Video Article

Rodent Behavioral Testing to Assess Functional Deficits Caused by Microelectrode Implantation in the Rat Motor Cortex

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Abstract

Medical devices implanted in the brain hold tremendous potential. As part of a Brain Machine Interface (BMI) system, intracortical microelectrodes demonstrate the ability to record action potentials from individual or small groups of neurons. Such recorded signals have successfully been used to allow patients to interface with or control computers, robotic limbs, and their own limbs. However, previous animal studies have shown that a microelectrode implantation in the brain not only damages the surrounding tissue but can also result in functional deficits. Here, we discuss a series of behavioral tests to quantify potential motor impairments following the implantation of intracortical microelectrodes into the motor cortex of a rat. The methods for open field grid, ladder crossing, and grip strength testing provide valuable information regarding the potential complications resulting from a microelectrode implantation. The results of the behavioral testing are correlated with endpoint histology, providing additional information on the pathological outcomes and impacts of this procedure on the adjacent tissue.

Video Link

The video component of this article can be found at https://www.jove.com/video/57829/

Introduction

Intracortical microelectrodes were originally used to map the circuitry of the brain, and have developed into a valuable tool to enable the detection of motor intentions which can be used to produce functional outputs[1]. Detected functional outputs can offer individuals suffering from spinal cord injuries, cerebral palsy, amyotrophic lateral sclerosis (ALS), or other movement-limiting conditions the control of a computer cursor[2,3] or robotic arm[4,5,6], or restore function to their own disabled limb[7]. Therefore, intracortical microelectrode technology has emerged as a promising and quickly growing field[8].

Due to the successes seen in the field, clinical studies are underway to improve and better understand the possibilities of BMI technology[5,9,10]. By realizing the full potential of communication with neurons in the brain, the rehabilitation applications are perceived as limitless[8]. Although there is great optimism for the future of intracortical microelectrode technology, it is also well-known that microelectrodes eventually fail[11], possibly due to an acute neuroinflammatory response following implantation. The implantation of a foreign material in the brain results in immediate damage to the surrounding tissue and leads to further damage caused by the neuroinflammatory response that varies depending on properties of the implant[12]. In addition, an implant in the brain can cause a microlesion effect: a reduction in glucose metabolism thought to be caused by acute edema and hemorrhage due to the device insertion[13]. Furthermore, the signal quality and the length of time that useful signals can be recorded are inconsistent, regardless of the animal model[11,14,15,16]. Several studies have demonstrated the connection between neuroinflammation and microelectrode performance[17,18,19]. Therefore, the consensus of the community is that the inflammatory response of the neural tissue that surrounds the microelectrodes, at least in part, compromises electrode reliability.

Many studies have examined local inflammation[11,20,21,22] or explored methods to reduce the damage to the brain caused by insertion[11,23,24,25], with a goal of improving the recording performance over time[14,26]. Additionally, we have recently shown that an iatrogenic injury caused by a microelectrode insertion in the motor cortex of rats causes an immediate and lasting fine motor deficit[27]. Therefore, the purpose of the protocols presented here is to give researchers a quantitative method to assess possible motor deficits as a result of brain trauma following the implantation and persistent presence of intracortical devices (microelectrodes in the case of this manuscript). The behavior tests described here were designed to tease out both gross and fine motor function impairments, and can be used in many models of brain injury. These methods are straightforward, reproducible, and can easily be implemented in a rodent model. Further, the methods presented here allow for a correlation of motor behavior to histological outcomes, a benefit that until recently, the authors have not seen published in the BMI field. Finally, as these methods were designed to test fine motor function[28], the gross motor function[29], and stress and anxiety behavior[29,30], the methods presented here can also be implemented into a variety of head injury models where the researchers want to rule out (or in) any motor function deficits.

Protocol

All procedures and animal care practices were approved by and performed in accordance with the Louis Stokes Cleveland Department of Veterans Affairs Medical Center Institutional Animal Care and Use Committees.

NOTE: To educate researchers on the decision about the use of a stab injury model as a control, it is recommended to review the work done by Potter *et al.*[21].

