monkeys moved directly from center to each of the two targets, and held it inside for at least 1 second. During experiments, the FTT task was performed daily to elicit task-related cell firing in motor cortex.

8.2.3 *Surgeries*

Surgical implantation of cranial microwire and cannulae arrays was performed during the same operative sessions for monkeys D and P. Monkey J underwent two separate procedures: the microwire array was implanted first for the purpose of other experiments while the cannulae array was implanted nine months later within the existing cranial chamber implant. In addition to cranial implant procedures, we subcutaneously implanted monkey P with three pairs of stranded stainless steel microwires penetrating triceps, biceps and deltoid muscles of the right arm. The subcutaneous wires delivered bipolar EMG to the cranial implant and Neurochip housed within it, for in-cage muscle-triggered BSR conditioning

sessions. All implantation procedures were performed under sterile conditions with monkeys under anesthesia.

Chapter 9

LOCATING AND CHARACTERIZING INTRACRANIAL SITES SUPPORTING SELF-STIMULATION BEHAVIOR

9.1 *Introduction*

9.1.1 *Intracranial self-stimulation as reinforcement*

With few exceptions, the vast majority of nonhuman primate research involving behavior has employed rewards in the form of food or water. Seminal studies by Olds & Milner (1954) and Olds (1958) demonstrated that rats would press bars and navigate mazes for Brain Stimulation Reward (BSR), and that BSR can be used to reinforce operant responding in place of more conventional food and liquid rewards [88, 87]. While the precise neural mechanisms underlying intracranial self-stimulation (ICSS) behavior are not well understood [56, 123], the reinforcing qualities of brain stimulation have been demonstrated in numerous behavioral investigations [110, 67, 10].

Several structures in the macaque brain support self-stimulation responding including the orbitofrontal cortex, lateral hypothalamus, amygdala, medio-dorsal nucleus of the thalamus and nucleus accumbens [12, 104, 75, 103]. These investigations employed simple bar-press tasks that required minimal motor precision or cognitive load. For this investigation, we initially sought reinforcement sites in the ventral tegmental area, but did not succeed in finding effective sites. Next, we chose the nucleus accumbens (NAc) as the locus of our search for ICSS-sustaining sites for several reasons, the first being its crucial involvement in processing reward-related information. Dopaminergic signals traveling along the mesolimbic pathway are likely essential to “stamping-in” of response-reward associations [123]. Secondly, unlike food and liquid rewards, electrical stimulation of NAc is not reduced by satiety [75, 103, 10]. Finally, comparatively lower current thresholds for ICSS responding have been observed in NAc than other ICSS-sustaining sites.

9.1.2 *Law of Effect model explains single-alternative operant responding*

In this investigation, we explored the quantitative relationship between response rate, in the form of target task force holds, and “amount” of stimulation delivered as reinforcement. Amount of stimulation can be varied by adjusting several independent parameters—we focused on pulse amplitude (current intensity), pulse frequency and number of pulses comprising the stimulation train—all of which vary the amount of reinforcement, r , paired with the operant response, R . In the subsequent description of the single-alternative Law of Effect model, we consider response rate R as a function of reinforcement magnitude r without distinguishing how r is adjusted. The parameterization findings that follow (results section 9.3.2 figure 9.3) demonstrate that regardless of choice of adjusted stimulation parameter, operant responding shows hyperbolic dependence on increasing ICSS stimulation intensity, characteristic of the single-alternative Law of Effect model.

In his seminal paper *On the Law of Effect* (1970), Richard Herrnstein describes a quantitative relationship between relative rates of responding and relative rates of reinforcement in subjects undergoing operant conditioning tasks [46]. The law implies correlative relation between behavior and environment, specifically: that an animal’s response rate R_i in an operant responding scenario is proportional to the amount of reinforcement delivered for that response, r_i . Mathematically, this can be expressed

$$R_i = \frac{kr_i}{\sum_{j=1, j \neq i}^n r_j + r_i} \quad (9.1)$$

where k is a constant of proportionality valued as the maximum possible responding, R_i is the level of responding in scenario i in which r_i levels of reinforcement were delivered. The sum in the denominator is the aggregate reinforcement associated with all other response alternatives available to the animal that may or may not have been accounted for by the experimenter. This term has important theoretical significance that will be addressed below. The above equation is valid for n concurrent schedules of reinforcement, for example: pellet delivery for every 3 presses of a left lever ($i = 1$) versus pellet delivery for every five presses of the right ($i = 2$).

The denominator in equation 9.1 contains the crucial aspect of the Law of Effect: “that the absolute rate of responding is a function of its reinforcement, but only in the context of the total reinforcements in the given situation.” [46]. Absolute responding R_i varies directly with the associated amount of reinforcement r_i , and varies inversely with reinforcement associated with other task alternatives (i.e. R_i where i does not equal j). When conditioning involves only one operant response, R_i in equation 9.1 becomes

$$R_1 = \frac{kr_1}{r_1 + r_e} \quad (9.2)$$

where R_1 and r_1 are the associated response-reinforcement pair for the single operant being conditioned and r_e is the “unknown aggregate reinforcement for other response alternatives” that comprises the sum in the denominator of equation 9.1. Herrnstein argued that no matter how stark the environment, the subject will always be exposed to distractions—other stimuli that “engage its activity and attention, even if those are no more than its own body, with its itches, irritations and other calls to service.” [46]. Competing demands for attention imply that at any moment of conditioning, the subject must choose whether to emit the operant response or an alternative. The combined total of all extraneous non-operant responses is represented as the r_e parameter in the single-operant Law of Effect model. More recent behavioral investigations have suggested slight modification to Law of effect for single-alternative operant responding such as

$$R_1 = \frac{kr_1^a}{r_1^a + \frac{r_e^a}{b}} \quad (9.3)$$

where parameters a and b correct for small deviations in observed responding compared to the purely hyperbolic function of equation 9.2 [8]. Such corrections did not appreciably affect our decisions to set stimulation settings for the purposes of cell and muscle conditioning experiments, thus we chose to ignore these parameters in model fits described below and presented in the results (section 9.3.2).

9.2 Methods

9.2.1 Placement of stimulating electrodes

To identify potential intracranial reinforcement sites, we co-registered a magnetic resonance image (MRI) and digitized brain atlas data to determine specific stereotaxic coordinates of prospective midbrain reinforcement loci. Monkey P underwent MRI scanning prior to surgical implantation. Monkeys D and J were of similar size and weights as atlas subjects, so MRIs were not deemed necessary. We selected coronal image slices of MRI data and a digitized brain atlas [80] located +3 mm rostral from the anterior commissure that contained the largest cross-section of the target region: nucleus accumbens (NAc). Using stretching/shrinking transformations, the digitized atlas image was morphed so that extremities of the map overlaid extremities of the brain tissue in the MRI slice. Since length scales of the MRI slice and atlas image were known, and the boundaries of the nucleus accumbens were identified in the atlas image, stereotaxic coordinates of the target locus could be measured relative to medial-lateral center and ear-bar zero.

Based on the location of the target locus, we determined intracranial positioning of the stimulating electrode(s) and the overlying array of cannulae for guiding them. We sought paths to the target locus (center of NAc), and surrounding region, that would not intercept major blood vessels or regions governing autonomic function. These were avoided by plotting a straight-line diagonal path 15 degrees lateral right with respect to the ventral dorsal axis in the right hemisphere. This course avoided electrode puncture of the dural venous sinus that resides along the longitudinal fissure. Intersection of the path with the animal's inner intracranial surface marked the desired location of array implantation in the coronal plane. Anterior-posterior position was taken to be the location of the MRI and atlas slices with respect to stereotaxic AP=0 (+3 mm). To counter the likely prospect of positioning error of electrode entry sites, we implanted an array of cannulae (10×10 mm grid of 16 parallel oriented cannulae spaced 1 to 1.5 mm apart) centered at the point of entry described above. Thus, in cases of slight angle misalignment or unintended shifts in lateral position of the center guide tube during surgical implantation, the target locus might still be reachable by inserting an electrode in one of the neighboring cannulae. Following

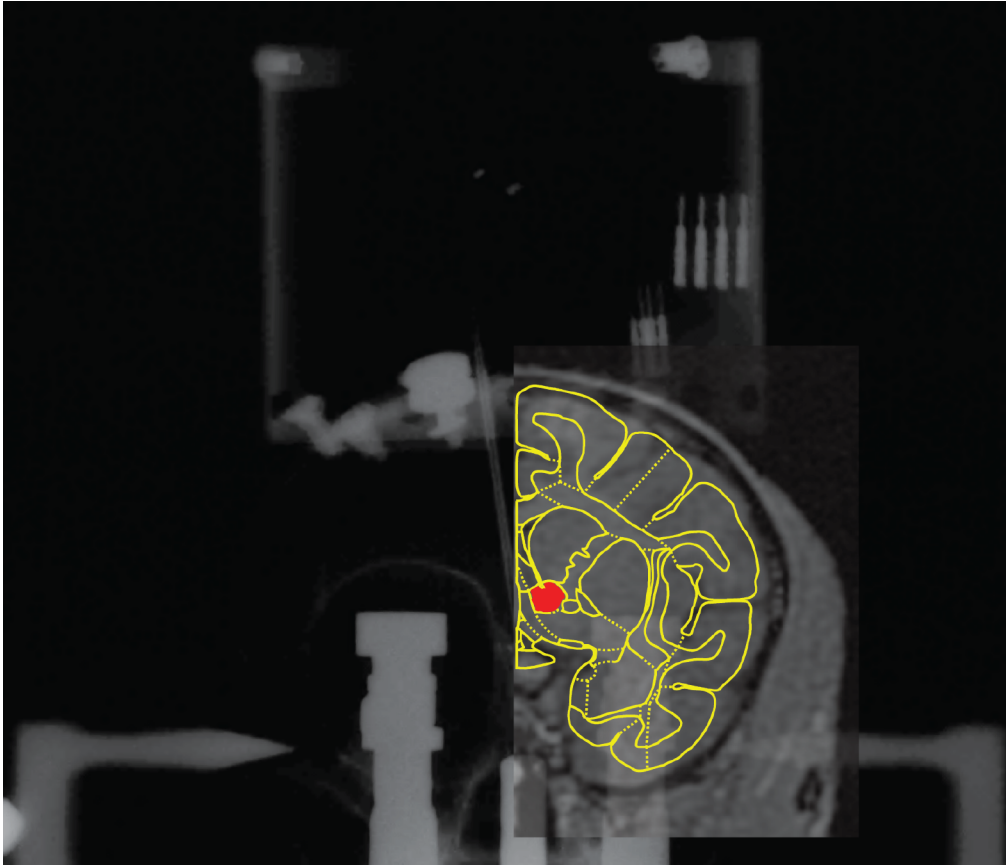


Figure 9.1: Co-registration of cranial X-ray, MRI and brain atlas images. A coronal map of the *M. Nemestrina* brain was morphed and positioned according to Monkey P's brain tissue boundaries discernible in an MRI image at the same anterior-posterior axis coordinate. The two were then positioned over a scaled X-ray image to show relative locations of the chamber and housed hardware. These served as landmarks for stereotaxic positioning the surgically-impanted cannulae array. The array guided and served as a platform for chronically implanted stimulating electrodes targeting nucleus accumbens and surrounding striatum (in red).

implantation, unused cannulae were occluded with stylets and sealed with silastic to block these vectors for potential cranial infection.

9.2.2 Implanting an array of cannulae for stimulating electrodes

In each of the three monkeys, we performed a craniotomy to accommodate the dimensions of the cannula array to be implanted. The location of each craniotomy was determined for each animal using the co-registration process described in methods section 9.2.1. Grooves machined into side surfaces of the array block promoted mechanical adhesion of the array to surrounding acrylic (base of chamber). Position and angular orientation of the array was guided using carriers calibrated to stereotaxic zero. Once positioned, any open space separating craniotomy and array boundaries was packed with antibiotic-infused gelfoam to fight potential pathogens. We built an acrylic base around the implantation site and surrounding cranial screws. Acrylic served as the base for a cylindrical titanium chamber placed to surround and protect both the microwire and cannula array implants. After acrylic had set, the stereotaxic carrier was removed and cannula-length stylets were placed in all guide tubes. The entire protruding surface of the array was sealed in silastic. Electrodes were inserted post-op in the laboratory following cold-sterilization of the chamber interior and electrodes.

9.2.3 Striatal site reinforcement verification procedure

To identify intracortical brain sites that sustain self-stimulation responding, we compared response rates during putatively reinforcing session periods (when each response triggered stimulation to a candidate reinforcement site) against non-reinforced periods (times when no stimulation was delivered, regardless of responding). During reinforced blocks of the schedule, each completed flexion or extension target hold triggered stimulation. Trains of symmetric biphasic square wave current pulses across poles of Rhodes SNEX-100 concentric bipolar electrodes targeting nucleus accumbens and surrounding striatum. In addition to step-tracking task auditory cues, a low frequency tone was generated during reinforced periods as a discriminatory stimulus. This tone was not present during non-reinforced

periods or time-outs. Candidate sites were declared “positively reinforcing” when monkeys performed wrist force target-tracking at significantly greater frequency than during non-reinforced periods.

Durations of reinforced periods, non-reinforced periods and time outs were randomly chosen; each drawn from uniform distributions spanning a specified time domain. Reinforced and non-reinforced periods: between 1 to 2 minutes, Time outs: between 30 and 90 seconds. Period durations were randomized to reduce the Monkey’s anticipation of schedule transitions (i.e. rewards following NR-R transitions and lack of rewards following R-NR transitions). The sequence of reinforced and non-reinforced schedule blocks were alternated (e.g. R,TO,NR,TO,R,...) or shuffled (e.g. NR,TO,R,TO,R,TO,NR,TO,NR,...) depending upon the Monkey’s familiarity with the paradigm.

9.2.4 *Fitting model curves to response rate data*

The Law of Effect model predicts a hyperbolic relation between the absolute responding and the amount of reinforcement paired with the operant response being conditioned (equation 9.4). The relationship also depends on two fixed parameters: k , the maximum possible rate of responding and r_e , the aggregate reinforcement for all non-operant alternatives. The behavioral apparatus of our investigation, the force target-tracking task, imposes a ceiling on k since delays were incorporated between intra-trial events (e.g. delays between subsequent target presentations, movement times) as well as in-target required hold times. For parameter r_e however, we have no a priori information regarding its value since a multitude of other responses are available to the monkey at any given moment, about the reinforcement value for each we can only speculate. Hence r_e is a fit parameter whose value can be adjusted to optimize fit of the single-operant Law of Effect model to empirical data. We incorporated a third parameter into the Law of Effect model, r_{th} , to account for the threshold properties of intracranial stimulation. Arvanitogiannis and Shizgal (2008) demonstrated that there are thresholds at which BSR supports self-stimulation responding in rats for pulse amplitude and frequency parameters [5]. The threshold-sensitive Law of Effect model used in this investigation is

$$R(r) = \frac{k(r - r_{th})}{(r - r_{th}) + r_e} \quad (9.4)$$

Unlike polynomial functions, equation 9.4 is not a linear combination of model parameters. To correctly optimize model parameters k , r_e and r_{th} we employed nonlinear regression incorporating weighted least squares. Weights for each average of data-points were calculated as the reciprocal of the range of their confidence interval. Confidence intervals were calculated using a simple percentile bootstrap method that incorporated Monte Carlo approximation via $R = 499$ random draws, with replacement, of each parameter-categorized distribution of response rates (gray points in figure 9.3).

9.3 Results

9.3.1 Task-triggered accumbens stimulation reinforces target-tracking behavior

We tested the efficacy of candidate ICSS-sustaining sites by measuring monkeys' rate of responding in a manual target-tracking task on which they had been well trained with applesauce reward. Trains of 25, 1 mA pulses delivered at 50 Hz upon completion of 1 second long force holds reinforced further responding at optimal electrode positions. As depicted in figure 9.2, target-tracking responding occurred at regular frequency (blue tick marks depict individual responses) during reinforcement (R) periods when target completions triggered trains of brain stimulation reward (BSR). Rates during R periods were significantly elevated ($p < 0.001$) compared to interleaved periods during which no stimulation was delivered (NR-periods). Compellingly, at the onset of R periods, target-tracking responding often quickly returned to that of the previous R-period, despite the interval of negligible, non-reinforced, responding that separated them.

9.3.2 Rate of target-tracking responding increases monotonically as a function of increasing BSR intensity

In order to determine stimulation settings suitable and effective for conditioning cortical spike activity, we observed rates of force target-tracking responses across a range of values

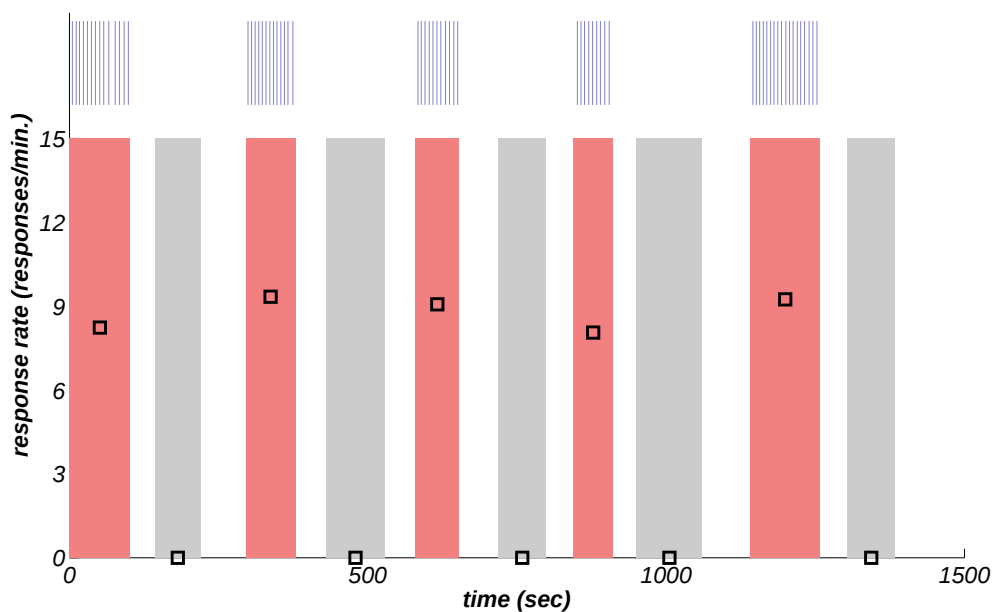


Figure 9.2: Average response rates (black squares) over reinforced (pink) and non-reinforced (gray) periods of the wrist force target-tracking task. Durations without backgrounds illustrate task-off periods. Blue ticks (*top*) mark trial completions. During reinforced (R) periods, each completed flexion or extension hold triggered behaviorally-reinforcing brain stimulation (BSR). To reduce anticipation of transitions in the task schedule, R and NR period durations were made variable, and were randomly chosen from a uniform distribution spanning 1 to 2 minutes on schedule generation. Task-off durations ranged between 30 sec. to 1 min. Typical FTT task response rates for applesauce reward in this animal (Monkey D) ranged between 10 and 13 responses per minute.

for three parameters: current intensity, pulse frequency and number of pulses per stimulus train. In these investigations, when each of these parameters was varied, the other two remained fixed. Fixed values for current intensity, pulse frequency and number of pulses per train were 1 mA, 50 Hz and 25 respectively. For each varied parameter, the set of values chosen to span the desired range were repeated 10 times, delivered in a randomized sequence, to prevent occurrence of possible “history effects” that might have otherwise arisen from repeated patterns in successive stimuli.

