A new rodent behavioral paradigm for studying forelimb movement

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ABSTRACT

The center-out task is a standard paradigm often used to study the neural control of reaching movements in human and non-human primates. However, there are several disadvantages to the use of monkeys, notably costs, infrastructural requirements, and ethical considerations. Here we describe a similar task designed to examine forelimb movements in rats. Rats were trained to grasp a joystick with their forepaw and use it to control the movements of a sipper tube in two dimensions. The rats learned to move the joystick in four directions with at least 70% accuracy after about 45 days of training. In addition, rats were able to learn a reversed mapping between joystick and sipper tube movement. This is a more complicated behavior than has been previously demonstrated for rats, and it could allow more motor behavior studies to be conducted in rodents instead of monkeys. We currently are using this behavior to decode the rats' forelimb movements from their brain signals.

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1. Introduction

Neurophysiologists have gone to great lengths to design experiments to elucidate how the brain controls movement. Beyond the basic scientific applications, this field may have clinical applications, in the development of neural prosthetics and brain machine interfaces (BMIs), which offer the hope of restoring motor output and sensory input to individuals with neurologic disorders. Since many BMI decoding algorithms are designed to make use of the modulation of brain signals (spikes or field potentials) with movement (Musallam et al., 2004; Serruya et al., 2002; Taylor et al., 2002), an improved understanding of how the brain controls movement is critical to improving BMIs. Many laboratories that study motor control use humans or monkeys as subjects, since primates are considered to be more dexterous than rodents and to have greater cognitive ability to learn complex tasks. One prominent paradigm is the use of a manipulandum (Fayé, 1986; Georgopoulos et al., 1982) or joystick (Wessberg et al., 2000) to study reaching behavior, for example in the center-out task (Georgopoulos et al., 1982). This paradigm provides a simple method to record kinematic information simultaneously with neural and muscle signals (Kalaska et al., 1989; Li et al., 2001; Scott, 1999; Shadmehr and Mussa-

Ivaldi, 1994). Here we describe a similar paradigm that can be used to study two-dimensional forelimb movement in rats. While this behavior is not quite the same as free reaching, it is similar. Indeed, Wessberg et al. (2000) showed that both joystick movements and free reaching movements can be decoded from a neural ensemble using the same types of models. We show that rats can learn the task relatively quickly, in approximately 45 short daily training periods.

2. Methods

2.1. Apparatus

The behavior apparatus, shown in Fig. 1(a), consisted of a polycarbonate box (4″ W × 10″ L × 15″ H) with a joystick mounted below the floor. The joystick (521F, Ultra Electronics Measurement Systems, Inc., Weldon Spring, MO) was spring-loaded so it would re-center after each movement. A sipper tube was mounted outside the box on two perpendicularly oriented servo motors which could position its tip anywhere within a circle of radius 2 cm (14° arc). The tube passed through a hole in a moveable polycarbonate disk in the front of the box which allowed free movement of the tube while preventing the rat from leaving the box. The height and depth of the tube into the box were adjusted to allow each rat to lick water from the tube while holding the joystick. A polycarbonate barrier was used to confine the rat to the right side of the box to encourage the consistent use of the left forepaw to move the joystick.