1. Microelectrode Implantation Surgical Procedure

1. **Pre-surgical animal preparation**
    1. Anesthetize the animal in an induction chamber using isoflurane (2 - 4%). While under anesthesia, continuously monitor the animal using a vital measurement system to monitor the heart rate and the blood-oxygen content.
    2. Move the animal to a nose cone to continue the anesthetic. Subcutaneously (SQ) inject a cephalosporin antibiotic, e.g. cefazolin (25 mg/kg) and a non-steroidal anti-inflammatory, e.g. carprofen (5 mg/kg) to prevent infection and manage the pain, respectively.
    3. Liberally apply ophthalmic ointment to the animal's eyes to prevent them from drying.
    4. Using small animal nail clippers, trim the toenails to prevent the animal from scratching the sutures during the wound healing. Ensure that the nails are not clipped too short, as this can lead to pain and bleeding for the animal.
    5. Shave the animal's head thoroughly from behind the ears to between the eyes using an electric razor trimmer.
    6. Provide a local analgesia with an SQ injection of bupivacaine (0.3 mL of 0.125% bupivacaine diluted from stock solution) at the top of the animal's head in the area of the incision.
    7. Mount the animal on a stereotaxic frame, using ear bars to keep the head from moving during the surgery. Place a circulating water heating pad under the animal to maintain the animal's internal temperature.
    8. Apply a sterile drape, e.g., institutionally-approved sterile plastic wrap, to isolate the surgical field.
    9. Scrub the surgical area using an alternating betadine solution and isopropanol scrubs.
    10. Perform a toe pinch according to the institutional protocol to ensure the animal is under the surgical plane.

2. **Prepare animal for implantation**
    1. Create an incision of approximately 1 in down midline exposing the skull using a No. 10 scalpel blade. Bluntly remove the periosteum using a cotton-tipped applicator, and stop any bleeding using a gauze pad. Retract the surrounding tissue using alligator clips and clean and dehydrate the skull with hydrogen peroxide.
    2. Place a few drops of cyanoacrylate-based tissue adhesive on the exposed skull to improve the dental cement bonding in later steps.
    3. In the chosen hemisphere, mark the region of the motor cortex corresponding to forepaw movement approximately 3 mm lateral to midline and 2 mm anterior to bregma by creating a nick in the bone.
    4. Remove a portion of the skull using a 1.75-mm rounded tip dental drill, taking special consideration not to drill too quickly or too deeply, and supporting one hand on the stereotaxic frame. The drill should be applied to the skull intermittently to avoid overheating[31].
    5. Reflect the dura using a fine hooked 45° dura pick.
    6. Clean any bleeding using a cotton-tipped applicator and saline, taking care to not directly touch the brain surface.

3. **Insertion of microelectrode in motor cortex**
    1. Carefully mount the sterilized microelectrode in the universal holder on the stereotaxic frame, taking caution not to bump the shank of the electrode. Ensure that the headstage interface connector of the electrode is firmly held by the holder.
    NOTE: Here, a non-functional Michigan-style silicon shank electrode measuring 2 mm x 123 µm x 15 µm was used, and the shank was inserted using fine forceps.
    2. Using the micromanipulators on the stereotaxic frame, carefully position the tip of the electrode over the open craniotomy.
    3. Gently lower the electrode approximately 2 mm into the brain using the micromanipulators as a measurement guide (depending on the choice of electrode, an automated insertion at controlled rates may be required.) Take caution to avoid any visible vasculature whenever possible. Once the electrode is in place, carefully release the connector from the universal holder and retract the insertion arm.
    4. Carefully clean any bleeding from around the electrode using a cotton-tipped applicator and saline.
    5. Seal off the craniotomy around the implanted electrode using a silicone elastomer.
    6. Fix the electrode to the skull using dental cement.
    7. Once the cement is completely dry, bring the edges of the incision together at the front and back of the cement headcap and suture them shut.

