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A rodent brain-machine interface paradigm to study the impact of paraplegia on BMI performance

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Abstract

Background: Most brain machine interfaces (BMI) focus on upper body function in non-injured animals, not addressing the lower limb functional needs of those with paraplegia. A need exists for a novel BMI task that engages the lower body and takes advantage of well-established rodent spinal cord injury (SCI) models to study methods to improve BMI performance.

New method: A tilt BMI task was designed that randomly applies different types of tilts to a platform, decodes the tilt type applied and rights the platform if the decoder correctly classifies the tilt type. The task was tested on female rats and is relatively natural such that it does not require the animal to learn a new skill. It is self-rewarding such that there is no need for additional rewards, eliminating food or water restriction, which can be especially hard on spinalized rats. Finally, task difficulty can be adjusted by making the tilt parameters.

Results: This novel BMI task bilaterally engages the cortex without visual feedback regarding limb position in space and animals learn to improve their performance both pre and post-SCI.Comparison with Existing Methods: Most BMI tasks primarily engage one hemisphere, are upper-body, rely heavily on visual feedback, do not perform investigations in animal models of SCI, and require nonnaturalistic extrinsic motivation such as water rewarding for performance improvement. Our task addresses these gaps.

Conclusions: The BMI paradigm presented here will enable researchers to investigate the interaction of plasticity after SCI and plasticity during BMI training on performance.

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Conflicts of interest

None.

Keywords

Brain machine interface; Neurorobotics; Tilt perturbation; Spinal cord injury; Balance; Water rewarding

1. Introduction

Brain machine interface (BMI)-driven neuroprosthetics have been successfully demonstrated in humans with severe neurological injury or disease (Gilja et al., 2015; Pandarinath et al., 2017) such as those with spinal cord injury (SCI) (Ajiboye et al., 2017; Collinger et al., 2013; Hochberg et al., 2012; Wodlinger et al., 2015). These systems, which directly access cortical neurons, restore lost function by allowing the brain to exchange information with an external device through a mathematical algorithm generally referred to as a decoder (Moxon and Foffani et al., 2015). To date however, demonstrations in humans have been restricted to upper limb function and, while important, do not address the lower limb functional needs of those with paraplegia. Additionally, these proof-of-concept demonstrations have not reached levels sufficient to fully restore function following injury (Baranauskas, 2014; Wodlinger et al., 2015). As such, more work is needed in understanding how to effectively implement BMI after injury or disease.

It is important to study restoration of lower limb function separate from that of upper limb function because the aid of visual feedback is greatly reduced when restoring lower limb function (Manohar et al., 2012). Demonstrations to date of lower limb BMIs in animals have worked to control stereotypical movements (Alam et al., 2014; Capogrosso et al., 2016; Donati et al., 2016) that can also be driven solely by training accompanied by stimulation below the level of the lesion (Cha et al., 2007; de Leon et al., 1998; Lovely et al., 1986). A major need of those with paraplegia is the ability to support their weight and coordinate balance of the upper trunk to maintain posture by learning new approaches to control muscle groups that span the level of the lesion (Manohar et al., 2017). To accomplish this, there is a need for a BMI task to study the impact of continuous changes in posture on neural encoding in a model of SCI. While non-human primates (NHPs) have served as excellent models for developing cortical BMIs (Athalye et al., 2017; Carmena et al., 2003; Churchland et al., 2012; Hwang et al., 2013; Jarosiewicz et al., 2008; Serruya et al., 2002; Taylor et al., 2002; Vargas-Irwin et al., 2010; Velliste et al., 2014; Wessberg et al., 2000), “humane” NHP SCI models are limited (Graham et al., 2013) and require “demanding protocols, extensive collaboration, considerable oversight, and major investments of fiscal and infrastructural resources” (Reier et al., 2012).

As an alternative, rodents can be used for developing lower limb BMIs in the context of SCI. From the SCI standpoint, rodents offer well-described reproducible controlled SCI-models, established histological, biochemical and molecular techniques, readily available behavioral outcome measure assays and are relatively inexpensive and available to most researchers (Zhang et al., 2014b). Additionally, rats have similar functional, electrophysiological and morphological outcomes compared to humans following SCI (Metz et al., 2000). From the BMI standpoint, researchers have found similar neural firing rate and timing property

changes in response to training in a BMI task as those found in NHPs (Arduin et al., 2014; Gulati et al., 2014; Knudsen et al., 2012, 2011; Knudsen et al., 2014; Koralek et al., 2012; Manohar et al., 2012).

To this end, we present a novel postural adjustment-related BMI task, driven by neurons in the hindlimb sensorimotor cortex, that bilaterally engages the cortex and can be performed before and after SCI. Since the task relies on the animal's natural abilities to respond to postural perturbations, animals only need to be familiarized with the experimental setup and training of a novel task is not required. Moreover, the task is naturally rewarding compared to studies that are entirely dependent on water or food reward signals, since water rewards were not required for this task. Results show that rats can perform above chance at the beginning of the experiment, improve performance with practice and continue to perform in the task following severe SCI.

2. Methods

2.1. Overview of experimental approach

The postural adjustment-related BMI task subjected animals to a randomized set of lateral tilt perturbations in the clockwise (CW) and counterclockwise (CCW) directions. The tilt perturbation-associated neural responses were used to classify tilt types before the completion of the tilt to its extreme inclination (Fig. 1). Correctly classified tilts immediately returned the platform to the 0° neutral position before the maximum tilt position was reached, providing a natural reward. Incorrectly classified tilts continued to the extreme tilt position with an increased tilt velocity, considered a "punish tilt", for 2–2.5 seconds while a bright light was turned on. The animals were tilted in the task for 25 days and were required to modulate their neural responses to improve the performance of the decoder, thus minimizing punishment and maximizing reward tilts. All animal procedures were conducted in accordance with Drexel University Institutional Animal Care and Use Committee-approved protocols and followed established National Institutes of Health guidelines.

Initially, we used a subset of animals (N = 10) to determine appropriate tilt angles and velocity that could be used in the BMI task (see 2.2 Selecting Tilt Types, below). Then the main experiment that consisted of 3 phases was conducted (Fig. 2). In the first phase, the Pre-BMI Phase, animals (N = 13) were handled, acclimated to the tilt platform, and stereotaxically implanted bilaterally with microelectrodes. After recovery, baseline neural recordings were made while the animals were tilted on the platform to collect data to train the classifier. Then, during the BMI Phase, animals were tested to understand the rate at which animals learned (improved performance in the task) and to determine if a water reward was required for animals to learn in the task. Animals were divided into 2 groups: BMI (N = 9) and naïve (N = 4), with each naïve animal yoked to one of the BMI animals such that they were subjected to the same tilts in the same order and received the same punishment and reward tilts. The BMI animals were further divided into those that were water restricted (No H_20) and given an added water reward (H_20) when their neural activity was correctly decoded to indicate the tilt type. Animals were tested in the task for 25 days. Finally, during the BMI SCI Phase, 6 of the BMI animals and 3 naïve animals were subjected to a complete spinal transection at the T10 level (N = 9 total), (re)introduced to the

BMI task and performance was evaluated for at least one day. Five animals were tested for at least eight days post-SCI to evaluate the effect of training in the BMI task on performance. Three of these five animals received BMI training pre-SCI and two were naïve animals that did not receive training pre-SCI (refer to Table 1 for details of animal numbers and neurons recorded).

2.2. Selecting tilt types

A major advantage of this task is that the speed of tilt can be modified to make the neural activity easier to classify the tilt type. This is especially important given that the neural implant surgery may produce a varying number of single neurons and the number of neurons may be more or less engaged in the task. If there are many neurons that are responsive to the tilts, then it may be easier to decode a set of tilts on a single trial than if there are fewer or less responsive neurons. We wanted to identify tilt speeds such that offline baseline performance was greater than 50% (chance performance was ~ 25%, see below for details) to ensure that animals were sufficiently motivated to work in the task but less than 80% so that there was room for improvement. Furthermore, it was important to ensure that reward tilts were executed quickly so as to maximize the rewarding effect and that punishment tilts were sufficiently uncomfortable but not so severe that the animal fell over.

To identify the proper tilts, a 5-step trial-and-error process was executed (Fig. 3). In step 1, test animals (N = 10) were tilted to determine a tilt angle for which animals showed visible signs of discomfort when being tilted but were still willing to participate in the experiment and not fall off the platform. We found that punishment tilts of at least 25° met this criterion for most animals. The punishment tilt angle from step 1 served as a reference for the maximum tilt angle determined in step 2. This angle was chosen such that it was small enough to be sufficiently different from the punishment angle, but large enough to require the animal to balance on the platform. Then in step 3, we chose the minimum tilt velocity such that its magnitude was the smallest our classifier could detect that a tilt occurred. This was approximately 27.3°/s. In step 4, the maximum peak tilt velocity needed to be small enough in magnitude such that the overall tilt duration was sufficient to record the neural activity necessary to decode the tilt (between 50–80% correct), classify the single trial, determine if it was correctly classified and either right the platform or allow it to continue to the punishment tilt (approximately 300 ms). The magnitude of the peak tilt velocity, however, needed to be sufficiently large to contrast the minimum velocity tilt found in step 3 to allow the classifier to discriminate between them. In step 5, we chose the maximum punishment tilt peak velocity such that it was larger in magnitude than the maximum tilt velocity found in step 4. Finally, the maximum and minimum tilts were applied in the clockwise (CW) and counter-clockwise (CCW) directions to allow discrimination between 4 tilt types. As a result, the average variance of the accuracy in offline performance across the 25 days of the experiment was 0.005 of the reported proportion of correct response with a minimum of 0.002 and maximum of 0.011. This worked well for the animals used in this study but, as the number of neurons recorded increase, one could either reduce the differences in speed or amplitude between tilts or increase the number of tilts to be classified with the maximum and minimum tilts identified to make the task more difficult.

