Advances in Occupational Ergonomics and Safety
A.C. Bittner Jr. et al. (Eds.)
IOS Press, 2001

Animal Models for Controlling and Quantifying Voluntary Muscle Performance of Rats Using Operant Conditioning

Oliver Wirth and Robert G. Cutlip
National Institute for Occupational Safety and Health (NIOSH),
1095 Willowdale Road MS 2027, Morgantown, WV, USA, 26505

Abstract: Two *in vivo* animal models for controlling and quantifying voluntary exertions of the rat upper and lower limbs are described. Using intact rats, operant conditioning with food rewards is used to produce repetitive and uniform patterns of volitional responding that can be maintained in daily sessions conducted up to several weeks duration. In the upper limb model rats are operantly conditioned to press on a force lever that records response force in real time. Response force and the pattern of responding can be controlled by programming force and other response criteria in the contingencies that determine when and how often responses are reinforced. In the hind limb model, rats are operantly conditioned to perform a voluntary lifting task to generate controlled movement of the plantar flexors. The apparatus allows the rat to enter a tube through an opening in the test chamber, insert its neck into a donut-shaped ring assembly, and lift the assembly. A load cell embedded in a platform at the bottom of the tube measures the dynamic force exerted by the plantar flexors. Weights can be placed on pans attached to the ring assembly to vary the load. The range of motion, velocity, and acceleration of the lift can be determined. Both upper- and lower-limb models are computer automated. There are several advantages of these models compared to invasive *in vitro* or *in situ* preparations of isolated muscle fibers or other *in vivo* models such as rodent dynamometry and treadmill running. Biomechanical parameters such as the force, duration, and rate of responding can be precisely controlled by manipulations of the reinforcement contingencies, while leaving the muscle-tendon complex and normal neuromuscular control processes intact. Also, limitations associated with anaesthesia or forced running that are common in other models are eliminated or minimized. When combined with biomechanical, biochemical, and histological analyses, these models can provide comprehensive methods for studying muscle pathomechanics and work-rest cycles that will broaden the scope of musculoskeletal research.

1. Introduction

Musculoskeletal disorders (MSDs) are a major health concern of the National Institute for Occupational Safety Health (NIOSH) and a National Occupational Research Agenda (NORA) priority. Musculoskeletal disorders represent a broad category of injuries and illnesses of the upper and lower extremities. Claims of MSDs in the workplace often are associated with repetitive manual work in business and industry. Known commonly as cumulative trauma disorders (CTDs), or repetitive strain injuries (RSIs), they represent a significant proportion of work-related injury claims. A survey conducted by the Bureau of Labor Statistics in 1994 reported that 705,800 cases of injury or illness resulted from overexertion or repetitive motion in the workplace [1]. Furthermore, the impact associated with MSDs in terms of lost work and lost productivity are enormous. Recent estimates place the cost of MSDs to the national economy at $13 to $20 billion annually [2,3].

It has been argued that quantitative exposure-response relations between physical load factors and MSDs are largely unknown [4] and, therefore, fundamental questions remain about the safe or acceptable limits of repetitive work and, for example, how many repetitions are too much. One problem concerns the difficulty in analyzing the factors associated with muscle injury in highly controlled, laboratory studies. Existing physiological models of muscle pathomechanics and adaptation are often limited by their requirements for invasive procedures or by their focus on muscle actions that are not applicable to real-world settings.

The scientific literature indicates that animal models are appropriate for the study of skeletal muscle, as the micro-architecture of rodent and human skeletal muscle are quite similar. The use of a rat model also can allow for controlled biomechanical exposures, rigorous histological and biochemical analysis of the muscle tissue, and controlled experimental conditions necessary to conduct a parametric investigations of factors associated with chronic contraction-induced injury. The present paper describes the application of operant conditioning to establish controlled voluntary movements in two animal models.

2. Operant conditioning

Operant conditioning is the process by which the frequency or strength of a learned response is modified by its environmental consequences. For example, if a hungry rat is presented with a food pellet immediately after it presses a lever, the probability of the rat pressing the lever again will increase. Appetitive reinforcers such as food or water are most common largely because they are effective across a wide range of species and situations [5].

Rats ordinarily do not come to the experimental situation ready to press a lever. The lever-press first must be acquired usually through training. The rat, however, brings with it a behavioral repertoire that approximates lever pressing (e.g., extending the forepaw or grasping). These approximations can serve as a basis by which lever pressing may be developed. "Shaping" is the process by which successive approximations of a target behavior are differentially reinforced until the target behavior occurs. In this way, shaping can be used to establish a variety of simple or complex forms of behavior (within genetic and biological constraints).

