Using a classic paper by Evarts as a platform for discussing cortical control of skeletal muscle in awake, behaving primates

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Cecala AL. Using a classic paper by Evarts as a platform for discussing cortical control of skeletal muscle in awake, behaving primates. Adv Physiol Educ 36: 246–250, 2012; doi:10.1152/advan.00103.2012.—A growing portion of premed curricula is being devoted to the study of physiological mechanisms underlying animal behavior. In the present article, I describe an activity centered around a classic Journal of Neurophysiology paper by Edward V. Evarts that lays the foundation for students to investigate common behavioral and physiological techniques used to study motor control in primates. Students will leave this activity being able to 1) critically assess behavioral, electromyographic, and single unit (extracellular) neurophysiological data typically acquired by behavioral neurophysiologists; 2) provide physiological evidence that the primate precentral gyrus (primary motor cortex) controls voluntary movements of the wrist; 3) intelligently discuss hypotheses concerning the role of the primary motor cortex in the generation of movement in mammalian species; and 4) discuss the ethical implications of using mammalian species as model organisms. The skills and background knowledge gained in this activity lay the platform for advanced study of scientific investigations into sensory, motor, and cognitive processes in undergraduate, graduate, or medical school curricula.

neuroscience; cortex; neurophysiology; electromyography

FOR MUCH OF OUR RECORDED HISTORY, humans have been interested in the behavior of their conspecifics as well as other animal species. Philosophers, physicians, psychologists, economists, and physiologists have all contributed to the development and testing of hypotheses concerning the behavior of animals. Evarts (5) provides a challenging, yet tangible, introduction to common behavioral, physiological, and analytic techniques used by neuroscientists to study the neural substrate underlying sensory, motor, and cognitive processes in primates. Furthermore, the timing of this particular report affords the opportunity to discuss 1) the historical and current controversies in the subdiscipline of neuroscience that focuses on the neural control of movement; 2) the contribution of the cerebral cortex, a well-developed structure in the primate brain, to behavior; and 3) the ethical use of animals in physiological research. I will briefly introduce each of these issues before discussing the target audience and lesson plan I have developed for this article.

Cortical Contributions to Primate Motor Control: a Historical Perspective

It is now taken for granted that the cerebral cortex is the extensive outer layer of gray matter (neurons) of the cerebral hemispheres, which is largely responsible for higher brain functions, including sensation, voluntary muscle movement, thought, reasoning, and memory. Modern scientific discussions of the cerebral cortex’s contribution to mammalian motor control began in the late 19th century when two German scientists, Eduard Fritsch and Gustav Theodor Hitzig, showed that movements of distinct body segments could be evoked via direct electrical stimulation of the anterior regions of the canine cerebral cortex (8). For example, stimulation of a distinct anterior region of the cerebral cortex might evoke movements of the hindleg, whereas stimulation of another, nonoverlapping region might evoke movements of the face. In support of these findings, Fritsch and Hitzig also showed that an incomplete lesion of the right cortical forelimb region resulted in uncoordinated left forelimb movements when their subjects were walking or running. From these observations, Fritsch and Hitzig concluded that the anterior half of the dog cortex “stands in immediate connection to muscular movements” and that these cortical centers were “a middleman…in which similar but better coordination of muscle movement takes place than in the gray substance of the spinal cord or brain stem” (8, 10). In other words, they believed that the anterior cerebral cortex’s functional role was to command specific movements (e.g., reach for a coffee cup) without providing commands to directly control functional properties of individual muscles; the latter was posited to be a function of the circuitry in the brain stem and spinal cord. Their observations led to a hypothesis that predicted that cellular activity in the motor regions of the anterior cerebral cortex represents specific movements of a limb but not the dynamic properties of the muscle (e.g., force and rate of change in force).

Cortical surface mapping experiments using direct electrical stimulation and lesion studies dominated investigations of the cerebral cortex from 1870 until the early 1950s. Throughout this period of time, Fritsch and Hitzig’s basic observations were replicated in guinea pigs, cats, rabbits, rhesus monkeys, chimpanzees, and humans (for a review, see Ref. 10). However, interpretations of these experimental results were not always in line with those of Fritsch and Hitzig. For example, Beevor and Horsley (3) put forth the hypothesis that the output of the motor cortex directly controlled the excitability of specific muscles, which resulted in the movements they observed. This hypothesis predicted that cellular activity in the anterior cerebral cortex would represent the dynamic properties of specific muscles and that it is the coordination of several “direct output lines” from the primary motor cortex that results in the smooth movements that animals make to interact with and navigate in their environment. It would be almost 60 yr before technology would be available for Edward Evarts to test these alternative hypotheses in awake, behaving primates.

