# EFFECTS OF REICHSTEIN'S COMPOUND S, STRYCHNINE AND LEPTAZOL PERFUSED THROUGH CEREBRAL VENTRICLES OF CATS

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Methods recently developed for perfusing the cerebral ventricles in the anaesthetized cat have made it possible to investigate the action of drugs on the nervous structures contained in the walls of the cerebral ventricles. With these methods tubocurarine was found to produce a number of central excitatory effects, some of which resulted from an action on structures in the walls of the third ventricle; others from an action on structures in the walls of either the anterior or the inferior horn of the lateral ventricle (Carmichael, Feldberg & Fleischhauer, 1962, 1964; Feldberg & Fleischhauer, 1962, 1965). None of these effects occurs when tubocurarine is injected intravenously because it does not cross the blood-brain barrier, or at least not in sufficient amounts.

In the present experiments the cerebral ventricles of the cat were perfused with either Reichstein's compound S ($17\alpha$-hydroxy-11-desoxycorticosterone), strychnine or leptazol, three substances which are able to cross the blood-brain barrier and produce convulsions when injected intraperitoneally or intravenously. The experiments were undertaken to determine what central effects are produced when these convulsants are confined in their action to the walls of the cerebral ventricles, and to what extent these effects contribute to the convulsive activity observed on their intraperitoneal or intravenous injection. In addition, the effect of hippocampal activation on the seizure threshold for intravenous leptazol was investigated.

## METHODS

The experiments were performed on cats of either sex, weighing 2.3–3.3 kg. In a few experiments Reichstein's compound S was injected into the cerebral ventricles of unanaesthetized cats through a Collison cannula implanted aseptically into the left lateral ventricle under pentobarbitone sodium anaesthesia 10 days previously. All other experiments were performed under chloralose anaesthesia after induction with ethyl chloride and ether to allow cannulation of the left femoral vein for injection of the chloralose (60 mg/kg). The trachea was cannulated and, with the cat lying on its abdomen, the head was fixed to the ear bars and mouthpiece of a Horsley-Clarke stereotaxic instrument.

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The methods of perfusing drugs through the different regions of the cerebral ventricles were those described by Carmichael *et al.* (1964). The perfusion fluid was the artificial cerebrospinal fluid of Merlis (1940). It had a pH of 8.4 and its composition was (g/l.): NaCl 8.1, KCl 0.25, $CaCl_2$ 0.14, $MgCl_2$ 0.11, $NaHCO_3$ 1.76, $Na_2HPO_4$ 0.07, glucose 0.61, and urea 0.13. The rate of perfusion from each cannula was either 0.1 or 0.05 ml./min.

Drugs were administered intravenously either by single injections or by continuous infusion into the femoral vein. For continuous infusion both femoral veins were cannulated so that intravenous injections could be made into one vein without interrupting the infusion into the other.

The electrical activity of the frontal and occipital regions of the cerebral cortex, the dorsal hippocampus, and amygdala was recorded with monopolar electrodes as described by Feldberg & Fleischhauer (1962) with an Offner Type R Dynograph or an Ediswan Pen Oscillograph. To prevent movements of the cat from interfering with the recording of the electrical activity of the brain, gallamine (20 mg) was usually injected intravenously and the cat was ventilated artificially, care being taken to avoid hyperventilation. Additional injections of gallamine were given as required in the course of the experiments. The gallamine did not affect the records of the electrical activity of the brain.

In some experiments the outflow from the cannulated aqueduct, blood pressure from the cannulated right femoral artery, and rectal temperature were recorded. The outflow was recorded with a hand operated signal marker, and the blood pressure with a Bell & Howell pressure transducer, both of which were coupled to the Dynograph. Rectal temperature was measured by a thermistor probe inserted 10–12 cm into the rectum and was monitored continuously by a Kent multi-channel recorder. Figure 3 is plotted directly from the tracing obtained in this way.

*Drugs.* These were the hemisuccinate salt of Reichstein's compound S (17α-hydroxy-11-desoxycorticosterone), kindly given to us by Dr H. R. Reinert, of Pfizer Ltd., strychnine hydrochloride, leptazol, gallamine triethiodide (Flaxedil, May & Baker) and d-tubocurarine chloride. Doses and concentrations refer to the salts except for leptazol.

<div align="center">RESULTS</div>

*Reichstein's compound S (17α-hydroxy-11-desoxy-corticosterone)*

Heuser & Eidelberg (1961) found that an intraperitoneal injection of the hemisuccinate salt (300–400 mg/kg) into the unanaesthetized cat caused convulsions and, on the electro-encephalogram, a seizure discharge which originated in the hippocampus. In the present experiments, injections of 1–1.5 mg into the cerebral ventricles of the unanaesthetized cat did not produce convulsions or any other visible changes. Larger amounts were not tested because they could not be dissolved at neutral pH in the small volume of saline solution (0.5 ml. or less) used for intraventricular injection.

The only effects observed in the anaesthetized cat on perfusion of the cerebral ventricles with compound S from left lateral ventricle to aqueduct were shivering and, on the electrocorticogram, a rhythmic discharge of abnormal high voltage surface negative spikes. These effects occurred only with strong concentrations of the compound.