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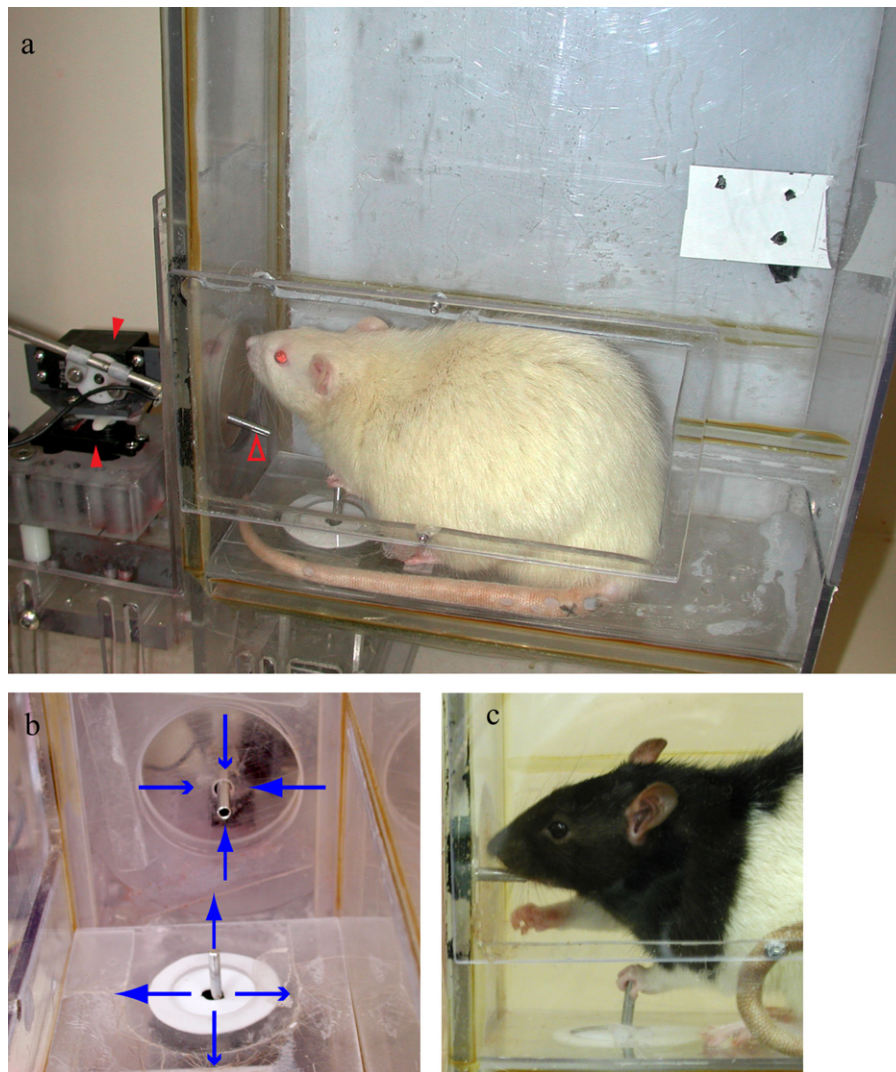*E-mail address:* mslutzky@md.northwestern.edu (M.W. Slutzky).

Fig. 1. Behavioral apparatus. (a) Entire apparatus is shown. Sipper tube (open arrowhead) is mounted to two servo motors (closed arrowheads) on the left. Polycarbonate barrier is not shown for clarity. (b) Rat's eye view of sipper tube and joystick and the respective directions of movement (small arrows for right and down, large arrows for left and up). (c) A rat moving the joystick down with the left forepaw.

2.2. Behavior controller

A custom-designed circuit using a programmable microcontroller (PIC 18F2620, Microchip Technology, Inc.) controlled the behavior. Joystick angular position was measured by two potentiometers, and converted in the controller circuit to control signals for the servo motors. The control signals were proportional to joystick position.

To start each trial, the rat had to position the joystick within 3° of the center position for 0.2 s. The sipper tube moved from the center position to one of four equidistant outer positions along the horizontal or vertical axes (Fig. 1(b)), and a "go" cue was given in the form of a tone. The rat moved the joystick about 2.3 cm (27°) in the direction opposite to the position of the sipper tube (Fig. 1(c)). The tube stopped when it was within 3° of the center position, and the rat received a second tone and a juice reward. If the rat did not successfully return the sipper tube to the center within 10 s (usually because it did not attempt a movement due to lack of motivation), the trial was aborted. If the rat initiated movement in the wrong direction, the trial was considered a failure.

