related to the instants of (test) stimulus presentation. We have shown that this time structure can be altered in various ways during presentation of our stimulus sequence. In some cases the alterations of synchronization are not accompanied by alterations of the firing patterns of the individual neurons as measured by the peri-stimulus-time histograms.

It is particularly evident in Fig. 1 that the alterations of the probability of nearly simultaneous firing can be either phasic or tonic with respect to the test stimulus. This suggests that there may be two dissociable sources that produce the nearly simultaneous firings. Although the spike train analysis described so far cannot uniquely determine such sources, we had available intracellular recordings. These showed, in addition to individual synaptic activity, that a large excitatory postsynaptic potential appeared almost simultaneously in all the observed neurons and was responsible for much of the nearly simultaneous firing. Various unsynchronized firings occurred at other times due to unshared excitatory postsynaptic potentials or to intrinsic pacemaker potentials.

Thus, the alterations of probability of nearly coincident firing are caused by alterations in the firing pattern of a source common to all the observed neurons. Under the in vitro conditions of these experiments this yet unidentified interneuron is able to partially synchronize the firings of a population of neurons. Synchronous firing in a neural population and plastic alteration of the time structure of such synchronization are of obvious theoretical interest. However, the detailed connectivity and the behavioral significance of these mechanisms remain to be examined, perhaps by methods used in recent studies of gill withdrawal habituation (*10*).

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Lack of Coincidence between Neural and Behavioral Manifestations of Cortical Spreading Depression

Abstract. *The presence of cortical spreading depression is typically inferred from the presence of hypesthesia. The electrocorticogram and slow-potential change were recorded during cortical spreading depression and it was found that hypesthesia remained long after the cortex recovered from neural depression. Hypesthesia, therefore, is an unreliable indicant of cortical spreading depression; if cortical spreading depression is used as a research tool, neural activity must be monitored. These data offer a special problem for memory transfer studies.*

Cortical spreading depression (CSD) has been used to investigate learning phenomena such as interhemispheric transfer of training (*1*). The assumption in such studies is that the CSD produced by the topical application of potassium chloride is a "reversible lesion" or a "functional decortication." Recently it has been demonstrated that CSD should not be considered to be a functional ablation during the entire depression period, since the cortex gives evidence of at least partial recovery of electrical activity from time to time during the course of the treatment (*2*).

Typically, the behavior criterion for CSD is the development of hypesthesia (diminished sensibility) of the limbs contralateral to the depressed hemisphere (*3*). It is assumed that this unilateral hypesthesia is the behavioral manifestation of an underlying unilateral neural CSD. The purpose of this study is to question the assumption that the presence of hypesthesia is necessarily indicative of the presence of CSD.

Six male hooded rats of the Long-Evans strain weighing 250 to 350 g were used in the first experiment. Animals were surgically prepared for CSD and for chronic electrocorticographic (ECoG) recording. Two pairs of cortical electrodes (stainless steel screws) were placed bilaterally in the skull about 5 mm on either side of the saggital suture: one pair was about 2 mm anterior to the coronal suture and the other pair was about 2 mm anterior to the lambdoid suture. A polyethylene cannula [0.070 inch (0.178 cm) inside diameter, 0.110 inch (0.279 cm) outside diameter] was inserted (*4*) over one hemisphere in the anterolateral parietal bone. In three rats the cannula was over the left cortex and in the other three it was over the right cortex. The cannula was filled with sterile 0.9 percent saline whenever the rat was in its home cage. The electrical activity of both hemispheres was recorded differentially between ipsilateral pairs of electrodes. This made it possible to compare the activity of the depressed hemisphere with the activity of the nondepressed hemisphere for the same animal. An animal was considered to exhibit CSD when the ECoG amplitude reduced by at least one-third of the baseline amplitude. This was ascertained by making a calibrated template of the baseline amplitude and continuously measuring

