become saturated, and the excess metal is ingested by the hepatic lysosomes. Saturation of the hepatic copper-binding sites results in a decreased uptake of the metal with a concomitant elevation in plasma copper not bound to ceruloplasmin. Whether the increased deposition of copper in extrahepatic tissues results from the elevated nonceruloplasmin copper or from the presence of the abnormal protein in these organs is not yet known (*13*).

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16 April 1973; revised 19 June 1973 ■

Spontaneous Whole Brain Slow Potential Changes during Recovery from Experimental Neurosurgery

Abstract. *Prolonged, nonlocalized brain slow potential changes, frequently associated with cortical spreading depression, occur spontaneously during 5 days following brain implant surgery in rats. These potentials are accompanied by reductions in multiple nerve cell activity and reductions in behavioral motility. The method used in this study provides a tool for evaluating recovery from neurosurgical trauma or other brain injuries, and for testing procedures that facilitate or impede this process.*

Injury effects are unavoidable complications of neurophysiological experiments when measurement of brain functions involves implantation of various devices in the brain. As a result, brain functions are often investigated in a previously injured brain. Thus far, effects of implants on the functioning of the brain, as well as the time necessary to recover from implantation trauma, have not been extensively investigated. Brain implantations result in prolonged increases in intracranial pressure, changes in brain impedance, and nonspecific electroencephalographic (EEG) abnormalities (*1*); however, more information is needed on the nature of events during recovery from implant surgery and the effect of such events on neuropsychological and neurophysiological functions. We now report that prolonged slow potential (SP) shifts, with superimposed episodes of the cortical spreading depression (SD) described by Leao (*2*), occur spontaneously during the first 5 days of recovery from brain implant surgery. These SP brain phenomena are associated with reductions in multiple nerve cell activity (MA) as well as depression of behavioral motility. The occurrence of these episodes thus influences neurophysiological and behavioral functions and represents an uncontrolled factor in many experiments.

Table 1. Peak amplitude of SP waveforms (mean ± standard deviation).

Area	SP amplitude (mv)	
	Preceded by SD	Not preceded by SD
Front cortex	− 3.5 ± 1.17	− 3.1 ± 0.90
Back cortex	− 3.4 ± 1.18	− 3.1 ± 0.86
Hypothalamus	− 2.5 ± 1.09	− 5.4 ± 1.33

Ten male Holtzman albino rats (350 to 400 g) were prepared under Nembutal anesthesia (40 mg per kilogram of body weight, intraperitoneally) for recording SP changes from two cortical areas and one subcortical area. The potentials were recorded in reference to the occipital bone marrow. The implant procedures were similar to those reported for cats (*3*) but with the electrodes miniaturized for rats. The two intracortical d-c electrodes were located 1 mm into the left cortex 2 mm from midline, with one 3 mm anterior to bregma and the other 10 mm posterior to the first. A third d-c electrode was implanted into the area of the anterior-lateral hypothalamus on the right side 2 mm posterior to bregma, 1.8 mm lateral to midline, and 7 mm below the dura. Placements were verified by postmortem examination.

Bipolar 30-gauge Nichrome-steel electrodes, insulated except for the cut ends, were implanted into the left anterior thalamus and right lateral hypothalamus for recording MA. Bilateral 22-gauge cannulas for guiding subsequent brain penetrations were placed 2 mm anterior to the front cortex d-c electrode and inserted just into the cortex at a 30° anterior-posterior angle. Stainless steel jeweler's screws were inserted into burr holes in the skull to help anchor the entire electrode assembly. Penicillin G procaine and penicillin G benzathine (15,000 units of each) were injected intramuscularly as a prophylactic measure. After surgery, the animals were placed in a recording cage with free access to food and water.

Brain potentials were fed through a movement artifact–free cable, suspended above the cage, to the recording amplifiers. In subsequent recordings of SP and SD, the output of Grass P-17 d-c amplifiers was fed to Grass 7P1 d-c chopper-stabilized amplifiers and displayed on a Grass model 7 polygraph. Recordings of MA were made through a Grass P-15 amplifier. The output of the amplifier was filtered activity in a band between 800 and 3000 hertz half-amplitude, eliminating EEG and other frequency signals from the MA records. The activity was fed into a Grass 7P3 integrator amplifier with the integrated output continuously displayed on the polygraph paper. Behavioral movement was continuously

recorded by means of a piezoelectric crystal upon which the cage rested. Movement of the cage resulted in small signals from the crystal which were amplified by the polygraph.

We continuously recorded from each animal for a total of 120 hours after surgery. Three of the ten animals could not be used, two because of data lost as a result of power failure in the laboratory building and one because of the development of epileptic seizures that were evident both behaviorally and electrophysiologically. In all of the remaining seven animals, we observed two types of spontaneous SP episodes. One type was in the form of one or two waves of SD from the intracortical leads; the first SD wave was followed immediately by a large negative monophasic SP wave that lasted more than 90 minutes and appeared simultaneously in the two intracortical and one subcortical locations. The second type of response was a large negative monophasic SP wave at all d-c electrodes without a preceding SD. The relative frequency of each type of episode was 50 percent. Examples of the two types of episodes are presented in Fig. 1. In more than 500 hours of recording SP's from rats recovered from implant surgery for at least 3 weeks, we have never observed spontaneous SD and SP waveforms like those reported here.

