INSTRUMENTATION & TECHNIQUES

An apparatus for behavioral and physiological study of aversive conditioning in cats and kittens

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An apparatus for aversive conditioning of adult cats and kittens is described. Animals are held by a cloth harness and head restraint device in a metal frame with a movable treadmill floor. They are supported in a natural standing position, with freedom to flex and move each leg and to walk or run on the treadmill. Various transducers record respiration, EKG, vocalization, leg flexion, and locomotion on the treadmill.

Several methods of restraining cats for neurobehavioral study have been described. Some investigators have enclosed cats in boxes, with or without holes for the limbs (Hobson, 1972; Humphreys, Menzies, and Hughes, 1974; Patterson, Olah, and Clement, 1977; Wolfe, 1969). Others have suspended the cats in a net or hammock (Adams, 1963; Brunner, 1969; Connor, Rossi, and Baker, 1966; Wickens, 1973) or used a frame and body harness (Rudy, 1974), or a molded plaster cast (O'Brien and Packham, 1973). In a review of these and other cat restraint techniques, Steinmetz, Romano, and Patterson (1980) noted that almost all were unsatisfactory in some way, either requiring prolonged adaptation, holding the cats in an unnatural posture, or excessively restricting movement. Recently, Romano, Steinmetz, and Patterson (1980) described a method which is easy to use, avoids surgery, and resolves many of the problems of earlier methods, but still restricts the cat's freedom of movement. There is no published report of a cat restraint method that allows leg flexion, walking, and running, maintains the animal in a natural position, and allows the recording of physiological responses. It should be noted that while restraint is often necessary to assure artifact-free recording of physiological data, excessive restraint leads to behavioral inhibition, especially during aversive conditioning (Martin and Fitzgerald, 1980). The study of aversively motivated behavior and its physiological correlates must be done in a situation that provides the subjects with as much freedom to move as possible, particularly, the freedom to ambulate.

In this report we describe a behavioral stand and methods of recording that provide considerable freedom for behavior, and permit the recording of neurophysiological, physiological, and behavioral data for the pur-

pose of a developmental study of classically conditioned leg flexion in cats. One large and one small stand permit kittens as young as 3 or 4 days of age to be treated the same as adult cats. Animals are positioned in the metal stand by means of a cloth harness and a cranial implant. The floor of the stand consists of a freely movable canvas belt, which allows the cat to walk or run while restrained. Various transducers and pickup leads record leg movements, locomotion, vocalization, heart rate, respiratory rate and amplitude. The system is stable enough for single nerve cell activity to be recorded during movements such as leg flexion or walking on the treadmill.

THE BEHAVIORAL STAND

The behavioral stand consists of a metal frame with a movable canvas treadmill floor, and a mechanism for positioning the animal's head in the frame. The animal's head is positioned by means of two rods rigidly mounted to the frame and passed through the sleeves in the implant; its body is supported and positioned in the center of the frame by a canvas harness. The larger stand, shown in Figure 1, accommodates both juvenile and adult cats, although harnesses of different sizes are needed to accommodate variations across the age range. Animals as young as ten weeks old can also be trained in this stand with the use of a smaller, lighter treadmill assembly, which when placed on the surface of the larger treadmill allows the younger animals to run more easily. The smaller stand is suitable for all kittens younger than 10 weeks of age. The important dimensions of the two stands are given in Table 1.

There are several important differences between the two stands. Naturally, the dimensions of each must match the leg length and body size of the animals for which they are designed. The treadmill on the smaller stand must be easy to turn (the treadmill assembly on

The authors wish to express their gratitude to Thomas Nicholas and W. Jeffrey Wilson for proofreading and suggestions. This research was supported by NIH Grant HD05958.

Figure 1. Side view of the larger stand.

the smaller stand is made of light plastic), while on the larger stand it must be fairly strong. Finally, the head restraint system must be very strong for the adult cats, particularly if single nerve cell recording is planned.

The Treadmill Assembly

On both stands, the treadmill assembly is an independent unit that can be positioned on the frame at any level of inclination. It consists of a pair of side rails with a roller mounted at each end, and a sturdy canvas

belt that fits over both rollers, providing a movable floor that supports the animal. The axle for one of the rollers moves forward and backward along the side rails so that the tension of the treadbelt can be adjusted. A polished aluminum plate, fixed between the side rails and positioned just beneath the belt, adds support for the animal and, if sufficiently smooth, hinders the movement of the belt very little. Thin, stiff plastic spokes sewn into the treadbelt at intervals of about 10 cm prevent it from sliding sideways or folding over itself.

Table 1
Dimensions (in Centimeters) of a Large and Small Stand

	Frame			Treadmill		
	L	H	W	L	H	W
Small Stand	35	25	18	30	5	15
Large Stand	75	40	38	70	5	31
Auxiliary Treadmill*				50	10	22

Note—L = length, H = height, and W = width.