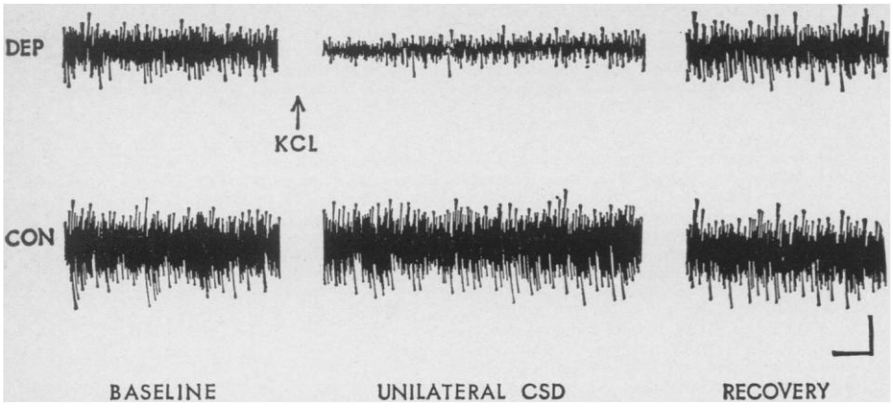

Fig. 1. Electrocorticogram (ECoG) from the hemisphere over which the cannula was placed (*DEP*) and from the contralateral hemisphere (*CON*). Prior to application of KCl, the baseline ECoG from the DEP hemisphere is of less amplitude than that from the CON hemisphere, probably due to a tonic mechanical depression caused by the cannula. Unilateral CSD is produced by application of 25 percent KCl. The ECoG amplitude of the DEP hemisphere is further reduced, indicating the presence of CSD, while the ECoG amplitude of the CON hemisphere is not reduced. In the recovery phase the ECoG amplitude of the DEP hemisphere has returned to baseline. During this phase marked unilateral hypesthesia was still present. Horizontal marker, 10 seconds; vertical marker, 100 μv.

