J.Neuropsychiat. 1, 185-190 (1960). LSD 788

EFFECTS OF LSD-25, SEROTONIN AND SERA FROM SCHIZOPHRENIC PATIENTS ON ADULT MAMMALIAN BRAIN CULTURES

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ABSTRACT

Morphological changes of nerve cells, glial cells and boutons were studied in cultivated brain tissue by the use of time-lapse phase contrast microcinemaphotography. The morphological changes of the above structures, as vital reactions of the living cells to LSD-25, serotonin and sera from schizophrenic patients, are described and discussed. The ability of water soluble components of normal brain to prevent the long-term changes induced in neurons by schizophrenic sera is noted.

The successful cultivation *in vitro* of neurons and neuroglia from the cerebral and cerebellar gray cortex, as well as subcortical white matter, from adult mammalian brain (rabbits, monkeys and humans), has been previously described.[9] Such brain cultures have been maintained up to two years, through six to seven subcultures. In subcultures, almost pure neurons, mixed cultures of neurons and glia, or pure glial cultures were obtained. Neurons, glia and other cells present can be identified by their characteristic size, shape and internal structure in the living state, with phase-contrast microscopy (Figure 1). Neurons generally show a structural polarity, as the axon hillock and axon can often be distinguished from the rest of the perikaryon and dendrites. Neurons retain their special staining properties for Nissl substance and neurofibrils; and axons may also show evidence of myelinization after fixing and staining.

By the use of time-lapse phase-contrast microcinemaphotography, it is possible to study continuously, for periods of hours or days, the activity of brain cells. Pomerat[22a] gave a detailed description of the pulsatile activity of oligodendroglia in human brain cultures. Over a period of six years, a considerable amount of data has been accumulated in the author's laboratory on the internal movements of subcellular structures within neurons: characteristic movements and changes in optical properties of the cells as a whole; the relationship developing between neurons, and between neurons and glia; the formation of synapses; and changes in morphology.[9]

The effect of drugs and of biogenic substances on these properties of the brain cultures has been studied during the past five years. This paper is concerned primarily with the direct effect of sera from acute schizophrenic patients (not receiving drugs), of LSD-25, and of serotonin, on mixed cultures and subcultures of neurons and glia, as observed with time-lapse phase-contrast microcinemaphotography. In each case, long sequences were photographed and movie films were prepared and presented.[10, 12, 13]

Technique

Cultures and subcultures used as test ob-

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This study was supported by a grant from the Mental Health Fund, Illinois State Department of Public Welfare.

Acknowledgments: The author wishes to express her thanks to Dr. Ivan Boszormenyi-Nagy, who obtained (from selected patients) a great part of the schizophrenic sera used in this work; to Dr. Dean Tasher for other schizophrenic sera tested; and to Dr. Percival Bailey for helpful observations and discussion. The microphotographs were made by Norman Bartley and Richard Cross.

Received for publication: February 15, 1960.

jects are maintained by the lying-drop double cover slip method of Maximow. Explants are embedded in a coagulum, enabling the cells to migrate out, form associations, grow processes and remain undisturbed in their associations over a period of months. The feeding solutions, always containing homologous sera in the supernatant, are changed every three days. Details of maintaining cultures have been previously described.[9] In a large number of experiments, sera from acute schizophrenic patients were substituted for the usual normal sera used in the media. In other experiments, lysergic acid diethylamide (LSD-25), in a concentration of 0.0002 to 0.001 γ/ml. supernatant, was added to the supernatant of normal cultures. Testing of serotonin was carried out in the same manner, using concentrations of 0.5 to 2.0 γ/ml. supernatant. Immediately on addition of the substance to the culture, time-lapse microphotography (using phase-contrast optics) was commenced at the rate of four frames per minute. In some cases, control films were taken on the same cell before addition of the substances being tested. In order to avoid complicating factors (such as prolonged exposure to light) before the addition of substances being tested, numerous controls were run on comparable cells in cultures from the same material.

Effect of LSD-25

The addition of LSD-25 has a visible action on structual elements of neurons in culture. Within 15 to 20 minutes after the addition of LSD-25 (0.001 γ/ml.), granules which have been concentrated around the nuclear membrane become dispersed throughout the surrounding cytoplasm (Figures 2 and 3); the body of the neurons as a whole shows some contraction, and the processes of the axonal endings and dendrites may retract; the movement usually seen at synaptic endings is slowed down; the nucleus shows an increase in optical density; the rate of movement of the nucleolus and nucleolar satellite increases; the nucleolus enlarges, becomes irregular in shape and shows sharp differences of refractivity.

Nuclear changes similar to those caused by LSD-25 occur also when the cells are stimulated by Metrazol, electric current,[8] etc., and are usually accompanied by increased production and accumulation in the cytoplasm of liponucleoprotein aggregates (Nissl bodies). However, under the effect of LSD-25, in spite of the changes in the nucleus, a diminution, instead of an accumulation, of Nissl substance is seen. The neurons gradually show a decrease in osmophilia (Figure 4), and, with prolonged exposure to this concentration of LSD-25, chromatolysis results. Application of LSD-25 at this concentration, during the first few minutes, causes contraction of oligodendroglia, which then gradually expand and remain in this state for several hours (Figure 5). When LSD-25 is washed out, and the feeding solution renewed, the observed effects are usually reversed within a half hour, often accompanied by an increased number of glia (Figure 3-c). A lower concentration of LSD-25 (0.0002 γ/ml.) also causes the observed effects on glia and changes in the neuron, which gradually reverse.