2.3. Behavioral task

2.3.1. Tilt platform—The tilt platform consisted of three Plasti Dip ® (Plasti Dip International, USA) coated plexiglass plates (one for each hindlimb and for the forelimbs collectively) coupled to a high performance brushless AC servo motor (J0400-301-4-000, Applied Motion Products, USA). Peak velocity, acceleration and final tilt inclination parameters were programmed using Si Programmer™ (v. 2.7.22, Applied Motion Products, USA) on a digital motor drive (SV7-SI-AE, Applied Motion Products, USA), which sent commands to the motor for tilting. Tilt parameters were chosen such that only one high and low peak velocity tilt existed in the CW and CCW directions for a total of four tilt types (see Fig. 1A, above). LabVIEW (2015, National Instruments, USA) provided top-level control by randomly determining the tilt type (100 trials each), tilt initiation time and the time between tilt trials (varied randomly between 2–3 seconds) by sending commands to the motor drive.

2.3.2. Sensor data acquisition—OEM style single point load cells (LCAE-600 G; Omega, USA) positioned underneath the platform plexiglass plates quantified hindlimb and forelimb ground reaction forces (GRFs). Inclination was measured using a single axis inclinomoter (H4 A2-70V-BR, Rieker Inc., USA) attached to the motor shaft and a triple axis MEMS accelerometer (ADXL335, Analog Devices, Inc., USA) was fixed underneath one of the plexiglass plates to quantify the change in inclination over time and start of tilt respectively. GRF, inclinometer and accelerometer sensor data were acquired using LabVIEW software (1000 s/s), which was filtered offline using a 2^{nd} order Butterworth zero-phase low-pass filter (inclinometer used a 20 Hz cutoff; load cells and accelerometer used a 200 Hz cutoff). Load cell cut-off frequencies are similar to commercial software used in animal research settings (Dimiskovski et al., 2017) and power spectral analyses revealed that a majority of sensor data signals fell below 1 Hz and 25 Hz for the inclinometer and accelerometer/load cells respectively. All sensor data were normalized to a period found in the 200 ms time window prior to tilt start for each trial. The inclinometer data (which is known to have a ≤ 50 ms lagged response time) was phase shifted such that it aligned with the start of tilt as indicated by the accelerometer.

2.4. Single neuron recordings

2.4.1. Cortical implantation—After platform acclimation, all rats (N = 13) received cortical implants. As previously described (Knudsen et al., 2012, 2014; Manohar et al., 2012), rats were stereotaxically implanted bilaterally in the hindlimb sensorimotor cortex (Leergaard et al., 2004) within the in- fragranular layer (1.3–1.5 mm) using two 16-channel arrays (i.e. 32 total)—one in each hemisphere, in a 4 × 4 configuration consisting of 50 μm Teflon-insulated stainless steel microwires separated by 250 μm (MicroProbes for Life Sciences, USA). All surgical procedures were performed under general anesthesia (2–3% isoflurane in O_2) via orotracheal intubation. Pain was managed using Buprenorphine SR™ LAB (0.5 mg/kg; Wildlife Pharmaceuticals Inc., USA) and animals were allowed at least a week to recover from the surgery before proceeding with any additional experimental manipulations.

2.4.2. Neural data acquisition—A Multichannel Neuron Acquisition Processor (MNAP, Plexon Inc., USA) system was used to record neural activity voltage waveforms

acquired from each electrode (signals high pass filtered and digitized at 400 Hz and 40 KHz respectively). These voltage waveforms were discriminated into single units (i.e. neurons firing) at the start of each experiment session using principle component cluster analysis and visual identification (Sort Client, Plexon Inc., USA) to define each neuron (Fig. 4A). The number of single neurons, averaged across days separately for pre- and post-SCI, recorded from each animal are listed in Table 1. The time that each voltage waveform crosses a threshold, which is also known as a spike time, was saved in a data file across the duration of the experiment (Fig. 4B). These spike times, in addition to the times corresponding to the initiation of a tilt were then used to create peri-stimulus time histograms (PSTHs) after full data collection and storage (Fig. 4C; see below section for details).

2.5. BMI training

2.5.1. Overview—BMI training consisted of 3 phases (refer to Fig. 2). In the Pre-BMI phase, animals were subjected to a total of four randomized lateral tilt perturbations in the clockwise (CW) and counterclockwise (CCW) directions while neuronal responses were recorded. These data were used to develop the decoder (see next). In the BMI phase, animals were tilted in the task for 25 days and were required to modulate their neural responses to improve the performance of the decoder, thus minimizing punishment and maximizing reward tilts. Approximately half the animals were water restricted and tested with an additional water reward. In the BMI SCI phase, animals received a complete spinal transection and were retested in the task.

2.5.2. Decoder—For the experiments shown here, the PSTH-based classifier (Foffani and Moxon, 2004; Knudsen et al., 2014) was used to classify neural responses to tilts and decode the type of tilt the rat was subjected to on a single trial (Fig. 5). However, any type of decoder could be used, including continuous decoders to better follow the temporal aspects of the behavioral and/or neural responses. For the PSTH-based classifier, single-neuron templates for each tilt type were built from the previous day's recording (average peri-event response for each neuron). During BMI testing, the single trial response of the neurons was compared to the average neural response (templates) for each tilt type. The single trial was classified as belonging to the tilt type with the smallest Euclidean distance between template and single trial. However, one could choose to perform a dimension reduction analysis of the single neuron activity (e.g. PCA-ICA in Laubach et al. (1999)) and use a single ICA that best classifies the tilt types, which could save computational time (Knudsen et al., 2014; Manohar et al., 2012) during BMI use. Care was taken to keep the definition of each neuron as similar as possible with the previous day's recording to avoid dramatic changes to the overall neuronal ensemble serving as an input into the decoder (Ganguly and Carmena, 2009). If a neuron, however, stopped firing (relative to the previous day's recording) it was not used in the decoder for that day.Similarly, if a new neuron began firing that was not present in the previous day's recording it was recorded and saved but not used by the decoder for that day. If the new neuron was still present the following day it was used.

The optimal bin size was determined by testing bin sizes between 1 and 100 ms and was determined to be 20 ms. The window for evaluating the performance of the decoder was optimized by testing post-stimulus windows from 100 to 500 ms on classifier performance

and was determined to be ± 200 ms (where time 0 ms is the start of tilt). These classifier parameters were used throughout the experiment, however, these are additional parameters that can be optimized for a particular experiment.

2.6. Experimental design

2.6.1. Pre-BMIphase—The Pre-BMI Phase consisted of handling the animals (at least one week), acclimating them to the platform (no titling, 3–5 days; tilting 1–2 days), cortical implantation and recovery (see above for details). Experiments were conducted on 12 female and one male Long Evans rats (250–275 g body weight at training outset). The one male rat demonstrated an unwillingness to participate in the experiment, was more likely to jump off the platform, and exhibited greater signs of distress when being tilted compared to the female rats tested. Therefore, we excluded males from future experiments. Further work needs to be done to understand the role of male rats in this task and determine if the task needs to be modified to accommodate them. Following at least a week of handling, animals were gradually acclimated to the tilt platform and encouraged to place their paws over the GRF sensors (~ 3–5 days). Animals were then familiarized with being physically tilted on the platform (~ 1–2 days).

Next, a baseline recording (i.e. “day 0”) was made such that rats were subjected to tilts of varying types to identify tilt types that would meet the 50%–80% starting criteria based on off-line analysis of the neural responses. When the final four tilts were selected, these data also served as a representation of the neural network before task training (i.e. Neural Response Properties). Finally, the data recorded during the baseline recording served to build the decoder used for the BMI phase.

2.6.2. BMIphase—In the BMI Phase, the goal was to assess the rate of learning (improvement in performance) and the necessity of a water reward. Animals underwent 25 consecutive days (except Saturdays) of BMI training. When the animals were first run in the task, if on-line performance fell below 30%, punishment tilts were restricted to 30% of the total experimental tilts so as to keep the animal engaged in the task.

Water reward is a common positive reinforcement signal used in rodent BMI studies (Arduin et al., 2013, 2014; Chapin et al., 1999; Gulati et al., 2014; Hira et al., 2014; Koralek et al., 2012). In this investigation, 5 animals were tilted in the task without water reward while the remaining 4 animals were tilted with water rewards in addition to punishment and reward tilts. Animals in the water reward condition had their water restricted and received water from a spout situated at the front of the platform after a reward tilt. Water was flavored with an oral rehydrator (Prang™, United States), which was intended to more strongly associate reward with task performance and distinguish the reward from unflavored water (given ad libitum outside task to maintain hydration).