On perfusion with compound S in a concentration of $10^{-3}$, which was subthreshold for eliciting the spike discharge, shivering occurred in both flanks after a latnecy of 3–20 min. It then spread within a few minutes to the trunk, shoulders and neck. In some experiments it occurred first in bursts at the end of each expiration, but later became continuous and more vigorous, only to abate again in the course of the perfusion. The site of action is probably the hypothalamus, because shivering did not occur when compound S was perfused through the inferior or anterior horn alone or, as happened in two experiments, had failed to enter the part of the third ventricle lying ventral to the massa

intermedia. This was verified at the end of these experiments when the dye bromophenol blue was perfused through the cerebral ventricles; the walls of the ventral half of the third ventricle remained unstained.

To obtain the rhythmic discharge of high voltage surface negative spikes, compound S had to be perfused through the cerebral ventricles in a concentration of $2.5 \times 10^{-3}$ or $2 \times 10^{-3}$. After a latency of 20–70 min spikes appeared in the left occipital lead at a frequency of 4–7/min, which increased slightly on continued perfusion. There was little spread of the discharge to other cortical leads. The spikes were single and usually remained so. In a few experiments they became multiple, but even then the after-discharge following each spike was short and consisted of only one or two deflections. In one experiment an episode—that is, a brief period of fast synchronous surface negative activity—interrupted the spike discharge shortly after its onset.

The spike discharge resulted from an action on structures lining the inferior horn, since it did not occur when compound S was perfused through either the anterior horn or the third ventricle, or both. It did occur, however, when the inferior horn alone was perfused with compound S. The discharge originated from the hippocampus, for, if the electrical activity were recorded simultaneously from the occipital cortex, hippocampus and amygdala it did not appear in the cortex until it had appeared in the hippocampus; it either appeared simultaneously in both leads or a few hippocampal spikes preceded the onset of the discharge in the occipital cortex. In the lead from the amygdala the spikes appeared later, sometimes several minutes after their appearance in the other two leads.

A typical experiment is illustrated in Fig. 1. In (b) are seen the first three spikes. They appear in the hippocampal lead, 66 min after the beginning of the perfusion with compound S ($2 \times 10^{-3}$). In (c), recorded a few minutes later, the size of the hippocampal spikes has increased approximately twofold; spikes have now appeared in the ipsilateral occipital cortex, and there is some spread to the contralateral occipital cortex, but no
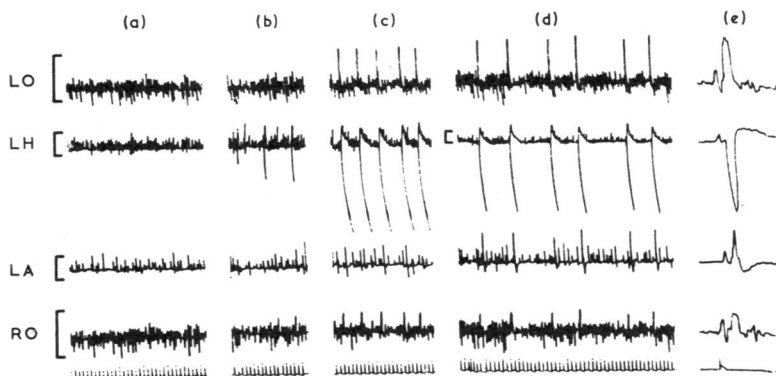


Fig. 1. Monopolar records of electrical activity from left and right occipital cortex (LO and RO), left dorsal hippocampus (LH) and left amygdala (LA) of 2.5 kg cat anaesthetized with chloralose. (a) during perfusion with artificial cerebrospinal fluid from both cannulated lateral ventricles to aqueduct; (b), (c), (d) and (e) 67, 69, 85 and 87 min after switching over to perfusion with Reichstein's compound S ($2 \times 10^{-3}$) from left lateral ventricle while perfusion with artificial cerebrospinal fluid from right lateral ventricle was continued. Infusion rate through each cannula 0.1 ml./min. Calibration 900 $\mu$V; negativity upwards; time marker in sec.

spikes have as yet appeared in the lead from the amygdala. They are present in (d), taken a few minutes after (c). In the meantime, the voltage of the hippocampal spikes has increased and the amplifier gain is therefore reduced. Record (e) shows a single spike in all four leads at a faster speed.

Carmichael *et al.* (1964) obtained a discharge of " slow waves " on the electrocorticogram when they perfused tubocurarine through the anterior horn ; the " slow waves " then acted as pacemaker and increased the frequency of the hippocampal spike discharge produced by tubocurarine perfused through the inferior horn. No discharge of " slow waves " occurred when compound S was perfused through the anterior horn ; further, when such a discharge had been established with tubocurarine it did not increase the frequency of the hippocampal spikes produced on perfusion of compound S through the inferior horn.

*Strychnine*

The only effect strychnine produced on perfusion through the cerebral ventricles, which was not obtained on intravenous infusion as well, was a rhythmic discharge of surface negative single spikes which originated in the hippocampus. On perfusion with strychnine ($10^{-4}$) from left lateral ventricle to aqueduct, the spikes appeared in the lead from the ipsilateral occipital cortex after a latency of 10–20 min, and quickly attained a frequency of 10–15/min. When perfusion was continued for over an hour, the spikes remained single, the frequency remained constant and episodes did not occur. The discharge did not spread to the other cortical leads. On perfusion with stronger concentrations, up to $2 \times 10^{-3}$, the only difference obtained was a spread of the discharge to the contralateral occipital and a very slight spread to the ipsilateral frontal cortex, but the spikes remain single, their frequency did not increase, and again episodes did not occur.

