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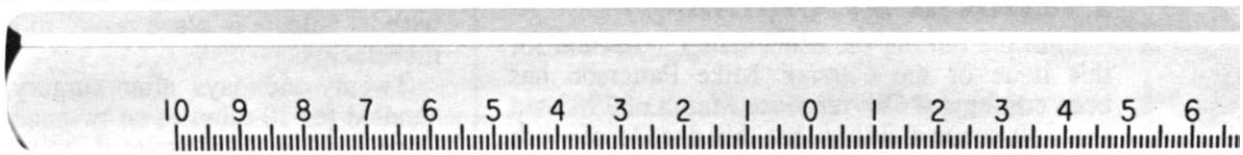

Eyeblink Conditioning In The Restrained Rat: A Novel Preparation

Nestor A. Schmajuk, Ph.D.
Beth A. Christiansen, M.S.

Department of Psychology
Northwestern University
2029 Sheridan Road
Evanston, IL, 60208-2710

Dr. Nestor Schmajuk studied under John Moore, PH.D. at the University of Massachusetts, Am-herst and received his Phi), in experimental psychology in 1986. He did a postdoctoral fellowship with Stephen Grossberg, PhD. at Boston University and joined the faculty at Northwestern University in 1988. In 1992, he was a Guest Fellow of the Royal Society in London. Beth Christiansen is a graduate student with Dr. Schmajuk, having received her Masters in 1992, and is currently working for her Doctorate. She did her undergraduate work at Iowa State University. Dr. Schmajuk can be reached at 708-491-5190

THE TECHNIQUE

Introduced by Gormezano, Schneiderman, Deaux, and Fuentes (1962), the rabbit nictitating membrane (MM) response preparation has become a prevalent and extensively used technique in classical conditioning. While many NM response studies have researched basic classical conditioning phenomena, others have explored the effects of brain ablations on associative learning, and yet others have focused on the neural substrates of classical conditioning. The extensive data base has proven invaluable in advancing classical conditioning theory (e.g., Schmajuk and DiCarlo, 1992).

Although possibly the most common experimental subject, the rat has rarely been used in eyeblink conditioning procedures. The rat is particularly appealing as an experimental animal, because of the massive amount of information that has been accumulated regarding its neuroan-atomical, neurophysiological, and behavior features. No less important is the fact that rats are inexpensive to acquire and maintain. Motivated by these considerations, we introduced a new technique to train rats and showed that reliable eyeblink conditioning is obtained with this new preparation with paired but not unpaired trials (Schmajuk and Christiansen, 1990).

Basically, the technique consists of placing a stainless steel bolt on the rat's skull and securing it with dental acrylic to six stainless steel machine screws implanted in the skull. Six holes are drilled on the skull, two located close to midline and 8 mm anterior to bregma, two placed on the lateral ridge and 2 mm anterior to bregma, and two situated 2 mm from the midline and 3 mm posterior to lambda. Each hole is large enough to accommodate a stainless steel fillister-head machine screw (1 mm in diameter and 3 mm in length from Small Parts Inc.). Cyanoacrylate cement is spread on the skull and around each of the screws so that a thin layer covers the entire exposed area. A stainless steel bolt (13 mm in length, 8 mm at the base of the bolt, and 4 mm a the shaft) is placed in between the six screws, perpendicular to the skull with the shaft of the bolt extending upwards. Dental acrylic is used to build a base around the screws and the bolt until 6-7 mm of the shaft of the bolt remains exposed.

