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Skilled hindlimb reaching task in rats as a platform for a brain-machine interface to restore motor function after complete spinal cord injury

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Abstract–**Current behavioral tasks utilized as models for decoding neural activity for use in brain-machine interfaces are constrained primarily to forelimb tasks or locomotion. We present here our methodology for training adult rats in a novel skilled hindlimb 'reaching' task in which the animal is trained to make different types of hindlimb movements. 6 adult Long-Evans rats were trained to make variable duration (<1 or >1.5 s) hindlimb presses cued by a spatially-independent visual cue. 5 of 6 animals (83.3%) were able to learn the task to proficiency. The training paradigm introduced here serves as a platform to investigate the ability of the animal to transfer motor cortical activity in response to a cue originally generated during normal movments, to a novel context in the absecense of movement and ultimately after complete mid-thoracic spinal cord transection. We also present preliminary results of offline classification of neural activity during trial performance for two trained animals.**

I. INTRODUCTION

THE ability to decode neural activity for use in a brain machine interface in animal models remains inexorably linked to the task that the animal is trained to perform. In monkeys, the majority of BMI applications are based around spatially-guided reaching and target acquisition tasks, such as the center out reaching task and random target smooth pursuit tasks [1],[2]. Until recently little attention has been given to decoding activity of motor cortical populations devoted to the control of hindlimb movements, and of these studies, the focus has been primarily on locomotion in in-tact animals [3],[4]. To explore the underlying neural processing that occurs during skilled hindlimb placements, it is necessary to decode the information about different types of skilled movements, both in healthy and spinally injured animals.

We previously introduced a novel skilled hindlimb reaching task in which rats were trained to make cued reaches as a platform for decoding the intention to reach from populations of motor cortex neurons. Rewards are delivered for each successful reach, while online neural activity is used to decode the animal's intent to perform the reach. Many aspects of the task, including reaction time and press velocity show good correlation to decoded neural activity. After a complete spinal cord transection, it was still possible to decode the intention to perform the reach. This suggests that the neural circuitry devoted to controlling areas of the body below the lesion site can still encode information about the reach.

The objective of this paper then, is to extend our previous work by modifying our training paradigm to include training for different durations of hindlimb press and to present preliminary offline decoding results from two animals trained in the task. We used operant conditioning methods to train rats to discriminate between two spatially-independent visual cues, and to make the appropriate type of press. Ensembles of neural activity recorded from hindlimb sensorimotor corex of two animals during the behavior were evaluated offline and decoded using PETH-based classification techniques [5] on a single trial basis. We hypothesize that the proposed training regime will serve as an improved platform to allow decoding of specific temporal features of the skilled hindlimb movement from populations of motor cortex neurons, and ultimately, after complete spinal cord transection.

II. MATERIALS AND METHODS

Six adult male Long-Evans rats (8 weeks old, 150-200g) were trained. Animals were housed separately, maintained on a 12/12 h light/dark cycle. One week prior to training, animals were allowed restricted access to water (100mL/kg/24 hours) to allow for training. Access to food was *ad libitum* though animals rarely ate in the absence of water. Healthy maintenance of weight was monitored. Animals were trained at least 6 days/week to maximize learning rate and to minimize losses of learning over time. All protocols were approved by the IACUC of Drexel University.

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Training Apparatus

Animals were trained in a custom-made Plexiglas chamber containing an inlet for water delivery and a moveable pedal connected to an amplitude sensor that uses a variable resistor (Fig 1). While learning the skilled hindlimb task, animals were rewarded for pressing the amplitude sensor via the pedal past a fixed threshold. An overhead LED array, either flashing or solid light (fixed duration), was used as the cue for the proper type of press.

Pre-training: Phase I and II

There were four phases of training involved in the skilled hind limb press task (see Table 1 for summary). In phase I, rats were introduced to the training chamber and water was released in to the receptacle at random intervals (free reward) until the location of water reward was learned.

In phase II of training, rats were trained to interact with and to make full hindlimb presses on the pedal. Initially, the numbers of rewards were greatly diminished to encourage exploration of the training enclosure. The size of the enclosure forced interaction with the pedal including sniffing, forelimb, and hindlimb contact. Initially, these interactions gained reward but behavior was shaped, as quickly as possible, toward hindlimb contact and then presses by restricting rewards.

