ACTA
BIOLOGIAE
EXPERIMENTALIS

FURTHER ANALYSIS OF SLOW SURFACE-NEGATIVE POTENTIALS OF THE CORTEX: ACTION OF X-RAYS AND ANALGESICS

Alexander I. ROITBAK

Institute of Physiology, Georgian Academy of Sciences, Tbilisi, U.S.S.R.

It is well known that a single electrical stimulus of liminal intensity applied to the surface of the cortex evokes an electrical reaction around the stimulating electrodes in the form of a negative potential of 20—30 msec duration — a dendritic potential (DP); it is an expression of the EPSP of apical dendrites. Upon intensification of the stimulation it is followed by a slow negative potential (SNP) of 500—3000 msec duration (under Nembutal anesthesia). The SNP was described in detail in the articles of Goldring and O'Leary (1960), Goldring et al. (1961) and Roitbak (1963, 1965). There are several hypotheses of the nature of the SNP. (i) The SNP is a result of the excitation of apical dendrites by a special system of synapses, which differs in its properties from the synaptic system that conditions the DP (Goldring and O'Leary 1960). (ii) The SNP is an "field effect" reflecting hyperpolarization (IPSP) of the cell bodies of pyramidal neurons (Li, Chou 1962). (iii) The SNP is a result of depolarization of afferent fibers which penetrate the cortex radially and ascend to its surface (Eccles 1963). These three hypotheses have one idea in common: the SNP is regarded as a postsynaptic potential. In one case it is considered a result of activation of axodendritic synapses, in the second case, axosomatic and in the third case, axoaxonal. (iv) The SNP is not a postsynaptic process, but is conditioned by accumulation of K^+ ions in the gliadendritic clefts and is associated with the activation of neuroglia (Roitbak 1963). Important facts recently obtained by American authors can be used in favor of the last assump-

tion. Firstly, in experiments with recording intracellular potentials of unresponsive cells of the cortex it has been established that stimulation of the surface of the cortex with an intensity which evokes a SNP leads to depolarization of the membrane of these cells; depolarization of the membranes of unresponsive cells corresponds in configuration and duration to the SNP on the surface of the cortex (Karahashi and Goldring 1966); these cells have been histologically identified as neuroglial (Kelly et al. 1967). Secondly, it has been discovered that stimulation of fibers of the frog's optic nerve depolarizes the membrane of glial cells in this nerve, the depolarization evoked by a single stimulus lasting several seconds; the amplitude of the response increases upon intensification of the stimulation, thus depending on the number of stimulated axons; while rhythmic stimulation results in a summation of responses. Theoretically the glial cell, whose membrane potential possesses greater sensitivity to changes in the external concentration of K^+ than does the neuron membrane, registers the amount of total activity in its neighbourhood: each impulse leaves a K^+ increment in the clefts and the level of the membrane potential of the glial cell thus indicates the number of impulses that have passed (Kuffler and Nicholls 1966). A similar mechanism may be assumed to be operating in the cortex during generation of the SNP.

Our experiments were performed on cats under Nembutal anesthesia (50—100 mg/kg). The cerebral cortex was exposed and its temperature was controlled. Ag-AgCl electrodes served to lead off the potentials from the cortex. The stimulating triple electrode consisted of silver wires 100 μm in diameter cemented into a triangle; two of the stimulating electrodes were connected with one pole of the stimulator and their potential was regulated by two potentiometers; the third electrode was connected with the other pole (Landau 1956). The stimulation was effected with square wave pulses from the stimulator with a radiofrequency output, a 400 ohm output resistance and an output for sweep synchronisation. The first ("active") electrode was placed near the stimulating electrode, the second on the bone. In the experiments with registering the potentials evoked by skin stimulation the "active" electrode was placed at an appropriate point of the somatosensory area I. The neuropharmacological substances were administered intravenously or were applied locally under the "active" electrode, for which purpose a cotton ball was soaked in a solution of the given substance heated to the temperature of the surface of the cortex. In the experiments studying the influence of X-rays mild Roentgen radiation with a mean energy value of 30 kev was used; the irradiation was effected on a PYM apparatus with a dose of 40 kr/min. The potentials were amplified by DC

amplifiers (УИПП-2 or Kossor) or an AC amplifier with a large time constant. The recording was done by means of a cathode-ray oscillograph (ЭНО-1 or C1-19).

Studies of the effect of X-rays on the direct response of the cortex (DP+SNP) assume particular interest since there are electron microscopy data indicating a special sensitivity of neuroglia to ionising radiation (Maxwell and Kruger 1965, Miquel and Haymaker 1965, Caveness et al. 1967). In our study (Roitbak et al. 1967) the first to weaken and disappear under the action of X-rays in a dose of 25—30 kr was the SNP. Intensification of direct responses could be observed under the action of a dose of about 10 kr (Fig. 1A1B). Fig. 1C shows that after a repeated irradiation (20 kr) the DP did not change, nor did the positive potential change noticeably, but the SNP was eliminated (no reaction of the pial vessels was observed). After the third irradiation (40 kr) the direct responses disappeared completely; they did not arise upon intensification of the stimulus and were not restored in the course of time (Fig. 1DEH). After irradiation of the left hemisphere the direct responses sharply increased in the right hemisphere (Fig. 1FG); this may be supposed to be the result of cessation or sharp weakening of the impulsation through the corpus callosum.

Our experiments have shown that the SNP may be selectively
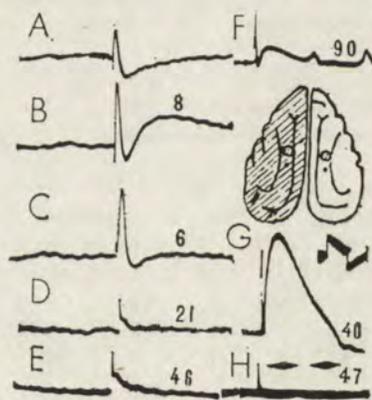
Fig. 1. Selective depression of the slow negative potential (SNP) by X-rays. Cat; Nembutal 80 mg/kg; AC amplifier with large time constant; arrangement of electrodes is shown in the diagram; intensity of stimuli — 10 v, duration — 0.05 msec. Left part of Figure shows responses in left hemisphere before and after X-ray action: A, before irradiation, B, after first irradiation (10 kr), C, after second irradiation (20 kr), D, E, H, after third irradiation (40 kr); figures above curves indicate time in minutes elapsed after irradiation. Right part of the Figure shows responses in right hemisphere: F, before irradiation of the left hemisphere, G, after third irradiation. Time markings for records A—E, 20 msec, for records F—H, 200 msec; voltage calibration — 0,5 mV (Roitbak et al. 1967)

eliminated by action of X-rays without an appreciable change in the DP (and other electrical reactions of the cortex, for example, the primary response). This was observed under the action of comparatively small doses of radiation (Fig. 1). In the course of postradiation action of comparatively large doses of radiation the SNP disappeared much sooner than the DP; in the course of the postradiation restoration of the responses the DP appeared much sooner than the SNP. Fig. 2A shows a record of a direct response (DP+SNP) made at a distance of 2.5 mm from the stimulating electrodes (the sweep of the ray used shows only
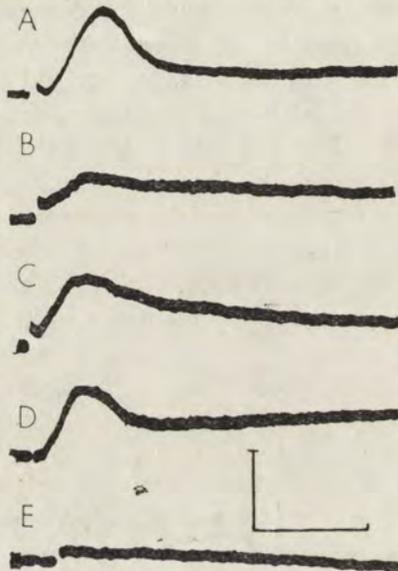
Fig. 2. Selective depression of SNP by X-rays. Cat; Nembutal 80 mg/kg; DC amplifier. Stimulating and recording ("active") electrodes at a distance of 2.5 mm from each other on gyr. suprasylvius dex. Duration of stimuli — 0.5 msec; intensity — 50 v. A, response consisting of DP and SNP before irradiation. B, 15 min after irradiation of the right hemisphere (dose — 25 kr). C, 34 min and D, 67 min after irradiation. E, after repeated irradiation (dose — 25 kr). Calibration: 20 msec, 0.25 mV (Roitbak et al. 1967)

the initial part of the SNP). Eleven minutes after irradiation (28 kr) the DP decreased and, characteristically, the SNP completely disappeared (Fig. 2B); the duration of the DP increased to 40—50 msec. Thirty-three minutes after irradiation the amplitude of the DP increased to 75—80% of the initial, but the SNP did not arise (Fig. 2C), 67 min after irradiation the direct responses were restored and the SNP became even more clearly marked than before irradiation (Fig. 2D). Repeated irradiation resulted in complete disappearance of direct responses in the irradiated hemisphere (Fig. 2E).

The fact of higher sensitivity of the SNP to radiation, compared with the DP, whose neuronal origin gives rise to no doubts, reinforces the hypothesis of the neuroglial origin of the SNP. Hardly improbable is the selective action of X-rays on definite synaptic structures in the cortex, which should be assumed on the basis of the hypothesis that postsynaptic potentials of neuronal elements underlie the SNP.

A striking effect is produced on the SNP by morphine. The mechanism of the analgesic action of morphine is still unknown (Valdman 1961, 1963, Martin 1963, Batrak 1965, Zakusov 1966). Despite the large number of studies of the influence of morphine on the spontaneous electrical activity and evoked potentials of the brain there is no information on the changes in prolonged electrical potentials. The initial fact discovered by us (Roitbak 1963) was that the SNP was weakened or eliminated by local application of morphine hydrochloride in the lead-off region (Fig. 3ABC); concentrations of 0.2% and higher were effective; the effect appearing in 3 min and lasted more than one hour. The prolonged negative deviations evoked by rhythmic stimulations of the cortex and the SNP in the somatosensory region of the cortex evoked by strong stimulations of the
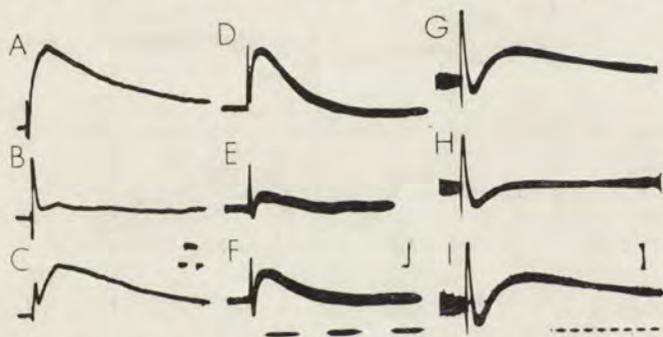
Fig. 3. Depression of SNP of the direct response of the cortex by local application of morphine, promedole and fentanyl. A—B, cat; Nembutal 100 mg/kg; stimulating and active recording electrodes on gyr. suprasylvius med., spaced 1 mm apart; DC amplifier. A, response to one stimulus (50 v. 0.05 msec) before poisoning. B, 3 min after removal of 1% morphine from the recording electrode (poisoning lasted 3 min). C, 23 min after its removal. Length of exposure — 1.5 sec, voltage calibration — 1 mV. D—F, cat, Nembutal 110 mg/kg, temperature of cortex — 33°C; stimulating and recording electrodes on gyr. sigmoid post., spaced 1.5 mm apart; AC amplifier with a time constant of 0.7 sec. D, response to one stimulus (50 v, 0.2 msec) before poisoning. E, 4 min after removal of 1% promedole (poisoning lasted 2 min). F, 13 min after its removal. Time marking — 200 msec, voltage calibration — 0.5 mV. G—I, cat, Nembutal 80 mg/kg, temperature of cortex — 34°C; stimulating and recording electrodes on gyr. suprasylvius med., spaced 1 mm apart; AC amplifier with a time constant of 0.7 sec. G, response to stimulus (30 v, 0.05 msec) before poisoning. H, 5 min after removal of fentanyl (0.005%), poisoning lasted 5 min. I, 60 min after its removal. Time marking — 20 msec, voltage calibration — 1 mV

corresponding portion of the skin were also suppressed (Roitbak 1965). Other types of evoked potentials did not change or were intensified (dendritic potentials, primary responses, secondary responses).

Thus the SNP was the only electrical reaction of the cortex to be eliminated as a result of local application of morphine to the surface of the cortex. Local application of a 1% solution of promedole (4-phenyl--4-propoxy-1,2,5-trimethyl-piperidine hydrochloride: analgesic) to the cortex also eliminated the SNP and, unlike morphine, also depressed the DP (Fig. 3DEF). Local application of a 0.005% solution of fentanyl (analgesic) eliminated the SNP and somewhat weakened the DP (Fig. 3GHI); restoration of the direct response took less than one hour.
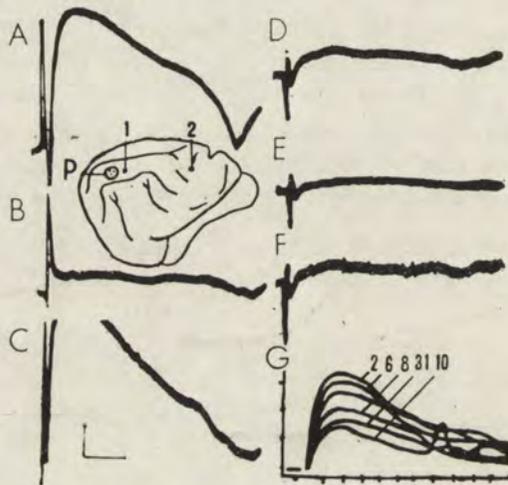
Fig. 4. Depression of SNP of the direct response and of SNP of the primary response by intravenous administration of morphine. A—F, cat, Nembutal 50 mg/kg, natural respiration, DC amplifier; electrode arrangement shown in diagram: recording electrode 1 on gyr. suprasylvius med. 1 mm from stimulating electrodes to record the direct response of the cortex — DP and the following SNP; recording electrode 2 on gyr. sigmoid post. serves to record the primary response and the following SNP evoked by strong stimulation of the skin. A, direct response to a stimulus of 50 v, 0.05 msec before administration of morphine. B, 7 min after intravenous administration of 3.6 mg/kg of morphine. C, 33 min after its administration. D, electrical reaction to stimulus of 50 v, 2 msec, applied to skin of contralateral foreleg before morphine administration. E, 13 min after morphine administration. F, 42 min after its administration. Time calibration 100 msec, voltage calibration — 0.2 mV. G, tracheotomised cat, Nembutal 60 mg/kg, controlled respiration, DC amplifier; stimulating and active recording electrodes on gyr. suprasylvius med., spaced 1 mm apart. Changes in SNP of direct response evoked by stimulus of 50 v, 0.05 msec, after morphine administration (5 mg/kg); figures above curves — time in minutes elapsed after morphine administration; time marking — 50 msec, voltage marking — 0.8 mV (Roitbak and Linenko)

In the experiments conducted jointly with V. I. Linenko on cats with natural and controlled respiration intravenous administration of morphine weakened and eliminated the SNP of the direct response of the cortex and the SNP of the somatosensory region evoked by a strong stimulation of the skin. Fig. 4 shows several records of one of these experiments. The stimulus applied to the surface of the cortex evoked a direct response in the form of an initial negative potential and an SNP of about 500 msec duration (Fig. 4A). The SNP was ten times as weak after administration of 3.5 mg/kg of morphine (Fig. 4B). The SNP was restored after 33 min (Fig. 4C). Records D—F illustrate the similar influence of morphine on the SNP after the primary response.

A dose of 10 mg/kg of morphine often completely eliminated the SNP several minutes after its administration; gradually weakening the effect lasted 60—180 min and longer. Fig. 4G shows the process of weakening of the SNP after administration of 5 mg/kg of morphine. In the second minutes no influence was as yet discovered; the weakening of the SNP could be clearly observed in the sixth minute; the maximum weakening of the SNP occurred in the tenth minute and remained at that level for 20 min. Restoration of the SNP began to be clearly observed in the 31st min.

In the studies conducted by Fujita et al. (1953) intravenous administration of morphine resulted in depression of the second negative potential of the direct response of the cortex, which in their records lasted about 30 msec; we cannot therefore with any certainty identify the second component of their record with the SNP which lasts 10—100 times as long. They considered the depression of the second component an indication that morphine blocks the excitation of intracortical neurons, which is at variance with a number of facts, including the well-known fact of the strychninelike effect of morphine (Longo and Chiavarelli 1962).

According to the data from A. K. Sangailo's laboratory (Strelkov 1963) and our own data, morphine in a dose of 2 mg/kg raises the threshold of pain in cats, the criterion for this being the animal's external reaction; the maximum effect of subcutaneous administration occurs between the 30th and 60th min and lasts 10—180 min. Thus there is a good correspondance between the dose, development and duration of the analgesic effect of morphine in experiments on normal cats, on the one hand, and its depressing effect on the SNP, on the other. The fact that the SNP is eliminated by morphine (and other analgesics) is apparently the key to understanding the mechanism of the analgesic action.

If this conclusion is justified, the factors eliminating the SNP should be expected to possess analgesic properties and, contrariwise, the factors

intensifying the SNP should intensify pain, i.e., possess antanalgesic properties. In our laboratory it has been established that the SNP is in some measure selectively depressed by strychnine, caffeine and bromine. Information is available to the fact that strychnine intensifies the analgesic effect of morphine and promedole (Chudakova 1955); the analgesic properties of caffeine have been demonstrated (Batrak and Stets 1966; Khrapov 1966); there are indications that bromine possesses analgesic action (Verkhovskaya 1962). The SNP is intensified and prolonged by nembutal (pentobarbital sodium) (Goldring and O'Leary 1960, Goldring et al. 1960, 1961); it is known that barbiturates have antanalgesic properties (Darbinyan 1967).

The hypothesis of the neuroglial origin of the SNP warrants the assumption that the point of application of morphine and other analgesics is the neuroglia and that the neuroglia is connected with the pain mechanism.

SUMMARY

In response to a stimulus applied to the surface of the cortex a dendritic potential, i.e. EPSP of apical dendrites and slow negativity (SN, 300—3000 msec) arise (Chang 1951, Goldring and O'Leary 1960). It was supposed (Roitbak 1963) that SN reflects depolarization of apical dendrites resulting from the activation of glia in their victinity. Experiments were carried out on cats under Nembutal anesthesia with Ag — AgCl electrodes and DC amplifier. Under the X-ray action (15 kr) SN disappeared; dendritic potentials did not alter at such dose, they disappeared at higher doses of X-rays. This fact might be considered as an evidence in favour of the neuroglial nature of SN, for there are morphological and physiological data asserting that neuroglia is more sensitive to the X-rays than the neurons. Under local application to the cortex morphine does not affect dendritic potentials, enhances primary and secondary responses. SN is the only evoked potential of the cortex which is eliminated as a result of local application of morphine. Analgesics promedole and fentanyl also eliminate SN. Intravenous administration of morphine (3,6 mg/kg) causes weakening and elimination of SN of the direct cortical response and SN of the somatosensory area elicited by the strong skin stimulation. Latency of this effect and its duration correspond to the course of the analgesic effect in the experiments on normal cats. It may be supposed that neuroglia is related to the mechanism of pain and is a site of action of morphine and other analgesics.

REFERENCES

BATRAK, G. E. 1965. Bol, shok, narkoz. Zdorovie, Kiev.

BATRAK, G. E. and STETS, V. R. 1966. On the modelling of some aspects of morphine action (in Russian). In D. G. Shefer, A. K. Sangailo, and S.S. Sokolov (ed.), Bol i borba s nei. Sverdlovsk, p. 41—43.

CAVENESS, W. F., CARSTEN, A. L., ROIZIN, L. and SCHADÉ J. P. 1967. Pathogenesis of X-irradiation effects in the monkey cerebral cortex. Brain Res. 7: 1—120.

CHUDAKOVA, G. A. 1955. Influence of analeptics on the action of analgetics (in Russian). Farmakol. toxikol. 28: 3—8.

DARBINYAN, T. M. 1967. Bol i anestaziya. Znanie, Moscow.

ECCLES, J. C. 1963. Progr. Brain Res. 1:263 (Discussion).

FUJITA, S., YASUHARA, M. and OGIU, K. 1953. Studies on sites of action of analgesics. Jap. J. Pharm. 3: 27—38.

GOLDRING S., and O'LEARY, J. L. 1960. Pharmacological dissolution of evoked cortical potentials. Federation Proc. 19: 612—618.

GOLDRING, S., O'LEARY J. L., HOLMES, T. and JERVA, M. J. 1961. Direct response of isolated cerebral cortex of cat. J. Neurophysiol. 24: 633—650.

KARAHASHI, Y. and GOLDRING, S. 1966. Intracellular potentials from "idle" cells in cerebral cortex of cat. Electroenceph. Clin. Neurophysiol. 20: 600—607.

KELLY, J. S., KRNJEVIĆ, K. and YIM, G. K. W. 1967. Unresponsive cells in cerebral cortex. Brain Res. 6: 767—769.

KHRAPOV, V. S. 1966. Pathogenesis of pain during neuralgia of the trigeminal nerve (in Russian). In D. G. Shefer, A. K. Sangailo, and S. S. Sokolov, (ed.), Bol i borba s nei. Sverdlovsk, p. 137—140.

KUFFLER, S. W. and NICHOLLS, J. G. 1966. The physiology of neuroglial cells. Erg. Physiol. 57: 1—90.

LANDAU, W. M. 1956. An analysis of the cortical response to antidromic pyramidal tract stimulation in the cat. Electroenceph. Clin. Neurophysiol. 8: 445—456.

LI, C. L. and CHOU, S. N. 1962. Cortical intracellular synaptic potentials and direct cortical stimulation. J. Cellular Comp. Physiol. 60: 1—16.

LONGO, V. G. and CHIAVARELLI, S. 1962. Neuropharmacological analysis of strychninelike drugs. In W. D. M. Paton (ed.), Proc. of the first international pharmacological meeting "Mode of action of drugs", Macmillan Co., New York, vol. 8, p. 189—198.

MARTIN, W. R. 1963. Strong analgesics. In W. S. Root, and F. G. Hofman (ed.), Physiological pharmacology. Academic Press, New York, vol. 1, p. 275—312.

MAXWELL, D. S. and KRUGER, L. 1965. The fine structure of astrocytes in the cerebral cortex and their response to focal injury produced by heavy ionizing particles. J. Cell. Biol. 25: 141—157.

MIQUEL, J. and HAYMAKER, W. 1965. Astroglial reactions to ionizing radiation: with emphasis on glycogen accumulation. Progr. Brain Res. 15: 89—114.

ROITBAK, A. I. 1963. On the nature of cortical inhibition (in Russian). Zh. Vyssh. Nerv. Deyat. 13: 859—869.

ROITBAK, A. I. 1965. Slow negative potentials of the cortex and neuroglia (in Russian). In Sovremennye problemy fiziologii i patologii nervnoi sistemy. Medicina, Moscow, p. 68—93.

ROITBAK, A. I., NADAREISHVILI, K. Sh. and MOISEYENKO, K. I. 1967. An attempt of analyse electrical potentials of the cerebral cortex by local X-ray irradiation (in Russian). In Sovremennye problemy deyatelnosti i stroenia tsentralnoi nervnoi sistemy. Metsniereba, Tbilisi, p. 173—192.

STRELKOV, R. B. 1963. Comparative estimation of analgesic activity of isopromedol and morphine (in Russian). Trudy sverdlovskogo medicinskogo instituta. Sverdlovsk, 35: 44—49.

VALDMAN, A. V. 1961. Influence of analgesics on transmission along the specific and diffuse afferent systems (in Russian). In V. A. Valdman (ed.), Issledovaniya po farmakologii retikuliarnoi formatsii i sinapticheskoi peredachi. Leningrad, p. 75—84.

VALDMAN, A. V. 1963. Pharmacology of the brain (in Russian). In V. A. Valdman (ed.), Aktualnye problemy farmakologii retikularnoi formacii i sinapticheskoi peredachi. Leningrad, p. 9—115.

VERKHOVSKAYA, I. N. 1962. Brom v zhivotnom organizme i mekhanizm ego deistviya. Acad. Sci. USSR, Moscow.

ZAKUSOV, V. V. 1966. Farmakologiya. Medicina, Moscow.

EEG FEEDBACK CONTROL OF MIDBRAIN ELECTRICAL STIMULATION INDUCING SLEEP OR AROUSAL IN RABBITS

Władysław Z. TRACZYK [1], David I. WHITMOYER and Charles H. SAWYER

Department of Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024 U.S.A.

It is now well established that in normal animals specific EEG patterns in the brain reflect the behavioral states of alertness and sleep. With electrical stimulation of basal brain structures, Hess (1929, 1956) induced several behavioral patterns including sleep and excitation in cats. From the work of Moruzzi and Magoun (1949) there is general agreement that high frequency electrical stimulation of the brain-stem reticular formation in sleeping animals evokes EEG arousal and behavioral arousal. Applying low and high frequency stimulation to the same medial thalamic and midbrain reticular regions Monnier et al. (1963) were able to induce synchronization or desynchronization of EEG patterns, respectively. More recently, brain-stem mechanisms related to sleep have been reviewed by Zanchetti (1967).

However, it is difficult to stimulate sustained EEG and behavioral patterns reproducibly in the same animal; the induced EEG arousal response undergoes habituation. This occurs during extended or repeated stimulation by external alerting stimuli acting through receptors (Sharpless and Jasper 1956) or by a direct electrical stimulation of the cortex and amygdala (Ursin et al. 1967) or midbrain reticular formation (Glickman and Feldman 1961). Habituation of EEG arousal elicited by brain-stem electrical stimulation appears not only in the neocortex but also in the hippocampus (Drewczyński 1968). Even during a 30 sec stimulation

---

[1] Present address: Department of Physiology, School of Medicine, Łódź, Poland.

period the resting EEG pattern returns after the first desynchronized phase (Bueno et al. 1968).

The purpose of this study was to determine the midbrain energy thresholds for inducing a hippocampal theta rhythm during relative alertness and spindle sleep and for the reversion of the EEG pattern from spindle sleep to a continuous arousal pattern. The differences in energy thresholds between two midbrain stimulating points and their stability over a few weeks of testing for individual animals were taken into consideration. The hippocampal theta rhythm was used as the most sensitive indicator of EEG arousal in the rabbits (Green and Arduini 1954; Petsche and Stumpf 1960). To eliminate the spontaneous fluctuations in the cyclic recurrence of sleep and wakefulness in intact animals during the determination of the energy threshold for the reversion of the EEG pattern, we have adapted the stimulus brain response feedback loop method (Mulholland and Runnals 1962, 1964) for direct electrical stimulation of the midbrain in rabbits.