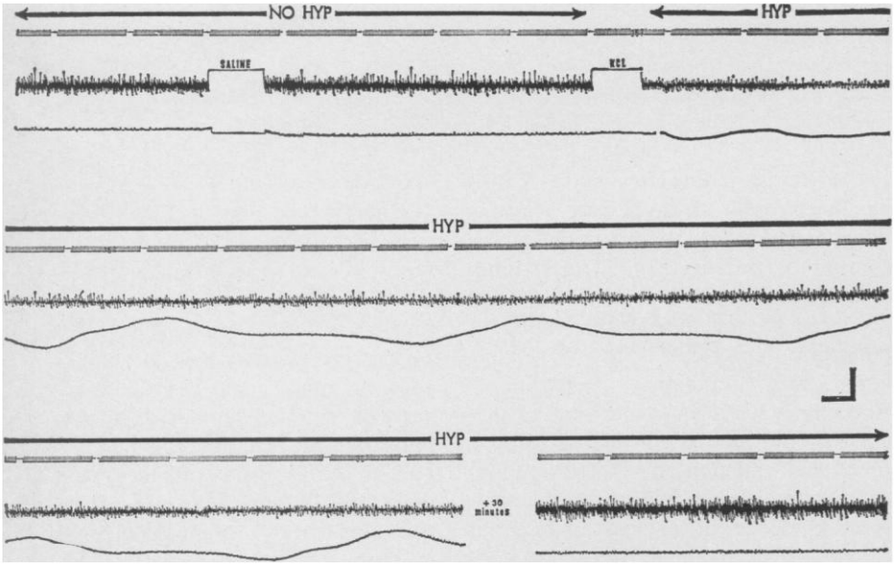

Fig. 2. ECoG (middle trace) was recorded from an ipsilateral pair of silver–silver chloride cortical electrodes, and direct current (lower trace) was recorded between the frontal member of the electrode pair and a reference electrode on the contralateral hemisphere. The upper trace (appearing as gray bars) is time calibration in seconds, with a 5-second pause once per minute. The first portion (2½ minutes) of the record is a sample of the baseline condition; 0.9 percent sterile saline was then injected into the cannula. The input leads to the preamplifier were shorted during injection of saline and KCl, but the chart motor was left running so that a continuous time record was available. As indicated by the arrow above the tracings, hypesthesia (*HYP*) was not present during either baseline or saline conditions. (Injection of saline ruled out the possibility that mechanical CSD was produced by cannular delivery of liquid to the cortex. Neither ECoG amplitude reduction nor slow-potential changes occurred following the saline injection.) Five minutes later 25 percent KCl was injected into the cannula. Hypesthesia was observed within 2 minutes after KCl. The first slow-potential change was simultaneous with the onset of hypesthesia, and within 30 seconds the ECoG amplitude diminished. During the next 20 minutes of continuous recording, marked hypesthesia was concomitant with both neural criteria for CSD: ECoG amplitude reduction and the occurrence of slow-potential changes. Thirty minutes after the ECoG amplitude had recovered and slow-potential changes had ceased (see the last 5 minutes of sample record), marked hypesthesia was still present. Horizontal marker, 25 seconds; vertical marker, 5 mv for lower trace and 500 μv for middle trace.

the ECoG amplitude "envelope" against the template.

The behavioral testing apparatus was a small, clear Plexiglas chamber (9 by 17 by 23 cm high) with bars 2.8 cm in diameter spaced 2 cm apart (center to center). The spacing of the bars required the rat to maintain a fair amount of postural tonus to remain erect within the chamber. When CSD was produced, unilateral hypesthesia resulted; the forelimb contralateral to the depressed hemisphere would fall through the bars and the rat would not retract the limb. If the animal's position prevented the limb from falling through, the experimenter would grasp the paw with a hemostat from beneath the bars and gently pull the limb through the bars. If the rat did not retract the limb, it was scored as hypesthesic. This procedure was followed every minute, thereby making it possible to obtain an almost continual record of whether or not hypesthesia was present. Whether the hypesthesia was unilateral or bilateral could be assessed by pulling on the limb ipsilateral to the cannula. If the rat was hypesthesic unilaterally, it would immediately retract the control limb. (If the CSD was bilateral both limbs would hang through the bars. This occurred in only one experimental run and the data for that rat are not included here.) The value of this method of assessing hypesthesia is that it minimizes the handling of the animal and thereby reduces the probability of inducing transient activation that would introduce artifact into the ECoG record. In a separate series of tests, whenever animals were judged to be hypesthesic by this method they were also found to be hypesthesic by the conventional behavioral tests (paw lifting and placing responses).

Twenty-four hours after surgical preparation, the rat was placed in the testing chamber (which itself was contained in a larger, electrically shielded recording chamber) and baseline ECoG was recorded for approximately 10 minutes. The chart speed of the polygraph was set at 1 mm/min. At the beginning of an experimental run, the 0.9 percent saline in the cannula was replaced with 12 percent KCl for three rats and with 25 percent KCl for the other three. When the animal was returned to the chamber after KCl injection, ECoG recording was continued until the termination of the experimental run. Both behavioral testing and

ECoG recording continued until 30 minutes after the ECoG envelope had regained its original (baseline) amplitude. Each rat was tested once on each of three successive days. Figure 1 shows typical ECoG tracings during baseline, during CSD, and after recovery for both the depressed and control hemispheres.

It was the consistent finding in *every* case that the behavioral indicant of CSD (hypesthesia) outlasted the neural manifestation of CSD (reduced ECoG amplitude), often by as much as 30 minutes. In the 12 percent condition, hypesthesia outlasted CSD by a median of 16 minutes (interquartile range = 10 to 30 minutes). In the 25 percent condition, hypesthesia outlasted CSD by a median of 30 minutes (interquartile range = 24 to 30 minutes). In all cases the onset of hypesthesia and the onset of ECoG depression occurred within 30 seconds and were usually simultaneous.

It is interesting to note that many rats showed reduced ECoG amplitude in the cortex over which the cannula was secured even before the application of KCl. Perhaps this occurred because the Tapp technique of cannular placement used in this study produced tonic mechanical depression of the cortex (see Fig. 1).