Figure 9.3 depicts dependence of target-tracking response rate on each varied stimulus parameter in two monkeys P and D. Specifically, we systematically varied each of three parameters—current pulse amplitude, pulse frequency and number of pulses per stimulus train—while holding the other two at fixed values: 1 mA, 50 Hz and 25 respectively. In all cases, nonlinear-regression fitted curves of the single response Law of Effect model well characterized observed response rates as they increased with increasing values of the tested stimulation parameter. Table 9.1 summarizes fit statistics for each of the plots. Curves indicate that ~80-90% of maximal responding (horizontal asymptote of each plot) occurred for stimulation parameters 1 mA and 50 Hz.

Varied parameter	Monkey	k	r_e	r_{th}	MSE
Pulse amplitude (mA)	P	22.45	1.47	0	0.15
	D	17.64	0.13	0.35	0.31
No. pulses per train	P	16.65	7.31	6.75	0.16
	D	23.72	37.00	0	0.04
Pulse frequency (Hz)	P	14.89	4.00	6.05	0.35
	D	17.74	6.86	7.32	1.46

Table 9.1: Single response Law of Effect model fit parameters and statistics. The fit parameters are: k , the maximal response rate asymptote (responses/min.), r_e , the aggregate reinforcement for all non-operant responses, and r_{th} , the threshold level, or lowest value at which the varied stimulus parameter supported self-stimulation. MSE: mean squared error of the model fit using non-linear regression.

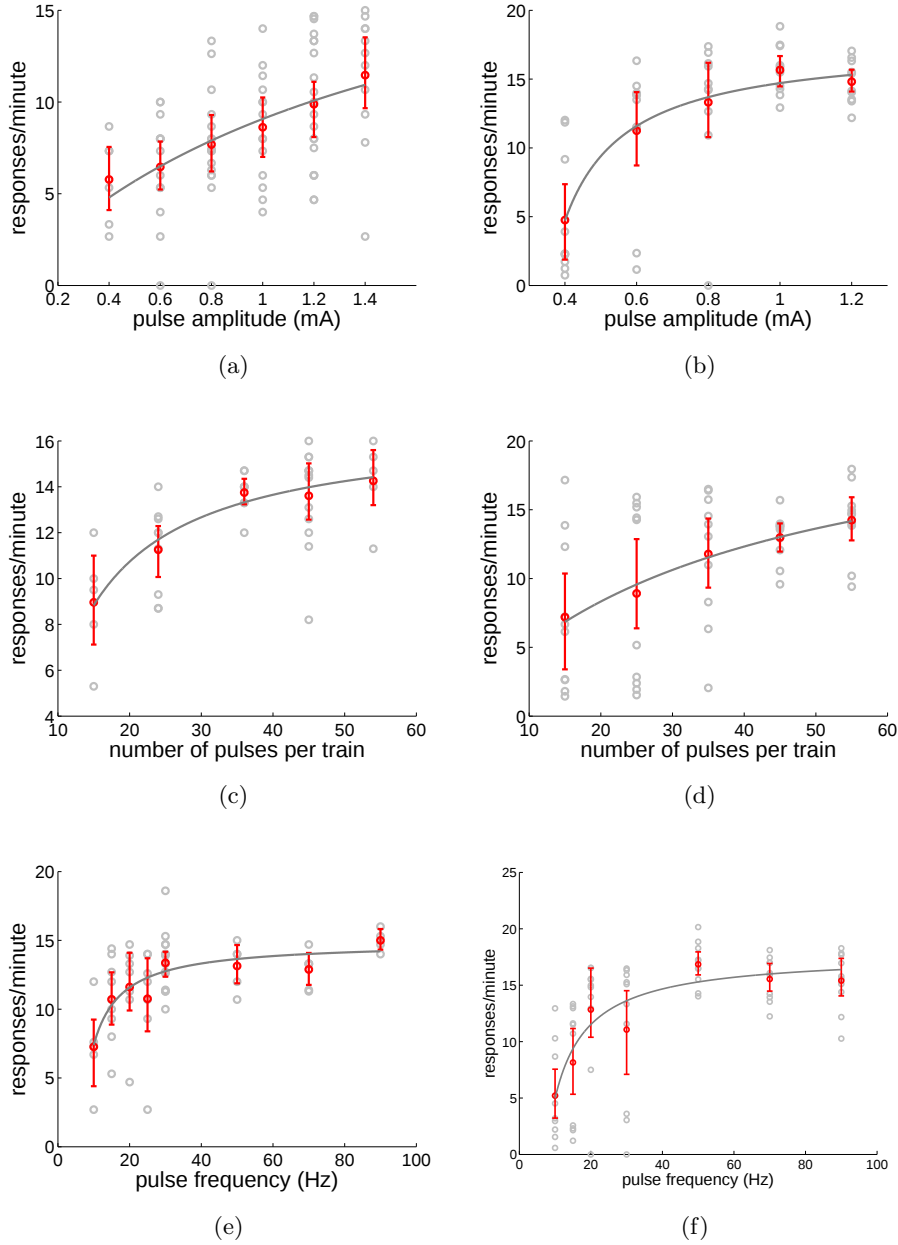


Figure 9.3: Force target-tracking response rates (gray circles) and response rate means and standard errors (red) are plotted as functions of each varied stimulation parameter: current intensity (*top*), pulse frequency (*bottom*), and number of pulses (*middle*). Response data from monkey P comprises the left column while plots on the right illustrate target tracking response dependence of monkey D. In these experiments, each of the three stimulation parameters was varied while the other two remained fixed. The fixed values of the stimulation intensity, pulse frequency and number of pulses per stimulation train were 1 mA, 50 Hz and 25 respectively. Continuous gray lines depict response rates predicted by regression-fitted Law of Effect model for single-alternative responding [46], [69].

In subsequent conditioning experiments, we set up spike-to-stimulation mapping such that bursts of elevated spike rates would trigger pulse trains at frequencies approaching 50 Hz. For slow cells (e.g. <10 Hz), multiple stimulus pulses (delivered at 50 Hz) were triggered for each RCST stimulus event. We set pulse amplitude to 1 mA for all conditioning attempts.

9.4 Discussion

9.4.1 *Precise mechanisms of Deep Brain Stimulation are not known*

In this investigation, we aimed to reinforce elevated rates of cortical cell discharge through delivery of rate-contingent, spike-triggered stimulation to ventral striatum. While self-stimulation responding using BSR is a well-established phenomenon across species, the physiological mechanisms underlying the effects of Deep Brain Stimulation (DBS) are not well-understood. Ranck et. al. (1975) found that myelinated axons are the most excitable neural elements and that their excitability depends nonlinearly on stimulus pulse width and amplitude [95]. Cell bodies are also activated by electrical stimulation but comparatively less than myelinated axons. One hypothesis suggests that stimulation from an electrode blocks somatic activity in proximal neurons, and only induces sub-threshold activation in distal cells, leaving myelinated axons and somata of intermediate cells the most likely to be affected. In this case, mono- and poly-synaptically connected brain structures would be activated as well. If this hypothesis is correct, the effects of stimulation that we delivered in our investigation were probably not confined to accumbens. In addition, the volume of the region surrounding the electrode tip is likely not strictly cylindrical or spherical but the shape varies depending on position of the electrode and properties of the surrounding tissue.

Presently it is unknown whether DBS inhibits or excites neurons and there are two competing principles possibly involved in the mechanism: synaptic inhibition and depolarization blockade [59]. For synaptic inhibition, electrical stimulation activates axon terminals that make inhibitory connections with neurons near the tip of the stimulating electrode; the nearby neurons are activated post-synaptically via neurotransmitter release from terminals of the activated axons. Depolarization blockade would oppose the above effect whereby

voltage-gated currents, activated by the stimulus, block neural output in the vicinity of the stimulating electrode. Neither of these two principles explains how stimulus-induced activity arises in distal axons. Evidence of DBS-elicited neurotransmitter release is conflicting and it appears that DBS activation of distal regions is mostly independent of local tissue effects.

The efficacy of DBS depends on a number of factors: 1) physiological properties of brain tissue—types of surrounding neurons and glial cells and their voltage sensitivity, 2) parameters of the electrical stimulation—width, amplitude, shape and frequency of pulses; parameters that are usually determined through trial and error, and 3) the geometric configuration of neural elements relative to the electrode—their proximity and orientation. With so many determinant factors of DBS efficacy, we employed a search strategy to locate subcortical sites would support robust self-stimulation responding. In our search, tangential location and depth of the electrode were systematically adjusted until monkeys would perform the force target-tracking task for stimulation in place of fruit sauce rewards. To set the stimulator, we used effective parameters (width, amplitude and frequency) that had already been discovered in previous work (Bowden D., personal communication, 2006), [42].

9.4.2 Complicated role of dopamine in ICSS behavior

Axon terminals of the medial forebrain bundle release dopamine within the nucleus accumbens on receipt of unconditioned rewards [122, 108]. Further, stimuli that are normally rewarding, such as food, water, several drugs of abuse and even stimulation of the medial forebrain bundle itself, are rendered ineffective as reinforcers in animals given dopamine antagonists [119]. Our use of spike-triggered DBS to sustain self-stimulation responding very likely evokes dopamine release during BSR events, so we will examine its possible role in ICSS behavior here. Both the priming and rewarding effects of BSR seem to be dopamine-dependent, at least partially. It should be noted that many investigators preferentially select the mesolimbic dopamine system and its terminal field in NAc over other dopamine field regions (i.e dorsal striatum, amygdala and orbitofrontal cortex) due to its association with locomotion; a requirement for operant responding in most experiments.

Under the dopamine hypothesis of reinforcement, the presence of dopamine serves to ‘stamp-in’ stimulus-reward associations in operant conditioning tasks. The hypothesis is supported by the finding that animals fail to learn operant tasks if training takes place while dopamine function is impaired. In the context of this project, ICSS-evoked dopamine release might serve to quicken acquisition of the cell activity-to-stimulus association compared to more conventional rewards. A second hypothesis, the dopamine theory of reward, asserts that dopamine contributes to pre-reward arousal; that dopamine effectively boosts motivation to perform the operant task. Rewarding stimuli that meet tissue need or hormonal level have an “energizing” effect on behavior and increase the probability of response initiation [123]. Proponents of the hypothesis cite decreases in response latency and increases in speed of an animal’s approach to a BSR-triggering lever following post-response stimulation. Also, the effect of priming—the precipitation of a learned response habit following receipt of an unearned reward—to elicit operant responding as evidence in support of the reward hypothesis as well. Though there are other contributors to priming behavior, dopamine amplifies the effect of pre-reward motivation [92, 101].

An alternative hypothesis, incentive motivation, explains the role of dopamine as conferring incentive-motivational value on stimuli that predict rewards. Such incentive-motivational stimuli are essentially the learned predictors of reward; incentive stimuli are effective in the present due to past association with dopamine release evoked by a primary reinforcer. When the dopamine system is blocked in animals that have already learned the task, established reward predictors lose their incentive-motivational efficacy. In addition, when an animal is tested for any length of time in the absence of a reinforcing stimulus (e.g. food reward, BSR), the ability of the incentive-motivational stimulus to elicit a response and energize the animal is extinguished. Dopamine not only helps establish incentive-motivational value of paired unconditioned stimuli during initial conditioning, it also helps to maintain that value through periodic reinforcement. Administration of dopamine agonists before responding can potentiate the incentive-motivational effects of conditioned stimuli (CSs) and can retard the extinction that normally develops when CSs are repeated in the absence of paired rewards [123].

Though dopamine is likely involved in mediating self-stimulation behavior, its release

dynamics [9, 41, 56] and the task-related phasic discharge of dopamine neurons [107] suggest the dopamine signal may actually reflect error in predicted reward as suggested by the temporal difference learning model [114], rather than reward delivery itself. This functional interpretation of dopamine release does not explain how ICSS behavior is sustained after it has been learned.

Schultz (1998) explains that in contrast to natural activation of dopamine neurons, dopamine-related self-stimulation does not activate all of the parallel neuronal systems responsible for coding different aspects of reward [106]. Information concerning liquid and food rewards is processed in a number of brain structures that do not contain dopamine neurons, including the dorsal and ventral striatum, sub-thalamic nucleus, orbitofrontal cortex and several others [82, 83, 4, 108]. In addition, when dopamine release is evoked as unconditional reinforcement, as opposed to being naturally elicited, the signal does not convey an error in reward prediction [106]. While predicted rewards are not reflected in the dopamine-release signal, evidence suggests that they are processed by the non-dopaminergic cortical and sub-cortical systems mentioned above. Apicella et al. (1991) found that a significant proportion of macaque nucleus accumbens neurons modulated their activity during task-contingent delivery of juice rewards [4]. Perhaps these neurons act to sustain learned ICSS behavior as documented in ICSS studies without an increase in extracellular dopamine in the nucleus accumbens [72, 60].

Chapter 10

CONDITIONING MUSCLE ACTIVITY PATTERNS DURING FREE BEHAVIOR

10.1 *Introduction*

Before attempting to condition cortical activity, we first sought to condition an operant behavior in-cage using intracranial brain stimulation as the paired reinforcer. Muscle EMG patterns were a logical candidate operant for several reasons. First, EMG signals are relatively easy to obtain compared to cortical single unit activity signals that require chronically-implanted, transdural electrodes. Second, patterns of EMG activity are strongly correlated with specific movements which would likely aid acquisition of the operant task. Thirdly, EMG activity spans a wide dynamic range, scaling monotonically with force of contraction, thus enabling discrimination of select patterns from which discrete BSR pulses can be triggered. Finally, to condition EMG activity patterns, we could employ a very similar operant-to-reinforcement mapping as we planned to use in future cortical spike-triggered BSR conditioning sessions. Though in-cage conditioning of muscle activity operants was a preliminary, not our primary aim, we gained valuable insights about reinforcement scheduling and across-session patterns in operant responding that would later guide cortical unit conditioning sessions.

10.2 *Methods*

10.2.1 *Surgical EMG implantation*

In monkey P, pairs of EMG wires were implanted subcutaneously in three proximal muscles of the monkey's right arm: the biceps brachii, triceps brachii and lateral deltoid. We chose to condition muscles due to their involvement in movements that the monkey routinely performed on his return to his cage following his daily training sessions. The EMG wires were routed subcutaneously around the shoulder, up the back and neck and terminated into

connectors located inside the cranial chamber for signal processing by the Neurochip.

10.2.2 Neurochip and Neurochip2-HV deliver activity-contingent stimulation in-cage

The Neurochip is an autonomous, battery-powered, computational device programmed to conduct cell and muscle activity conditioning while monkeys moved freely about their cages [68]. It is capable of simultaneously discriminating cortical cell and EMG activity patterns using dual time-amplitude window discrimination while delivering stimulation triggered from discriminated events in real time. We set the Neurochip and Neurochip2-HV to implement the alternating R/NR reinforcement schedule as was performed for in-booth conditioning using rack-mounted equipment. Depending on battery life and activity rates, either from EMG modulation frequency or cell spike rates, the Neurochip could record and stimulate for up to 24 hours in-cage (48 hours for the Neurochip2-HV). The Neurochip2-HV is an in-house, second-generation extension of the original Neurochip that benefits from improved storage, processing and stimulation intensity capabilities [126].

10.2.3 Time series analysis detects acquisition of the alternating R/NR task

Monkeys undergoing the alternating R/NR conditioning paradigm produce a set of ordered observations—specifically, a temporal sequence of activity averages, one for each schedule interval—each of which has an associated observation time. To detect temporal structure within a series of activity measurements, we computed autocorrelation and von Neumann ratio test statistics on the empirically-observed sequence and compared it to distributions of the same statistics calculated after the series has been randomized R times. Our use of time series randomization is justified here because statistics of the activity signals being conditioned (i.e. EMG modulations and later cell spike events) obey the interchangeability requirement: that is, the mechanism generating the data is such that any observed value is equally likely to have occurred at any position in the series. Serial correlation and the von Neumann ratio measure temporal structure in time-series data—we calculated both, as well as their statistical significance compared to random, for each example session analyzed.

Serial correlation

In a time series, data points that are independent in time give no information about the next value that will occur. They may give information about the probability distribution from which they were sampled, but they give no direct information about the next value. Alternatively, points correlated (or anti-correlated) in time give information that can be used to predict the next data point in the series. Autocorrelation, informally stated, measures similarity between observations in a series as a function of the time lag between them. It is obtained by cross-correlating a signal with itself to detect repeated patterns. Data points positively autocorrelated in time are more like their near neighbors in the series and less like points further away in time while negatively autocorrelated data points are less like their near neighbors and tend to jump from high to low to high from sample to sample. When employing the alternating R/NR schedule, strong negative autocorrelation between neighboring rate averages in the series would indicate acquisition of the activity-driven conditioning task. The autocorrelation at lag k is the correlation between x_i and x_{i+k} , which can be expressed mathematically as

$$r_k = \frac{\sum_{i=1}^{n-k} \frac{(x_i - \bar{x})(x_{i+k} - \bar{x})}{n-k}}{\sum_{i=1}^n \frac{(x_i - \bar{x})^2}{n}} \quad (10.1)$$

where $\bar{x}$ is the mean of all n datapoints in the time-series.

von Neumann ratio

The von Neumann ratio tests for the presence of auto-correlation in a time-series. More specifically, it tests if sequential data points in a series are random against the alternative that it was generated from a Markov process—one of the simplest alternatives to randomness—whereby a given data point in the series depends solely on the data point that preceded it, but no other points before that. Markov processes are “memoryless”, and are expressed

$$x_i = \tau x_{i-1} + \epsilon_i \quad (10.2)$$

where x_i is a series of sequential samples, τ is a constant and ϵ_i values are independent random variables with mean zero and constant variance [64]. With the aim of detecting a Markov processes, the von Neuman ratio v is calculated from a series of sequential samples x_i using

$$v = \frac{\sum_{i=2}^n (x_i - x_{i-1})^2}{\sum_{i=1}^n (x_i - \bar{x})^2} \quad (10.3)$$

where i indexes the location of the sample in the sequence and $\bar{x}$ is the group mean. For a random, strictly Markovian series, the von Neumann ratio takes a value of 2; indicating no auto-correlation. Positive serial correlation between successive values yield ratios $0 < v < 2$ while a series with successive values consistently different from each other will fall in the range $2 < v < 4$ [65]. A successful conditioning session using the alternating R/NR schedule would have a statistically significant von Neuman ratio greater than 2, indicating anti-correlation between NR period rate means and their R period neighbors. We use randomization testing to evaluate the statistical significance of v whereby the ratio calculated from the observed series is compared against a distribution of ratios calculated after the series is randomly permuted.

10.3 Results

10.3.1 Upper limb muscle activity reinforced during free behavior using contraction-contingent BSR

After finding sites that sustained robust self-stimulation responding via the target-tracking task, we tested the in-cage paradigm using EMG activity as the operant. Discrimination time-stamps mark detection of large biphasic patterns in the multi-unit EMG signal indicating muscle contraction. More frequent discrimination events indicate more intense contractions as prevalence of large-amplitude components in the EMG signal increases with increased contractile intensity. In the above depicted sequence of time-averaged means, rates of phase-discriminated EMG (figure 10.1) evoked rate increases during reinforcement periods compared to non-reinforced intervals that separated them. During bicep condition-

ing, strong, statistically significant anti-correlation occurred between neighboring averages as measured by serial correlation ($\Delta x_1 = -0.88$, $p < 0.002$) of the alternating R/NR schedule while next-one-over averages comprised of either all R means or all NR means showed significant positive correlation ($\Delta x_2 = 0.76$, $p < 0.002$). Significant, but smaller, correlation occurred during in-cage tricep conditioning as well ($\Delta x_1 = -0.82$, $p < 0.002$, $\Delta x_2 = 0.67$, $p < 0.002$). Our chosen alternating statistical measure, the von Neumann statistic, computed for these two sessions also indicate temporal structure significantly different than random for both muscles conditioned (bicep: $v = 3.74$, $p < 0.002$ and tricep: $v = 3.63$, $p < 0.002$).