METHODS

Fourteen mature female New Zealand White rabbits weighing 3.4 to 4.4 kg were used. Under pentobarbital anesthesia several electrodes were implanted stereotaxically into the brain according to the atlas of Sawyer et al. (1954). Two silver-ball electrodes were placed epidurally over the frontal and limbic cortex of the right hemisphere and bipolar concentric stainless steel electrodes were implanted into the right dorsal hippocampus (P4, R5, H+5), into the midbrain reticular formation (RF) on the right (P8, R2, H-3) and into the periaqueductal central gray (CG) on the left (P10, L1.5, H-1). Five large microelectrodes were also introduced into deep subcortical regions but recordings from these will be reported separately. An indifferent stainless steel screw and plate electrode was placed over the frontal bones and three grounding screws were fixed to occipital and parietal bones. Wires from the electrodes were crimped to female contacts for insertion into a 17 receptacle Amphenol Socket which was later fixed to the skull with acrilic dental cement. Antibiotics were administered locally around the assembly and injected intramuscularly for a few days following surgery. Two or three weeks later the rabbits were individually familiarized with the large experimental sound-attenuated chamber by placing them in the chamber for 24 hr every 7—10 days.

Energy thresholds for theta rhythm and for somatic responses. Stimulation and recording experiments began one month after implantation. In unrestrained free moving animals electrical activity was recorded from dorsal hippocampus, frontal and limbic cortex with a Grass Model III-D Electroencephalograph. With Tektronix Type 162 Waveform Generators and a Grass Model S4 Stimulator the RF was stimulated for 5 sec every 30 sec. The electrical pulses were monitored constantly on a Tektronix Type 502 Oscilloscope and readings of current in microamps and of amplitude in volts were noted on EEG paper. The effects of 5 sec trains of square wave pulses were tested at frequencies of 10, 20, 30, 40, 80, 150 and

300 c/sec and durations of 0.05, 0.1, 0.5, 1.0 msec were tested. Complete trials were given the frequencies of 30 and 300 c/sec, constant duration 0.1 msec and amplitudes gradually increased by 0.1 or 0.2 V steps. In all trials polarity of the pulses was the same: the barrel of the concentric electrodes, cathode and the tip, the anode.

In each trial the first train was applied while the animal was sitting quietly but fully alert according to the EEG records and behavior which was observed through a one-way window and noted on the EEG paper. The energy threshold per train for the hippocampal theta rhythm in alert animals (THTA) was established as the lowest energy which changed the hippocampal electrical activity slightly. This threshold stimulus was usually repeated ten times. By increasing the amplitude of the pulses in successive trials it was possible to establish the energy threshold for the hippocampal theta rhythm during spindle sleep (THTS): the theta rhythm appeared each time the stimulus was applied. Further increases in amplitude induced somatic responses such as contralateral or ipsilateral turning of the head, relaxation of neck muscles with the head dropping to the floor and finally general behavioral excitation with stamping, circling or running. The thresholds of these manifestations could be measured (TSR).

The energy of the square wave electrical pulses was calculated from the formula:

Energy per pulses in $m\mu Ws = U \times i \times t$

U = amplitude of pulse in volts

i = current of pulse in microamperes

t = duration of pulse in milliseconds

$m\mu Ws$ = millimicrowatt-seconds

The energy of the train was calculated from the formula:

Energy per train in $m\mu Ws = m\mu Ws/pulse \times f \times T$

f = frequency of pulses

T = duration of train in seconds

The energy threshold for continuous EEG arousal pattern. After initial experiments had been completed to obtain the energy thresholds for inducing the hippocampal theta rhythm during alertness (THTA) and spindle sleep (THTS) and for somatic responses (TSR), automatically modulated stimulation was applied. For this purpose an electrical circuit was developed containing two Tektronix Type 162 Waveform Generators, a Grass Model S4 Stimulator with stimulation isolation unit, a Grass Model III-D Electroencephalograph, Tektronix Type 502 Oscilloscope and our own cathode follower, feedback control device and stimulus modulator. The generators and stimulator provided automatic stimulation of the RF with 1.5 sec or 2 sec trains separated by 1.5 sec or 2 sec intervals. Rectangular wave pulses of 30 or 300 c/sec frequency and 0.1 msec duration were used. The current and voltage of the pulses were constantly monitored on the oscilloscope.

While hippocampal activity was being recorded electroencephalographically the stimulus modulator was controlled through a cathode follower and feedback control device containing 7.5 c/sec band pass filters for the hippocampal electrical activity. The amplitude of pulses applied to the RF decreased automatically when the hippocampal theta rhythm appeared and increased when it disappeared. The range of amplitude of the pulses was adjusted for each animal with respect to the EEG arousal (hippocampal theta rhythm) threshold during alertness (THTA) and spindle sleep (THTS). The amplitude of the pulses was constantly modified by the modulator between minimum and maximum amplitude depending on the EEG pattern. The threshold was established for continuous EEG arousal pattern, TCAP,

i.e., the lowest energy train which repeatedly converted the sleep EEG pattern into a hippocampal theta rhythm and cortical desynchronization and maintained them for 10 min.

Two-way reversion of the EEG pattern. After the threshold energy for continuous EEG arousal had been established the pulse amplitude range was shifted downward to a level consistent with recording the deepest spindle EEG pattern. When the density of EEG spindles from the cortical leads was sufficiently high the pulse energy was elevated to the threshold for a continuous EEG arousal pattern (TCAP) for 30 sec periods and then lowered again to the initial level.
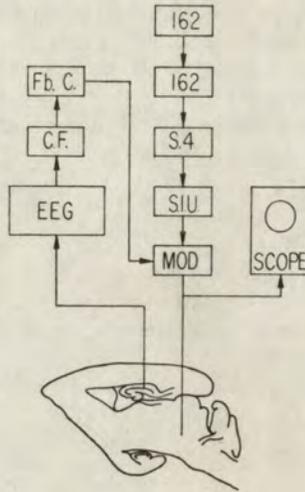
Fig. 1. Block diagram of feedback loop controlling EEG arousal. The stimulating electrode lies in the midbrain reticular formation and the recording electrode in the dorsal hippocampus. Boxes represent: 162, Tektronic Type 162 Waveform Generators; S. 4, Grass Model S4 Stimulator; S. I. U., Grass Stimulation Isolation Unit; these were the instruments generating the trains of square wave electrical pulses. EEG, Grass Model III-D Electroencephalograph; C. F., Cathode Follower; Fb. C., Feedback Control Device for recording and filtering hippocampal electrical activity; MOD, Stimulus Modulator which modulated the amplitude of square wave electrical pulses; SCOPE, Tektronix Type 502 Oscilloscope for monitoring voltage and current of each pulse

Similar trials were given CG stimulation to establish energy thresholds for theta rhythm, for somatic responses, for the continuous EEG arousal pattern and for two-way reversion from spindle sleep to EEG arousal pattern.

At the completion of the experimental program direct current was passed between indifferent and active electrodes and each rabbit was killed with an overdose of pentobarbital i.v. The brain was perfused with isotonic saline followed by 10% formalin containing a solution of potassium ferro- and ferri-cyanide for the Prussian Blue reaction with iron ions. Coronal histological frozen sections were made to determine the electrode positions in the brain.

RESULTS

Energy thresholds for hippocampal theta rhythm and somatic responses

RF stimulation. Histological verification of the position of the electrode tips showed that stimulation had been applied to the midbrain reticular formation in all 14 rabbits (Fig. 2). During 5 sec stimulation of the RF at a frequency of 30 c/sec in relatively alert animals a theta rhythm (THTA) appeared at a stimulus energy level of 705±165 mµWs//train (mean ± standard error) in 13 animals. Repetition of the 5 sec stimulation trains at 30 sec intervals led to a gradual decrease in EEG arousal, and spindles appeared in the cortical leads during the intervals as well as during stimulation. Increasing the pulse energy restored EEG arousal but spindles appeared more frequently and were more pronounced during the intervals. The excitability of the brain fluctuated constantly and whereas some trains evoked EEG arousal during stimulation most of them eventually failed.

STIM.:
30 c/sec, 0.1 msec, 5 sec	△	RELAXATION
300 c/sec, 0.1 msec, 5 sec	▼	RELAXATION
– " – – " – – " –	◖	IPSILAT. HEAD MOV.
– " – – " – – " –	◼	CONTRALAT. HEAD MOV.
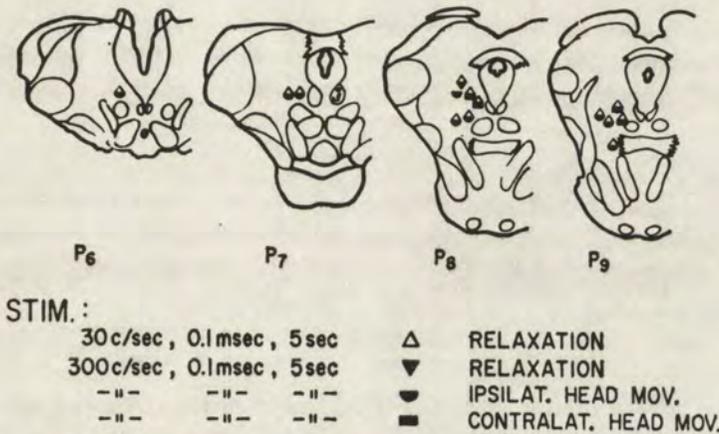
Fig. 2. Coronal sections of rabbit brain stem with the distribution of stimulation points in the reticular formation (RF) of 14 animals. Stimulation of each point with 5 sec trains of pulses of 30 or 300 c/sec induced a theta rhythm in the dorsal hippocampus. With further increase of energy in the trains somatic reactions were observed including relaxation of neck muscles, and ipsilateral and contralateral tonic head movements. One of the 14 animals was not studied at 30 c/sec

Reaching a mean energy of 6,675±795 mµWs/train, each train induced an EEG arousal pattern in 13 animals but during the intervals the spindle sleep reappeared. This is the energy threshold for EEG arousal during spindle sleep (THTS). With further increase in the stimulus energy the EEG arousal pattern was maintained throughout the whole interval and some somatic responses were observed.

During the spindle sleep episodes the rabbits were lying on the floor with their ears elevated. If they stood up and moved the trains were not applied again until a minute after they sat or lay down on the floor. This type of behavior was like that observed in unstimulated animals.

The somatic response for which the threshold was established in 13 animals was a slow lowering of the head probably due to relaxation of neck muscles. The mean energy threshold for this response (TSR) was higher than that for EEG arousal during spindle sleep: 11,880± ±1,260 mμWs/train. With an increased energy train in one animal the downward movements of the head were accompanied by an ipsilateral tonic turning of the head and in another animal with rapid rebound movements when the stimulus train terminated. With still higher levels of stimulation the animals usually stood up and moved or stamped.
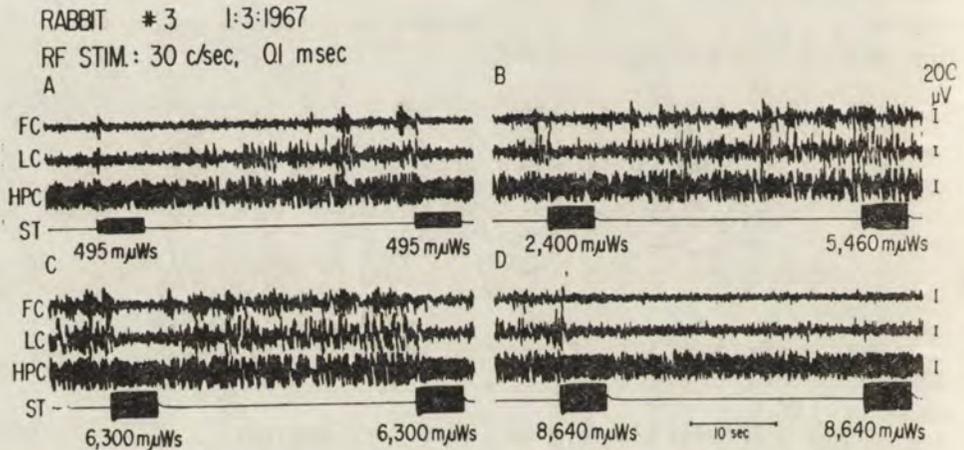
Fig. 3. EEG records in frontal cortex (FC), limbic cortex (LC), and dorsal hippocampus (HPC) during stimulation of the reticular formation (RF) for 5 sec, with 30 c/sec trains (ST). A, stimulation at threshold energy — 495 mμWs/train induces HPC theta rhythm in a relatively alert animal. B and C, during spindle sleep energy levels 2,400 and 5,460 mμWs/train were below and 6,300 mμWs/train was approximately at the threshold for EEG arousal (THTS). D, the energy level 8,640 mμWs/train was below the threshold for a somatic response (TSR) — relaxation of neck muscles

At a stimulation pulse frequency of 300 c/sec in the same animals the hippocampal theta rhythm and cortical desynchrony (THTA) appeared at an energy level of 1,800±195 mμWs/train. The mean energy threshold for EEG arousal during spindle sleep (THTS) was 7,500± ±1,095 mμWs/train. The trains evoked somatic responses at the mean energy threshold (TSR) 10,650±1,500 mμWs/train. The somatic responses

were multiform. Only one animal lowered its head while nine showed ipsilateral and three contralateral tonic head turning. The thresholds for the somatic responses (TSR) and for EEG arousal during spindle sleep (THTS) were closer together than during the 30 c/sec stimulation. In three animals the thresholds were identical.
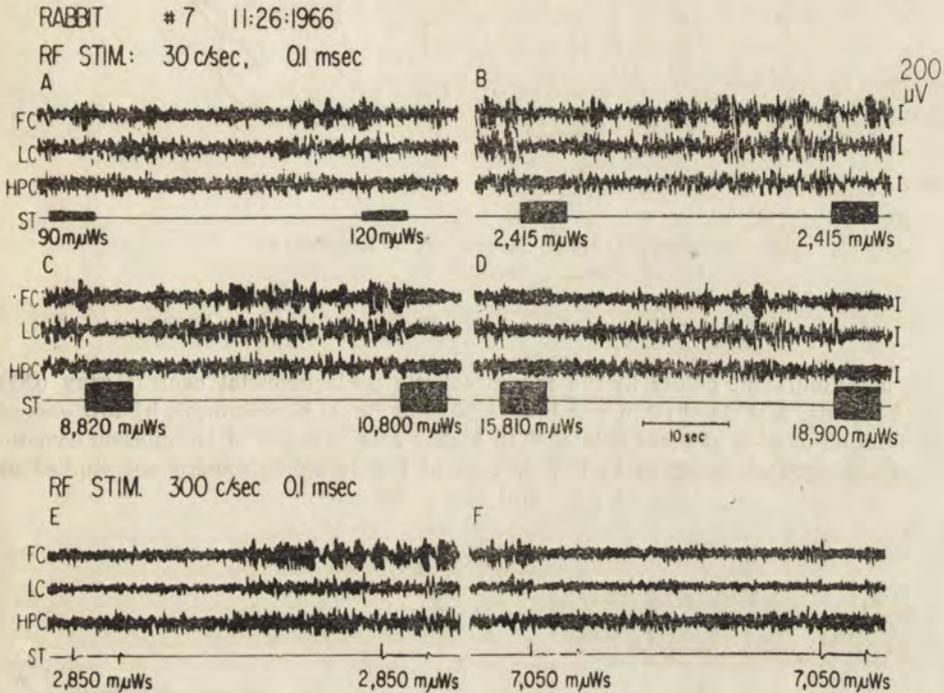
Fig. 4. EEG records during RF stimulation at different frequencies. From A to D the pulse frequency was 30 c/sec and E and F, 300 c/sec. A, in the relatively alert rabbit 120 mμWs/train was the threshold for HPC theta rhythm (THTA). B and C, during spindle sleep the energy level 2,415 mμWs/train was below and 8,820 mμWs/train was close to threshold for EEG arousal (THTS) D, the threshold (TSR) for a somatic response (relaxation of neck muscles) was 15,810 mμWs/train. E and F, energy level 2,850 mμWs/train was below and 7,050 mμWs/train was above the threshold for HPC theta rhythm during spindle sleep but still below the threshold (TSR) for a somatic response (contralateral tonic head movement)

The energy threshold for EEG arousal during spindle sleep (THTS) was more definite than the other two. There was little difference between the energy levels applied to the RF at 30 and 300 c/sec to produce this response (p = 0.6—0.5).

CG stimulation. Histological study of the electrode positions showed that in 12 of 14 animals the electrode tips were in the mesencephalic periaqueductal central gray matter and in the other two animals, close

STIM.:

30 c/sec , 0.1 msec , 5 sec	△	RELAXATION
300 c/sec , 0.1 msec , 5 sec	▼	RELAXATION
– '' –	– '' –	– '' –	⏷	IPSILAT. HEAD MOV.
– '' –	– '' –	– '' –	▥	CONTRALAT. HEAD MOV.
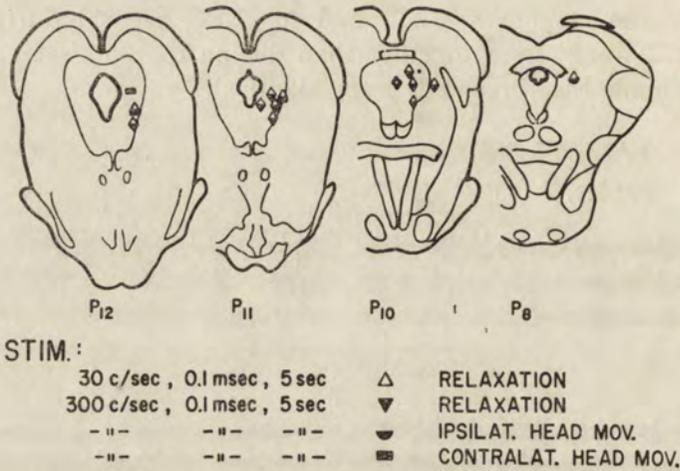
Fig. 5. Stimulation points in the mesencephalic periaqueductal central gray (CG) in 14 rabbits. A theta rhythm was induced in the dorsal hippocampus by low energy stimulation of each of these points. With higher energy trains of stimulation somatic reactions were observed as in Fig. 2. Two of the 14 animals were not studied at 30 c/sec and one at 300 c/sec
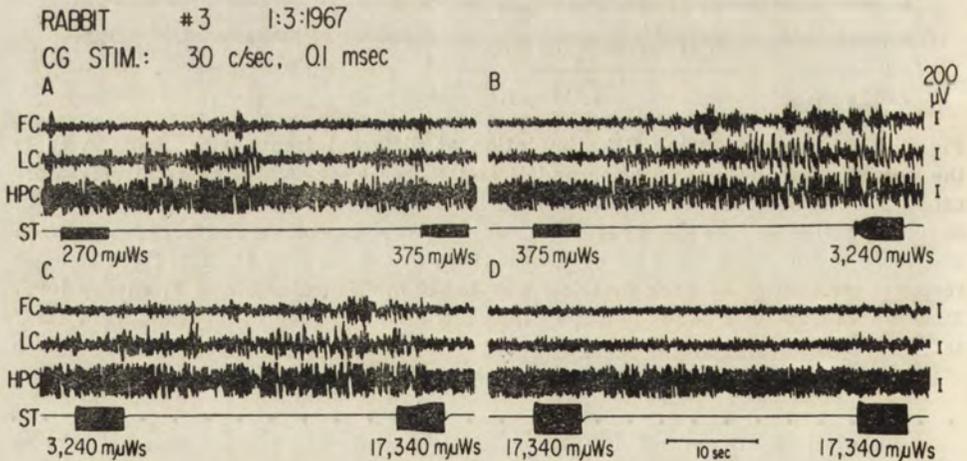
Fig. 6. EEG records during CG stimulation. A, in the relatively alert rabbit 270 mμWs/train was below and 375 mμWs/train was at threshold for HPC theta rhythm (THTA). B and C, during spindle sleep an energy level higher than 3,240 mμWs/train was needed to induce the HPC theta rhythm (THTS). D, the energy level of 17,340 mμWs/train was above threshold (TSR) for the somatic response (relaxation of neck muscles)

to this region. Stimulation with this electrode placement induced EEG arousal in all 14 animals.

The mean energy threshold for inducing hippocampal theta rhythm by CG stimulation in alert animals (THTA) was 900±230 mμWs/train for 30 c/sec and 2,700±825 mμWs/train for 300 c/sec stimulation. Repetition of the trains resulted in spindle sleep with RF stimulation. The mean energy threshold for theta rhythm during spindle sleep (THTS) was 5,715±600 mμWs/train for 30 c/sec and 13,350±4,200 mμWs/train for 300 c/sec stimulation. The difference was not significant (p = 0.1—0.05).
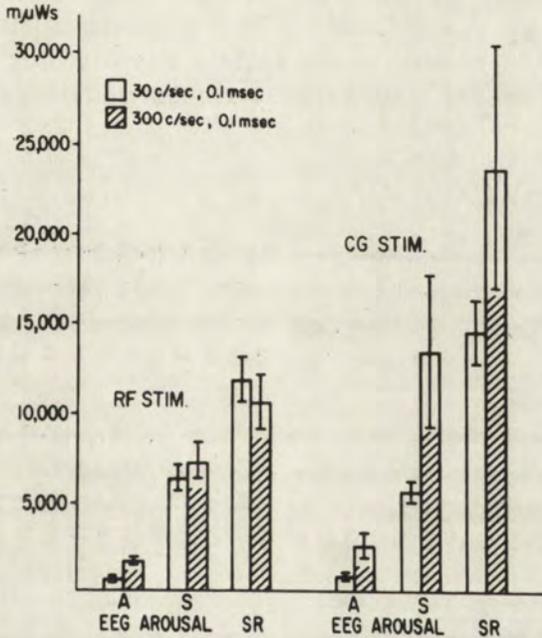
Fig. 7. Thresholds of EEG arousal (HPC theta rhythm) during arousal (A = THTA) and sleep (S = THTS) and for somatic responses (SR = TSR). Bars represent mean energy in mμWs/train and brackets, the standard error of the mean

The mean threshold for somatic responses (TSR) was 14,460±±1,815 mμWs/train for 30 c/sec and 23,550±720 mμWs/train for 300 c/sec; the difference was not significant (p = 0.3—0.2). The 30 c/sec trains caused relaxation of the neck muscles and a slow lowering of the head in 12 animals in which this threshold was studied. Somatic responses evoked by 300 c/sec were as follows: lowering of the head in 9 animals,

2*

in 3 of which tonic contralateral head movement was also observed. In 4 animals tonic head movements appeared alone, contralateral in 3 cases and ipsilateral in the other.

The energy threshold for continuous EEG arousal pattern

The energy thresholds established above for hippocampal theta rhythm were taken into consideration during trials of the automatically controlled modulated stimulation. The range was set so that the minimum modulated stimulus energy was close to the threshold in the alert animal (THTA) and the maximum stimulus energy was below the threshold during spindle sleep (THTS).

RF stimulation. In automatically controlled and modulated RF stimulation spindle sleep was induced by both frequencies (30 and 300 c/sec) in all animals. The density of the spindle sleep records in the cortical leads usually increased after each 30 sec in which the minimum and
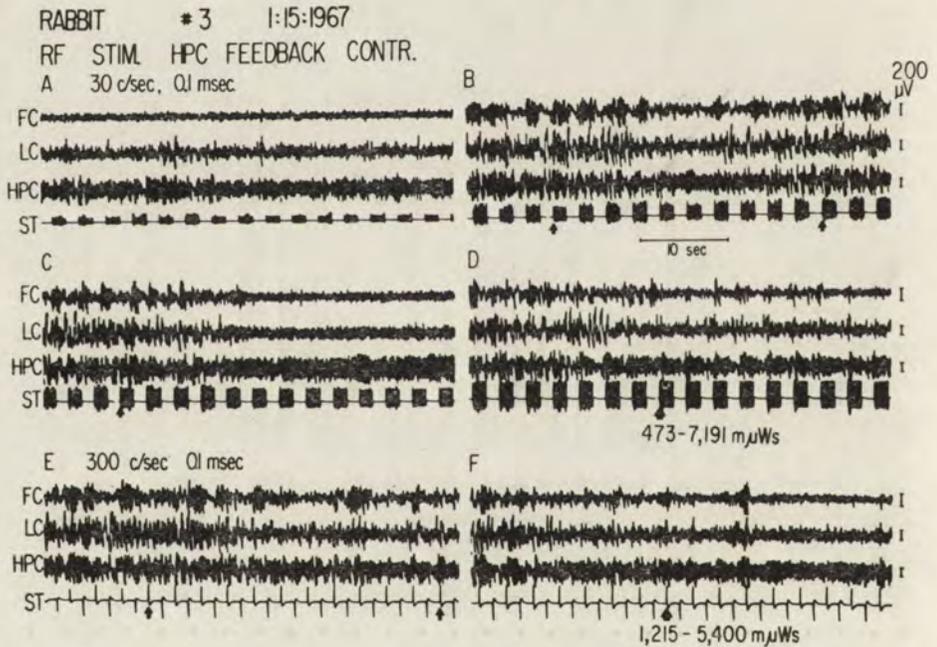
Fig. 8. EEG records during modulated RF stimulation under HPC feedback control with 1.5 sec trains and 1.5 intervals (ST). The arrows indicate the stimulation energy was increased. In A to D the pulse frequency was 30 c/sec. C, episodes of paradoxical sleep. D, The energy which induced a continuous EEG arousal pattern ranged from 473 mμWs/train minimum to 7,191 mμWs/train maximum (TCAP). In E and F the pulse frequency was 300 c/sec. F, A continuously aroused EEG pattern was induced by a range of 1,215 mμWs/train to 5,400 mμWs/train maximum (TCAP)

maximum amplitude of the pulses were jointly shifted upward. In some animals paradoxical sleep appeared giving way to spindle sleep if RF stimulation was continued after 20—150 sec of paradoxical sleep. If the minimum and maximum amplitude of the pulses was adjusted still higher a continuous EEG arousal pattern appeared, i.e., the reversion threshold (TCAP) had been reached. The arousal pattern could be maintained in immobile animals for 10—60 min or longer. The mean energy threshold for the continuous EEG arousal pattern (TCAP) was 4,185± ±590 mμWs/train for 30 c/sec and 3,285±540 mμWs/train for 300 c/sec stimulation. Again the difference between these thresholds was not significant (p = 0.3—0.2). The reversion threshold (TCAP) was calculated only for maximal energy trains since during spindle sleep the hippocampal theta rhythm did not appear and the unmodulated pulses reached their maximum.
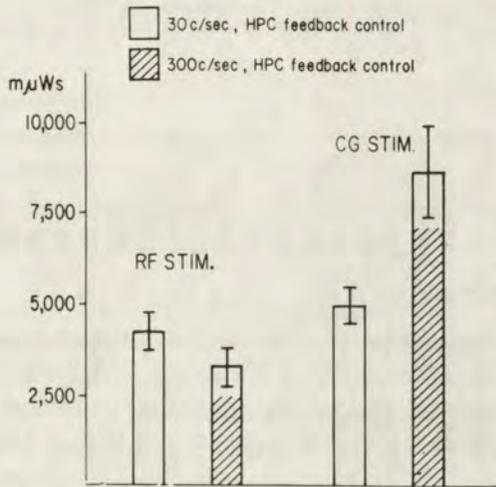
Fig. 9. Maximum energy levels (TCAP) required to convert induced spindle sleep into continuous EEG arousal (mean ± standard error in 12 animals). The stimulus was modulated under HPC feedback control with 1.5 sec trains and 1.5 sec intervals and pulses of 30 and 300 c/sec frequency were delivered to the midbrain reticular formation (RF) and periaqueductal central gray (CG)

CG stimulation. Stimulation of the central gray with the automatically controlled and modulated system with 30 and 300 c/sec trains gave results similar to those following stimulation of the RF. The range between minimum and maximum amplitude of pulses was again established according to the theta rhythm thresholds. The mean energy threshold for conversion from spindle sleep into the continuously aroused EEG pattern (TCAP) was 5,009±463 mμWs/train for 30 c/sec and 8,685±

$\pm 1,215$ mμWs/train for 300 c/sec. This difference between thresholds is significant (p $<$ 0.05).