Over time the rats begin to make sufficiently large amplitude hind limb presses that pass a threshold (step 2, Fig 2B-C). As rats began to make consistent (>80%), self-directed presses from a stationary stance position, they were rewarded only for the full press past threshold, lasting less than 1 second. Once proficiency in the motor skill was achieved (>85% of presses single motion of on-and-off movement), phase III cue training began.

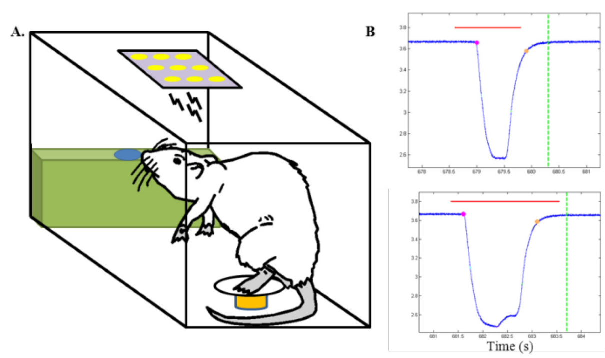

Fig. 1. Schematic illustration of training apparatus and example amplitude sensor output for short and long presses (B and C respectively). Horizontal line indicates press duration. Vertical line indicates average time of reward. Red dot indicates start of press. Orange dot indicates end of press. Reward is presented 0.7 s after end press.

TABLE I

Training schedule for skilled hindlimb press task

	Phase	Level	Training Criterion	Mean # of sessions
I	Water Reward		Awareness of water reward	3.2±0.4
II		1	FP and HP presses rewarded	
		2	Press w/HP from any position	
		3	Press w/HP from starting stance	26.8±2.4
		4	Single motion press	
III	Cue Training	1	Introduce cue 1 coincident	20.2±1.9
		2	Lights out condition introduced	31±2
		3	Proficency I	
		4	Introduce cue 2 at median press duration until 1.5 s	32.4±3.1
		5	Proficiency II	
IV	Cue choice training	1	Randomized 1:1 cues	29.4±4.1
V	Implantation		Proficiency III	

Phase III: Cue Training

In phase III of training, rats were trained to perform the learned skilled hindlimb reach task only when instructed by a visual cue. There were five intermediate steps as shown in Table 1.

In step 1, cue 1 was delivered coincidentally with animals making a self-directed press about half the time. To reinforce cueing behavior and to reduce the number of self-paced presses, a house lights-off condition was introduced in step 3. In this step, any presses up to 2 seconds prior to a cued trial resulted in the overhead lights turning off for up to four seconds in which no rewards were delivered and no cues were given (false alarm, FA). This condition was kept in place over training days until animals reached proficiency in this step.

The second half of phase III asked animals to alter their learned hindlimb press by increasing the duration of the press until animals reached proficiency (>80% TP for both short and long presses independently and <20% false alarms). This was accomplished by the introduction of cue 2 and house lights-off conditions. To start, median press times were found and set as the minimum press duration required for reward. As training progressed the minimum duration was increased on average 70 ms per session. This continued until the minimum duration was 1.5 seconds.

Phase IV: Cue-choice Training

In phase IV, animals were proficient in both short and long duration presses, and performed both types of the task in random order.

Performance Metrics

Performance of animals was based on the numbers of correct and incorrect trials. The proportion of correct presses (TP) divided by the total number of trials was defined as the true positive rate (TPR). The false alarm rate was defined as the number of incorrect presses in the 2 second window before cue onset (false alarm: FA) divided by the total number of trials (FAR). Separate measures of TPR for short and long presses were tabulated upon introduction of cue 2. Proficiency for all phases of cue training was considered a TPR >80% for all press types and <20% FAR.

Offline Decoding: PSTH-based classifier

Two animals were implanted bilaterally with 4x4 microwire arrays (Neurolinc Inc., NY) in the hindlimb sensorimotor cortex under aseptic conditions. The duration of press for two animals trained to proficiency in the described task were classified using the PSTH-based classifier approach described in [5]. Briefly, maximal

