Towards a Method to Study Neurorobotic Control in a Rat Model of Spinal Cord Injury

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***Abstract*—Neurorobotic control of prosthetic devices may be a viable therapeutic intervention that provides spinal cord injury patients with the ability to use the neuronal activity of populations of single neurons to control an external device (i.e. cursor on a computer screen or robotic arm). However, we are limited by our understanding of how spinal cord injury alters the ability of these neurons to convey information about the intention to move. Therefore, there is a need to develop animal models that 1) describe how population of single neurons encode information about different behavioral tasks (skilled vs. unskilled), 2) determine how this encoding is modulated by spinal cord injury and 3) perform neurorobotic control after spinal cord injury. To address the first question, we developed a rat model of spinal cord transection to evaluate the effects of the injury on the neuronal activity related to hindlimb activity. The model consists of training the rat to press a pedal with its hindlimbs. This paper describes the method that defines both the magnitude and latency of a neuron's activity in terms of its Peri-Event Histogram relative to the animal's movements during the task. The method provides a means by which changes in neural activation can be correlated with changes in behavior.**

I. Introduction

Neurorobotic control of prosthetics devices has been used in healthy rats and monkeys to control a robotic devices and move a cursor on a computer screen. While it has been used in a limited number of humans with spinal cord injury (SCI) to control a cursor on a computer screen, to study the problem of how SCI alter the ability of populations of neurons to encode sensorimotor events requires an appropriate animal model. Rats are good models because much of what is known about SCI and the effect of therapeutic interventions has been done in rats. A complete transect model at the T8/T9 level that block all sensorimotor information transfer between the brain and spinal cord caudal to the lesion is useful because it demonstrates an important clinical state and an unambiguous experimental model. However, neuronal activity has not been studied in awake animals after SCI. Moreover, little is known about how hindlimb sensorimotor regions of the brain encode sensorimotor tasks. To overcome these limitations, we present here methods to study how neurons in the hindlimb sensorimotor cortex encode information during different behavioral tasks (skilled and unskilled). Two important issues arise when measuring the neuronal response to kinematically relevant events. First, since a freely moving animal can experience multiple such events in parallel rather than sequentially, there is a need to evaluate a cell's principle response or the event to which the cell responds most strongly, when that cell also responds at some level to other events associated with the behavior under study. Second, there is a long and variable latency between the behavioral event of interest and the increase in neuronal activity. Both of these issues are addressed by basing the method on the standard Peri-Event Histogram (PEH).

Our method is tested in awake, freely moving rats that have been chronically implanted with bilateral arrays of microwires in the cortex at layer V in the area of the somatotopic forepaw and hindpaw representations and trained to perform a skilled reaching task with their hindlimbs.

II. Methods

Step one: Identify PEH peak response region

The ability to determine a cell's principle response event, as well as quantify its response rests on determining the peak region of the PEH. In brief, PEH are calculated by summing the activity of a cell within a set of time windows created around repeated occurrences of an event. The occurrences of the event under study are aligned at time zero so a theoretical instantaneous response should exhibit a peak centered at zero. However, there are inherent transmission latencies between the cortex and the periphery, so it is not appropriate to assume that only searching an area centered on zero will capture the peak of the histogram. Instead, the peak region is identified by using a threshold based on the mean firing rate of each neuron. The mean firing rate of each cell is determined as the number of spikes occurring during the entire record divided by the overall time period of the record. This mean rate is used to find the 99% confidence bounds appropriate for a random Poisson process with the same overall mean as the cell's mean firing rate. In Fig. 1A below there is an example of a cell's PEH and the confidence bound that was calculated for that cell.

In order to determine the extent of the PEH's peak region, the 99% confidence bound threshold is applied to a smoothed PEH waveform, obtained by applying a 25 msec sliding rectangular window average to the PEH (Fig. 1B). The peak of this smoothed histogram is identified as the single bin with the highest value. The peak region is defined as the continuous set of bins surrounding this peak that also

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exceed the confidence bound threshold (Fig. 1C). The same cell can be evaluated for several different behavioral events.