2.6.3. BMI SCI phase—The BMI SCI Phase investigated rats’ ability to perform in the task following a complete spinal transection (SCI). Rats were completely transected at the T8/T9 mid-thoracic level. Anesthesia (via a nose cone), pain management and recovery time were identical to that used for cortical implants described above, and care for spinalized animals was performed in a manner similar to our previous work on full transects (Ganzer et

al., 2016; Manohar et al., 2012). After recovery from the SCI surgery, animals underwent a second baseline recording session where neuronal response properties were recorded while the animal was subjected to the same set of tilts done in the first baseline recording. The resulting neuronal response properties were compared with those found at the end of the Pre-BMI Phase to assess changes in neuronal response properties (see Neuronal Response Property Changes under Data Analysis, below). Using the data from the post-SCI baseline to initiate the decoder, animals were re-introduced to the BMI task using the same pre-SCI tilt types. Since post-SCI animals no longer had lower body and hindlimb control, their feet were taped to the platform and hindquarter, weight-support was provided using a sash attached to the platform.

2.7. Data analysis and statistics

2.7.1. Neuronal response properties—To assess how the task modulated neural responses, the responsiveness of neurons during baseline recordings was evaluated before and after SCI in animals that had at least 1 neuron in each hemisphere (N=5 animals). PSTHs were first generated in Matlab (version 2012b, The MathWorks, Inc.) for each neuron and tilt type, using 2 ms bins and a ± 200 ms window centered on the start of tilt. The PSTH was first smoothed using a 6 ms span moving average lowpass filter. A threshold was then set at 1.65 SD above the background firing rate, which was calculated as the average spikes/trial within the background window (−200 to 0 ms prior to tilt start). Neurons that had at least one set of 5 consecutive bins that rose above this threshold and for which the response window was significantly greater than the background window (one-sided paired t-test, $p > .001$) were considered responsive. If more than one set of consecutive bins were considered responsive, the most responsive set for each neuron across all tilt types was used for subsequent analyses. This ensured that each neuron only contributed one observation per experimental session.

Similar to our previous work, (Kao et al., 2011; Manohar et al., 2012) the following neuronal response parameters were then extracted from the unsmoothed PSTHs for each set of consecutive bins (refer to Fig. 4C): (a) Response Magnitude (RM): sum of the spikes within a set(s) of consecutive bins in the response window divided by the total number of trials after subtracting the average background activity (i.e. background firing rate; BFR). (b) First bin latency (FBL): time at which the first bin exceeds threshold (c) Last bin latency (LBL): time at which the last bin exceeds threshold. (d) Background Variance (BFRvar): the standard deviation of the BFR. Responses with a RM less than 0.002 spikes/trial, a LBL equal to 0 and/or a BFR less than 0.01 spikes/trial were removed.

2.7.2. Performance—Online performance was calculated as the total number of correctly classified trials divided by the total number of trials using templates created from the previous day's recording. Since the rats' ability to improve task performance (i.e. learn) was the primary interest, all online performance values were normalized such that they represented changes from the first day of the experiment. Though animals were tested for 400 total trials (~ 40–60 min duration), the last 100 trials were dropped in an effort to minimize fatigue effects from the tilt task.

Chance level performance (i.e. bootstrapped performance) was calculated by executing the classifier using the same neural response data and classifier templates used to calculate online performance but with the event trial timestamps randomly shuffled. This operation was performed 50 times, which provided an asymptotically stable estimate of random single neuron information within 0.001 bits, and was applied to the data from the first day of the 9 pre-SCI and post-SCI animals.

2.7.3. Evaluation of the effect of learning in the task—Animals that learned in the task were defined as those that displayed online performance greater than the first day for at least 5 consecutive days. Animals that did not meet this criterion were classified as non-learning animals. To validate the criteria, the learning and non-learning animals were compared during the early (days 2–6) and late (days 21–25) phases of the task.

2.7.4. Statistical analysis—For the neuronal measures, to account for the fact that multiple neurons were recorded from the same animal, a three-way hierarchal linear model (HLM), with two levels (neuron nested within animal) was used to assess effect of hemisphere (left or right), tilt direction (CW or CCW) and SCI (pre- or post) using a variance components variance- covariance structure for the random effect. Hemisphere, tilt direction and SCI groups were treated as fixed effects while animal was modeled as a random effect. A separate HLM was applied for RM, FBL, BFR and BFRvar measures (see Fig. 4C). We discriminated an average of 44.10 ± 10.46 pre-SCI neurons and an average of 29.01 ± 10.27 post-SCI neurons across animals. To account for imbalances in samples sizes between hemispheres across animals, however, a random subsets of neurons (range: 3–12) were used so that an equal number of neurons existed for each hemisphere before and after SCI. This random sampling procedure was applied to each animal independently. In total, for the neuronal response measures, 148 neurons were studied from each hemisphere in 5 animals before (N = 74) and after (N = 74 SCI).

To assess whether online performance was significantly above chance (see Section 2.6.2 Performance for definition of chance performance), a two-way mixed ANOVA was used to compare online performance with chance level performance (performance group; factor 1) before and after SCI (SCI group; factor 2). Since online performance and chance level performance was calculated for each animal, the performance group independent variable was modeled as a within-subjects factor. In contrast, not all animals belonged to both pre and post-SCI conditions or a significant amount of time passed between the pre-SCI and post-SCI condition such that they could be treated as independent measures. As such, the SCI group independent variable was treated as a between-subjects factor.

To assess the learning criteria, a two-way HLM (neuron nested within animal) was then used to compare experiment phase (early, late) with learning group (learning and non-learning).

To assess the impact of water reward, the normalized performance (relative to day 1) of animals that received water reward and punishment tilts [H_20] was compared with animals that only received punishment tilts [No H_20]. Experiment days were grouped into early and late phase time periods and a two-way HLM applied; experiment phase (early, late) with experiment condition (H_20, no H_20) in a manner similar to that used to asses learning. To

determine if animals could perform above chance post-SCI, statistical comparisons were made using the mixed model ANOVA described for reward evaluation.

For all HLMs, a simple effects analysis was used to make pairwise comparisons of the estimated marginal means when appropriate. All p-values were Bonferroni-corrected to adjust for multiple comparisons as appropriate. All statistics were performed using SPSS (v. 24, IBM SPSS Statistics; USA), with a significance criterion of 0.05.

3. Results

3.1. Task is bilateral

The behavior of the animal was similar for CW and CCW tilts (Fig. 6). For CCW tilts, in approximately the first 100 ms (0–100) the platform pushes into the right hindlimb and away from the left hindlimb. In the next 100 ms (100–200), the animal extends the left hindlimb and flexes the right hindlimb in an effort to place her center of gravity over the base of support. This effectively causes increases and decreases in the left and right hindlimb GRFs respectively (Fig. 6B). The converse is true for CW tilts. The low forelimb GRF levels are likely due to the fact that both right and left forelimbs were recorded on a single load cell sensor—one is pushing into the sensor while the other is pulling away. As expected, GRFs decrease following SCI, however, spinal circuits below the level of the lesion provide reasonable reflexes such that GRFs are not entirely eliminated. The time course of GRF changes are similar to those before SCI.

The magnitude and latency of neural responses from the left hemisphere (n = 37 neurons from 5 animals) was similar to that of the right hemisphere (n = 37 neurons from 5 animals) [$F(1,291.914) = 1.543$, $p = .215$, $F(1,290.299) = 0.498$, $p = .485$, $F(1,291.600) = .558$, $p = .456$, $F(1,291.358) = .027$, $p = .869$ for the RM, FBL, BFR and BFRvar respectively] regardless of whether the tilts were CW or CCW [$F(1,291.914) = 1.794$, $p = .187$, $F(1,290.299) = 0.564$, $p = .453$, $F(1,290.362) = .250$, $p = .618$, $F(1,291.600) = .000$, $p = .984$ for the RM, FBL, BFR and BFRvar respectively].

As expected, the magnitude of the response was attenuated after SCI (pre-SCI: n = 74 neurons from 5 animals; post-SCI: n = 74 neurons from 5 animals) [$F(1,291.914) = 19.138$, $p < 0.001$] while the latency of the response was increased [$F(1,290.299) = 49.682$, $p < 0.001$]. BFR [$F(1,291.600) = 1.665$, $p = .198$] and BFRvar [$F(1,291.358) = .111$, $p = 0.739$], however, remained unchanged following SCI

In summary, the left and right hemispheres responded similarly overall but there was a decrease in firing rate and increase in latency following SCI (Fig. 7). Therefore, this task can be used to study impact of SCI on a BMI for control of both lower limbs.

3.2. Animals learn in the task

The neural responses recorded above were used to define a neural decoder for online BMI training. On the first day of BMI platform control, pre-SCI animal performance was 50.8% +/− 14.8% (range 25%–73%), which was significantly above chance [$F(1,16) = 67.410$, $p < 0.00$]—approximately 25%. Approximately half of the animals improved their online

performance (N = 5 learners, N = 4 non-learners) and met the learning criteria for the task. Learning animals demonstrated rapid performance increases within the early phase (within 5–6 days) of the experiment followed by slow to asymptotic performance increases (after around day 10) which is consistent with other learning tasks (D'Amours et al., 2011; Koralek et al., 2012; Qian et al., 2015). The fact that some animals learned and improved their performance in the task while others did not, allows this BMI paradigm to be used to study mechanisms of BMI learning after SCI.

Comparisons of performance between the early and late phase showed a significant effect [early (N = 45, 9 animals); late (N = 43, 9 animals)]: $F(1,79.046) = 10.723$, $p = .002$) such that the performance increase of learners (N = 49; 5 animals) was greater than that of non-learners (N = 39; 4 animals) [$F(1,9.001) = 16.694$, $p = .003$; Fig. 8]. A significant interaction between phase and group [$F(1,79.046) = 21.006$, $p < 0.001$] justified follow-on simple effects pairwise comparisons which revealed: 1) early phase performance differed from late phase performance for the learning group ($p < 0.001$), 2) non-learning animals did not exhibit this effect ($p = 0.383$) and 3) the learning group differed from non-learning group in both the early ($p = 0.047$) and late ($p < 0.001$) phase. These results therefore suggest that the learning group task criteria appropriately separated animals that learned and did not learn in the task. Therefore, the parameters of this task can be titrated so that a control group of non-learners can be included.