4. **Post-operative care**
    1. Allow the animal to recover on a circulating water heating pad while continuing to monitor its vital signs. Avoid using heat lamps as the temperature from lamps is more difficult to control and animals can overheat.
    2. Once the animal is fully awake, move the animal to a clean cage with easy access to food and water.
    3. During post-operative days 1 - 3, provide the animals with SQ cephalosporin antibiotic (25 mg/kg) and a non-steroidal anti-inflammatory (5 mg/kg) to prevent infection and manage their pain.
    4. Monitor the animals daily for the signs of pain or discomfort, bleeding, weight change, or suture issues through at least post-operative day 5.

2. Behavioral Testing

1. For all behavior testing, test the animals 2x per test in the week prior to the electrode implantation surgery to calculate their pre-surgery baseline scores. Following the surgery, allow the animals to rest for 1 week before beginning behavior testing 2x per week on each test. Consistent testing conditions should be used throughout the study for both pre- and post-surgical testing to minimize the effects of stress on the performance, which could result in a measurement of anxiety.
    1. Clean all testing equipment with a chlorine dioxide-based sterilant at the beginning of each testing session and after each animal.
    2. Film the open field grid and ladder testing. These tests require a video camera (1080p, minimum of 15 fps, 78° diagonal field of view), a laptop, and room to store the video data.
    3. At the beginning of each testing day, bring the animals to the testing room and allow them to acclimate for at least 20 min before beginning the testing. The room should be light and temperature-controlled, and the same personnel should complete all testing. Ideally, the same room will be used for all animals throughout the course of the testing with no changes to the room.
    4. Use food rewards to encourage the animals to complete the tasks, especially during the ladder training. Cereals or small pieces of banana chips or crackers make good rewards.
    5. Normalize all weekly testing performances to the pre-surgery scores for each individual animal (**Equation 1**).

        Equation 1: $$\%\ change\ in\ performance = \frac{baseline\ score - weekly\ test\ score}{baseline\ score} * (100)$$

2. **Open field grid testing**

    NOTE: The open field grid test was built in-house and has a running surface of 1 m^2 with approximately 40-cm high opaque side walls. The bottom running surface of the grid is divided into 9 equal squares from the underside using tape (**Figure 1A**). The recording camera is permanently mounted above the center of the grid on scaffolding.
    1. To begin open field grid testing, place the animal in the center of the grid facing away from the tester.
    2. Allow the animal to run freely for 3 min while recording a video.
    3. When the animal has completed testing, remove the animal from the grid and return it to the cage. Clean the grid thoroughly with chlorine dioxide-based sterilant.
    4. Test each animal 1x per testing day.
    5. Analyze the number of gridlines crossed, the total distance traveled, and the maximum velocity of the animal as metrics of the gross motor function using a video tracking software.

        NOTE: The data presented here were quantified manually by trained researchers, but it is preferred to use a recently developed in-house tracking algorithm[32].

3. **Ladder testing**

    NOTE: The ladder test was built in-house and consists of 2 clear acrylic side walls, each 1 m in length, connected by 3-mm diameter rungs spaced at 2 cm apart (**Figure 2A**). Ladder testing is a skilled test, and therefore requires 1 week of training prior to recording the pre-surgery baseline scores. The protocol for the training and testing is the same.
    1. Move the animal to a temporary clean holding cage to begin ladder testing.
    2. Set the ladder up so that it bridges 2 cages. The start end of the ladder rests on a clean cage, and the finish end rests on the animal's home cage to serve as a motivation to complete the run.
    3. Position the same (or similar) video camera on a tripod at the center of the ladder. The position of the camera should be at rung height and allow for the whole ladder to be seen.
    4. With the video camera running, hold the animal to the starting line of the ladder, allowing their front paws to touch the first rung.
    5. Allow the animal to cross the ladder at their own pace. The time elapsed between the moment when the animal's paw touches the first rung and the finish line at the third to last rung will determine the animal's time to cross.
    6. If the animal turns around on the ladder or does not move for a period of 20 s, consider the animal to have failed the run. Assign the animals a penalty score time for each failed run. Determine the penalty time by the slowest performance recorded during pre-surgery testing[27].
    7. Allow each animal to cross the ladder 5x per testing day with approximately 1 min rest in between each run.
    8. Average the fastest 3 runs per day as a metric of fine motor function. Additionally, record the number of times each of the front paws slips off the rungs using a video tracking software.

