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CHRONIC HEAD RESTRAINT PROCEDURES IN THE CAT AND RAT

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INTRODUCTION

There are many studies where it is desirable to perform experiments in the unanesthetized animal. Investigations often mandate that anesthesia not be used due to the confounding effects of the anesthetic agent in various organ systems. With regard to studies involving the nervous system, this is an especially important consideration. In some experiments, the capability of the animal to perform a behavioral task is an essential element. The use of anesthesia would make such a study impossible to carry out. Over the past several years we have developed a head restraint system that has been used in a number of different investigations. Initially, it had been developed for use in the cat (McElligott & Waterhouse, 1977) involving oculo-motor and vestibular studies. This system employs only head restraint and does not require other parts of the body to be restrained or confined. Thus, it allows the cat to participate in a number of limited behavioral investigations where complete freedom of motion is not needed.

CAT HEAD RESTRAINT

The basic procedure involved preparing the cat for attachment of a head block implant onto its skull. This is a standard procedure that has been used in neurophysiological investigations in awake unanesthetized animals (monkeys, cats, rabbits etc.). The particular approach we used was to thread skull screws into the cranium of the animal in order to secure the head block to the animal's head. The block consists of dental cement and hardware that is used to attach the animal's head to the restraining apparatus. This 'hardware1 consists of 4 elongated nuts (i.e., hexagonal standoffs) that are usually used to mount electronic circuit boards. The cat's head is then bolted to a Plexiglas rectangular box that is open at both ends. We have found with such a system it was not necessary to 'bag' or restrain the cat in any other way in order to carry out behavioral and/or chronic single unit electrophysiological recording experiments.

Cats rapidly habituate and come to rest comfortably in such a situation. In some studies this can create another difficulty since cats often fall asleep in the apparatus. This is fine for sleep studies but it presents a definite problem for other behavioral studies. In these cases liquid rewards for behavioral performance or mildly arousing stimuli help to keep the animal attentive.

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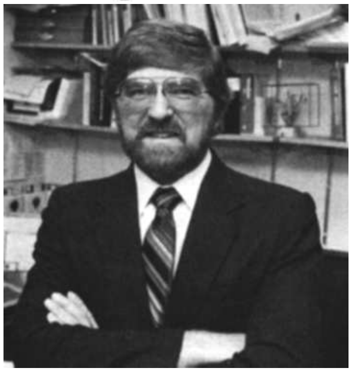

Editor's Column

It hardly seems possible that it is almost the end of summer as I write this column. As many of you will recall, we moved to Kansas City this summer. The move went very smoothly, with almost no hitches or problems. Mother Nature was the biggest problem, with the flooding around the Mississippi River area. We drove to Iowa on July 10 and crossed the river bridge just one hour before it was closed for the rest of the flood. Out here, both the Osteopathic School and our new home were high and dry, but two small towns just three miles from us were almost wiped out. The river showed again how powerful and unforgiving it is. Things are getting fairly well cleaned up now, but a lot of people lost their homes and belongings, and many businesses will not reopen. We are really enjoying our new surroundings.

We feel like tourists, going to festivals, seeing football (we even saw Joe Montana play in the first pre-season Chiefs game) and baseball games, and enjoying KC barbecued ribs. Not long ago, we even saw Peter, Paul and Mary in concert in a lovely outdoor theater. For an aging hippie who imprinted on them in college, it was a truly wonderful experience to finally get to see them in person. Both my wife and I left feeling like there was hope for the world after all.

Please look at the business reply card included in this issue of the Carrier. A new David Kopf Instruments catalog is almost ready, and you can get your copy by peeling off the label and sending the card in. Please correct your address if it is not the right one. Don't miss the opportunity to get this valuable reference to the best stereotaxic instruments available.

Please make a note of our new address. I hope to see you at the Society for Neuroscience Meetings.

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When the cat was adapted to this head restraint apparatus, attempts were made to increase the general comfort of the animal. First, the floor of the Plexiglas box was adjusted to the proper height for each individual cat. This was accomplished by placing boards of different thicknesses on the floor of the box to accommodate cats of varying sizes. Second, in some cases the Plexiglas box was made long enough so that the animal could not grab or hold onto anything solid in order to pry itself loose. An additional advantage of the Plexiglas is that it is slippery and does not provide any surface on which to hold. In a well adapted cat this is less of a problem. Third, it is important not to carry out any procedure that would cause discomfort to the animal in the restraint apparatus. For example, if an antibiotic injection is to be given, this should be done outside of the. restraint and experimental situation. Finally, we have found that adaptation to restraint is facilitated if it was initiated as soon as possible after the surgery. In addition, we have found that feeding or giving the cat milk in the restraint will also help adaptation. In general, anything that will reduce stress to the cat and make it feel at ease in the apparatus will accelerate the period of adaptation.