3.3. Animals do not require a water reward

To determine the necessity of water rewarding, we made comparisons between animals that received a water reward (N = 4) and those that did not (N = 5). Statistical outcomes show that performance differences do not exist between the two groups (Fig. 8C,D). Specifically, there was no effect of experimental condition [H_20 (N = 39; 4 animals) versus noH_20 (N = 49; 5 animals) $F(1,9.099) = 0.008$, $p = 0.932$], but an effect of experimental phase [early versus late $F(2,212.008) = 4.982$,$p = 0.008$] with no interaction [$F(2,2.384) = 2.384$, $p = 0.095$]. In fact, animals were equally likely to learn in the task (2 out of 4 H_2O animals and 3 out of 5 no H_2O animals) regardless of whether they received a water reward. Therefore, for tasks that might be sensitive to external rewards, the desire to remain in the neutral position is sufficient to allow BMI control and induce learning.

3.4. Animals can perform in the BMI task post-SCI

Next, animals (N = 9) received a complete spinal transection and were tested in the BMI task for at least one day following SCI. Six of the nine were BMI animals (4 learners, 2 non-learners) pre-SCI. The remaining three animals experienced tilt types and reward/punishment events identical and in the same order as those received by BMI animals but whose neural response did not influence task outcomes (naïve animals). Performance of the nine animals was statistically above chance [raw performance, bootstrapped performance: $F(1,16) = 67.410$, $p < 0.001$] the first day of the experiment (52.8%+/− 10.2%; range 41%–69%). Additionally, post-SCI animal performance did not differ from that of pre-SCI animals [pre-SCI (N = 9; animals = 9), post-SCI (N = 9, animals = 9): $F(1,16) = 0.006$, $p = 0.938$] with no interaction between SCI and performance groups [$F(1,16) = 0.315$, $p = 0.582$]. Of the nine post-SCI animals, five were tilted in the task for at least eight days (as

pre-SCI animals three received BMI training—two of which learned and one did not; remaining two were naive animals). Three of the 5 animals learned post-SCI suggesting that similar to pre-SCI, post- SCI approximately half of the animals can learn BMI control and improve performance. Therefore, learning can be studied post-SCI by comparing learning animals to non-learning animals. One of the animals that learned was a naïve rat, suggesting that pre-SCI BMI training is not necessary, although more work should be done to confirm this. Moreover, if there was a break in training (Fig. 9), performance declined but could be restored with additional practice. Therefore, all animals can perform the task post-SCI and approximately half improve with practice.

4. Discussion

This study demonstrates a novel BMI task that bilaterally engages the cortex and that animals can learn to improve their performance both pre- and post-SCI (Table 2). The task is relatively natural such that it does not require the animals to learn a new skill (e.g. reaching to a target or some other unnatural behavior) and is self-rewarding such that the need for additional rewards like food or water is not required although it appears there is a small yet insignificant effect of water reward.

This is important because the animals will not need food or water restriction, which can be especially hard on spinalized rats. Importantly, the types of tilts can be titrated so that both pre- and post- SCI, animals can perform just above chance the first day of BMI so that BMI learning can be studied both pre- and post-SCI. Task difficulty can be adjusted for each rat by making the tilt parameters more similar or adjusting the total number of tilts to be classified. In future experiments, investigators could adjust tilt difficulty dynamically much like N-back tasks (Kirchner, 1958) performed in humans. While only females were used in this study, it is likely that males could also learn to perform in this task despite requiring more care to acclimate, but more work would need to be done to confirm this observation.

The rodent model of spinal cord injury is well suited for preliminary studies on SCI (Sharif-Alhoseini et al., 2017; Zhang et al., 2014a). The advent of BMI in the rat and the rapid translation to non-human primates and humans with spinal cord injury does not eliminate the need for important rodent studies. BMI technology is in its infancy and more work needs to be done before it can be proposed as a viable therapeutic intervention. One important area of investigation is electrode technology, especially regarding biocompatibility of microelectrodes and their reliability over long periods. While the number of neurons recorded post-SCI was reduced in this study, this is not typical [refer to (Knudsen et al., 2014; Manohar et al., 2012)]. In addition, similar to our previous work, the magnitude of the response decreased post-SCI (Manohar et al., 2012). However, the reduction is simply to baseline levels noted before BMI training and not to pathologically low response rates. More importantly, there is no reduction in performance in the task, as reported here, and we have previously shown that this is due to temporal changes in the neuronal firing patterns (spike-timing information), not changes in the magnitude of the response. Finally, although the latency of the response was initially shifted later post-SCI, our previous work demonstrates that this shift normalizes with time in the BMI task, post-SCI. Therefore, the changes in neuronal firing patterns after SCI are similar to previous studies using more traditional BMI

paradigms (simple, single limb movements) and support our conclusion that this task is a good paradigm to study BMI after SCI.

4.1. Challenges of BMI for paraplegia

BMI training for paraplegia has several challenges that are difficult to overcome with non-human primates or even humans. A BMI for paraplegia that aims to restore functioning of one's own limbs by controlling functional electrical stimulation (BMI-FES) will likely require that subjects undergo extensive physical therapy to maintain muscle mass and then extensive learning to control the system (Harkema et al., 2011; King et al., 2015). To that end, we selected a tilt task that aims to decode the animal's intention to maintain balance. There are several benefits to this approach. First, balance management after severe SCI has proven to be one of the most challenging deficits after SCI as all the clinical trials with standing in paraplegia show that balance is never achieved with FES and never induced by local central pattern generators (CPGs) or reflexes in complete spinal cord injured patients, although it is continuously being studied (Guiraud et al., 2014; Harkema et al., 2011; Nataraj et al., 2016; Rouhani et al., 2017). Therefore, the study of BMI or especially, BMI-FES to restore balance after SCI could provide insights into novel approaches to restore this difficult function.

Second, after complete transection studied here, just as in humans with paraplegia might stumble and drag their legs during training with parallel bars or some other upper body support, with extensive therapy (Manohar et al., 2017), animals can take one or two weight-supported steps but then lose their balance and begin to drag their hindlimbs (Manohar et al., 2017). The approach presented here allows us to examine signals generated in the brain during this moment when sensorimotor information indicates how much the animal was tilted. Human subjects learning to regain independent locomotion, whether with a closed-loop BMI-FES system or other approach, would start with using their upper arms to prevent failing over, which is analogous to the quadrupedal system studied here (Harkema et al., 2011; King et al., 2015). In fact, it is possible that even with BMI-controlled FES, severe SCI subjects may continue to use a walker (for advances in this area see the PARASTEP system for example, (Bazo Hac, 2015; King et al., 2015)). Sensory information coming from the trunk and upper limbs above the level of the lesion would provide sensorimotor information for the BMI. This experimental paradigm can be used to study the brain signal needed to control stimulation to limit forces on the upper body. Therefore, while the results of studies with this task might not be directly translatable, they will provide important insight into how sensorimotor systems can learn to encode for balance after SCI, early in training when the subject will be using both upper and lower limbs.

Third, this approach could, in the very long term, allow for the study of the role of the central pattern generator (CPG) after severe SCI in maintaining balance (Harkema et al., 2011). The CPG is a component of the mammalian spinal cord system, including rats and humans (Guertin, 2009; Haghpanah et al., 2017; Nielsen et al., 2007), that generates stereotypical locomotion. The BMI paradigm demonstrated here, that studies repeated response to imbalance after SCI could test the effect of controlled timing and amplitude of stimulation to the spinal cord via a BMI-controlled FES, addressing questions as to whether

simply stimulating the CPG is sufficient or whether more precise, interspinal stimulation is required. Of course, one possibility is that balance will not be restored after severe SCI without the use of an exoskeleton and these brain signals could be used to control the exoskeleton.

And, finally, human subjects cannot be expected to follow the movement of their lower limbs to ensure effective movements. Therefore, as in this tilt task, animals could not visualize the position of their hindlimbs during the task.

4.2. Combining study of plasticity after SCI with plasticity in BMI training

It is well established that therapy, after SCI, induces extensive brain reorganization (Antri et al., 2005, 2003; Endo et al., 2007; Ganzer et al., 2016, 2013; Graziano et al., 2013; Kim et al., 1999; Oza and Giszter, 2014, 2015), but it is not clear how to combine this type of therapy-induced plasticity with BMI-induced plasticity. A major problem is the difficulty in performing severe injuries that will allow for exploration of therapeutic approaches after severe injury. For example, Capogrosso et al. (2016) recently reported brain-controlled epidural stimulation to alleviate gate deficits in non-human primates, however, the lesion was unilateral and the animal experienced extension recovery, including ‘residual hip or knee oscillations’. Moreover, improvements in stepping quality and quantity could be achieved without any training. Therefore, it is not clear that movements of the limb in response to stimulation represent the intended volitional movement of the limb or simply modified steps in response to the stimulation based on the on-going cyclic behavior. Because performing more severe injuries in primates is difficult, the rat model of severe injury offers important insight. In this study, rats were subjected to a complete, mid-thoracic spinal transection that disconnected the hindlimb periphery completely from the cortex. This task is an important model for investigating learning versus non-learning because approximately half the animals learn to improve function after SCI. This is an important component of the recovery of function after SCI.