Animals are restrained in a Fisher Scientific restraining cage (Figure 1). A small metal sheet with a hole in the middle serves as the place of attachment for the restraining bolt on the rat's head. Additional pockets and air holes provide adequate ventilation. A flexible tubing installed in a hole located in the front of the cage, serves to deliver an airpuff to the eye. Eyeblink is measured through a window on the side of the cage, with a phototransistor that detects alteration in reflectance of infrared light from a light-emitting diode aimed at the eye (Disterhoft, Kwan, and Lo, 1977). After the animal is placed in the restraining cage, head movements are eliminated by fastening the restraining bolt to the cage and

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President's Column

I am pre-emting the usual Editor's Column for this issue of the *Carrier*. Mike Patterson has been editing the *Carrier* since March of 1983-ten years. It seemed fitting that this decade of work be brought to the attention of *Carrier* readers. I have been pleased with the regularity with which the *Carrier* has been produced and with the wide variety of articles which have appeared in it. It will continue to be a medium through which David Kopf Instruments will offer to our valued readers and customers information about techniques and issues of general interest. I look forward to Dr. Patterson's continued service as Editor and consultant.

There is another reason for taking over the column for this issue. As many of you know, Dr. Patterson has been associated with the Osteo-pathic Medical profession since 1971 when he took a position at the Kirksville College of Oste-opathic Medicine. In 1977, he moved to his present position at the Ohio University College of Osteopathic Medicine in Athens, Ohio. He has been active in research and teaching in the Osteopathic Profession during that time and has also become increasingly involved in its professional activities, in addition to continuing his activities and involvement in such organizations as the Society for Neuroscience.

Last November, at the National Convention of the American Osteopathic Association, Dr. Patterson was awarded the Gutensohn/Denslow Award. This award is granted once a year to recognize outstanding contributions to the Osteopathic Profession in research and education. Dr. Patterson was the eighth recipient of this prestigious award, which is named in honor of J. Sted Denslow, D.O. and Max Gutensohn, D.O. Den-slow started the modern era of research in the Osteopathic Profession and Gutensohn is one of its outstanding Osteopathic educators. At the same time, Dr. Patterson was appointed to the American Osteopathic Association's Bureau of Research, the national body which oversees research activities and initiatives in the profession and provides grant funds for research in Osteopathic Medicine.

I want to publicly congratulate Dr. Patterson on these significant recognitions of his work and thank him for his contributions to David Kopf Instruments through his ten years of *Carrier* Editorship. I look forward to his continued association with our company.

J. David Kopf

securing it with a stainless steel nut. The cage with its tailgate in place serves to eliminate body movements. Twenty-one days after surgery, animals are handled for 30 minutes on two consecutive days. Once handling is completed, animals are habituated to restraint in a darkened conditioning chamber for 50 minutes on 2 consecutive days. After being handled and habituated, rats show few signs of stress and voluntarily enter the restraining cage.

We have recently used the technique to study the effect of hippocampal lesions (HL) on acquisition and extinction of classical conditioning (Christiansen and Schmajuk, 1991a). After the six stainless steel machine screws are implanted and the cement is spread on the skull, the hippocampus is removed by aspiration following Isaac-son and Woodruffs (1975) method. Two small holes are made approximately 2 mm posterior to bregma and 2 mm lateral to the sagittal suture and enlarged with ronguers so that the final holes extend 2 mm from the sagittal suture to 1 mm from the lateral ridge, and 2-3 mm anterior to lambda. After the dura is cut, aspiration proceeds. Following apsiration, porous protein (Gel-foam) soaked in antibiotic is packed into the wounds. Finally the bolt is placed on the skull, anterior to the skull openings, and the exposed area is covered with dental acrylic.

In addition to using the rat preparation to study HL effects on classical condition, we have recorded hippocampal neural activity during classical conditioning by positioning the bolt close to the two most anterior skull screws in order to allow chronic electrodes to be implanted.