The stimulation of RF or CG in fully alert and freely moving animals with modulating trains of either frequency at energy levels below the threshold for continuously aroused EEG pattern markedly changed the animal's behavior. A few seconds after the onset of the modulated stimulation the rabbits sat down or lay down and spindle sleep appeared in the EEG.
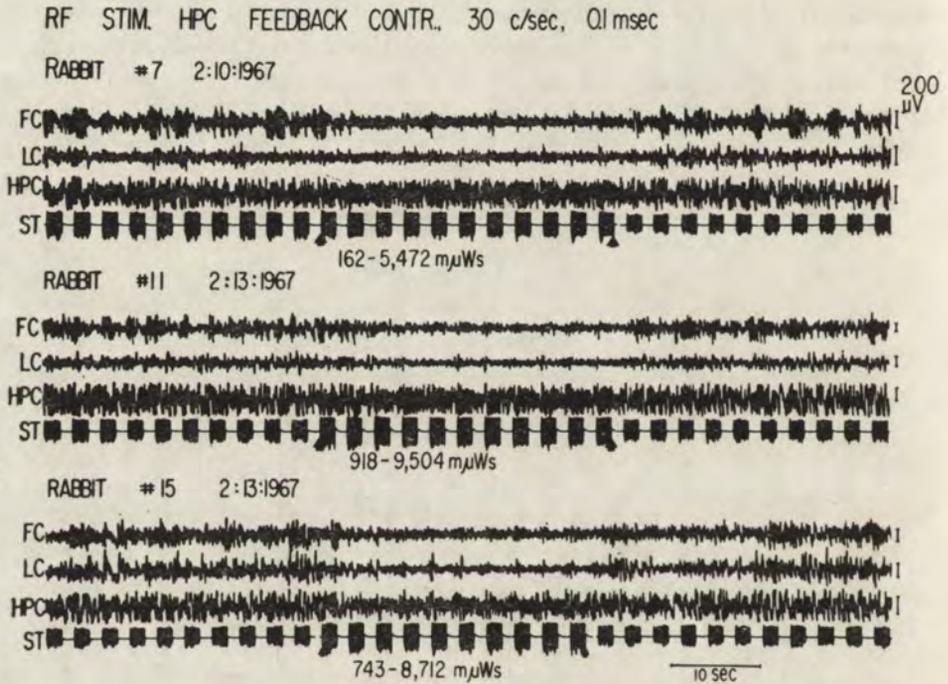
Fig. 10. EEG records of three rabbits during modulated RF stimulation under HPC feedback control with 1.5 sec trains and 1.5 sec intervals and a pulse frequency of 30 c/sec. The arrows indicate 30 sec periods during which the stimulation energy was increased to the threshold for continuous EEG arousal (TCAP). The figures under each record indicate the range from minimal to maximal energy per train needed to convert spindle sleep to the induced continuous EEG arousal pattern

Behavioral responses to the modulated stimulation showed two phases. In the first phase stimulation of the RF produced sleep spindles, but the animals occasionally stood up and moved around the chamber as during nonstimulation periods. During the second phase of stimulation, in which the continuous EEG arousal pattern was maintained, the animals usually remained motionless on the floor; they stood up and moved much less

frequently than during the first phase. In some animals rhythmic pal-pebral movements could be seen during this motionless alert state coin-cident with stimulation.

Two-way reversion of the EEG pattern

After the energy threshold for the continuous EEG arousal pattern (TCAP) had been established the two-way reversion of EEG pattern was instituted. The minimum and maximum amplitudes of pulses were join-tly adjusted to the range at which the deepest spindle sleep was recorded. Shifting the amplitude up to the level for continuous arousal immediately evoked desynchronization of the cortical record and a theta rhythm in the hippocampus. After 30 sec the amplitude of the pulses was shifted back down to the initial level and spindle sleep reappeared in a few seconds.

This two-way reversion of the EEG pattern was repeated several times in each animal using 30 c/sec trains of stimulation of either RF or CG. The energy levels of the trains inducing the reversion remained quite constant for individual animals over a few weeks of testing.

DISCUSSION

Behavioral sleep and/or EEG sleep patterns have been elicited in many species by direct stimulation of various regions of the brain. Many of the experiments were done in cats in which hypnogenic areas were found in the thalamus (Hess 1929, 1956, Akert et al. 1952; Hess Jr. et al. 1953; Hernández-Peón and Chávez Ibarra 1963; Parmeggiani 1964), mid-brain reticular formation (Favale et al. 1961; Parmeggiani 1964), basal forebrain (Clemente and Sterman 1963), preoptic region and amygdala (Hernández-Peón and Chávez Ibarra 1963), mammillary bodies and hip-pocampus (Parmeggiani 1964) and pontine reticular formation (Rossi 1963). Sleep was also produced by stimulation of the thalamus in dogs (Akimoto et al. 1956), midbrain reticular formation in monkeys (Proctor et al. 1957) and in rats (Caspers and Winkel 1954). In rabbits sleep was induced by electrical stimulation of rhinencephalic-hypothalamic areas (Faure 1957, Kawakami and Sawyer 1959), and the medio-central in-tralaminar thalamus and midbrain reticular formation (Monnier et al. 1963).

Our results are in agreement with their reports implicating the midbrain reticular formation as a hypnogenic area but they differ signi-ficantly in the parameters of electrical stimulation. To induce spindle sleep in rats Caspers and Winkel (1954) used a stimulus frequency of

12 c/sec; in cats, Favale et. al. (1961) used 12 c/sec and Parmeggiani (1964), 4, 8.5 or 17 c/sec; in rabbits Faure (1957) Kawakami and Sawyer (1959) and Monnier et al. (1963) all used low frequency stimulation of 4—6 c/sec. For triggering paradoxical sleep in rabbits Kawakami and Sawyer (1964) and Faure (1964) applied 5 c/sec to the central gray.

However, Kaada et al. (1967) reported that the optimal stimulation frequency for producing synchronization in anesthetized cats was about 100—300 c/sec and it also could be obtained with 30 c/sec. Prince and Shanzer (1966) also obtained rhythmic slow activity in the neocortex at moderate levels of anesthesia by stimulating the mesencephalic tegmentum at high frequency (40—250 c/sec) in cats. High frequency stimulation of the midbrain reticular formation has also been employed in the spindle stage of sleep to trigger paradoxical sleep in cats (Jouvet et al. 1960, Rossi et al. 1961, Rossi 1963, Lissák et al. 1965).

Our results indicate that the stimulus frequency is not the most critical parameter for elicitating behavioral or EEG sleep or arousal patterns, but the amount of energy applied per second or per train. Stimulating RF at frequencies of 30 and 300 c/sec, no significant differences attributable to frequency were found in the amount of energy per train for producing EEG arousal or reversion of spindle sleep into a continuous EEG arousal. The threshold energies for somatic responses elicited by the low and high frequency stimulation differed only insignificantly. From the same stimulation point in one animal two different somatic responses were elicited by low and high frequency stimulation of about the same amount of energy per train. Low frequency stimulation caused a lowering of the head with relaxation of the neck muscles, and high frequency did the same but with additional ipsilateral or contralateral tonic head movements which were more prominent. Such somatic reactions have also been observed in cats (Hess 1956, Skultety 1962) and in rabbits (White and Himwich 1957). Our observation did not confirm Ward's (1958) interpretation that stimulation of the medial part of the midbrain reticular formation elicited ipsilateral and the lateral part, contralateral head turning. The somatic response to midbrain stimulation is more a function of stimulus frequency than of position of the electrode tips.

Habituation did not occur to modulated trains in our experiments. This agrees to a certain extent with findings of Ursin et al. (1967) but does not confirm the findings of Glickmann and Feldman (1961) or Drewczyński (1968). However, lack of habituation in our experiments does not prove that this phenomenon cannot be elicited from stimulation of the midbrain. It seems rather that continual variations of pulse

and train energy interfered with habituation. Variations in the energy of sequential pulses in one train as well as the energy of sequential trains eliminated the repetitiveness of stimuli needed for habituation.

In our experiments it has been rather difficult to differentiate separate neural mechanisms for behavioral and electrical sleep patterns. Trains of both frequencies, with energy levels below the threshold for somatic responses, caused alert and moving animals to lie down after a few seconds and show spindle sleep. The animal's behavior and the induced electrical activity of the brain could not be differentiated from spontaneous sleep.

It is likely that the neural mechanisms for spindle sleep as well as paradoxical sleep require a certain low amount of energy. The lowest amount of energy per second or per train induces spindle sleep followed sometimes by paradoxical sleep. Increases in energy per train step by step induce EEG and later behavioral arousal. The energy required for the induction of sleep can be applied by direct electrical stimulation of the midbrain or other brain structures or by peripheral nerve stimulation (Pompeiano 1963).

The induced continuous EEG arousal pattern is a peculiar stage from both behavioral and bioelectrical points of view: the stage of immobilization with an aroused EEG pattern is difficult to classify. The position of the animal's body remains as during spindle sleep, prone on the floor with the head rather low and ears elevated. The frequency of the hippocampal theta rhythm during each train is slower than during paradoxical sleep (Kawamura and Sawyer 1964). In the 1.5 sec intervals between stimulation the theta rhythm was even slower. When the modulated stimulation was switched off there was usually a short phase of spindle sleep after which the animal alerted, stood up and moved. This suggests that the induced continuous EEG arousal pattern differs significantly from both paradoxical sleep and full alertness. It can be characterized as an induced cataleptic state with a desynchronized cortical electrical pattern and a low frequency hippocampal theta rhythm.

According to Bonvallet and Newman-Taylor (1967) there is evidence for differential organization in the midbrain reticular formation. Stimulation of the dorsal part and the region immediately adjacent to the central gray is most effective for self-sustained reticular discharges in cats. The trains of energy applied to the midbrain in our unanesthetized rabbits were probably high enough to activate the neuronal intrareticular circuits producing constant EEG arousal, but was perhaps too low to produce behavioral arousal or in some way suppressed it by an inhibitory action on the motivation centers.

SUMMARY

An automatic feedback control system has been developed for maintaining electrically stimulated spindle sleep or EEG arousal in unrestrained, unanesthetized rabbits with chronically implanted electrodes. Hippocampal electrical activity was used to modulate the amplitude of electrical pulses automatically applied to the midbrain reticular formation or periaqueductal central gray in 1.5 sec trains with a frequency of 30 or 300 c/sec and 1.5 sec intertrain intervals. In the development of this system energy thresholds were established for inducing a hippocampal theta rhythm during relative alertness (THTA) and spindle sleep (THTS) and for evoking somatic responses (TSR) (5 sec trains, pulse duration 0.1 msec, 30 sec intervals, frequencies 30 and 300 c/sec). The energy thresholds at frequencies of 30 and 300 c/sec were generally similar, but arousal from spindle sleep with the stimulus applied to the central gray required a significantly higher energy for 300 c/sec trains than for 30 c/sec trains. With the automatically modulated system low energies of stimulation induced behavioral and EEG sleep patterns. Raising the energy level caused a reversal of the EEG pattern to arousal (TCAP) which could be maintained for several minutes; spindle sleep could be restored by merely lowering the energy of the stimulation train below arousal threshold.

REFERENCES

AKERT, K., KOELLA, W. P. and HESS, R. Jr. 1952. Sleep produced by electrical stimulation of the thalamus. Amer. J. Physiol. 168: 260—267.

AKIMOTO, H., YAMAGUCHI, N., OKABE, K., NAKAGAWA, T., NAKAMURA, I., ABE, K., TORII, H. and MASAHASHI, K. 1956. On the sleep induced through electrical stimulation on dog thalamus. Folia Psychiat. Neurol. Jap. 10: 117—146.

BONVALLET, M. and NEWMAN-TAYLOR, A. 1967. Neurophysiological evidence for a differential organization of the mesencephalic reticular formation. Electroenceph. Clin. Neurophysiol. 22: 54—73.

BUENO, J. R., BOST, K. and HIMWICH, H. E. 1968. Lower brain-stem EEG synchronizing mechanisms in the rabbit. Electroenceph. Clin. Neurophysiol. 24: 25—34.

CASPERS, H. and WINKEL, K. 1954. Die Beeinflussung der Grosshirnrindenrhythmik durch Reizung im Zwischen — und Mittelhirn bei der Ratte. Pflügers Arch. Ges. Physiol. 259: 334—356.

CLEMENTE, C. D. and STERMAN, M. B. 1963. Cortical synchronization and sleep patterns in acute restrained and chronic behaving cats induced by basal forebrain stimulation. Electroenceph. Clin. Neurophysiol. Suppl. 24: 172—187.

DREWCZYŃSKI, A. 1968. Extinction of hippocampal arousal during repetitive midbrain electrical stimulation in rabbits. Bull. Acad. Polon. Sci. Ser. Biol. 16: 311—315.

FAURE, J. 1957. Participation du rhinencéphale à la régularisation hormonale. Rev. Path. Gen. 690: 1029—1053; 691: 1263—1283; 692: 1445—1463.

FAURE, J. 1965. Hormones in relation to sleep-wakefulness mechanisms. Proceedings, 2nd International Congress of Endocrinology London 1964, Exc. Med. Int. Congr. Ser. 83: 606—611.

FAVALE, E., LOEB, C., ROSSI, G. F. and SACCO, G. 1961. EEG synchronization and behavioral signs of sleep following low frequency stimulation of the brain stem reticular formation. Arch. Ital. Biol. 99: 1—22.

GLICKMAN, S. E. and FELDMAN, S. M. 1961. Habituation of the arousal response to direct stimulation of the brain stem. Electroenceph. Clin. Neurophysiol. 13: 703—709.

GREEN, J. D. and ARDUINI, A. A. 1954. Hippocampal electrical activity in arousal. J. Neurophysiol. 17: 533—557.

HERNÁNDEZ PEÓN, R. and CHÁVEZ IBARRA, G. 1963. Sleep induced by electrical or chemical stimulation of the forebrain. Electroenceph. Clin. Neurophysiol. Suppl. 24: 188—198.

HESS, R. Jr., KOELLA, W. P. and AKERT, K. 1953. Cortical and subcortical recordings in natural and artificially induced sleep in cats. Electroenceph. Clin. Neurophysiol. 5: 75—90.

HESS, W. R. 1929. Lokalisatorische Ergebnisse der Hirnreizversuche mit Schlafeffekten. Arch. Psychiat. Nervenkr. 88: 813—816.

HESS, W. R. 1956. Hypothalamus und thalamus. Experimental Dokumente. Georg. Thieme Verlag, Stuttgart.

JOUVET, M., DECHAUME, J. and MICHEL, F. 1960. Étude des mécanismes du sommeil physiologique. Lyon Med. 204: 479—521.

KAADA, B. R., THOMAS, F., ALNAES, E. and WESTER, K. 1967. EEG synchronization induced by high frequency midbrain reticular stimulation in anesthetized cats. Electroenceph. Clin. Neurophysiol. 22: 220—230.

KAWAKAMI, M. and SAWYER, C. H. 1959. Induction of behavioral and electroencephalographic changes in the rabbit by hormone administration or brain stimulation. Endocrinology 65: 631—643.

KAWAKAMI, M. and SAWYER, C. H. 1964. Conditioned induction of paradoxical sleep in the rabbit. Exptl. Neurol. 9: 470—482.

KAWAMURA, H. and SAWYER, C. H. 1964. D-C potential changes in rabbit brain during slow-wave and paradoxical sleep. Amer. J. Physiol. 207: 1379—1386.

LISSÁK, K., KARMOS, G. and GRASTYÁN, E. 1965. A study of the so-called "Paradoxical Phase" of sleep in cats. Progr. Brain Res. 1: 424—428.

MONNIER, M., HÖSLI, L. and KRUPP, P. 1963. Moderating and activating systems in medio-central thalamus and reticular formation. Electroenceph. Clin. Neurophysiol. Suppl. 24: 97—112.

MORUZZI, G. and MAGOUN, H. W. 1949. Brain stem reticular formation and activation of EEG. Electroenceph. Clin. Neurophysiol. 1: 455—473.

MULHOLLAND, T. and RUNNALS, S. 1962. Evaluation of attention and alertness with a stimulus-brain feedback loop. Electroenceph. Clin. Neurophysiol. 14: 847—852.

MULHOLLAND, T. and RUNNALS, S. 1964. Cortical activation during steady and changing visual stimulation. Electroenceph. Clin. Neurophysiol. 17: 371—375.

PARMEGGIANI, P. L. 1964. A study on the central representation of sleep behaviour. Progr. Brain Res. 6: 180—189.

PETSCHE, H. and STUMPF, CH. 1960. Topographic and toposcopic study of origin an spread of the regular synchronized arousal pattern in the rabbit. Electroenceph. Clin. Neurophysiol. 12: 589—600.

POMPEIANO, O. 1963. EEG synchronization induced by peripheral nerve stimulation. Progr. Brain Res. 1: 429—432.

PRINCE, D. A. and SHANZER, S. 1966. Effects of anesthetics upon the EEG response to reticular stimulation. Patterns of slow synchrony. Electroenceph. Clin. Neurophysiol. 21: 578—588.

PROCTOR, L. D., KNIGHTON, R. S. and CHURCHILL, J. A. 1957. Variations in consciousness produced by stimulating reticular formation of the monkey. Neurology 7: 193—203.

ROSSI, G. F., FAVALE, E., HARA, T., GIUSSANI, A. and SACCO, G. 1961. Researches on the nervous mechanisms underlying deep sleep in the cat. Arch. Ital. Biol. 99: 270—292.

ROSSI, G. F. 1963. Sleep inducing mechanisms in the brain stem. Electroenceph. Clin. Neurophysiol. Suppl. 24: 113—132.

SAWYER, C. H., EVERETT, J. W. and GREEN, J. D. 1954. The rabbit diencephalon in stereotaxic coordinates. J. Comp. Neurol. 101: 801—824.

SHARPLESS, S. and JASPER, H. 1956. Habituation of the arousal reaction. Brain 79: 655—680.

SKULTETY, F. M. 1962. Circus movements in cats following midbrain stimulation through chronically implanted electrodes. J. Neurophysiol. 25: 152—164.

URSIN, H., WESTER, K., and URSIN, R. 1967. Habituation to electrical stimulation. of the brain in unanesthetized cats. Electroenceph. Clin. Neurophysiol. 23: 41—49.

WARD, A. A. 1958. Efferent functions of the reticular formation. In H. H. Jasper et al. (ed.), Henry Ford Hospital International Symposium, Reticular Formation of the Brain, Boston—Toronto, p. 263—273.

WHITE, R. P. and HIMWICH, H. E. 1957. Circus movements and excitation of the striatal and mesodiencephalic centers in rabbits. J. Neurophysiol. 20: 81—90.

ZANCHETTI, A. 1967. Brain stem mechanisms of sleep. Anesthesiology 28: 81—98.

STUDIES ON THE VISUAL FIXATION REFLEX
III. THE EFFECTS OF FRONTAL LESIONS IN THE CAT

Bogdan DREHER and Bogusław ŻERNICKI

Department of Neurophysiology, Nencki Institute of Experimental Biology,
Warsaw, Poland

The fixation reflex plays an important role in the perception processes in the vertebrates with a part of retina specialized for detailed vision. However, the behavioral and particularly the neural properties of the fixation reflex are unsatisfactorily known. This is mainly due to the technical difficulties: the fixation reflex is absent under narcosis while the recording of the eye movements in an unanesthetized animal is difficult. To avoid these difficulties the cats with pretrigeminal brain stem transection (Batini et al. 1959, Żernicki 1968a) were used in the present series of studies. In the pretrigeminal cat the fixation reflex is preserved (Batini et al. 1959, Affanni et al. 1962) while due to the restraining of the cat in a stereotaxic apparatus the accurate recording of eye movements is possible.

In the first paper of the series (Żernicki and Dreher 1965) the behavioral properties of the fixation reflex were studied. In the second paper (Dreher et al. 1965) some facts concerning the reflex arc of the fixation were established. It was found that the fixation reflex is absent after removal of the superior colliculi (even without damage of the pretectal area), and is seriously impaired after bilateral ablation of the visual cortex. On the other hand, after bilateral lesion of the frontal oculomotor cortex the fixation reflex was not impaired, and in some cats it appeared to by very resistant to habituation. The further analysis of the last finding is the objective of the present paper.

METHODS

Apparatus. The same experimental chamber as in the preceding papers of this series was used. The chamber was optically isolated and illuminated with a strong diffused light (the illumination at the eyes was 650 luxes). The cat was placed in the chamber after pretrigeminal transection, restrained in a stereotaxic apparatus.

For evoking the fixation reflex 3 sec rotation of a black "X" shaped figure of angular diameter 12° was used. Two "X" figures were located on a tangent white screen. One of the figures was 25° above the horizontal plane through the nodal point of the animal's eyes (see Vakkur et al. 1963) and the other 25° below this level.

As the control stimuli, the odors of valeriane and butyric acid were used (see Żernicki et al. 1967). The odors was introduced into both nostrils of the cat using a modified blast injection method: for about 3 sec, 400 ml of the air was blown in three strokes by a pump; the air passed through a 100 ml bottle containing 20 ml of the odorous liguid.

The right eyeball was filmed (for technique of film analysis see the first paper of the series). The ECoG activity was recorded bipolarly with an inkwriter from visual areas.

Procedure. 1. Preoperative general examinations. The locomotor behavior based on visual cues was tested in an experimental room. During the examination of ocular activity, the head of the cats was restrained by hand. The visual stimuli were a piece of white cotton wool and a piece of meat. They were moved rhythmically in front of the cat's eyes at distance of about 40 cm with a speed 30°—50°/sec.

2. Bilateral cortical ablation. The operation was done by suction in sterile conditions under Nembutal narcosis.

3. Postoperative general examinations (see above). They were started three days after the cortical operation.

4. Pretrigeminal transection. This was done 7—17 days after the cortical ablation. The transection was performed in tracheotomized cats under ether anaesthesia (for technique see the first paper of the series and Żernicki 1968a). To allow better observation of the eyeballs, the nictitating membranes and the upper eyelids were partially removed. For ECoG activity recording, silver electrodes were implanted supradurally in visual areas.

5. Session I. This began 2 hr after the pretrigeminal transection. The session consisted of two parts. In the first part, the fixation reflex to the rotation of one or the "X" figure (the "X" figure producing a stronger response was used) was habituated. Maximal number of trials was 250. The first 30 intertrial intervals lasted 60 sec, and the following ones, 30 sec. The fixation reflex was considered habituated when it was absent in three succesive trials. For checking dishabituation, the rotation of the opposite "X" figure was applied.

In the second part of the session, the ocular responses (eye movements, pupillary dilatation) and ECoG responses to the odor of valeriane were habituated. Maximal number of trials was 60. The first 30 intertrial intervals lasted 2 min and the following ones, 1 min. Similarly as with the visual stimulus, the criterion of habituation was the lack of responses in three succesive trials. For testing dishabituation, the odor of the butyric acid was used.

6. Session II. This started 90 min after the end of the session I. During this the fixation reflex usually fully recovered. The fixation reflex was rehabituated in the same way as in the session I. However, due to the slow time course of the spontaneous recovery of the response to the odor of valeriane (see Żernicki et al. 1967), the olfactory stimuli were not applied in the session II.

7. Testing of the following reflex. The reflex was tested to the movement of a piece of white cotton wool as described above. In addition, in some preparations the following reflex was tested carefully in an optically isolated chamber. The stimulus was a beam of light moving on the perimeter. The eye movements were recorded with a technique developed by Dreher and Kozak (to be published): the movement of the beam of light, reflected by a mirror connected to the eyeball, was recorded with a photokymograph.

Nursing care. During experiments the temperature of the cat was maintained from 38° to 39°. Between the experimental sessions the eyes were closed with bandages soaked with saline.

Statistics. Only the Principal Experiment was analysed statistically. In the Tables the differences between the experimental Groups and the control Group above $p < 0.05$ were marked. For all statistics two-tailed tests were used.

RESULTS

Principal experiment

The experiment was performed on 32 cats. The control group (Group Con) consisted of 11 cats. The extent of lesions in the experimental groups was based on our recent finding (Dreher, Santibañez-H. and Żernicki, in preparation) that in unaneasthetized cats (pretrigeminal and encephale isolé preparations) the eye movements may be obtained from a large frontal region comprising the whole anterior sigmoid gyri on the lateral and medial aspects of hemisphere. In the first experimental group (12 cats) the frontal oculomotor area on the lateral aspect of the hemispheres was only removed (Group LOM) (Fig. 1). In the second experimental group (9 cats) the frontal oculomotor area was totally removed (Group TOM) (Fig. 2).
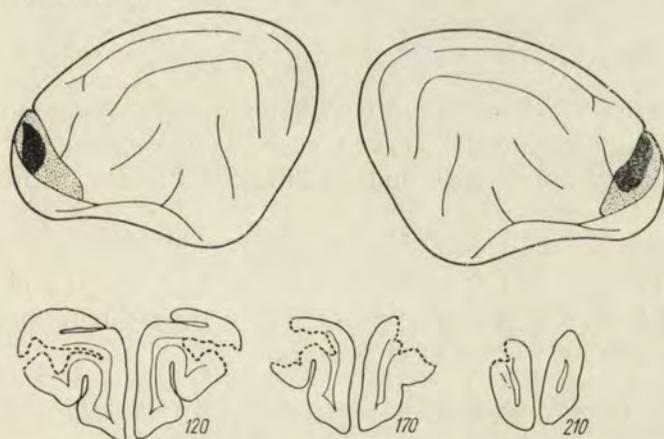
Fig. 1. Lateral frontal oculomotor (LOM) ablations. Above, reconstructions of maximal (stippled area) and minimal (shaded area) lesions. Below, representative cross sections (stippling indicates gliosis)
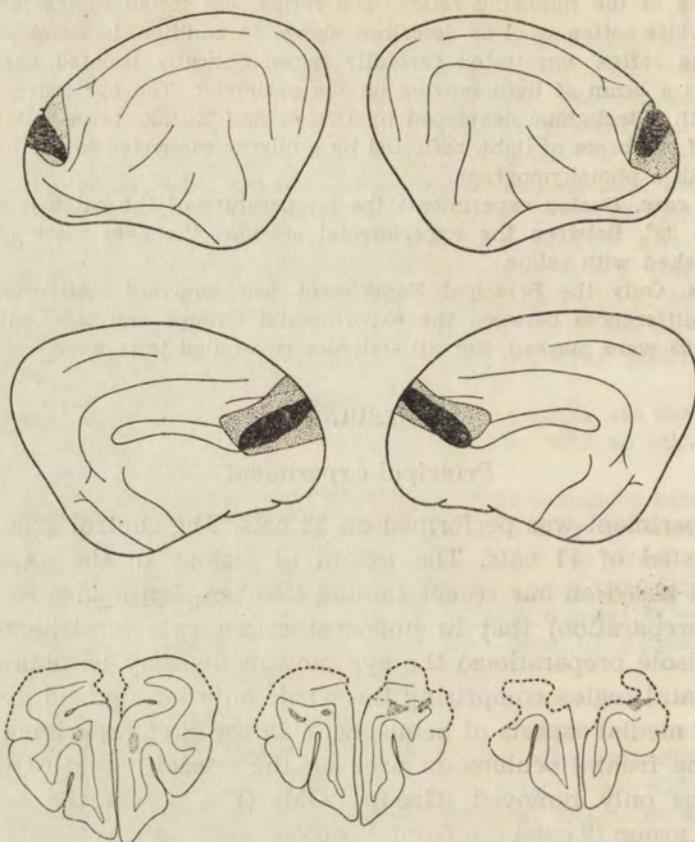
Fig. 2. Total frontal oculomotor (TOM) ablations. Other denotations as in Fig. 1

Observations before pretrigeminal transection

The behavior of the cats with oculomotor lesions was only slightly changed. Their movements showed some stiffness, and they jumped down from boxes unwillingly and unskillfully. Besides, both visual and tactile placing responses were absent.