        NOTE: The data presented here were quantified manually by trained researchers, but it is preferred to use a recently developed in-house tracking algorithm using Dona *et al.*[32].

4. **Grip strength testing**
    1. Calibrate the grip strength meter before each testing session, and measure the strength in grams.
    2. Position the grip strength meter on the edge of a counter with the grip handlebars extended over the floor.
    3. Allow the animal to grab the handlebars with both front paws while holding the animal by the base of the tail (**Figure 3A**).
    4. Once the animal has a firm grip with each paw, pull the animal away from the meter by the base of the tail with slow and steady force.
    5. Record the maximum grip strength exerted by the animal which is displayed on the digital output by the grip strength meter.
    6. Test each animal 3x per testing day with approximately 2 min rest in between each test.
    7. As a metric of fine motor function, record and average the maximum grip strength output from each of the 3 trials.

3. Post-behavioral Protocol

1. Following all behavioral testing (*e.g*, 8 - 16 weeks after the implantation), anesthetize the animals deeply using ketamine (160 mg/kg) and xylazine (20 mg/kg), transcardially perfuse them, harvest their brains and cryo-slice them, and stain the tissue using immunohistochemical markers to quantify the cellular response around the site of implantation[33,34,35,36,37,38].

4. Statistical Analysis

NOTE: A prospective power analysis is strongly suggested for any studies seeking to answer a particular research question. The power analysis, which informs the number of animals required to achieve a statistical significance for a particular study design, should be based on the particular research hypothesis, the design of the experiment, the estimated effect size and variability of the intended treatments, as well the effect size required to achieve clinical or scientific relevance.

1. Conduct statistical analyses using common statistical software.
2. Tabulate the descriptive statistics and display them as Mean ± Standard Error.
3. Analyze the behavioral performance [in the open field grid (step 2.2), ladder (step 2.3), and grip strength testing (step 2.4)] at each weekly time point to compare the control *vs.* implanted groups using a two-sample t-test. Consider each weekly time point an independent measure.
4. Quantify the longitudinal performance using a mixed effect linear model. The week and the group are fixed factors and an experimental animal is nested within the group as a random effect. An analysis of variance (ANOVA) is used to determine the factor effect with a significance level of $p < 0.05$.
5. Compare the ladder performance with immunoglobin G (IgG) intensity using a linear regression analysis. Calculate the correlation coefficient by a Pearson's method.

Representative Results

Using the methods presented here, a microelectrode implantation surgery in the motor cortex is completed following established procedures[39,40,41,42], followed by open field grid testing to assess the gross motor function and ladder and grip strength testing to assess the fine motor function[27]. Motor function testing was completed 2x per week for 16 weeks post-surgery in implanted animals, with no surgery non-implanted animals as a control. All post-surgery scores were averaged per week and normalized to each individual animal's pre-surgery baseline scores. All error is reported as standard error of the mean (SEM).

To measure their gross motor function and stress behavior, animals were allowed to run freely in an open field grid test for 3 min (**Figure 1A**). Various metrics from this test can be recorded, including the number of grid lines cross, the total distance traveled, and the maximum speed achieved by the animal. In this previously reported data, the number of grid lines crossed is presented[27]. In the first week following the recovery period (the 2-week timepoint), a significant difference was seen in the open field grid performance between the 2 groups. However, there was no further significance throughout the rest of the study (**Figure 1B**). The control and microelectrode-implanted animals scored similarly throughout testing, and the variance in performance was relatively high in both sets of animals. No significance was seen when comparing the open field grid performance in both sets of animals across the entire experimental time. Because there was no difference in performance between the 2 groups of animals, this result was interpreted to indicate that there is no gross motor deficit or severely limiting stress caused by a microelectrode implantation in the motor cortex[27]. When interpreting the data, a decrease in the number of grid lines crossed, the total distance traveled, or the maximum speed achieved by the animal all indicate a decrease in its gross motor function (**Table 1**).