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Electrophysiological Techniques Used to Study the Neural Control of Movement

By the middle of the 20th century, electrophysiological techniques had been developed to record both extracellular electrical potentials generated by neurons and muscle (2, 21). Electromyography (EMG) is an electrical recording of muscle activity that aids in the diagnosis of neuromuscular disease. In the undergraduate laboratory environment and most medical facilities, surface EMGs are used to get a gross approximation of muscle activity during behavior. Piper (21) was the first person to use metal surface electrodes to detect human muscle activity, and his technique, aside from the common addition of an electrolyte gel to enhance skin conductivity, has differed little over the past century (2). Surface electrodes were a wonderful addition to clinical and teaching laboratories because they were noninvasive, cheap, and required little training to use. However, it was quickly surmised that surface recordings only acquired electrical activity from large, superficial muscles and, depending on electrode size, might detect electrical activity from multiple muscles. These limitations led Adrian and Bronk (1) to develop needle electrodes that could be inserted directly into the belly of a single muscle and could be used to record electrical potentials from muscles and/or motor neurons. The latter technique is often used in scientific studies that investigate correlations between cortical activity and muscle activity (10, 22) and in the clinic to dissociate nerve from muscle contributions to limb weakness or paralysis (24). However, it should be noted that Evarts (5) used surface electrodes to get a first approximation of the relationship between activity in the primary motor cortex and the muscles, and this is one of the limitations that students should discuss in their assignment (see Sample Questions for Discovery Learning below).

Edgar Adrian and his students were also some of the first to classify the all-or-none characteristic of neuronal action potentials in the sensory and motor systems of anesthetized animals (21). Anesthetized animals were the first subjects used to correlate action potentials from single neurons (“single units”) in the cerebral cortex with phenomena outside of the nervous system. Vernon Mountcastle, David Hubel, and Torsten Wiesel were able to record extracellular action potentials from the posterior regions of the cat cerebral cortex while their subjects were presented either somatosensory (18) or visual stimuli (13). These authors were able to demonstrate changes in neural activity (e.g., number and rate of action potential production) in direct response to changes in stimulus properties (e.g., direction of visual object motion across the subject’s visual field). Hubel and Wiesel (14) were able to replicate and extend their observations in anesthetized primates and shared the 1981 Nobel Prize in Physiology and Medicine for their efforts. It was around this time that Edward Evarts began to develop a method for recording single-neuron activity from unanesthetized monkeys (6, 7), performing operantly conditioned movements that would allow him to address the hypotheses born out of the cortical mapping studies of the 19th century (3, 8).

Teaching Points

The following teaching points illustrate some of the important physiological principles that can be explored using this classic paper:

1. Data collected from rhesus macaques producing wrist movements provided support for a hypothesis that states that neuronal activity within the primary motor cortex (precentral gyrus) is related to the dynamics of individual muscles.

   A. Direction-specific increases in single unit activity were observed when there was no load applied to the hand during flexion-extension movements of the wrist. A particular single unit increased its activity for either a flexion or extension movement and was either reduced or quiescent during movement of the opposite direction (Fig. 6 from Ref. 5). This observation alone is consistent with both the “muscle dynamics” and “displacement” hypotheses.

   B. Regardless of movement direction, the absolute firing rate for a given single unit was variable but predictable given the direction, magnitude, and rate of change of force opposing wrist movement (see Figs. 7, 9, and 11 and Table 2 from Ref. 5). This observation is consistent only with the muscle dynamics hypothesis.

2. Although this report addresses the function of a specific region of the cerebral cortex using a specific set of techniques (EMG and single unit neurophysiology) during a specific behavior (wrist flexion/extension), it provides insights into a potential general mechanism by which the cerebral cortex could control skeletal muscles throughout the body. A great deal of classic neurophysiology has taken a view that there are discrete control modules throughout the cerebral cortex, and perhaps the brain as a whole, which perform specific computations. A discussion of this philosophy, its use in determining the regionalization of brain function, and its implications for the diagnosis of neurological diseases that affect the initiation, accuracy, and precision of movements is warranted.

3. Breakthroughs in scientific research are often the result of the integration of technical or theoretical knowledge from a number of academic fields. To address the aforementioned scientific hypotheses, Evarts was required to engineer an apparatus that would record and reward a stereotyped behavior and be conducive to electrophysiological recordings. A discussion of the technical limitations and breakthroughs in mid-20th century would interest some students (see Ref. 21 for details) and be a platform for discussing the limitations of modern studies using similar techniques. This discussion will undoubtedly include in-depth discussions of the basic cellular mechanisms underlying action potentials in neurons and muscles, mechanisms that students of physiology should be able to recite at a moment’s notice.

