ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 697-701

Apparatus for studying behavior and learning in restrained rats

Stefan Soltysik[1], **Richard Jackson**[1] and **Piotr Jeleń**[2]

[1]University of California Los Angeles, Neuropsychiatric Institute, Mental Retardation Research Center, Los Angeles, CA 90024, USA; [2]Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Technical communication

Abstract. A new treadmill/stand apparatus for a rat is described. It will be useful in behavioral experiments when control of the animal's position in the training chamber is required while considerable freedom of movement, including locomotion, is desired. The position and distance relative to sources of stimuli are thus kept constant. The speed and the distance of ambulation are easily monitored. The rats are sufficiently restrained to enable easy recording of various measures such as EKG, EMG or EEG, etc. Classical and instrumental conditioning procedures are easily implemented. An example of the data from acquisition training during Pavlovian conditioning is shown (diaphragm EMG, heart rate (HR), locomotion and vocalization).

Key words: treadmill/stand, rats, learning

In the past decade we used successfully in cats and kittens (Soltysik et al. 1982, 1983, 1984, 1988, Nicholas et al. 1983, Wilson and Soltysik 1985, Dess and Soltysik 1989, 1993) an apparatus that combines features of a Pavlovian stand and a treadmill, allowing the animal to move, ambulate and assume a natural standing position (Wolfe and Soltysik 1981). The relative freedom of movement does not negate the fixed localization of the subject and easy access to its body and brain (for recording and experimental manipulations). Fast and uniform learning, particularly when respiratory rate and amplitude were used as indices of conditioned responses, made this apparatus an attractive alternative to such traditional procedures as e.g., conditioned emotional response (CER), shuttle or Skinner boxes, or total restriction of movement in a plastic tube.

In this paper we describe a smaller and modified version of such an apparatus for use with a different species, the rat. The prototype of the rat treadmill/stand was built by one of the authors (RJ), and then modified, tested and used on laboratory rats in a study on the effects of neuropharmacological drugs on learned aversive responses and their inhibition (Jeleń et al., in preparation).

As in the original apparatus for cats, the rats are relatively free to move and their ambulation on the treadmill is monitored (distance and speed). At the same time they are sufficiently restrained to allow for easy recording from EKG, brain (EEG) and muscle (EMG) electrodes. Unlike the cats, their heads are not rigidly fixed but are left free to move. This latter change was required in our design by the relative weakness of the rat's cranium and the danger of braking off any cranial implants. It is through such implants that the cats' heads are attached to the apparatus frame. One of the convenient aspects of totally immobilizing the head of the cat was the possibility of placing a nasal thermistor in front of the naris for recording the rate and amplitude of respiratory activity. On the other hand, as reported elsewhere (Soltysik et al., in preparation), replacing the nasal thermistor with implanted diaphragmatic electrodes provides an even more precise method for recording respiratory activity which is not dependent on immobilizing the subject's head.

The "treadmill/stand" consists of two parts: the treadbelt mechanism and the Plexiglas frame. The treadbelt portion is constructed on the platform with two cylinders of 3.5 cm external diameter and an interaxial distance of 20 cm (small adjustment of this distance is provided for the purpose of stretching the cloth belt). The width of the belt (= length of the horizontally placed cylinders) is 8.5 cm. The cloth belt rests, between the cylinders, on the plate which provides support for the weight of the animal. The entire belt mechanism is easily disassembled for changing and cleaning the belt. The cylinders turn easily on four ball bearings and in one of the cylinders, a photocell, activated through the holes in the circular sidewall of the cylinder, allows monitoring of the speed and distance of the rat's locomotion.

The other part of the apparatus consists of two side walls, the rear vertical plate with the tail holder and the cover plate, which opens for placing and removing the rat from the treadbelt. The cover plate has a semicircular concave opening in front, providing access to the rat's head. The cover plate is located 7.5 cm above the surface of the treadbelt.

On the interior side of the walls, there are two smaller plates (13 x 5 cm) attached by bolts which are exteriorized on the outer side of the apparatus, so that their distance from the wall can be adjusted to the girth of each individual animal. This prevents rats from turning around. The lower edges of these plates are placed 1 cm above the (treadbelt) floor, allowing rats their natural locomotion with the hands and feet turned slightly to the side and extending laterally from the vertical contour of their bodies. A horizontal transverse metal bar in the rear of the apparatus restricts backward locomotion. It is placed ca 2.5 cm above the belt and the subject's tail is located under it in such a way that the tail base is placed just in front of the bar. The tail itself is taped to the Plexiglas rectangular bar attached longitudinally to the treadbelt assembly. The schematic drawing (Fig. 1) and the photo of the treadbelt/stand with the rat in it (Fig. 2), illustrate the proportions and details of the apparatus.