To measure the coordinated grasp and fine motor function, animals took part in a horizontal ladder test (**Figure 2A**) where the time it took the animal to cross the ladder and the frequency of paw slips were recorded. Post-surgery ladder crossing times were normalized for each animal to each individual animal's pre-surgery scores. Therefore, a positive percentage coincides with a decrease in time to cross the ladder and an increased performance, and a negative percentage coincides with an increase in time to cross the ladder and a decreased performance (**Figure 2B**, **Table 1**).

In this previously reported data, the control animals, having received no implant, displayed the slowest performance times (82.6 ± 26.0%) during the first week of post-surgery testing immediately after the recovery phase[27]. Beginning in the second week of post-surgery ladder testing, the control animals resumed their baseline performance times and maintained scores comparable to their baseline scores over the course of the study with very little variance.

The animals receiving an intracortical microelectrode saw a reduced performance straightaway following surgery. These animals demonstrated an increased ladder crossing time compared to their baseline of 199.1 ± 61.4% in the first week of post-surgery testing. The implanted animals displayed a reduced performance for the duration of the study and their performance did not return to their baseline scores. At their worst, implanted animals decreased in performance during week 11 to an average of 526.9 ± 139.4% compared to their baseline performance. Additionally, the implanted animals showed a higher variance compared to the control animals. There was no significant difference between the control and implanted animals during the first week of testing. However, a significant difference in the percent change compared to the baseline times was seen between the groups at all subsequent weeks in the study ($p < 0.05$) (**Figure 2B**).

Further evidence of fine motor impairment was demonstrated by the frequency of front right paw slips between the 2 groups of animals. The performance of the front right paw was of particular interest because microelectrodes were implanted in the left hemisphere of the brain in the region of the motor cortex responsible for front paw control. By meticulous video analysis, paw slips were chronicled and quantified (**Figure 2C**). While no significant differences were seen in the frequency of left paw slips, it was found that the implanted animals experienced significantly more front right paw slips as compared to the control animals (an average of 0.54 ± 0.07 front right paw slips per week in the implanted animals as compared to an average of 0.32 ± 0.02 front right paw slips per week in the control animals). When interpreting the data, an increase in the time to cross the ladder or an increase in the number of paw slips indicates a decrease in fine motor function (**Table 1**).

As a secondary measure of coordinated grasp and fine motor function, the animals completed a grip strength test (**Figure 3A**) where the maximum grip strength exerted by the animals was recorded. The individual animal's weekly grip scores were normalized to their pre-surgery baseline grip strength. It was seen that the implanted animals' post-surgery grip strength was significantly reduced compared to the control animals' at almost every post-surgery time point. (**Figure 3B**). The control animals' grip strength improved following pre-surgery testing, likely due to the training effect. Further, the control animals' grip strength was significantly greater than the baseline throughout the course of the study ($p < 0.05$). Interestingly, the implanted animals' grip strength performance was significantly worse than the baseline ($p < 0.01$) in the first week of testing following the recovery phase, but slowly returned to their baseline performance. Of note, a decrease in the maximum grip strength achieved by the animal indicates a decrease in fine motor function (**Table 1**).

Various histological markers can be used to visualize the microenvironment near a brain implant, including neuronal nuclei, astrocytes, and blood-brain barrier stability. Here, we performed immunohistochemical staining for IgG, a common blood protein not commonly found in the brain. Earlier work has shown that IgG is a useful indicator of blood-brain barrier integrity as it is an antibody found in the blood, and not normally present in the brain[16,18], and therefore the presence of IgG in the surrounding brain tissue can be correlated to the integrity of the blood-brain barrier[43]. Here, IgG fluorescence intensity was normalized to background brain tissue and quantified starting at the boundary of the electrode explantation hole and moving out in concentric bins until IgG was no longer present in the tissue. The implanted animals showed a significant increase in IgG intensity near the hole out to 150 µm as compared to the control animals. The IgG intensity in the implanted animals gradually returned to background intensity over the distance radiating from the implanted microelectrode hole. In the control animals, having never been implanted with a microelectrode, the normalized IgG intensity was not present in significant quantities above background intensity as the blood-brain barrier was not damaged in these animals.

