Spreading depression vs lesion effects of KCl on classical conditioning in the rabbit[1]

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KCl induction of spreading depression (SD) results in lesions. The relative effects of such lesions and of SD per se on retention of the classically conditioned eyeblink response were assessed in two groups of rabbits. A KCl-lesioned control group was compared with a similarly lesioned experimental group; the latter, however, was tested while under the influence of SD. Lesions alone resulted in no behavioral deficit, while performance was markedly decreased under the influence of SD.

The induction of cortical spreading depression (SD) is a widespread technique for temporarily inactivating the cortex in studies of memory in animals (Bureš & Burešová, 1960; Bureš, Burešová, & Fifková, 1964). Potassium chloride (KCl) has become a standard agent for the induction of SD, and its use is predicated on the assumption that its effects are reversible. Recently, Hamburg et al (1968) have shown, however, that repeated applications of KCl to the dura of rats produce cortical lesions, the size varying with the concentration of KCl, the locus of application, and the number of applications. This finding suggests that it is at least possible that some of the effects of SD may be due to cortical damage rather than to SD per se. Examination of the literature on the effects of cortical lesions on classical conditioning (e.g., Polytrew & Zeliony, 1930; Marquis & Hilgard, 1936; Allen, 1945; Raab & Ades, 1946) gives little indication of exactly what to expect of lesions of the type and location that would generally be involved in SD studies. That is, the effects of such lesions have varied depending on CS, responses conditioned, and brain area lesioned. Furthermore, such studies have employed carnivores, while SD studies have generally employed rodents.

Papsdorf, Longman, & Gormezano (1965) reported that rabbits trained in a classical eyeblink-conditioning paradigm showed temporary loss of the conditioned response under KCl-induced SD. Whether or not any lesions were produced by their procedure is unknown. The purpose of the present investigation, therefore, was to determine whether or not cortical lesions produced by multiple applications of KCl have any effect on the retention of the classically conditioned eyeblink response of the rabbit independent of the effects of SD per se.

METHOD

The Ss were eight adult male New Zealand albino rabbits, weighing between 2 and 3 kg.

During training and testing, animals were restrained in a small wooden box with an adjustable stock through which S's head and ears protruded (Gormezano, 1965). Ss were placed in an electrically shielded enclosure. Electrical stimulating and recording leads were connected to S's eyelid for evoking and recording eyeblinks (Schwartz & Marsh, 1964). A Grass Model 7 polygraph was used to monitor the onset of the CS and UCS as well as the eyelid response (one channel per S). Lehigh Valley modular circuitry was programmed to generate a trial randomly on the average of once every 30 sec. The UCS was produced by a Grass S8 stimulator.

The Ss were given injections of atropine sulfate, anesthetized with sodium pentobarbital, and placed in a stereotaxic instrument. A 2-in. midline incision was made in the scalp; the skin and underlying muscle tissue were retracted and two fenestrae, 6 mm in diam, were bilaterally trephined in the bone over the motor cortex about 1 mm anterior and 6 mm lateral to the bregma suture (Swadlow et al, 1968). Care was taken not to disturb the underlying dura; animals showing evidence of dural damage, including discoloration or edema, were discarded. Cannulae made of 1.59-mm polyethylene tubing (inside diam, 6 mm) about 6 mm long were placed over the openings and embedded in dental acrylic secured to the skull by four stainless-steel screws. The cannulae were closed by cotton pledgets soaked in mineral oil throughout the experiment to prevent drying of the dura.

After suturing the scalp wound, four 2.5-cm lengths of 26-ga nichrome wire, doubled and twisted, were implanted in the lids of one eye, one near the nasal corner and one in the medial portion of the upper lid near its edge, and two in corresponding locations in the lower lid. Nasal leads were used for recording, while medial leads were used for stimulating. Following surgery, Ss were given a 50-mg intramuscular injection of tetracycline HCl and allowed a 5-day postoperative recovery.