The vertical and horizontal "spontaneous" ocular activity was present in all cats. The eye and head components of the vertical and horizontal fixation and following reflexes, and the eye and head movements toward the auditory stimuli were also unaffected.

Observations after pretrigeminal transection

Ocular and ECoG background activity. The oculomotor lesions did not affect the position of the eyeballs. The pupillary diameter was also similar in all groups of animals (Table I).

Table I
Background diameter of pupils (in millimeters)

Groups		
Con	LOM	TOM
0.2	0.3	0.3
0.3	0.4	0.3
0.4	0.4	0.4
0.5	0.5	0.4
0.6	0.5	0.7
0.8	0.5	1.0
1.0	0.5	1.0
1.6	0.5	1.1
1.8	0.6	1.6
2.2	0.7	
2.3	1.0	
	1.1	
Median 0.8	0.5	0.7
Mean 1.1	0.6	0.7

In accordance with the previous description of the ECoG activity in the pretrigeminal cat (Żernicki et al. 1967), in some cats of the control Group the ECoG activity was continuously desynchronized, while in other cats the desynchronized ECoG activity was mixed in different proportion with synchronized activities. Similar results were obtained in the experimental Groups (Table II).

Table II
Percentage of cats with permanent desynchronization of ECoG activity

Groups		
Con (N = 11)	LOM (N = 12)	TOM (N = 9)
54	42	44

General character of the fixation reflex. The oculomotor ablations also did not seem to change the essential properties of the fixation reflex (see the first paper of the series). In all Groups of animals both single and serial fixation reflexes were observed. In the single reflex four phases (saccadic movement toward the "X" figure, maintenance of fixation, return movement and tonic fixation) were present. The phase of

http://rcin.org.pl

maintenance was usually longer-lasting than the "X" figure rotation. In the serial reflex the characteristic train of "after-fixations" (the cats fixated fully or abortively the already motionless "X" figure) was observed.

Duration of the fixation reflex. Because of the habituation of the reflex (see below) the first trial of the sessions only was analysed in this respect. In both experimental Groups the time when cat fixated the

Table III
Duration of fixation (in seconds) in the
first trial (means from both sessions
are given)

Groups		
Con	LOM	TOM
2.8	2.3	5.6
5.3	3.1	6.1
5.7	3.3	7.9
6.0	5.6	8.0
6.4	5.8	10.5
6.7	7.7	14.2
7.3	8.2	14.6
7.8	10.6	19.8
8.1	11.8	27.0
8.3	15.9	
11.0	18.5	
	20.3	
Median 6.7	8.0	10.5
Mean 6.9	9.4	12.6*

* p<0.05 (Mann-Whitney U test).

Table IV
Percentage of cats with serial fixation reflex in the
first trial

	Groups		
	Con (N = 11)	LOM (N = 12)	TOM (N = 9)
In one session only	18	50	33
In both sessions	0	25	22
Totally	18	75*	55

* p<0.025 (Fisher exact probability test).

stimulus was increased. This was manifested in two ways. First, the phase of maintenance of fixation was longer-lasting (Table III). Second, the after-fixations were more common (Table IV). Both these effects were positively correlated (Table V).

Table V

Duration of fixation (in seconds) in the first trial in cats of Groups LOM and TOM with single reflex in both sessions (on left) and serial reflex in both sessions (on right) (means from both sessions are given)

Groups	
Cats with single reflex	Cats with serial reflex
2.3	10.5
3.1	11.8
3.3	15.9
5.6	18.5
6.1	19.8
7.9	
14.2	
Median 5.6	15.9
Mean 6.1	15.3*

* p = 0.01 (Mann-Whitney U test).

Table VI

Dilatation of pupils (in millimeters) to the odor of valeriane in the first trial[a]

Groups		
Con	LOM	TOM
0.3	0.4	0.5
0.5	0.4	0.7
0.8	0.5	0.9
1.4	0.5	
1.8	0.6	
	1.2	
	1.4	
	1.6	
	2.0	
Median 0.8	0.6	0.7
Mean 0.8	0.9	0.7

[a] In some cats the data were not available.

In some cats for control in the end of experiment the rotation of the "X" figure was much prolonged: to 20 sec or even to 7 min. In response to such stimulus the phase of maintenance of fixation was usually also much prolonged in the experimental Groups (some cats fixated the rotating "X" figure even for 7 min) but not in the control Group (see also the first paper of the series).

The pupillary and ECoG components of the reflex to the rotation of the "X" figure were also recorded. However, the comparison of their strength in different groups of cats was difficult for two reasons (i) under our experimental conditions the accurate estimation of the pupillary diameter during eye movements was not possible, and (ii) there were considerable individual differences in the background ECoG activity.

On the other hand, the pupillary dilatation to the olfactory stimuli could be accurately measured. Table VI shows that the dilatation to the odor of valeriane was similar in all groups of animals.

Habituation of the fixation reflex. The general character of the habituation of the fixation reflexes seemed to be similar in all groups and

in accordance with the original description of habituation in the pretrigeminal cats given in the first paper of the series. The following features of the habituation may be noted: (i) the after-fixations usually habituated within a few trials, (ii) the fixation reflexes became gradually abortive (iii) the tonic fixation became gradually larger, (iv) a pause of 5 min resulted in a partial recovery and a pause of 90 min usually in a full recovery of the fixation reflex, and (v) a dishabituation of the fixation reflex could be always evoked by a single application of the rotation of the opposite "X" figure.

Table VII

Resistance to habituation (in trials) of fixation reflex (means from both sessions are given)

Groups		
Con	LOM	TOM
3	16	16
6	17	17
7	18	33
12	20	50
14	21	61
14	32	71
15	45	73
24	79	151
38	82	245
52	90	
	91	
	235	
Median 14	38	61
Mean 22.9	73.1*	84.7*

* p<0.02 (Mann-Whitney U test).

Table VIII

Resistance to habituation (in trials) of fixation reflex in cats of Groups LOM and TOM with single reflex in the first trial of both sessions (on left) and with serial reflex in the first trial of both sessions (on right)

(means from both sessions are given)

Groups	
Cats with single reflex	Cats with serial reflex
16	45
17	79
18	151
20	235
50	245
73	
82	
Median 20	151
Mean 39.4	151*

* p = 0.03 (Mann-Whitney U test).

On the other hand, in both experimental Groups the resistance to habituation of the fixation reflex was strongly increased; the mean number of trials needed for habituation was approximately three times higher than in the control Group (Table VII). In addition, this difference was obviously much reduced by the shortening of the intertrial intervals from 60 sec to 30 sec after the first 30 trials. Even under these conditions in two cats (one cat from Group LOM and one from Group TOM) during one session the fixation reflex was not habituated within 250 trials. The increase of resistance to habituation was positively correlated with the presence of after-fixations (Table VIII).

Table IX presents the data concerning the habituation of the un-specific responses (pupillary dilatation and ECoG arousal) to rotation of the "X" figure. Their habituation is presented jointly because in some cats the pupillary dilatation was more resistent to habituation and in some cats the ECoG arousal. We see that in both experimental Groups some trend for an increase of resistance to habituation was present. However, it could be simply a secondary phenomenon due to the increase of resistance to habituation of the fixation reflex itself: the change of visual field during the eye movement may be an additional arousal producing stimulus (see the first paper of the series).

Table IX

Resistance to habituation (in trials) of pupillary dilatation and ECoG arousal to rotation of "X" figure

(means from both sessions are given)

Groups		
Con[a]	LOM	TOM
4	1	3
5	2	5
5	5	11
8	12	12
14	14	16
16	16	23
	16	24
	17	35
	29	59
	30	
	45	
	150	
Median 6.5	16	16
Mean 8.7	28	20.9

[a] In some cats the data were not available.

Table X

Resistance to habituation (in trials) of pupillary dilatation and ECoG arousal to odor of valeriane[a]

Groups		
Con	LOM	TOM
8	1	4
10	1	4
11	3	5
12	7	5
25	9	6
37	9	9
	10	52
	10	
	26	
	60	
Median 11.5	9	5
Mean 17.2	13.6	12.1

[a] In some cats the data were not available.

In both experimental Groups the resistance to habituation of pupillary dilatation and ECoG arousal to the odor valeriane was not increased (Table X). The same was true for the habituation of a small dawnward eye movement produced by the odor; it usually disappeared a few trials earlier than the pupillary dilatation and ECoG arousal.

Following reflex. The oculomotor lesions did not seem to affect the following reflex. The eyes of the preparations followed adequately the beam of light moving with a speed up to $30°—35°$/sec and frequency up to 0.6 c/sec.

Additional experiment

The aim of this experiment was to obtain further data on the localiza-
tion of the cortical area affecting the fixation reflex and on the me-
chanism involved. The experiment was performed on 20 cats. The animals
were divided in four equal groups. In the first group the oculomotor
area on the medial aspect of the hemispheres was removed (Group MOM)
(Fig. 3). In the second group the prefrontal amputation was done (Group
PF) (Fig. 4). In the third group the posterior sygmoid gyri on the lateral
aspect of hemisphere were removed (Group PS) (Fig. 5). In the fourth
group no ablation was done, but 30 min before the session I, 1 mg/kg
d-amphetamine sulphate was intravenously administrated (Group AN).
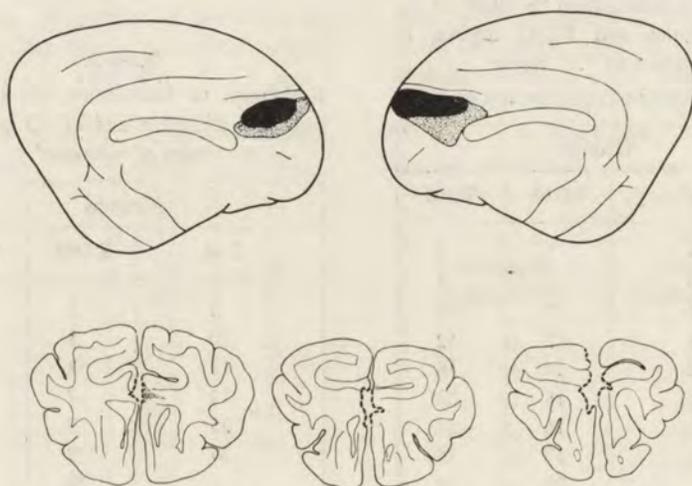
Fig. 3. Medial frontal oculomotor (MOM) ablations. Other denotations as in Fig. 1

Observations before pretrigeminal transection

The general behavior of the cats of Groups PF and PS seemed to be
completely normal, while the behavior of the cats of Group MOM was
similar to that described for the Groups LOM and TOM in the Principal
Experiment.

"Spontaneous" ocular activity and the ocular responses to visual and
auditory stimuli seemed to be unaffected in all cats.

Observations after pretrigeminal transection

The data are presented in Table XI—XVIII. The Tables are paralled
to the Table I—IV, VI, VII, IX and X for the Principal Experiment, and
for comparison they include the data for the control Group. Mainly on
the basis of such comparison the following conclusions may be derived:

Fig. 4. Prefrontal (PF) amputations. Other denotations as in Fig. 1

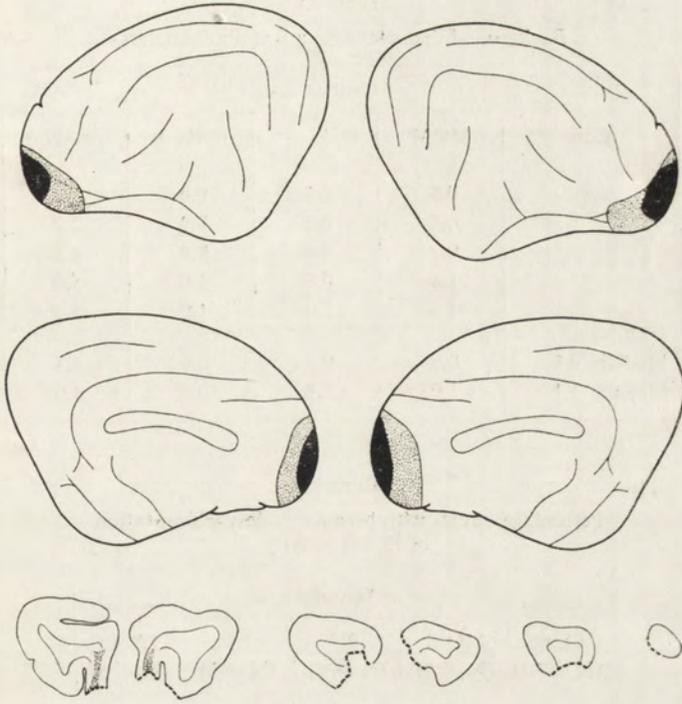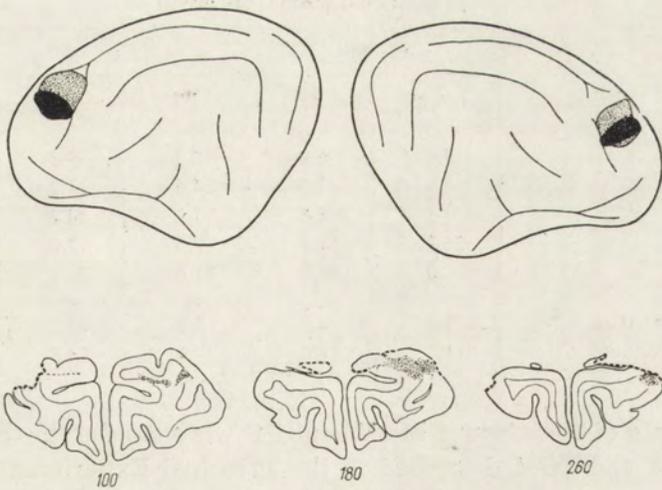Fig. 5. Posterior sygmoid gyri (PS) ablations. Other denotations as in Fig. 1

Table XI

Background diameter of pupils (in millimeters)

Con		Groups		
	MOM	PF	PS	AN
	0.5	0.3	0.4	2.4
	0.5	0.5	0.9	2.7
	0.7	0.6	0.9	4.8
	1.5	2.0	1.0	5.0
	1.6	3.0	1.0	5.3
Median 0.8	0.7	0.6	0.9	4.8
Mean 1.1	1.0	1.3	0.8	4.0

Table XII

Percentage of cats with permanent desynchronization
of ECoG activity

Con (N = 11)	MOM (N = 5)	Groups PF (N = 5)	PS (N = 5)	AN (N = 5)
54	20	80	20	100

Table XIII

Duration of fixation (in seconds) in the first trial
(means from both sessions are given)

Con		Groups		
	MOM	PF	PS	AN
	3.4	6.9	2.5	9.3
	4.5	7.5	4.6	11.2
	7.5	8.6	7.2	14.2
	25.3	11.8	9.5	17.0
	28.0	20.0	11.6	22.1
Median 6.7	7.5	8.6	7.2	14.2
Mean 6.9	13.7	11.0	7.1	14.8

1. The cats of the Groups MOM and PF were similar to those of the Groups LOM and TOM described in the Principal Experiment.

2. The removal of the posterior sygmoid gyri (Group PS) did not seem to have any effect in the tests applied in our study.

Table XIV

Percentage of cats with serial fixation reflex in the first trial

	Groups				
	Con (N = 11)	MOM (N = 5)	PF (N = 5)	PS (N = 5)	AN (N = 5)
In one session only	18	20	60	20	20
In both sessions	0	20	40	40	80
Totally	18	40	100	60	100

Table XV

Dilatation of pupils (in millimeters) to the odor
of valeriane in the first trial

	Groups		
Con	PF	PS	AN[b]
	0.3	0.6	0.3
	0.4	0.7	0.7
	0.5	0.8	0.9
	1.0	1.3	1.0
	1.1	1.5	1.2
Median 0.8	0.5	0.8	0.9
Mean 0.8	0.7	1.0	0.8

[a] In Group MOM the data were not available.

[b] The background diameter of pupils was much larger (see Table XI).

3. In the Group AN the duration of fixation and its resistance to habituation seemed to be even more increased than in the cats with oculomotor and prefrontal ablations. We could expect, however, that in cats under amphetamine action the background arousal would also be increased. In fact, in all cats of Group AN the pupils were considerably dilated (which was obviously partially of the central origin) and the ECoG activity was continuously desynchronized. In addition, the presence of pupillary dilatation to the olfactory stimulus against a background of a large pupillary diameter (Table XV) may suggest that the general responsiveness of the cats was increased.

Table XVI

Resistance to habituation (in trials) of fixation reflex
(means from both sessions are given)

	Groups			
Con	MOM	PF	PS	AN
	18	17	9	54
	32	19	21	62
	40	34	23	109
	78	115	25	128
	97	119	48	230
Median 14	40	34	23	109
Mean 22.9	53	60.8	25.2	116.6

Table XVII

Resistance to habituation (in trials) of pupillary
dilatation and ECoG arousal to rotation of "X" figure
(means from both sessions are given)[a]

	Groups		
Con	MOM	PF	PS
	3	1	4
	13	4	9
	23	5	10
	25	8	17
	50	100	22
Median 6.5	23	5	10
Mean 8.7	22.8	23.6	12.4

[a] In Group AN the data were not available.

DISCUSSION

First of all, the difference in the character of data obtained before
and after pretrigeminal transection should be stressed. Before the tran-
section our observations were only qualitative. They showed that in cats
with frontal lesions the vertical and horizontal fixation and following
reflexes, and the directionary eye and head movements toward the
auditory stimuli, are present. After pretrigeminal transection the presence
of the vertical fixation and following reflexes was confirmed. Then,
however, the *quantitative* analysis of the fixation reflex could be done.

Table XVIII

Resistance to habituation (in trials) of pupillary dilata-
tion and ECoG arousal to odor of valeriane[a]

Groups			
Con	MOM	PF	PS
	9	3	8
	11	9	19
	16	10	36
	21	15	40
	26	19	52
Median 11.5	16	10	36
Mean 17.2	16.6	11.2	31

In Group AN the data were not available.

In this analysis, three effects of the frontal oculomotor and prefrontal lesions (Groups LOM, MOM, TOM, PF) were found. The strong increase of the resistance to habituation of the fixation reflex was the main effect. The longer-lasting phase of maintenance of fixation and the more common after-fixations were the further ones. All these effects seemed to be correlated each with other, and they all contributed in the increase of the *fixation* time during the experiment. This result is in good agreement with recent observation by Jeannerod et al. (1965) that frontal cats show persistent fixation reflex, and with clinical data that humans with frontal lesions may show a "spasmodic fixation" (Gowers 1879, Holmes 1938, Teuber 1964).

Although the *fixation time increase* was clearly manifested only in some animals it may be considered as a syndrome for the frontal oculomotor and prefrontal cats. We know that in pretrigeminal cats there are considerable individual differences in responsiveness. They were clearly seen in the control Group and they were also the subject of a special study (Żernicki et al. 1967). It may be assumed, therefore, that in some cats the syndrome could not be observed because of their low responsiveness to visual stimuli.

The problem of specificity of this syndrome should be considered. We know that the increase of fixation time may be simply due to the general increase of animal responsiveness. The latter may be produced by electrical stimulation of the brain stem reticular formation (Affanni et al. 1962, Żernicki et al. 1969) and in this paper it was obtained by amphetamine application (Group AN). However, the frontal syndrome of fixation time increase may not be explained in this way. First, the background

pupillary diameter and ECoG activity were unchanged. Second, the pupillary responses to olfactory stimuli and their resistance to habituation were unaffected. Third, the resistance to habituation of the unspecific responses associated with fixation reflex (pupillary dilatation and ECoG arousal) seemed to be also normal.

In the pretrigeminal cat the fixation reflex is represented only by the vertical eye movement. It is tempting to think, however, that the fixation time increase syndrome concerns the whole fixation reflex, i.e. also (i) the horizontal fixation reflex and (ii) the head component of the reflex. As far as the frontal oculomotor cortex is concerned it may be noted that its electrical stimulation produces both the vertical and horizontal eye movements (Shipova 1965, Dreher et al., in preparation) and the evoked by stimulation eye movements are associated with the movements of the head (Delgado 1952, 1953, Hassler 1960). The question "whether or not the syndrome concerns the following reflex" may be answered by the appropriate quantitative investigations on the pretrigeminal cat. At the moment it may be only noted that such a possibility is suggested by the recent finding of Bizzi (1968) that in the monkey's frontal oculomotor area some neurons fire during voluntary saccades while others during pursuit eye movements.

Another limitation of our study should be noted. The fixation reflex was investigated qualitatively only in one day (in different cats from 7th to 17th day after the cortical ablation), and we do not know how much was the fixation time increase a permanent syndrome. In this respect it is important the observation of P. L. Marchiafava (personal communication) that when in the acute pretrigeminal cat the frontal oculomotor lesion is performed, the fixation reflex is also very resistant to habituation. This result suggests that in our cats the fixation time increase syndrome was not of the supersensitivity origin (see Stavraky 1961, Żernicki and Santibañez-H. 1961).

Our results strongly suggest that frontal lobes have an inhibitory influence on the fixation reflex. It may be assumed that the information about the stimulus evoking the fixation reflex goes to the frontal lobe and then the latter inhibits this reflex (the phase of maintenance of fixation becomes shorter-lasting, the after-fixations are reduced and the habituation develops quicker). As far as the appropriate neural pathways are concerned, the anatomically well established projection from the paravisual cortex to the frontal oculomotor area (McCulloch 1949, Kuypers et al. 1965), and perhaps the recently suggested direct projection from the lateral geniculate body to the frontal cortex (Bignall et al. 1966), might be utilized for the visual information mediation.

On the other hand, little may be said about the pathways mediating

inhibition from frontal lobes to the fixation reflex arc. First, this arc is complex and only partially known (see the second paper of the series). Second, according to a number of data obtained mainly on monkeys and cats there is abundant projection from the frontal cortex. The projection from the oculomotor cortex to the well recognized links of the fixation reflex arc (superior colliculi, pretectal area, visual cortex) as well as to the possible links (e.g. the head of the caudate nucleus, see Mettler and Mettler 1942, Forman and Ward 1957, Thompson 1959, Laursen 1963, Wagman 1964, Starr 1967) should be particularly noted [1].

The fact that the fixation time increase syndrome was obtained after both frontal oculomotor and prefrontal ablations is of interest. The simplest explanation of this similarity would be that only the frontal oculomotor cortex ablation is responsible for the syndrome and the prefrontal amputations produced some lesions in the oculomotor cortex, or vice versa, the prefrontal cortex would be only important in this respect and it was accidentally lesioned by the oculomotor ablations. However, the comparison of the individuals with ablations of different size rather did not suggest such a possibility. It is probable, therefore, that the oculomotor and the prefrontal ablations affected specifically the fixation reflex. It is well known that the oculomotor and prefrontal areas have different physiological meaning. It is tempting to think, therefore, that after ablations of both kind the mechanism of the syndrome was different. In the first case it could be of more specific origin being connected with the meaning of the ablated area for the ocular activity, while in the second case the syndrome might be the manifestation of the well known impairment of inhibition in prefrontal animals (see for review Brutkowski 1965). This problem, however, apparently needs further experimental analysis.

The fixation reflex is a representative (possibly the main one) of the important category of reflexes called orienting reflexes [2]. The problem arises, therefore, whether or not other orienting reflexes are also affected

---

[1] The frontal oculomotor are a projects to the superior colliculus and pretectal region (Beevor and Horsley 1890, Mellus 1907, Mettler 1935, Levin 1936, Crosby et al. 1952, Astruc 1964, Sprague 1965), to paraprojective visual cortex (Crosby and Henderson 1948, McCulloch 1948), to the nuclei of the oculomotor nerves (Beevor and Horsley 1890, Mettler 1935), to the midbrain reticular formation (Pearce 1961, Sprague 1965), and to the head of the caudate nucleus (see for review Divac 1968). The caudate nucleus projects in turn via globus pallidus to the nuclei of the oculomotor nerves (Mettler 1935).

[2] To avoid terminological confusion it should be noted that by the orienting reflex (or targeting reflex, see Konorski 1967) we understand the directional response. It should be distinguished from the associated arousal response manifested by pupillary dilatation, ECoG arousal, etc. (for further discussion of this problem see Żernicki 1968b).

in frontal animals. It would be particularly tempting to think that the convergence and accomodation reflexes, usually associated with fixation reflex, are also affected. Alvarado-P (1967) has recently found that in frontal cats the resistance to habituation of the orienting reflexes to auditory stimuli is increased; and Butter (1964), measuring indirectly the strength of the responses to visual and auditory stimuli by their effects on lever-pressing for food rewards, found that the resistance to habituation of the responses to these stimuli is increased in frontal monkeys. On the other hand, in our frontal cats the resistance to habituation of the eye movement to the odor of valeriane, which perhaps is an orienting response to the olfactory stimulus, seemed to be unaffected. In this respect it is also interesting the recent finding of I. Stępień and her associates (Stępień and Stępień 1965, Stępień et al. 1966, Stępień and Stamm, in preparation) that in cats, dogs and monkeys with selective frontal lesions the orienting reflexes are increased to positive conditioned stimuli but not to negative or to neutral stimuli.

It may be also noted that in frontal cats not only the orienting reflexes may be increased. It is well known that the lesions of the motor cortex increase the stretch reflex and the prefrontal lesions disinhibit the conditioned reflexes. Besides, in frontal rats the resistance to habituation of the cardiac response (Glaser and Griffin 1962) and the flexor reflex (Griffin and Pearson 1968) to nociceptive stimuli were increased. Apparently for the evaluation of the role of frontal inhibitory mechanism for different kinds of reflexes further experimental data are needed.

SUMMARY

1. The properties of the fixation reflex (the important representative of the orienting reflexes) to the rotation of the "X" shaped figure were studied in cats with frontal lobes lesions or after d-amphetamine application. To obtain quantitative data the investigations were mainly done on the cats with the pretrigeminal brain stem transection.