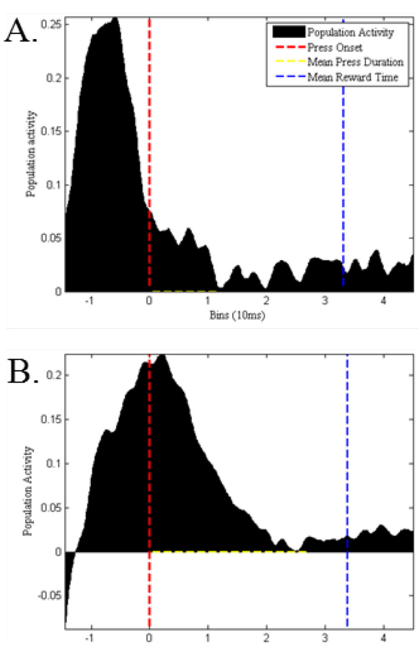

Fig. 3. Example neural population functions of short (A) and long (B) presses over single recording session (64 short, 69 long presses included). First dotted line indicates cue onset, second dotted line represents time of reward delivery. For a given single trial, peri-event time histogram was compared to templates as in A and B and was correctly classified if the trial matched the corresponding template.

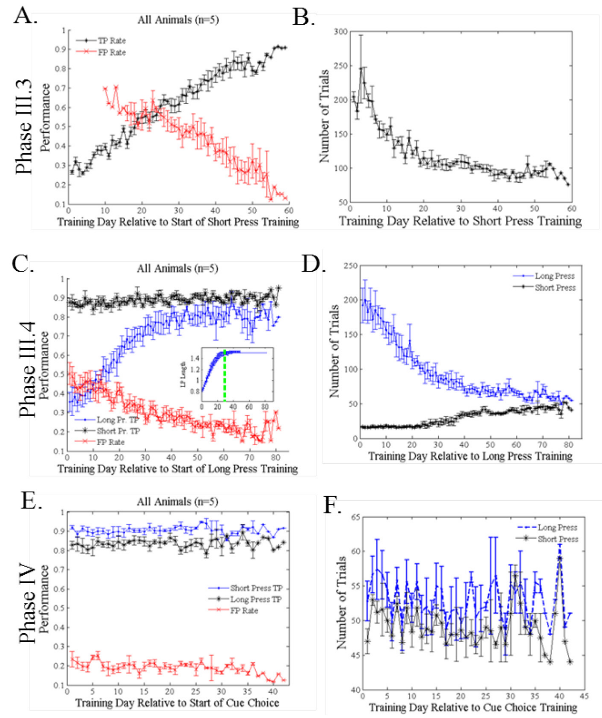

Fig 3. Performance (TP rate and FA rates) and numbers of trials over short (A-B) and long press cue training (C-D) and cue choice training (E-F). Data presented as mean and SEM performance (number of trials) relative to onset of phase of training (III.3: short cue; III.4: long cue; IV: cue choice).

sources (weights) of neuronal variance were estimated through a PCA/ICA analysis of neural firing rates during the task. To classify, single trials were randomly removed from a given recording day and templates for long and short presses were created by weighting average firing rates for all remaining trials of each cue type (Fig. 2A, short; 2B long template). The PETH of the selected trial was then decomposed into pre- and post- cue windows (pre: -1.5s bg; post: 0 to 4.5s). Four comparisons were made on each window (pre and post cue) to both templates. A true positive was considered to be a correctly classified trial, where a given trial matched most closely the distribution of the correct template. Conversely, a false positive was defined as any misclassification of either window.

III. Results

Table 1 shows the average number of training sessions it took animals for each phase.

Phase III –levels 1-3: short duration press

It took, on average, 31 ± 2.56 days for rats to complete the pre-training (Phases 1 and 2) and to be ready for Phase III, cue training. During cue training, rats required an additional 51.2 training sessions to reach proficiency in the initial, short duration hindlimb press. Animals learned to perform the task after cue presentation (correct trials) and reduced the number of self-paced skilled movements