Seminar Characteristics and Assignments

Before reading Evarts’ paper (5), a student should have knowledge of the cellular mechanisms underlying the generation of an action potential, the technology used to record extracellular action potentials, and the general pathways connecting the motor cortices with muscles. These topics are typically covered in physiology, neuroscience, or other seminar courses involving animal behavior, which are the target audience for the use of this particular activity. However, as a safeguard, students will review pertinent material in these topics as part of a writing assignment to be completed before an in-class discussion of the classic paper. The primary goals of the written assignment are to 1) make sure students have thoroughly read the paper and have a basic understanding of
Resistance could be applied to movements of one direction or between 400 and 700 ms in duration, between the two “stoppers.” The subject was rewarded with a droplet of juice for restricting rotation of the forearm when the manipulandum was grasped. The subject inserted one of their arms into a tube (manipulandum) attached to the monkey’s home cage as it would have been found during the subject’s initial training. The implementation of this system was important because 1) previous experiments with awake primates (e.g., Ref. 4) did not attempt to place restrictions on the animal’s behavior and 2) it allowed the author to attribute changes in neural activity to movement direction or changes in muscle dynamics (changes in EMG activity → changes in force production). The remainder of the classic paper’s METHODS describes the techniques used to gain access to the neural vault and restrain the head during recording sessions and the type of electrodes used during neural recording.

Figure 2 of the present article shows typical raw neural and potentiometer traces obtained during flexion and extension movements when no load was opposing the movement in either direction. In this case, the neuron produces no action potentials when the subject extends the wrist (potentiometer deflecting toward its lowest point) but becomes active when the subject is holding the wrist stable in the extended position and flexing the wrist. From these data alone, it cannot be determined whether “the activity is related to the flexion displacement per se or to the slight flexor force associated with [the] displacement.”

Figure 3 of the present article shows raw neural and potentiometer records during trials with 400-g opposing flexion (top), no load (middle), and 400-g opposing extension (bottom). It is clear from these records that at least some units located in the precentral gyrus (primary motor cortex) have discharge frequencies related to the force generated by a particular set of muscles that gives rise to movement about the wrist joint, not specifically the direction of displacement. This is particularly evident when contrasting flexion movements occurring in the bottom set of traces of Fig. 3, in which the unit is virtually silent, with the top set of traces in Fig. 3, in which the unit fires at a high rate. Evarts went on to show that the majority of neurons he recorded from the precentral gyrus had discharge frequencies related to force and rate of change of force (see Figs. 8 and 9 in Ref. 5) and was “only secondarily related to the direction of displacement.” These results are consistent with the view that the neurons in the precentral gyrus are essentially muscle controllers.

Animal Ethics

As many readers of the present article will confess, with the exception of a few paragraphs in a course syllabus or at the beginning of a prelaboratory handout, little time is allotted to the discussion of animal ethics in an introductory physiology course. Should animals be used in biomedical research? If
animals are used for research, should there be restrictions on which or when specific species can be used? Can students name examples of how they, or their family members, have benefited from discoveries made using animals in scientific research? The assignment below includes a single question meant to prompt students to discuss these questions and many more. Also, I have provided a brief, by no means exhaustive, list of references for both students and faculty members since an in-depth discussion of the philosophical arguments for and against the use of animals in biomedical research is beyond the scope of this article (12, 17, 19, 23). Instructors can decide if they want to spend class time discussing the various ethical frameworks (e.g., deontology, utilitarianism, etc.), provide a brief handout or supplementary reading describing these frameworks for use during discussion or the written assignment, or simply let students discuss their feelings on the subject of animal use in research without allusion to formal ethical theories. Articulate answers to ethical questions may be difficult for students to generate early in their career, but they need practice making decisions using an ethical framework if they are seriously considering pursuing a career in research or medicine, where ethical dilemmas abound.

Student Learning Outcomes

After completing this activity, students will be able to:

1. Critically assess behavioral, EMG, and single unit (extracellular) neurophysiological data typically acquired by behavioral neurophysiologists.

2. Provide physiological evidence that the primate precentral gyrus (primary motor cortex) controls voluntary movements of the wrist.

3. Intelligently discuss hypotheses concerning the role of the primary motor cortex in the generation of movement in mammalian species.

4. Discuss the ethical implications of using mammalian species as a model organisms.

Sample Questions for Discovery Learning

The following questions should be completed before students attend the discussion period.

Question 1. Based on your knowledge of neuro- and muscular anatomy, describe the descending pathway(s) whereby the primary motor cortex could produce voluntary flexion and extension of the wrist.

Question 2. Evarts briefly described two prevailing hypotheses concerning primary motor cortex function dominating the field of neurobiology in the 1960s. Describe these hypotheses and the behavioral task that Evarts used to dissociate these alternatives (see Fig. 1 of the present article).