The combined frequency of occurrence of these spontaneous waveforms during recovery was determined for ten consecutive 12-hour periods after surgery. There were 2 waveforms in the first period, 6 in the second, 7 in the third, 12 in the fourth, 9 in the fifth, 4 in the sixth, 1 in the seventh, 0 in the eighth, 1 in the ninth, and 2 in the tenth. Characteristics of the SP waveforms were as follows (mean $\pm$ standard deviation): rise time to peak, 14.7 ± 1.48 minutes; half-amplitude fall time, 90 ± 9.7 minutes; and total duration, 166 ± 18.7 minutes. These characteristics did not substantially vary as a function of recording location or of the presence of cortical SD. The mean total duration should be considered as an approximation only. The fall of the waveform is essentially exponential and, hence, total duration is difficult to measure objectively.

There were differences in the amplitudes of the SP waveforms as a function of electrode location in the brain. Table 1 shows that the SP shift from the subcortical electrode was larger than the shifts from the intracortical

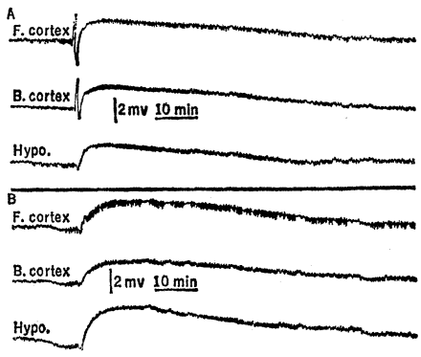

Fig. 1. Examples of two types of spontaneous SP episodes during recovery from neurosurgery. (A) Simultaneous recordings from the front cortex (*F. cortex*), back cortex (*B. cortex*), and hypothalamus (*Hypo.*) are shown for a negative SP episode that is preceded by a spreading depression at the intracortical electrodes. (B) The SP episode shown is not preceded by spreading depression at the intracortical electrodes.

electrodes when the SP shift was not preceded by an SD. The subcortical magnitude was larger in all of nine episodes for which magnitudes from all three electrode locations could be compared (sometimes the pens were driven off scale by the SP shift on one of the electrode channels and magnitude could not be measured). In contrast, during 15 of 18 episodes in which SD preceded the SP shift, the magnitude of the subcortical shift was smaller than that of either of the intracortical locations.

In 51 percent of the episodes, marked depression of MA (lower than any during the preceding or succeeding 2 hours) occurred during the first 5 minutes of the SP change. The lateral hypothalamus exhibited such depressions only under conditions in which no SD preceded the SP change. The anterior thalamus exhibited depressions only when cortical SD preceded the SP change. Since the recording location in the lateral hypothalamus was contralateral to the cortical d-c electrodes and that in the anterior thalamus was ipsilateral, this may indicate that the event that resulted in the SP change and nerve cell inhibition was restricted to the hemisphere where the inhibition took place.

For each animal a measure was derived of the average behavioral motility for 2 hours before and 4 hours after the onset of spontaneous SP episodes. An analysis of variance indicated that behavioral motility was significantly reduced ($P < .05$) for the 2-hour period after the onset of an SP episode. Behavioral motility during the next 2 hours was not significantly lower than that before SP episodes. In addition, there were indications that behavioral and situational events would predispose the animals to the occurrence of an SP episode. Fifty-five percent of the episodes were directly preceded by brief periods of intense motor activity, eating, or drinking, or by a disturbance in the environment (such as the experimenters entering the room). This suggests that the spontaneous episodes are more likely to appear under conditions of general stress or behavioral activation.

Spreading depression and prolonged negative potentials, identical to those occurring spontaneously during neurosurgical recovery, were directly elicited by a 3.0-mm penetration wound of the cortex in 20 other rats. These animals were prepared identically to those described above, and the penetration wound was made 3 weeks after surgery through a previously implanted canula. Penetration of the cortex produced SD waves in that cortex but not in the contralateral cortex. The prolonged negative SP occurred on both cortices as well as in the hypothalamus. These data indicate that spontaneously occurring SP episodes following neurosurgical implantations represent discrete injuries taking place during the recovery process. Similar brain SP events occur after vascular occlusion and anoxia of the brain (*2, 4*), which suggests that the spontaneous SP episodes represent "strokelike" events.

We conclude that the method used in this study provides an experimental paradigm for studying the process of recovery from neurosurgical intervention or other brain injury, and for testing procedures that may facilitate or impede the recovery process. Many neurophysiological and neurobehavioral studies may run the risk of having outcomes influenced by spontaneous brain disruptions if care is not taken to assure adequate recovery from neurosurgical intervention before data are collected.

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5. Supported in part by a grant from the graduate school, Southern Illinois University, Carbondale. We thank T. Irwin and J. Kakolewski for help in preparing the manuscript and Mrs. Pearl Miller for histology. Address reprint requests to J.W.K.