Once acquired, new forms of responding can be maintained for long periods of time through the scheduling of intermittent reinforcement. As in the natural world, reinforcement does not have to occur after every instance of behavior. Reinforcement can be infrequent or intermittent. The scheduling of intermittent reinforcement and its effects on behavior have been studied and described extensively [6]. Reinforcement can be made contingent on various response parameters (e.g., number, rate, and force), temporal features (e.g., duration of each

response or time since last response) and, depending on how and when reinforcers are delivered, characteristic and repeatable patterns of responding may be obtained. For example, under a fixed-ratio (FR) schedule of reinforcement a fixed number of responses are required for each reinforcer. The characteristic pattern of behavior that occurs under an FR schedule can be described as a burst-and-run pattern. Following each reinforcer, the organism pauses briefly before responding once again resumes and, once it does, responding continues without interruption until the next reinforcer is delivered. A fixed–interval (FI) schedule, on the other hand, requires that a specified amount of time elapse before a response is reinforced. The pattern of responding under this schedule is characterized by a gradually accelerating rate of response until reinforcement. Other simple schedules of reinforcement include the variable-ratio (VR) and variable-interval (VI) under which reinforcement is made contingent respectively upon a specified number of responses or after an interval around some average. VR and VI schedules tend to produce high, steady rates of responding with generally somewhat higher rates under the VR schedule.

Schedule control over behavior may be restricted to certain situations. For example, reinforcement can be presented for responding during the presence of a red light only, but it is withheld during the absence of the light regardless of responding. In a situation where the presence and absence of a red light alternates, discriminative control over responding will be established; the organism soon will learn to respond only in the presence of the red light. Discriminative stimuli can be used to precisely control the timing and amount of behavior emitted by an organism, allowing a parametric study of work-rest cycles.

3. Upper limb model

3.1 Apparatus

Figure 1 shows the upper limb apparatus. Rats are studied using standard operant test chambers (Med Associates) located in a separate room adjacent to the home quarters. The test chamber is about the size of the rat's living cage, and is enclosed within a light- and sound-attenuating, ventilated cubicle to minimize distractions. Low-wattage lamps provide light for general room illumination and discriminative stimuli. Auditory stimuli, in the form of "white noise" (a random mixture of frequencies in the audible range), are between 80 and 85 decibels to mask extraneous noise. Food rewards consist of nutritionally complete 45-mg food pellets (Noyes).

A custom-designed lever (Med Associates) serves as the response operandum and allows the continuous, real-time recording of isometric force exerted on the lever [7,8]. The lever consists of a 0.6 cm by 0.6 cm aluminum bar attached to a strain gage to measure the isometric force exerted between 0.1 N and 5.0 N. The bar initially protrudes approximately 1.75 cm into a small opening on the far right side of the front panel. The lever is mounted to a bracket that allows the lever to be retracted manually during training to a maximum distance of 3 cm outside the chamber. The retracted position requires the rat to reach through the small opening and extend its right fore limb to contact the lever. All experimental events, such as the presentation of visual or auditory stimuli, are controlled and monitored by computer throughout the test session.

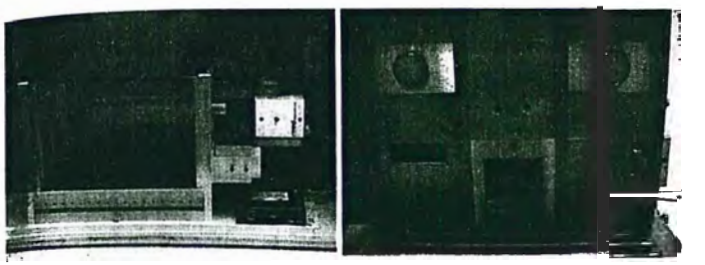

Figure 1. The panel on the left shows a side view of the operant test chamber with the isometric force lever mounted in the foreground; the pellet dispenser appears on the right behind the lever. The panel on the right shows the front panel as seen by the rat. The isometric force lever appears on the far right protruding through a small opening in the panel. A stimulus light is mounted above the lever and a trough through which pellets are delivered appears in the center.

In addition, each chamber is equipped with a mini-video camera for remote surveillance during experimental sessions.

3.2 Procedure

The rat initially is magazine trained until it reliably retrieves food pellets as they are delivered. The rat then is trained to press the lever for food with the lever protruding into the chamber. When the force exerted on the lever reaches a predetermined criterion (0.25 N during training), a food pellet is delivered according to an FR 1 schedule of reinforcement. Under this schedule, one response is requiring for each pellet. The stimulus light is turned on whenever the force lever is active, and turned off when a pellet is delivered.