The task is well suited to studying BMI learning post-SCI. First, the fact that neurons recorded from the hindlimb sensorimotor cortex can perform above chance in the BMI task and learn post-SCI (which deafferents the hindlimbs) suggests that rats use information from body areas still transmitting sensory information about the tilt to the cortex. Such areas likely include the forelimbs and trunk above the lesion, which are still engaged during the tilt task. Researchers have shown in particular that forelimb stimulation can evoke hindlimb sensorimotor cortex responses following SCI {Humanes-Valera, 2013 #1469;Aguilar, 2010 #835} Specifically, investigators in these studies found an increase in the probability of hindlimb cortex long-latency activations, which was defined as a > 50 ms somatosensory response, when providing peripheral stimulation to the forelimb following SCI due to enhanced coherence between forelimb and hindlimb cortex. Given that our electrode site and SCI model are similar as found in these studies, that the response latency to tilts are generally greater than 50 ms, and that the forelimbs are engaged in this task, it is likely that long-latency activation probability increased following SCI in this study. Such an increase may explain the increased overall response latency found here.

Finally, this task is well-suited to investigate the interactions of SCI therapy and BMI learning. While progress has been made on both fronts, little work has been devoted to understanding the interaction between BMI-learning and increased neuroplasticity caused by SCI and SCI-based therapies (Foffani et al., 2015; Manohar et al., 2017). Our tilt paradigm could be used to investigate this question by comparing SCI animals with and without therapy

4.3. Studying effects of learning in a BMI task

We also found that a water reward, which is used in most BMI rodent studies, is not necessary for animals to increase their performance in this task. This unique feature suggests that negative reinforcement signals provided by the punishment tilts sufficiently invoke the necessary neuroplasticity-related changes required to improve decoder accuracy. When combined with positive reinforcement signals, the task parallels “real-world” conditions as both signals will likely play a role as the “teacher signal” that drives BMI performance improvements. Alternatively, given that positive and negative reinforcement engages different pathways in the brain (Frank et al., 2004; Hikida et al., 2016; Kravitz and Kreitzer, 2012; Kravitz et al., 2012; Namburi et al., 2015) this task could be used to investigate the independent role of positive and negative reinforcement in the BMI context by separating animals into punishment and water reward only groups. Researchers might additionally find this task particularly useful for post-SCI animals as they are not as resilient to water deprivation schedules.

In addition, since both hemispheres are engaged in this task one could use one hemisphere as an input into the decoder (i.e. direct neurons) while simultaneously recording from the contralateral hemisphere that does not input into the decoder (i.e. indirect neurons; (Fetz and Baker, 1973; Ganguly et al., 2011). Questions about the strategy the brain uses to improve task performance could then be explored (e.g. global strategy using direct and indirect neurons vs. an individual neuron strategy using only direct neurons; (Hwang et al., 2013). The relatively large distance between hemispheres might also provide clarity towards the role of indirect neurons for which a consensus has not yet been reached (Arduin et al., 2013, 2014; Clancy et al., 2014; Fetz and Baker, 1973; Ganguly et al., 2011; Gulati et al., 2014; Hwang et al., 2013; Law et al., 2014), by reducing the confounds associated with using direct and indirect neurons from the same hemisphere. It is our hope to continue such investigations in future research.

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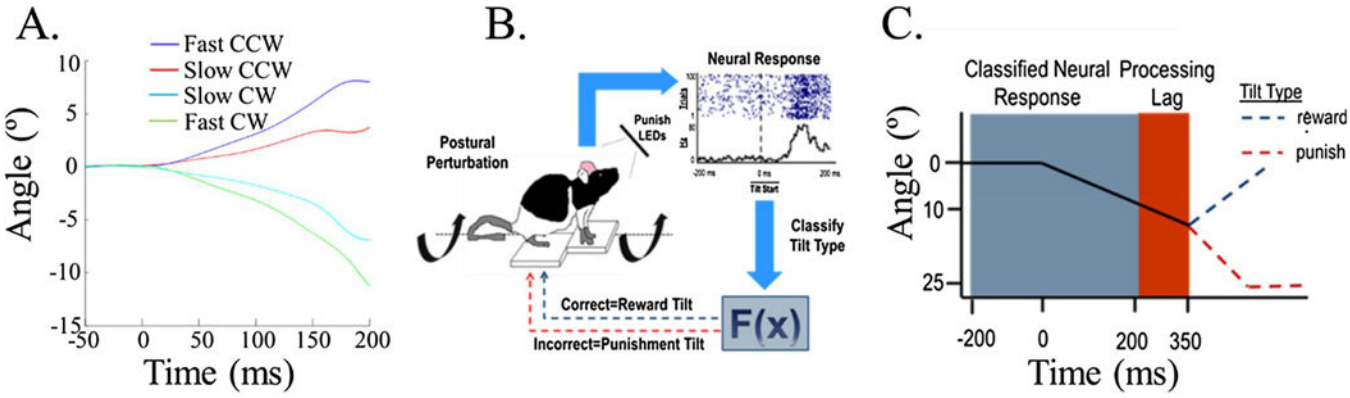

Fig. 1. Task Overview.

(A) Example tilt profiles. (B) Experimental paradigm. (C) Illustration of “reward” and “punish” tilts.

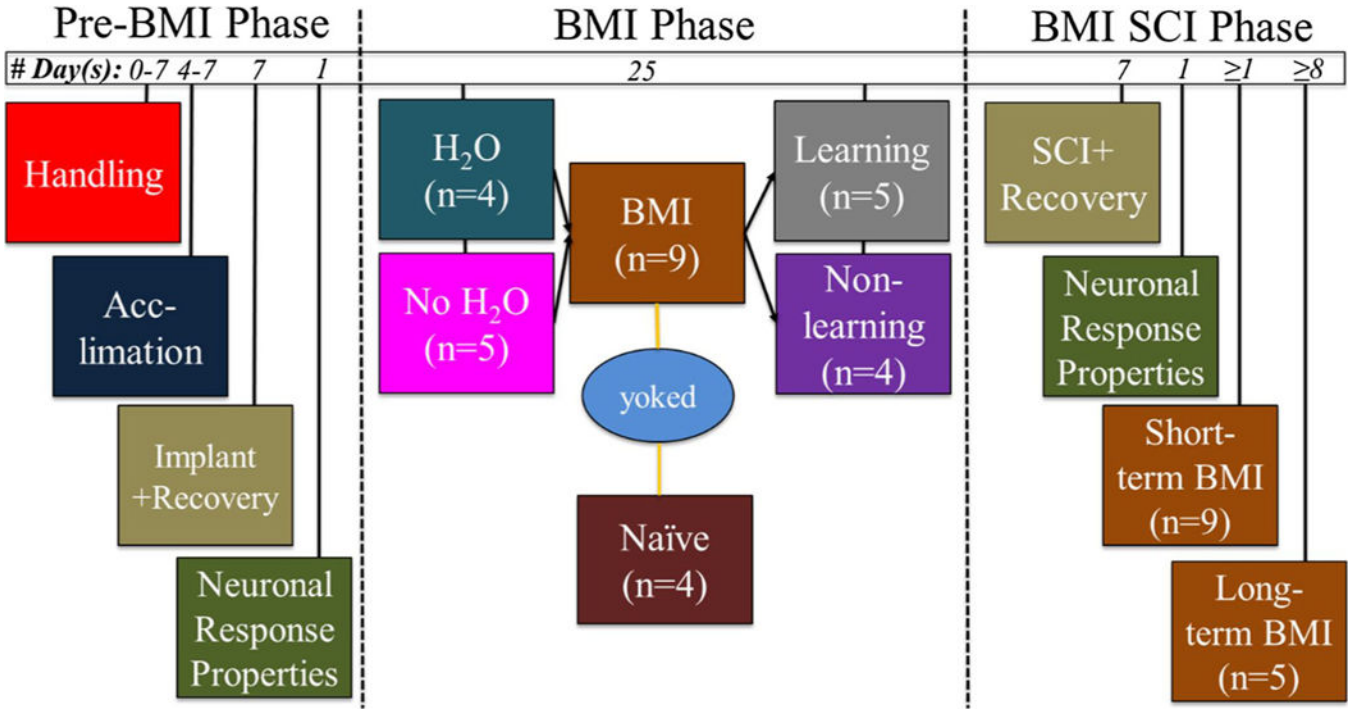

Fig. 2. Experimental Timeline.

Depiction of the progression of the experiment from the pre-BMI, to the BMI and finally to the BMI SCI Phase.