BEHAVIORAL STUDIES

Figure 2 shows that when trained in delay conditioning, albino rats achieve a criterion of 8 conditioned responses (CRs) in a block of 10 consecutive trials in approximately 250 trials. The rate of acquisition of eyeblink conditioning in rats seems to be somewhat slower than that of NM response conditioning in rabbits, which attain a comparable level of responding in around 120 trials. While acquisition might be slower in rats, they seem to extinguish faster than rabbits. An extinction criterion of 10% CRs in a block of 10 trials is reached by rats in around 40 trials, while rabbits reach a less stringent criterion in 500 trials (Gormezano, et al., 1972). However, these comparisons should be taken with caution since the rat and rabbit studies described here differ in

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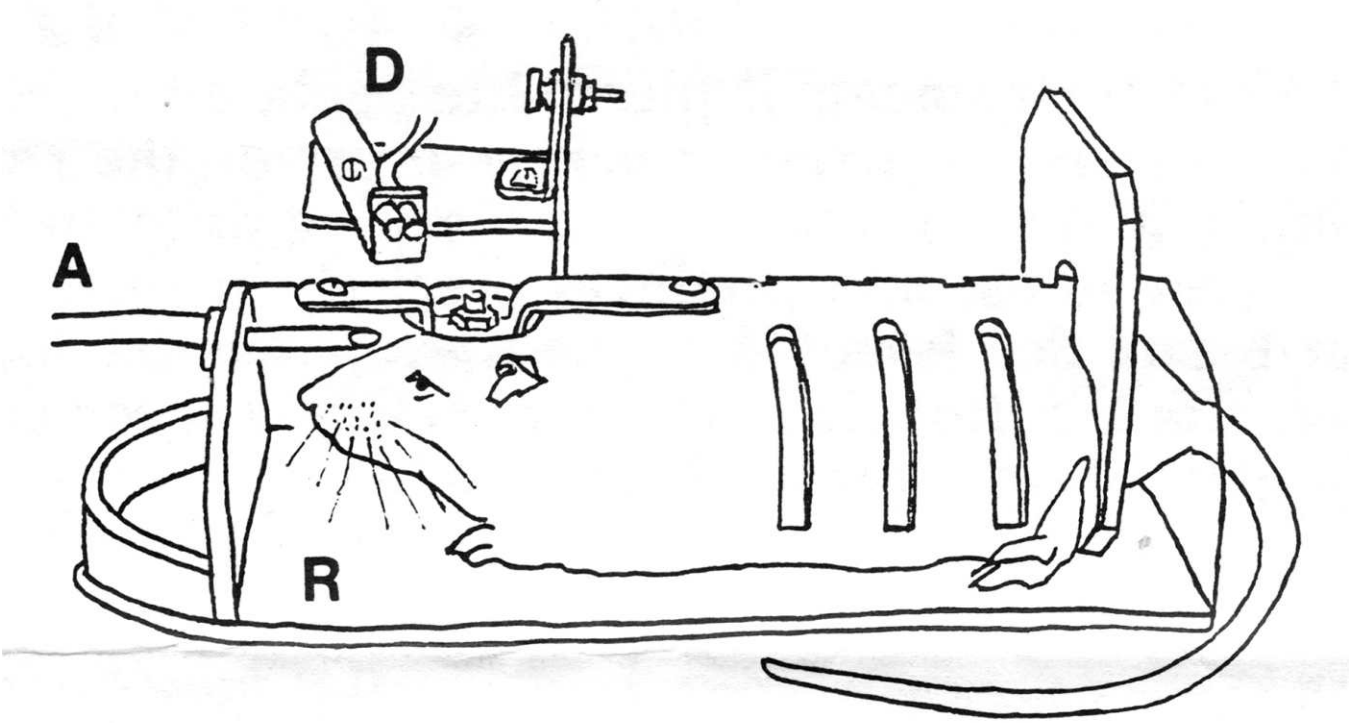

Figure 1. A rat in the restraining cage (R), with phototransistor and light-emitting diode (D) mounted on a bracket arranged for recording the eyeblink, and tubing for delivering air puff (A).