The spike discharge differed from that obtained by Feldberg & Fleischhauer (1962) with tubocurarine similarly perfused because in their experiments the spike discharge was interrupted by episodes, the spikes became multiple—that is, each spike was followed by a long lasting after-discharge—and their frequency increased to 30–40/min. These results were obtained on perfusion with tubocurarine in strong concentrations ($2 \times 10^{-4}$ or $5 \times 10^{-4}$). However, in the present experiments, when tubocurarine was perfused in weaker concentration ($5 \times 10^{-5}$) the spikes remained single, their frequency scarcely increased, and episodes did not occur. This spike discharge thus resembled that obtained with strychnine perfused in either weak or strong concentrations.

The spike discharge occurred on perfusion with strychnine through the inferior horn alone, but it did not occur when strychnine was perfused through the anterior horn or the third ventricle, or both. A discharge of " slow waves " was not produced with strychnine perfused through the anterior horn.

Figure 2 shows the effect of strychnine ($2.5 \times 10^{-4}$) when perfused from left lateral ventricle to aqueduct, on the electrical activity recorded from the left occipital and frontal cortex, hippocampus and amygdala. Seventeen minutes after beginning the strychnine perfusion the first spikes appeared simultaneously in the hippocampal and occipital leads (at (b)). It took several minutes before they appeared in the lead from the amygdala
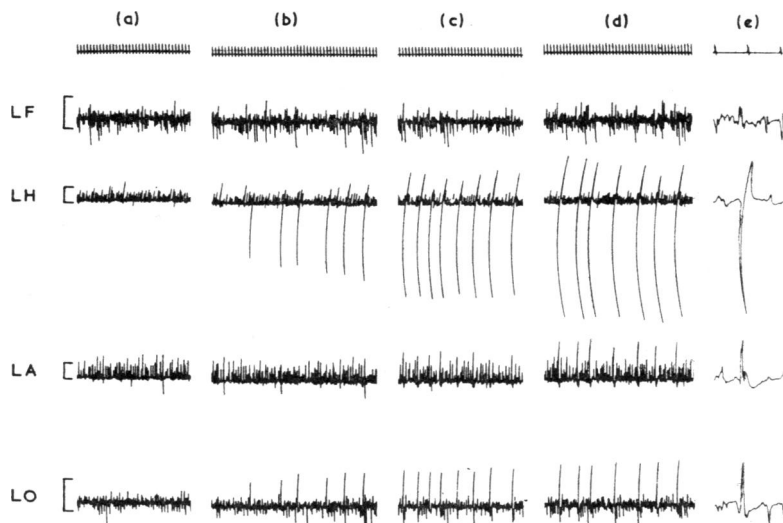
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Fig. 2. Monopolar records of electrical activity from left frontal and occipital cortex (LF and LO), left dorsal hippocampus (LH) and left amygdala (LA) of 2.5 kg cat anaesthetized with chloralose, paralysed with gallamine and artificially ventilated. (a) during perfusion with artificial cerebrospinal fluid from both cannulated lateral ventricles to aqueduct; (b), (c), (d) and (e) 17, 22, 33 and 38 min after switching over to perfusion with strychnine ($2.5 \times 10^{-4}$) from left lateral ventricle while perfusion with artificial cerebrospinal fluid from right lateral ventricle was continued. Infusion rate through each cannula 0.1 ml./min. Calibration 500 $\mu$V; negativity upwards; time marker in sec.

(at (c)) and even 11 min later (at (d)) there was scarcely any spread to the left frontal cortex. An indication of such spread is perceptible with the faster recording at (e). In other similar experiments a few spikes were recorded in the hippocampal lead before they appeared ·in the cortical one.

Other effects seen on perfusion of strychnine from a lateral ventricle to aqueduct were also obtained on its intravenous infusion, and might therefore result from the absorption of strychnine into the blood stream. These effects were increased sensitivity to auditory and tactile stimuli such as a loud clap or tapping the spine with a finger. This hyper-excitability developed in a characteristic sequence. First, each tap or clap produced a single positive spike synchronous in all cortical leads. When these stimuli were repeated about 2 sec apart only the first few evoked spikes, then there was extinction of the response for several seconds. As the sensitivity increased, the taps, and later the claps, evoked jerks which also became more violent as perfusion with strychnine continued. The jerks when fully developed took the form of a forceful propulsion of the trunk of the cat due to a sudden extension of the limbs, which lifted the body several mm. from the table. On repeated tapping, there was at first extinction of the motor response, but as excitability increased this no longer occurred, and there was also no extinction of the spikes evoked on repeated stimulation. In this condition previously ineffective stimuli, such as snapping the fingers or simply touching the cat's eyelid or the skin over its lumbar spine, resulted in violent jerks. A few minutes later similar jerks occurred

without the application of external stimuli. Before this happened there were in some experiments occasional limb movements or twitching of the tail, again without application of external stimuli.

Positive spikes independent of the application of external stimuli appeared early in the development of the increased excitability. They occurred synchronously on all cortical leads at irregular intervals, and did not differ from the positive spikes evoked by auditory or tactile stimuli. On continued perfusion with strychnine they became more frequent and biphasic—that is, the surface positive deflection was followed by a small surface negative one.

An intravenous injection of gallamine had no effect on the positive spikes, whether evoked by external stimuli or not, although it prevented the motor effects.

In a few experiments in which strychnine ($10^{-3}$) was perfused through the third ventricle alone, none of the motor or electrocortical effects occurred. They developed, however, when perfusion with strychnine was confined to either horn of a lateral ventricle.