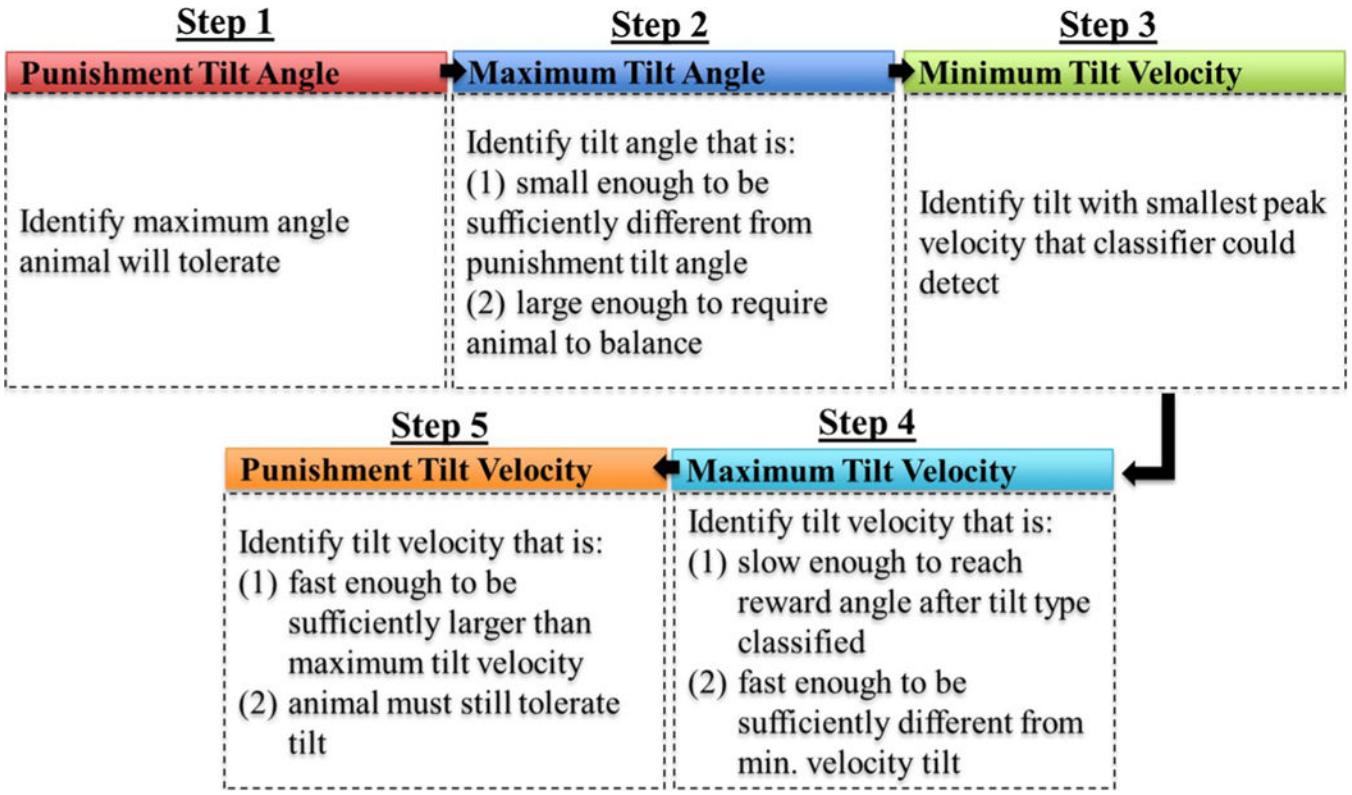

Fig. 3. Selecting Tilt Types Procedure.

The steps used to determine the parameters used for the four different tilts used in this experiment are described above.

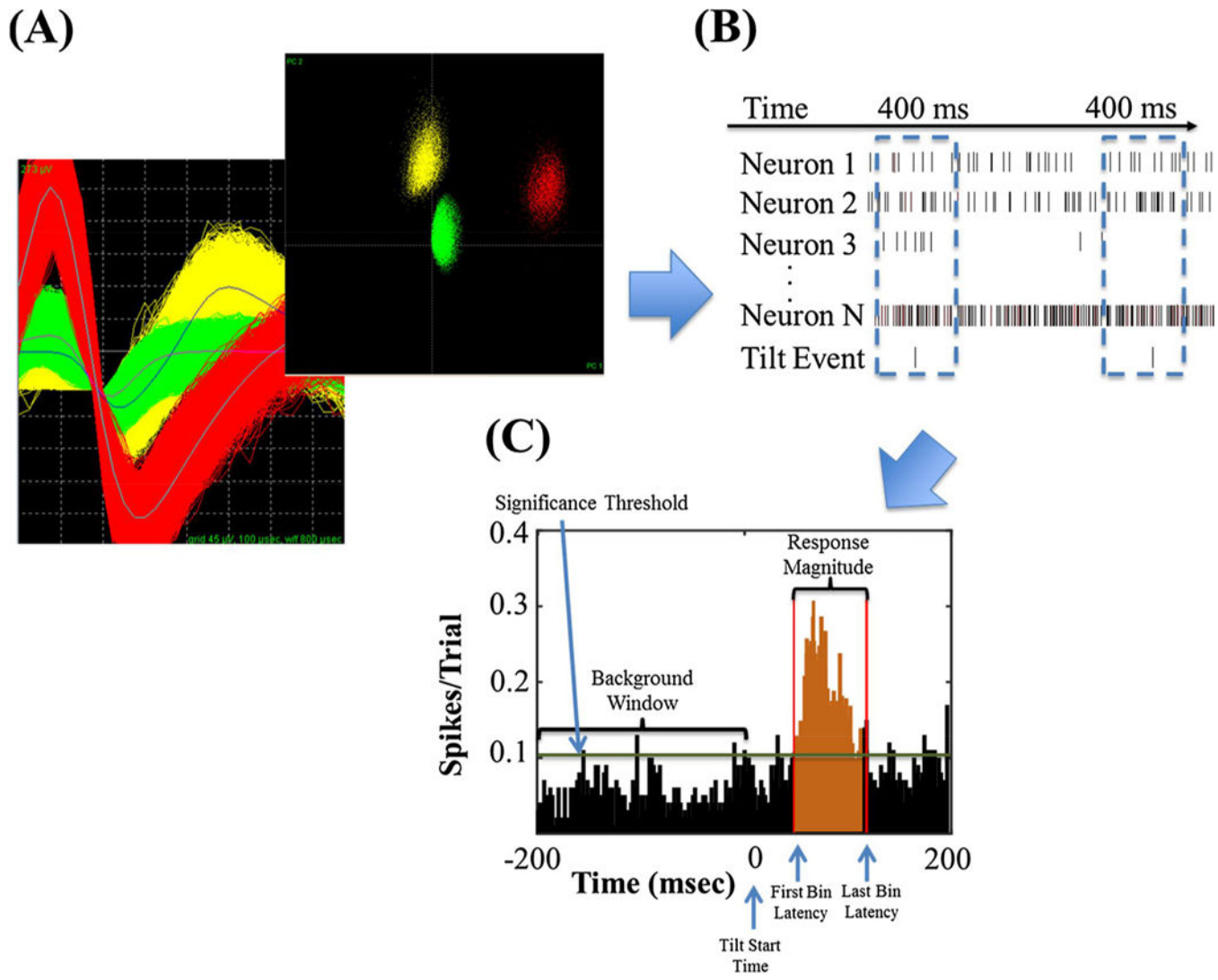

Fig. 4. Neural Data Acquisition.

(A) Raw voltage waveforms corresponding to action potentials recorded by implanted multi-electrode arrays and principal component analysis clusters used to separate voltage waveforms into individual neurons. (B) Raster plot depiction of voltage waveforms converted into discrete timestamps as well as timestamps representing the initiation of each tilt. (C) Example peri-stimulus time histogram (PSTH) for a single neuron using ± 200 ms window and 2 ms bin size.