RAT HEAD RESTRAINT

Recently, we have successfully adapted this restraint procedure for use in the rat in studies involving cardiovascular regulation by the central nervous system (Parry & McElligott, 1993). The object of these studies was to investigate cerebel-lar regulation of blood pressure in the absence of anesthetic agents. In addition, restraint of the animal was necessary to minimize movement and to allow on-line measurement of blood pressure and neurotransmitters using the microdialysis technique. The restraint procedures employed here are similar to that used with the cat and are designed to minimize discomfort and to reduce stress to the rat. Skull and head block preparation are similar to that used previously in the cat. For the rat, these are described in detail in Parry & McElligott (1993).

a) *Initial Surgical Procedures*

After anesthetizing Sprague-Dawley rats, they are placed in a standard rat stereotaxic apparatus (David Kopf, Tujunga, CA) with the upper incisor bar set at 11 mm below the interaural line. A midline incision is made in the skin overlying the skull. The skin is retracted and

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the muscle attached to the dorsolateral and posterior aspects of the skull, is dissected away. Three small holes are then drilled into the lateral aspect of each parietal bone into which stainless steel skull screws (#M80, 1/8 "; Small Parts, Inc., Miami, FL) are placed. It should be noted that the holes are smaller than the screws in order to affix the screws to the skull firmly.

The dorsal and lateral aspects of the skull are scraped clear of blood and debris and dried with 95% ethanol. Two hexagonal standoffs (#4-40 thread x 1/2", Small Parts, Inc., Miami, FL) are glued together using cyanoacrylate (Super Glue; DURO™) and are attached to a metal plate template (5 cm x 10 cm) by a threaded bolt. This metal plate is held in place and positioned by a stereotaxic electrode carrier. The two standoffs are aligned in the rostro-caudal direction above the mid-sagittal suture. They are located at a position to allow access to the brain region of interest. For our studies in the cerebellum, the standoffs are located at the rostral end of the skull. These are firmly attached to the cranium and skull screws by dental acrylic. Scoring a groove in the aluminum standoffs allows them to be held firmly by the dental cement. When the cement dries, the bolt holding the standoff assembly to the horizontal metal plate is removed. The acrylic is then built up over the standoff assembly. The use of two standoffs prevents rotation of the animals head about the vertical axis which can occur if only one standoff is used. A schematic rendition of the head implant is presented in Figure 1.

b) *Catheter for Blood Pressure*

Following the adaptation period (see next section), animals are equipped with a femoral catheter filled with heparinized saline (200 U/ml). The catheter is run subcutaneously to the nape of the neck and cemented to the head block with additional dental acrylic (Figure 1). On the day of the experiment, the femoral catheter is attached to a pressure transducer (Statham Model P23Db) for continuous monitoring mean arterial pressure and heart rate. We have used the blood pressure and heart rate measure as an indicator of the animals level of stress.

c) *Restraint Apparatus*

The restraining apparatus (1 = 55 cm, h = 7 cm, w = 19.5 cm) is a Plexiglas (wall thickness = 0.25 inch) rectangular box open at both ends. A rectangular aluminum rod (1 cm x 0.5 cm x 15 cm), drilled to accommodate two head restrained animal's (inter-head block distance = 6.5 cm), was bolted across an opening (1 = 15 cm; w = 12.5 cm) in the top of the restrainer. This serves as a rigid support to which two rats are held in place. A stereotaxic electrode carrier bar is anchored lengthwise to the Plexiglas restraining apparatus.

d) *Adaptation Procedures*

After one day of recovery from surgery, rats are attached to the rigid bar via their aluminum standoffs. The rats are adapted to this restraint for eight hours per day for three days. During the first hour of adaptation, the restrained animals exhibit overt signs of stress (struggling, back hunching, etc.). However, after the first hour of the initial habituation period, this is reduced considerably. After the third day of habituation, rats show minimal signs of stress.