Once reliable responding is established, training continues as the lever is gradually retracted until the bar is positioned approximately 2.5 cm behind the chamber panel. The final position of the lever requires that the rat reach through the opening to contact the lever (see Figure 2). Placement of the lever against the far right side of the chamber also facilitates the acquisition of an isometric lever press that is isolated to the right fore limb, allowing the contra-lateral limb to be used as a control. Occasionally, it is necessary to bait the lever with a food pellet to encourage reaching through the opening.

Once responses occur reliably, the number of responses required for each pellet is increased gradually up to 15, and then delivered intermittently every 30 s according to a VI schedule. This type of intermittent schedule of reinforcement is used because it produces a rapid and steady rate of responses. Total training takes approximately four days, with sessions lasting one hour on each day.

Further requirements on the topography of the response can be imposed after training. For example, the apparatus and control software allow reinforcement to depend on such factors as the number, rate, force, duration, or temporal pattern of lever presses.

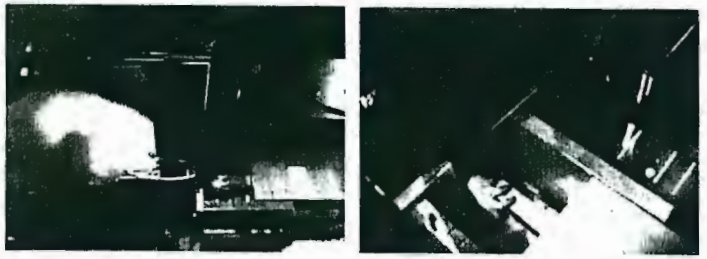

Figure 2. The panel of the **left** shows a rat reaching through the opening on the front panel and pressing the lever, which has been retracted 2.5 cm from the opening. The panel on the **right** shows the fore paw of the rat as it contacts the retracted lever.

4. Lower limb model

4.1 Apparatus

Figure 3 shows the lower limb apparatus. A standard operant chamber is adapted to accommodate a lifting response. An opening in the front panel of the operant chamber allow the rat to enter an acrylic tube that is mounted vertically to restrict the movement of the rat. A load cell is embedded in a platform at the bottom of the tube to measure the dynamic force exerted by the plantar flexors. Inside the tube, a neck ring is supported by a yoke that moves along two vertical shafts via linear bearings. A displacement transducer (LVDT) is attached to the ring assembly to measure the range of motion of the lift, and allow determinations of velocity and acceleration of the lifting motion. An infrared nose-poke response device, positioned near the top of the tube, records each lift response. An LED lamp is positioned above the nose-poke response device. When lit, this lamp serves as a discriminative stimulus to signal when the device is active. The apparatus allows the rat to enter the tube through the opening, insert its neck into the ring, and lift the ring assembly until its nose breaks the infrared beam. Concentric and eccentric muscle contractions of the plantar flexors can be produced by the lifting and lowering the ring. Weights can be placed on pans attached to the ring assembly to vary the load. The entire process is computer automated, and vertical displacement, time during each lift, and dynamic forces exerted during each lift are sampled at 100 Hz via a computer-controlled data acquisition system.

4.2 Procedure

Training begins with magazine training as described above. Initially, access to the vertical tube is blocked off and a nose-poke response device, which is identical to the one mounted above the tube, is positioned inside the chamber on the front panel. After magazine training, nose pokes are trained under a FR 1 schedule of reinforcement. The LED lamp is turned on whenever the nose-poke device is active,.and turned off briefly during pellet delivery. This response is acquired rapidly as the rat has an affinity for poking its nose into small openings. Training continues for several more sessions until nose pokes occur reliably.