*Note that the auxiliary treadmill, which is used with a large stand to accommodate small cats, has increased height; therefore, the smaller subjects with shorter legs are raised to the level of the headholder designed for larger cats.

Head Restraint

Holding a kitten's head in the smaller frame is not difficult, because kittens are relatively weak. Any kind of mechanical linkage that mounts on the frame, holds a positioning rod, and allows its adjustment with respect to the frame is adequate for the kittens. However, holding the head of an adult cat is much more difficult because of its strength. Figure 2 illustrates our method of restraining the head of adult cats, in which the main stabilizing force is provided by a thick metal yoke that bolts firmly to the frame.

Figure 2. Apparatus for head restraint. The positioning rods (R) are made in two parts so that one part inserts through a sleeve in the cranial implant and the other part screws onto the shaft of the first, clamping firmly onto the sleeve. The rods are made of drill steel for stiffness. The position of the front rod in the frame can be adjusted as needed by the clamps shown in the photograph. The rear rod is mounted on a thick metal yoke (Y) by a bracket (B) that slides up and down on the yoke in grooves.

The Harness

The harness is a particularly important part of the apparatus. It must not restrict the animal's movement too much or be undesirably tight, and yet must be inescapable. We use a light canvas suit with holes for the legs that fits snugly around the body and neck and is held closed with Velcro fasteners. Two additional flaps, each the length of the harness, are sewn along the lower margins to serve to attach it to rods installed for that purpose on the frame (Figure 1 shows the rods). Each harness accommodates cats of a fairly restricted size range, so we have found it necessary to make harnesses of different sizes.

SURGERY: THE CRANIAL IMPLANT AND ABDOMINAL ELECTRODE

A cranial implant made of acrylic plastic serves several important functions in the restraint system. Metal sleeves contained within the implant allow the animal's head to be positioned in the stand by means of positioning rods attached to the frame. Implanted pickup leads are exteriorized on its surface, permitting EEG and EKG to be conveniently recorded. Also, the implant provides a base for the attachment of a probe that records respiration, and allows access to the brain through stainless steel recording wells. The implant is built up around stainless steel cranial anchoring screws while the cat is anesthetized. The surgical procedure is described in detail below after a note about our method of recording the EKG.

The EKG is recorded by means of an implanted lead that passes subcutaneously from the cranial implant to the peritoneal cavity. A fine, flexible, Teflon-insulated,

braided stainless steel wire (adults: Bersen Wire Rope Co., San Diego, Calif., Microline 2.39; kittens: Cooner Sales Co., Chatsworth, Calif., AS 632) must be prepared in advance by removing 2-3 cm of insulation from the end and unbraiding the exposed wire to form a brush. It must then be sterilized. It can be implanted with a minimum of physical trauma by the following procedure. The wire is passed backward through an 18-ga hypodermic needle until about half of the exposed, bushy end emerges; the bare end is folded back against the shaft of the needle. The needle and wire may then be pushed through the abdominal wall into the peritoneal cavity. The wire protruding from the hypodermic needle is forced farther into the cavity with forceps, and the needle is withdrawn. A long, blunt needle (e.g., a Tucky Lumbar Arthrography Needle, 17 ga, 175 mm) is then passed from an opening made in the skin covering the cranium subcutaneously to the point where the wire emerges from the skin covering the abdomen, and forced through the same hole. The EKG pickup lead can then be passed through the long needle to the head, and the long needle withdrawn.

The implant is anchored by stainless steel screws in the calvarium. If the screws are directed toward the center of the cranial cavity the completed implant is firmly anchored by the orientation of their shafts, rather than by their threads. The permanence of the implant depends strongly on this point. When implanted properly, adult cats can be expected to retain their implants and remain healthy for several years.

Very young kittens require special treatment because their frontal and parietal bones are too soft to retain the anchoring screws. The calvarium is covered with a layer of an especially adhesive dental cement (Grip Cement, L. D. Caulk Co., Milford, Delaware) which sticks to the soft cartilaginous plates of neonatal cranium and hardens quickly. The screws can be set in the cement, and become anchored in the calvarium later.

The phono jack (or other mounting device) that supports the probe for recording respiration should be cemented to the cranium about 1.5 cm above the glabella pointing forward but oriented at an angle of 45 deg from the vertical. Grounding the jack to the anchoring screws reduces the artifacts that occur when an animal touches the probe (e.g., by licking). Details of the construction of the probe are discussed in the section on methods of recording.

When the EKG lead, reference and ground connections, and other wires are in place, the final connections to a multipin female connector can be made. For 4-week-old kittens and older animals, a miniature seven-pin connector (Amphenol 126-196) is used. With younger kittens, the smaller Amphenol 39F Reli-tac series is better.