Initially, at the beginning of these studies, we restrained and adapted a single animal. A rat when restrained alone will struggle more during the initial adaptation period and will take longer to adapt than animals restrained in pairs. We came upon this dual restraint system by chance. Since our study involved the use of a number of rats, we found that it was more efficient to surgically prepare two rats and adapt them for subsequent experimentation in groups of two. We then noted that rats when adapted in pairs exhibit less overt stress and appear to remain more calm. Thus, we decided to restrain the animals in pairs not only during the adaptation but also during the actual experiment. In this situation one animal is used as the experimental animal and the other is used as a companion to reduce stress (Figure 2). We have not subjected this observation to an actual experiment since this was not the object of our studies. However, we have continued to use this pairing procedure throughout our studies and have noted that animals paired in this manner are indeed calm, struggle less, and have normotensive levels of mean arterial pressure and heart rate. However, when two rats are restrained and adapted together, there is considerably less struggling and overt signs of stress during the initial adaptation period.

e) *Concluding Remarks*

Similar to the procedures described for the cat, care is taken to reduce each animal's discomfort by adjusting the height of the floor so that each animal is not in an awkward or uncomfortable position during restraint. In addition, a Plexiglas restraining box is used that provides a slippery surface to minimize the ability of the rats to grab onto some object and use leverage to pry

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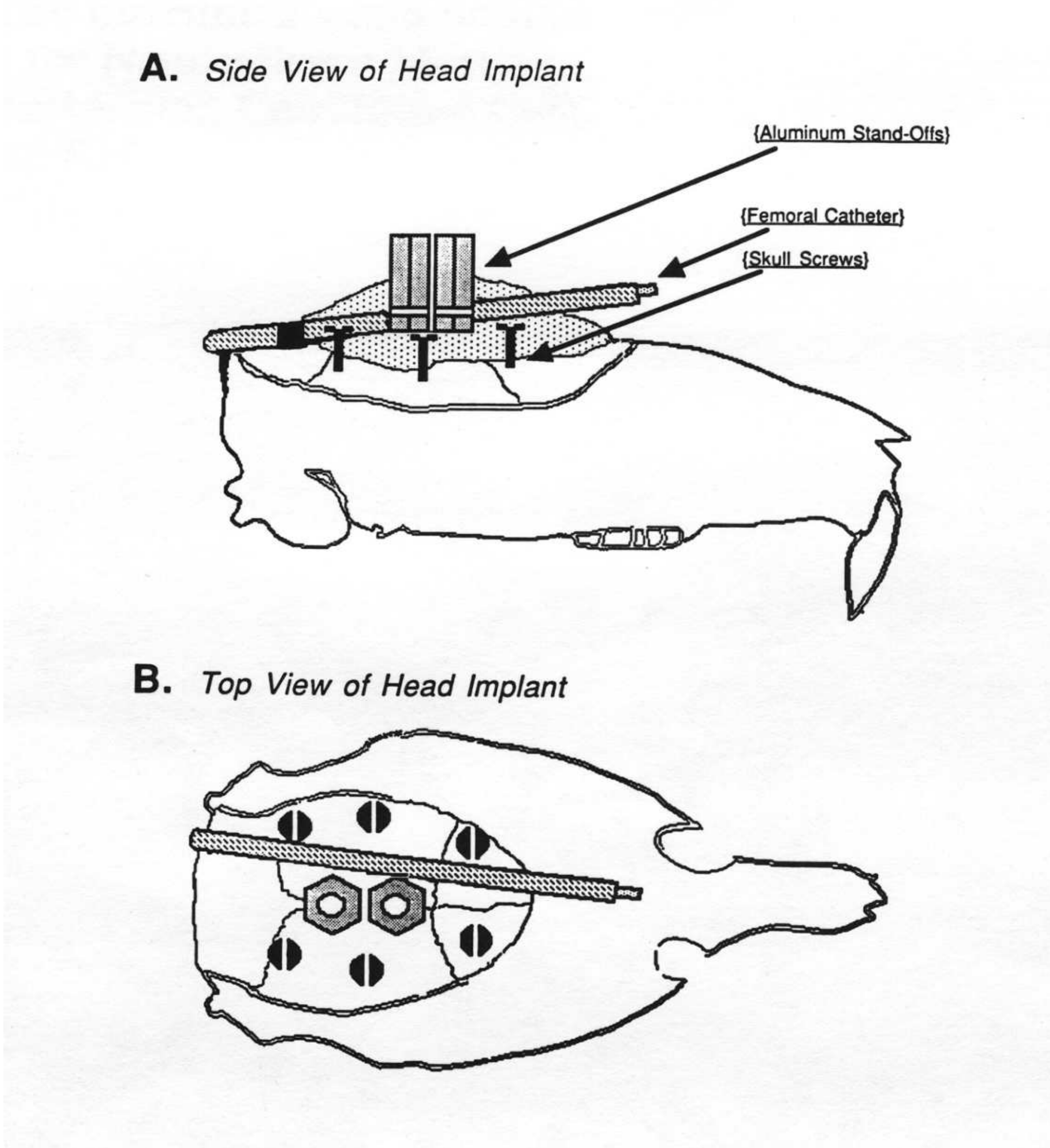

Figure 1. This is a schematic rendition of the side (A) and the top (B) view of a rats skull. Selected parts of the head implant are indicated in (A).

itself loose. Finally, the other key element is the restraining of rats in pairs which helps to reduce the stress of head restraint for the rat. As with the cats, no other body restrain is needed. With the procedures described we have found it possible to restrain rats for extended periods of multiple sessions without the use of anesthesia, muscle relaxants, sedatives or other drugs.