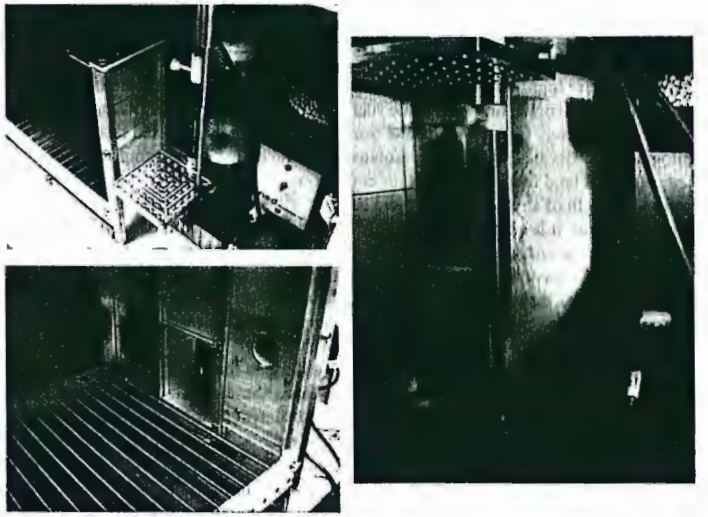

Figure 3. The panel on the **top left** shows details of the lifting apparatus including the ring assembly (inside the vertical tube), vertical support shaft with LVDT positioned near the top, weight pans (foreground), and nose-poke response device (black box above the tube). The panel on the **bottom left** shows the front panel with the pellet trough (left), access opening to the vertical tube (center), and the nose-poke response device with LED lamp used for training (right). The panel on the **right** shows a rat performing the lift.

Thereafter, sessions are conducted in which the nose-poke response device is removed from the front panel, and access to the vertical tube is established. The nose-poke response device that is mounted at the top of the tube is activated and responses reinforced according to an FR 1 schedule (one pellet for each completed lift). Initially, the ring assembly is fixed at its highest position such that the rat enters the tube, stands erect, and is able to make a nose poke response at the top of the tube without having to lift the ring assembly. This procedure takes advantage of the rat's tendency to explore and the transfer of discriminative control to the LED lamp above the nose-poke response device.

After the rat reliably enters the tube, stands erect, and breaks the infrared beam of the nose-poke device, the ring assembly is gradually lowered, each time requiring the rat to lift the ring assembly a greater distance. Eventually, the rat lifts the ring assembly the full vertical distance of the tube. Total training takes approximately seven to ten days, with sessions lasting one hour each.

As with the upper limb model, the schedule of reinforcement can be varied to depend on such factors as the number, rate, temporal pattern, force exerted on the force plate, duration, or displacement of lifts. Weights up to 1 Kg can be place on the ring assembly to increase the load. Furthermore, the ring assembly can be fixed at a vertical distance to generate isometric muscle actions of the plantar flexors. Under this condition, the force exerted by the rat on the force plate can be used to control when and how often reinforcement is delivered.

5. Advantages of the Models

There are several advantages of the proposed models compared to invasive *in vitro* or *in situ* preparations of isolated muscle fibers or *in vivo* models such as rodent dynamometry and treadmill running. Conventional techniques used to study the contractile properties of skeletal muscles are highly invasive [9]. In most cases, the procedures separate muscles or muscle groups from connecting soft tissue such as tendon and bone. For instance, *in vitro* preparations completely isolate the muscle from the animal and conducted tests in a physiologic baths. Because muscles are isolated from the body or other connecting tissue, these preparations lend themselves well for conducting the force-frequency experiments, and to control the length of stretch, velocity, and force and the activation pattern with great precision. However, it is extremely difficult to isolate muscles without damaging other functional structures, and viability limitations of isolated muscles prohibit chronic studies. The present models leave the muscle-tendon complex and normal neuromuscular control processes intact. Less invasive models such as *in vivo* rodent dynamometry [10,11] leave the muscle-tendon complex and neural and vascular supply intact; however, these models still require anaesthesia making chronic study difficult.

Although noninvasive, the present models still allow for control over the movement topography. Biomechanical parameters such as the force, duration, and rate of responding can be precisely controlled by manipulations of the reinforcement contingencies and, within some limits, the rat's behavior will conform to those contingencies. Treadmill exercise models [12] are relatively noninvasive and allow for chronic study; however, they are total body physical activities in which it is difficult to assess the damage to the muscle because of the diversity of actions being performed. Forces exerted by the muscles using these techniques cannot be measured accurately.

Like *in vitro* and *in situ* preparations, *in vivo* models such as dynamometry produce involuntary muscle contractions via electrical stimulation to produce supramaximal contractions of muscles. Stimulation in which all muscle fibers are activated limits direct comparisons to the submaximal contractions of voluntary movements.

Furthermore, because the present models produce voluntary responding through operant contingencies, some nonphysical factors may be investigated experimentally. According to recent reviews [13], there is increasing evidence that nonphysical factors related to work and the work environment play a role in the development of MSDs. These include work organization factors (e.g., workload, repetitiveness, job control, mental demands, etc.), temporal aspects of the work and task (e.g., cycle time and shift work), economic aspects (e.g., pay and benefits), and individual characteristics of individual workers (e.g., personality traits and attitudes). It is thought that interactions among these factors constitute a "stress process" that impacts both job performance and health status.