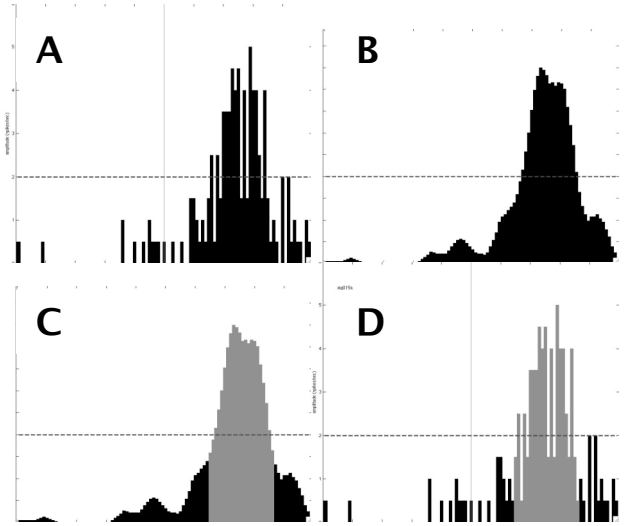

Fig. 1: Process for determination of peak response region and firing statistics. The dotted line in each frame represents the upper bound of a 99% confidence interval around the cell's mean firing rate based on a Poisson distribution.

Step two: Identify significantly modulating cells

Before evaluating the response of a neuron to a particular behavioral event, we use the information contained within the PEH's peak region to evaluate whether the cell exhibits activity that is significantly increased from its mean or baseline value in association with or in response to an event. First, the peak region determined in Step One, above, is applied to the original PEH (Fig. 1D). If there are three bins within the peak region that exceed the upper 99% confidence bound, the cell is classified as being significantly modulated by the event used to construct the PEH. Only cells thus classified are included when population means are calculated for a particular statistic.

Step three: calculate neural parameters

The response parameters in which we are interested can be calculated by quantifying neuronal activity in the peak region applied to the original PEH (Fig1D). These parameters are summarized in table (1) below. A graphical depiction of the latency parameters that are calculated is presented in fig. 2.

Value	Description
Response Magnitude (RM)	sum of bin values within the peak region - mean firing rate
Peak Response (PR)	value of maximum bin within peak region - mean firing rate
First Bin Latency (FBL)	time to first bin of peak region
Last Bin Latency (LBL)	time to last bin of peak region
Peak Latency (PL)	time between peak response and zero

Table 1. Definitions for neural parameters

The quantities listed in Table1 allow us to compare the activity of large groups of single neurons across animals, across behavioral tasks and before and after spinal cord injury. A graphical depiction of the latency parameters that are calculated is presented in fig. 2.

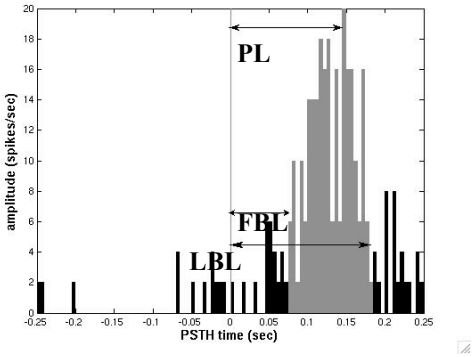

Figure 2. Depiction of latency parameters

In an anesthetized preparation or in a controlled environment of serial movements, specifying the neural parameters for each neuron in response to every event independently would be appropriate. In an unrestrained movement environment, events of interest often occur close enough together that the windows used for the calculation of cells' PEH overlap. Thus the various PEH calculated from the cells' activity can be classified as significantly different from baseline in association with multiple events. When this happens, it is necessary to determine which event is primarily responsible for changes in the cell's spike activity. This event will be called the Principle event and any others will be called the surround events, a nomenclature borrowed from our somatosensory mapping studies. A cell's principle event identity is the event for which the cell's PEH exhibits the highest peak response, together with the shortest peak latency. These methods are applied to rats trained to perform a skilled reaching task.