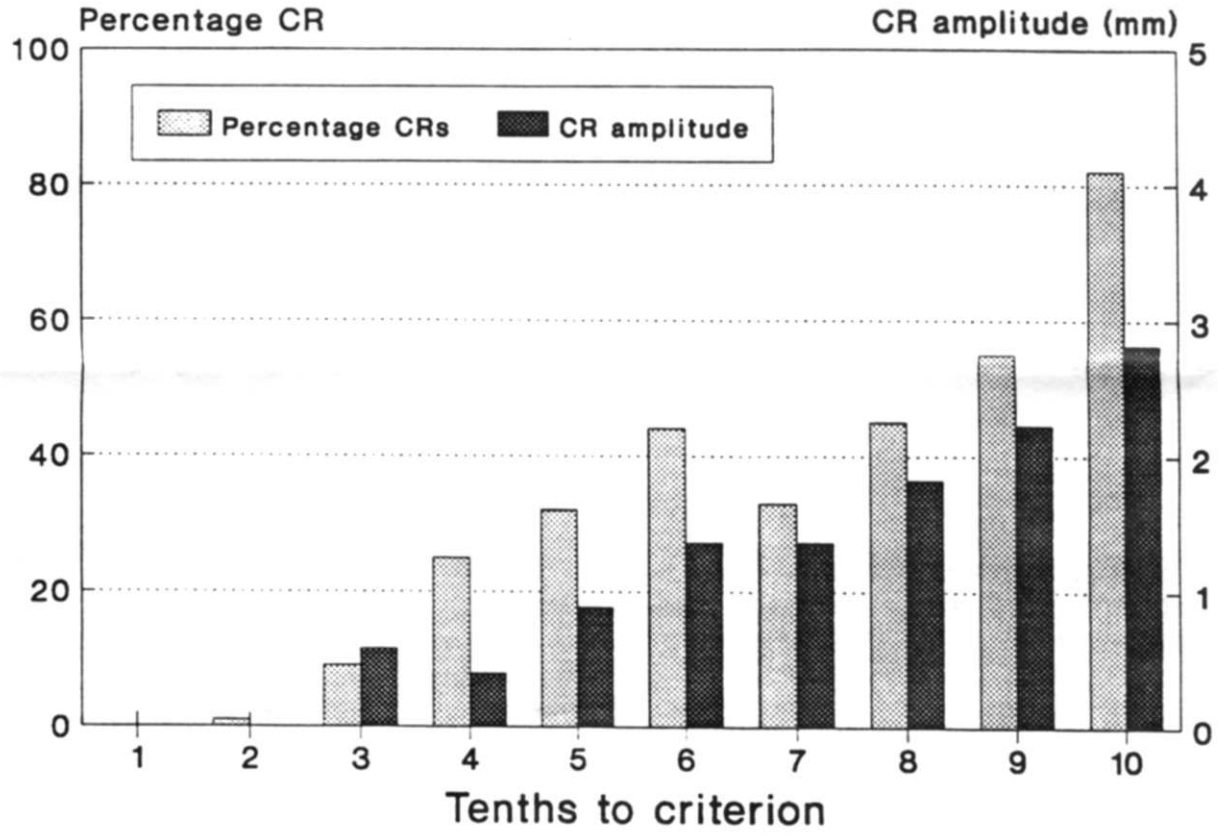

Figure 2. Percentage of CRs and CR amplitude during the CS-US period as a function of Tenths to Criterion (TTC).

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ithe rat and rabbit studies described here differ in a number of important conditioning parameters including interstimulus interval, intertrial interval, conditioned stimulus (CS) intensity and frequency, as well as in the number of daily trails.

Figure 3 shows the topography of a typical eyeblink. As do rabbits, rats acquire the conditioned eyeblink with an orderly sequence of changes: percentage of CRs generated in each session increases, CR amplitude increases, and CR onset latency decreases. At the beginning of training, the first CRs are initiated just before the US, but initiation moves to progressively earlier portions of the CS-US interval with an asymptotic latency occurring at about the midpoint of the ISI. The maximal response amplitude (CR peak) tends to be located around the time of the US occurrence. Interestingly, whereas CR amplitude and percentage of CRs are positively correlated, CR amplitude and CR onset latency are inversely correlated.