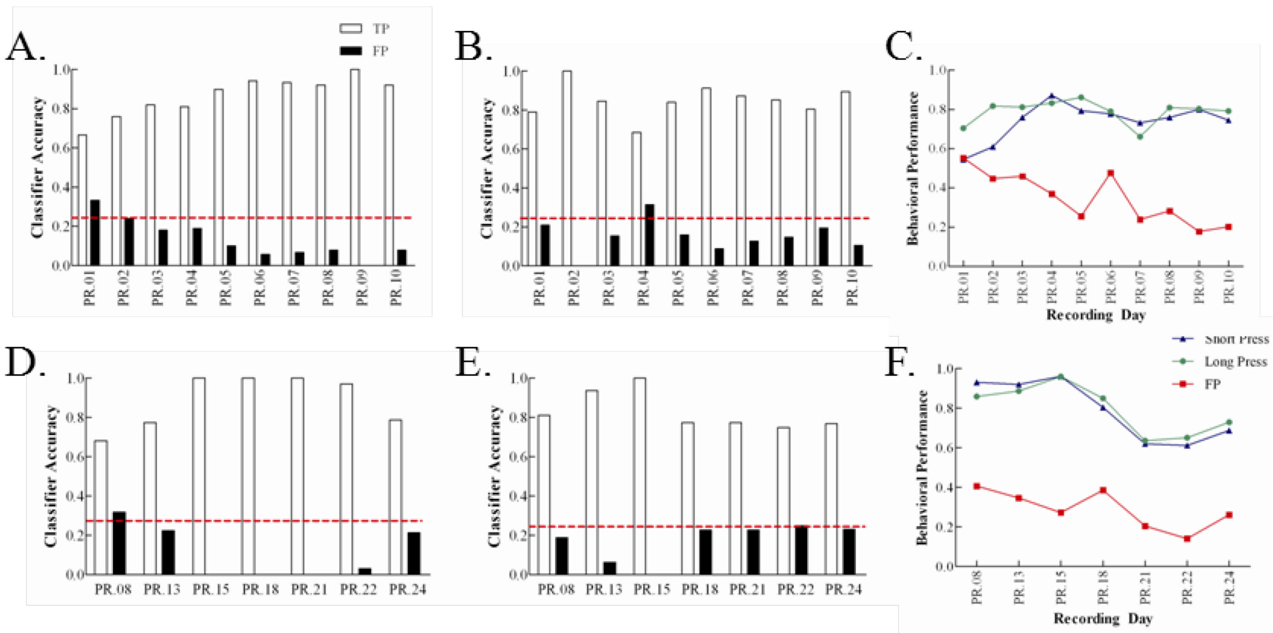

Fig 4. Results of offline classification for two trained animals (A-C, NRC.01; D-F, NRC.02). Correctly (white bars) and incorrectly (black bars) classified trials for short presses (A, D) and long presses (B,E) from neural activity recorded during task performance. Corresponding behavioral performance for respective recording sessions shown in C, F. Correct classification of trials is significantly above chance (25%) on all days, suggesting preliminarily that M1 ensembles differently encode kinematics of press.

outside the trial periods (Fig. 2A). The number of correct trials per session increased almost daily (Fig. 2B).

Phase III –levels 4-5: long duration press

Animals proficient in the short cued press moved to the long cued press. The median short press time (0.824 ± 0.027 sec, n=5) increased on average 0.07 seconds per training day until the final minimum press time of 1.5 seconds was reached (inset of Fig. 3C). Rats learned to make a full duration press (1.5 s) within 28±3.32 training sessions (minimum 19 sessions, maximum 36 sessions). As performance reached proficiency (>80%TP,<20%FA; Fig. 2C), the ratio of long to short press cues were decreased to approximately 1:1 before moving to phase IV (Fig 2D).

Phase IV: Cue-choice training

Cues were presented in random order over the course of each training session at roughly 1:1 (Fig. 2F). On average, animals trained in phase IV for 29.4±4.07 sessions before performance criteria were met (Fig. 2E) and animals moved to cortical implantation.

Phase V: Cortical Implantation and Offline Decoding

Fig. 4 shows PSTH-based classifier accuracy over all recording sessions for two animals (NRC.01, n=32±3 cells; NRC.02, n=37±2 cells). We first evaluated whether we could discriminate the press from background activity, which was readily discriminable from non-press periods, (>90%, not shown). Then we discriminated short press or long press from each of the four possible event windows. Classifier performance averaged 82% but was always better than 60% (Fig. 4, bars). Behavioral performance over recording sessions is shown in Fig. 4C and F.

IV. Discussion

The results presented here demonstrate our ability to train rats in different variations of a skilled hindlimb press task for use as a platform for brain machine interface. As an extension of our previous work, we have trained animals under our same operant conditioning paradigm to perform multiple duration presses. Furthermore, we have shown preliminarily that the neural substrates of variable duration hindlimb presses to a single reach target. Using PSTH-based ensemble classification methods [5], we were able to highlight differences in cortical activity between press types on a single trial basis, allowing us to both accurately decode an animal's intention to move to discriminate the temporal features of the behavior for use in a BMI following complete thoracic spinal transection.

Acknowledgements

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