2.3. Rat training protocol

The behavioral protocol was approved by the Northwestern University Animal Care and Use Committee. Rats were water restricted for approximately 20 h before each session. After each session, they were allowed at least an hour of unlimited access to water. Rats were trained 20–45 min per day, up to 5 days per week, and allowed free access to water over the weekends. Rats were first acclimated to the box, then operantly conditioned to hold the top of the joystick handle with their left forepaw and then to move the joystick to control the sipper tube. We first trained the rats to move the joystick in a single direction (e.g., left), with an acrylic "channel" mounted to the floor restricting joystick movement to that direction. We proceeded to train them, sequentially, to move in the opposite direction, then both directions along a single axis. We repeated the process for the second axis and finally for all 4 directions. We removed the guide channels after all 4 directions were learned. We chose a criterion of 70% successful trials (over the entire session, cumulative over all directions) to define successful learning of the task. The sipper tube position was presented in a random order in all sessions in which more than one direc-
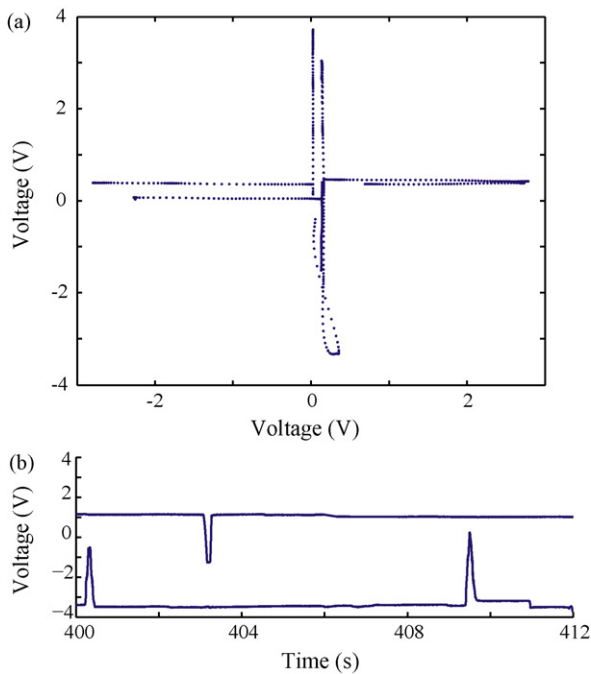
Fig. 2. (a) Joystick movement trajectories for eight consecutive trials (two in each direction) by one rat. The first 200 ms of each reach is shown. (b) Time courses of *x* and *y* components (top and bottom traces, respectively) of three consecutive joystick movements. Each movement lasts about 200 ms.

tion was presented. We trained a subset of rats to perform the task with the gain between joystick and sipper tube movement inverted, i.e., the sipper tube moved in the direction opposite to the joystick ("reverse" paradigm, see Results and Discussion for further details).

2.4. Signal recording methods

All behavioral and physiological signals were recorded using an RZ5 Bioamp System (Tucker Davis Technologies [TDT], Alachua, FL). We recorded joystick position signals, digital behavior signals (trial onset, reward/abort/failure codes), and brain signals. After a rat had achieved the 70% success criterion, we implanted 16-channel electrode arrays over the forelimb area of the right sensorimotor cortex. We recorded field potentials from electrodes placed either intracortically (50 μm-diameter tungsten microwire arrays, TDT) or epidurally (custom-built using 100 μm-diameter platinum wires with 700 μm interelectrode spacing). Signals were bandpass filtered from 1 to 240 Hz and digitized at 500 Hz. Forelimb muscle EMG signals were also recorded from two rats during the task using intramuscular braided stainless steel wire electrodes 25 μm in diameter (A-M Systems, Inc., Sequim, WA).