III. Results

Skilled hindlimb reaching task

In the skilled reaching task, animals are trained (pre-implantation) to press a pedal embedded in the floor of an experimental chamber with either hindpaw in response to an auditory cue for a water reward. Paw preference differs among individuals but remains consistent for a single animal from initial training through the end of the study. The pedal is attached to an amplitude sensor which records the press activity; this record serves as the basis for assigning behaviorally relevant events for the task. In an experiment, the trials that resulted in the successful completion of the trained task in response to the auditory stimulus (called the "chime") are known as the valid trials. The validChime events are therefore those stimuli that resulted in a successful trial. This event, along with the beginning of the downward deflection of the pedal (startPress), the point of maximum pedal displacement (maxPress), and the relaxation of force applied to the pedal surface (lift), which is also the beginning of a return to stance, where the animal is required to maintain a quiet posture for a period of 3-5 seconds before a new trial can begin as signaled by the next auditory cue. Rats can learn to perform this task at a 90% success rate with less than 10% false positives (pedal depression in the absence of the chime), achieving 50-100 trials per session. Animals in this study received training in this apparatus for

30-45 minutes per day, 6 days per week until they acquired the desired level of performance. They then underwent bilateral implantation of chronic microwire arrays into the hindlimb somatotopic representation area of the cortex. Post implantation recordings were performed, events relevant to the task were identified, and calculation of neural parameters were determined as described above. Cells that were modulated principally in association with each of the given events can be described in terms of their Response Mangnitude, Peak Response, First Bin Latency, Last Bin Latency, and Peak Latency. Of the 102 recorded cells with signficant response to an event, 18 were associated with the validChime event, 35 with startPress, 18 with maxPress, and 31 with lift (fig. 4). Thus the beginning and end of the period of placing dynamic pressure onto the pedal seemed to engender a larger representation in the cortex than points before and after these times did.

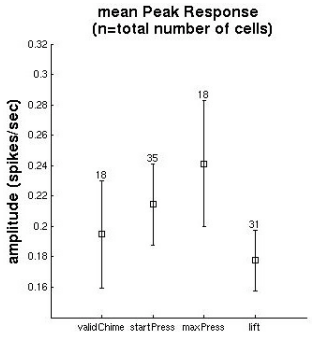

Figure 4. Global mean response to skilled reaching events. Numbers accompanying each errorbar are cells responding to that event.

Correlation of neural parameters with performance

The frequency of repetition of the skilled reaching task can be highly irregular. This happens when, for instance, the animal pauses from the task to groom itself, or if it becomes distracted by sounds or smells circulating through the external environment. Data collection proceeds during these times but no valid trials are likely to be recorded. Does the presence of interruptions in the steady performance of the task lead to a higher, lower, or unaffected level of neural activity when attention returns to the task? To answer this question, we correlated the neural parameters with the behavioral performance for that day. First, the performance was defined as the number of valid trials for that day divided by the total number of trials. As expected, there was a significant positive correlation between the response magnitude of startPress cells and performance on the task.

A second way of measuring performance is to examine the regularity with which trials occurred. This was obtained by subtracting the time stamp of an event that takes place early in a press, such as the time of the audible cue, from a later event in the same press, such as the actual beginning of pedal depression. This produced a vector of values that express the reaction time of the animal to the tone (for the two events chosen in this example). Calculating the standard deviation of this vector provides an expression of the regularity of the animal's response time on a given day. So that this measure can be more readily comparable across days, it was calculated as a percentage of the mean for that day. This value was negatively correlated at a significant level with response magnitudes for startPress cells and uncorrelated with response magnitudes of other cells. The negative correlation means cells that were activated around startPress do so with lower firing rates when the animal's performance was more irregular. On the other hand, cells that were modulated chiefly during the lift phase seem to behave oppositely, as they are positively correlated with the irregularity in the time elapsed for the pedal downstroke.