The question might be raised whether or not hypesthesia is associated with another neuroelectrical effect of CSD, the slow-potential change (SPC). To investigate this possibility, another experiment was conducted using the SPC (as well as ECoG reduction) as a measure of CSD, in order to confirm and extend the findings of the first study. The surgical and behavioral procedures were the same as described earlier, with the exception that nonpolarizable silver–silver chloride electrodes were used so that slow potentials as well as ECoG could be recorded from the same electrodes. The electrocorticogram was recorded differentially between ipsilateral pairs of electrodes and direct current was recorded monopolarly between each electrode and a nonpolarizable reference electrode. Typical ECoG and d-c tracings taken during baseline, during CSD, and after recovery from a depressed hemisphere are shown in Fig. 2. Slow-potential changes usually consisted of an initial brief (1 to 2 minutes) negative shift of approximately 5 to 10 mv followed by a longer lasting positive shift. During the maximum negativity of the SPC, ECoG depression was usually most dramatic.

Each experimental run included 10 minutes of baseline recording, after which the 0.9 percent sterile saline in the cannula was replaced with either 12 percent or 25 percent KCl. Three animals received 12 percent and three received 25 percent. Behavioral testing and electrographic recordings were continued for 30 minutes after the cessation of slow-potential changes. Each animal was tested once on each of three successive days.

The results confirm the findings of the first experiment. In *every* case the animals exhibited hypesthesia long after slow-potential changes had ceased. In 17 out of 18 observations, hypesthesia outlasted the slow-potential change criterion for CSD by the full 30 minutes. In the remaining observation (an animal in the 12 percent condition) hypesthesia outlasted slow-potential changes by 21 minutes.

Both experiments suggest a dissociation between the commonly accepted behavioral sign of CSD (hypesthesia), and two neural correlates of CSD (reduction of ECoG amplitude and the occurrence of slow-potential changes). We of course do not believe that there is *no* electrophysiological event correlated with hypesthesia. We merely stress that the two neuroelectrical properties of CSD that are usually assumed to indicate the presence of a *hypothetical* neural state ("functional decortication") can be dissociated from the behavioral criterion for CSD.

The implications of these findings for "transfer" studies should be pointed out. A typical transfer paradigm requires that animals be trained on day 1 to some behavioral criterion on a learning task while undergoing unilateral CSD. On day 2, the animals are given a single "transfer" trial with both hemispheres intact, and on day 3 these animals are retrained on the same task with the originally intact (donor) hemisphere depressed, and the originally depressed (recipient) hemisphere intact.

If the animal learns the task in significantly fewer trials on day 3, interhemispheric transfer of learning is said to have taken place. If hypesthesia is used as the behavioral criterion for unilateral CSD on day 1, and if some proportion of those animals which showed this behavioral evidence of CSD may actually have recovered during the training session (or perhaps never even were cortically depressed), many of the "training" trials on day 1 would in fact be "transfer" trials. This question cannot be satisfactorily answered as long as behavioral criteria such as hypesthesia or locomotor impairment are the sole measures employed as indicants of CSD. It is incumbent upon anyone who would use CSD to produce a "functional decortication" (as in memory transfer studies) to monitor neural activity during the behavioral training and testing.

The use of CSD as a research technique for the investigation of learning phenomena is based on a number of assumptions about the electrophysiological consequences of that treatment (*5*). In spite of the fact that most of these assumptions have been called into question in the literature (*6*) the technique continues to be a popular tool in such research. Although CSD has very attractive hypothetical properties (such as the production of a "functional lesion") for investigating learning, it remains to be established that those properties have reliable neuroelectrical counterparts in the brain.

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5. For example: (i) CSD is assumed to be restricted to a single hemisphere upon unilateral topical application of an electrolyte; (ii) some investigators [for example, Albert (see *1*)] claim to terminate CSD by application of saline to the depressed cortex; (iii) it is commonly assumed that as long as KCl is present, the cortex to which it is applied is quiescent; and finally, (iv) a general assumption is that CSD can generate a "reversible lesion," that is, that there are no lasting effects from the application of KCl.