The metal sleeves used for holding the head are added to the implant when all anchoring and other screws are fixed in the calvarium. These are short, hollow stainless steel tubes, beveled on the ends, made so that the

positioning rods fit rigidly against them when the rods are inserted and screwed together. Sizes of sleeves are: 1- to 4-week-old kittens, 15 mm long x 6 mm o.d.; 12-week-old kittens and juveniles, 25 mm x 8 mm; and adults, 45 mm x 8 mm.

METHODS OF RECORDING

Electrocardiogram

The EKG is recorded by means of the lead implanted in the abdominal cavity. In our experience, the EKG is more free of movement artifacts with this method than when an external pickup lead is used. Moreover, prolonged adjustment is often required to get good EKG records with chest or other external electrodes, while this method requires no more than the insertion of a connector into an implanted socket.

We use a conventional differential preamplifier for amplification. The ground input is connected (through the implanted socket) to several of the stainless steel anchoring screws at the front of the cranium, one reference input is connected to several anchoring screws at the back of the cranium, and the second reference input is connected to the implanted abdominal electrode. These ground and reference points can be used for other preamplifiers (e.g., for nerve cell activity and EEG). If the reference on the cranium is rostral rather than caudal, the EKG will be contaminated with EEG spindles during sleep. This is a problem, particularly in kittens. We have found that a larger, bushier expanse of wire in the peritoneal cavity results in a larger, more stable EKG signal.

This method is particularly successful in kittens, with which we generally obtain almost artifact-free recording in every situation. In adults, the EKG is usually obscured by movement artifacts during vigorous behavior like struggling, but is satisfactory during movements like leg flexion.

Respiration

Respiration is monitored by recording changes in air temperature just outside an external naris. These measurements are made with a thermistor (Victory Engineering Co., 51A30) held in the airpath by a thin rod that mounts on the cranium. The thermistor must be extremely small so that the time constant of its temperature response will be low enough for it to follow rapid respirations. Also, it must be minimally connected to the supporting rod, or the mass of rod will serve as a heat source/sink and also impair response time. However, a delicate thermistor so mounted is easily damaged (e.g., by a lick). We found that suspending the thermistor inside a short, narrow piece of brass tubing (Figure 3) solved all of these problems. Anchoring the thermistor probe on the cranium insures that the thermistor will maintain its position outside the external naris even if the animal moves its head in the stand.

Figure 3. Method of mounting the thermistor for recording respiration. Left: a side view of the shield (made of brass tubing: length = 10 mm, o.d. = 7 mm). Right: the thermistor mounted inside the shield. It is supported by its fine connecting leads, which pass through small holes drilled on either side of the shield and are anchored with cement.

This method of recording respiration has the advantages of being simple, noninvasive, sensitive, and uncontaminated by body movements. The raw data accurately identify the time of occurrence of inhalations and exhalations, and thus provide precise information about respiratory rate. The volume of air exchanged determines the size of thermistor resistance changes, and so a measure of volume is also available, but it must be considered a relative measure and of only moderate accuracy. Figure 5 shows a strip-chart record of respiration data taken during a classical tone-foot shock trial in an adult cat.

Hindlimb Position

The position of each hindlimb is measured along two axes, one perpendicular to the plane of the treadbelt floor (up-down), and another parallel to the plane (forward-backward) by a system of light steel rods and precision potentiometers, illustrated in Figure 4. The potentiometers (Bourn 35105-1-203, 20K, 3 turn) act both as hinges for the rods and as transducers, monitoring position changes with corresponding changes in resistance according to the following equations:

$$D = L[\sin(360 \times \text{DELTA}/R)], \quad (1)$$

where D = height of leg from floor of stand, L = length of rod, DELTA = change in resistance due to leg displacement, and R = change in resistance per full turn, which for angles less than 20 deg simplifies to

$$D = L[.1745(360 \times \text{DELTA}/R)] \quad (2)$$

Figure 4. A rear view of the apparatus showing the system of potentiometers and rods used to monitor leg position.

Figure 5. A sample of a record of leg flexion (lower tracing) and respiration (upper tracing). A vocal response is superimposed on the respiration record as a high-frequency signal. Duration of the CS-US interval is 5,000 msec. These data are entered into the computer via a digitizing tablet.

The linearity of the relationship between D and Δ in Equation 2 means that, in the linear range, the resistance of a potentiometer is directly proportional to the leg flexion (or displacement forward or backward from a central position). Thus, the voltage drop across the potentiometer can be amplified and calibrated, providing an easily recorded measure of leg position. A Velcro strap on the end of the up-down rod allows it to be connected easily to the animal's leg. The apparatus is modified slightly for the kittens, in that the rods are shorter and made of plastic to reduce their weight. This system of measuring hindlimb position allows the cats to move their hindlegs freely in all directions, is light enough to permit walking and running, is sensitive enough to detect any limb movement that is visually observable, and provides a quantitatively accurate record of limb position. Figure 5 shows a strip-chart record of flexion of the shocked limb during a classical tone-foot shock trial in an adult cat.