2. The syndrome of *fixation time increase* was described in cats with ablation of frontal oculomotor or prefrontal cortex. The syndrome consists in: (i) prolongation of the phase of maintenance of the fixation reflex, (ii) the presence of after-fixations, and (iii) the strong resistance to habituation of the reflex.

3. In the cats with ablation of the posterior sygmoid gyri the syndrome was absent.

4. In all groups of operated animals the background arousal (pupillary diameter, ECoG activity), the arousal components of the fixation reflex

(pupillary dilatation, ECoG arousal) and the responses the olfactory stimuli were in principle unaffected.

5. Under amphetamine action the syndrome of *fixation time increase* was present but simultaneously the arousal of the animals was considerably increased.

6. The role of the frontal lobes in various types of orienting reflexes is discussed.

REFERENCES

AFFANNI, J., MARCHIAFAVA, P. L. and ŻERNICKI, B. 1962. Orientation reactions in the midpontine pretrigeminal cat. Arch. Ital. Biol. 100: 297—304.

ALVARADO-P., M. 1967. Habituaction del reflejo de orientacion en gatos. Ph. D. Thesis. Univ. Chile, Santiago de Chile.

ASTRUC, J. 1964. Corticofugal fiber degeneration following lesions of area 8 (Frontal Eye Field) in Macaca Mulatta. Anat. Rec. 148: 256.

BATINI, C., MORUZZI, G., PALESTINI, M., ROSSI, G. F. and ZANCHETTI, A. 1959. Effects of complete pontine transections on the sleep-wakefulness rhythm: the midpontine pretrigeminal preparation. Acta Ital. Biol. 97: 1—12.

BEEVOR, C. E. and HORSLEY, V. 1890. A record of the results obtained by electrical excitation of the so-called motor cortex and internal capsule in an orangoutang. Phil. Trans. Roy. Soc. London 181: 129—158.

BIGNALL, K. E., IMBERT, M. and BUSER, P. 1966. Optic projections to nonvisual cortex of the cat. J. Neurophysiol. 29: 396—409.

BIZZI, E. 1968. Discharge of frontal eye field neurons during saccadic and following eye movements in unanesthetized monkeys. Exp. Brain Res. 6: 69—80.

BRUTKOWSKI, S. 1965. Functions of prefrontal cortex in animals Physiol. Rev. 45: 721—746.

BUTTER, CH. M. 1964. Habituation of responses to novel stimuli in monkeys with selective frontal lesion. Science 144: 313—315.

CROSBY, E. C. and HENDERSON, J. W. 1948. The mammalian midbrain and isthmus regions. II. Fiber connections of the superior colliculus. B. Pathways concerned in automatic eye movements. J. Comp. Neurol. 88: 53—92.

CROSBY, E. C., YOSS, R. E. and HENDERSON, J. W. 1952. The mammalian midbrain and isthmus regions. II. The fiber connections. D. The pattern for eye movements of the frontal eye field and the discharge of specific portions of this field to and through midbrain levels. J. Comp. Neurol. 97: 357—383.

DELGADO, J. M. R. 1952. Hidden motor cortex of the cat. Amer. J. Physiol. 170: 673—681.

DELGADO, J. M. R. 1953. Responses evoked in waking cat by electrical stimulation of motor cortex. Amer. J. Physiol. 171: 436—446.

DIVAC, I. 1968. Functions of the caudate nucleus. Acta Biol. Exp. 28: 107—120.

DREHER, B., MARCHIAFAVA, P. and ŻERNICKI, B. 1965. Studies on the visual fixation reflex. II. The neural mechanism of the fixation reflex in normal and pretrigeminal cats. Acta Biol. Exp. 25: 207—217.

FORMAN, D. and WARD, J. W. 1957. Responses to electrical stimulation of caudate nucleus in cats in chronic experiments. J. Neurophysiol. 20: 230—244.

GLASER, E. M. and GRIFFIN, J. P. 1962. Influences of the cerebral cortex on habituation. J. Physiol. 160: 429—445.

GOWERS, W. R. 1879. Note on a reflex mechanism in the fixation of the eyeballs. Brain 2: 39—41.

GRIFFIN, J. P. and PEARSON, J. A. 1968. The effect of lesions of the frontal areas of the cerebral cortex on habituation of the flexor withdrawal response in the rat. Brain Res. 8: 177—184.

HASSLER, R. 1960. Thalamo-corticale systeme der körperhaltung und der augen-bewegungen. In D. B. Tower and J. P. Schadè (ed.), Structure and function of the cerebral cortex. Elsevier Publ. Co., Amsterdam, p. 124—130.

HOLMES, G. 1938. The cerebral integration of the ocular movements. Brit. Med. J. 2: 107—112.

JEANNEROD, M., MOURET, J. et JOUVET, M. 1965. Étude de la motricite oculaire au cours de la phase paradoxale du sommeil chez le chat. Electroencephal. Clin. Neurophysiol. 18: 554—566.

KONORSKI, J. 1967. Integrative activity of the brain. An interdisciplinary approach. Univ. Chicago Press, Chicago. 530 p.

KUYPERS, H. G. J. M., SZWARBART, M. K., MISHKIN, M. and ROSVOLD, H. E. 1965. Occipitotemporal corticocortical connections in the rhesus monkey. Exp. Neurol. 11: 245—262.

LAURSEN, A. M. 1963. Corpus striatum. Acta Physiol. Scand. 59, Suppl. 211: 1—116.

LEVIN, P. M. 1936. The efferent fibers of the frontal lobe of the menkey, Macaca Mulatta. J. Comp. Neurol. 63: 369—419.

McCULLOCH, W. S. 1948. Some connections of the frontal lobe established by physiological neuronography. Res. Publ. Ass. Res. Nerv. Ment. Dis. 27: 95—105.

MELLUS, E. L. 1907. Relations of the frontal lobe in the monkey. Amer. J. Anat. 7: 227—243.

METTLER, F. A. 1935. Corticifugal fibers of Macaca Mulatta. The frontal region. J. Comp. Neurol. 61: 509—542.

METTLER, F. A. and METTLER, C. C. 1942. The effects of striatal injury. Brain 65: 242—255.

PEARCE, G. W. 1961. Some cortical projections to the midbrain reticular formation. In D. B. Tower and J. P. Schadè (ed.), Structure and function of the cerebral cortex. Elsevier Publ. Co., Amsterdam, p. 131—137.

SHIPOVA, N. V. 1965. Motor cortical representation of muscle system of the visual analyser in the cat (in Russian). Fiziol. Zh. SSSR 51: 293—299.

SPRAGUE, J. M. 1965. Visual, acoustic and somesthetic deficits in the cat after cortical and midbrain lesions. In D. Purpura and M. Yahr (ed.), The thalamus. Columbia Univ. Press, New York, p. 391—417.

STARR, A. 1967. A disorder of rapid eye movements in Huntington's chorea. Brain 90: 545—564.

STAVRAKY, G. W. 1961. Supersensitivity following lesions of the nervous sytem. Univ. Toronto Press, Toronto, 210 p.

STĘPIEŃ, I. and STĘPIEŃ, L. 1965. The effects of bilateral lesions in precruciate cortex on simple locomotor conditioned response in dogs. Acta Biol. Exp. 25: 387—394.

STĘPIEŃ, I., STĘPIEŃ, L. and SYCHOWA, B. 1966. Disturbances of motor conditioned behavior following bilateral ablations of the precruciate area in dogs and cats. Acta Biol. Exp. 26: 323—340.

TEUBER, H. L. 1964. The riddle of frontal lobe function in man. In K. Akert and J. M. Warren (ed.), The frontal granular cortex and behavior. McGraw-Hill Book Co., New York, p. 410—444.

THOMPSON, R. L. 1959. Effects of lesions in the caudate nuclei and dorsofrontal cortex on conditioned avoidance behavior in cats. J. Comp. Physiol. Psychol. 52: 650—659.

VAKKUR, G. J., BISHOP, P. O. and KOZAK, W. 1963. Visual optics in the cat, including posterior nodal distance and retinal landmarks. Vision Res. 3: 289—314.

WAGMAN, I. H. 1964. Eye movements induced by electric stimulation of cerebrum in monkeys and their relationship to bodily movements. In M. B. Bender (ed.), The oculomotor system. Harper Row Publ., New York.

ŻERNICKI, B. 1968a. Pretrigeminal cat. Brain Res. 9: 1—14.

ŻERNICKI, B. 1968b. Reflex as the basic unit of neuro-behavioral activity in higher animals: a theoretical multidisciplinary approach to the reflex activity. Acta Biol. Exp. (in press).

ŻERNICKI, B., DOTY, R. W. and SANTIBAÑEZ-H., H. 1969. Isolated midbrain in cats. Electroenceph. Clin. Neurophysiol. (in press).

ŻERNICKI, B. and DREHER, B. 1965. Studies on the visual fixation. I. General properties of the orientation fixation reflex in pretrigeminal and intact cat. Acta Biol. Exp. 25: 187—205.

ŻERNICKI, B., DREHER, B., KRZYWOSIŃSKI, L. and SYCHOWA, B. 1967. Some properties of the acute midpontine pretrigeminal cat. Acta Biol. Exp. 27: 123—139.

ŻERNICKI, B. and SANTIBAÑEZ-H., G. 1961. The effects of ablations of alimentary area of the cerebral cortex on salivary conditioned and unconditioned reflexes in dogs. Acta Biol. Exp. 21: 163—176.

SYNCHRONIZED SLEEP IN THE CHRONIC PRETRIGEMINAL CAT

Magdalena ŚLÓSARSKA and Bogusław ŻERNICKI

Department of Neurophysiology, Nencki Institute of Experimental Biology,
Warsaw, Poland

As was shown in the cat by Bremer (1935), the transection at the midbrain level (*cerveau isolé* preparation) produces a pathologic state of the isolated cerebrum consisting in permanent synchronized sleep: the ECoG shows high voltage slow activity, the pupils are myotic, and the preparation cannot be awake by visual and olfactory stimuli. A contrasting state is obtained when the brain stem transection is done a few millimeters more caudally, namely, at the midpontine pretrigeminal level (Batini et al. 1959). Except for the somatic states (see Żernicki et al. 1967), the isolated cerebrum of the pretrigeminal cat is continuously awake: high voltage slow ECoG activity is absent; pupils are of a few millimeters diameter; visual and olfactory stimuli produce orientation reflexes consisting in ECoG arousal, pupillary dilatation and eye movements (Affanni et al. 1962a, Żernicki et al. 1967); and pupillary conditioned reflexes to visual stimuli may be elaborated (Affanni et al. 1962b). Recently, Żernicki et al. (1967) have shown that when the transection is done with a thin spatula (which seems to produce smaller lesion than electrocoagulation used originally by Batini et al.), an awake preparation can also be obtained by transection at the rostropontine level. The difference between the *cerveau isolé* and the pretrigeminal preparation is apparently due to the presence of the midbrain reticular formation within the isolated cerebrum of the latter.

Recently *cerveau isolé* cats (Villablanca 1965, 1966, Serkov et al. 1966) and *cerveau isolé* dogs (Batsel 1960) were observed chronically. With

time the function of the isolated cerebrum was gradually improved: in the ECoG activity the long-lasting episodes of desynchronization appeared, the pupils became moderately dilated (Villablanca 1966), and olfactory (Villablanca 1965, Serkov et al. 1966) and visual (Serkov et al. 1966) stimuli could produce ECoG arousal response. So far, however, in the chronic *cerveau isolé* preparation the ocular responses to visual and olfactory stimuli were not described and, therefore, the problem "whether or not this preparation may be awake" is left for further investigations.

The observations of chronic pretrigeminal preparations have been less abundant. The original study of Batini et al. (1959) did not suggest any difference between acute and chronic pretrigeminal cats. The later study of Żernicki and Osetowska (1963) was exclusively behavioral and showed that in the isolated cerebrum of the chronic pretrigeminal cat both positive and inhibitory conditioned reflexes may be elaborated.

In the light of these observations two interesting possibilities may be considered in respect to the isolated cerebrum of the chronic pretrigeminal cat: (i) this cerebrum does not sleep (and by analogy with chronic *cerveau isolé* preparation may show even more desynchronized ECoG activity) but is functionally good, and (ii) in this cerebrum the sleep function is restored. The present paper was undertaken to answer which of these possibilities is true.

MATERIAL AND METHODS

The experiments were carried out on ten pretrigeminal cats. Histological verification showed that in five cats the transection was at the midpontine and in five cats at the rostropontine level. In eight cats the transection was complete, whereas in two cats a small tectal or pontine remnant was present, respectively.

The brain stem transection was done is aseptic conditions under Nembutal anaesthesia. The transection was performed with a thin spatula. To avoid the tentorium the spatula was oriented sterotaxically at an angle 30° from the vertical plane (for details of technique see Żernicki 1968). In four cats the electrodes for ECoG recordings were implanted immediately after the transection, and in six cats 7—9 days later. Four pairs of silver electrodes were placed in both sensorimotor and visual areas. The interelectrode distance of a pair was 10 mm.

The animals were kept in incubators (for nursing care see Żernicki 1968). As a visual stimulus the movement of a piece of white cotton wool in front of animal's eye was used. To observe the ocular activity more accurately, the cats were occasionally moved to a small experimental chamber and placed in a holder. In the chamber the rotation of an "X" shaped figure and the vertical movement of a disc were used as visual stimuli. Both figures were black and moved against a white screen. Occasionally the odors of valeriane and butyric acid were applied (for technique of olfactory stimulation see Żernicki et al. 1967).

The cats survived from 5 to 22 days. Seven cats died during the observation

period while three cats were killed after survival of 17, 19 and 22 days, respectively. Because of the long-lasting effect of the Nembutal narcosis, the observations started on the third day after the brain stem transection. The ECoG data were collected mainly on six cats because four cats died soon after electrode implantation.

The ECoG activity and responsiveness to visual stimuli were tested twice a day (in the morning and in the evening) and occasionally more often. Besides 20 sessions lasting from 7 hr to 32 hr were done. Because in our EEG machine (Alvar Reega-VIII) the lowest speed was only 15 mm/sec, during the sessions continous recordings were not made but the ECoG activity was recorded every 15 min for at least 1 min. Nevertheless, due to the rather infrequent spontaneous changes of type of ECoG activity some reliable information could be obtained. In the majority of sessions the eyes were kept closed (with bands attached to the lids by Mendeleiev's wax), and in others the responsiveness to visual stimuli was also checked.

RESULTS

Spontaneous ECoG and ocular activity. Three types of ECoG activity could be distinguished:

Type I. Almost continously desynchronized ECoG activity, interrupted less frequently than 1/min by the synchronization episodes lasting shorter than 1 sec.

Type II. Synchronized ECoG activity of moderate amplitude mixed with desynchronized activity. In the records the long-lasting episodes of 6—16 c/sec activity (Fig. 1, Type IIA) or the small spindles (Type IIB) were usually observed.

Type III. High voltage activity mixed with a small amount of the desynchronized activity. In the records the fully developed spindles (Fig. 1, Type IIIA) or delta waves (Type IIIB) dominated.

The criterion of this division was to make the ECoG activity of the chronic pretrigeminal cat comparable to that of both the acute pretrigeminal preparation and the intact cat. It may be assumed that the Type I and Type II activities are present in the acute pretrigeminal cat (Żernicki et al. 1967) and are comparable to those present in the intact cat during activation and relaxed wakefulness or drowsiness, respectively (Hess et al. 1953, Dement 1958, Sterman et al. 1965, Ursin 1968). On the other hand, the Type III activity is absent in the acute pretrigeminal cat while the Type IIIA and Type IIIB activities are comparable to those present in the intact cat during "light slow wave sleep" and "deep slow wave sleep", respectively (Ursin 1968).

These three types of ECoG activity were observed in all preparations. However, considerable individual differences in their distribution were present (Table I). All types of activities usually appeared as long episodes, lasting from several minutes to a few hours. The Type III episodes

sen.mot.   Type I

vis.

Type IIA
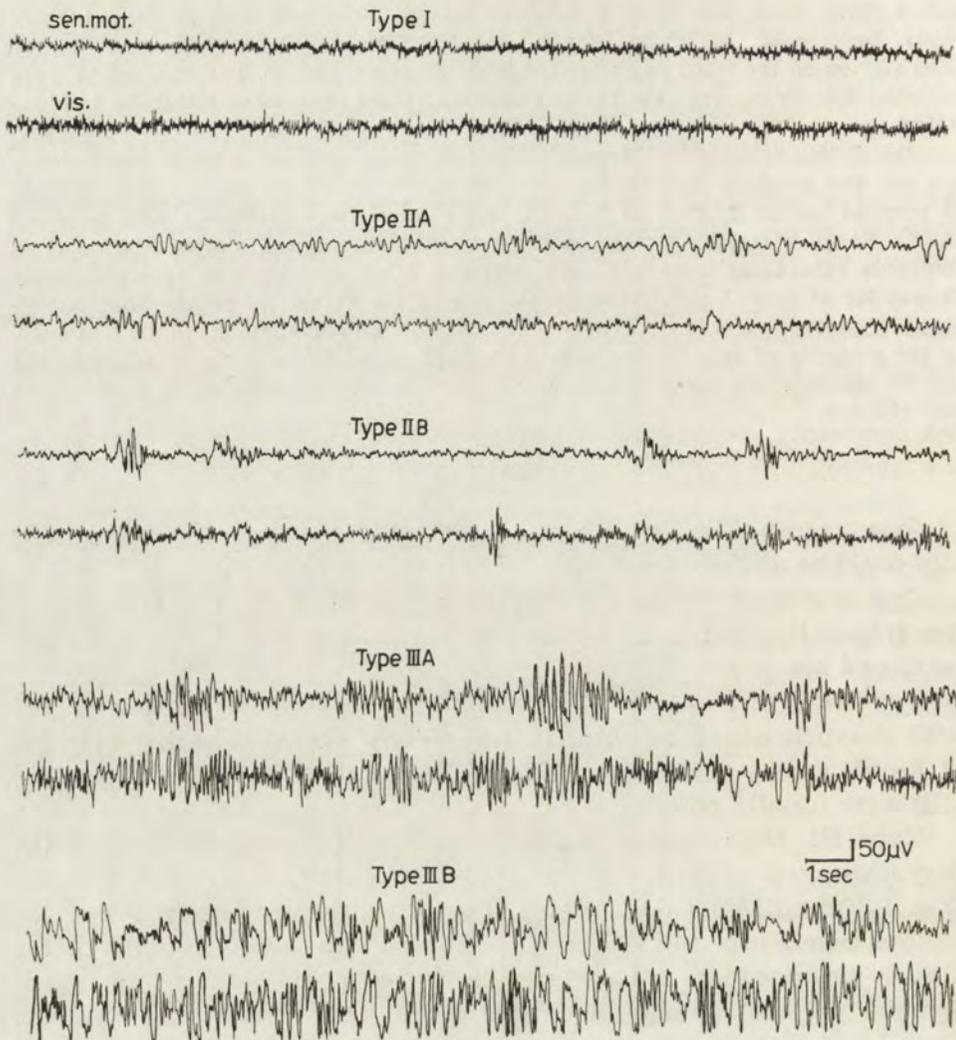
Type II B

Type IIIA

]50μV
1sec
Type III B

Fig. 1. Types of ECoG activity in chronic pretrigeminal cat

were on average shorter, and never lasted more than 2 hr. These episodes
developed gradually (Fig. 2A), but terminated abruptly (Fig. 2B). The
ECoG activity was usually more desynchronized in the visual areas than
in the sensorimotor ones.

There was no satisfactory amount of records made on individuals to
answer definitely the question whether or not the percentage distribution
of the identified types of activities changed with time. However, the mean
figures from all cats (Table II) did not suggest any change. In particular,
the episodes of Type III activity were observed already on the third

day after the transection (the first day of observation) and afterwards they did not seem to appear more frequently or become longer-lasting.

Although under our experimental conditions the observation of eyes was not accurate, some information was collected. There was some correlation between pupillary diameter and ECoG activity. During Type I and Type II activities the pupils were of a few millimeters diameter.

Table I

Percentage distribution of the types of ECoG activity
in different cats

Cat	Type I	Type II	Type III	Number of 1 min samples
Cat 1	50.5	36.4	13.1	396
Cat 2	43.0	36.0	21.0	244
Cat 3	18.5	44.0	37.5	164
Cat 4	67.2	15.5	17.3	164
Cat 5	64.0	30.4	5.6	160
Cat 6	4.0	67.0	24.0	128
Mean 42.0	37.0	21.0	

The episodes of Type III activity were accompanied by a pupillary constriction, which sometimes was full. Besides, during Type III episodes the eyeballs were rotated downwards and the nictitating membranes enlarged. The pattern of rapid eye movements typical for the desyn-

Table II

Percentage distribution of the types of ECoG activity in different
time after the pretrigeminal transection
(means from six cats are given)

Days	Type I	Type II	Type III	Number of 1 min samples
3—5	63	27	10	344
6—7	37	42	21	152
10—11	60	23	17	104
13—14	46	41	13	144
16—20	35	40	25	56

chronized sleep was never observed. However, in two cats small eye movements of frequency from 2 to 30 a minute were almost continously present. These movements were associated with all types of ECoG activity.
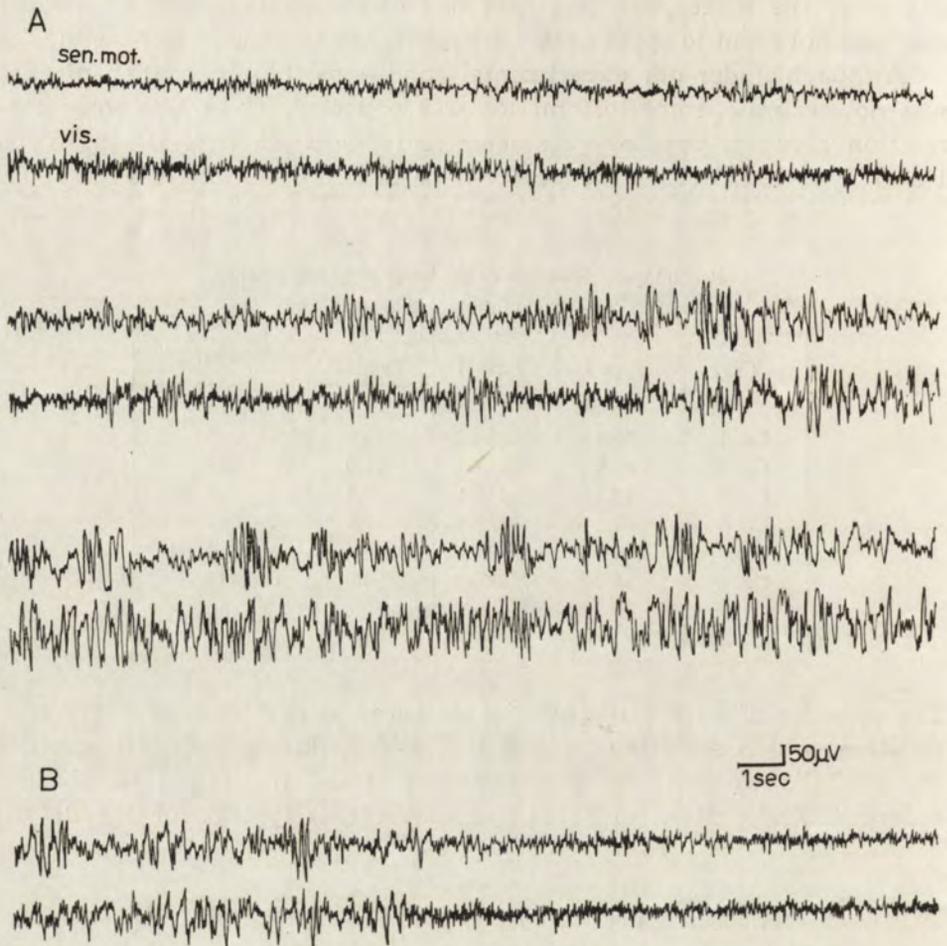
A

sen. mot.

vis.

B

50μV

1sec

Fig. 2. Gradual development (A) and abrupt ending (B) of the Type III activity episode

Responsiveness to visual stimuli. During Type I and Type II ECoG activities the preparations usually reacted vigorously to visual stimuli with clear ocular responses (fixation reflex, pupillary dilatation) and with ECoG arousal (on the background of ECoG desynchronization the latter could be undetectable). However, in all preparations the episodes of unresponsiveness, which occasionally lasted a few hours, were observed.

On the other hand, during the Type III ECoG activity the preparations were usually unresponsive. In the case when the visual stimulus was effective, it produced either a short-lasting effect (this usually hap-

pened in the begining of the episode), or a definite termination of the Type III episode (the animal was awaken).

When the visual stimuli were inefficient, the olfactory stimuli and the changing of the position of the preparation (particularly the repetitive changes of the head position) could sometimes evoke the ECoG and ocular responses. Particularly during the Type III ECoG episode, these stimuli could awake the preparation.

DISCUSSION

Chronic pretrigeminal cat versus acute pretrigeminal cat. Our results showed that in contrast with the acute preparation, in the chronic pretrigeminal cat the synchronized sleep is present. The most likely reason for this difference seems to be that simply the long-lasting lack of sleep forces sleep episodes even in the pretrigeminal cat. However, some other reasons may be considered. First of all, for a while after pretrigeminal transection the isolated cerebrum may "feel" unusually. Furthermore, the acute preparation is restrained in the sterotaxic apparatus and this may interfer with sleep. In fact, in the present paper we observed that proprioceptive stimuli may have an arousing effect, and Villablanca (1965) found that in *cerveau isolé* cat both proprioceptive and nociceptive stimuli may evoke ECoG desynchronization. On the other hand, it seems to be improbable that by analogy with possible sensitization of the "desynchronization" center in the *cerveau isolé* preparation (see Moruzzi 1963, Villablanca 1965), the sensitization of the sleep center would take place in the pretrigeminal cat. In fact, in contrast to the gradual development of desynchronization episodes in the *cerveau isolé* preparation, in the chronic pretrigeminal cat the episodes of sleep were present already on the third day and afterwards they did not seem to appear more frequently or become longer-lasting.

It is interesting that the presence of the synchronized sleep in the chronic pretrigeminal cat was overlooked in our previous study (Żernicki and Osetowska 1963), in which the conditioned reflexes were elaborated in this preparation. Although then the ECoG activity was not recorded, the ocular manifestations of synchronized sleep might be seen. The most likely explanation seems to be in the arousing effect of the proprioceptive stimuli. These stimuli were in operation, for example, when the animals were moved in hands by a technician from the animal house to the conditioned-reflex chamber. During the experiment, furthermore, the electrical stimulation of the hypothalamic perifornical area (used as the unconditioned stimulus) obviously interferred with the development of sleep episodes.

Chronic pretrigeminal cat versus chronic cerveau isolé cat. When the obtained by us ECoG records are compared with those from the chronic *cerveau isolé* cat (Villablanca 1965, Serkov et al. 1966), we see that the general character of the ECoG activity, the amount of the synchronized activity, the duration of the desynchronization and synchronization episodes, and the way as they were replaced each by other are in both preparations similar.