Because significant differences were seen in both the ladder performance and IgG intensity, the two were correlated (**Figure 4**). Here, the normalized fluorescent intensity of the IgG area under the curve from 0-50 µm from the tissue-electrode interface for each animal was correlated with the average of each animal's ladder performance over the course of the study. A correlation coefficient of 0.90 was determined, demonstrating a very strong correlation between the fine motor performance and damage to the blood-brain barrier.

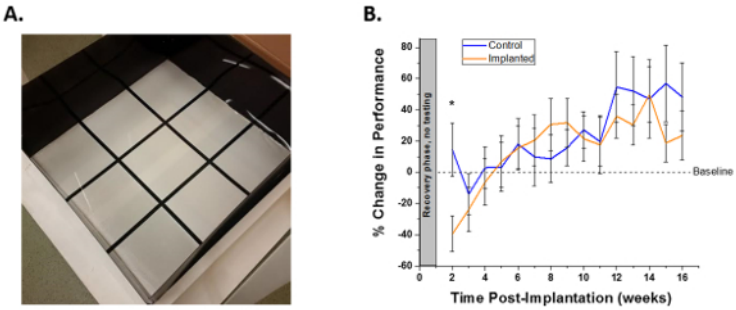

Figure 1. Representative open field grid test results. (**A**) This panel shows a behavioral testing setup for an open field grid test (for gross motor and anxiety testing). The open field grid test consists of a 1 m^2 acrylic sheet with 4 opaque walls of 40 cm in height, and square bottom sections of approximately 33 cm each. (**B**) This panel shows a gross motor function performance measured by the number of grid lines crossed, compared to the baseline performance. A significant difference in performance was seen between the control ($n = 10$) and the implanted ($n = 17$) groups at 2 weeks post-surgery ($p < 0.05$). All error is reported as SEM. This figure is reprinted from Goss-Varley *et al.*[27] with permission from the Nature Publishing Group. Please click here to view a larger version of this figure.