3. Results

Both Sprague–Dawley (*n* = 7) and Long–Evans (*n* = 5) rats were trained to perform the behavioral task. Fig. 2(a) shows an example of joystick position trajectories from several different reaches. The trajectories were very linear, probably due to mechanical restoring forces in the joystick. The time courses of the horizontal and vertical components of these trajectories are shown in Fig. 2(b). The mean reach duration was approximately 200 ms. On average, the rats took 1.2 s from the start of each trial to earn a reward. The rats would often take breaks to turn around or groom themselves. Thus the vast majority of aborts were the result of a rat not trying rather than an unsuccessful attempt.
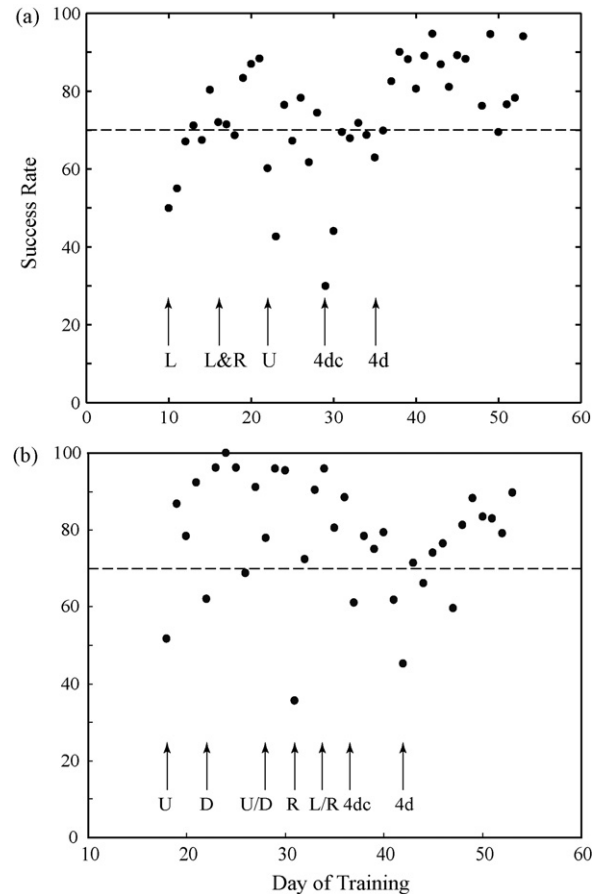

Fig. 3. Performance of two rats during training on the (a) forward and (b) reverse tasks. No data points are shown for the first 9 (a) or 17 (b) days of training, which involved teaching the rat to move the joystick with its forepaw. Arrows indicate the start of a new task: L, left reaches only; R, right only; L&R, left and right; U, up only; D, down only; U/D, up and down; 4dc, four directions with channel restraint; 4d, four directions without channel. These rats achieved 70% success at four directions after only 37 and 43 days of training for forward and reverse tasks, respectively.

Once most rats learned to grasp the joystick, they learned to move the sipper tube fairly readily, as seen in Fig. 3(a) for one exemplary rat. The performance increased steadily, with regular drops as new directions were added (arrows). The mean time that it took the 12 rats to reach the 70% success criterion for the initial direction was 14 ± 4 days. It took 6 additional days (20 ± 7 total) to achieve 70% success in two directions and 25 additional days (46 ± 11 total) for four directions (without movement restrictions). Once learned, rats routinely achieved 150–200 successful reaches per session; one rat achieved as many as 500 successes per session. Performance was largely the same regardless of direction once the task was learned; however, a few rats took longer to perform one direction with high accuracy than the other directions. The direction that these rats had trouble with was not consistent across rats. There was a statistically insignificant trend of faster learning of four directions in the Long–Evans rats (42 ± 7 days vs. 48 ± 12 days, *p* = 0.31, unpaired *t*-test). If the effect were significant, it would fit with prior observations that these two strains of rats have similar skilled reaching success (Whishaw et al., 2003).