Not only can schedules of reinforcement produce the kinds of long-term response patterns that simulate repetitive movements found in occupational settings, but the reinforcement conditions themselves may be arranged to simulate occupational situations. For example, rats can be trained to respond repetitively under high-work demands with little payoff by arranging a "lean" schedule of reinforcement in which the amount or rate of behavior required per reinforcement is high. The effects of these kinds of factors on the occurrence of MSDs and associated physiological mechanisms have not yet been examined experimentally.

6. Conclusions

The application of operant conditioning to the development of two animal models expands the armamentarium of techniques that can be used for the study of muscle pathomechanics. The models produce voluntary movements by the animal that more closely approximate the movement topographies encountered in work settings. The training procedures also take advantage of the animal's behavioral repertoire, thereby minimizing training requirements and allows for chronic study of repetitive loadings. The application of these models will lead potentially to a better understanding of the mechanisms that underlie the pathogenesis of MSDs and associated factors and better preventative strategies for minimizing the occurrence of injury in the workplace.

References

1. BLS. (1995). *Workplace injuries and illness in 1994* (USDL 95-508). Washington, DC: U.S. Department of Labor, Bureau of Labor Statistics.
2. NIOSH. (1996). *National occupational research agenda* (DHHS (NIOSH) No. 96-115). Washington, DC: National Institute for Occupational Safety and Health.
3. AFL-CIO. (1997). *Stop the pain.* Washington, DC: AFL-CIO.
4. Viikari-Juntura, E. (1997). The scientific basis for making guidelines and standards to prevent work-related musculoskeletal disorders. *Ergonomics, 40*, 1097-1117.
5. Gleeson, S. (1991). Response acquisition. In I. Iversen & K. Lattal (Eds.), *Experimental Analysis of Behavior, Part I* (Vol. 6, pp. 63-86). Amsterdam: Elsevier.
6. Ferster, C. B., & Skinner, B. F. (1957). *Schedules of Reinforcement*. New York: Appleton-Century-Crofts.
7. Fowler, S.C. (1974). A microcomputer system for recording the dynamic properties of individual operant responses. *Behavior Research Methods & Instrumentation*, 6, 288-292.
8. Notterman, J. M., & Mintz, D. E. (1965). *Dynamics of Response*. New York: Wiley.
9. Warren, G. L, D. A. Lowe and R. B. Armstrong. (1999). Measurements of functional muscle injury: Measurement tools used in the study of eccentric contraction – induced injury. *Sports Med.* 27, 44-59.
10. Ashton–Miller, J.A, Youda, He, V. A. Kadhiresan, D. A. McCubbery, and J. A. Faulkner. (1991). In vivo measurement of mouse ankle properties: An apparatus to measure in vivo biomechanical behavior of dorsi – and plantar flexors of the mouse ankle. Biophysics, 59.
11. Cutlip, R. G, W. T. Stauber, R. H. Willison, T. A. McIntosh and K. H. Means. (1997). Dynamometer for rat plantar flexor muscles *in vivo*. Communication. *Med. Biol. Eng. Comput.*, 35, 540-543.
12. Gregor, R. J, R. R. Roy, W. C. Whiting, R. G. Lovely, J. A. Hodgson and V. R. Edgerton. (1988). Mechanical output of the cat soleus during treadmill locomotion: In vivo vs in situ. *J. Biomechanics*, 21(9), 721-723.
13. Bernard, B. P. (1997). *Musculoskeletal disorders and workplace factors: A critical review of epidemiological evidence for work-related musculoskeletal disorders of the neck, upper extremity, and low back*. Cincinnati, OH: U.S. Department of Health and Human Services.

Advances in Occupational Ergonomics and Safety

Edited by

Alvah C. Bittner Jr.
Battelle Seattle Research Center, Seattle, WA, USA

Paul C. Champney
Paul Champney Consulting, Prosser, WA, USA

and

Stephen J. Morrissey
Oregon OSHA, Portland, OR, USA

IOS Press
Ohmsha

Amsterdam • Berlin • Oxford • Tokyo • Washington, DC

Advances in Occupational Ergonomics and Safety

Volume 4

Previously published in this series:

Volume 1. Advances in Occupational Ergonomics and Safety, B. Das and W. Karwowski (Eds.)
Volume 2. Advances in Occupational Ergonomics and Safety, S. Kumar (Ed.)
Volume 3. Advances in Occupational Ergonomics and Safety, G.C.H. Lee (Ed.)

ISSN: 1384-2269