A third measure can also be obtained from the mean of the vector of elapsed times between sets of events for a day's recording. The mean elapsed time value provided an indication of simply how slowly or quickly the animal was executing the task on a given day. There was an interesting duality in how the neurons' responses varied with this statistic. Generally, Response Magnitudes were negatively correlated with mean elapsed trial time, but the Peak Responses were negatively correlated. This indicates a narrowing of the PEH response region when the animal performs more slowly, which can be caused when the neural responses become more tightly time-locked to the event with which they're associated.

Discussion

A wealth of data can be obtained from neural recordings of awake behaving animals through the methods outlined above. The challenge that follows is to understand the physiological significance of the parameters chosen, and what their changing values mean in the context of complex behaviors and how spinal cord injury alters these neuronal responses.

Representation of reaching movements in the cortex

There are competing theories regarding what aspects of movement are encoded by the cells of the motor cortex. The debate in its classic form pits adherents of directionally coded population vectors [3],[7],[8] against proponents of biomechanical activation of muscle forces, joint torques, etc [1],[2],[10],[11]. Here, when sequential phases of a skilled movement are executed, we are able to observe two phenomena of interest. The first is the distribution of cells' principle event association identities, and the second is the comparative average firing rates among cells affiliated with each of the events.

Regarding cell distribution, the majority of recorded cells were associated with the startPress and lift movements, events occurring at the beginning and end of the skilled reach. This arrangement could reflect the investment of cortical resources necessary to perform a challenging hindlimb reach task, since attempting to manipulate the environment with their hindlimbs seems to be novel concept for rats, a statement supported by the diffficulty of training them to perform the skilled hindlimb reach. The hypothesis of correlation between cortical resource investment and skill learning is supported by studies indicating that acquiring new behaviors enhances the activation of cortex in the area representing the limbs involved in the newly learned behavior [4]-[6],[9]. Whether there is an expansion of the hindlimb representation during training is as yet unknown,

but could be tested by implanting animals at the beginning of their training, rather than waiting until they have achieved proficiency.

The second interesting phenomenon apparent in these results is that the firing rates of cells associated with the maximum application of force during the task is greater than those of cells associated with any other phase. Taken together, these results raise the intriguing possibility that large populations of cells are necessary to execute the phases of the task requiring the most coordination, but high firing rates are more appropriate for the exertion of higher degrees of force. Consistent with this notion is the observation that the firing rates of cells associated with the lift phase of the task, which is in fact assisted by a spring that returns the pedal to its starting position, have lower rates of firing than any of the other groups of cells recorded during this task.

The event selection approach used to determine principle association identities bears some resemblance to the construction of population vectors from preferred directions of individual cells pioneered by Georgeopoulos *et al.* [3]. In the context of our experiments, however, the population activity is examined in a temporal reference frame, rather than the spatial reference frame employed by Georgeopoulos *et al*. We feel that this is appropriate because of the simultaneous, or short-delay sequential, coordinated movements of multiple limbs involved in unrestrained behavior. These movements tend to incorporate synergy in direction among limbs so that the animal may move about successfully. It becomes necessary, therefore, to understand which limb movement, or other relevant behavioral event, is of primary importance to a given cell before investigating other questions.

The skilled task performed by the animals in the recordings described here is similar to tasks carried out in an extensive body of work that involves rats performing skilled reaching movements with a forepaw. The use of the hindpaw is novel, however, and allows a new chapter of investigation to be opened into cortical activity resulting from learned movements, using a limb that is unaccustomed to performing such movements ordinarily.

The methodology discussed here will be incorporated into spinal cord injury studies currently ongoing in our laboratory, allowing us to address questions such as how the brain's ability to encode for movement is affected by the loss of its efferent targets in the periphery.

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