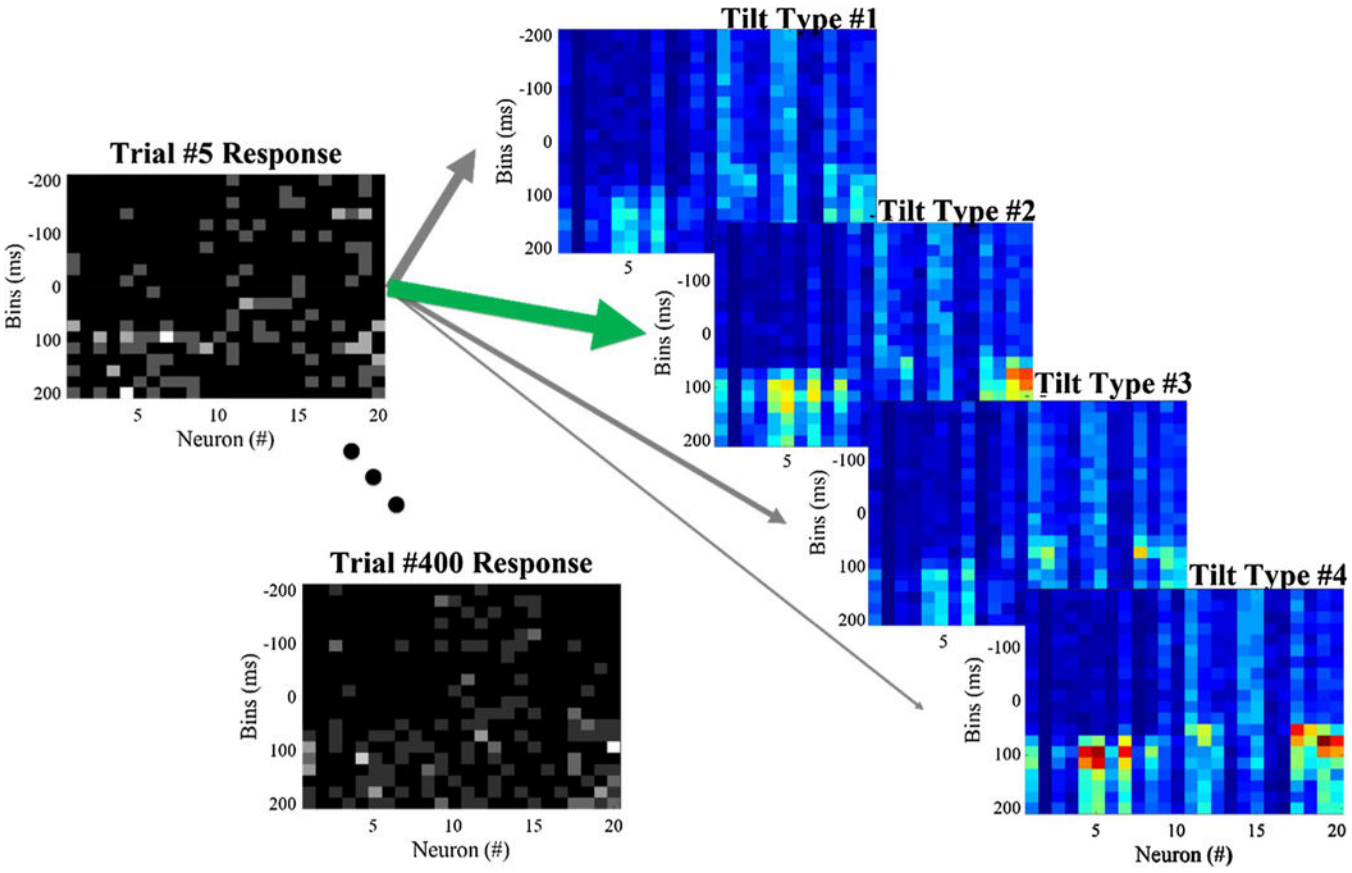

Fig. 5. Decoder.

Depiction of the PSTH-based classifier method used to decode which tilt types experienced by rats using neural responses. The classifier calculated the Euclidean distance between the single trial response (left side) and a template created for each tilt type (right side) from the previous day's recording. The classified tilt type corresponded to that whose template had the smallest Euclidean distance (green arrow) with the single trial response (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

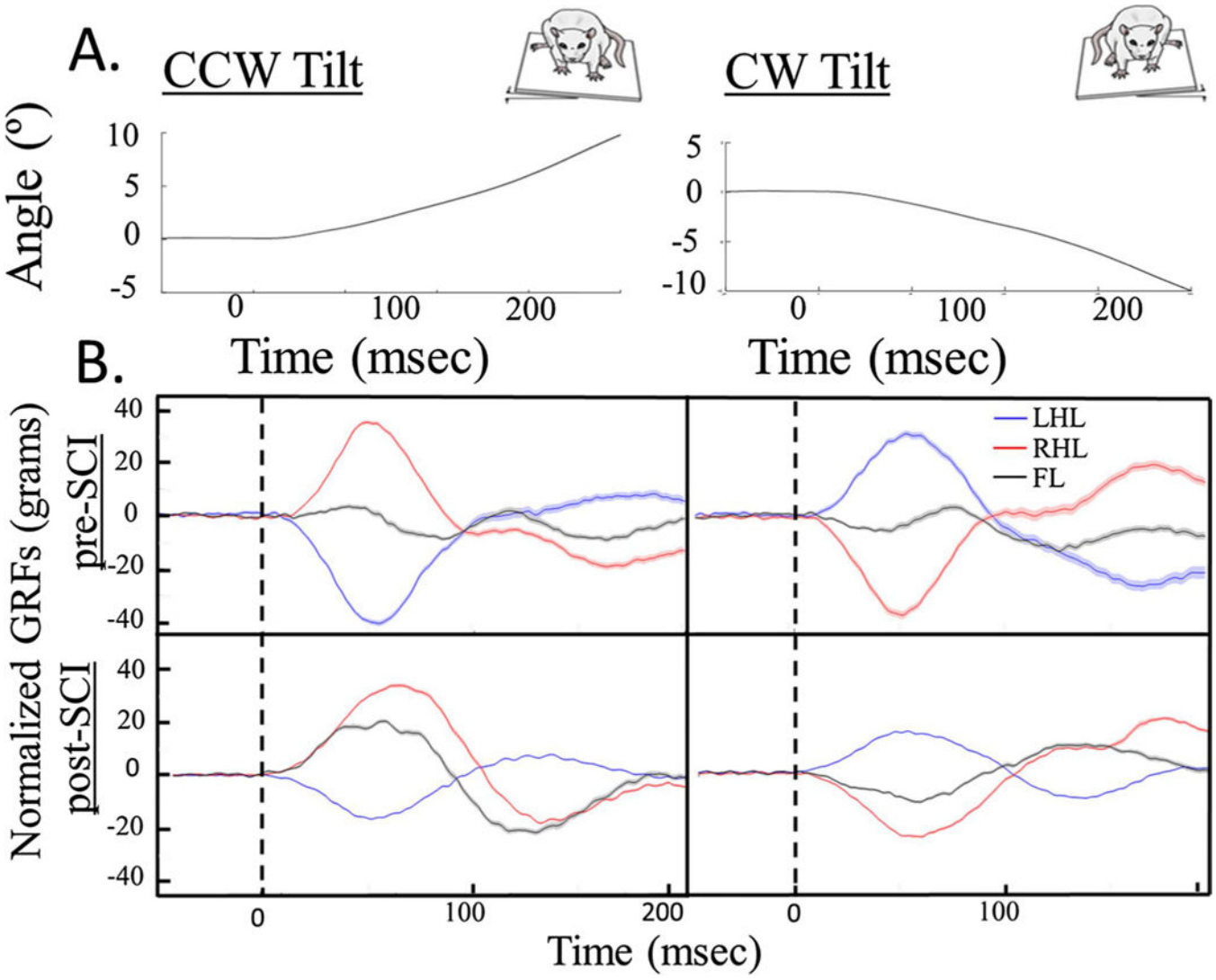

Fig. 6. Behavioral response during tilt.

(A) Inclinometer data show position of the platform during tilt. Time zero is determined from the accelerometer. (B) Ground reaction forces (GRFs) captured from the left hindlimb (LHL) right hindlimb (RHL) and forelimb during clockwise (CW) and counter-clockwise (CCW) tilts. Top panels represent GRFs before SCI (pre-SCI) while bottom panels represent GRFs following SCI (post-SCI). The dotted line represents the start of the tilt (time = 0).

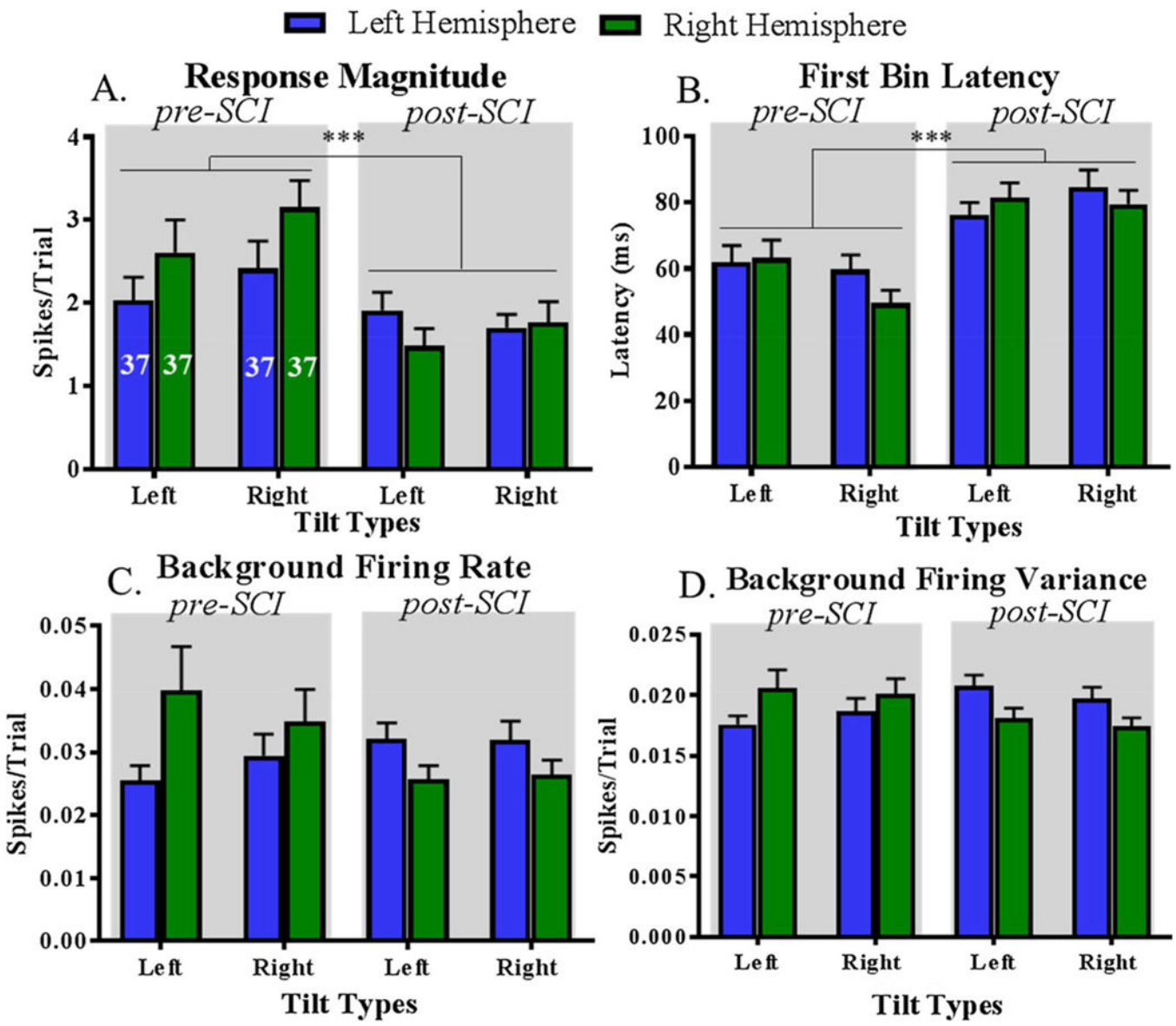

Fig. 7. Response Property Changes.