The Ss were trained (two at a time) in a classical eyeblink-conditioning paradigm. The CS consisted of a 2,000-Hz, 100-msec tone. The UCS was shock at 70 V for .3 msec. The CS-UCS interval was 500 msec. Training continued for 100 trials/day to a criterion of at least 80% CRs/session for 2 successive days.

After reaching criterion, each S was treated with daily bilateral applications of KCl to the dura for 3 days, the protective cotton pledget was removed, the dura was cleaned with isotonic saline, a few crystals of KCl were placed on the dura, and a fresh cotton pledget soaked in mineral oil was inserted. On the 4th day, the dura was simply cleaned and further application of KCl was omitted. The 5th day following training was the test day. Four animals (Group E), randomly selected, were treated with KCl as on Days 1-3 and tested for retention by means of 100 retraining trials, 1 h after application of KCl. The remaining four animals (Group LC) served as lesion controls. They were treated on the test day exactly as on Day 4 and, thus, were not under SD while being tested.

Immediately following the test period, Ss were sacrificed and perfused with saline followed by 10% formalin; brains were removed and prepared for histology. Frozen sections (Humason, 1967; Davenport, 1960), 75 microns thick, were taken every 300 microns. Sections showing the deepest portion of the lesion were strained with cresyl violet and mounted on slides.

RESULTS

Figure 1 shows the mean percentage of CRs made by Ss in each group on the combined criterion days and the test session. The figure indicates that performance decreased markedly for Ss in Group E on the test day. These data were transformed for analysis with an arcsin square-root transformation. Analysis of

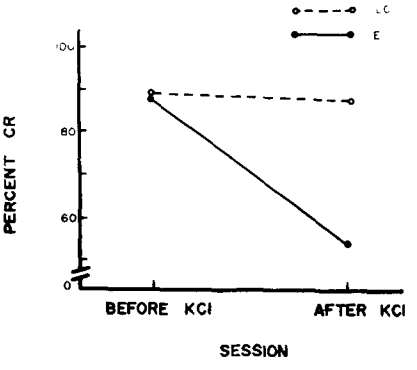

Fig. 1. **Conditioned eyeblink performance in KCl-lesioned rabbits (E under SD, LC without SD).**

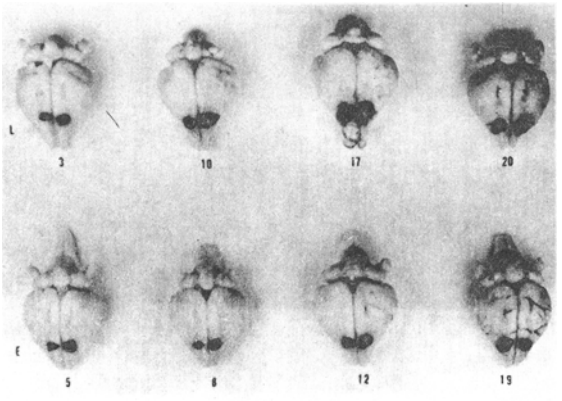

Fig. 2. KCl-produced lesions in rabbit cortex.

variance revealed statistically significant differences between Groups E and LC ($F = 17.11$, $df = 1/6$, $p < .01$) and between sessions ($F = 48.50$, $df = 1/6$, $p < .01$). More importantly, the Groups by Sessions interaction was also statistically significant beyond the .01 level ($F = 41.55$, $df = 1/6$). Further analysis indicated a statistically significant difference in performance of Group E animals on the test day compared to performance at criterion ($t = 6.90$, $df = 3$, $p < .01$), while the performance of Group LC animals did not show a corresponding drop on the test day ($t = 1.10$, $df = 3$). There was also no indication that animals in Group E differed from those in Group LC prior to treatment ($t = 0.24$, $df = 6$). Histological examination revealed that all animals did indeed suffer bilateral lesions in the motor cortex. Figure 2 shows the intact brains with lesioned areas marked in black. The approximate lesion diameters ranged from 2.4 to 6.0 mm; the location of the lesions corresponded with the fenestrae in the skull. Lesion depth ranged from .9 to 4.8 mm; they were generally cup-shaped, in accord with previous findings (Hamburg et al, 1968). Lesion size was estimated by the formula, $\bar{V} = 1/6h(h^2 + 3a^2)$, where h is the height of the spherical segment and a is the radius of its base; when a is equal to h, the formula reduces to that for the volume of a hemisphere. The mean $\bar{V}$ for the combined lesions in each S in Group E was compared to that of Group LC by means of a t test. The LC mean $\bar{V}$ was actually greater than that for Group E, but the difference was not statistically significant. This result indicates that Group E's suppression of CRs cannot be attributed to any difference in size of their cortical lesions.