Effect of Serotonin

Serotonin, in concentrations of 0.5 γ to 2.0 γ/ml., has a marked effect on cytoplasmic movements in the neuron. Normally, in the perikarya of neurons, slight pulsatile movements of the cytoplasm can be inferred by the slow rhythmic movements of granules, when observed with time-lapse microphotography at the rate of four frames per minute. However, when serotonin is added, a slow rhythmic expansion and contraction of the whole perikaryon develops, imparting a massive rhythmic pumping movement to the neuron. The time required for one cycle of contraction and expansion varies between 45 minutes and 1½ hours (Figure 6). During the slow contraction phase, cytoplasm is seen to be forced down into the axon. When this occurs, the proximal portion of the axon bulges, and the axon as a whole becomes more rigid (Figure 7). In the perikaryon and dendrites, the liponucleoprotein becomes more aggregated and the thionine stain more dense, accompanied by an increased osmophilia. Fine undulating membranes may appear at the

edges of the neuronal perikaryon or its processes.

Irreversible changes occur in certain neurons after prolonged exposure to serotonin (24-48 hours of 2 γ/ml.), even if the drug is subsequently washed out of the culture. These changes are accompanied by loss of staining properties of the nucleoplasm, which normally stains a reddish color with May-Grünwald-Giemsa stain, thus indicating a change in the form or content of the usually highly polymerized desoxyribonucleic acid. Within one to three days, the nerve cells undergo chromatolysis and completely degenerate. However, if, instead of the usual feeding solution, a feeding solution containing a Tyrode's extract of brain tissue is added, the neurons recover form the prolonged effect of serotonin—thus far, after 48 hours. The rhythmical pulsating movement of oligodendroglia, when present, is gradually arrested in the contracted state (Figure 8). Murray [21] has described a similar arrest in the contractile state of oligodendrocytes in cultures of human fetal brain with a higher concentration (5 γ/ml.). Microglial motility (whether the cells are in a rod form or in gitter cell form) is unaffected, as are certain movements of protoplasmic astrocytes (Figure 9).

Effect of Sera from Schizophrenic Patients

When, in place of normal human sera, sera from patients with acute schizophrenia are used over a period of ten days in the feeding solution of cultures or subcultures of adult human cerebral cortex, a series of changes occurs in the morphology of the neuron and in the nature of its cytoplasm. Glial motility is enhanced throughout this period. During the first few days after the addition of schizophrenic serum, the Nissl granules appear more aggregated—at first around the nuclear membrane. This effect is seen one to two hours after the addition of the schizophrenic serum. During this period, the cytoplasm of the neurons shows increased pulsatile and pumping movements; fine undulating membranes appear on cell surfaces as well as on axons and dendrites; the spines on the dendrites become more noticeable and retract and extend (Figure 10); the degree of pumping movement varies with the type of neuron and the area form which it originates; the boutons appear more dense; the oligodendroglia increase their rate of movement, and, when present as perineuronal satellites, their transfer of material to the neuron becomes more evident (Figure 11). Along the length of the axon, the oligodendroglia show an increased rate of spiderlike movement. Protoplasmic astrocytes and microglia also show increased motility (Figure 12). The increased acceleration of glial movements persists throughout the ten-day period—the longest period in which an individual serum has been studied.

The increased cytoplasmic activity, manifested initially by the neurons, gradually disappears and is rarely manifest after 72 hours. At this later stage, the dendrites show a tendency to flatten out, and may retract. The liponucleoprotein aggregates gradually become smaller and more diffuse (as seen with LSD-25), and tend to disappear. By the tenth day, or even sooner, the shape of the neuron and its staining properties can undergo a complete change (Figure 13).* Sera tested thus far have retained their activity up to three weeks when kept at 4° C. When a Tyrode's extract of brain (not necessarily homologous) has been added to the supernatant of the culture along with the schizophrenic sera, this has prevented the major long-term changes otherwise caused by the schizophrenic serum.

Effect of Brain Extracts

It was observed previously [8] that brain tissue contains a substance or substances which can protect the neurons against moderate amounts of drugs or other damaging agents. These protecting factors can be extracted from the brain by Tyrode's solution. Well-centrifuged Tyrode's extracts of rabbit brain effectively protect not only rabbit brain cells, but also those obtained from monkeys

* These findings may have some bearing on changes in brain cells of schizophrenic patients described in the literature.[16, 19, 23]

or humans, showing that this factor(s) is not species specific. Although all cells in the culture (even those still in the explant) react to LSD-25, serotonin and schizophrenic serum, cells which have migrated from the explant show greater sensitivity, particularly those which have been long in culture or are in subculture. All of the photographs presented in this paper are of cells in subcultures.