Fig. 4 shows an example of simultaneously recorded epidural field potentials and EMGs along with joystick and behavior signals (go cue, movement onset time, and reward signal) during three reaches. The epidural signals were used offline to decode the direction of joystick movement (Slutzky et al., 2009, and work in preparation).
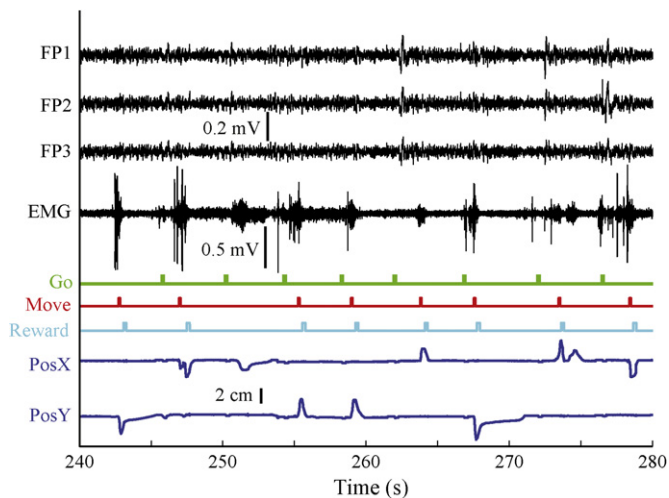
Fig. 4. An example of behavioral and brain signals recorded during eight consecutive reaches. Epidural field potentials from three neighboring channels are shown at the top. Intramuscular EMG signal from the biceps is shown in the middle (EMG). PosX and PosY are the X and Y position signals of the joystick, respectively. Go, move, reward are the behavioral cues. Scale bars indicate voltage for EFPs and EMG signals and distance for position signals.

Four rats were trained on a "reverse" paradigm; that is, the movement direction of the joystick was opposite to that of the sipper tube. This more complicated task was done as part of a control to ensure that the signals we recorded were related to forelimb and not head movements. Two rats learned only the reverse paradigm. As seen in Fig. 3(b), the rats learned this reverse paradigm at a rate similar to that of the rats learning the forward paradigm: 19 days to achieve 70% success for the initial direction, 30 ± 1 days for two directions, and 41 ± 3 days for four directions. The other two rats first learned the normal paradigm and then the reverse paradigm. These two rats learned both paradigms equally well, and after learning both paradigms they were able to switch between paradigms with 12 days of retraining. This confirmed that they really did retain some understanding of the relationship between joystick and sipper tube in both paradigms.

4. Discussion

This study demonstrates a new behavioral platform for investigating neural control of movement in rats. We have shown that rats are capable of learning more complex motor tasks than has been demonstrated previously. On average, rats learned a two-directional task within 20 days and a four-directional task within 45 days, requiring only 30–45 min of daily training. A few groups (Chapin et al., 1999; DiGiovanna et al., 2009; Olson et al., 2005) use rats as their primary model for researching BMIs. However, the motor or BMI tasks in these studies have involved relatively simple, one-dimensional, lever-presses and did not require rats to use the same forepaw for the task. In one behavioral psychology study, two rats were trained to use a joystick to move a computer cursor in two dimensions, but they were allowed to use their mouths, paws, or other body parts indiscriminately (Washburn et al., 2004). Further, the rats in that experiment were able to move the cursor into a target in only 25% of all trials despite 24-h access to the behavioral setup. In another study, rats were trained to move a computer cursor in two dimensions using a trackball, again with the ability to use any body part (Vato and Mussa-Ivaldi, 2007). Francis and Chapin (2004) demonstrated that rats could push a one-dimensional manipulandum past a position or force target with a forepaw to get liquid rewards. Our study demonstrates that rats are capable of more complex behavior. Not only were they able to move

a joystick in two dimensions to control a sipper tube, they also were able to reverse the association between joystick and sipper tube.

There were some limitations to the current design of the reaching paradigm. As seen in Fig. 2, the spring-loaded joystick constrained the movements largely to movements along the cardinal axes. As a consequence, the force exerted by the rat may had a component in a different direction than the movement of the joystick. This could cause errors in decoding forelimb movements, if the field potentials correlated more closely with the rat's intended movement direction, rather than the joystick direction. Despite this possibility, we have been able to decode forelimb movements with accuracies far above chance (Slutzky et al., 2009). If less constrained movements were desired, an alternative joystick could be used. We chose this one because it was relatively inexpensive, readily available, and the spring-loading avoided the need to teach rats to bring the joystick back to the center to start each trial. We did not require the rats to hold the tube in the target position. This simplified the task, but it was still useful for examining forelimb movement and EMG patterns. Finally, the rats' heads and bodies were not restrained, and some rats tended to move their head with the sipper tube. The correlation between head and forelimb movements could potentially confound the decoding of forelimb movements from brain signals. We controlled for this movement by introducing the reverse paradigm, described above, which decoupled the head and forelimb movements. Our predictions corresponded to forelimb, not head, movements (data not shown).