Question 3. Describe Evarts’ use of EMG and single unit neuronal recordings in awake, behaving monkeys to test hypotheses concerning the role of the primary motor cortex in primate motor control. These methods are often described as “correlative techniques.” What does this mean? What common neurobiological methods could be considered “causal” techniques?

Question 4. Using the data provided by Evarts, describe, in detail, the evidence for or against each of the following statements:

A. Pyramidal tract neurons (PTNs) may be active while the wrist is either in motion or in a static position.

B. Activity of PTNs is related to the displacement of the hand at the wrist, not the magnitude of the force generated by the movement of the hand at the wrist.

C. The primary motor cortex, via PTNs, may issue a command to change limb position (hint: think about the relative timing of a PTN burst).

D. The size of the load opposing limb motion may influence the relationship (i.e., an increase or a decrease in the firing rate) between cell activity and movement onset.

E. Under the conditions of this study, a cell that modulates its activity during wrist movement is directly influencing muscles of the forearm.

The following questions should be completed before students attend the discussion period:

Question 6. List as many problems with the experimental design, data analysis, or interpretation in Evarts (5) that you can think of.

The following is a potential list of issues to discuss:

1. There was no discussion of EMG electrode placement beyond being positioned in the wrist flexors or extensors. In its purest form, the muscle controller hypothesis states that the output of a particular neuron (i.e., action potential number or rate) should be directly related to the activity of a specific muscle. Later experiments fixed this problem (for a review, see Ref. 10).

2. Movements of the wrist were restricted to the horizontal plane in this experiment, and it was assumed that a neuron correlated with the magnitude of flexion force in one plane wall be correlated to flexion force in all planes. Kakei and col-
leagues (16) challenged this assumption and found that the mapping from cortical neurons to wrist muscles can change if the arm is placed in different configurations.

3. Movements of the wrist (and fingers) are highly specialized in primates. Furthermore, rhesus monkeys and apes have a large number of corticospinal neurons that project directly to motor neurons in the ventral spinal cord that control these body segments compared with other mammals (for reviews, see Refs. 10 and 15). In fact, many fewer corticospinal neurons project to motor neurons that control movements of the upper arm and shoulder (22). Therefore, it would be inappropriate to conclude that Evarts’ results translate to all regions of the primary motor cortex or other regions of the anterior primate cortex involved in motor control.

4. No statistics were used in this study. Would statistics be necessary to support Evarts’ conclusions if this study were being published today? Why or why not?

Question 7. In addition to single unit recording, neurophysiologists have used microstimulation and chemical inactivation to test hypotheses concerning the primary motor cortex’s role in the voluntary and involuntary movement of primate fingers, wrist, forearm at the elbow, and upper arm at the shoulder joint. With your group, design an experiment to test the following hypotheses:

A. The discharge of PTN activity is related to the force exerted by specific muscles of the upper arm (e.g., biceps brachii or triceps brachii) during reaching movements.

B. The discharge of PTN activity is related to a body segment’s movement vector (direction and distance) during reaching movements, not the activity of a specific muscle (e.g., biceps brachii or triceps brachii).

Question 8. Although monkeys had been used in anesthetized and behavioral experiments for a number of years before Evarts’ study, his ability to show that neural activity could be recorded from awake, behaving monkeys assured their place as one of the animals of choice when studying motor control. The ethical treatment of subjects is a topic that must be addressed by any investigator interested in performing experiments in physiology. What are the pros and cons of using mammalian animals to study the nervous system?

Conclusions

The use of awake, behaving primates as a model for studying neural substrates underlying sensory processing, the neural control of movement, and cognition (e.g., decision making, emotional processing, memory formation, and recall) grew significantly after Evarts’ landmark 1968 *Journal of Neurophysiology* publication. However, students reading a recent behavioral neurophysiology study in an American Physiological Association journal or participating in a summer research experience in a primate neurophysiology laboratory studying these processes today would note that the core techniques described in Evarts’ classic paper (operant conditioning, single unit recording, electromyography, and data-analysis techniques used to correlate physiological activity of excitable cells with behavior) remain virtually identical! Therefore, Evarts’ experimental design can be considered a template for modern studies that use a variety of physiological techniques (single-unit or multiunit neural recording, electroencephalography, functional magnetic resonance, etc.) to study the cortical and subcortical contributions to these processes. This makes Evarts (5) a “must read” for any student interested in pursuing advanced study in the biomedical sciences. Indepth study of sensorimotor processing can be achieved by supplementing this paper with more recent primary sources or textbook chapters and with laboratory activities in vertebrate and invertebrate models (e.g., Refs. 9, 11, and 25).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.L.C. conception and design of research; A.L.C. drafted manuscript; A.L.C. edited and revised manuscript; A.L.C. approved final version of manuscript.

REFERENCES