On the other hand, the pretrigeminal and the *cerveau isolé* preparations seem to differ importantly in their responsiveness to visual and olfactory stimuli. The difference is particularly striking when the ECoG records do not show high voltage slow activity. Then the pretrigeminal cat is only occasionally unresponsive while in the *cerveau isolé* preparation at least the ocular responses seem to be continously absent.

The mechanism of the unresponsiveness, which was not associated with high voltage slow ECoG activity, is not clear. Possibly its mechanism is similar in the pretrigeminal and the *cerveau isolé* preparation. Although this unresponsiveness is often associated with fully desynchronized ECoG activity, it is probably not the manifestation of the desynchronized sleep because there is some evidence that the center responsible for this stage of sleep is located in the ponse behind the pretrigeminal transection (see Jouvet 1967). Besides the pattern of rapid eye movements characteristic for the desynchronized sleep was observed neither in the pretrigeminal nor in the *cerveau isolé* preparation. Concerning the latter preparation it should be noted that in the case of high mesencephalic transection the lack of ocular responses, particularly the lack of fixation reflex, may be due to the oculomotor nucleus lesion. The lesion of the occipital lobe (but only unilateral) necessary to perform the midbrain transection may also affect the fixation reflex (see Dreher et al. 1965).

Chronic pretrigeminal cat versus intact cat. In the intact cat the ECoG activity and its relation to the behavior have been quantitatively described by a number of authors (Hess et al. 1953, Delorme et al. 1965, Sterman et al. 1965, Ursin 1968). In the present paper the quantitative data had only a preliminary character. However, some important differences between the chronic pretrigeminal and the intact cat may be noted:

1. The amount the synchronized sleep is less in the pretrigeminal cat. This is probably due to the absence of the deactivating influences of the medulla (see Moruzzi 1964).

2. Desynchronized sleep is probably absent in the pretrigeminal cat (for discussion of the mechanism of the desynchronized sleep see Jouvet 1967).

3. The episodes of a given type of ECoG activity are much longer-lasting in the pretrigeminal cat: in the intact cats the episodes last minutes and in the pretrigeminal cats may last hours. This may be due to the probable prevalence of the extraneural control of sleep in the isolated cerebrum.

4. In the pretrigeminal cat the unresponsiveness to visual stimuli may be associated with any type of ECoG activity.

SUMMARY

1. In chronic cats with pretrigeminal brain stem transection the ECoG activity and ocular activity were observed.

2. In all preparations the periods of wakefulness and synchronized sleep were alternatively present. They lasted from several minutes to a few hours. The synchronized sleep occupied about 20% of the recording time.

3. Some conclusions concerning the differences in the wakefulness/ /sleep function between the pretrigeminal, the *cerveau isolé* and the intact cat are drawn: (i) the main difference between the acute pretrigeminal cat and the chronic pretrigeminal cat is that in the latter the synchronized sleep is present, (ii) the main difference between the chronic *cerveau isolé* cat and the chronic pretrigeminal cat is that the latter is behaviorally responsive to visual and olfactory stimuli, and (iii) the main differences between the intact cat and the chronic pretrigeminal cat is that in the latter the episodes of wakefulness and sleep are longer-lasting, and the desynchronized sleep is probably absent.

REFERENCES

AFFANNI, J., MARCHIAFAVA, P. L. and ŻERNICKI, B. 1962a. Orientation reactions in the midpontine pretrigeminal cat. Arch. Ital. Biol. 100: 297—304.
AFFANNI, J., MARCHIAFAVA, P. L. and ŻERNICKI, B. 1962b. Conditioning in the midpontine pretrigeminal cat. Arch. Ital. Biol. 100: 305—310.
BATINI, C., MORUZZI, G., PALESTINI, M. ROSSI, G. F. and ZANCHETTI, A. 1959. Effects of complete pontine transections on the sleep-wakefulness rhythm: the midpontine pretrigeminal preparation. Arch. Ital. Biol. 97: 1—12.
BATSEL, H. L. 1960. Electroencephalographic synchronization and desynchronization in the chronic "cerveau isolé" of the dog. Electroenceph. Clin. Neurophysiol. 12: 421—430.

BREMER, F. 1935. "Cerveau isolé" et physiologie du sommeil. C. R. Soc. Biol. (Paris) 118: 1235—1241.

DELORME, F., VIMONT, P., JOUVET, D. and JOUVET, M. 1964. Etude statistique du cycle veille-sommeils chez le Chat. C. R. Soc. Biol. (Paris) 158: 2128—2130.

DEMENT, W. 1958. The occurrence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. Electroenceph. Clin. Neurophysiol. 10: 291—296.

DREHER, B., MARCHIAFAVA, P. L. and ŻERNICKI, B. 1965. Studies on the visual fixation reflex. II. The neural mechanism of the fixation reflex in normal and pretrigeminal cats. Acta Biol. Exp. 25: 207—217.

HESS, R. Jr., KOELLA, W. P. and AKERT, K. 1953. Cortical and subcortical recordings in natural and artificially induced sleep in cats. Electroenceph. Clin. Neurophysiol. 5: 75—90.

JOUVET, M. 1967. Neurophysiology of the states of sleep. Physiol. Rev. 47: 117—177.

MORUZZI, G. 1963. Active processes in the brain stem during sleeping. In Harvey lectures, Series 58. Academic Press, New York.

MORUZZI, G. 1964. Reticular influences on the EEG. Electroenceph. Clin. Neurophysiol. 16: 2—17.

SERKOV, F. N., MAKULKIN, R. F. and TYCHINA, D. N. 1966. Electrical activity of the cerebrum following mesencephalic transection under chronic experiment (in Russian). Fiziol. Zh. SSSR 52: 837—846.

STERMAN, M. B., KNAUSS, B. A., LEHMANN, D. and CLEMENTE, C. D. 1965. Circadian sleep and waking patterns in the laboratory cat. Electroenceph. Clin. Neurophysiol. 19: 509—517.

URSIN, R. 1968. The two stages of slow wave sleep in the cat and their relation to REM sleep. Brain Res. 11: 347—356.

VILLABLANCA, J. 1965. The electrocorticogram in the chronic cerveau isolé cat. Electroenceph. Clin. Neurophysiol. 19: 576—586.

VILLABLANCA, J. 1966. Ocular behavior in the chronic "cerveau isolé" cat. Brain Res. 2: 99—102.

ŻERNICKI, B. 1968. Pretrigeminal cat. Brain Res. 9: 1—14.

ŻERNICKI, B., DREHER, B., KRZYWOSIŃSKI, L. and SYCHOWA, B. 1967. Some properties of the acute midpontine pretrigeminal cat. Acta Biol. Exp. 27: 123—139.

ŻERNICKI, B. and OSETOWSKA, E. 1963. Conditioning and differentiation in the chronic midpontine pretrigeminal cat. Acta Biol. Exp. 23: 25—32.

THE CIRCADIAN SLEEP OF RABBITS

Juliusz NARĘBSKI, Jadwiga TYMICZ and Wiesława LEWOSZ

Department of Neurophysiology, Copernicus University, Toruń, Poland

The usage of long-lasting EEG records together with the EMG of neck muscle and the eye ball movements records (EOG) led to the discovery of two essentially different kinds of sleep (Dement and Kleitmann 1957, Dement 1958). The first was named slow sleep or synchronized sleep (SS), the second paradoxical sleep (PS) (Jouvet et al. 1959). The question of SS overlaps considerably the neurophysiological problem of the synchronization (SN) of the EEG. On the other hand, PS has a number of specific and surprising features (Jouvet 1967). Two kinds of events appear in PS (i) long-lasting tonic ones, and (ii) within them, intermittently present, phasic ones. Among the tonic events there are: desynchronization of the cortical EEG pattern and theta hippocampal rhythm. Unexpectedly they are identical with the bioelectrical pattern of intense arousal (AR) (Green and Arduini 1954). The specific symptom of PS is a remarkable drop of the antigravital muscular tonus (Jouvet et al. 1959), and in rabbits a decrease of the spontaneous electrical activity of the olfactory bulb (Kawakami and Sawyer 1962). From among the phasic PS phenomena, eye movements are easy to record.

Khazan and Sawyer (1963), working on 10 castrated female rabbits found that SS occupied 10—12 hr in numerous episodes of every 24 hr. In this period of time PS appeared 20—30 times. Every PS episode lasted from 30 sec to 6 min. Faure (1965) on the basis of experiments performed on 40 rabbits of both sexes reports that each PS episode lasts from 40 sec to 4 min, and within 3 hr experiments appear 2 to 15 times. SS occupies about 60% of the record, and PS respectively 3 to 16%. This makes an average of 7%. For the AR there remained therefore 23%.

According to experiments on 5 rabbits of Weiss and Roldán (1964) every episode of PS lasts on the average 3 min 42 sec ±11 sec and occupied a total 15.1% of the record; it appears always as an immediate sequence of SS and terminates in the transition into AR. The individual AR episode lasts on the average 1 min 48 sec ±39 sec. The intervals between adjacent PS episodes last on the average 20 min 48 sec ± 1 min 18 sec, which, after the subtraction of the AR time gives 19 min 39 sec for an average episode of SS. According to Weiss and Roldán in rabbits in average 67 episodes of PS appear during 24 hr. On the other hand, the circadian combination of the summarized time of each SS, PS and AR looks according to these authors as follows: SS 77.5%, wakefulness with AR 7,5%, and PS 15.1%. Finally, Kawakami et al. (1965) working on 38 castrated female rabbits found the following data: the individual PS episode lasted from 5 sec to 6 min 30 sec, but on average 1 min 12 sec; PS occupied altogether 2.7% of the circadian rhythm, SS 36.7%, and wekefulness with AR about 60%, respectively.

From the data presented above it can be deduced that there is similarity only concerning the range of values of the duration of a single PS episode. However, the data concerning the mean values of duration of one PS show, like the remaining data, considerable divergencies, which are difficult to explain.

Among the authors only Faure (1965) defines more precisely the conditions in which sleep (SS and PS) occurs in rabbits. Sleep appeared in his laboratory in females only after 2—8 days adaptation. In male rabbits this time was longer, lasting 1—8 months. For the facilitation of the occurrence of PS Faure advises silence, a temperature of 17°—20°, illumination of only 100 lux and in the vicinity of the animal the smell of its excrement, but not the other sex, and sufficient food of adequate moistness.

The purpose of the present work was to explain some incompatibilities among the data described above. We wanted to find how the SS and PS would appear in our laboratory conditions, and then to calculate and combine the episodes of sleep (PS and SS) and wakefulness during a given 24 hr period. Moreover, we were trying to calculate the correlation between the time elapsed between two consecutive PS episodes (comprising AR and SS) and the length of the succeeding PS. A positive correlation would be some confirmation of the Jouvet (1967) hypothesis about the monoamine-metabolic dependance of PS.

MATERIALS AND METHOD

Experiments were performed on nine rabbits of both sexes, five male and four female, weighting between 2.5 and 3.8 kg. Each animal had chronically im-

planted electrodes as described before (Narębski et al. 1966). The EEG was recorded from the motor cortex epidurally by means of two silver electrodes placed bilaterally on the points A4 and L4; from the dorsal hippocampus by means of one concentric bipolar electrode placed in the point V6.5, P4.5 and L4.5. The platinum wire electrodes each 0.2 mm in diameter were implanted into neck muscles, their bare tips placed 6 cm caudally from the skull. The reference electrode for the monopolar leads was made from silver wire of diameter 0.3 mm, its bare ending being placed under the skin on the nasal bone 5 cm rostrally from bregma. Moreover, two silver electrodes were implanted extraorbitally on both sides of the eye, in the horizontal plane. All electrodes were soldered to the miniature valve holder which was attached to the calvarium by means of acrilic plastic. The position of the electrodes was checked by X-rays, and after the end of experiments histologically and by dissection. The experiments started about three weeks after surgery. During the experiments animals were kept in a cage 30×45×40 cm in which they could move freely and were joined to the recorder by means of a long multiconductor cable hanging up on elastic rubber. Some amount of food was put into the cage and it was cleaned only when the experiment was over.

A continuous simultaneous 24 hr record (in some cases 12 hr record) was taken. The Kaizer 55 ink electroencephalograph with a time constant of 0.3 sec for EEG and EOG, and 0.03 sec for EMG was used. The laboratory room was slightly darkened, isolated from noise and kept at the temperature of 25°.

RESULTS

The experimental data comprise twentytwo polygraphic 24 hr records and six 12 hr nocturnal ones. Out of nine, five rabbits were twice the subject of 24 hr experiments, and the remaining four were three times the subject of such experiments. At least 24 hr adaptation of the animals for the laboratory conditions preceded the actual experiments. This time seems to be sufficient because in each experiment full SN and PS appeared many times.

The differentiation in polygraphic recors of three states (AR, SN and PS) did not present difficulties. Particular attention was paid to the transition from each of the mentioned three states into the subsequent ones. Each such particular transition is an essential component of the diagram of the spontaneous course of sleep and wakefulness named the hypnogram.

The transition of SN into AR (Fig. 1A) is characterized by a suddenly appearing desynchronization of the cortical leads and simultaneously by the regular theta waves in hippocampus. There is a full and clear desynchronization only in the cortical bipolar lead. In the cortical monopolar lead there are superimposed waves with a frequency of about 2 c/sec, but they are not very distinct in all rabbits. The source of these slow waves is the reference electrode implanted in the vicinity of the olfactory bulb. This frequency has a respiration rhythm. EMG, beside artifacts, demonstrates a considerable increase in amplitude.
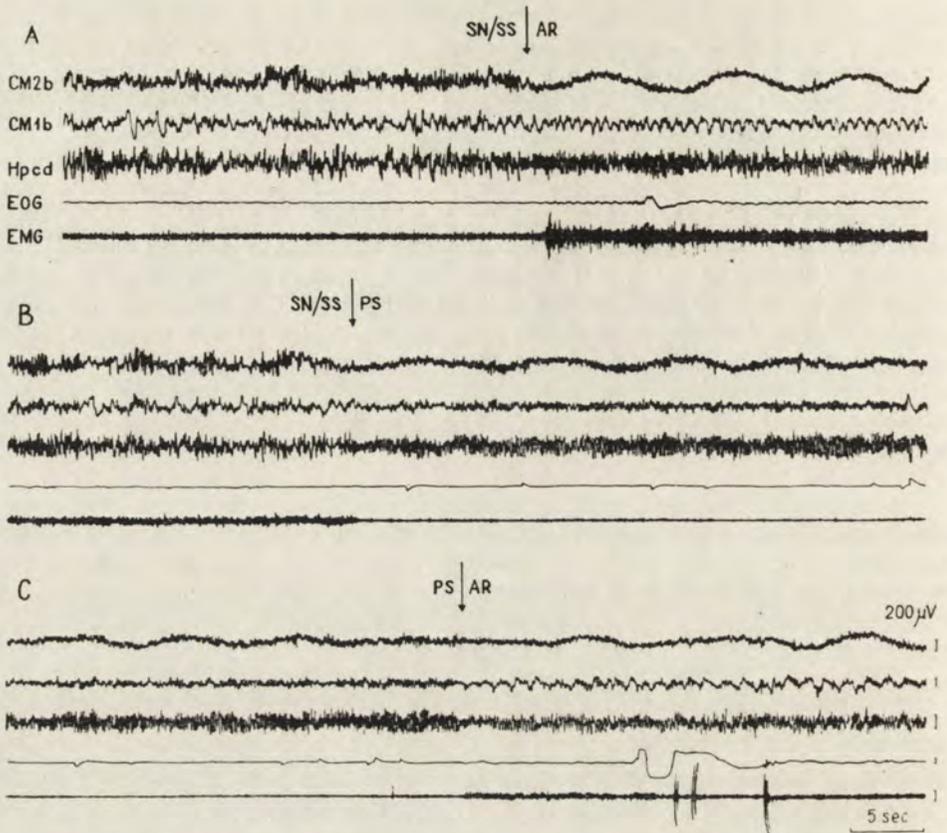
Fig. 1. A, transition from synchronization into arousal. B, transition from synchronization-slow sleep into paradoxical sleep. C, transition from paradoxical sleep into arousal. CM2b, motor cortex, bipolar. CM1b, motor cortex, monopolar. Hpcd, dorsal hippocampus. EOG, electrooculogram. EMG, neck muscles. AR, arousal. SN/SS, synchronization-slow sleep. PS, paradoxical sleep

The transition from SN, which may be here regarded as SS, into PS (Fig. 1B), is an electroencephalographic phenomenon like the one described above. The first difference is the gradation of this transition; it lasts about 10 sec. The next difference is the lack or hardly visible presence of the slow respiration rhythm during PS in the monopolar cortical lead. Moreover, during PS the hippocampal lead has both a somewhat greater regularity and amplitude than is seen in AR. This pertains especially to the period of eye movements. But first of all the EMG and EOG demonstrate the essential difference between PS and AR. In the EMG there is a decrease of amplitude. The EOG demonstrate extensive eye movements; they are mostly single, have a rapid and a slow phase and each movement has almost always the reverse direction to

the preceding movement with an interval of 5—10 sec, and more rarely in short bursts of intervals of about 1 sec.

The end of each PS, which nearly always passes into AR, is more difficult to distinguish than the transition described above (Fig. 1C). In the cortical bipolar lead there is no change. On the other hand, in the monopolar one, in the majority of rabbits, the slow respiration rhythm appeared just after awaking. In the hippocampus, theta waves appear less regular, and have a slower rhythm. In the EOG eye movements disappeared. In the EMG the amplitude evidently increased.

The transition from SN into AR, as well as the beginning and the end of each episode of PS may be determined exactly. Unfortunately, the transition from AR into SN may be established only according to conventional criteria and therefore inevitable subjectivism. This process passes gradually and lasts some minutes. Often the EEG is only partially synchronized and often there exists an alternation of the AR and SN. Consequently, in the estimation of the moment of the transition discussed, we used the following principles (i) all episodes of partial SN were grouped as wakefulness and therefore the same as AR, because partial SN cannot be considered as manifestation of SS; (ii) only such segments of records which lasted at least one minute were accepted as episodes of SN; (iii) short episodes of AR lasting less than 20 sec, preceded and succeded by SN, were not taken into account, but were included into SN.

On the basis of the criteria described above, a complicated, polygraphic picture of alternating episodes of wakefulness, having variable intensity, and the two states of sleep, may be considered as the continuous sequence of three states: (i) wakefulness with arousal (AR), (ii) synchronization with slow sleep (SN/SS), (iii) paradoxical sleep (PS). The diagram representing this sequence was called a hypnogram. Fig. 2 represents one of twentytwo circadian hypnograms executed.

From this hypnogram one may notice that in rabbits wakefulness with AR considerably predominates over the two kinds of sleep together. The periods of activity and rest, the latter also including sleep, are repeated many times and are dispersed unequally during the day and night. Not every SN episode, which may be the manifestation of the SS, is followed by PS. In 22 hypnograms executed, 1887 episodes of SN were followed by 775 of PS and 1112 by AR. The proportion is about 5:2:3. In the hypnogram illustrated (Fig. 2), the PS is always followed by AR. But in all rabbits, 15 times out of 775 PS episodes registred, it was otherwise: a direct sequence of PS episodes was SN and not AR. Moreover, it happened 8 times in two hypnograms of one rabbit (no. 4S ♀) and 6 times in one of these two hypnograms. In the remaining 7 hypno-
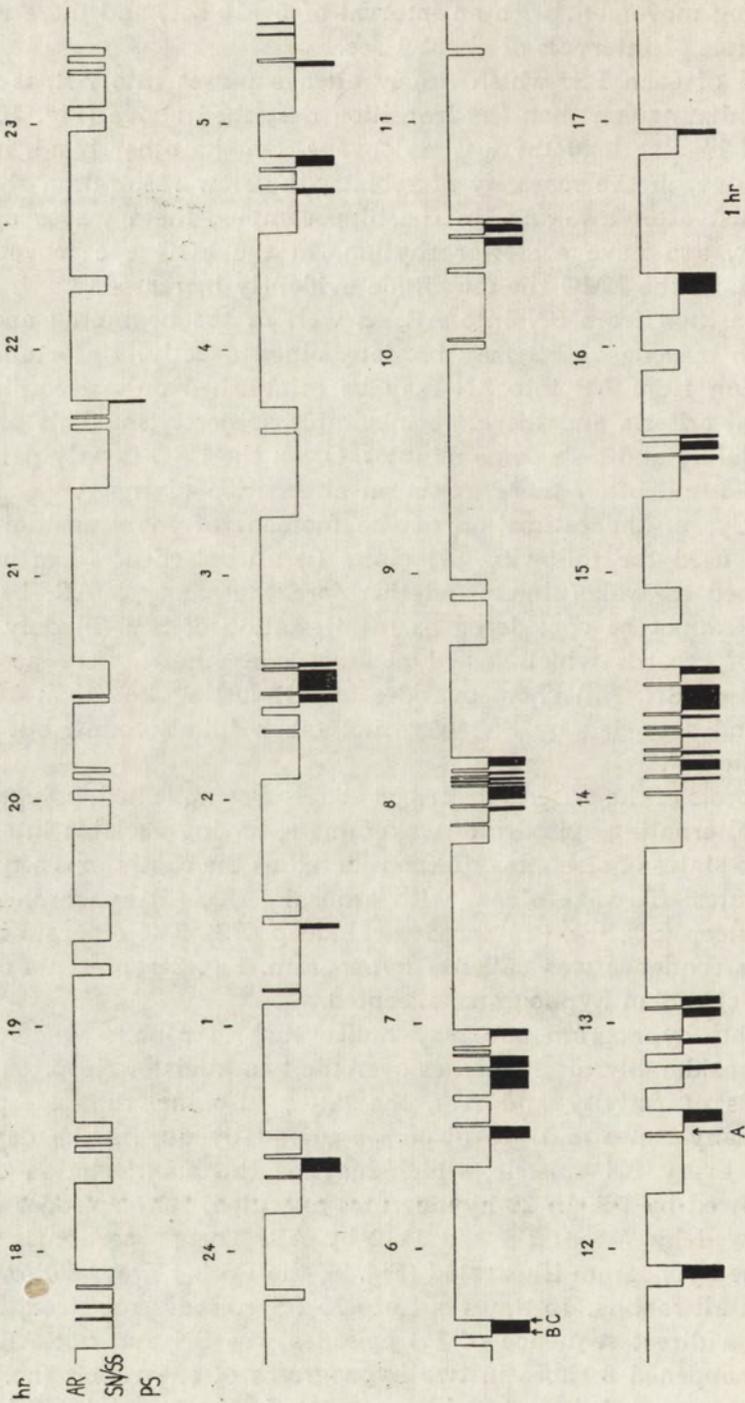
Fig. 2. Circadian hypnogram of rabbit S8O' from 15/16.II.1968. A, B, C, parts of hypnogram which are the subject of Fig. 1. For other explanations see Fig. 1

grams such a sequence appeared in 5 of them only once, and in two, twice. In consequence, the transition of PS into SN is a rather rare phenomenon and takes place more frequently only in some rabbits. If the PS appears after long lasting wakefulness and SN, than it either lasts a comparatively long time as an uninterrupted episode, or a burst of several short PS episodes with short intervals takes place.

The diagram representing the 24 hr number of episodes of AR, SN/SS and PS is illustrated in Fig. 3.
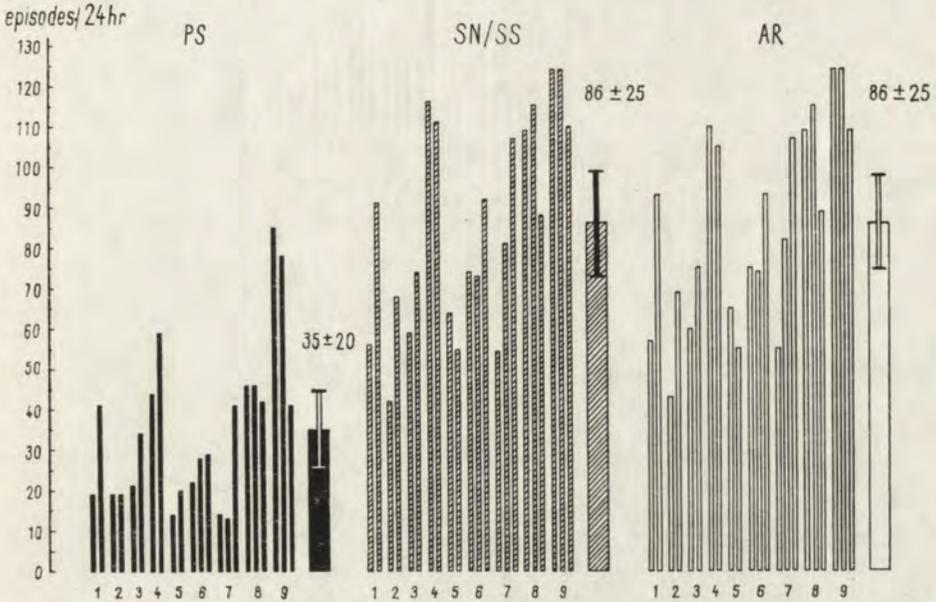

Fig. 3. The circadian number of episodes of PS, SN/SS and AR. Each column denotes the number of episodes for 24 hr. Arabic numbers no. of rabbits. On right of each group the mean value with standard deviation. For other explanations see Fig. 1

It is evident from Fig. 3 that PS occurs in a rabbit during 24 hr about 35 times. The circadian number of SN/SS episodes is equal to the number of AR episodes and is 86, about two and a half times greater than PS. Every SN episode is followed either by PS or AR. When SN is followed by PS, the episode of PS is put between SN and AR. The departure from this rule, in the form of the lack of AR after PS, appeared not more often than, as mentioned above, once for about 50 PS episodes.

As one may see from Fig. 4, the average time of a single PS in our rabbit is about 82 sec. The variations of an average duration of PS for

5*

each hypnogram was rather great. The longest PS episode recorded was 10 min 20 sec. As to the shortest PS episodes, the limitation has to be conventional, because a duration shorter than 8 sec is impossible for certain identification according to criteria used in this paper. The single average SN episode, which in part is the SS, is about four times longer than PS.
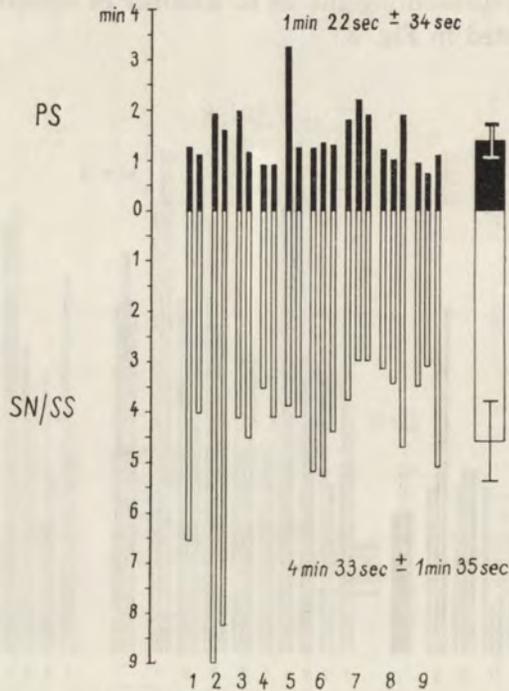

Fig. 4. The mean duration time of the single PS and SN/SS episode, as the diagram of the arithmetical mean of all hypnograms. Each column denotes one hypnogram. For other explanations see Fig. 1 and 3

The duration of the average episode of wakefulness with AR is longer and lasts 13 min 16 sec, but the deviations are huge, from a fraction of a minute to some hours. The added times of the single average lengths of the three different states discussed are: AR + SN/SS + PS = 19 min 37 sec ±7 min 14 sec.