On the intravenous infusion of strychnine increased excitability developed in the same way as on intraventricular application, but the abnormal discharge of surface negative spikes in the occipital leads did not occur. In one cat the increased excitability developed after 20 min intravenous infusion of 0.05 $\mu$g/kg min, in another after 15 min infusion of 0.5 $\mu$g/kg min. Intravenous infusion of a much larger amount (80 $\mu$g/kg min) did not produce either the abnormal discharge of surface negative spikes in the occipital leads, or seizure-like activity in the electrocorticogram, but positive spikes occurred at a high frequency, over 90/min, and became biphasic.

*Leptazol*

The only effect obtained with leptazol perfused from left lateral ventricle to aqueduct, even in concentrations as strong as $5 \times 10^{-3}$ to $2.5 \times 10^{-2}$, was shivering. No other motor effects, no autonomic responses and no changes in the electrocorticogram were produced.

Shivering began in the flanks within a few minutes of the leptazol perfusion, spread quickly over the whole body, became more and more vigorous, and then persisted, usually unabated, for as long as the leptazol perfusion was continued—that is, up to 90 min.

The effect could be attributed to an action on the hypothalamus, because it occurred when the leptazol was perfused through the third ventricle alone, and not when the third ventricle was excluded from the leptazol perfusion—that is, on perfusion of leptazol through the left ventricle or through either its anterior or inferior horn. The shivering produced a rise in rectal temperature as illustrated in the experiment of Fig. 3. In this experiment shivering began within a minute of perfusion of the third ventricle with leptazol ($10^{-2}$) and rectal temperature began to rise within 2 min. On perfusion of the third ventricle with weaker concentrations, ($10^{-3}$) of leptazol, some shivering, confined to the flanks, was obtained.

The shivering was reduced or abolished by adrenaline perfused through the third ventricle. In the experiment of Fig. 3 vigorous shivering, which had been going on all over the body during the leptazol perfusion, was greatly reduced and became restricted to the flanks when adrenaline ($10^{-5}$) was added to the perfusion fluid as well. Shortly afterwards temperature began to fall.
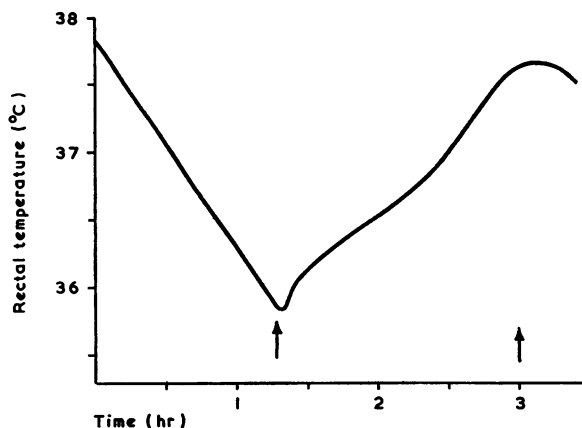
Fig. 3. Record of rectal temperature of 3.5 kg cat anaesthetized with chloralose injected about 1 hr before beginning of record. About 0.75 hr after beginning of record, third ventricle and aqueduct were cannulated, and perfusion of third ventricle with artificial cerebrospinal fluid was begun. From first arrow, perfusion was continued with leptazol ($10^{-2}$), from second arrow with leptazol ($10^{-2}$) plus adrenaline ($10^{-5}$). Infusion rate, 0.05 ml./min. throughout.

When the inferior horn had been perfused for 1 hr with leptazol ($2.5 \times 10^{-2}$), a subsequent perfusion with tubocurarine ($2 \times 10^{-4}$) brought on the typical spike discharge, interrupted by episodes, recorded from the occipital cortex. Thus perfusion with strong concentrations of leptazol does not affect the excitability of the hippocampus, at least not as far as stimulation by tubocurarine is concerned.

*Effect of hippocampal activation on seizure activity in electrocorticogram produced by intravenous leptazol*

As shown by Feldberg & Fleischhauer (1962, 1965) hippocampal activation occurs when tubcurarine is perfused through one or through both inferior horns. The procedure adopted in the present experiments was to perfuse one (the left) or both horns for 1 hr with tubocurarine ($2 \times 10^{-4}$) or with artificial cerebrospinal fluid before leptazol was given intravenously either by repeated injections or by continuous infusion, while perfusion of the cerebral ventricles was continued. The tubocurarine perfusion caused, within 10–15 min, the appearance of large surface negative spikes interrupted by episodes in the left, or in both, occipital leads. The spikes, initially single, became multiple, their frequency increased and they spread to the frontal leads during the hour preceding the first leptazol injection.

*Repeated intravenous injections of leptazol.* Intravenous injections of 30 mg of leptazol made at 20 min intervals to cats weighing 2.4–2.7 kg resulted in the appearance, within seconds, of surface positive spikes on the electrocorticogram. The voltage and frequency of the spikes decreased during the intervals but with each injection the spikes became larger and more frequent and continued for a longer time. Often they became biphasic, the surface positive deflection being followed by a surface negative one. In the control experiments, no further changes developed on the electrocorticogram with the first five injections, but the sixth injection precipitated a seizure discharge—that is, a