NEUROPHYSIOLOGICAL STUDIES

The neurophysiological basis of classical conditioning of the rabbit's NM response has been the subject of numerous studies in past decades. Recently, we tried to replicate with our preparation results previously obtained with the rabbit regarding the effect of HL on different conditioning paradigms. Using the restrained rat technique, we have studied HL effects on acquisition and extinction of eyeblink conditioning (Chris-tiansen and Schmajuk, 1992a). Although HL affected neither acquisition nor extinction rates, HL animals showed significantly shorter CR onset latency during acquisition and extinction, and larger CR peak amplitude during acquisition. Figure 3 shows that HL animals display shorter CR onset latency, larger CR amplitude, and more prolonged UR than sham lesioned (SL) animals. The results are similar to those reported by Ber-ger and Orr (1983) and Port and Patterson (1984) using the rabbit NM response preparation.

Most recently, we have studied the effect of HL on latent inhibition (Christiansen and Schmajuk, 1992b), Figure 4 shows that, as in previous studies using the rabbit NM response (e.g., Solomon and Moore, 1975), aspiration lesions of the hippocampus impair latent inhibition of the rat eyeblink conditioning

A PROMISING TECHNIQUE

The restrained rat eyeblink preparation seems to be a promising behavior technique. As in the case of the rabbit NM response, positive features of this preparation are (a) the passive behavior of that rat during restraint, (b) the reliability of the extension of the eyelid under stimulation by an air puff, (c) the inability of the rat to close its eye for long periods of time and, consequently, to avoid the air puff, (d) the almost negligible spontaneous blinking, and (e) the possibility of an accurate temporal recording of the eyeblink that permits the establishment of correlations between neural firing and behavior.

In addition, neurophysiological studies suggest similar neural substrates in rats and rabbits. For instance, in both rabbits and rats acquisition of the delay conditioned eyeblink/nictitating membrane response is preserved after HL (Christian-sen and Schmajuk, 1992a; Port and Patterson, 1984) but impaired after cerebellar lesions (Skel-ton, 1988; Thompson, 1989). Similarly, latent inhibition is impaired by HL in both species (Christiansen and Schmajuk, 1992b; Solomon and Moore, 1975).

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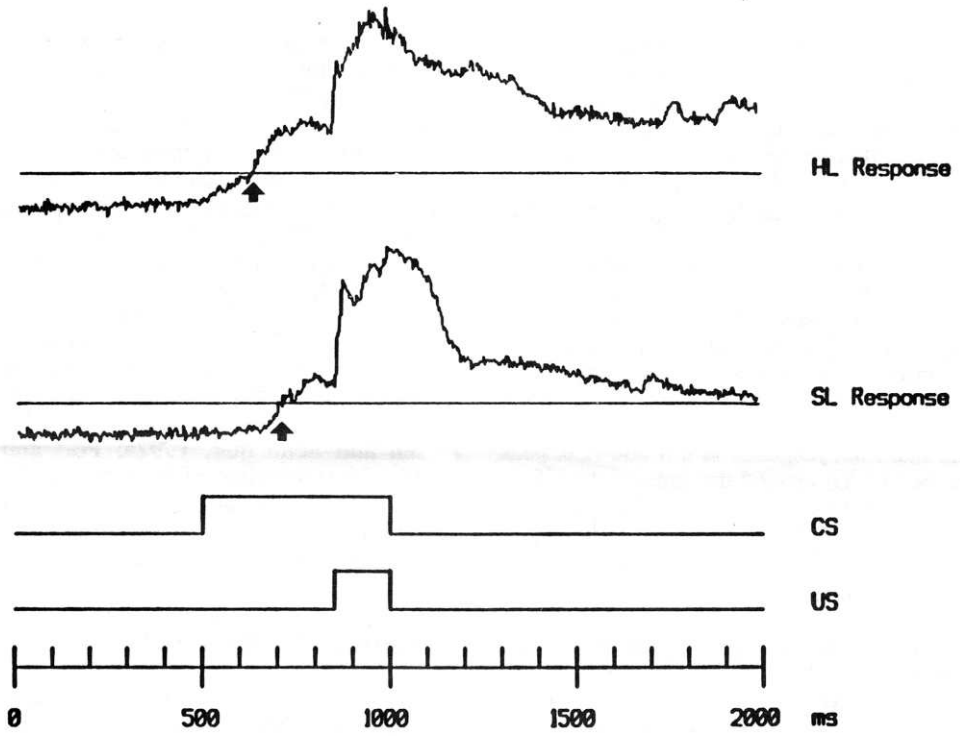