10.3.2 Peri-transition bicep activity reveals associative learning across in-cage conditioning session

Further inspection of intra-period activity averages (figures 10.2 and 10.3) reveal that progressive increases in contractile responding occurred over the course of conditioning: comparatively low and slow increases during the first 6 hours, moderate during the middle period, with greatest and fastest increases during the last 6 hours. The smoothed-raster in figure 10.3 confirms that the progression is not an artifact of how these peri-transition sweeps were grouped, but an actual phenomenon with relatively long lags separating NR-R transitions and onsets of increased density in early sweeps, with shortest lags and greatest intensities near the end.

An intense, brief increase in EMG responding occurred just following R-NR transitions during the first and middle thirds of conditioning as shown in peri-transition rate averages (figure 10.2) and raster plots (figure 10.3). The transient increase subsided during the last third of the bicep conditioning session. We suspect this increase to be extinction bursting consistent with classical models of operant responding as the schedule dependent phenomenon itself extinguished over the course of conditioning. In summary, changes in time-average and intra-period responding over the course of conditioning likely indicate learning of the conditioning paradigm with the monkey's increased exposure to the alternating R-NR schedule.

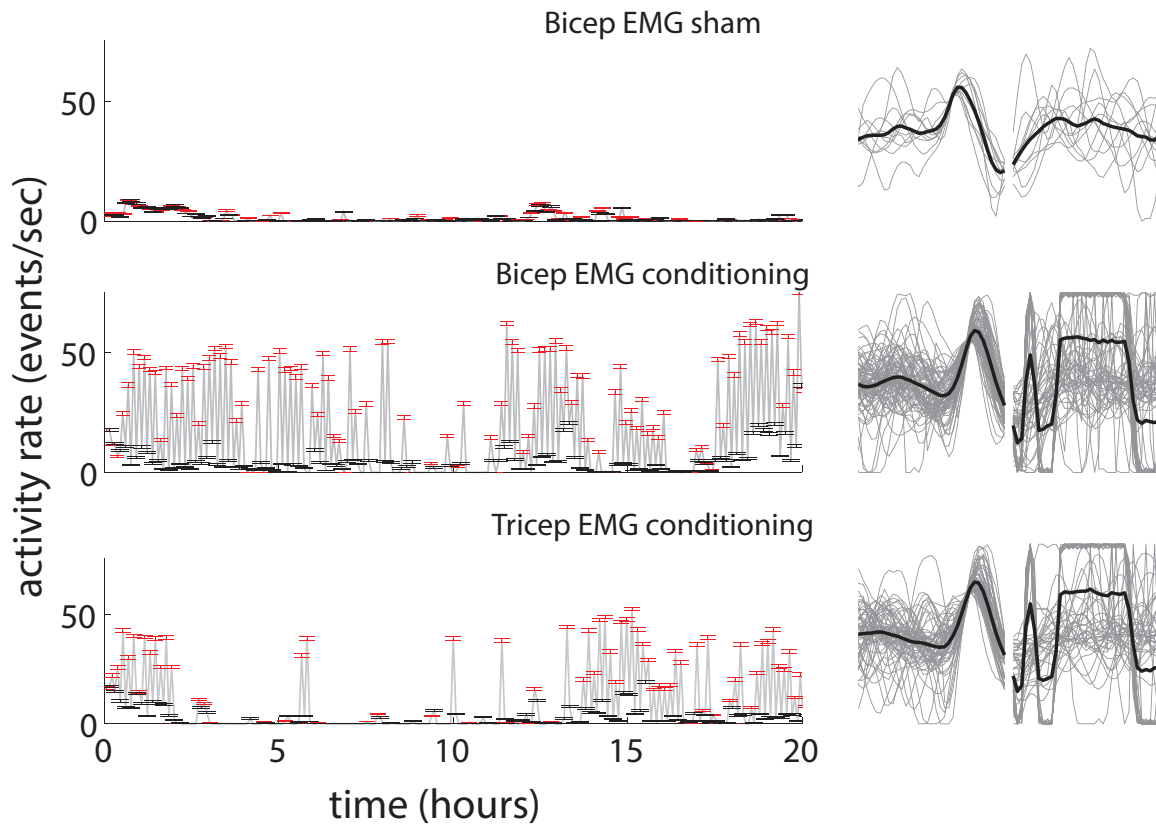


Figure 10.1: Contractions-contingent BSR reinforces muscle activity increases in-cage. *Top-left*: Baseline session in which no stimulation was delivered during either pseudo-reinforced periods (red) or non-reinforced (black) periods. Each data-point indicates mean EMG activity over 5 minutes, and surrounding whiskers mark standard error boundaries. *Middle* and *Bottom-left*: contractions-contingent current pulses delivered to accumbens during R-periods. *Right*: samples of EMG profiles that triggered stimulation over each 20-hour session (gray), and their averages (black). Accepted biphasic patterns occupy the first half of these example sweeps, while artifacts from triggered stimulation events can be seen in the last half.

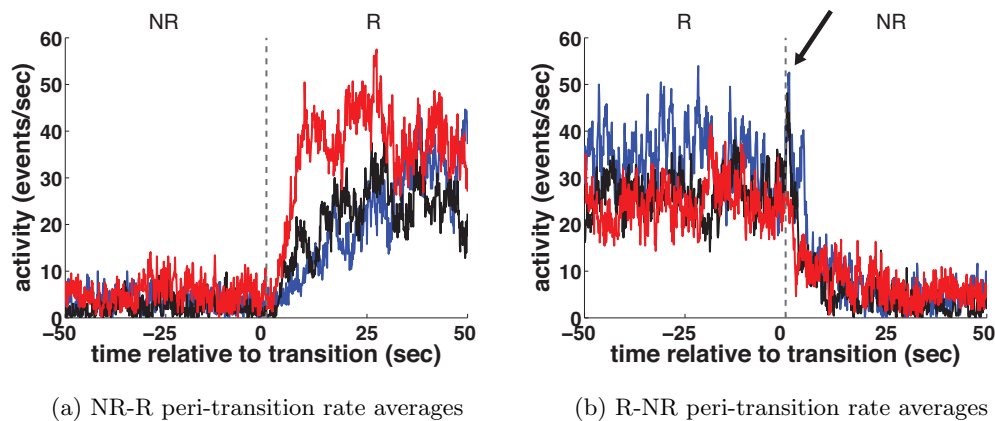


Figure 10.2: Peri-transition muscle activity reveals associative learning across in-cage conditioning session. Averages of bicep EMG rate surrounding NR-R schedule transitions (*left*) and R-NR transitions (*right*). Activity increases across NR-R transitions above illustrate that muscle activity rose more rapidly in the average of the last third of the transitions (red) compared to the average profiles from the first third (blue) and middle third (black). This suggests the animal learned to associate biceps activity with reinforcing brain stimulation over the course of the 20-hour conditioning session. The rate trace across the initial third of R-NR schedule transitions shows a transient increase in muscle activity immediately after termination of reinforcement (arrow), characteristic of extinction behavior. This post-transition peak disappeared in the last third of the session.

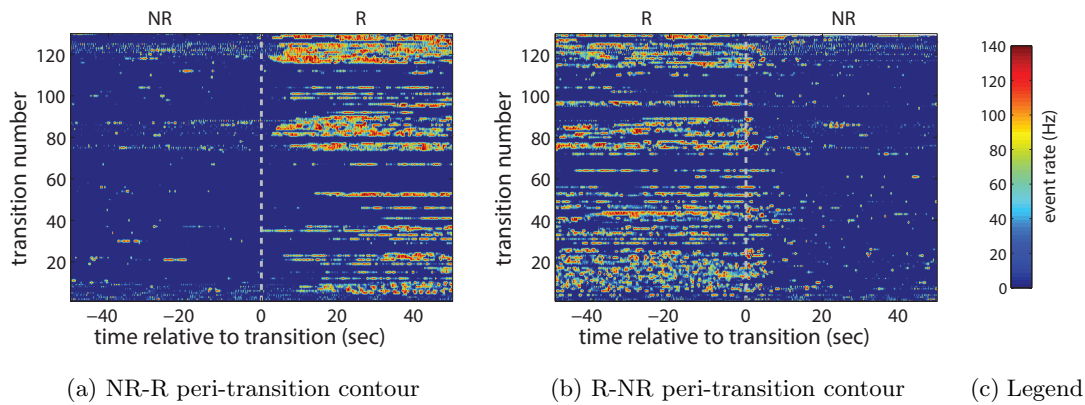


Figure 10.3: Peri-transition biceps activity during the 20-hour conditioning session. In the two plots, the abscissa measures time relative to transitions in the alternating reinforcement schedule, i.e. NR-R (*left*) and R-NR (*right*). Ordinates count the transitions over the course of the 20-hour session. For a given transition, at a particular instant in time relative to transition, the plotted color indicates the rate of bicep EMG activity (see scale). Notice in NR-R transitions, bicep contraction begins earlier during later transitions as compared to earlier transitions indicating acquisition of muscle-brain stimulation association. Across R-NR transitions, contractile activity continues for longer after the stimulator is shut off during early transitions as compared to later transitions (extinction).

Chapter 11

CONDITIONING SINGLE-UNIT CORTICAL ACTIVITY USING RATE-CONTINGENT, SPIKE-TRIGGERED INTRACRANIAL STIMULATION

11.1 *Introduction*

11.1.1 *Reinforcement during free behavior*

Conditioning cortical single-unit and muscle activity in the cage requires a means of delivering reinforcement that is temporally precise, since durations of cell bursting events may be short-lived and difficult to distinguish without explicit feedback. For this reason, we delivered electrical current pulses to a self-stimulation sustaining site in the mesolimbic reward pathway as a means to rapidly reinforce instances of elevated cell discharge. A single pulse or series of pulses were triggered from spike events occurring when the real-time spike rate rose above some threshold value. Timing theory [39] suggests that the most rapid acquisition of elevated spike-rate behavior will occur with brief “presentations” of the conditioned stimulus, for example, auditory clicks on cell-triggered stimulation events, and comparatively longer periods separating successive reinforcements.

11.1.2 *Previous work: Contrasting against Hiatt, (1972)*

In his 1972 doctoral dissertation, David Hiatt, a student of James Olds, attempted to condition increases in single-unit activity using burst-triggered BSR in rats [47]. The investigators sought non-movement-related cells in hippocampus, cerebellum, midbrain and superior colliculus as candidates for conditioning. Rats had been pre-trained, using food rewards, to modulate burst rate prior to cell-triggered BSR sessions. Discriminative stimuli were incorporated in conditioning for all sessions for all animals. While our investigation also employed activity-dependent delivery of BSR, our investigation differed from Hiatt’s in several other respects: 1) we used macaques rather than rats, 2) we preferentially selected

motor cortex neurons that modulated their activity with manual force generation, 3) BSR, in the form of single pulses, was triggered from single-unit spike events, often with an imposed rate-threshold, rather than trains of pulses triggered from cell spike bursts, and 4) except for cases when we were able to condition the same cell over repeated days, monkeys had no practice in modulating a candidate cell’s activity patterns prior to BSR conditioning sessions. We ran sessions both with and without discriminative stimuli and both in the training booth as well as the monkey’s home cage. Finally, while Hiatt compared coarse-scale average response rates of reinforced and non-reinforced conditioning blocks, we compiled peri-event spike histograms about schedule transition events to detect fine timescale changes firing rate within reinforced and non-reinforced conditioning periods in addition to block averages.

11.2 Methods

11.2.1 Microwire array

Chronically implanted microwire arrays enabled relatively stable, long-term recording of single-cell action potentials in motor cortex [51]. Microwires were positioned to advance along the caudal bank of the precentral gyrus, layer V where somata of many force-correlated cells (including corticomotoneuronal (CM) cells) have been identified [98, 113]. Though corticomotoneuronal connectivity was a requirement for a parallel investigation using monkeys D and J (part I), we preferentially selected cells only demonstrating modulation with motor behaviors for this investigation, a far less stringent criterion.

11.2.2 Rate-contingent, spike-triggered (RCST) stimulation

During alternating R/NR conditioning, we approximated instantaneous firing rate in real-time using two similar methods, depending on the conditioning environment. For most in-booth sessions, time-amplitude window discriminated spike events each triggered a 1 ms wide square pulse. The pulse train output was low-pass filtered, $\tau = 50$ ms, and amplified using a simple in-house analog device. We set signal amplification so that maximum firing rate of the specific cell to be conditioned reached a level of 5 volts, the signal requirements of the task computer. These operations produced a continuous signal suitable for controlling

cursor movements on the display screen in front of the animal in the training booth to serve both as visual feedback and control signal for rate-contingency. When the activity-controlled cursor crossed into the interior of the presented target, all subsequent in-target spike events triggered stimulation of the reinforcement site. Stimulation events were often used to trigger auditory click stimuli. Position of the target on the screen, relative to cursor-inactive position determined threshold of the rate-contingency. We initially set target position just above baseline firing rate, and gradually elevated its position over the course of conditioning in efforts to boost conditioned spike rates. Only during R periods of the alternating R/NR task were targets presented for available RCST stimulation.

For in-cage sessions, we pre-programmed the Neurochip2-HV to perform a real-time sliding window operation to estimate instantaneous spike rate. Specifically, the device counted the number of spike-acceptance pulses recorded within a 500 ms wide window for time-averaging. The window was then advanced by 10 ms and spike events were re-counted in the new domain to determine time-averaged rate at the next time sample. We set the Neurochip2-HV to deliver spike-triggered stimuli on spike events that occurred when this rate estimate surpassed a set threshold frequency. Threshold was determined from force target-tracking or in-booth R/NR task response averages that revealed baseline and maximum firing rates of the particular cell. Typically, in-cage RCST stimulation thresholds were set at 75% of the observed maximum firing rate of the candidate cell. In later sessions, the Neurochip2-HV was also set up to govern operant conditioning sessions in the training booth as well.

11.2.3 Designing the in-booth reinforcement schedule

Prior to conditioning, durations of reinforced (R) and non-reinforced (NR) periods were randomly-selected, with replacement, from uniform distributions spanning 3 to 5 minutes for NR-periods and 1 to 2 minutes for R-periods. Using the two sets of period durations, the reinforcement schedule was compiled by placing drawn R and NR durations in alternating sequence and then computing their cumulative sum. We incorporated randomness of period durations, within limits, to reduce the monkeys' anticipation of transitions in the

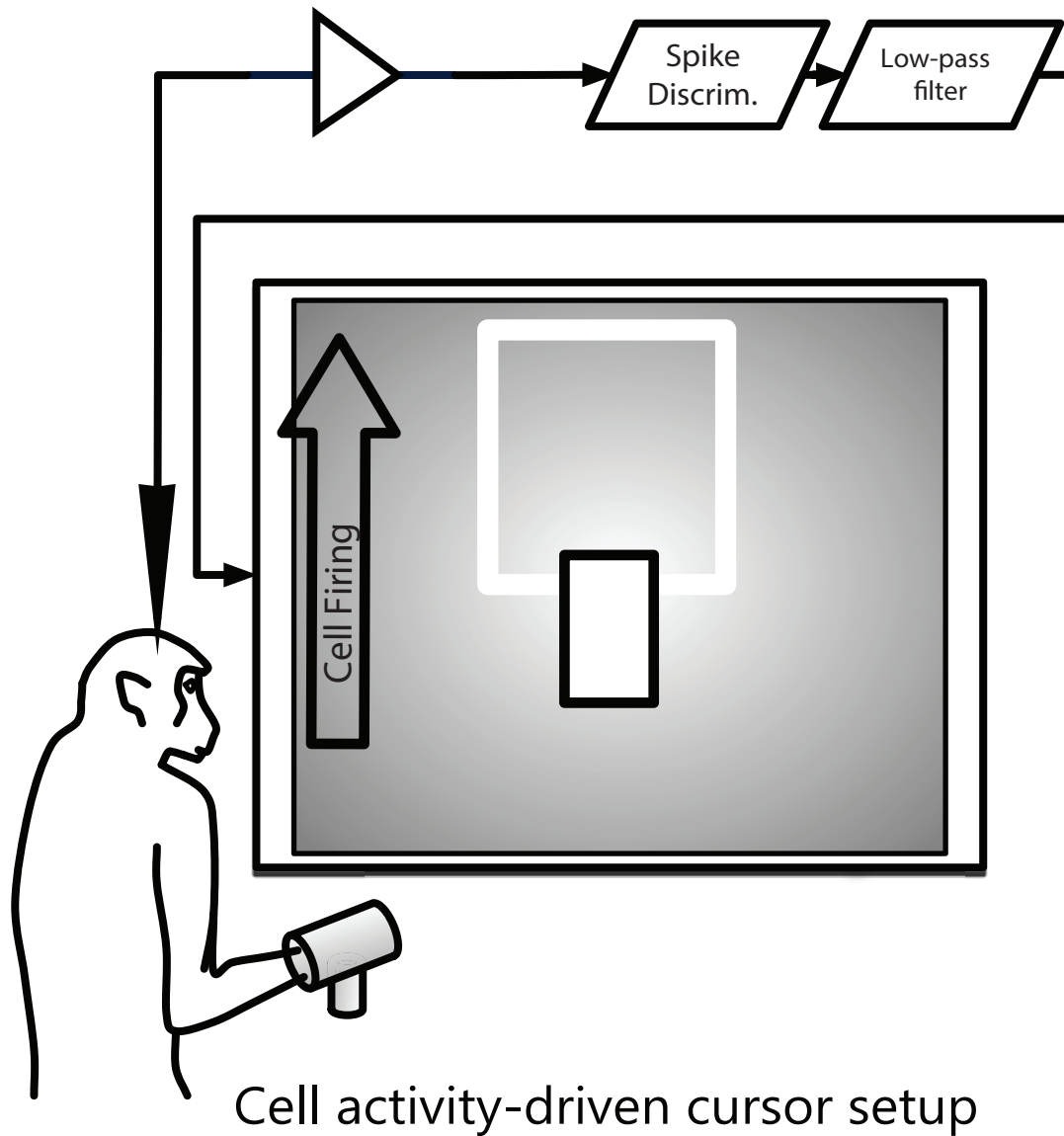


Figure 11.1: Schematic representation of in-booth activity-driven cursor setup. During conditioning, we amplified cortical activity signals to discriminate single neuron spike events. Discriminator acceptance pulses were low-pass filtered to produce a continuous, rate-dependent voltage signal suitable for cursor control. The activity-driven cursor provided visual feedback of cortical cell firing rate. Lower boundary of target marked the rate-threshold for spike-triggered intra-cortical stimulation.