period of very fast high voltage activity which consisted of biphasic but mainly surface positive deflections. A typical experiment is illustrated in Fig. 4. The fifth injection (at the arrow in (b)) resulted in considerable augmentation of the spiking residual from the fourth injection. The sixth injection (at the arrow in (c)) precipitated a seizure discharge after several seconds of increased spiking, some of the spikes being biphasic. Seizure activity began in the frontal but later became more pronounced in the occipital leads. This sequence was observed in most experiments. Seizure activity ended abruptly and simultaneously in all leads, after several seconds of slower and more regular activity.
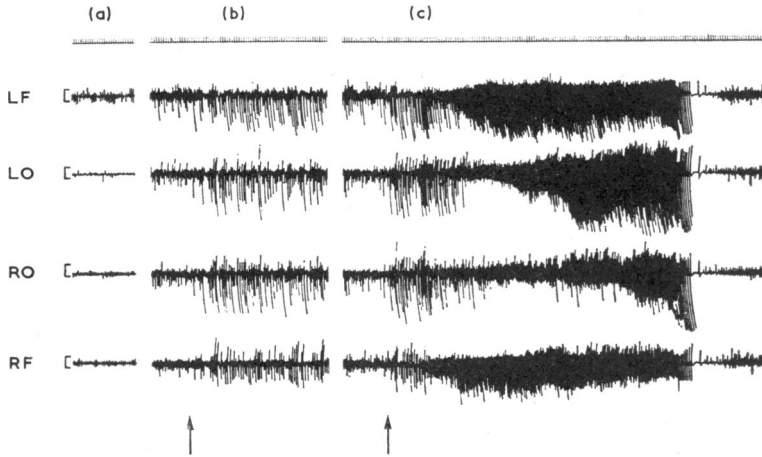


Fig. 4. Monopolar records of electrical activity from left and right frontal (LF and RF) and occipital (LO and RO) cortex of 2.5 kg cat anaesthetized with chloralose, paralysed with gallamine triethiodide and artificially ventilated. Intravenous injections of leptazol (30 mg) were made at 20 min intervals. (a) taken before the first, (b) and (c) during the fifth and sixth injection given at the arrows. Perfusion from both cannulated lateral ventricles to aqueduct with artificial cerebrospinal fluid was begun 1 hr before the first injection and continued throughout the experiment. Infusion rate through each cannula, 0.05 ml./min. Calibration 500 $\mu$V ; negativity upwards ; time marker in sec.

Figure 5 illustrates with faster recording the different phases of a leptazol seizure in another experiment: the seizure activity initially more pronounced in the frontal and later in the occipital leads (in (a) and (b)) ; the slower and more regular activity before the end of the seizure (in (c) and (d)) ; afterwards small regular oscillations recorded from all leads and continuing for about 20 sec at a frequency of about 10/sec (in (d)) ; finally, the return to normal background activity with intermittent spiking (in (e)).

Each seizure was associated with a rise in arterial blood pressure of 30–50 mm Hg, profuse salivation and a pronounced reduction or cessation of outflow from the cannulated aqueduct. Since the heart rate usually increased greatly with repeated leptazol injections before a full seizure developed, a further increase did not always occur during the seizure unless it was a severe one.

The rise in blood pressure and the cessation of outflow from the perfused ventricles are illustrated in the experiment of Fig. 6. As seizure activity began and the blood
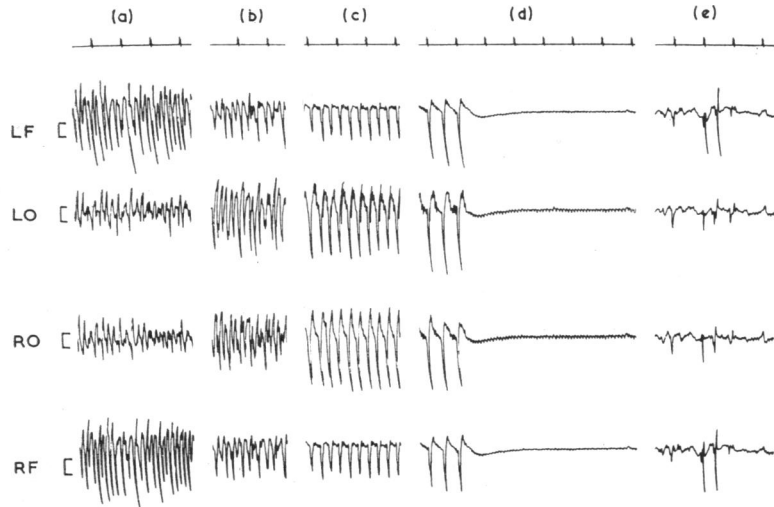
Fig. 5. Monopolar records of electrical activity from left and right frontal (LF and RF) and occipital (LO and RO) cortex of 2.6 kg cat anaesthetized with chloralose, paralysed with gallamine triethiodide and artificially ventilated. Intravenous injections of leptazol (30 mg) were made at 20 min intervals. (a), (b), (c), (d) and (e), taken 80, 131, 155, 167 and 207 sec after eighth injection. Perfusion from both cannulated lateral ventricles to aqueduct with artificial cerebrospinal fluid was begun 1 hr before the first injection and continued throughout the experiment. Infusion rate through each cannula, 0.1 ml./min ; calibration 500 $\mu$V ; negativity upwards ; time marker in sec.
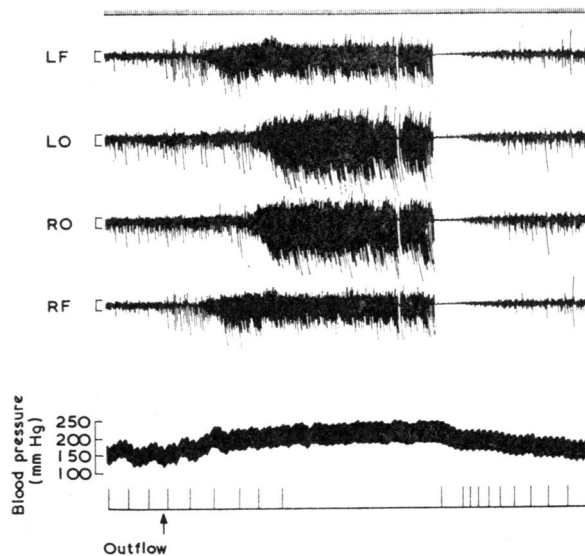