The changes in the neurons brought about by LSD-25, serotonin and schizophrenic sera vary in degree with different types of neurons and seem to depend also on the area from which the cells derive, as well as on the condition of the neuron in culture prior to the addition of the drugs. For example, the Nissl substance of neurons from the cerebellar cortex underwent more rapid changes than the Nissl substances in neurons from the cerebral cortex under the influence of serotonin. However, chromatolysis was less evident in the former. Pumping movements were most marked in neurons from the occipital area, with or without the presence of glia, under the influence of serotonin. On the other hand, schizophrenic sera seem to induce the greatest increase in pulsing and pumping movements in neurons obtained from the temporal lobe.

It has been suggested that a lack or excess of serotonin may play a role in mental disturbance.[28, 29] Depressed conditions are treated with monoamine oxidase inhibitors (iproniazid, etc.),[17, 25] which allegedly act by increasing the concentration of serotonin or noradrenaline in the brain.[4, 5, 7] The evidence for this action, however, is highly contradictory;[6, 27] there is also evidence that serotonin may facilitate axonodendritic synaptic transmission. The present experiments show that serotonin increases the size and density of fine fibers and endings of dendrites. Moreover, the initial effect of serotonin on the nuclear apparatus, Nissl granules and osmophilia of the neuron is somewhat similar to that observed during electrical or chemical stimulation of the neurons (Metrazol, adrenaline, noradrenaline).[8, 14] In fact, a neuron, after prolonged exposure to serotonin, resembles a fatigued neuron. Oligodendroglia in the contracted state were often observed to extrude cellular material into the surrounding environment, and possibly into the neurons. One of the most striking effects of serotonin—namely, the induction of marked pumping movements—may be an exaggeration of the normal slow cytoplasmic movements in the neuron. Rhythmic contraction of the neuron may be part of the normal mechanism by which it supplies its processes with those metabolites which are synthesized in the nucleus and perikaryon.

When a long time-lapse movie is taken of a neuron under the usual culture conditions (over a period of several days) at a slow rate of two frames per minute, and the movie is then projected at a speed of 24 frames per second, it is seen that the neuron normally shows some pumping movement, each cycle of which requires six to eight hours. It is likely that neurons, like glia, also give off substances to their milieu during the phase of contraction, and during expansion take up material from the environment which includes the glia. The appearance of fine undulating membranes on the neurons in the presence of serotonin, and even more so after the addition of schizophrenic serum, is also conceivably associated with a change in surface permeability and with uptake of substances from the environment. The increased movements of the spines on the apical and basal dendrites, as well as the increase in their optical density and osmophilia, may also indicate an increased metabolism at these sites, accompanied by an exchange of substances with their surroundings. One effect of all the substances tested here was the increased sensitivity of the neurons to light. Those treated with LSD-25 became most sensitized.

Evidence has been accumulated in the past, describing a number of specific changes in the properties of blood,[24] red cells,[2, 26] the accumulation of substances in the blood[18] and urine,[3, 20] etc., of schizophrenic patients. Of most recent interest are the extensive studies of Heath *et al.*,[15] who were able, by injecting into normal individuals sera (or fractions of serum proteins) prepared from

blood of schizophrenic patients, to induce for short periods some of the symptoms of the disease.

During the past six years, hundreds of different sera and cord sera from normal individuals have been used for making up feeding solutions for human brain cultures. None of these "normal" sera induced the kinds of changes caused by sera from schizophrenic patients (none of whom were under drug treatment). Since the content of ceruloplasmin is unusually high in cord sera, and cord sera did not produce any such changes, we can rule out the possibility that these changes are due to ceruloplasmin.

In addition, the factors present in schizophrenic sera which induce the above-described changes in brain cells are much more stable than ceruloplasmin in sera.[1] The fact that a water-soluble component of normal brain is able to prevent the long-term changes induced in neurons by schizophrenic sera suggests that normal brain contains substances which are able to neutralize certain specific substances present in the schizophrenic sera; or that brain cells in culture may not contain these protecting substances in sufficient quantity, and are therefore dependent on their constant supply by the added serum. This need of the neurons may be greater when metabolic stimulants are present. The reversal of early chromatolysis (caused by overstimulation of the neuron by Metrazol, adrenaline, serotonin, etc.), by the addition of Tyrode's extract of normal brain to the feeding solution, also points to the possibility of the supply of additional necessary compounds.

Osmond and Hoffer[22] have collected evidence indicating that some disturbance in the metabolism of adrenaline may be connected with schizophrenia. They suggest that accumulation of certain adrenochromes or adrenolutin may be responsible for the symptoms of schizophrenia. In the author's experiments, adrenochrome (prepared by L. G. Abood) was used in the same low concentration (0.001 γ/ml.) as that of adrenaline and noradrenaline; and the effects of these compounds were compared. Adrenochrome induced much more rapid and drastic changes in neurons than did either adrenaline or noradrenaline in the same concentrations: Within 24 hours it killed those neurons which are outside the area of the explant. However, it should be kept in mind that in all the above studies we were dealing with direct effects of drugs, etc., on neurons and glia without the presence of the blood-brain barrier.

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