The histogram, demonstrating the relationship between the duration of single PS and the frequency of appearing PS episodes of determined duration is illustrated in Fig. 5.

Fig. 5 shows that in rabbits the time interval of most frequently appearing PS is 20 sec. The one directional slope of the histogram shows that shorter PS episodes are more frequent than the medial ones. If the

PS episodes are short, they are greater in number and vice versa. The smallest number of PS in one hypnogram was 13. The arithmetical mean of one PS was then 2 min 13 sec. In another rabbit 14 PS during 24 hr appeared, having a mean duration of 3 min 14 sec. The greatest number of PS episodes in one hypnogram was 85. The mean time of one PS was then only 52 sec. The same rule concerns the episodes of SN/SS.
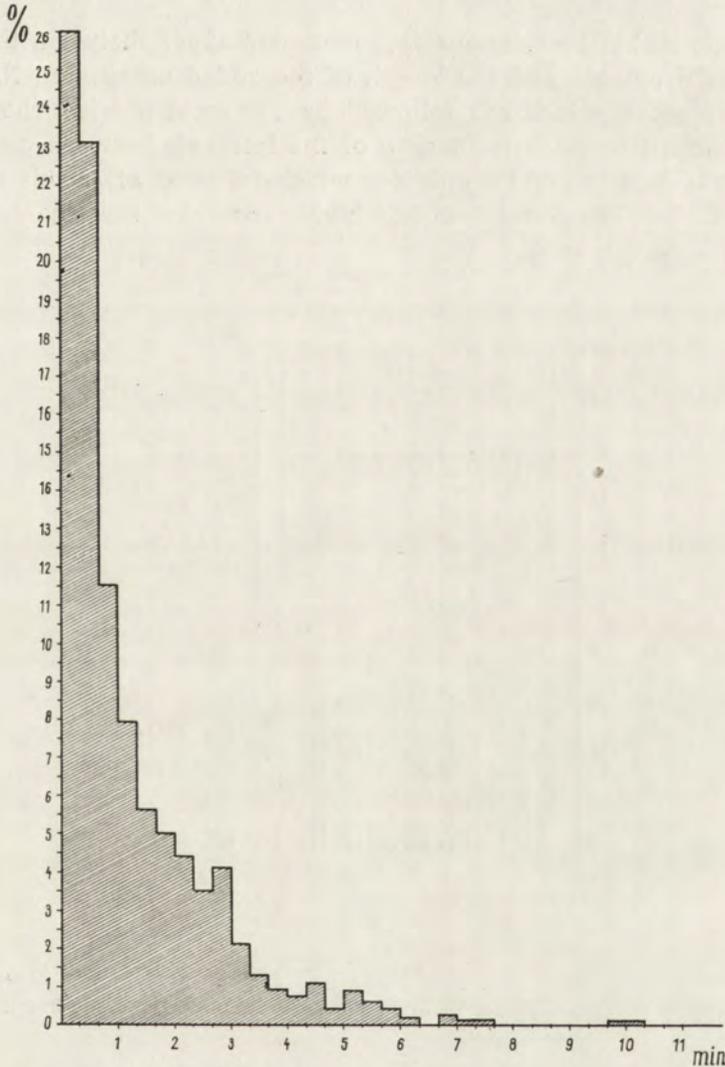

Fig. 5. Histogram of the occurrence of the frequency of PS according to duration of single PS episodes. Abscissa, duration of the particular PS, grouped in intervals of 20 sec. Ordinate, the occurrence of frequency of the individual PS having a definite duration, grouped in classes every 20 sec, and expressed in percentage of the total number of PS episodes observed (n = 775)

The summarized times of three states studied, appearing in one day and night life of rabbits, is the subject of Fig. 6. The data of Fig. 6 expressed in units of time are as follows:

PS	45 min 03 sec ±	16 min 08 sec	
SN/SS	6 hr 07 min 51 sec	±1 hr 36 min 25 sec	
AR	17 hr 08 min 06 sec	±1 hr 42 min 19 sec	

So as to make the relationship, mentioned above, between the length of single PS episode and the length of the added up single SN/SS with the AR episodes, which are followed by PS, most precise, the correlation coefficient between the length of the intervals between consecutive PS and the duration of PS episodes which followed after this intervals, was calculated. The result was 0.24 ± 0.03, and show that the correlation exist but is rather weak.
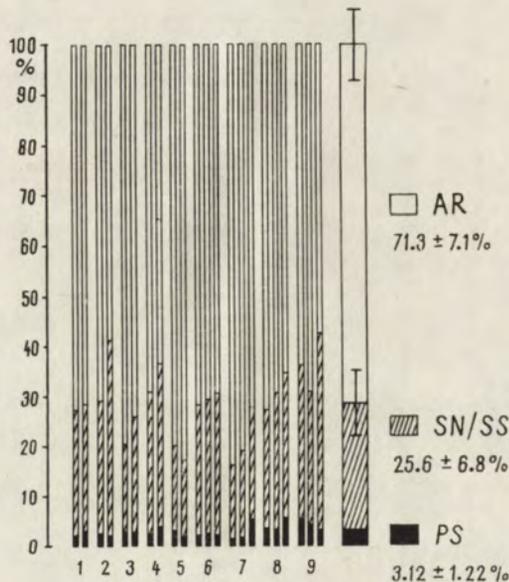

Fig. 6. Summarized 24 hr duration of PS, SN/SS and AR for each of 22 hypnograms of 9 rabbits expressed in percentage. For other explanations see Fig. 1 and 3

The experiments during the nightly 12 hr gave results which do not differ in any essential data from those presented above of 24 hr. They were not presented here, because it was evident that 12 hr sleep experiments had a considerably lower value.

DISCUSSION

The simultaneous recording of the cortical electrical activity using bipolar and monopolar leads, from the dorsal hippocampus together with the EOG and EMG of the neck muscles allowed us to assign any part of the circadian continuous record performed to three states: wakefulness with arousal (AR), synchronization with slow sleep (SN/SS) and paradoxical sleep (PS).

The proper recognition of the PS during the whole duration of one PS episode is possible only when besides the EEG, one records the EMG of the neck muscles and the EOG. In this diagnosis also the registartion of the respiratory-olfactory wave is helpful. This was recorded from the reference electrode situated close rostrally to the olfactory bulb; its amplitude decreases significantly during PS. We always were able to determine with a precision of the order of seconds, the beginning and the end of each PS episode. Owing to that, the PS may be easily measured *quantitatively*.

The phasic phenomenon was revealed in our experiments in the form of typical eye movements accompanied by the amplitude and regularity increase, and by acceleration of the hippocampal theta waves. During PS episodes of long duration this rhythm has not a uniform frequency. The exact analyses of the hippocampal rhythm fluctuation and the comparison of the theta rhythm in PS and AR, because it is not identical in these two states, would be justified only when using an automatic frequency analyzer, which was not at our disposal.

The PS longer than 6 min took place rather exceptionally, there being only 7 out of 775 such cases. It was not possible to exclude the

Table I

Quantitative characteristic of rabbits circadian sleep as reported by different authors

Author	AR %/24 hr	SN/ SS %/24 hr	PS %/24 hr	PS number/24 hr	Duration of one PS episode fluctuations	mean
Khazan and Sawyer	—	50	—	20—30	30 sec— —6 min 30 sec	—
Faure	23[a]	60[a]	7[a] (3—16)	16—120[a]	40 sec— —4 min	—
Weiss and Roldán	7.4	77.5	15.1	67	—	3 min 42 sec ±11 sec
Kawakami et al.	60.6	36.7±3.1	2.7±0.6	24—75	5 sec— —6 min 30 sec	1 min 12 sec
Narębski et al.	71.3	25.5±6.8	3.1±1.2	35±20 (13—85)	8 sec— 10 min 20 sec	1 min 22 sec ±34 sec

[a] Calculated from 3 hr experiments.

possibility of PS shorter than 8 sec, but in techniques used it was not possible to identify them.

The data presented in Table I show that our results are compatible only with those of Kawakami et al. (1965). The results of Faure (1965) were obtained only in 3 hr experiments and therefore they will not be discussed. On the other hand, the data of Weiss and Roldán (1964) differ essentially from the other ones. The circadian quantity of PS, according to these authors, is almost three times greater, the average PS episode lasts three times longer, and the circadian number of PS episodes is about twice bigger. In the light of the method of experimentation of Roldán and Weiss (1963), which was without EOG and EMG recording, it is possible to suppose that these authors accepted some episodes of AR as PS.

Table II
Greatest circadian PS quantity

Rabbit	Date	PS%/24 hr
S7 ♂	20/21.II.1968	5.45
S8 ♂	15/16.II.1968	5.67
S9 ♂	18/19.II.1968	5.79
	19/20.II.1968	4.13

It is possible that the race and age of rabbits have some influence on the circadian PS. The majority of authors working on sleep in this species used only female rabbits, considering that sex had an essential part in such chronic experiments. According to our data, essential differences concerned with sex in the circadian number of sleep episodes and their spread during 24 hr, do not exists. The natural sleep of male rabbits has a little greater fluctuation of the circadian number of PS episodes, from 13 to 85 (female respectively 14—59), and slightly greater spreading of the circadian quantity of PS, from 1.72% to 5.79% (female respectively 1.7—3.6%.

A surprisingly great circadian quantity of PS appeared in our experiments only a few times in three out of five male rabbits used.

On the basis of our experimentation experience this phenomenon seems to be connected with the season, and its explanation needs further experiments.

In general, the EEG of rabbits is characterized by really great difference between the resting, synchronized pattern, and AR. However some difficulties exist only with the exact recognition of the moment of the transition from AR into SN. This difficulty is caused by the gradation of this transition. The subjectivity, therefore, of the choice of this

moment is unavoidable. Than, the conclusions drawn from circadian summarized quantity of SN/SS has a considerably lower value than PS respectively. Therefore, though the differences concerning these data among authors cited seem to be understandable, they are not easy to remove without uniformity of criteria here used.

The important problem is the physiological interpretation of the state of the animal when its EEG is fully synchronized. It may be both relaxed wakefulness and SS. The SS exists certainly some time just before the PS. There is a lack of objective criteria which would enable one to detect the moment of falling asleep during the SN. Falling asleep always takes place during relaxed wakefulness (in the normal conditions) when the EEG is already for some times synchronized. Therefore, in this paper we do not use the term SS but SN/SS. We wish to be in agreement with the objective possibilities of the experimentation on sleep.

The typical sequence suggested by many authors: AR-SN/SS-PS and again AR does not repeat every time. As presented above (Fig. 3) PS occurs 35 times during 24 hr, but SN/SS 86 times. It means that the "triad" AR-SN/SS-PS occurs only about 41 times out of 100, and the sequence AR-SN/SS without PS more often, 59 times out of 100. Almost every PS is followed by AR. In experiments described, only 15 times out of 775 PS passes directly into SN/SS. This phenomenon, though not frequent, brings also some limitation to this rule. It seems to be a feature of an individual animal. Pellet and Béraud (1967) do not describe this fact, but in published hypnograms of the rat and guinea pig the presence of such a sequence is shown.

As was described above, the summarized average time of three kinds of episodes: AR+SN/SS+PS is 19 min 13 sec±5 min 14 sec. But taking into account that AR and SN/SS are two and a half times more numerous than PS episodes, such summarized length of the "triad" is an artificial creation. It is suitable to calculate the average time between two consecutive PS episodes more precisely: from the end of the preceeding to the beginning of the succeding PS episode. This mean time in our experiments is 39 min 36 sec. According to Weiss and Roldán (1964) this time is nearly twice shorter, being 20 min 48 sec. The comparison of this data may justify the assumption that authors cited above probably recognized some AR episodes as PS.

The dispersion in time of the individual PS episodes is not accidental. The changeable time between the consecutive PS may be described as follows: When this time is long, then the succeding PS is also long, as a single one or multiple, divided with short episodes of AR and SN/SS. When this time is short, the succeding PS is also short. Such a rule of

the resolution in time of the individual PS permits one to bring forward the supposition that during the hours without PS it seems to appear an augmented, with the elapsed time, "need" for PS. It is possible that out of the "need" there exists another factor which delays the appearance of PS or causes a partition of the already existing PS into several segments. The factor of the need of PS seems to be postulated by Jouvet (1967) and Dement et al. (1967), a monoamine of metabolic origin, accumulated in some parts of the brain proportionally to the time elapsed without PS and processed during PS (Pujol et al. 1968). The factor involved in the delay of PS and its fractionation, remain till now unknown. Probably it has a nature of a rapidly acting neural reflex. The quick transition from PS into AR, which is the rule, is physiologically well understandable. PS is a deep sleep, and though it is indispensable for living puts the animal into danger. The spontaneous frequent and easy transition from PS into AR, means awakening in the state of immediate readiness for defense or agression, serves without doubt for the individual safety of the animal.

The sequence in time of particular PS episodes, though not incoherent, is complicated and without sharp limits of classification. Therefore, it is not easy to describe it mathematically. An attempt was made to calculate correlation between the length of the segment of time without PS and the duration of the following PS episode, but with uncertain results. We think it takes place therefore, because the relation investigated by the method of correlation has in the experiments of spontaneous sleep (without sleep deprivation) a character too complex for the method used. However, the obtained positive, but very weak correlation may be recognized as support of monoamine metabolic theory of PS.

SUMMARY

Twentytwo 24 hr experiments were performed on nine rabbits with chronically implanted electrodes. The purpose of the work was to establish the temporal sequence of the spontaneously appearing episodes of wakefulness with arousal (AR), synchronization with slow sleep (SN/SS), and paradoxical sleep (PS). The continuous recording of the EEG from cortex and hippocampus, the EMG of neck muscles and the EOG permits one to determine the beginning and end of these episodes. However, a really exact determination is possible only with the PS. Therefore, this kind of sleep may be treated quantitatively. The sex of rabbits does not play an essential role in the circadian processes of wakefulness and sleep in its two kinds. The quantitative data concerning the sleep

of rabbits are as follows: in 24 hr the number of PS episodes is 35 ± 20 with fluctuations from 13 to 85, while the number of SN/SS episodes is 86 ± 25. The single average PS episode lasts 1 min 22 sec±34 sec, but oscillates from 8 sec to 10 min 20 sec. PS occupied $3.1\pm1.22\%$ of the 24 hr period, and respectively SN/SS 25.6%. The remainer 71.3% belongs to wakefulness with AR. The duration of one PS episode depends, in some degree, on the length of the preceeding segment of time filled up by AR and SN/SS.

REFERENCES

DEMENT, W. C. 1958. The occurrence of low voltage fast electroencephalogram patterns during behavioural sleep in the cat. Electroenceph. Clin. Neurophysiol. 10: 291—296.

DEMENT, W. C. and KLEITMANN, N. 1957. Cyclic variations in EEG during sleep and their relation to eye movements, body motility and dreaming. Electroenceph. Clin. Neurophysiol. 9: 673—690.

DEMENT, W. C., HENRY, P., COHEN, H. and FERGUSON, J. 1967. Studies on the effect of REM deprivation in humans and animals. Assoc. Res. Nerv. Ment. Dis. 45: 456—468.

FAURE, J. 1965. Le sommeil "paradoxal" du lapin dans ses aspects anatomo-fonctionnels et hormonaux. In M. Jouvet (ed.), Aspects anatomo-fonctionnels de la physiologie du sommeil. Centre Natl. Rech. Sci. Paris. 106: 241—283.

GREEN J. D. and ARDUINI, A. A. 1954. Hippocampal electrical activity in arousal. J. Neurophysiol. 17: 533—557.

JOUVET, M. 1967. Neurophysiology of states of sleep. Physiol. Rev. 47: 117—177.

JOUVET, M., MICHEL, F. and COURJON, J. 1959. Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. Compt. Rend. Soc. Biol. 153: 1024—1028.

KAWAKAMI, M. and SAWYER, C. H. 1962. Effects of hormones on "paradoxical" sleep in the rabbit. Federation Proc. 21: 354.

KAWAKAMI, M., NEGORO, H. and TERESAWA, E. 1965. Influence of immobilization stress upon the paradoxical sleep (EEG afterreaction) in the rabbit. Japan. J. Physiol. 15: 1—15.

KHAZAN, N. and SAWYER, C. H. 1963. "Rebound" recovery from deprivation of paradoxical sleep in the rabbit. Proc. Soc. Exptl. Biol. Med. 114: 536—539.

NARĘBSKI, J., TRACZYK, W. and HURYNOWICZ, J. 1966. Spontaneous electrical activity of the cortex and subcortical centers during reversible anaphylactic shock in rabbits. Acta Physiol. Polon. 17: 609—622.

PELLET, J. and BÉRAUD, G. 1967. Organisation nycthémérale de la veille et du sommeil chez le cobaye. (Cavia porcellus). Comparaison interspécifiques avec le rat et le chat. Physiol. Behav. 2: 131—137.

PUJOL, J. F., MOURET, J., JOUVET, M. and GLOWIŃSKI, J. 1968. Increased turn-

over of cerebral norepinephrine during rebound of paradoxical sleep in the rat. Science 159: 112—114.

ROLDÁN, E. and WEISS, T. 1963. Excitability changes of the reticular ascendent activating system during sleep cycle in the rabbit. Arch. Int. Physiol. Biochem. 71: 518—527.

WEISS, T. and ROLDÁN, E. 1964. Comparative study of sleep cycles in rodentes. Experientia 20: 280—282.

THE AVOIDANCE REFLEX REINFORCED WITH FOOD

Stefan SOŁTYSIK

Department of Physiology of the Nervous System, Institute of Psychoneurology, Pruszków, Poland

A well trained avoidance reflex in the dog consists of three acquired responses integrated into a self stabilizing behavioral unit. The first is the classical fear conditioned reflex (CR) elicited by the warning conditioned stimulus (CS); one index of it is heart rate acceleration (Sołtysik and Kowalska 1960, Sołtysik 1960b) and it has been shown that this cardiac change precedes the motor instrumental response. Second comes the motor avoidance response itself, occurring as an overt response to the fear (a drive component) and to conditioned stimuli which include both the specific warning CS and the situational cues. This convergence of drive and directing cues was reflected in the early model of the instrumental CR postulated by Wyrwicka (1952), though the drive nature of the unconditioned stimulus (US) center in her model was clearly stated only later by Sołtysik (1960a) and Konorski (1967). For the avoidance reflex a similar model was considered in a series of papers (Sołtysik and Kowalska 1960, Sołtysik and Zieliński 1962, Sołtysik 1963). Finally, the instrumental response generates stimuli (afferent and central feedback of the response) which, being paired with the termination of the noxious US (in the case of the escape response) or with the termination of the CS (in the case of each avoidance response), becomes a conditioned inhibitor (CI) of the fear response. Experimental data supporting this view were presented elsewhere (Sołtysik and Kowalska 1960, Sołtysik 1960b, c) and the implications of this concept are discussed in the paper of Sołtysik (1963) and in Konorski's monograph (1967). In brief, this inhibitory feedback is believed to play an important role in respect to the fear CR as

well as to the instrumental response. Inhibition of the fear CR protects this emotional response from extinction; such a role of the CI presented in compound with the CS was shown first by Chorążyna (1957) for food instrumental training and confirmed by Sołtysik (1960c) in a situation resembling the avoidance reflex performance. The second role played by this conditioned suppression of fear is to provide an additional "reward"; the fear is not merely decreasing due to the termination of the fear-eliciting CS but is also actively inhibited. Thus, our model postulates not only drive reduction but even a drive inhibition as a source of reward. Owing to this double role the inhibitory feedback stabilizes the avoidance behavior and makes it virtually independent of the noxious US.

An interesting question arises as to what would happen if such a well trained avoidance CR was systematically reinforced with food? The first guess might be that the avoidance response would be transformed into a food instrumental CR, according to the rule that any motor response which is rewarded by food will become an "alimentary instrumental" response. However, our model offers an alternative prediction. To become a food instrumental CR, the avoidance CR should have been transformed in its drive component, leaving the CS and motor response the same. This is very unlikely, as the drive CR (i.e., a fear reaction) is well shielded from the following events by the conditioned inhibition. And as the hunger drive and the fear drive are antagonistic activities of the brain, it is not conceivable to assume any easy way for their mutual replacement. One could rather imagine a coexistence of the conditioned inhibition of fear with the classical consummatory food CR as these both need not be antagonistic and even may corroborate with each other in suppressing the fear reaction. Is there, then, a possibility that we might obtain a "hybride" CR complex, in which an avoidance reflex will end with the food CR? In other words, would the motivation of the instrumental movement remain a fear while the movement itself becomes, besides the CI of fear, also the conditioned signal for food?

This paper presents some new data obtained with dogs trained first in avoidance CR and then systematically reinforced with food after each avoidance response.

MATERIAL AND METHOD

The experiments were carried out on six dogs (adult male mongrels) in a sound proof conditioned reflex chamber. The animals were experimentally naive but accustomed to stand in the harnesses on the Pavlovian stand. In three dogs (no. 1—3) the food instrumental reflexes were established by rewarding a passive movement (pressing a bar) with a portion of food delivered from the automatic feeder. First, all the spontaneous responses were rewarded but after a few days

a conditioned stimulus (a buzzer) was introduced and only the responses occurring during its presentation were rewarded. Soon the intertrial responses disappeared and the bar-pressing response to the buzzer was well established.

In the three remaining dogs (no. 4—6) an avoidance bar pressing response was taught by the following method. The buzzer was reinforced with electric shock to the hindpaw, but occasionally, 5 or 6 times in a session, a passive movement (pressing a bar) was performed during the presentation of the buzzer and the movement was rewarded by the termination of the CS and omission of the shock. When some active or semiactive responses appeared a shaping procedure enabled us to train the perfect bar press response to the buzzer. In dog 3 the bar pressing occurred as a "random" response to the second application of the shock and was immediately fixed as the escape or avoidance response; thus, in this dog no passive movements and shaping procedure were necessary.

After the response to the buzzer was well overtrained (during four months of daily training with ten trials a day) so that virtually no shock reinforcements were necessary to maintain the avoidance performance, a new stimulus, a rhythmic tactile stimulation of the dog's chest, was introduced and paired with shock. The transfer of the avoidance response was almost immediate and after 10 days of training using both stimuli in equal proportions though randomly intermixed no detectable differences in reacting to both stimuli were seen. At that moment a new procedure was started. Three or four times a week the dogs had sessions in which only the tactile stimulus was used and after each response a portion of food was offered from the automatic feeder. The animals were not fed before these sessions and soon learned to accept the food. In the two remaining sessions (at the beginning and in the middle of a week) the animals were fed before the session and only the buzzer was used as before. It should be mentioned that the shock US was never used on the sessions with the tactile CS but it was rigorously applied on the sessions with the buzzer if the dog failed to respond within 10 sec. After 40 sessions with food reinforcement several "satiation sessions" were carried out in which the trials with food reinforcement were repeated until the dogs were fully satiated and consistently refused to eat. The changes in motor responding during the satiation sessions were compared with the changes of responses in food-instrumental dogs subjected to the similar procedure of gradual satiation by presenting a long series of trials. In some of the dogs carotid arterial loops were prepared surgically by the van Leersum (1911) method, so that the recording of heart rate during the instrumental performance was possible. The method of recording was described elsewhere (Sołtysìk et al. 1961).

RESULTS

I. The effect of applying the food after an avoidance CR

Presenting the food immediately after the dog has performed the avoidance movement aroused in the beginning a marked fear reaction. The noise from the feeder and the unexpected appearance of the full foodtray elicited repeated pressing of the bar accompanied often with barking. The animals did not accept the food and it took several sessions before they habituated to these new events and learned to eat. The training was continued for three months and no evident changes in behavior

occurred. Latencies of the movement on both the avoidance sessions (to the buzzer) and "avoidance-food" sessions (to the tactile CS) were similar. The casual observer could not tell whether the dogs performed the movement to the tactile stimulus because it has been previously a danger signal or because it was rewarded presently with food. True, the animals never looked at the foodtray in response to the CS, but they did so after they pressed the bar, and this food-approach reaction was occasionally seen also on the avoidance sessions with the buzzer.

II. The effect of gradual satiation during the prolonged session

Fig. 1 and 2 show the typical course of changes in the instrumental CR in dogs 1, 2 and 3 during the satiation session. In each case the gradual diminution of hunger caused the lengthening of latencies of bar presses. In dog 1 the increase of latency starts early in the session and soon the responses disappear altogether; this coincides roughly with the refusal to eat. The animal might have not responded but still accept the food [1] or, equally likely, might after having responded refuse to feed (cf. the eighth and ninth trials). Dog 2 shows a different "satiation curve". During the first six trials the latencies of bar presses remain the same and only on the seventh trial the sudden and steep deflection of the curve is seen. The dog often ate after not having responded during the 20 seconds CS–US interval. The third dog shows a gradual increase of latencies throughout the session but in distinction to the two other dogs he responded on many trials after he had stopped to feed (from the tenth trial on). The dogs exhibited different behavior in response to the CS in the later part of the session when, having eaten ad libitum they refused to accept food. Thus, dog 2 was quiet and seemed to disregard the CS. Dog 3 always reacted positively, pressed the bar and only after having looked at or even sniffed the food turned away from the feeder. Very distinct and strange behavior was observed in dog 1. The animal, when satiated, stood qietly during the intertrial intervals but responded with struggling, barking and general restlessness to the CS. In this dog we kept the record of the pulse rate, and cardiac responses to the CS are shown in Fig. 1.