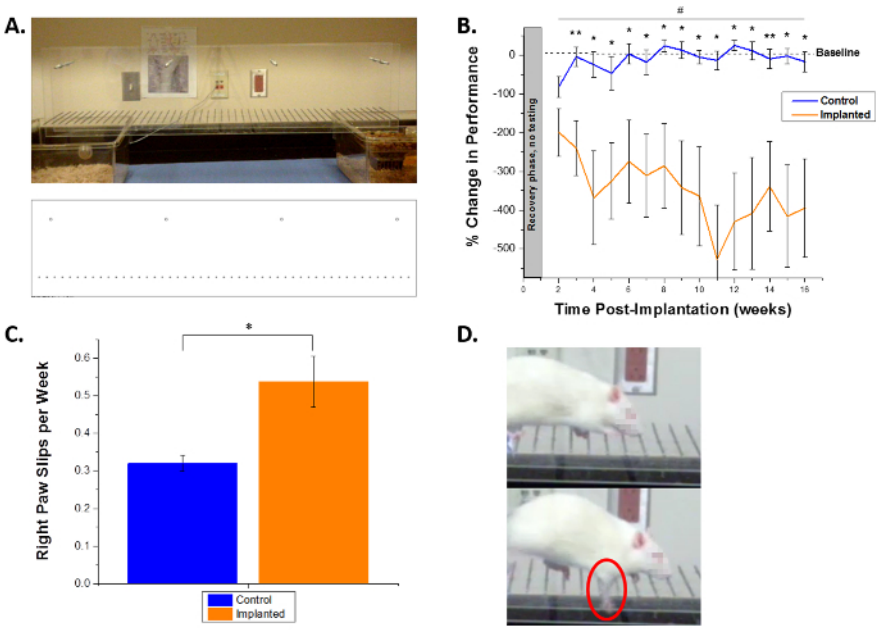

Figure 2. Representative ladder test results. (**A**) This panel shows a behavioral testing setup for a ladder test (for fine motor function testing). The ladder consists of 2 clear acrylic sides of 1 m in length and 25 cm in height, joined by stainless steel rungs spaced at 2 cm with a 3-mm diameter. (**B**) This panel shows fine motor function performance measured by time to cross the ladder, compared to the baseline performance. The results below the dashed line indicate a decrease in performance as compared to the baseline performance. A significant difference in performance was discovered between the control (n = 10) and the implanted (n = 17) groups for the post-surgery weeks 3 - 16 (* = $p < 0.05$, ** = $p < 0.01$) and longitudinally across the entire study (# = $p < 0.05$). (**C**) This panel shows a quantified instance of right front paw slips. A significant difference was discovered in the occurrence of right front paw slips per week when comparing the control and the implanted groups (* = $p < 0.05$). (**D**) This is an example of a paw slip. All error is reported as SEM. This figure is reprinted from Goss-Varley *et al.*[27] with permission from the Nature Publishing Group. Please click here to view a larger version of this figure.

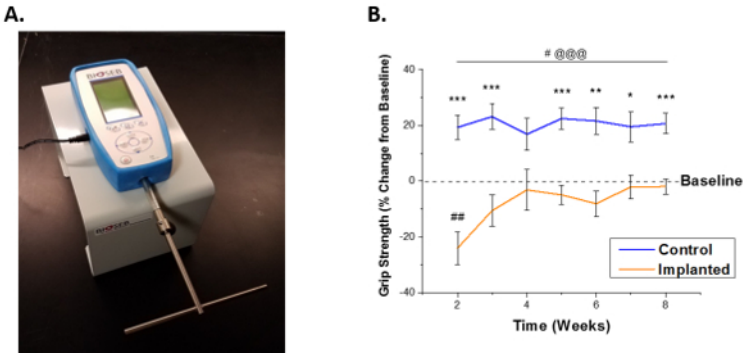

Figure 3. Representative grip strength test results. (**A**) This panel shows a behavioral testing setup for grip strength (for fine motor function testing). The grip strength meter consists of a weighted base with a mounted strength gauge connected to a grip handlebar. (**B**) This panel shows the fine motor function performance, measured by the maximum grip strength exerted compared to the baseline performance. The results below the dashed line indicate a decrease in performance as compared to the baseline performance. Significant differences were seen between the control (n = 5) and the implanted (n = 6) animals for almost all post-surgical weeks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Further significance was seen between the control animals' weekly and baseline performances (# = $p < 0.05$) and between the implanted animals' weekly and baseline performances (## = $p < 0.01$). The control and the implanted animals performed significantly different longitudinally across the entire study (@@@ = $p < 0.001$). Please click here to view a larger version of this figure.

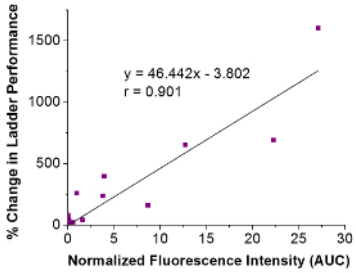

Figure 4. Correlation of IgG and ladder performance. A normalized IgG fluorescence intensity around the site of implantation was correlated with a change in ladder performance, and a correlation coefficient of 0.901 was found ($p < 0.001$). Please click here to view a larger version of this figure.