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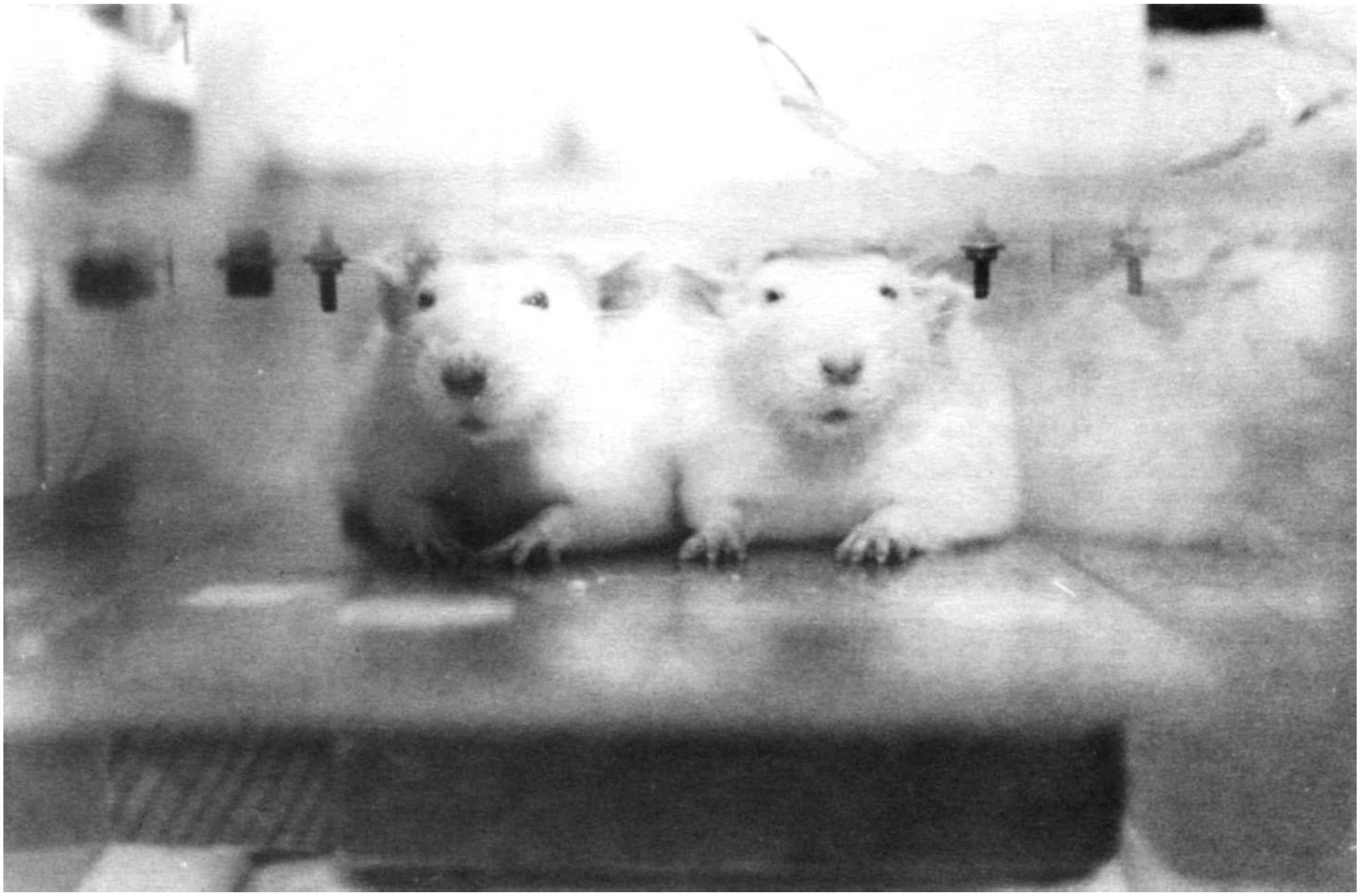

Figure 2. Shown is a front view of a pair of rats in the Plexiglas head restrainer. Note that the rats are restrained only by securing their heads to the rectangular aluminum bar by two bolts. The rat at the left of the photograph is the experimental animal and is implanted with a microdialysis-electrode probe and with a femoral catheter to measure blood pressure and heart rate. The other rat acts as a companion animal in order to reduce stress in the experimental animal.