The Velcro strap which attaches to the animal's left leg is also provided with a silver disk electrode through which a foot shock unconditioned stimulus is delivered. The other disk electrode for the 300-msec ac foot shock stimulus is placed on the proximal part of the tail. It is effectively very large, because it is placed over gauze

soaked in electrode paste and wrapped around the shaved portion of the tail. This stimulus elicits vigorous and reliable hindleg flexion in 100% of the subjects of all ages.

Treadmill Movements

The movement of the treadmill is monitored by means of a photodiode mounted on the side rails of the treadbelt assembly and extending about .2 cm into the interior of the roller. A circle of small holes drilled through the wall of the roller allows light from a light source outside the roller (between the upper and lower parts of the belt) to strike the photodiode as each hole passes between the light source and the diode. A Schmitt trigger converts the photodiode's signal to a +5 V TTL logic pulse that can be recorded or used to activate control circuits.

Training Procedure and Data Processing

Although this apparatus does not exclude appetitive reinforcers and operant learning procedures, it has been used on nearly one hundred cats and kittens trained with the procedure of aversive classical conditioning. The training generally is easy, requires only 5 days for the acquisition of conditioned responses, and can be continued almost indefinitely, if necessary. The results of our studies on the development of learning abilities in cats and on the blocking of extinction are published elsewhere (Soltysik & Wolfe, 1980; Soltysik, Wolfe, Garcia, & Nicholas, Note 1). The interested reader is referred to these publications for more details of the procedures and data processing available with this apparatus. Figure 6 illustrates the computer display of data from four sessions in one cat after the acquisition training; The format of the graph allows immediate visual evaluation of several response measures averaged from a single session or several sessions. The frequency of intertrial responding is negligible, perhaps because no unsignaled shocks are used and the intertrial interval is randomly varied.

DISCUSSION

This apparatus is very easy to use. With the rare exception of an especially aggressive animal, animals can be put into the harness without difficulty by one person. Most animals require only one or two sessions of adaptation, and then stand quietly on the treadmill. Normally, cats accept sessions of 1-2 h with little or no struggling and daily sessions of this length can be continued for weeks or months with no increase in struggling or evidence of trauma. Sessions can be extended to 3- or 4-h duration for a procedure like unit recording, but we do not schedule such lengthy sessions on a daily basis.

The method combines many techniques used by others. Benevento and Kandel (1967) and Romano et al. (1980) recorded leg flexion with a level-potentiometer

Figure 6. A computer display of several averaged response measures. Data from 40 postacquisition trials are shown for one cat. The conditioning consisted of pairing a 5-sec air puff directed to the sacral region (CS) with a 300-msec ac shock of 60 Hz and 3.0 mA, delivered to the left foot and tail (US). The top curve is an averaged heart rate sampled at .25-sec intervals. The acceleratory change during the CS is highly significant. The 95% confidence limits for the averaged values are also displayed for this curve, but they are omitted, for clarity, on the remaining ones. The second curve shows the leg flexion; it can be scored in absolute units (e.g., centimeters) or as a percentage of the response amplitude at the shock. The third curve is a change in the amplitude of respiration, expressed as a percentage of the average amplitude of respiration during the 10-sec period just preceding the onset of the CS. Upward deflection means a reduction in the amplitude, which is typical anticipatory response to an aversive CS. The last two curves show changes in the rate of respiration (sampled at .5-sec intervals) and vocalization, expressed as a percentage of occurrence.

combination, but our implementation provides much greater freedom for leg movement and provides a two-axis record of leg position. Our method of recording the EKG was modified from the technique of Sonnenschein (Note 2). The use of a thermistor to record respiration was adapted from McGinty (Note 3).

When this apparatus is compared with that recently described by Romano et al. (1980), one finds that each has advantages. The method of Romano et al. is very convenient because no surgery is involved, but it is limited because the overall behavior of the cat inside the body box is somewhat restrained. Moreover, their use of a bite bar precludes food or liquid reinforcement. The apparatus described here has several advantages: (1) The cats are held in a natural position, with the freedom to walk, run, and move their limbs normally. (2) The cranial implant permits physiological and neurophysiological recordings to be conveniently taken. (3) The method has been designed for developmental

studies. The main disadvantage of this method is the need for a cranial implant. A choice between the two methods would be determined by the specific need of the investigator. In a study lasting only a few days, the extra time required for surgery may be impractical; however, the need for physiological data may override time considerations. Perhaps the major elements of our stand could be combined with the bite bar system of head restraint in Romano et al. for yet a third approach to behavioral studies of the cat.

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