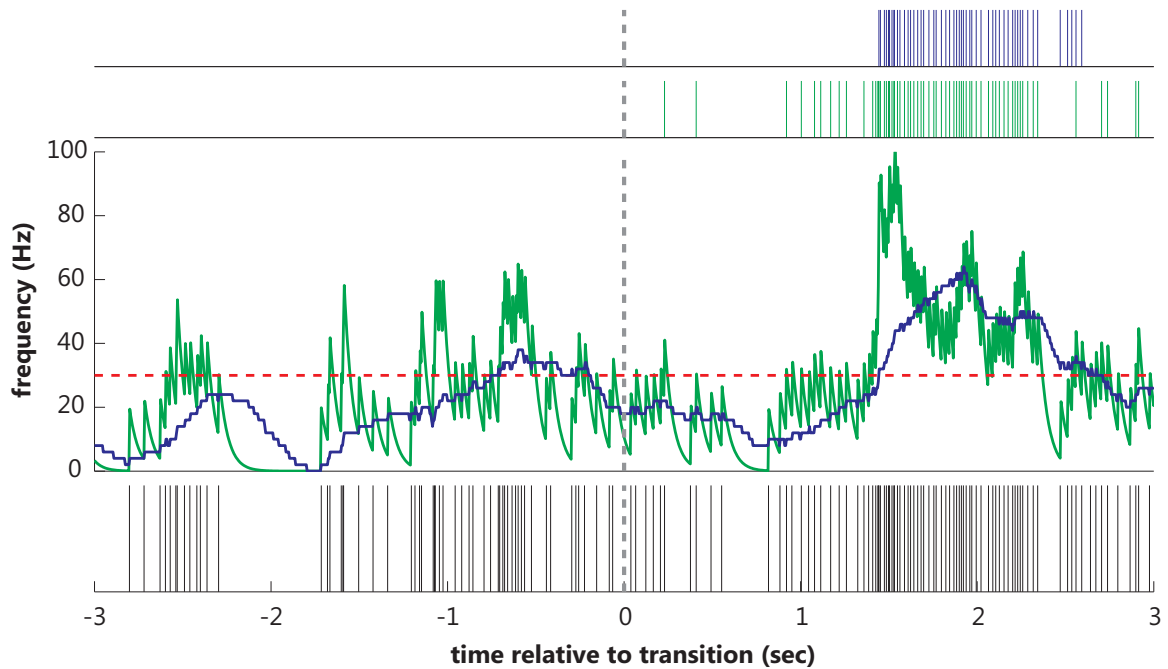


Figure 11.2: Two procedures used to deliver Rate-Contingent, Spike-Triggered (RCST) intracranial stimulation. *Bottom*: Discriminator accepted spike events from a motor cortex neuron. If a spike occurs when the rate trace is supra-threshold (above red dashed line) during a reinforcement period (right of gray dashed line), that spike triggers BS—one to three 1 mA pulses delivered to the striatal reinforcement site. *Top*: Spike-triggered stimulus events contingent upon low-pass filtering (green) and sliding window (blue) rate approximation methods. In-booth experiments employed low-pass filtering. In-cage Neurochip experiments used the sliding window method.

reinforcement schedule.

11.2.4 Confidence intervals for time-averaged rates

Much empirical evidence indicates that neuronal spike event sequences can be accurately characterized by Poissonian point-process statistics [24]. That is, the durations separating successive spike events, or interspike intervals (ISIs), are independent of each other and are exponentially distributed. To compute confidence intervals for time averaged means, we developed a non-parametric bootstrap method based on this Poissonian property of independent ISIs. First, for each reinforced, and non-reinforced period during conditioning, we calculated $n - 1$ ISIs from the list of n spike events that occurred and then sorted ISI values in ascending order. Second, from the ordered set, ISIs were randomly drawn with replacement until their cumulative sum just surpassed the duration of the actual interval containing the observed spike events. Third, the number of ISIs comprising the cumulative sum was divided by the period duration to provide a simulated time average rate value. Repeating steps one through three a total of R times generated a bootstrap distribution of time-averaged rate values from which confidence intervals were determined.

11.2.5 A bootstrap method to evaluate confidence in peri-event spike activity averages

This project tests for changes in neuronal activity patterns elicited through spike-triggered BSR—to first order, as changes in average spike rate. To validate visually-appraised features, or structure, in spike histograms, we quantitatively evaluated their statistical significance using time-varying rate estimates by compiling event-relative spike timestamps into peri-transition spike histograms and kernel-smoothed averages. We then used the method of Davison & Hinkley to compute confidence intervals about these point density estimates of instantaneous firing rate [23, Chapt. 8.2]. In a nutshell, the method employs a Taylor series expansion of the spike rate expected value function, and its known asymptotic convergence for sufficiently large number of samples n and small bandwidth h (Delta method), to properly scale smoothed rate trace estimates of bootstrap-sampled spike events. From these bootstrapped estimates, accurate confidence intervals about the empirically-observed rate

trace could be estimated.

Spike rate, as determined from a time series of discrete neuronal discharge events, is always approximate since heuristics must be imposed (e.g. histogram binning or kernel shape) to transform a discrete variable (i.e. list of event times) into a continuous one, $r(t)$, a completely-defined time-dependent rate function. To gauge the validity of spike rate approximates, and also detect statistically significant changes, we computed 95% confidence intervals about kernel-smoothed estimates of firing rate from peri-event spike trains. We applied the method of Davison & Hinkley as follows:

1. *Extract peri-transition spike events*: Empirically-observed spike events occurring within 75 seconds before and 75 seconds after each R-to-NR transition event, as well as each NR-to-R schedule transition, were extracted and set relative to the transition event at $t = 0$. The extra 15 seconds before and after the desired $[-60, 60]$ second domain of interest prevented margin effects in subsequent smoothing procedures. The number of transitions, total number of spike events, and interval durations were stored for kernel width calculation in step 2.
2. *Calculate kernel bandwidth*: The method employs a spike train smoothing process (i.e. convolution of a spike event list with a zero-mean Gaussian of bandwidth h) to approximate instantaneous firing rate $\hat{\lambda}$ as a function of time y . In general, the kernel-smoothed estimate of instantaneous firing rate can be mathematically expressed

$$\hat{\lambda}(y; h) = \left(\frac{1}{nh} \right) \sum_{j=1}^n w \left(\frac{(y - y_j)}{h} \right) \quad (11.1)$$

where $w(\cdot)$ is a symmetric density function with mean zero and unit variance. To ensure that the method does not introduce bias into the bootstrap of subsequent steps, a suitable bandwidth must be chosen. Note that $\hat{\lambda}(y; h)$ estimates $\int w(u) (y - hu) du$, not the actual spike rate $\lambda(y)$. According to the delta method, the expected value of the smoothed rate function $\hat{\lambda}(y; h)$ and its variance relate to the actual spike rate function $\lambda(y)$ by the relations

$$\mathbb{E} \left\{ \hat{\lambda}(y; h) \right\} = \lambda(y) + \frac{1}{2} h^2 \lambda''(y) \quad (11.2)$$

$$\text{var} \left\{ \hat{\lambda}(y; h) \right\} = \frac{c}{nh} \lambda(y) \quad (11.3)$$

for sufficiently large n and small h , with c as the density function normalization factor. Analytical methods and simulations confirm that choice of $h \propto n^{-\gamma}$ where $\gamma = \frac{1}{3}$ renders the bias term negligible compared to $\lambda(y)$ in equation 11.2 for $n > 50$ [23, Chapt. 5, p. 228]. For our application, bandwidth h is calculated

$$h = a T n^{-1/3} \quad (11.4)$$

where T is the duration of the peri-transition domain (120 sec.), n is the total number of observed spike events and a is a unitless scale factor applied to ensure “ h stays smaller than is commonly used for point estimation of the intensity” [23, Chapt. 8, p. 420]. Because all cell and muscle conditioning experiments were well-sampled, resulting in sufficiently small h values (typically 0.3 sec), we left $a = 1$.

3. *Performing the resampling procedure:* For $R = 199$ repetitions, n spike events were randomly drawn from the empirically-observed collection of extracted spike events (step 1). From this resampled collection, a bootstrap estimate of the spike rate function $\hat{\lambda}^*(y; h)$ was then computed using the kernel smoothing process and bandwidth h calculated in step 2. For each bootstrapped rate trace, the standardized quantity

$$Z(y; h) = \frac{\left\{ \hat{\lambda}^*(y; h) \right\}^{1/2} - \left\{ \hat{\lambda}(y; h) \right\}^{1/2}}{\frac{1}{2} (nh)^{-1/2} c^{1/2}} \quad (11.5)$$

was calculated to generate the bootstrap distribution over R repetitions.

4. *Calculating confidence band limits:* Simulated values of Z^* were used to estimate 95% quantiles ($\alpha = 0.025$), the lower and upper limits of which we denote $z_{L,\alpha}(h)$ and

$z_{U,\alpha}(h)$ respectively. Specifically, $z_{L,\alpha}(h)$ and $z_{U,\alpha}(h)$ were taken as the $(R+1)\alpha$ th ordered values of $\min_Y z_r^*(y; h)$ and $\max_Y z_r^*(y; h)$ respectively. Lower and upper confidence bands were then computed using these values using the following respective operations:

$$\left\{ \left[\hat{\lambda}(y; h) \right]^{1/2} - \frac{1}{2} (nh)^{-1/2} c^{1/2} z_{U,\alpha}(h) \right\}^2 \quad (11.6)$$

$$\left\{ \left[\hat{\lambda}(y; h) \right]^{1/2} - \frac{1}{2} (nh)^{-1/2} c^{1/2} z_{L,\alpha}(h) \right\}^2 \quad (11.7)$$

noting the switch in $z_{U,\alpha}(h)$ and $z_{L,\alpha}(h)$ values with respect to band-limit designation.

11.2.6 Spike event shuffling as a control measure

In this investigation, we sought to determine if there existed statistically significant features in spike activity that recurred over the course of the alternating R/NR schedule. To evaluate if modulations in rate traces are non-spurious, robust and recurring, we contrasted traces from the observed sequence of spike events against a collection of rate traces computed after those spike events had been shuffled. Peri-transition snippets were extracted 75 seconds prior to 75 seconds following each NR-R transition and each R-NR transition over the full course of conditioning. Snippets of spike trains were combined into peri-event spike histograms, binwidth = 50 ms, as well as consolidated into a single dense train that was convolved with a Gaussian kernel of calculated bandwidth (methods section 11.2.5) to approximate instantaneous spike rate relative to the transition event. Rate traces from shuffled sets were computed using identical procedures and parameters, as was performed on the original unperturbed sets so that traces from observed and shuffled trains could be directly compared. Domains in which the observed set diverged outside the confidence interval of the shuffled set indicate that such features in peri-transition spike activity cannot be explained as random fluctuation, and therefore arise as a consequence of the conditioning paradigm.

To shuffle spike events, inter-spike intervals (ISIs) were computed within each peri-transition train. The model of stochastic neuronal firing is a Poisson point process with the property that intervals separating successive event are independent and interchangeable [24, p. 25]. Under this model, the sequence of ISIs can be randomly permuted and then cumulatively summed to give a new sequence of spike event values in ascending order. We added a randomly drawn “jitter” component to each shuffled train of the set so that first spike events did not all reside at $t = -75$ sec. Shuffling interval-by-interval, rather than shuffling the entire combined list of ISIs, is valid for our purpose since sets of shuffled spike trains would be consolidated into a single (dense) train in the next step, prior to rate estimation via kernel convolution.

Reshuffling the observed set of spike trains $R = 199$ times generated a distribution of shuffled train rate traces from which average and confidence intervals could be computed. These Monte Carlo estimates of confidence interval boundaries were computed using the procedure outlined in methods: A bootstrap method to evaluate confidence in peri-event spike activity averages (section 11.2.5). The procedure was performed for peri-transition spike activities for both NR-R and R-NR transitions in each of the in-booth and in-cage sessions analyzed in-depth that are presented in the results.

11.3 Results

11.3.1 Force-modulated cortical neurons were preferentially selected for conditioning

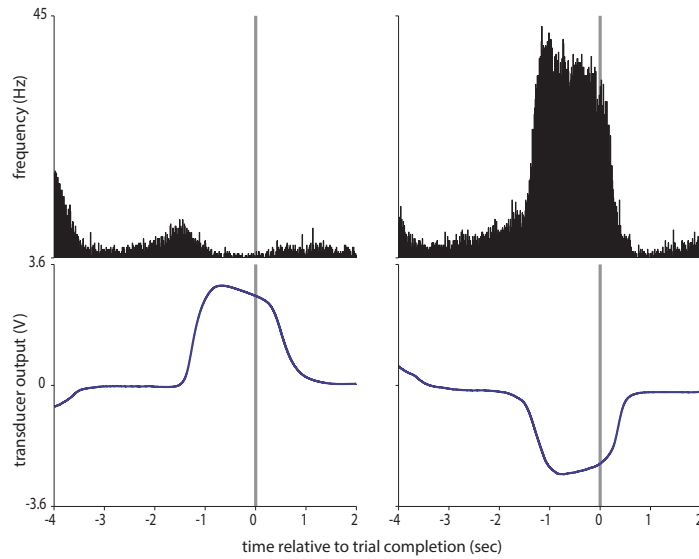
Task-related motor cortex neurons altered their firing in a consistent manner over repeated trials of the manual target-tracking task. We used an isometric task whereby the manipulandum remained static while the attached transducer measured forces exerted against it. Because hand position remained stationary, we can conclude task event modulations in cells’ activity were correlated with force generation. Cells could elevate or depress spike rates during force holds. Other cells changed their activation only during dynamic torque events (i.e. when the force-driven cursor moved into or out of the target). It is also important to note that cell candidates for conditioning often exhibited direction selectivity of concurrent wrist torque, whereby cells would be more active during torque generation in one direction (e.g.

extension) and suppressed for the opposite (flexion). Force target-tracking was performed using the same recording configurations, and just prior to, subsequent RCST stimulation sessions after the cell candidate had been selected for conditioning.

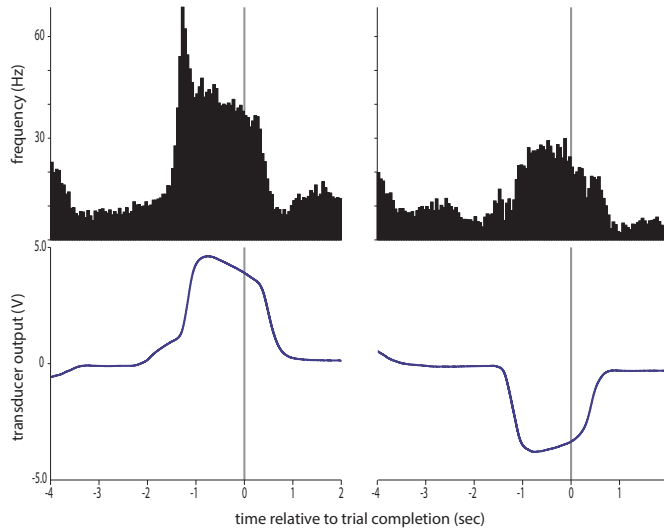
11.3.2 Significant spike rate effects conditioned in many cortical cells from three monkeys

Table 11.1 summarizes results from all cortical cell conditioning sessions, categorized by conditioning environment, for the three monkeys used in this investigation. Depending on stability and isolation of the cortical cell candidates, we often conditioned the same cell over repeated sessions. In the case of monkey J, the same strongly-correlated motor cortex neuron remained isolated for more than 6 months, enabling its repeated conditioning in several environments: in-booth with visual and auditory feedback, in the monkey’s home cage using the Neurochip2-HV as well as Neurochip-guided conditioning in both environments during the same session for comparison of environmental effect. We attempted many more in-cage Neurochip sessions on the three animals than is reported above; the majority of in-cage attempts, $\sim 70\%$, were deemed invalid for one or more of the following reasons: 1) insufficient intensity of the BSR pulses often arising from impedance increases in the stimulating electrode, 2) interruption in Neurochip functioning (e.g. recording leads disconnected, chamber exudate interfered with circuitry), 3) loss of single-cell spike signal due to isolation loss or cell death, and 4) what we later learned to be improper setting of conditioning parameters (e.g. rate-threshold set too low, NR-periods set too short).

Conditioning single cortical cell spike activity in-cage is a novel paradigm involving many parameters whose importance and relevant value ranges were almost completely unknown when we began this project. To facilitate conditioning of significant, let alone robust, spike rate effects, we had to explore the parameter space for interval durations in the alternating R/NR conditioning schedule, rate-thresholding levels conducive for eliciting robust spike rate increases, means to approximate instantaneous firing rate in real time both in the training booth and using the Neurochip2-HV as well as methods for delivering sensory feedback during conditioning in both environments. Of course, the effectiveness of many of these parameters could only be explored in the fully intact preparation which in and



(a) D20100903 FTT task spike rate and torque sweep averages



(b) J20121110 FTT task spike rate and torque sweep averages

Figure 11.3: Cells exhibiting force-modulatory effects were preferentially selected for conditioning. Above we show averaged cortical cell spike activity and concurrent wrist torque aligned by trial completions of the two-target manual force-tracking task. Activity plots, from an example session with monkey D, are plotted on top while the four plots from monkey J's example session occupy the bottom. In each set of four, histograms and event-triggered torque averages on the left show those for the wrist flexion target; plots on the right depict extension box activity averages. Peri-completion spike histograms plot time-averaged spike rate in 20ms (*top*) and 100ms (*bottom*) bins within the -4 to 2 second time domain spanning each target vanish event.

of itself presented formidable technical challenges (i.e. successful implantation of a stable, pathogen-free microwire array, reinforcement site verification through behavior, stimulus artifact exclusion strategies, etc.).

		in-booth				in-cage			
		effect type				effect type			
		+	0	-	% success	+	0	-	% success
Monkey P	cells:	7	2	1	70.0	–	–	–	–
	sessions:	7	4	4	46.7	–	–	–	–
Monkey D	cells:	21	3	2	80.8	3	1	0	75.0
	sessions:	38	12	4	70.4	3	2	0	60.0
Monkey J	cells:	20	2	0	90.9	6	2	1	66.7
	sessions:	42	14	1	73.7	18	9	1	64.3
Total	cells:	48	7	3	82.8	9	3	1	69.2
	sessions:	87	30	9	69.0	21	11	1	63.6

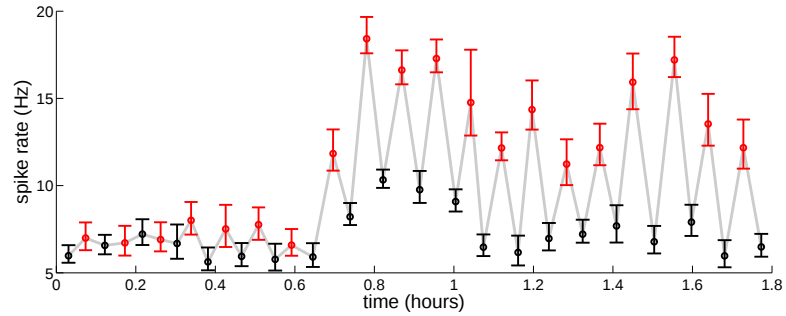
Table 11.1: Summary of all effects from cortical spike-triggered BSR conditioning attempts across subjects, cells, sessions and conditioning environments. Key: “+” symbols denote statistically significant increases during R-periods compared to NR-periods, “0”s mark instances where no significant changes were observed between R and NR activities and “–” symbols categorize occasions when NR-period spike rates were significantly greater than R-period rates. Often, the same cell underwent conditioning in multiple sessions. In this table, a given cell was tallied as generating a positive effect though it may have produced null or negative effects in other conditioning attempts. If a cell showed null (0) and negative (–) effects over sessions, it was categorized as null.