13 November 1972; revised 21 May 1973

Human Embryonic Kidneys in Organ Culture: Abnormalities of Development Induced by Decreased Potassium

Abstract. *Human embryonic kidneys of 5 to 12 weeks of gestation were grown in organ culture. Potassium concentrations of 3 to 6 milliequivalents per liter produced decreased ureteral bud branching, failure of nephron induction, and occasional cystic dilatations of the ureteral bud. Normal development of the kidney occurred at potassium concentrations of 6.5 to 10 milliequivalents per liter. These studies confirm the importance of relative stability of the potassium concentration in the development of the embryonic kidney.*

It is important to determine the relevance of experimental renal teratogenesis in animals to human disease. Studies in human embryonic or fetal tissue would be the most reliable index of mechanisms of growth abnormalities in man.

Perey *et al.* (*1*) produced a disease similar to human polycystic kidney disease by injecting high doses of steroids into newborn rats. Their data suggested that hypopotassemia, induced by the mineralocorticoids, was responsible for the morphologic changes. Crocker and Vernier (*2*), using 1000 whole organ cultures of fetal mouse kidney, showed that alteration of the potassium concentration was one of the likely causes of this renal maldevelopment. They emphasized the necessity of constant maintenance of potassium concentration for normal renal differentiation in the early stages of development. Stewart and Welt (*3*) and Serrano *et al.* (*4*) showed that when the maternal serum potassium concentration in the pregnant dog was lowered by dialysis to the range of severe hypokalemia, potassium concentration in the fetus was not affected. Similarly, Dancis and Springer (*5*) showed that high plasma potassium in fetal rats was maintained when the mothers were fed potassium-deficient diets. In the crucial early stages of renal differentiation, the embryonic or fetal plasma potassium may be kept stable by a pump, probably at the placental level.

We report the development of a whole organ culture model for human kidney, in which we studied the teratogenic effects of potassium concentrations of 3 to 6 meq/liter. Oxygen concentration and acid-base stability must be closely regulated, because changes in nephron development patterns may be caused by these factors (*6*). Our observations emphasize the requirement of high embryonic serum potassium concentrations for human renal development.

Whole human kidney pairs from 75 embryos 5 to 12 weeks of gestation were obtained by vacuum curettage. After curettage, the material in the vacuum apparatus was washed rapidly in Hanks balanced salt solution through a multiple mesh screen system. This removed most conception products other than the embryo. Portions of embryos or whole embryos of 5 to 12 weeks of gestation were usually obtained. The kidneys were removed rapidly with cataract knives while the embryo was viewed under a Zeiss stereomicroscope, and were placed in Hanks balanced salt solution.

Kidneys were placed on a Nuclepore membrane filter supported by stainless steel wire mesh in a Falcon plastic organ culture dish, or were grown submerged on a Nuclepore filter in the dish. All cultures were oscillated (2 min^{-1}) on a Bellco rocker to improve circulation of the tissue culture medium. Human kidney of this size and gestational age was chosen because (i) small kidneys are easy to handle; (ii) their use minimizes the problem of central necrosis, which is apparently caused by hypoxia during prolonged organ culture; and (iii) human metanephric kidneys begin differentiation at 35 days of embryonic life and, as judged from previous animal studies in this laboratory (*2*), the first weeks of renal embryonic development would be the period of maximum sensitivity to potassium concentration variations.

The cultures were incubated in a water-jacketed incubator at 37°C in 80 or 95 percent oxygen with 5 percent carbon dioxide. Oxygen was monitored with a Beckman model D oxygen analyzer. Media were changed every 48 hours. Medium 199, prepared virtually free of potassium (0.01 meq/liter) with Hanks base (*7*), was supplemented with extract of 9-day chick embryos (*8*), 4 percent; horse serum, 10 percent; penicillin, 200 unit/ml; streptomycin (*7*), 200 mg/ml; and Mycostatin (*7*), 100 units/ml. Electrolyte concentration of the final medium was measured by flame photometry after a known quantity of potassium solution was added. The *p*H of the medium was controlled by titration with 7.5 percent sodium bicarbonate solution. Cultures were grown for 2 to 5 days and kidneys were then processed by standard histological fixation techniques. Normal human embryonic kidneys at similar gestation age were also studied and compared with experimental organs to be certain of organ growth and development. Of each kidney pair, the control organ was grown in medium with a potassium concentration of 6.5 to 10 meq/liter, while the other was grown with potassium of 3 to 6 meq/liter.

In 38 embryonic kidneys grown with potassium concentrations of less than 6 meq/liter, the following defects were seen (Fig. 1B): (i) a decreased number of branches of the ureteral bud, (ii) failure of nephron induction at the site of branching, and (iii) occasional dilatation of the ureteral bud. The paired control embryonic kidneys grown in media containing 6.5 to 10 meq/liter grew normally and were free of the defects seen in those grown at lower potassium concentrations. The renal pelvis and calyces were normal if kidneys of later gestational age were grown in culture. Severe signs of previous anoxia or damage (thought to be due to the vacuum extraction procedure) made 37 pairs of kidneys unsuitable for evaluation. The teratogenic