Figure 3. Eyeblink topography of a sham (SL) and hippocampal lesioned (HL) animal.

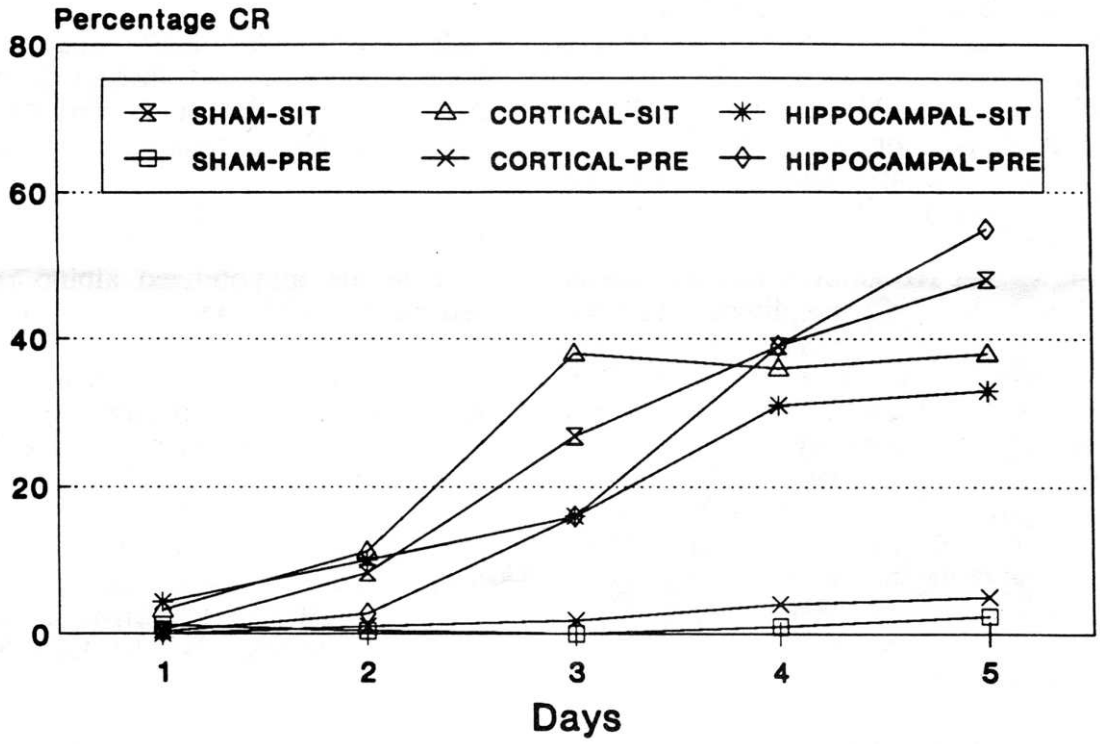

Figure 4. Percentage of CRs as a function of trials in sham (SL), cortical control (CL), and hippocampal lesioned (HL) rats preexposed (PRE) and not preexposed (SIT) to the CS.

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