Fig. 6. Monopolar records of electrical activity from left and right frontal (LF and RF) and occipital (LO and RO) cortex as well as records of arterial blood pressure and outflow (in drops) from cannulated aqueduct in 2.5 kg cat anaesthetized with chloralose, paralysed with gallamine triethiodide and artificially ventilated. At the arrow intravenous injection of leptazol, 30 mg (during the preceding 2 hr, 175 mg of leptazol had been injected intravenously in doses of 5–40 mg at various intervals). Artificial cerebrospinal fluid was perfused from both cannulated lateral ventricles to aqueduct. Infusion rate through each cannula, 0.1 ml./min ; calibration 500 $\mu$V ; negativity upwards ; time marker in sec.

pressure was rising, the outflow slowed down and then stopped. When the seizure activity ended the outflow recurred, initially at a greatly increased rate, while the blood pressure was falling. Motor effects associated with the leptazol seizures were prevented because the cats had received intravenous gallamine.

In those experiments in which the hippocampus was first activated by tubocurarine, the only differences observed were that seizures developed after fewer injections and occurred more frequently than in the control experiments. This facilitation of leptazol seizure activity is illustrated in Fig. 7, which shows diagramatically the seizures which occurred on the electrocorticogram and their duration following repeated injections of 30 mg of leptazol.

In the first four control experiments the first seizure developed after the sixth injection, but each subsequent injection did not bring on another seizure. Most of the seizures began within seconds of the injection. Twice they began later, and on several occasions more than one seizure occurred after an injection. In the last three experiments with hippocampal activation a seizure occurred after the third or fourth injection, and each subsequent injection brought on at least one and often several seizures in rapid succession. Facilitation of seizure activity by hippocampal activation is also seen in the fourth control experiment in which perfusion with tubocurarine through the left inferior horn
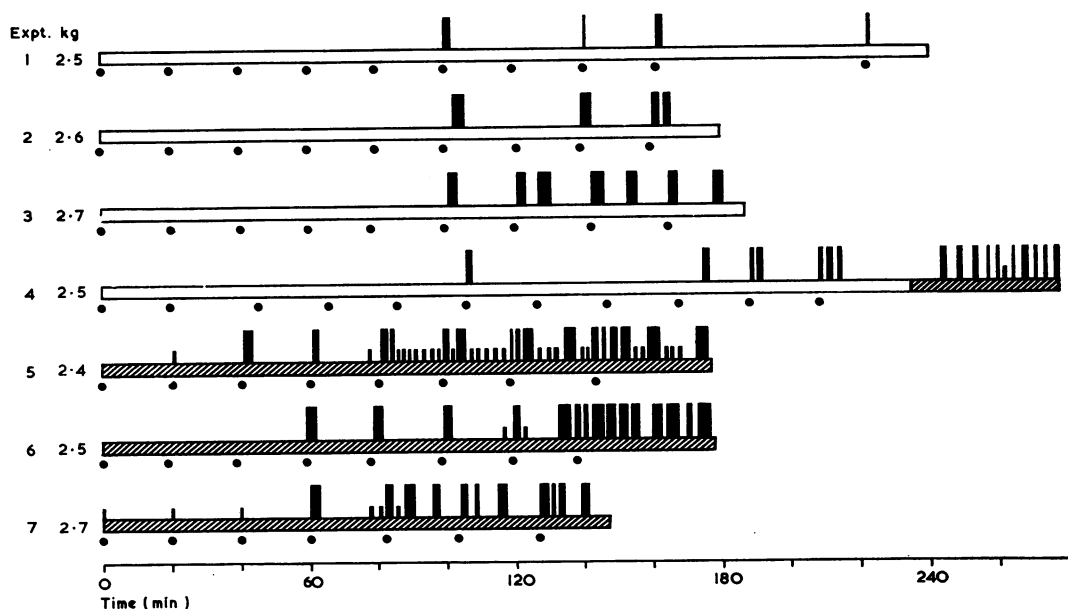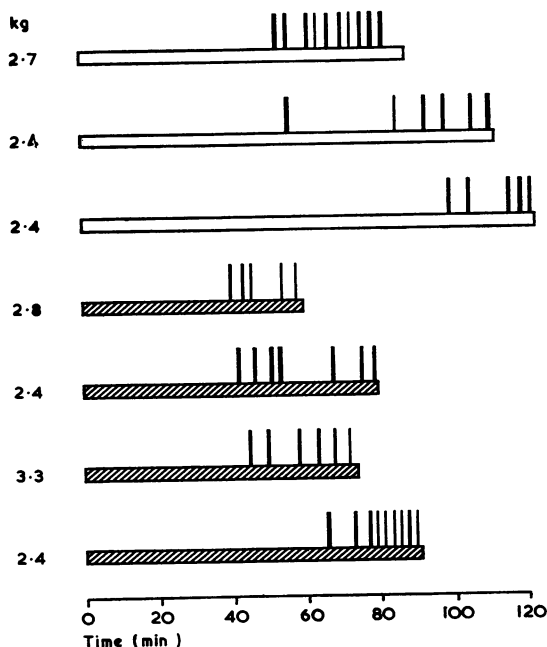