(A) Response magnitude, (B) first bin latency, (C) background firing rate and (D) variance between left and right hemisphere before (pre-SCI) and after (post-SCI) spinal cord injury. *** $p < .001$.

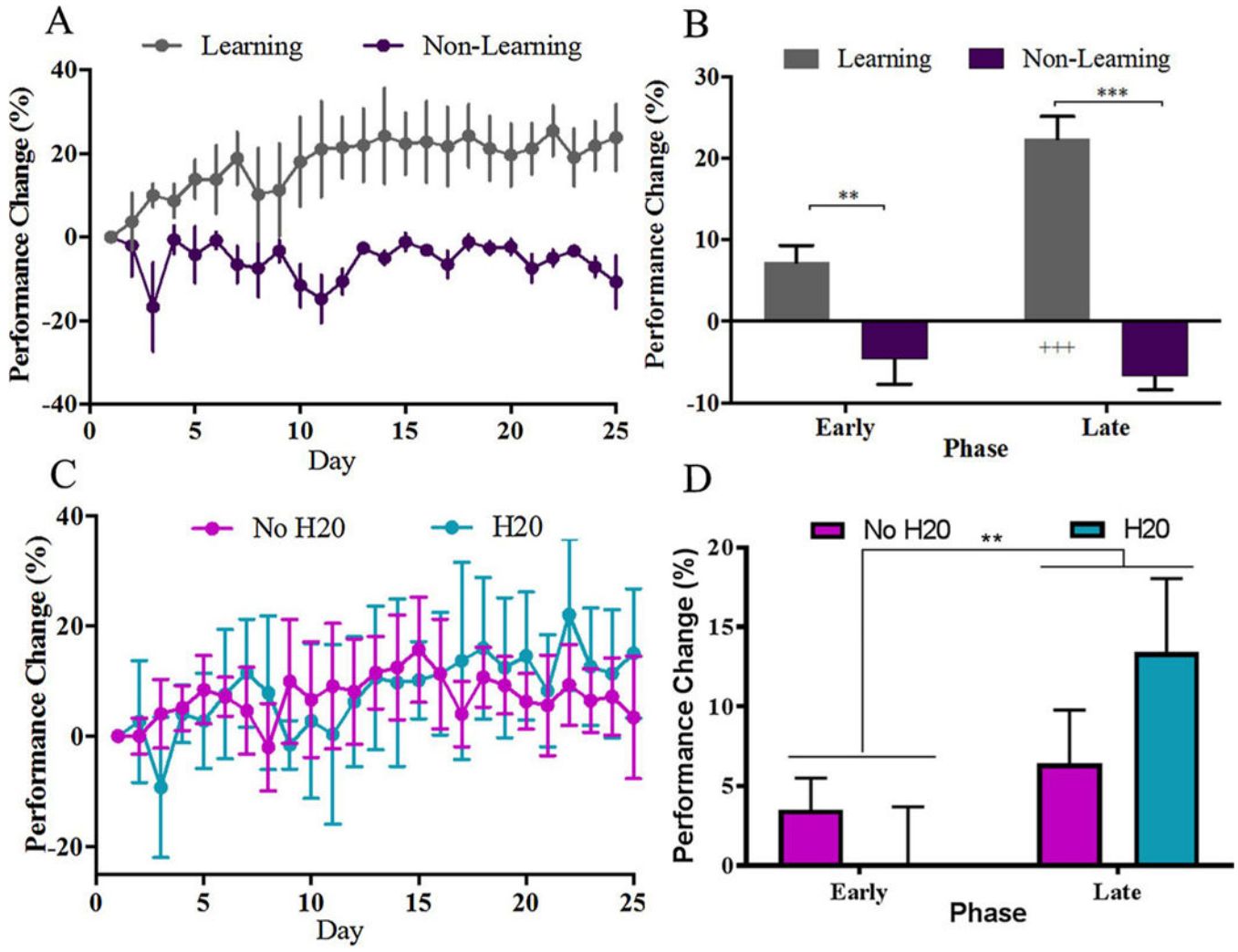

Fig. 8.

A subset of animals improved performance with practice in the task. (A) Performance normalized to day 1 plotted across the 25 days of the BMI experiment for learners and non-learners separately. (B) Learning and non-learning performance values compared during early (days 2–6) and late phases (days 21–25). (C) Raw online performance of animals with punishment tilt and no water reward (No H20) compared to animals with both punishment tilt and water reward (H20) across 25 days of experiment. (D) H20 and No H20 performance values compared during early (days 2–6) and late phases (days 21–25). *p < .01,**p < .05, ***p < .001 (Bonferroni corrected). +++ p < .001 Bonferroni corrected comparison between early and late phase.

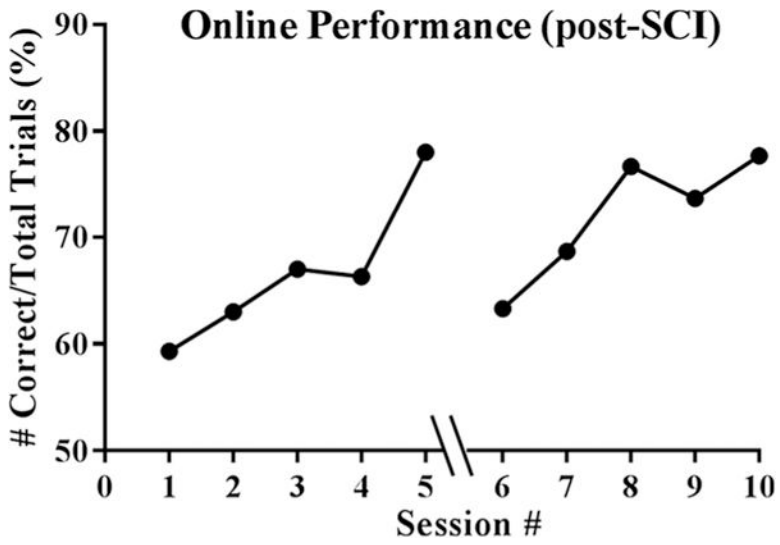

Fig. 9. Learning in post-SCI Rat.

Raw performance of one post-SCI rat that learned after SCI. There were two days of no tilting between recording sessions 5 and 6. Aside from one day of no tilting between sessions 7 and 8, all other data points represent consecutive days of tilting.

Table 1

Summary of the mean number of neurons and their standard deviations for the right and left hemispheres before and after SCI across days of the BMI task. While there is a loss of neurons recorded post-SCI compared to pre ($F[1, 681] = 11.1$, $p < 0.01$), this is likely due to the unreliability of the electrodes and not due to the SCI (Knudsen et al., 2014; Manohar et al., 2012).

Number of Neurons										
	pre-SCI Hemispheres					post-SCI Hemispheres				
		Right		Left			Right		Left	
Animal	# Days	Mean	Std. Dev	Mean	Std. Dev	# Days	Mean	Std. Dev	Mean	Std. Dev
1	25	6.57	0.70	21.57	2.63	26.00	17.81	1.47	14.33	1.88
2	25	21.39	1.54	26.99	0.95	30	13.50	2.29	19.87	1.80
3	25	31.37	3.94	28.39	2.35	8	19.50	0.53	24.25	1.04
4	25	13.00	3.08	34.03	2.17	4	–	–	30.00	3.46
5	25	15.76	1.96	17.47	2.34	–	–	–	–	–
6	25	19.11	1.16	16.04	3.86	1	17.00	–	2.00	–
7	25	14.90	3.42	22.62	2.01	–	–	–	–	–
8	24	8.97	0.97	12.81	2.10	–	–	–	–	–
9	25	19.96	2.28	27.85	4.49	14	–	–	16.71	0.47
10	25	28.94	0.80	16.04	0.20	2	15.00	0.00	5.50	0.71
11	25	11.05	3.01	29.55	0.91	3	4.00	0.00	16.00	0.00
12	25	28.77	3.33	26.80	0.85	–	–	–	–	–
13	25	22.73	3.56	23.23	3.77	24	15.38	1.01	24.67	1.40

Table 2

Summary. Summary of the results and conclusions from this study.

Phase	Comparison	Measure(s)	Result	Conclusion
Pre-BMI	left vs. right tilts	RM, FBL, BFR, BFRvar	=	task is bilateral
	left vs. right hemisphere	RM, FBL, BFR, BFRvar	=	
BMI SCI	pre-SCI vs. post-SCI	BFR, BFRvar	=	
	pre-SCI vs. post-SCI	RM, FBL	–	SCI changes firing rate and timing response properties
BMI	learning	online performance	+	task-learning possible
	non-learning		NC	some animals do not learn
	learning vs. non-learning		+	learning animals SSoutperform non-learning animals
	water	online performance	+	positive reinforcement improves performance
	no water		+	negative reinforcement improves performance
	water vs. no water		=	water reward is not necessary
BMI SCI	Short-term BMI	online performance	*	SCI cortex contains sufficient information to discriminate tilts
	Long-term BMI		+	60% of SCI animals learned