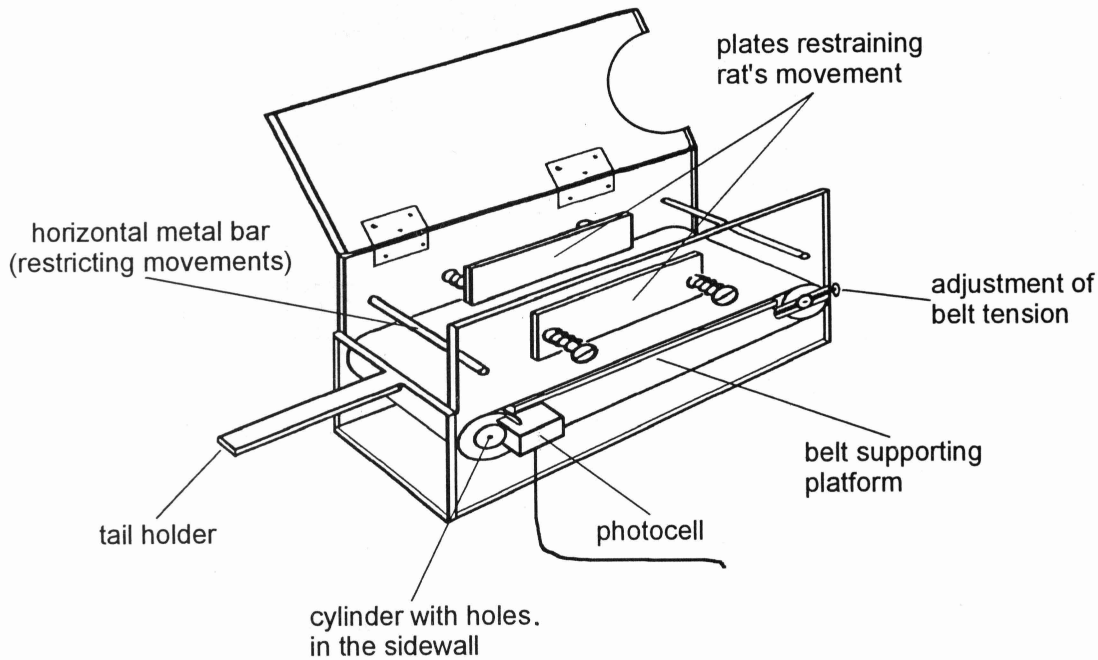

Fig. 1. A scheme of the apparatus.

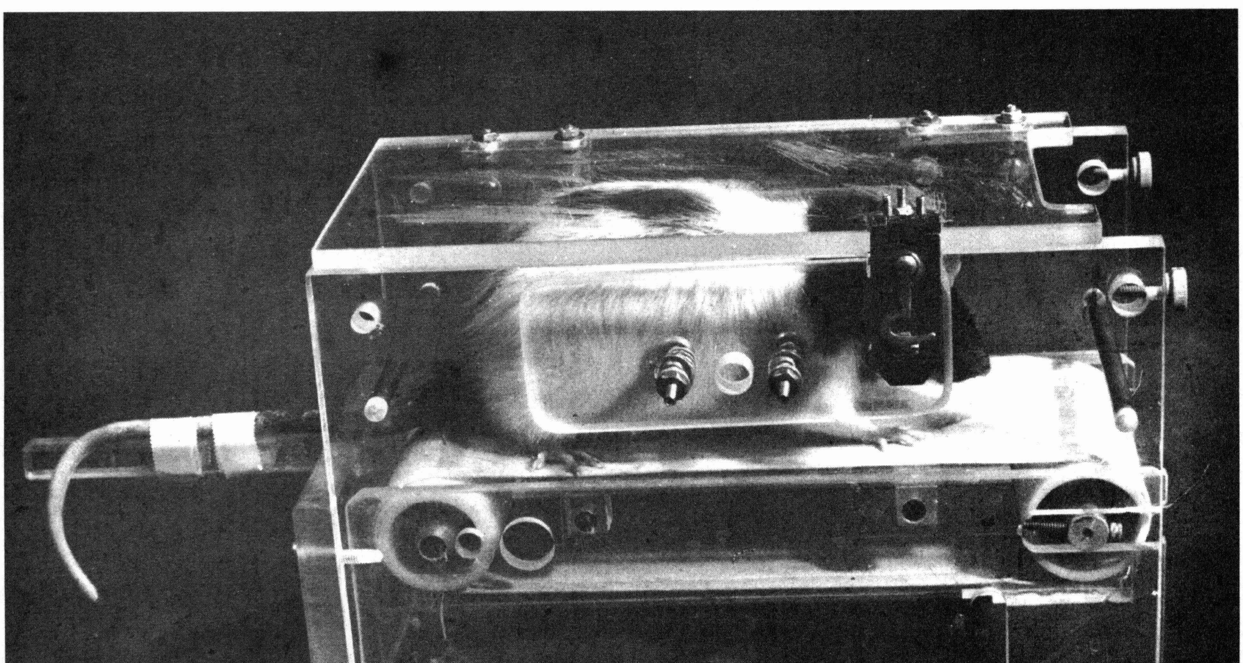

Fig. 2. A photo of the apparatus with a rat inside.