Fig. 7. Diagrammatic presentation of incidence and duration of electrocortical seizure discharges produced by repeated intravenous injections of 30 mg of leptazol (at ● ) in seven cats anaesthetized with chloralose, paralysed with gallamine trethiodide and artificially ventilated. Perfusion from both cannulated lateral ventricles to aqueduct started in each experiment 1 hr before the first leptazol injection. Empty horizontal bars, perfusion with artificial cerebrospinal fluid; hatched horizontal bars, with tubocurarine ($2 \times 10^{-4}$) from both ventricles (experiments 5 and 6) or from the left lateral ventricle experiment 7). Infusion rate from each cannula, 0.1 ml./min. High vertical bars, seizure discharges; low vertical bars, episodes.

was begun 25 min after the tenth injection of leptazol—that is, about 20 min after the last seizure evoked by this injection. At 6.5 min later, large surface negative spikes appeared on the left occipital lead—a sign of hippocampal activation—and 3 min later a series of seizures began following each other at intervals of 1–4 min, although no further injections of leptazol were made.

*Intravenous infusion of leptazol.* To produce surface positive spikes on the electro-corticogram leptazol had to be infused at a much greater rate than strychnine. Infusion of leptazol, 0.1 mg/kg min, for instance, was subthreshold. To produce seizures leptazol was infused at a rate of 1.5 mg/kg min and in these experiments surface positive spikes and hyperexcitability developed before the first seizure was recorded. The seizures were of shorter duration and their biphasic deflections of smaller magnitude than in the experiments with intravenous injections of 30 mg of leptazol. Further, they were not always associated with salivation and a reduction in outflow from the cannulated aqueduct.

As in the experiments with intravenous injections of leptazol, hippocampal activation facilitated the seizure activity. This is illustrated diagrammatically in Fig. 8. In the first three experiments without hippocampal activation, the first seizure appeared after 54, 57 and 99 min (mean 74 min, corresponding to 105 mg/kg of leptazol). In the last



Fig. 8. Diagrammatic presentation of incidence and duration of electrocortical seizure discharges produced on continuous intravenous infusion of 1.5 mg/kg min of leptazol into cats anaesthetized with chloralose, paralysed with gallamine and artificially ventilated. Perfusion from both cannulated lateral ventricles to aqueduct, at a rate of 0.1 ml./min through each cannula, started 1 hr before zero time when intravenous leptazol infusion was begun. Empty horizontal bars, perfusion with artificial cerebrospinal fluid; hatched horizontal bars, with tubocurarine $(2 \times 10^{-4})$ from the left, and artificial cerebrospinal fluid from right lateral ventricle. Vertical bars, seizure discharges.

four experiments with hippocampal activation, the first seizures appeared after 40, 42, 45 and 66 min (mean 48 min, corresponding to 72 mg/kg of leptazol).

### DISCUSSION

Reichstein's compound S, strychnine and leptazol, which are convulsants when applied systemically, were relatively inactive when perfused through the third and lateral cerebral ventricles. Of the many central excitatory effects produced with tubocurarine perfused through the cerebral ventricles, only two were obtained with the three convulsants: shivering, which resulted in a rise in body temperature, and a discharge of abnormal surface negative spikes recorded on the electrocorticogram. With Reichstein's compound S both effects were produced, with strychnine the spike discharge only, and with leptazol shivering only.

Shivering can be attributed to an action on the hypothalamus, since to obtain the effect compound S and leptazol had to be perfused through the third ventricle and, as shown for compound S, had to pass through its ventral half.

The spike discharge produced by compound S and strychnine is of hippocampal origin. It occurred only when the inferior horn was included in the perfusion with these substances and, when recordings were made simultaneously from the hippocampus, amygdala and occipital cortex, the discharge did not appear in the occipital cortex, before it appeared in the hippocampus. It either appeared simultaneously in both regions or first in the hippocampus, whereas in the amygdala it always appeared last.

The hippocampal spikes produced by compound S and strychnine remained single and their frequency did not increase on continued perfusion. As far as compound S was concerned, an explanation for this finding might be that the strongest concentration in which this steroid could be perfused on account of its solubility was just threshold for its stimulating effect on the hippocampus, because on perfusion with a threshold concentration of tubocurarine the hippocampal spikes also remained single and their frequency did not increase. This explanation, however, does not account for the results obtained with strychnine as it was perfused not only in threshold concentration but also in concentrations up to twentyfold stronger. Since this neither increased the frequency of the spike discharge nor produced multiple spikes or episodes, it appears that there is a genuine difference between the action of strychnine and tubocurarine on the hippocampus.

No experiments were performed to find out if a discharge of slow waves elicited on perfusion of the anterior horn with tubocurarine would increase the frequency of the hippocampal spike discharge produced by strychnine. It had no such effect on the spike discharge produced by compound S. It may therefore be that the pacemaker effect of the "slow waves" is exerted on the hippocampal spikes only when they have become multiple.