11.3.3 *Rate-contingent, spike-triggered accumbens stimulation elicited increased motor cortex cell spike rates*

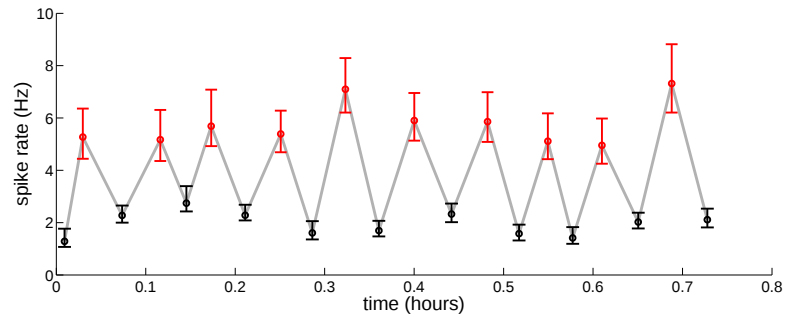
During reinforced periods the three monkeys received spike-triggered BSR when instantaneous spike rate surpassed a pre-determined threshold. Figure 11.4 depicts progressions of time averaged rates across RCST BSR conditioning sessions of monkeys J and D in the training booth, while figure 11.5 shows across-session averages from monkey J during conditioning in his home cage. Table 11.2 summarizes conditioning parameters used for each of the sessions. We employed an alternating R/NR reinforcement schedule to explore the effect, if any, of spike-triggered accumbens stimulation produced in the firing rate of the triggering motor cortex cell. We expected this recurrent closed-loop paradigm to induce elevated spike rates during “time-in” or reinforced periods (R) when RCST BSR was delivered compared to “time-out” or non-reinforced periods (NR) during which no stimulation was delivered regardless of spike activity.

Figure 11.4 shows time-averaged motor cortex neuron spike rates, and surrounding 95% confidence intervals, over the course of RCST BSR conditioning in the training booth. Results of representative sessions from monkeys D and J are shown (D20100524: 48,257 total spikes, 6,588 stim. pulses over 1.8 hours, D20100903: 8,598 total spikes, 1,585 stim. pulses over 0.75 hours and J20121110: 91,511 total spikes, 25,581 stim. pulses over 2.7 hours). Distributions of R-group averages were significantly greater than their NR-group counterparts in the three sessions (D20100524: $p = 4.22 * 10^{-5}$, D20100903: $p = 1.08 * 10^{-4}$, J20121110: $p = 3.45 * 10^{-12}$, Kruskal-Wallis). Robust increases in spike rates were observed during R-periods as compared to the NR-periods that separated them; these alternating activities between R (high) and NR (low) reveal successful acquisition of the spike-activity operant, and volitional control over the trigger cell. In all plots, reinforced rates were significantly greater—as indicated by non-overlapping confidence intervals—than their two non-reinforced neighbors for the majority of points (D20100524: 14/20, D20100903: 10/10, and J20121110: 32/35).

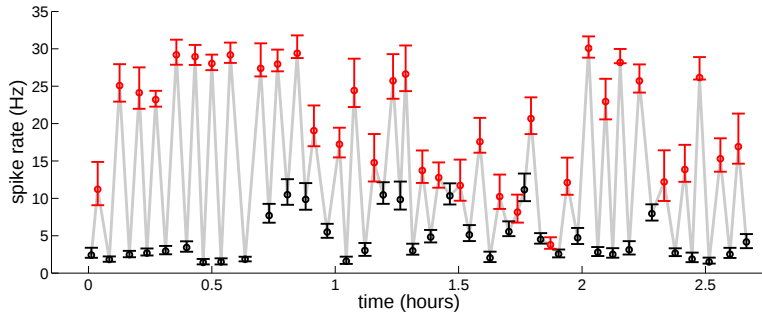
The example session conditioned in-cage (figure 11.5) also shows statistically significant increases in R-period distributions of session time averages compared their NR counterparts



(a) D20100524 in-booth conditioning



(b) D20100903 in-booth conditioning



(c) J20121110 in-booth conditioning

Figure 11.4: In-booth-conditioned sequences of time-averaged cortical spike rates show robust, anti-correlated, alternating rate patterns over the course of the alternating R/NR reinforcement schedule. In the two sessions depicted above, for each animal: monkey D (*top, middle*) and monkey J (*bottom*), circles mark time-averaged rates during reinforced periods (red) when RCST stimulation was available and during non-reinforced periods (black) when no stimulation was delivered regardless of the cell's activity. Whiskers straddling these averages mark 95% confidence intervals of the time averages. Instances in which the confidence band of a given average does not overlap the range of values bounded by the confidence intervals surrounding nearest-neighbor averages indicate statistical difference.

Monkey	Date	Cell / Muscle	Preferred direction	Environ- ment	Equipment	R-period (min.)	NR-period (min.)	Rate thresh. (Hz)	Trig. : Pulse	Feed- back
P	20071124	bicep	elbow flexion	cage	Neurochip1	$t = 5$	$t = 5$	none	1 : 1	none
P	20071125	triceps	elbow extension	cage	Neurochip1	$t = 5$	$t = 5$	none	1 : 1	none
D	20100524	w8c6	wrist extension	booth	rack (Neuro1)	$1 \leq t \leq 2$	$3 \leq t \leq 5$	20	1 : 1	VC, AC1
D	20100903	w8c9	wrist extension	booth	rack (Neuro1)	$1 \leq t \leq 2$	$3 \leq t \leq 5$	15	1 : 3	VC, AC1
D	20110222	w8c11, (w8c12)	wrist ext., (none)	booth	rack (Neuro1)	$1 \leq t \leq 2$	$3 \leq t \leq 4$	12	1 : 2	VC, AC1
J	20120614	w4c8, (w4c10)	wrist ext., (flexion)	booth	rack (Neuro1)	$1 \leq t \leq 2$	$2 \leq t \leq 4$	21	1 : 1	none
J	20121110	w2c4.1	wrist flexion	booth	rack (Neuro1)	$1 \leq t \leq 2$	$3 \leq t \leq 5$	30	1 : 1	VC, AC1
J	20121128	w2c4.1	wrist flexion	cage	Neurochip2-HV	$t = 1$	$t = 5$	50	1 : 1	AC2
J	20130222	w2c4.1	wrist flexion	booth & cage	Neurochip2-HV	$t = 2$	$t = 5$	60	1:1	AC2

Table 11.2: Summary of conditioning parameters used for each example conditioning session. In the table above, we list monkey identities, session dates, conditioning environments, sensory feedback used, schedule durations, rate thresholds and trigger-to-stimulus pulse ratios used for each of the example sessions depicted in the results. In all sessions, BSR stimuli were delivered in 0.2 ms wide, biphasic square pulses at 1 mA intensity. The feedback types are: VC: visual cues in the form of a rate-guided computer cursor, AC1: auditory cues in the form of spike-triggered clicks produced by rack-mounted equipment, AC2: clicks on BSR pulse delivery generated by the Neurochip2-HV onboard clicker.

(J20121128: $p = 1.59 * 10^{-10}$, Kruskal-Wallis) though these changes were smaller, relative to NR period levels, than those observed for typical in-booth-conditioned sessions. During this session, 820,501 total spikes were recorded and 107,770 stimulus pulses were delivered over the course of more than 8 hours.

Table 11.3: Time-series statistics for D20100524 in-booth conditioning example

	k					
	1	2	3	4		
x_k serial corr.	-0.156	0.873	-0.261	0.697	x_k von Neumann	2.27
p-value	0.2	< 0.002	0.07	< 0.002	p-value	0.812
Δx_k serial corr.	-0.95	0.938	-0.907	0.885	Δx_k von Neumann	3.83
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002

Table 11.4: Time-series statistics for D20100903 in-booth conditioning example

	k					
	1	2	3	4		
x_k serial corr.	-0.893	0.8596	-0.885	0.5534	x_k von Neumann	3.5969
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002
Δx_k serial corr.	-0.955	0.919	-0.938	0.9586	Δx_k von Neumann	3.6738
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002

The alternating rate patterns described above give rise to robust, statistically significant, time series measures—serial correlation and von Neumann ratio—that are listed in tables 11.3, 11.4, 11.5 and 11.6. Correcting for large-scale rate drift over the course of conditioning, serial correlation in changes between successive samples, Δx_k values reveal strong anti-correlation

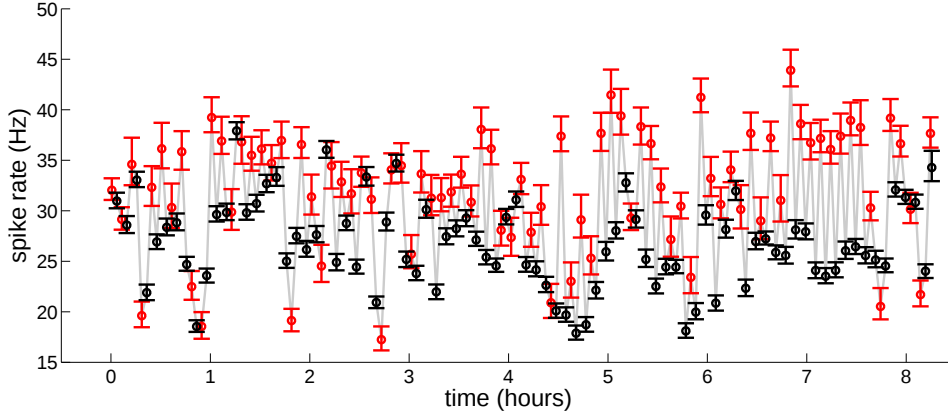


Figure 11.5: Anti-correlated, alternating cortical spike rates conditioned as monkey J moved freely about his home cage. Over the course of 8 hours, the Neurochip2-HV controlled conditioning of a motor cortex neuron that triggered RCST accumbens stimulation in an alternating R/NR schedule. As in figure 11.4, circles and surrounding bars plot time-averaged spike rates and their corresponding 95% confidence intervals during R-periods (red) and NR-periods (black).

Table 11.5: Time-series statistics for J20121110 in-booth conditioning example

	k					
	1	2	3	4		
x_k serial corr.	-0.679	0.81	-0.716	0.731	x_k von Neumann	3.31
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002
Δx_k serial corr.	-0.948	0.9	-0.882	0.853	Δx_k von Neumann	3.86
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002

Table 11.6: Time-series statistics J20121128 in-cage conditioning example

	k					
	1	2	3	4		
x_k serial corr.	-0.059	0.293	-0.283	0.284	x_k von Neumann	2.11
p-value	0.25	< 0.002	< 0.002	< 0.002	p-value	0.764
Δx_k serial corr.	-0.671	0.445	-0.541	6.493	Δx_k von Neumann	3.33
p-value	< 0.002	< 0.002	< 0.002	< 0.002	p-value	< 0.002

between nearest and succeeding odd-valued neighbors: $k = 1$ and $k = 3$, as well as tight positive serial correlation with “next-one-over” averages: $k = 2$ and $k = 4$. Similarly, von Neumann ratios calculated for the four example sessions reveal that neighboring R and NR averages were consistently different from each other at levels significantly greater than chance. Significant von Neumann ratios indicate that patterns in time-averaged rates are not attributable to random Markovian fluctuations; the alternative is that observed alternation in rate levels between R and NR periods arose from the conditioning paradigm. While alternating patterns are obvious from inspection of in-booth conditioning sessions, they are not immediately apparent in sequences of activity averages conditioned in-cage. Serial correlation and von Neumann ratios measure pattern in time series from which statistical significance can be approximated through randomization and Monte Carlo approximation methods. These analysis techniques can detect patterns in these series of time-averages that might otherwise go unnoticed.

Box plots (figure 11.6d) summarize distributions of reinforced and non-reinforced time averages across each session. For both monkeys, non-reinforced distributions have lower medians and were less variable than the reinforced group distributions of time-averaged spike rates to which they were compared. These differences are statistically significant in all four examples as assessed by Kruskal-Wallis and unpaired Student’s t-tests; the p-values from which are listed in table 11.7.

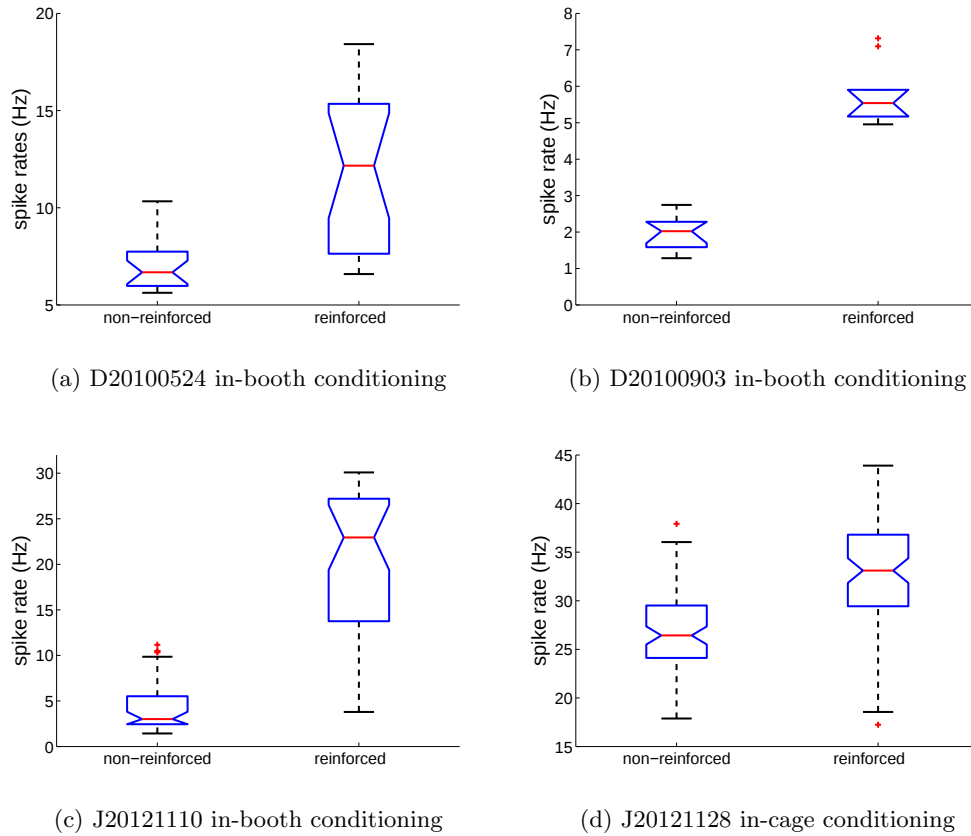


Figure 11.6: Significant increases in cortical cell spike rate during reinforced periods compared with rates during non-reinforced periods. Distributions of cortical cell time-averaged spike rates are portrayed as box plots of reinforced (R) and non-reinforced (NR) period groups pooled for in-booth conditioning sessions of monkeys D (*top row*) and J (*bottom left*). The distribution comparison for the example in-cage session is depicted on the lower right. In each box, the central horizontal line marks the distribution median while blue box extremities depict upper (75%) and lower (25%) quartiles. Red crosses plot rate instances with values outside of whisker boundaries—the value of the outermost datum within 1.5 times the inter-quartile range as measured from box boundaries. Notch height shows approximate limits of confidence intervals about their median at the 5% significance level.

Statistical test		
Session	Kruskal-Wallis, p	Student's unpaired-t, p
D20100524	$4.22 * 10^{-05}$	$3.17 * 10^{-06}$
D20100903	$1.08 * 10^{-04}$	$3.11 * 10^{-07}$
J20121110	$3.45 * 10^{-12}$	$2.58 * 10^{-13}$
J20121128	$1.59 * 10^{-10}$	$2.78 * 10^{-12}$

Table 11.7: Tabulated p-values from statistical tests of the four example sessions presented in this section 11.3. Table entries are probabilities that R-period and NR-period groups of time averages were drawn from a common distribution. Two statistical tests were employed to assess statistically significant differences: Kruskal-Wallis and Student's unpaired t.

11.3.4 *Peri-transition averages reveal fine time scale of schedule-dependent activity patterns*

In figures 11.7, 11.8 and 11.9 above, we show histograms and overlying kernel-smoothed rate traces of consolidated motor cortex neuron spike trains extracted about NR-R and R-NR schedule transitions. Regions where the red rate trace and its surrounding confidence boundaries emerged outside of the “chance band” indicate statistically significant deviations from chance—that is, the rate trace and surrounding confidence limits of the spike data after interspike intervals had been shuffled. Note that statistically deviant features in these plots can arise from different scenarios. One possibility is a rate deviation that recurred at the same time relative to schedule transition events over the course of conditioning. Alternatively, since intra-period spike histograms are compiled from many peri-transition sweeps, fluctuations in the average could be the result of constructive or destructive interference when phase-shifted spike density patterns are superposed. Regardless, intra-period deviations in spike density in peri-transition histograms imply changes in cortical cell spike probability that occur with respect to the conditioning schedule. Deviations with amplitudes outside the 95% confidence interval of the shuffled data reached significance in reinforced domains and either remained within, or dropped below the “chance interval” in NR domains. Two sets of peri-transition averages, one for monkey J and one for monkey D exemplify robust

rate increases observed across NR-R transitions while animals underwent RCST stimulation conditioning while under restraint in the training booth. During in-booth sessions, activity peaked early, usually within 10 seconds following the NR-R schedule transition, and then decayed over the remainder of each reinforced period. Reinforced period activity peaked later during in-cage conditioning. Spike activity quickly declined following R-NR transitions both in-booth and in-cage. However, as shown in figure 11.10, non-reinforced spike activity tended to be more variable in the cage than in the booth, when monkeys were restrained.

Instrumentation in the training booth allowed us to record wrist torque during RCST conditioning. In all examples, motor cortex neurons modulated their activity during dynamic and/or static phases of the force target tracking task; task-related cell activities from two example cells are shown in figure 11.3. Peri-transition averages of the torque signals depict increased torques during R periods that accompanied spike rate increases and corresponding diminution of torque generation during non-reinforced intervals with lower cortical spike rates (figures 11.7, 11.8 and 11.9). Overall increase in torque was greatest early on during conditioning and declined thereafter.

Consistent with the parallel analysis of sequential time-averages (figures 11.4 and 11.5), across NR-R transition increases in spike rates were greater in cells conditioned in-booth than in cells conditioned in-cage. At this stage it is tempting to conclude that in-booth conditioning under restraint elicits greater effects than in-cage sessions. However, since each of the cells conditioned in these examples are unique, and the reinforcement paradigms differed, such a statement remains inconclusive. For a definitive comparison, we conditioned the same cell, using identical conditioning parameters, both in the training booth and as monkey J moved freely about his cage (results section 13.1.1).