Fig. 3. An example of several responses from one trial of Pavlovian fear conditioning. A, heart beats; B, amplified, rectified and integrated EMG of the rat's diaphragm; C, vocalization of the rat (amplified, rectified and integrated signal from the bat detector); D, signals from the photocell showing the rat's locomotion - the distance between two consecutive pulses denotes time during which the belt moved approximately 2.7 cm (the shorter is the distance the higher is the speed of rat's locomotion); CS, conditioned stimulus - sound (5 s, 500 Hz); US, unconditioned stimulus - electric current (100 ms, 3 mA); t, time - two consecutive vertical bars denote 5 s interval.

Unlike the cats in the original version of the apparatus, there is no need to restrict the movements of the rat's head, and no surgery is required for immobilizing the animals. However, in our subjects some standard and previously described operations were performed in order to record heart rate and respiration. The former was recorded by monitoring the EKG signal from electrodes implanted in the cranium and in the lower part of the body. For monitoring the respiratory activity an operation was performed according to the procedure used and described by Chang and Harper 1989. Briefly, it consisted of placing chronic miniature EMG electrodes (1-2 mm apart) in the diaphragm above the liver and connecting them *via* a soft EMG wire running under the skin to the socket placed on the cranial implant.

Fifteen rats were tested in the apparatus. All animals adapted easily to the conditions of the apparatus and were subjected to a Pavlovian conditioning procedure. After acquisition training in which auditory and visual stimuli were paired with a shock as the unconditioned stimulus, the vocal, heart rate, locomotor and respiratory responses were analyzed and the effects of several anxiolytic and anxiogenic drugs (diazepam, midazolam, pentylenetetrazol) were tested. The exact methods and data processing are provided in other papers (Jeleń et al., Soltysik et al., in preparation). Figure 3 illustrates the record of several types of responses in one trial. In all operated rats the data were artifact-free and usable on every trial during 14 days of initial training and 30 days of pharmacological testing.

We conclude from this series of experiments that our new apparatus provides a useful and convenient method for chronic, physiological and pharmacological studies on adult rats.

Authors wish to thank Dr. Huifang Ni and Dr. Rebecca Harper at UCLA for demonstrating surgical procedure used in this study, and Dr. Tomasz Werka for drawing the scheme of the apparatus. The paper was supported by a statutable grant from the State Committee for Scientific Research to the

Nencki Institute and by the Mental Retardation Research Center at the University of California Los Angeles, which permitted us to use some laboratory equipment from Dr. S. Soltysik's Lab.

Chang F.-C.T., Harper R.M. (1989) A procedure for chronic recording of diaphragmatic electromyographic activity. Brain Res. Bull. 22: 561-563.

Dess N.K., Soltysik S.S. (1989) Ontogeny of conditioned inhibition of conditioned respiratory suppression in kittens. Dev. Psychobiol. 22: 257-269.

Dess N.K., Soltysik S.S. (1993) Associative properties of a conditioned inhibitor as a function of age in kittens. Anim. Learn. Behav. 21: 138-144.

Nicholas T., Wolfe G., Soltysik S.S., Garcia J.L., Wilson W.J., Abraham P. (1983) Postnatal development of heart rate patterns elicited by an aversive CS and US in cats. Pavlov J. Biol. Sci. 18: 144-153.

Soltysik S.S., Dess N.K., Wilson W.J., Matochik J.A., Berg S. (1988) Procedure and reliability of conditioned respiratory suppression. Anim. Learn. Behav. 16: 177-184.

Soltysik S.S., Nicholas T., Wilson W.J. (1984) Postnatal development of respiratory and vocal responses during aversive classical conditioning in cats. Pavlov. J. Biol. Sci. 19: 169-181.

Soltysik S., Wolfe G., Garcia-Sanchez J., Nicholas T. (1982) Infantile and adult heart rate patterns in cats during aversive conditioning. Bull. Psychon. Soc. 19: 51-54.

Soltysik S.S., Wolfe G.E., Nicholas T., Wilson W.J., Garcia-Sanchez J.L. (1983) Blocking of inhibitory conditioning within a serial conditioned stimulus-conditioned inhibitor compound: maintenance of acquired behavior without an unconditioned stimulus. Learn. Motiv. 14: 1-29.

Wilson W.J., Soltysik S.S. (1985) The effects of pharmacological manipulations of nucleus accumbens on classically conditioned responses in cats. Acta Neurobiol. Exp. 45: 91-105.

Wolfe G., Soltysik S. (1981) An apparatus for behavioral and physiological study of aversive conditioning in cats and kittens. Behav. Res. Methods Instrum. 29: 637-642.

Received 28 February 1996, accepted 7 May 1996