The reflex hyperexcitability, the surface positive spikes and the jerking movements which occurred when strychnine was perfused through the cerebral ventricles can be attributed to an action exerted after its absorption into the bloodstream. This conclusion is based on the finding that similar effects developed when strychnine was infused

intravenously at a rate of 0.05–0.5 $\mu$g/kg min. No data are available for the rate of absorption into the blood stream of strychnine from the perfused cerebral ventricles of the cat, but if the rate is comparable to that for histamine it would be adequate to account for these effects. Draškoci, Feldberg, Fleischhauer & Haranath (1960) found in cats that on perfusion with histamine ($10^{-3}$) through the cerebral ventricles 1.2 $\mu$g/min was absorbed into the bloodstream. The finding that strychnine did not produce the effects when perfused through the third ventricle alone suggests that the rate of absorption of strychnine from this region is much lower than from the other regions of the ventricular cavities.

With leptazol, hyperexcitability and surface positive spikes were not produced when it was perfused through the cerebral ventricles, although they developed when it was administered intravenously. However, the amounts required to produce these effects on intravenous administration were much greater than those of strychnine. Although the effects were produced on intravenous infusion of 0.05 $\mu$g/kg min of strychnine, an infusion of even 100 $\mu$g/kg min of leptazol was insufficient. It is unlikely that in the present experiments the rate of absorption of leptazol into the bloodstream from the perfused cerebral ventricles approached this figure.

The reduction or cessation in outflow from the cannulated aqueduct which accompanied the seizure activity in the electrocorticogram produced on intravenous administration of leptazol does not result from muscular effects since the experiments were carried out in animals treated with gallamine. Nor can it be attributed to the rise in arterial blood pressure which accompanied each seizure since changes in blood pressure do not affect the aqueductal outflow (Bhattacharya & Feldberg, 1958). The interference with this outflow may result from cerebral vasodilatation during the seizure, the dilated vessels exerting a valve-like effect at the rostral end of the aqueduct and causing obstruction.

The effects produced on perfusion of the three convulsants through the cerebral ventricles appear to bear little or no relation to the seizures obtained on their systemic application. There is no association between shivering and seizures. According to Heuser & Eidelberg (1961) the seizure discharge on intraperitoneal injection of compound S into unanaesthetized cats originates in the hippocampus; however, the hippocampal discharge produced by its action when perfused through the inferior horn in the anaesthetized cats was not sufficient to precipitate a seizure. Not all parts of the hippocampus are necessarily activated by compound S on its perfusion through the inferior horn, and it may be that seizure is only precipitated when this happens, or, alternatively, that the main action of compound S when precipitating seizures is on the cerebral cortex. This appears to be the site of action of leptazol which did not produce hippocampal spikes on perfusion through the inferior horn. There was also no evidence that the seizures following its intravenous administration originated in the hippocampus since in most experiments seizure activity began in the frontal and not in the occipital cortex to which hippocampal activity spreads first.

It has been suggested that some features of temporal lobe epilepsy point to the possibility of two separate abnormalities of cerebral activity during this disorder: a focal discharge recorded as spike activity in the electroencephalogram and an increased excitability of other brain structures. The focal discharge may continue firing for long periods

without spread of the electrical disturbance to other regions. Such a focal discharge, which resembles the spike discharge produced in cats by tubocurarine acting on the hippocampus, apparently requires an increase in the excitable state of other structures of the brain before it spreads to precipitate an overt seizure (Carmichael *et al.*, 1964). This hypothesis is supported by the present findings in that hippocampal activation with intraventricular tubocurarine reduced the threshold amount of leptazol required to produce seizures in the electrocorticogram although the reduction was not great. In this connection it is interesting to note that smaller amounts of leptazol are required to produce clinical seizures in epileptic than in non-epileptic patients, but again the difference in dosage is not great (Kaufman, Marshall & Walker, 1947 ; Cure, Rasmussen & Jasper, 1948).

## SUMMARY

1. In cats anaesthetized with chloralose the method of regional perfusion of the cerebral ventricles as developed by Carmichael *et al.* (1964) was used for studying the effects produced by three convulsants: Reichstein's compound S, strychnine and leptazol.

2. Two effects were obtained from an action of the convulsants on structures situated in the walls of the cerebral ventricles: shivering, which led to a rise in body temperature, and an abnormal rhythmic discharge of surface negative spikes recorded from the occipital region of the cerebral cortex. With Reichstein's compound S both effects were produced, with strychnine the spike discharge and with leptazol shivering only.

3. The shivering could be attributed to an action on the hypothalamus since the effect occurred only when the third ventricle, or its ventral half alone, was perfused with the convulsants.

4. The abnormal spike discharge resulted from an action on the hippocampus, since the effect occurred only when the inferior horn was included in the perfusion with the convulsants. Further, when recordings were taken simultaneously from the cerebral cortex, the dorsal hippocampus and amygdala, the abnormal discharge never appeared in the occipital cortex before appearing in the hippocampus. It either appeared simultaneously in both regions or first in the hippocampus. It always appeared last in the amygdala.

5. In addition to the spike discharge of hippocampal origin the perfusion with strychnine through the cerebral ventricles produced reflex hyperexcitability, muscle jerks and positive spikes recorded in the electrocorticogram. These effects are probably fully accounted for by absorption of small amounts of strychnine into the bloodstream, for the same effects were obtained when strychnine was infused intravenously at a rate of 0.05 $\mu$g/min.

6. The effects that the three convulsants produced by their actions on structures in the walls of the cerebral ventricles apparently bear little or no relation to the electrocortical seizures obtained when these convulsants are administered intraperitoneally or intravenously.

7. Hippocampal activation brought about by perfusion with tubocurarine through the inferior horn of one or both lateral ventricles reduced the threshold dose of leptazol required to produce electrocortical seizures on its intravenous administration.

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