Article Author:

Article Title: Suda I, Kito K, Adachi C; Bioelectric discharges of isolated cat brain after revival from years of frozen storage

Imprint: Amsterdam; Elsevier/North Holland,

ILL Number: 35579528
Bioelectric discharges of isolated cat brain after revival from years of frozen storage

ISAMU SUDA, KYOKO KITO AND CHIZUKO ADACHI

Department of Physiology, Faculty of Medicine, Kobe University, Ikuta-ku, Kobe (Japan)

(Accepted January 21st, 1974)

We reported previously that cat brains which had been isolated, frozen, and stored showed spontaneous electrical activity after thawing and perfusion with blood. The isolation of a brain was performed during an initial perfusion with a cooled balanced salt-dextran solution, frozen after subsequently employing a glycerol perfusion technique, and stored in vitro at —20 °C (refs. 3–5). The electrocorticogram obtained from the revived frozen brain resembled that of the normal in situ brain. However, the longer the period of preservation the slower was this basic rhythm. This paper reports results obtained in our attempt to investigate the effects of long-term storage, many years of maintenance in the frozen state, on spontaneous activity and structural integrity of brain tissues. This was determined by electrical recording and histological study.

Fig. 1 shows sample records of spontaneous discharges recorded from the right cerebellar culmen with a needle electrode insulated except at the tip. Isolation of the brain, glycerol treatment and storage were performed on August 19, 1965 and the tissue preserved in a deep freezer at —20 °C until November 11, 1972. After slow thawing over a period of more than 12 h as described before, the brain was revived by recirculation with diluted fresh cat blood at 39 °C under a pressure of 80/60 mm Hg and a pulsation rate of 120/min. Following exposure of the brain by removal of portions of the surrounding skull, well synchronized discharges of Purkinje cells were observed (Fig. 1A). This activity became desynchronized gradually (Fig. 1B and C) and finally transformed to an "avalanching" type of discharge (Fig. 1D) which continued for several minutes.

Spontaneous electrical activity could be recorded from the thalamic nuclei and cerebellar cortex for more than 4 h after the beginning of the reperfusion of warm blood. In this brain, however, an attempt to record unit activity from the cerebral cortex after an hour or so ended in failure.

Fig. 2A and B show the electrocorticograms of a brain revived after 7.25 years of storage in the frozen state. For comparison sample strips of electrocorticograms, shown in Fig. 2C and D, were obtained from a revived brain which had been kept for 777 days in the frozen state (—20 °C). In this latter case electrocortical activity could
be recorded for more than 5 h but in the former it could be observed for only 15–20 min. The activities observed in these revived brains have some features in common; electrical activities as a whole are much simpler than normal; they are characterized by large solitary deflections, slow waves, and rhythmic but continuing uniform wavelets which appear suddenly and then fade away for a while. This type of electrocortical activity resembles closely that of the neonate dog observed by Fox.

It is important to determine what the principal differences are in electrocortical activity of brains stored for 2 years and those maintained in the frozen state for 7.25 years. Dissimilarities in rhythmicity and in the mode of transition in frequencies of the brain waves were observed. In order to categorize and compare these differences in activity characteristics an autocorrelogram was devised as shown in Fig. 3. The abscissa of each band A to E in Fig. 3 represents time as proceeding from left to right. The numbers of stripes designate frequency per 0.95 sec and the intensities of stripes roughly correspond to the positive value of correlation functions where $\Delta \tau$ was taken as 10 msec. Each of the 5 bands shown corresponds to an electrocorticogram record of 15 sec duration. Thus, the pattern of fluctuation of rhythmic behavior of the
Fig. 2. Comparison of electrocorticograms recorded from a brain frozen for 7.25 years (A, B) and one thus maintained for 777 days (C, D). These are electromagnetic oscillograph recordings through appropriate bandpass amplifiers. A and B are recordings obtained with a monopolar electrode from the middle ectosylvian gyrus. C and D are bipolar records from sigmoid-suprasylvian gyri. Double headed vertical arrow designates 100 µV for A, B, 200 µV for C and 500 µV for D. With due consideration for modes of recording, it was concluded that neuroelectrical activity in A and B is much smaller than in C and D despite the analogous pattern.

electrocortical activity is seen at a glance. Each band A to E in Fig. 3 is representative of the most rhythmic portion of each autocorrelogram obtained from: A, a control brain anesthetized with Nembutal in situ; B, a 5-day frozen brain; C, a 203-day frozen brain; D, a 777-day frozen brain; and E, a brain frozen for 7.25 years. Fig. 3A and B are autocorrelograms obtained from the same brain before and after freezing. They differ little from each other. In Fig. 3C dark blotches are present indicative of irregularities of pattern and stripes show a loss of lateral continuity. These deteriorations in rhythmic pattern which had developed in the brain giving the record Fig. 3C were intensified as the period of freezing was extended. This is shown in Fig. 3D to E. In Fig. 3E the entire band shows a blotchy appearance with only faint short stripes. Therefore, it can be concluded that the frozen brain stored at —20°C after glycerol treatment gradually lost the ability to display a rhythmicity of electrocortical activity with the lapse of time. Moreover, it seemed that the magnitude of rhythmic activity decreased significantly during long-term frozen preservation (note the difference indicated by magnifications required in Fig. 2A, B and C, D). Histological evidence substantiates the presence of deterioration suggested by the abnormality in electrocortical
activity. It was found that the sections of the brain stored in a frozen state for 7.25 years show extensive reduction of cell density accompanied by evidence of severe hemorrhage. This could be found throughout the brain tissues but the more significant fact is that areas of well preserved cells and structures were also present and adjacent to regions where hemorrhage had occurred.

When one hemisphere of the brain tissue stored for many years (5–7 years) was examined histologically without reperfusion and compared with the other hemisphere which was reperfused, it was found that the latter revealed many cracks in tissues while the former did not. It seems that reperfusion for revival brought about enlargement of microclefts in tissues which might have been produced during freezing or thawing of the long-term frozen tissues. This may be a cause of the bleeding and the loss of activity in the revived brain.

The efficacy of cryoprotective agents such as glycerol, dimethyl sulfoxide (DMSO), polyvinylpyrrolidone (PVP, mol. wt. 40,000) and hydrogenated dextran (mol. wt. 63,000), and of storing temperature (−20 °C, −60 °C and −90 °C) was also examined for weeks of frozen preservation of the brain. Revival from −20 °C storage
with glycerol was the best condition for obtaining of integrative neuroelectrical activity and DMSO at —20 °C was the second best condition. Comparison experiment with glycerol vs. DMSO over years of preservation has still been continuing. No electrical discharge, however, could be observed at —90 °C preservation under employment of any cryoprotective agents and in use of PVP at any storing temperature.

From the above described observations, it can be concluded that: (1) it was possible to preserve viability of nervous tissues during many years in storage in the frozen state; (2) integrated neuroelectrical activity, such as brain waves, may be affected significantly by the dissolution of functions resulting from the fractures and associated damage to the tissues; (3) individual nerve cell activity, on the contrary, may be well preserved in spite of the degenerative processes.

We are grateful to Dr. Chandler McC. Brooks of the Downstate Medical Center, State University of New York, for kindly reading the manuscript and improving the English.

This work was supported by grants from the Ministry of Education (1969), the Waksman Foundation of Japan Inc. (1970) and USAR and D (DA-CRD-Ag-S92-544-67-G52).