Using rats instead of primates to study reaching movements could be advantageous for several reasons. Compared with rats, monkeys require a significantly larger investment for both institutions and investigators. Clearly, there is an ethical desire to minimize use of higher-order animals for research. While monkeys can perform more complex behaviors than rats, and have more clearly defined motor cortices, rats may provide a quick and relatively inexpensive experimental model for studying the neural control of movement as well as the development of BMIs.

References

Chapin JK, Moxon KA, Markowitz RS, Nicolelis MA. Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. Nature Neuroscience 1999;2:664–70.
DiGiovanna J, Mahmoudi B, Fortes J, Principe JC, Sanchez JC. Co-adaptive brain–machine interface via reinforcement learning. IEEE Transactions on Biomedical Engineering 2009;56:54–64.
Fayé IC. An impedance controlled manipulandum for human movement studies. Department of Mechanical Engineering, M. I. T; 1986.
Francis JT, Chapin JK. Force field apparatus for investigating movement control in small animals. IEEE Transactions on Biomedical Engineering 2004;51:963–5.
Georgopoulos A, Kalaska J, Caminiti R, Massey J. On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. Journal of Neuroscience 1982;2:1527–37.
Kalaska JF, Cohen DA, Hyde ML, Prud'Homme M. A comparison of movement direction-related versus load direction-related activity in primate motor cortex, using a two-dimensional reaching task. Journal of Neuroscience 1989;9:2080.
Li C-SR, Padoa-Schioppa C, Bizzi E. Neuronal correlates of motor performance and motor learning in the primary motor cortex of monkeys adapting to an external force field. Neuron 2001;30:593–607.
Musallam S, Corneil BD, Greger B, Scherberger H, Andersen RA. Cognitive control signals for neural prosthetics. Science 2004;305:258–62.
Olson BP, Si J, Hu J, He JP. Closed-loop cortical control of direction using support vector machines. IEEE Transactions on Neural Systems and Rehabilitation Engineering 2005;13:72–80.
Scott SH. Apparatus for measuring and perturbing shoulder and elbow joint positions and torques during reaching. Journal of Neuroscience Methods 1999;89:119–27.

Serruya MD, Hatsopoulos NG, Paninski L, Fellows MR, Donoghue JP. Instant neural control of a movement signal. Nature 2002;416:141–2.

Shadmehr R, Mussa-Ivaldi FA. Adaptive representation of dynamics during learning of a motor task. Journal of Neuroscience 1994;14:3208.

Slutzky MW, Jordan LR, Miller LE. Evaluation of epidural and intracortical field potentials as control inputs for brain–machine interfaces. In: Society for Neuroscience Annual Meeting; 2009.

Taylor DM, Tillery SI, Schwartz AB. Direct cortical control of 3D neuroprosthetic devices. Science 2002;296:1829–32.

Vato A, Mussa-Ivaldi FA. Cortical stimulation for artificial proprioception: a preliminary study in behaving rats. San Diego, CA: Society for Neuroscience Annual Meeting; 2007.

Washburn DA, Rulon MJ, Gulledge JP. A new breed of computer users: rats control a cursor via joystick manipulation. Behavior Research Methods 2004;36:173–9.

Wessberg J, Stambaugh CR, Kralik JD, Beck PD, Laubach M, Chapin JK, et al. Real-time prediction of hand trajectory by ensembles of cortical neurons in primates. Nature 2000;408:361–5.

Whishaw IQ, Gorny B, Foroud A, Kleim JA. Long–Evans and Sprague–Dawley rats have similar skilled reaching success and limb representations in motor cortex but different movements: some cautionary insights into the selection of rat strains for neurobiological motor research. Behavioural Brain Research 2003;145:221–32.