11.4 Discussion

11.4.1 The operant model

Operant-contingent rewards, in the form of RCST brain stimulation, maintain or increase above-baseline probability that the operant response, a motor cortex cell action potential or biphasic fluctuation in EMG, will recur. Without reward pairings, the likelihood

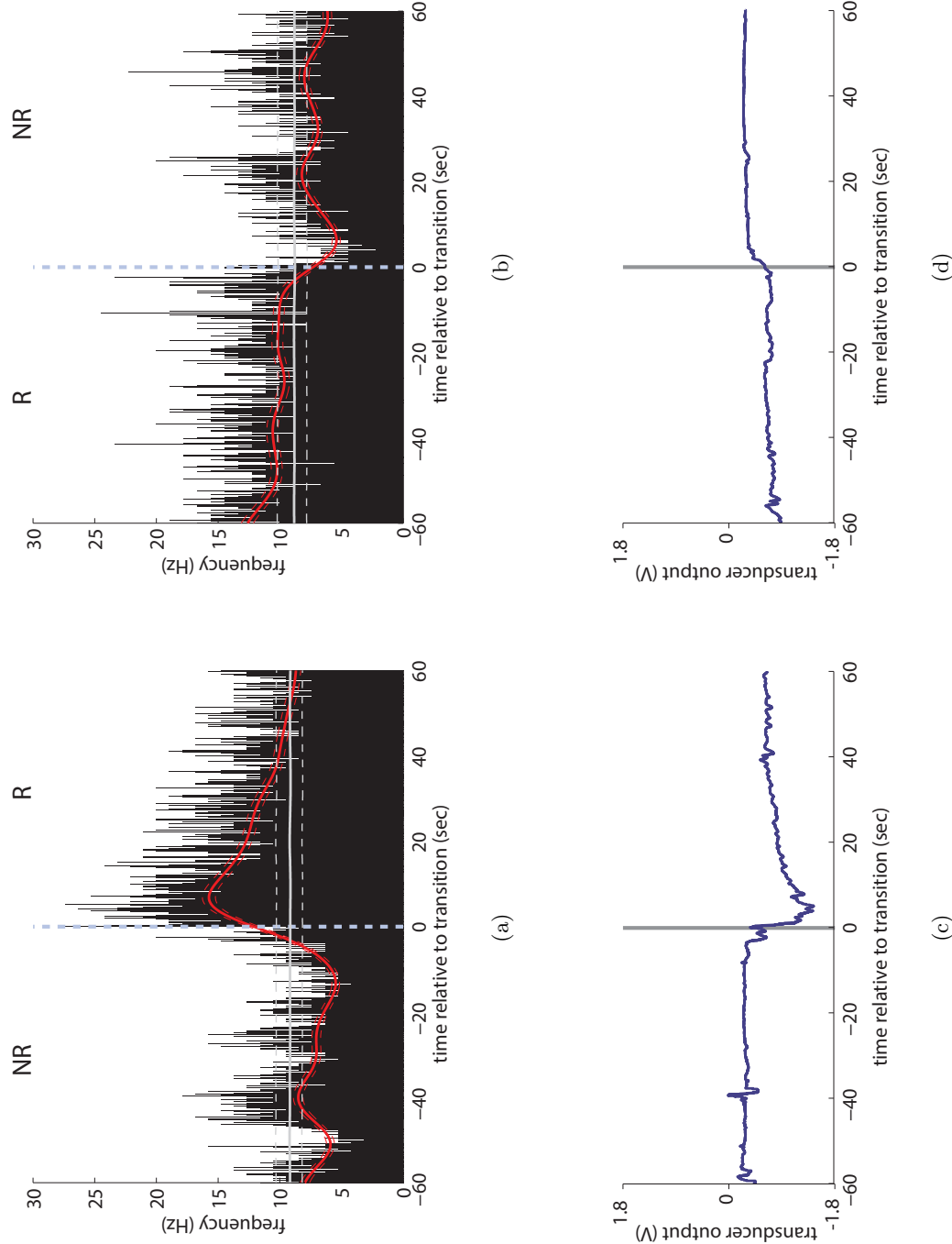


Figure 11.7: Peri-transition spike histograms of motor cortex neuron spike activity during alternating periods of rate-contingent, unit-triggered BSR during in-booth conditioning. Spike time stamps occurring during two minute intervals straddling repeated NR-R transitions (*left*) and R-NR transitions (*right*) are pooled and binned into histograms above. Light-blue dashed vertical lines at $t = 0$ mark onset and offset of activity-dependent BSR. The point-density estimate of spike rate (thick red line) and its 95% confidence band limits (dashed red lines) overlay corresponding histograms. Horizontal gray lines show averages (solid) of sweeps, and surrounding 95% confidence intervals (dashed) after spike shuffling. Averages of flexion-extension torque traces are placed below each pair of spike activity histograms recorded concurrently. Peri-transition activities from session D20100524 are depicted above. In this example, monkey D produced a four-fold increase in the firing rate of a motor cortex neuron during R-periods relative to NR-periods.

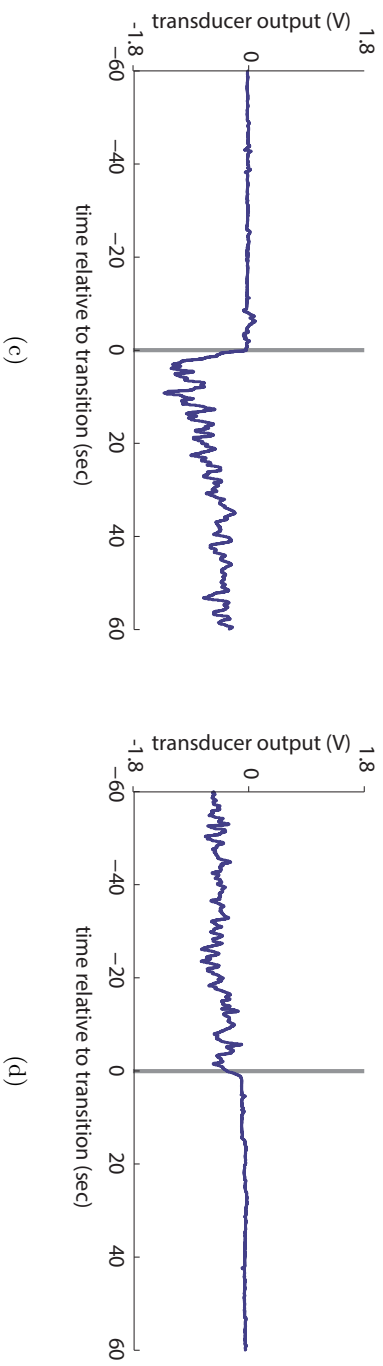
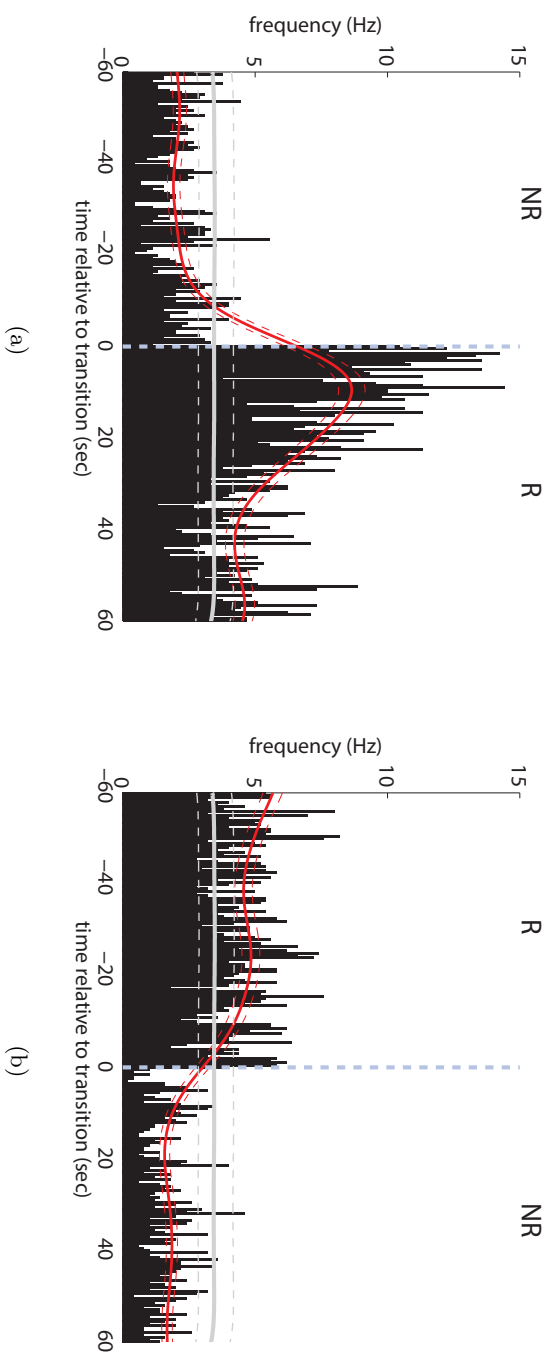


Figure 11.8: Peri-transition spike histograms of motor cortex neuron spike activity during alternating periods of rate-contingent, unit-triggered BSR during in-booth conditioning. Spike time stamps occurring during two minute intervals straddling repeated NR-R transitions (*left*) and R-NR transitions (*right*) are pooled and binned into histograms above. Light-blue dashed vertical lines at $t = 0$ mark onset and offset of activity-dependent BSR. The point-density estimate of spike rate (thick red line) and its 95% confidence band limits (dashed red lines) overlay corresponding histograms. Horizontal gray lines show averages (solid) of sweeps, and surrounding 95% confidence intervals (dashed) after spike shuffling. Averages of flexion-extension torque traces are placed below each pair of spike activity histograms recorded concurrently. Peri-transition activities from session D20100903 are depicted above. This example shows that monkey D could keep cell spike rates elevated, on average, for the full duration of reinforcement.

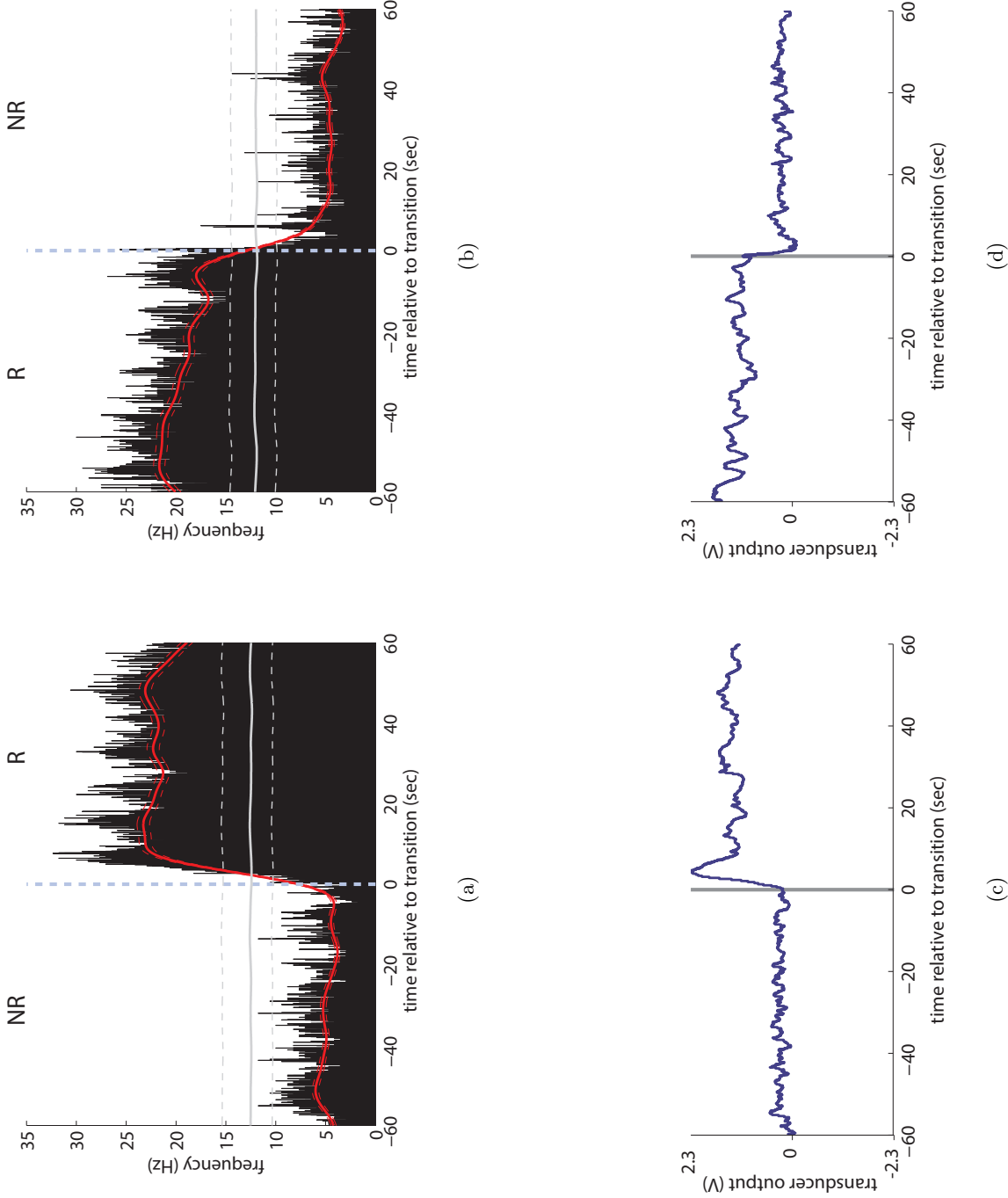


Figure 11.9: Peri-transition spike histograms of motor cortex neuron spike activity during alternating periods of rate-contingent, unit-triggered BSR during in-booth conditioning. Spike time stamps occurring during two minute intervals straddling repeated NR-R transitions (*left*) and R-NR transitions (*right*) are pooled and binned into histograms above. Light-blue dashed vertical lines at $t = 0$ mark onset and offset of activity-dependent BSR. The point-density estimate of spike rate (thick red line) and its 95% confidence band limits (dashed red lines) overlay corresponding histograms. Horizontal gray lines show averages (solid) of sweeps, and surrounding 95% confidence intervals (dashed) after spike shuffling. Averages of flexion-extension torque traces are placed below each pair of spike activity histograms recorded concurrently. Peri-transition activities from session J20121110 are depicted above. This example shows monkey J produced a four-fold increase in motor cortex cell spike rate and kept rates elevated, on average, for the full duration of reinforcement.

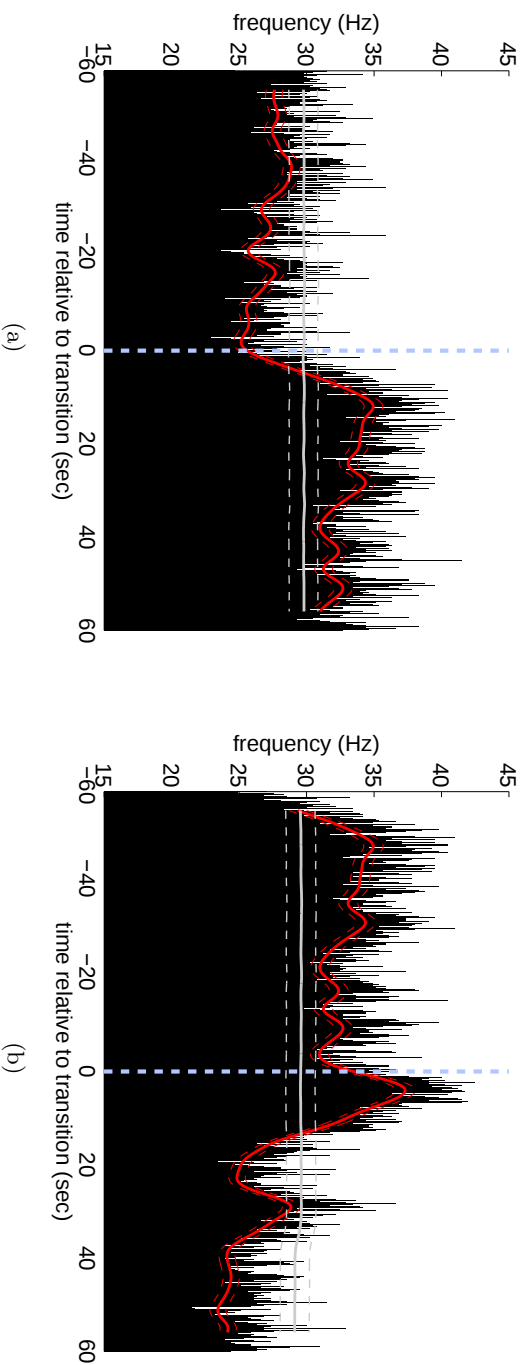


Figure 11.10: Peri-transition spike histograms of motor cortex neuron spike activity conditioned in-cage. The Neurochip2-HV delivered rate-contingent, spike-triggered BSR in the alternating R/NR schedule. Spike time stamps occurring during two minute intervals straddling repeated NR-R transitions (*left*) and R-NR transitions (*right*) are pooled and binned into histograms above. Light blue dashed vertical lines at $t = 0$ mark onset and offset of activity-dependent BSR. The point-density estimate of spike rate (thick red line) and its 95% confidence band limits (dashed red lines) overlay corresponding histograms. Horizontal gray lines show averages (solid) of sweeps, and surrounding 95% confidence intervals (dashed) after spike shuffling. This example illustrates schedule dependent changes in motor cortex cell firing during an in-cage conditioning session with monkey J. Notice transient extinction bursting immediately following R-to-NR transitions in the alternating schedule.

that a spike will recur during the next unit of time decreases as can be observed in alternating sequences of R-NR time-averaged means (figures 11.4 and 11.4) as well as peritransition histograms aligned about R-NR transitions of the reinforcement schedule (figures 11.7, 11.8, 11.9 and 11.10). While the means by which monkeys emit increased spike rates of a single cortical neuron or produce increased EMG power we have not documented, we propose that during the early stages of conditioning, monkeys explore their motor repertoire and discover behaviors that recruit activation of the triggering cell or muscle. Concurrent recordings of wrist torque during in-booth unit conditioning sessions and isolated biceps contraction observed during in-cage muscle conditioning sessions support this idea. Through repeated exposure to the response-reward pairing, with reward-eliciting responses occurring spontaneously during the early stages, monkeys “learn” to select more efficient means to recruit the BSR-triggering activity patterns so as to intensify their responding to increase delivery of reward. In this model, cognition may or may not be involved in determining whether or not the operant response is generated at a given instant in time; operant responses being either the operant activity itself or an activity-recruiting behavior.

Conditioning sessions incorporating discriminative stimuli, in the form of activity-dependent visual and auditory feedback, often showed greater effects, as did conditioning environments with fewer non-task-related stimuli. These observations suggest that attention played a crucial role in the efficacy of this conditioning paradigm. An alternative explanation that could also explain these enhanced conditioning effects might be that sensory feedback drove sensory-motor transformations functionally parallel to the association formed via the response-reward pairing.

11.4.2 Functional relationships between motor cortex and striatum

In a recent investigation, Koralek et. al. (2013) found that temporally precise coherence between output-relevant neuronal populations in motor cortex and dorsal striatum developed during learning of a cortically-guided operant response task [57]. The findings suggest that coherence serves to promote communication between task-relevant populations and that temporal precision between these populations modulates the induction and direction

of long-lasting synaptic plasticity. While ventral, not dorsal, striatum was stimulated for this investigation, it is possible that cortical spike-triggered stimulation of the region affected coherence between striatum and motor cortex and hence any correlated learning, and/or plasticity effects. In addition, the activity of motor cortex neurons may affect striatal integration of relevant sensory cues with reinforcement [120] since the motor cortex is one of the cortical areas from which the ventral striatum receives input [115, 118].