	Open Field Grid Lines Crossed	Open Field Total Distance Traveled	Open Field Maximum Speed	Ladder Crossing Time	Ladder Paw Slips	Grip Strength Maximum Pull
Increase from Baseline	Improved Performance – No Gross Motor Function or Anxiety Deficits	Improved Performance – No Gross Motor Function or Anxiety Deficits	Improved Performance – No Gross Motor Function or Anxiety Deficits	Reduced Performance – Fine Motor Function Deficit	Reduced Performance – Fine Motor Function Deficit	Improved Performance – No Fine Motor Function Deficit
Decrease from Baseline	Reduced Performance –Gross Motor Function or Anxiety Deficits	Reduced Performance – Gross Motor Function or Anxiety Deficits	Reduced Performance – Gross Motor Function or Anxiety Deficits	Improved Performance – No Fine Motor Function Deficit	Improved Performance – No Fine Motor Function Deficit	Reduced Performance – Fine Motor Function Deficit

Table 1. Overall representative behavior data showing increase and decrease in performance compared to baseline scores for each testing metric. The green boxes represent an improved performance which makes a motor deficit unlikely, and the red boxes represent a reduced performance which makes motor function deficits likely.

Discussion

The protocol outlined here has been used to effectively and reproducibly measure both fine and gross motor deficit in a model of rodent brain injury. Additionally, it allows for the correlation of fine motor behavior to histological outcomes following a microelectrode implantation in the motor cortex. The methods are easy to follow, inexpensive to set up, and can be modified to fit a researcher's individual needs. Further, the behavior testing does not cause great stress or pain to the animals; rather, the researchers believe the animals grew to enjoy the exercise and rewards that came with testing. Previous studies have suggested that motor cortex damage can cause motor, memory, and functional damage[44,45]. However, despite this knowledge, there is limited information on the functional impact caused by a microelectrode implantation in the motor cortex[27], which could negatively impact the clinical outcomes in patients.

Modifications can be made throughout the protocol, both in the surgical procedure and in the behavior testing. This protocol outlines the procedure to implant microelectrodes in the motor cortex of animals in the region affecting the forepaws. This procedure can be easily adapted to vary the implant, including electrodes for electrical stimulation[46] or cannulas for drug delivery[47], or the type of injury, including a TBI model[48]. Further modifications can be made to the scoring metrics used on the open field grid test, and to the ladder testing apparatus. In addition to the number of gridlines crossed, the total distance traveled, and the maximum velocity achieved by the animal, the time spent stagnant and the number of right and left turns can also be recorded as additional parameters of motor performance[32]. In the ladder test, removing rungs[49] or placing the ladder on an incline[50] can increase difficulty, although with the current implants the authors did not find this necessary to tease out fine motor deficits in this application. Finally, although the testing apparatus presented here were designed to be used with rats, the units could be scaled up or down to be used with various-sized rodents. It is important to note that if issues arise where an animal is not able to complete the pre-surgery testing consistently, the animal should be removed from the study.

As with all behavioral testing, it is critical to remain as consistent as possible over the course of the study. It has been shown that test results can vary based on the researcher working with the animals[51], the location in which the testing is performed[52], and environmental factors including animal housing and husbandry procedures[53]. Additionally, research has shown great variability in producing a brain injury by way of skull heating during a craniotomy procedure[31] and models of TBI including the weight-drop model[54] and mechanical variation in a controlled cortical impact model[55]. Researchers should, therefore, take special care to maintain consistency in the surgical procedure, testing and housing conditions, and in the testing personnel, among others.

Future directions of these behavior testing methods could expand upon the testing presented here to provide more thorough results. For example, a water maze test or a rotor rod test could be incorporated to further extract anxiety[56] or gross motor function[57] deficits, respectively. Additionally, future work might also aim to reduce the tissue damage caused by a device insertion in the brain. Current work in this area has focused on inflammation mitigation through anti-oxidant treatments[42,58], mechanically compliant implants[41,59,60], the inhibition of the innate immunity signaling pathway[14,15], and reducing vascular damage during a device implantation[31,61].

Lastly, it must be considered that the current work was completed using healthy, juvenile, male rats that do not necessarily embody the characteristics of the typical human patient receiving a brain implant. Additional research exploring further fine and gross motor function tasks in characteristic disease models is required to ratify the findings presented here. In varying disease models, differences between implanted and non-implanted sham animals may require the above-mentioned modifications to test conditions.

Disclosures

The authors have nothing to disclose.

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