6. With regard to each assumption stated above: (i) Unilateral application of KCl has been reported to reduce EEG bilaterally [M. Gollander and S. Ochs, *Amer. Psychologist* **18**, 431 (1963)], and to reduce evoked responses in both depressed and control hemispheres [N. Freedman and A. Langford, *J. Comp. Physiol. Psychol.* **69**, 362 (1969)]. (ii) Application of saline does not terminate CSD (as measured both by ECoG reduction and slow-potential changes) within the time limits assumed by Albert (T. J. Carew, T. J. Crow, L. F. Petrinovich, report to the Western Psychological Association, Los Angeles, 1970). (iii) Electrocortical and behavioral correlates of CSD, rather than remaining constant over the period of KCl application, actually wax and wane. It has been demonstrated that locomotor activity increases during the "recovery" phase of CSD, and that training under different amplitude phases of CSD produces differential performance [N. Freedman, R. Pote, R. Butcher, M. Suboski, *Physiol. Behav.* 3, 373 (1968)]. (iv) Application of electrolytes such as KCl to cortical surfaces has been reported to cause injury to cortical and even subcortical structures (see *4*), and to cause extensive cortical damage as a function of concentration [M. Hamburg, P. Best, R. Cholewiak, *J. Comp. Physiol. Psychol.* **66**, 492 (1968)].

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"Behavior Induction" or "Memory Transfer"

A recent report by Golub *et al.* (*1*) concerning "Behavior induction" or "memory transfer," produced by injections into naive animals of extracts from brains of trained animals, could conceivably give rise to a new wave of studies on this issue. This comment is an attempt to aid present and future workers.

Near the end of their report, Golub *et al.* say, "When additional treatments, such as extended overtraining or the interpolation of extinction training between acquisition sessions, are introduced into the donor-training phase of transfer paradigms, these incubation periods are probably unnecessary." Although this statement is not particularly strong, it might give the reader the impression that he could use several variations of the procedure used by Golub *et al.* (*1*) and still expect to obtain an effect on the recipient rat's behavior. However, it is my opinion that future research should start with the procedure (identical in all details) used by Golub *et al.* (*1*) and continue with this procedure until the replicability of the results is clearly determined.

As a supporting case on the point of extended overtraining being a sufficient substitute for "incubation periods," I refer the reader to a recent report from our laboratory (*2*) which described a series of attempts to obtain a transfer effect. The eighth experiment in this series involved considerable overtraining; however, the naive recipient animals showed no effect of the donor training.

This experiment differed from those reported by Golub *et al.* (*1*) in many details, any one of which could conceivably be blamed for our failure to obtain a transfer effect. However, most of the details of our study were identical to those of previous studies by other workers which had yielded positive results. The fact that not all of the details were the same (because we lacked access to identical equipment) led some critics to say that our work did not constitute a true replication attempt.

To reiterate the point of the communication—workers who enter this area would do well to copy the technique of Golub *et al.* (*1*) in all details to determine the replicability of the effect before going on to examination of the phenomenon.

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8 May 1970

We believe, and have so stated on several occasions (*1*), that the "transfer" experiments should always be repeated as carefully as possible in order to determine the critical variables necessary for the effect to occur. We assuredly did not intend to give readers the impression that variations in our procedures would necessarily yield results identical with those found in our laboratory. In fact, we stated [see reference 7 in (*2*)] that "Detailed procedures are available to investigators interested in repeating these studies." Our intention was to provide interested colleagues with the information necessary to repeat our experiments as exactly as possible and thus to discourage variations of the procedure.

On the other hand, we do not believe that small discrepancies typically have been responsible for the failure of some investigators to replicate the "transfer" phenomenon. Clearly, in some attempts to repeat successful "transfer" studies, investigators have used different paradigms (*3*), different injection routes or dosages (*4*), or different behavioral or chemical procedures (*4, 5*) from those used in the experiments they purported to replicate, and such studies are not legitimate replications.

Again, we wish to express agreement with the intent of Corson's message and to apologize to the readers if what we believed was a clear plea for careful replication was misinterpreted.

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7 August 1970

Bat-Guano Cave Environment

Poulson and White, in a recent discussion on the utilization of caves as natural laboratories, suggested that the relatively constant cave environment, together with the comparative simplicity of cave communities, facilitates the study of evolutionary and ecological problems (*1*). This approach to biospeleology certainly gives emphasis to a particularly interesting aspect of caves (*1, 2*), but it tends to give the impression that all cave organisms live in environments of high constancy. It also detracts from the potential interest of those caves that contain animal communities of relatively high diversity—