Chapter 12

FURTHER ANALYSIS OF CONDITIONED CORTICAL SPIKE PATTERNS

12.1 Results

12.1.1 Rate patterns of motor cortex cell spike activity conditioned in-cage resemble in-cage-conditioned muscle activity averages

As occurred in averages of conditioned EMG activity, relative increases in BSR-reinforced spike activity were smallest, compared to NR-period activity, during the first third and greatest during the final third of the in-cage unit-conditioning session (figure 12.1). A transient increase in spike rate followed R-NR transitions in the alternating reinforcement schedule, when high-frequency spike bursts no longer triggered accumbens stimulation. The same post-extinction burst effect can be seen in R-NR peri-transition sweeps of in-cage conditioned bicep activity (figure 10.2) of the first and middle third session averages. Unlike muscle conditioning however, the extinction burst in spike activity, though markedly reduced, did not completely subside during the final third of the unit-conditioning session.

12.1.2 Concurrent activity patterns of synaptically-linked neighbor neurons imply circuitry recruitment

In each of the two sessions depicted in figure 12.2, we recorded the spike train produced from an additional correlated cortical neuron alongside that of the conditioned cell that triggered RCST accumbens stimulation. The resulting schedule-aligned effects produced by the correlated cell suggest involvement of multi-neuronal circuits in driving rate modulations of the trigger cell to which they both belonged. Equivalently, though only the activity of the trigger cell affected reinforcement, many other neurons, from which it received both direct and indirect input, were also recruited during reinforced periods of the alternating R-NR schedule. These hidden neural contributors affected, either directly or indirectly, the

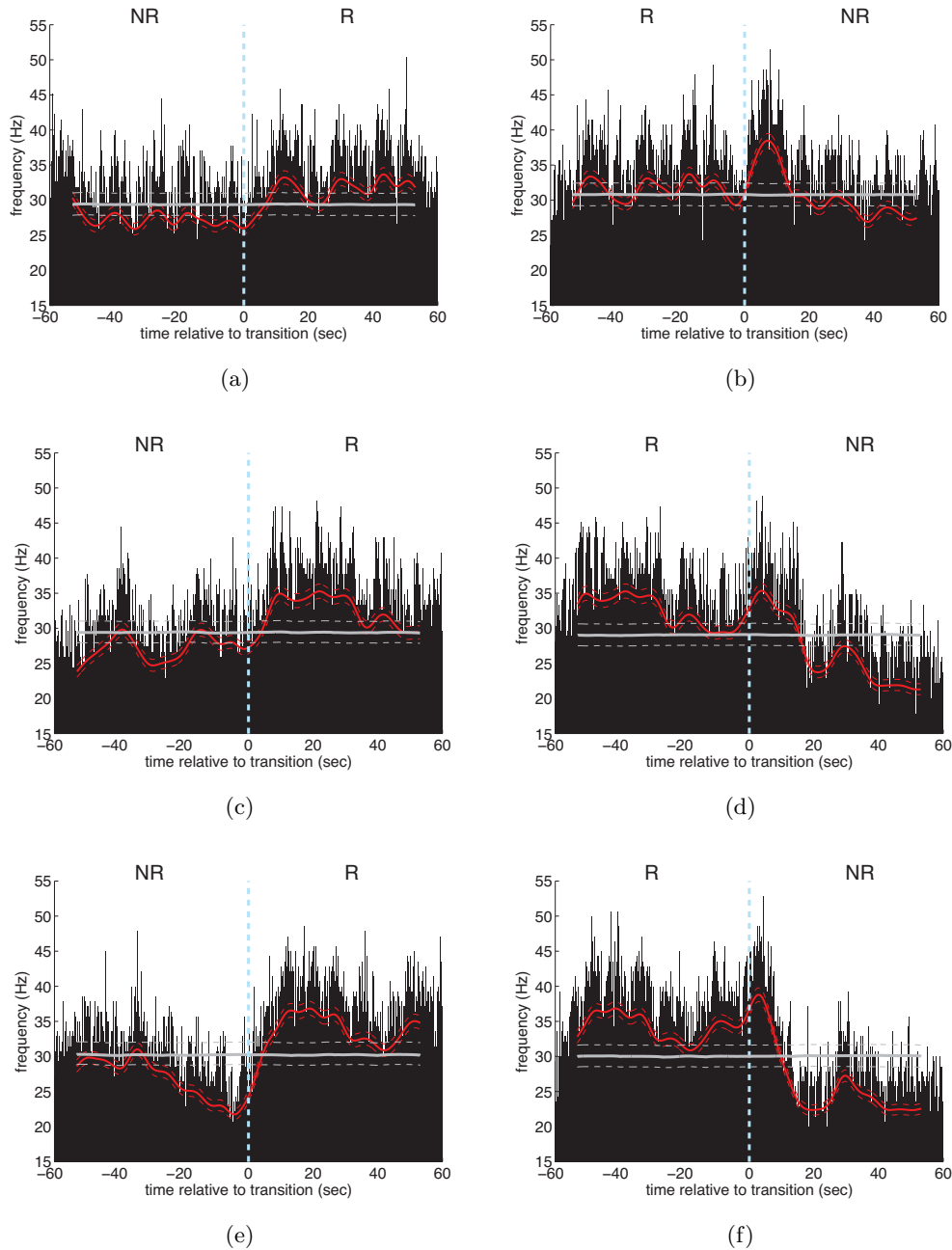


Figure 12.1: In-cage-conditioned spike activity grouped by session time. Histograms and overlying kernel-smoothed averages (red) and 95% confidence limits (red dashed) of peri-transition spike activity from the example shown in figure 11.10; this time after separating trains into subsets comprising the first third (*top*), second third (*middle*) and final third (*bottom*) of session time. As usual, spike rate averages in the left column show activity changes across NR-R schedule transitions while those on the right illustrate peri-R-NR transition rates and light blue dashed lines mark transition events in the alternating R/NR schedule. Horizontal gray traces show rate averages, and surrounding 95% confidence interval limits (dashed), after the spike trains had been shuffled. Notice R-period increases in spike rate were greatest during the final third of the conditioning session and least during the first, indicative of learning.

modulatory firing rate changes of the triggering neuron.

In the first example (figure 12.2, left), activity produced by the correlated neuron shows a rate increase over NR-R transitions resembling the same conditioned increase as the trigger cell. The cross-correlation spike histogram of the two spike trains peak near zero time-lag, indicating their common drive from upstream sources. Thus, the neural circuitry driving increased R-period activity of the trigger cell affected increases in its correlated neighbor as well. In the second example (figure 12.2, right), while the trigger cell and correlated neuronal neighbor also share input from a common upstream source, the correlated neuron produced schedule-varied rate changes opposite to that of the trigger cell. That is, elevated firing probability during non-reinforced schedule intervals and comparatively reduced spike activity during reinforced periods.

12.2 Discussion

12.2.1 Evidence of learning during conditioning sessions

To examine across-session changes in operant responding during conditioning, we separated cortical and muscle activities into subsets comprising the first, second and final thirds of session time to detect possible differences in peri-transition activation patterns. Reinforced activity that peaked late during a conditioning session compared to early in each session lends evidence toward gradual acquisition of the reinforced behavior (i.e. elevated motor cortex neuron spike rate) and the alternating R-NR reinforcement schedule. The opposite trend—greatest activity early followed by diminution of R-period activity later—would suggest satiety, or loss of interest in the conditioning task.

Large amplitude, regular alternation between high R-period and low NR-period time-averaged spike rates also supports acquisition of the activity-triggered BSR task. These patterns can be seen in the final hours of the in-cage muscle conditioning (figures 10.2 and 10.3) and in-cage motor cortex cell conditioning (figure 12.1) examples reported in the results as well as for in-booth conditioned examples.

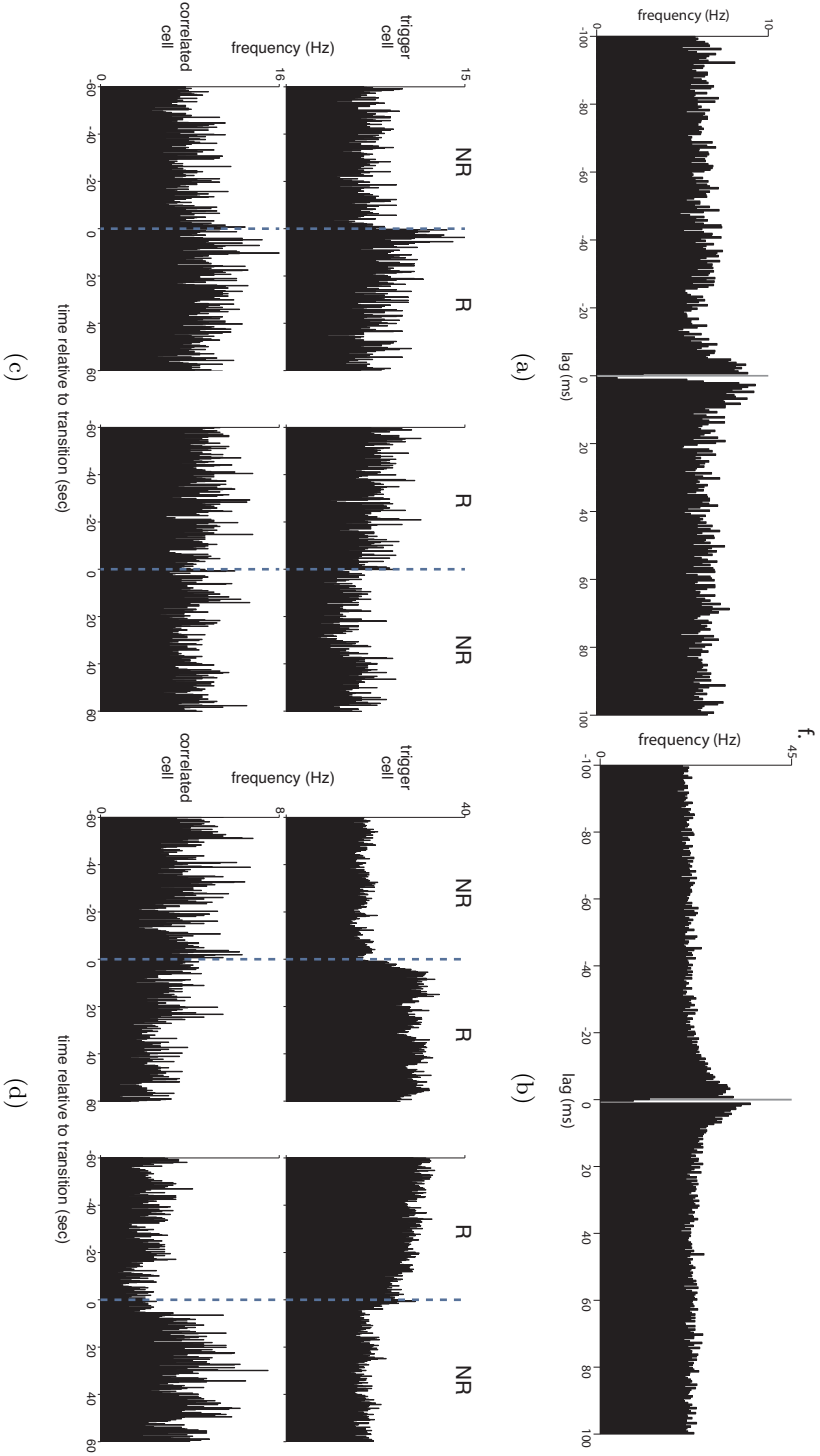


Figure 12.2: Concurrent activities of neurons that were correlated with trigger cells also modulate their activity during RCST conditioning. Cross-correlation (top row) and peri-transition (middle and bottom rows) histograms compiled from spike trains of two neighboring neurons, one of which triggered rate-contingent, spike-triggered BSR over the alternating R/NR schedule. Sub-figures on the left were compiled from a conditioning session using monkey D during which NR-R transition increases occurred in concurrent spike trains from both the trigger cell (left middle) and correlated cell (left bottom). Firing of the correlated neuron (right bottom) reciprocally activated across R-NR schedule transitions compared to the reinforced increases of the trigger cell (right middle) during an example conditioning session with monkey J.

12.2.2 *Effect of conditioning on correlated neighbor neurons of the trigger cell*

In each of the two sessions depicted in figure 12.2, the spike train produced from an additional, correlationally-linked, cortical neuron was recorded concomitantly with activity from a cell triggering RCST accumbens stimulation. Schedule-synched activity patterns observed from the correlated neuron–activity patterns that did not directly affect stimulation—suggests involvement of upstream multi-neuronal circuits in driving firing rate modulations of the trigger cell receiving common synaptic input. This is to say, though only the activity of the trigger cell affected reinforcement, many other neurons, from which it received direct and indirect input, were also recruited during BSR reinforcement intervals to mediate rate increases of the triggering neuron. These examples closely resemble previous work of Fetz and Baker (1973) in which the concomitant activity of an adjacent neuron was recorded along with that of the conditioned cell whose burst patterns triggered food rewards [30]. In that study, non-reinforced neighbor cells could also elevate their firing rates, relative reinforcement events, in patterns resembling those from the conditioned cell. In contrast to our approach however, possible correlational-linkage between cell pairs, as assessed through cross-correlation of their spike trains, was not reported.

In the first example (figure 12.2, left), activity produced by the correlated neurons shows a rate increase over NR-R transitions resembling the same conditioned increase as the trigger cell. The cross-correlation spike histogram of the two spike trains peak very close to zero time-lag indicating their common drive from upstream sources. Thus, the neural circuitry driving increased activity of the triggering cell during R-periods likely affected increases in its correlated neighbor as well. While this is the most straight-forward explanation, there are possible alternatives. It might be that the correlated cell relieves inhibition of the trigger cell through some polysynaptic pathway. Also, it could be that the similar rate increase of the correlated cell might be recruited for a motor behavior that the monkey associated with delivery of brain stimulation reward.

The second example (figure 12.2, right) requires more extensive interpretation. While the trigger cell and neighboring correlated neuron also share input from a common upstream source in this example, as indicated by a central peak in their cross-correlogram,

the correlated neuron produced schedule-synched rate changes opposite to those from the trigger cell; that is, elevated firing probability during non-reinforced periods and comparatively reduced spike activity during reinforced periods. One possible explanation might be that the correlated cell functionally inhibits the trigger cell, during NR intervals, while that inhibition is released during reinforced intervals. Inhibition of the trigger cell by the correlated cell in this case is likely indirect as a direct monosynaptic connection would give rise to asymmetry in the cross-correlogram. Another explanation could be that the correlated neighbor neuron exerts no influence on the trigger cell and received greater upstream inhibition during R-periods, regardless of the input shared by the trigger cell. Finally, another explanation might be that the trigger cell acts to inhibit the correlated cell through some polysynaptic circuit. The primary point of these examples is that they implicate involvement of other cells in the trigger cell's network in producing its modulated activity patterns during alternating R/NR conditioning.

12.2.3 Thoughts on volition

Thus far, we have made every effort to describe results from the behaviorist perspective resisting the temptation to imagine monkeys' subjective experience or hypothesize "what the monkeys were thinking" in anthropomorphic terms. Our primary finding however, that paired reinforcing brain stimulation results in conditioned increases in physiological activity patterns—patterns that were often correlated with motor behaviors—does leave room for interpretation the role of volition beyond the incentive theoretic description presented thus far. Why we have avoided mention of volition up until now stems from the fact that the term is loosely-defined and inextricably bound with philosophic notions of will, consciousness and/or awareness all of which are difficult to define, measure and test using objective techniques.

Several studies monitoring brain activity during decision tasks show that at least some actions, such as moving a finger, are initiated unconsciously at first then enter consciousness afterwards [63, 38]. These studies decode brain activity to predict decision outcomes before the subject reports the urge to act. Proponents of these studies believe that they "challenge

any versions of ‘free will’ where intention occurs at the beginning of the human decision process” [111].

In the context of our experiments, to be consistent with findings described above, neurophysiological circuits upstream from primary motor cortex could have driven increased spike rates of the trigger cell—or could have prompted cell-recruiting motor behaviors prior to a the monkeys’ “awareness” of the decision to respond. Awareness is placed in quotes because it assumes monkeys do in fact possess awareness consistent with the common notion (e.g. ability to perceive, to feel, or to be conscious of events, objects, or sensory patterns). According to the findings from Fried et. al. (2011) on epileptic humans, any possible awareness that monkeys experience during RCST BSR conditioning would not influence simultaneous cell responding and lag behind it by at least several hundred (~ 800) milliseconds [38]. Such “illusory” influence of conscious volition could at best affect responding hundreds of milliseconds into the future. Thus, according to the Fried model, the role of conscious awareness driving conditioned increases in BSR-paired cortical cell and muscle activities remains nebulous at best.

Critics have pointed out that neuroscientific investigation of volition, particularly the studies cited above, lacks consensus on the specific definition of “volition” and “free will” and that preparatory brain activity does not necessarily infer a final decision as brain activity is processed largely in parallel, through complex network connectivity, rather than in a serial step-wise progression [111]. They further point out that human reporting is very subjective. For these findings to be strengthened investigators must develop methods to objectively determine the timing of conscious decisions to actions.

Chapter 13

COMPARING CONDITIONING EFFICACY OF A CELL ACROSS IN-BOOTH AND IN-CAGE ENVIRONMENTS

13.1 Results

13.1.1 Common cell conditioned in both environments reveals superior efficacy of in-booth conditioning

In figure 13.1, spikes from a force-modulating motor cortex neuron triggered single-pulse stimulation to the accumbens during instances of elevated firing rate under the RCST stimulation paradigm. Stimulation was available during reinforced periods of an alternating 2 min. R / 5 min. NR conditioning schedule. During the first hour, the monkey underwent unit conditioning while he moved freely about his cage after which he was transferred to his familiar training booth and restrained. The Neurochip2-HV guided conditioning in both environments, ran continuously during the 6 minute transfer interval, and continued uninterrupted throughout the entire 2 hour and 10 minute session. During reinforcement periods, single 1mA biphasic pulses to accumbens were delivered on each event that exceeded 30 counts within a 500 ms wide sliding window updated every 10 ms. Cell spike activity we plot above as time-averaged rates and their surrounding 95% confidence intervals. Group means of reinforced and non-reinforced intervals for each environment are plotted and have been coded by red and black respectively. The Neurochip2-HV generated an auditory click on each stimulation pulse event to provide a discriminative stimulus. No visual feedback was provided in either environment.