DISCUSSION

This study clearly indicates that while there was a considerable performance decrement attributable to SD, there was no behaviorally manifested consequence of lesions to the cortex produced by the prior application of KCl. Control animals' responses during the test sessions were nearly identical to their performance on criterion days, in spite of a 4-day interval between training and the test day. Ss in Group E, on the other hand, exhibited severely debilitated performance under the immediate influence of SD.

These results cannot be explained simply in terms of a motor impairment during SD as has been suggested might be the case for some tasks (Tapp, 1962) since there was no change in the UCRs evoked during retention testing, indicating that Ss were physically able to make the appropriate response. They also indicate that limited lesions of the type found here have little or no effect on the classically conditioned eyeblink response in the rabbit.

REFERENCES

ALLEN, W. F. Effects of destroying three localized cerebral cortical areas for sound on correct conditioned differential responses of the dog's foreleg. American Journal of Physiology, 1945, 144, 415-428.

BUREŠ, J., & BUREŠOVÁ, O. The use of Leao's spreading depression in the study of interhemispheric transfer of memory traces. Journal of Comparative & Physiological Psychology, 1960, 53, 558-563.

BUREŠ, J., BUREŠOVÁ, O., & FIFKOVÁ, E. Interhemispheric transfer of a passive avoidance reaction. Journal of Comparative & Physiological Psychology, 1964, 57, 326-330.

DAVENPORT, H. A. *Histological and histochemical techniques.* Philadelphia: Saunders, 1960.

GORMEZANO, J. Classical conditioning. In J. B. Sidowski (Ed.), *Experimental methods and instrumentation in psychology.* New York: McGraw-Hill, 1966.

HAMBURG, M., BEST, P., & CHOLEWIAK, R. Cortical lesions resulting from chemically induced spreading depression. Journal of Comparative & Physiological Psychology, 1968, 66, 492-494.

HUMASON, G. L. *Animal tissue techniques.* San Francisco: Freeman, 1967.

MARQUIS, D., & HILGARD, E. Conditioned lid responses to light in dogs after removal of the visual cortex. Journal of Comparative Psychology, 1936, 22, 157.

PAPSDORF, J. D., LONGMAN, D., & GORMEZANO, I. Spreading depression: Effects of applying potassium chloride to the dura of the rabbit on the conditioned nictitating membrane response. Psychonomic Science, 1965, 2, 125-126.

POLYTREW, S. S., & ZELIONY, G. P. Grosshirnrinde und Assoziationsfunktion. Zeitschrift für Biologie, 1930, 90, 157-160.

RAAB, D., & ADES, H. Cortical and midbrain mediation of a conditioned discrimination of acoustic intensities. American Journal of Psychology, 1946, 59, 59.

SCHWARTZ, M., & MARSH, G. R. Evoked responses during eyelid conditioning in the rabbit. Paper presented at the annual meeting of the American Psychological Association, 1964.

SWADLOW, H., SCHNEIDERMAN, E., & SCHNEIDERMAN, N. Classical conditioning of a discrimination between electrically stimulated lateral geniculate bodies in the rabbit. Proceedings of the 76th Annual Convention of the American Psychological Association, 1968.

TAPP, J. T. Reversible cortical depression and avoidance behavior in the rat. Journal of Comparative & Physiological Psychology, 1962, 55, 306-308.

NOTE

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