The progression of alternating time-averages of reinforced and non-reinforced cortical cell firing rates show statistically significant increases during periods of BSR reinforcement compared to the neighboring time out periods that separate them, both in the training booth as well as the latter intervals during in-cage conditioning. Series correlation and von Neumann ratios indicate strong anti-correlation in the alternating pattern, well beyond

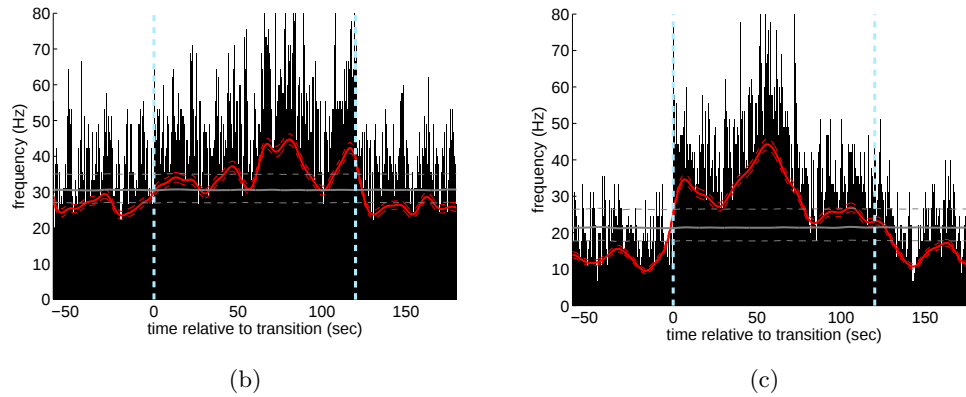
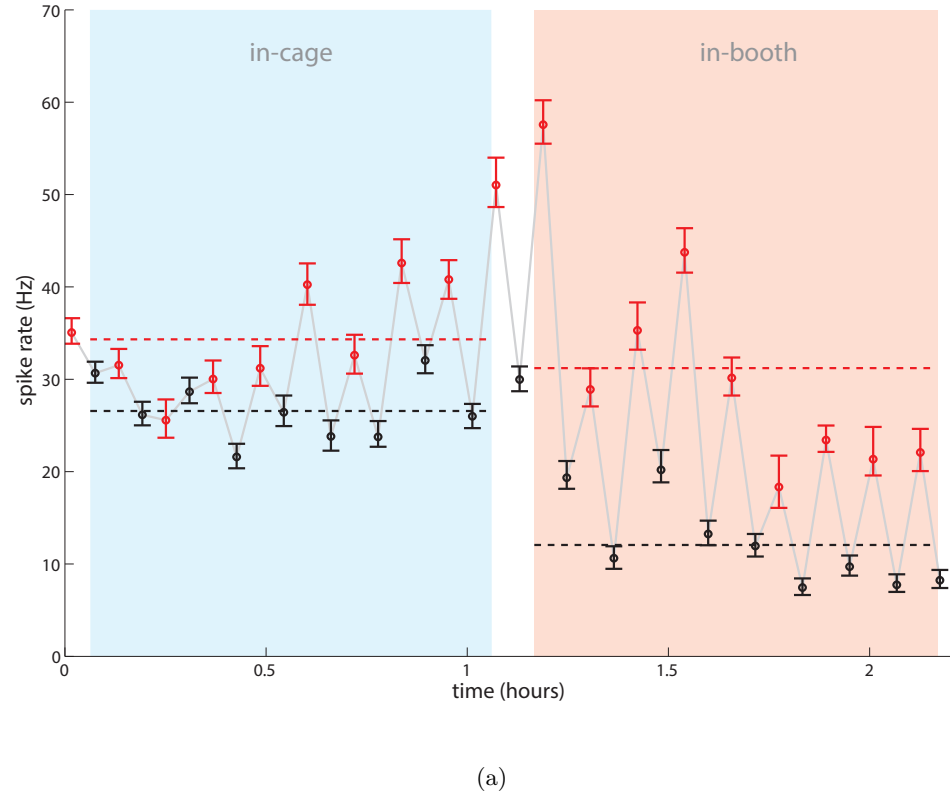


Figure 13.1: Comparing rate changes of a motor cortex neuron conditioned both in-cage and in-booth. (*Top*): Reinforced (red) and non-reinforced (black) time averages (circles) and surrounding 95% confidence intervals of motor cortex cell firing rate over the course of conditioning. The monkey was placed in his home cage (tan background) during the first hour and then quickly transferred to the training booth during hour two (light blue). (*Bottom*): Peri-transition spike activity averages from the same spike train compiled during conditioning periods in-cage (*left*) and in-booth (*right*). The Neurochip-guided conditioning in both environments; it ran continuously during the 6 minute transfer interval, and continued uninterrupted throughout the entire 2 hour 10 minute session.

chance levels as reported in tables 11.5 and 11.6, for both conditioning environments. In addition, comparisons between distributions of pooled R and NR time averages show statistically significant increases during reinforcement both in-booth and in-cage. While the medians of R groups in both environments were roughly equal (between 28 and 32 Hz), the group median of NR period averages during in-cage conditioning (25 Hz) was substantially greater than the median of the NR group during in-booth conditioning. Despite the difference in means between the two environment-coded NR distributions, their variability did not change appreciably. Peri-transition firing activity averages of the conditioned cell (figure 13.1) also reflect increased NR activity during in-cage conditioning compared to in-booth conditioning. Intra-period activity averages reveal that cortical cell firing peaked midway through the two-minute reinforcement interval. Other above-chance local maxima occurred within 10 seconds after NR-R transitions for in-booth-conditioned rates and 10 seconds prior to in-cage R-NR transitions.

13.2 Discussion

13.2.1 Possible causes of disparity between spike rates conditioned in-booth and in-cage

According to the learning metrics described above (results section 13.1.1), learning progressed slower during in-cage conditioning sessions compared to those we conducted in-booth. In addition, in-cage-conditioned R-period increases were smaller and harder to discern when comparing against NR-period activities. Several differences between conditioning environments could have contributed to this disparity. First, monkeys were restrained during in-booth sessions; their head chambers and right arms were secured relative to the booth. We believe such restraint effectively reduced activity of the often movement-correlated cell during NR periods, and served to better focus monkeys' attention on the conditioning task. Second, we had stronger discriminative stimuli at our disposal during most in-booth sessions (in the form of auditory clicks and a rate-responsive computer cursor) than the barely-audible clicks produced by the battery-powered Neurochip2-HV that guided in-cage conditioning sessions. More intense discriminative stimuli are more likely to draw monkeys' attention back to the conditioning task when they became distracted. Third, the lack

of restraint during in-cage conditioning permitted monkeys to explore a much broader set of activity patterns and associated motor behaviors when acquiring the effective response-reward association between the triggering motor cortex neuron and BSR pulses. The expanded response repertoire meant monkeys had more choices to filter out when forming effective response-reward associations and consequently took longer to demonstrate acquisition. By contrast in the training booth, where monkeys had already spent hundreds of hours performing precise task-guided wrist-force responses to which the training booth was already strongly associated, monkeys likely drew from a much smaller pool of potential reward-eliciting responses when forming response-reward associations.

Finally, consistent with the reasoning behind the r_e parameter of the single-response Law of Effect model (section 9.1.2), in-cage environments introduced additional reinforcers—in the form of food, toys, neighbor monkeys and grooming activities to name a few—that each served to increase probability of recurring behaviors other than the spike rate operant generated by the particular triggering motor cortex neuron chosen for conditioning. As the collective contribution from all non-task reinforcers, r_e , increases, the influence of the task-associated reinforcer, r (BSR in our case), on operant responding is effectively reduced. One can see in the mathematical expression of the single response Law of Effect model that the sum of the two terms $r + r_e$ comprise the denominator of the proportion governing response rate (section 9.1.2, equation 9.2). As r_e increases, the slope of the responding-reward hyperbolic curve decreases, so that the response-paired rewards must be intensified to maintain equal levels of operant responding.

When conditioned in their home cage environs, monkeys can choose from many potentially rewarding responses when opportunities arise; they are not limited to increasing the firing rate of the trigger cell for brain stimulation reward. According to the Law of Effect model, other response choices available to the monkey compete against the single cell spike rate operant that we investigators have chosen to condition. Since fewer non-task-reinforced response alternatives are available to monkeys in the training booth, the Law of Effect predicts the effectiveness of the rewards paired to the operant response (RCST brain stimulation) should be greater than in the cage where distractions are plenty.

Chapter 14

REINFORCED INCREASES IN CORTICAL SPIKE RATES ARE NOT ELICITED THROUGH DIRECT SYNAPTIC LINKAGES

14.1 Introduction

14.1.1 Evidence of anatomical projection from nucleus accumbens to primary motor cortex

Recent anatomical investigations [74, 73] suggest a more direct pathway through which input from the nucleus accumbens could reach primary motor cortex than the well-established striatal-pallidal-thalamo-cortical circuit demonstrated by Alexander et al. (1990) and Parent and Hazrati (1995) [2, 90]. Shorter than expected time-course of transynaptic, retrograde tracer implicates an alternative pathway, possibly from the ventral striatum, directly to the basal forebrain which sends widespread projections to the cerebral cortex [55, 44]. This mechanism might underlie observations of post-stimulus facilitation of proximal arm muscles elicited from accumbens stimulation (Eaton, Zanos & Fetz, 2007 unpublished data), though activation of the internal capsule due to proximity of the stimulating electrode might also be responsible for observed stimulus-evoked muscle activation. To address this possible confound (direct striatal-cortico linkage) for behaviorally-mediated increases in cortical activity via spike-triggered BSR, we delivered continuous 5 Hz pulses to the BSR site while we recorded spike activity of the candidate cell prior to each conditioning session to detect stimulus-evoked effects in cell firing probability.

14.2 Results

14.2.1 Absence of evoked effects from accumbens stimuli eliminates direct synaptic linkage as possible mechanism

To investigate one possible contributing mechanism underlying observed rate increases during RCST stimulation periods, we compiled peri-stimulus spike histograms of each cell that underwent conditioning. The presence of statistically significant features emerging at appro-

appropriate latencies (5-50 ms) would indicate presence of mono- or poly-synaptic linkage between the stimulation site and the motor cortex cell. Of all the cell candidates conditioned, none exhibited statistically significant increases in firing probability at any latency (between 0 and 200 ms) following single-pulse stimulus delivery to accumbens at the current intensity (1 mA) set for RCST stimulation conditioning. Significant stimulus-evoked effects would be characterized by deviation of the rate trace outside the 95% confidence interval straddling the average of shuffled spike data. Each subfigure in figure 14.1 shows that 95% confidence intervals surrounding kernel-smoothed traces of the observed spike event sequences (red) did not exceed chance levels (gray); indicating that the modest and transient fluctuations in spike probability seen in these histograms did not achieve statistical significance. The absence of observed stimulus-evoked effects in motor cortex cell activity eliminates the possibility that striatal-cortico linkage contributed to cortical cell spike activity increases during RCST conditioning.

14.3 Discussion

14.3.1 *Verifying discrimination of cortical single-unit spike activity*

During in-booth and in-cage conditioning sessions, single-unit cortical activity was recorded for many hours. Stability of the microwire implants enabled a single cell, based on its signature spike profile, to be discriminated within the cortical signal over multiple hours or even days (up to 48 hours). When RCST stimulation was delivered, we were concerned that the presence of stimulus artifacts following trigger spikes could be incorrectly identified as cell spike events if an artifact happened to pass through discriminator windows. These experiments required continuous discrimination of on-going cell discharge events without mistaken discrimination of subsequent stimulus artifacts and we employed strategies to reduce this possibility. In addition, we verified that discriminator acceptance events were indeed accurate using two techniques to detect instances where stimulus artifacts were misclassified as spikes: 1) by inspecting samplings of peri-discriminator-acceptance sweeps of the recorded cortical signal to visualize possible errant instances and to appraise cell stability and, 2) compiled inter-spike interval histograms of the spike train event record whereby

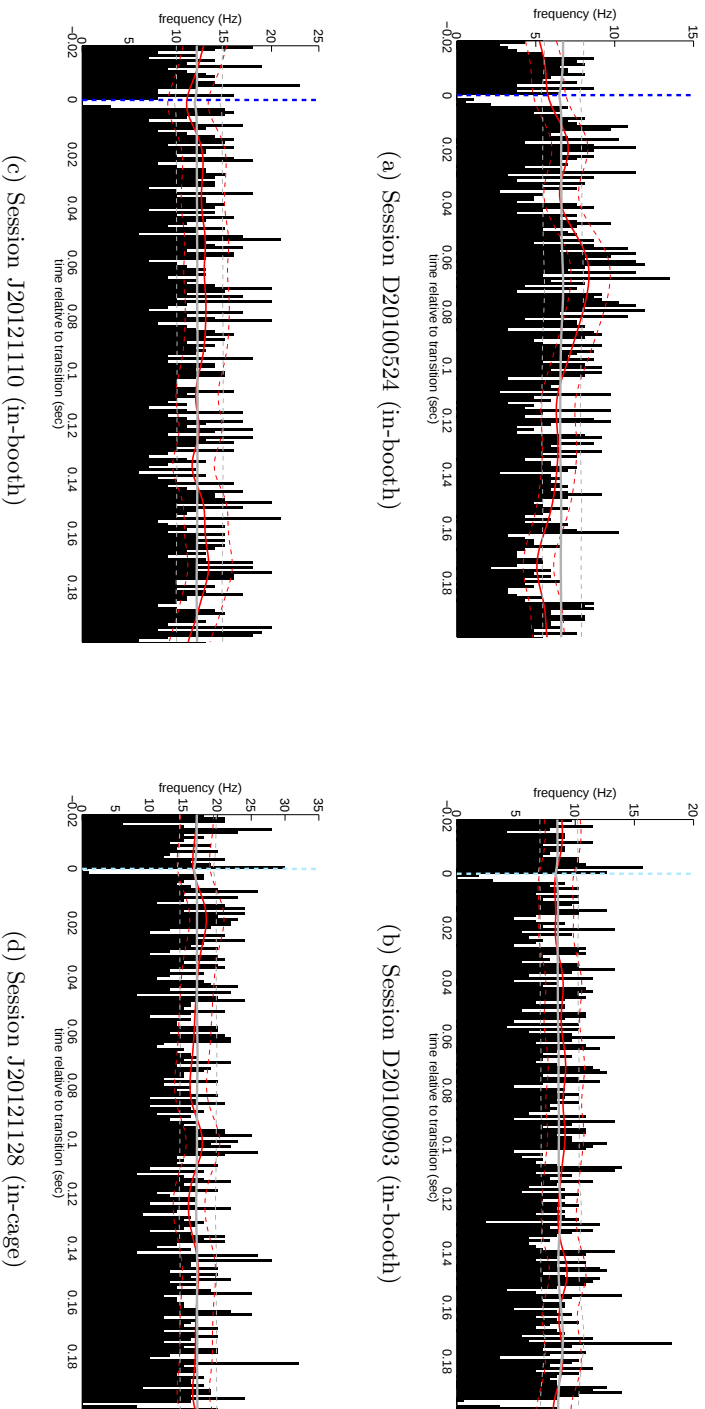


Figure 14.1: Peri-stimulus spike histograms compiled from spike trains surrounding single-pulse stimulation of striatal rein-
forcement sites. Histograms above were compiled just prior to example experiments depicted in figures 11.4 and 11.6d using
the same recording and stimulating configurations. Vertical blue dashed lines depict stimulus delivery. Histogram bin width:
2ms. Point density average (thick red line) and limits of the 95% confidence band (red dashed lines) depict kernel-smoothed
approximates of peri-stimulus mean spike rates.

possible misidentified spikes would arise as localized increases in ISI probability at latencies of pulse delivery. After inspecting in-booth sessions, neither spike profile sweeps nor the corresponding ISI histograms showed any indication that stimulus artifacts were falsely detected as spike events as more stringent spike discrimination and more sophisticated artifact suppression techniques were available than for in-cage neurochip conditioning sessions.

The example neurochip-guided in-cage session (figure 11.5) was minutely contaminated by false events whereby some stimulus artifacts were incorrectly identified as cortical spikes. Within the collection of peri-acceptance sweeps, these were evident as irregularly shaped, non-spike sweep traces that were aligned with discriminator windows and contributed to abrupt, transient jumps in frequency at latencies of pulse delivery in ISI histograms. Misclassification in these sessions was rare occurring in only 1.5% of discriminated events during R-periods when stimulation was delivered. We calculated this proportion as the number of counts comprising the spike above the ISI histogram trend—as interpolated from heights of surrounding bars—divided by the total number of acceptance events during R-periods. These small proportions of false events contributed negligibly to RCST stimulation conditioning, and observed resultant rate increases, in the example session described above.

Chapter 15

FUTURE DIRECTIONS**15.1 Investigating neural coding**

The high temporal precision of spike-triggered BSR could permit precise reinforcement of selected spike patterns generated by neurons. Increased probability of chosen operant patterns, when paired with BSR, could provide evidence toward the temporal coding hypothesis; crudely stated here as the possibility that additional information might be contained in the relative timing of spikes during synaptic transmission, beyond the information conveyed through changes in average firing rate over comparatively broader time-scales [100]. Successful conditioning of temporal spike patterns would implicate temporal coding as a mechanism in neural processing as would be implied by its being subject to modification through operant conditioning. Candidate strategies for conditioning temporal spike patterns include, but are not limited to:

1. Selecting particular inter-spike intervals to trigger BSR stimulation as generated from an ongoing spike-train of a single neuron. Investigators would need to correct for probability increases of the selected ISI(s) attributable to overall firing rate increases.
2. Reinforcing beyond-chance instances of synchrony between concurrent spike trains produced by two or more neurons.
3. Reinforcing “higher-order” patterns in a single-cell spike-train, such as inter-spike intervals between two spikes separated by at least one or more intermediaries in the sequence (e.g. $ISI_2 = t_{i+2} - t_i$, or $ISI_n = t_{i+n} - t_i$, where i indexes each spike event in the list of all time-stamps).
4. Conditioning changes in oscillations of spike rate by selecting to reinforce ISIs marking peaks or troughs in the cell’s auto-correlation distribution.

Unfortunately, failure to demonstrate conditioned changes in single-cell or multiple-cell spike patterns would not yield fruitful insights as absence of effects in this paradigm could be attributed to other factors; for example, if inter-spike intervals are not subject to change through performance of any motor behavior but are nevertheless involved in some other aspect of neural processing.

15.2 Clinical applications of cortically-triggered striatal brain stimulation

Deep brain stimulation is currently used to treat tremor and obsessive-compulsive disorder in humans. The treatment paradigm is “open-loop” as the stimulation is either delivered continuously, externally-controlled or self-administered [59]. Closed-loop, cortically-driven striatal stimulation to accumbens, to our knowledge, has not been performed in humans, likely due to inaccessibility of stable, long-term cortical signals. Single cell action potentials, at present, are particularly ill-suited for human treatment models due to the invasiveness of conventional electrode recording procedures used in this, and most other traditional in-vivo electrophysiological investigations using non-human primates as subjects. Less-invasive procedures monitoring epidural cortical field potentials, in place of intra-cortical single-cell action potentials, would be one strategy to extend this cortically-driven closed-loop paradigm to humans; in particular, to those suffering from impaired motor function as the result of spinal injury or stroke, who use BCIs to restore lost function. Recent work has demonstrated increased in oscillations of cortical field potentials can be induced through operant conditioning in non-human primates [25]. Though a suitable activity-to-stimulation mapping would have to be developed, autonomous cortically-driven accumbens stimulation could enable a person to hone control over her own cortical activity without the substantial investments of time, effort and attention required by singly-focused sessions of BCI skill practice. In the ideal scenario, activity-driven BSR runs as the person carries out her normal behavior throughout the day. When the time comes to use her neuroprosthetic, she would benefit from enhanced control over the device as her skill in modulating her own cortical signals had improved through acquisition of the field-to-BSR pairing and subsequent schedule-correlated increases in field activity.

We focused on conditioning single units in this study for three reasons: 1) single-cell

action potentials, while relatively difficult to obtain, are easier to analyze and interpret compared to oscillations in cortical field potentials, 2) the discreteness of neuron action potentials enables conceptually simple activity-to-stimulus relationships; specifically, that supra-threshold increases in spike frequency imply increased frequency of BSR in a direct one-to-one relationship, and finally 3) single cell action potentials have been well-documented to play a functional role in neural processing underlying motor behaviors; doubt still exists about the functional, as opposed to epiphenomenal, role of field potentials in conjunction with motor behaviors [29].

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