These cardioacceleratory responses diminish as the dog becomes satiated but then they paradoxically increase and reach amplitudes far exceeding the size of responses at the beginning of the session. Characteristically, the resting level of the heart rate gradually diminishes during

---

[1] During the satiation sessions the food was presented on each trial even if the dog has not pressed the bar.
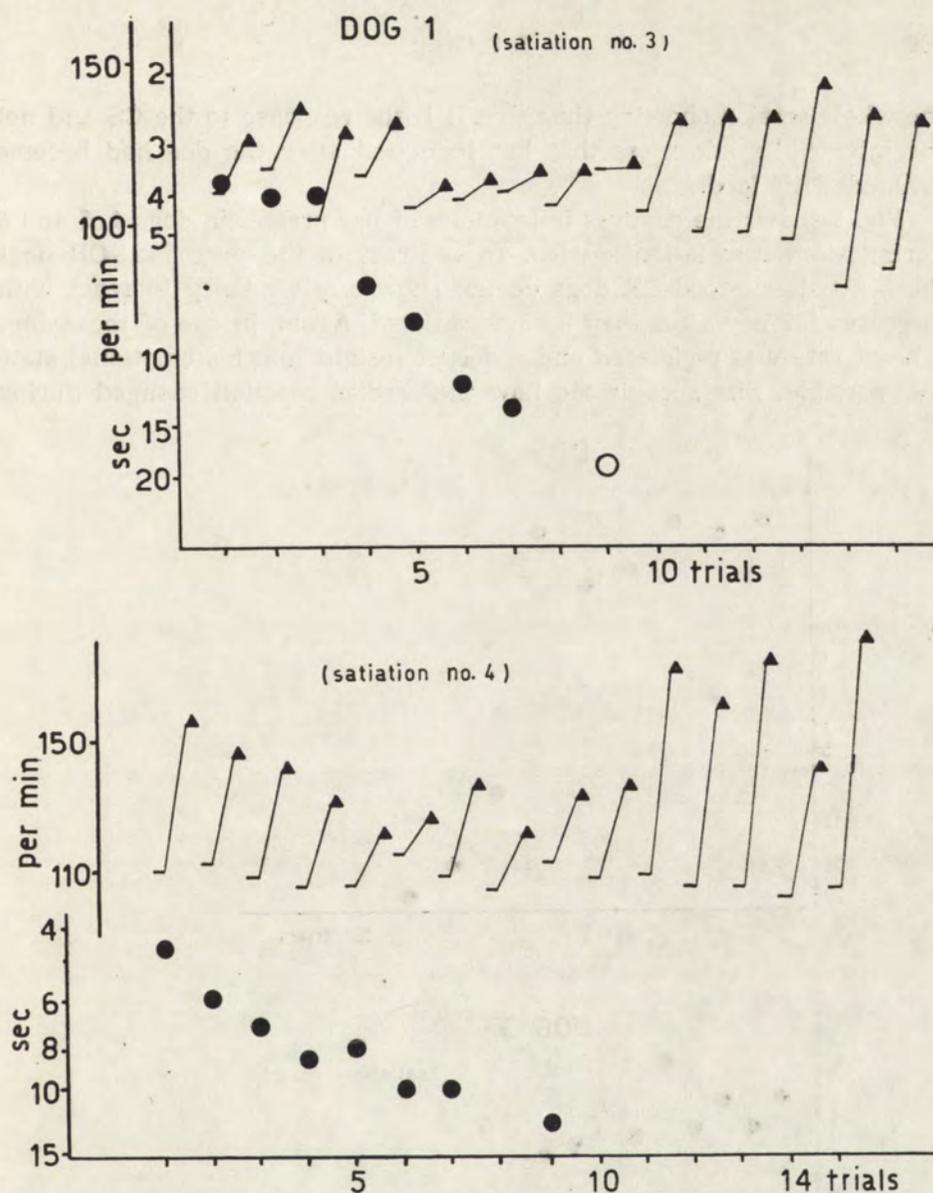
Fig. 1. Changes in cardiac responses and latencies of motor instrumental CRs in Dog 1 during two prolonged sessions. Abscissae: consecutive trials in the session. Ordinates, upper part on each graph: heart rate in beats per minute. Ordinates, lower part: latency of motor response in seconds. Note that the order of number is reversed so that the lengthening of latency corresponds to a downward shift on the graph. Filled dots show the latencies of responses followed by eating; open circle shows the latency of a response after which the dog refused to eat. Heart rate responses are shown in the following way: the short horizontal "foot" is a pre-CS level and the filled triangle shows the heart rate during the CS. Heart rate was measured during the 10 sec immediately preceding the onset of the CS and during the second 5 sec of its operation. Note the marked increase of the heart rate response after the dog has become satiated

the whole session showing thus that it is the response to the CS and not the intertrial restlessness that has increased after the dog had become satiated with food.

Fig. 3 shows the changes in latencies of bar presses in dogs 4, 5 and 6 during similar satiation session. In contrast to the pure food-CR dogs these avoidance-food-CR dogs do not show any tendency to react with increased latency after they become satiated. Again, in one of these dogs a heart rate was registered and a deeper insight into his emotional state was possible. First, let us see how the cardiac reaction changed during
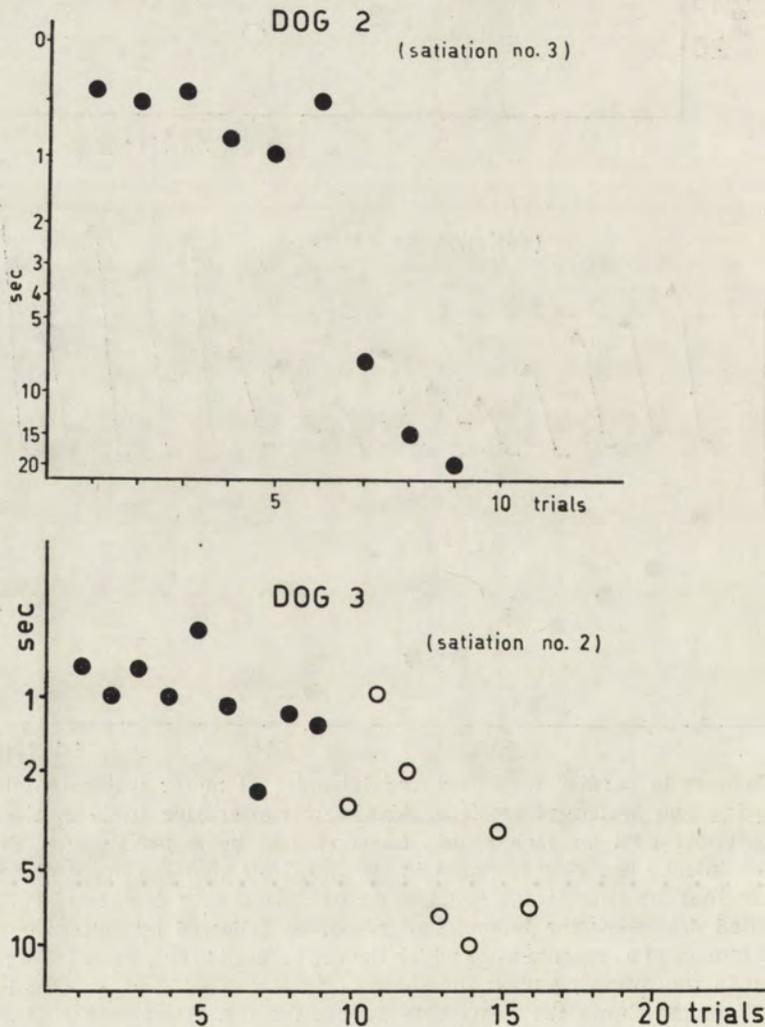
Fig. 2. Changes in latencies of the food instrumental responses in two dogs during the prolonged sessions. Explanations as in Fig. 1

DOG 4   (satiation no. 2)

DOG 5

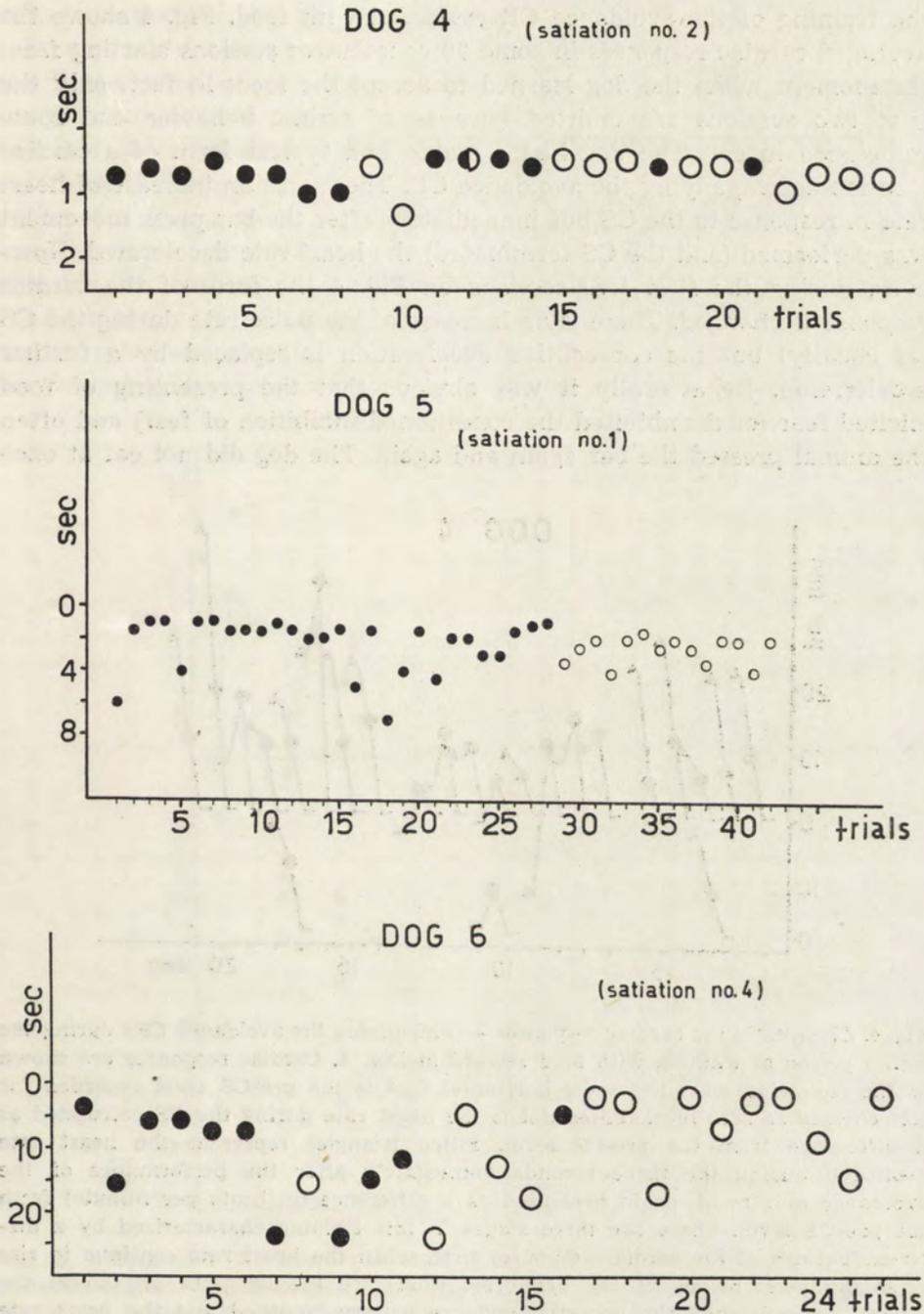(satiation no.1)

DOG 6

(satiation no. 4)

Fig. 3. Lack of changes in the latencies of avoidance CR reinforced with food in three dogs during the prolonged sessions. Explanations as in Fig. 1

the training of the avoidance CR rewarded with food. Fig. 4 shows the averaged cardiac responses in some 20 consecutive sessions starting from the moment when the dog learned to accept the food; in fact, only the first two sessions are omitted because of erratic behavior and some reluctance to eat. The dog had a stable and typical form of a cardiac reaction accompanying the avoidance CR. There was an increase of heart rate in response to the CS but immediately after the bar press movement was performed (and the CS terminated) the heart rate decelerated. However, during the first five sessions on Fig. 4 the form of the cardiac response is changed. There is an increase of the pulse rate during the CS (as usually) but the consecutive deceleration is replaced by a further acceleration. Behaviorally it was obvious that the presenting of food elicited fear (or disinhibited the conditioned inhibition of fear) and often the animal pressed the bar again and again. The dog did not eat at once
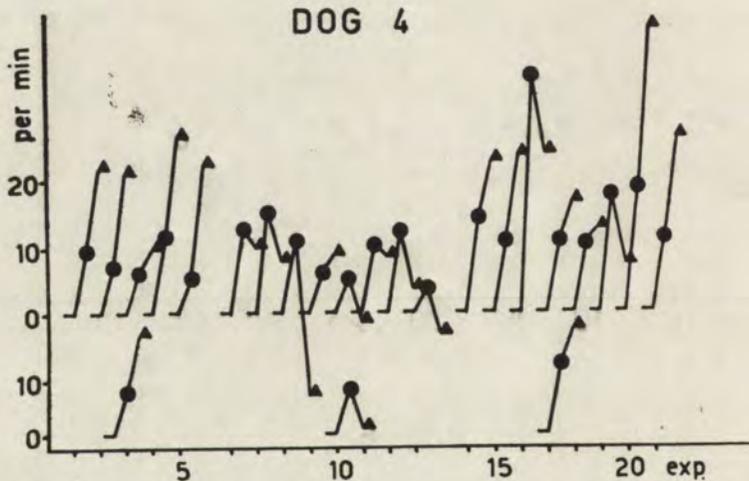

Fig. 4. Changes in the cardiac responses accompanying the avoidance CRs during the initial period of training with food reward in Dog 4. Cardiac responses are shown in the following way: the short horizontal foot is the pre-CS level regarded for convenience as zero level. Filled dot is the heart rate during the CS calculated as a difference from the pre-CS level. Filled triangles represent the heart rate measured during the three seconds immediately after the performance of the avoidance movement, again presented as a difference (in beats per minute) from the pre-CS level. There are three stages in this training characterized by a different pattern of the cardiac response: first, when the heart rate continue to rise after the performance of the bar press (therefore the triangles are above the circles), second when the normal avoidance pattern returned and the heart rate drops after the motor response is performed, and third, when again the heart rate increases after the avoidance response because it has become a food CS. Beneath each stage is shown the average cardiac response

being startled by the not yet familiar noise of the feeder. During the following eight sessions the signs of distress caused by the operation of the feeder subsided and the form of the heart rate reponse returned to its previous normal pattern: an increase of rate to the CS and an immediate decrease after the bar press response. Finally, the cardiac deceleration following the bar press was again replaced by the cardioacceleration, but this time no signs of distress were its behavioral counterpart but the more less evident "alimentary reaction". The dog would turn towards the foodtray, lick it and seize the food without hesitation.

As mentioned before, at this phase of training the satiation sessions were started and revealed that the latency of the bar press was resistant to the satiation factor. Fig. 5 shows that while the motor responses
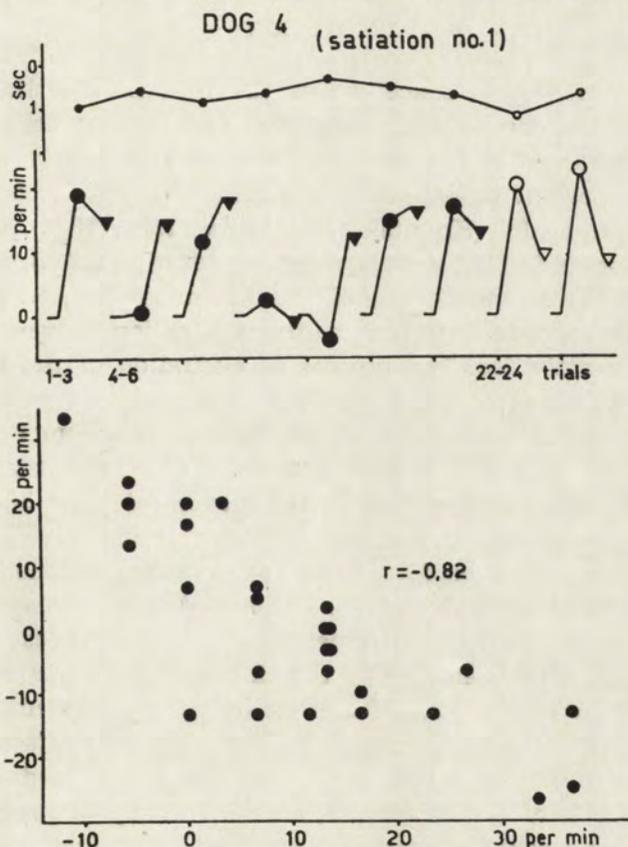

Fig. 5. Changes in the heart rate reactions of the Dog 4 during the prolonged satiation session. Upper graph: upper curve shows the latencies (in blocks of three trials) and beneath are the cardiac reactions. Explanations as in Fig. 4. On the last six trials the dog did not eat. Lower graph: negative correlation between cardiac responses to the CS and heart rate changes immediately after the motor responses (Ordinates). Data are taken from the session presented on the upper graph.

remain constant, the cardiac changes accompanying them undergo
a series of fluctuations. At the very beginning the cardiac responses to
the CS were large and followed by some slight deceleration — a picture
resembling the original pattern when the avoidance response has not
been reinforced with food. Soon, however, the deceleratory response was
replaced by the acceleration and at the same time the responses to the CS
tended to diminish. At last, when the dog became satiated the full original
avoidance pattern of the cardiac reaction reappeared. The apparent
reciprocal relation of the size and direction of these two cardiac responses
is shown on the lower graph of Fig. 5 where the responses to the CS are
plotted against the responses to bar presses.

DISCUSSION

This experiment was conceived as the first in a series devoted to
studying the relations between defensive and feeding behaviors in the
context of instrumental conditioning. We are particularly interested in
verifying our present model of the instrumental CR. In contrast to the
old Konorskian model (Konorski and Miller 1933, 1936) and Pavlov's
model (1936) or its further development by Asratian (1967), the emphasis
is laid not on the signalling role of the movement (i.e., on some sort of
proprioceptive conditioning) but on the distinction between drive and
consummatory activities. The process of elicitation of the instrumental
movement depends according to the old concept' of Konorski and Miller
on the proprioceptive feedback of the movement becoming a *differen-
tiated* signal of food. In Pavlov's and Asratian's formulations also the
proprioceptive stimuli generated by the movement must become a food
CS but the mechanism postulated by them is entirely different and
concerns the so-called backward or "reverse" conditioned connection
between the "food center" and the "representation of movement". Our
conditioned drive model of instrumental CR (as we propose to call it in
distinction from models employing the classical consummatory CR as, at
least partially, a motivational factor [2] eliciting the movement) assumes
that the instrumental movement is elicited only by the convergent ex-
citation from the drive center and from the conditioned stimuli. The
afferent feedback of the motor response inevitably acquires an appro-
priate signalling meaning, for example, of food CS in alimentary condition-
ing or fear CI in avoidance training, but it has no direct relation to the

---

[2] Formulations such as Seward's "tertiary motivation" (1950), Mowrer's "hope"
(1960), or Hullian and neoHullian "fractional antedating goal responses" belong to
this class of models.

mechanism of eliciting the movement. Fig 6 summarizes this reasoning. The paradigm 1 represents the view of Pavlov: movement generates the stimuli which are the food CS but the food center is also connected with the movement center and thus the movement is elicited whenever the food center is excited. Paradigm 2 represents our present concept of the instrumental food CR. Instead of a single "alimentary center" there two centers: a hunger center through which the movement is elicited and the food center which inhibits the hunger drive center. Paradigm 3a shows the avoidance reflex; the movement is elicited through the fear center
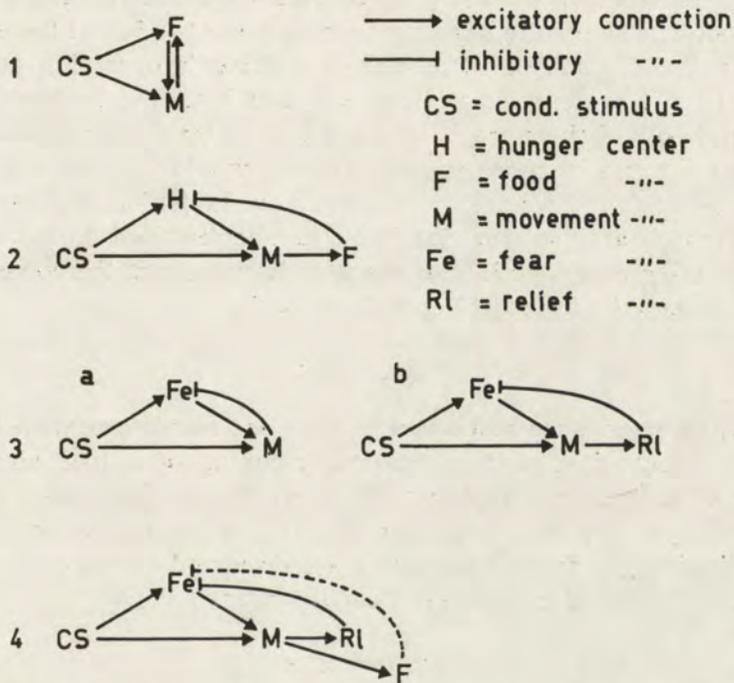

Fig. 6. Simplified paradigms of the instrumental conditioned reflexes. Explanations in the text

and the movement-produced stimuli inhibit the fear center. Paradigm 3b is modified according to Konorski's concept (1967) of a relief center which is an inhibitory counterpart of the fear center; thus the inhibition by the feedback of the motor response is executed through this relief, or security center. Finally, a paradigm of the behavior described in the present experiment is given. It is the avoidance reflex whose afferent feedback has become a signal of food. As we see it is quite feasible to imagine such a product of our procedure (reinforcing the avoidance response with food), since food consummatory CR is not

colliding with the conditioned inhibition (or relief CR) of fear. On the contrary, eating and fear are relatively incompatible, so we may assume that this food classical CR corroborates in inhibiting the fear.

We should admit, however, that we do not know what would happen if the training of the avoidance CR reinforced with food was continued for a longer period, and if the sessions of pure avoidance conditioning were discontinued. For the time being we are only able to ascertain that the avoidance response may easily become a classical CS of food and that this does not alter its performance.

There is one practical aspect of this experiment that should be mentioned. The use of the heart rate changes as an index of the emotional state is relatively simple when only one drive is operating during the session. In the present experiment we used both the hunger and fear drives, and both of them are manifested in dogs by the cardioacceleration. Therefore the use of more specific indices would be desirable. Unfortunately no such indices are conveniently available. Hunger contractions of the stomach are too slow for intervals shorter than 30 sec intervals. Probably, the direct recording of the unit discharges of the corresponding centers seems to be the most hopeful approach

SUMMARY

When the avoidance responses in dogs are reinforced with food, the response-generated stimuli acquire the meaning of a food signal. This does not change the performance of the avoidance CR, which continues to depend upon the fear drive and therefore is insensitive to manipulation on the hunger drive. The results are discussed in the context of the Konorskian model of instrumental conditioning.

REFERENCES

ASRATIAN, E. A. 1967. Functional architecture of instrumental conditioned reflexes. Proc. XVIII Int. Psychol. Congr. Symp. 4: Classical and Instrumental Conditioning, Izd. Nauka, Moscow, p. 35—53.

CHORĄŻYNA, H. 1957. Some data concerning the mechanism of conditioned inhibition. Bull. Acad. Pol. Sci. 5: 387—392.

KONORSKI, J. 1967. Integrative activity of the brain. An interdisciplinary approach. Univ. Chicago Press. Chicago, xii+531 p.

KONORSKI, J. and MILLER, S. 1933. Podstawy fizjologicznej teorii ruchów nabytych. Ruchowe odruchy warunkowe. (in Polish, French summary). Warszawa—Lwów, Książnica Atlas, TNSW. 168 p.

KONORSKI, J. and MILLER, S. 1966. Conditioned reflexes of the motor analyzer (in Russian, Engl. summary). Trans. Physiol. Lab. I. P. Pavlov 6: 119—278.

van LEERSUM, E. C. 1911. Eine Methode zur Erleichterung der Blutdruckmessung bei Tieren. Pflügers Arch. Ges. Physiol. 142: 377—395.

MOWRER, O. H. 1960. Learning theory and Behavior. John Wiley and Sons, Inc. New York, xiii+555 p.

PAVLOV, I. P. 1936. Physiological mechanism of the so-called voluntary movements (in Russian). Trans. Physiol. Lab. I. P. Pavlov, 6: 115—118.

SEWARD, J. P. 1950. Secondary reinforcement as tertiary motivation. A revision of Hull's revision. Psychol. Rev. 57: 362—374.

SOŁTYSIK, S. 1960a. On the intercentral connections in the reflex arc of the alimentary conditioned reflex (in Russian). In E. A. Asratian (ed.), Tsentralnye i pericheskie mekhanizmy dwigatelnoi deyatelnosti zhivotnykh. Izd. An SSSR, Moscow. 58—60 p.

SOŁTYSIK, S. 1960b. Studies on the avoidance conditioning II. Differentiation and extinction of the avoidance reflexes. Acta Biol. Exp. 20: 171—182.

SOŁTYSIK, S. 1960c. Studies on the avoidance conditioning III. Alimentary conditioned reflex model of the avoidance reflex. Acta Biol. Exp. 20: 183—192.

SOŁTYSIK, S. 1963. Inhibitory feedback in avoidance conditioning. Boll. Inst. Estud. Med. Biol. Mex. 21: 433—449.

SOŁTYSIK, S., JAWORSKA, K., KOWALSKA, M. and RADOM, S. 1961. Cardiac responses to simple acoustic stimuli in dogs. Acta Biol. Exp. 21: 235—252.

SOŁTYSIK, S. and KOWALSKA, M. 1960. Studies on the avoidance conditioning I. Relations between cardiac (type I) and motor (type II) effects in the avoidance reflex. Acta Biol. Exp. 20: 157—170.

SOŁTYSIK, S. and ZIELIŃSKI, K. 1962. Conditioned inhibition of the avoidance reflex. Acta Biol. Exp. 22: 157—167.

WYRWICKA, W. 1952. Studies on motor conditioned reflexes. 5. On the mechanism of the motor conditioned reaction. Acta Biol. Exp. 16: 131—137.

http://rcin.org.pl

MORRIS G. R., [ed. Supervisor] .... Ibm 29, Ibm Morris M gov ....
ro, Vol. 2, 23–29.

PAYNE J. A. ....

SUNKEL W. .... A. rydek

AILSON K. ....

POLITOWS ....
....
....

ROZYNSKI .... Glosa 11–22. sumkebs.
....

SCHWALK K. ....

SOKOLWSKI ....
....

SCHMIDT K. AND ....
....

STRZ ....
....
tyta vollac. 978 ....

ROZYNSKI A. ....
Jalan der ....

WYBICKA W. ....
....

CONNECTIONS OF THE INTERMEDIATE (TUBERAL) PART OF THE HYPOTHALAMUS IN THE DOG

Antoni ŚMIAŁOWSKI

Department of Comparative Neuroanatomy, Jagiellonian University, Cracow, Poland

MATERIAL AND METHOD

The paper is based on observations of eight continuous series of sections of dog brain. Three series of sections, stained by Weigert-Wolters method (frontal, horizontal and sagittal) and transected every 50 µ, and five series, stained by the Klüver-Barrera, Nissl, Schultze method (two frontal, two horizontal and one sagittal) and transected every 20 µ, were used for observations.

RESULTS

Hypothalamo-hypophyseal tract

This is a system of fibres connecting the hypothalamus with the pituitary gland. In the dog brain the following hypothalamic nuclei were found to have connections with the pituitary gland: the paraventricular and anterior supraoptic nucleus in the anterior part and the posterior supraoptic and ventromedial nuclei in the intermediate part. All the fibres of this tract are well myelinated and stain well in Weigert-Wolters and Klüver sections. The fibres of this system take rise in the above mentioned nuclei of the hypothalamus and tend through the infundibulum to the posterior (neural) part of the pituitary gland.

This tract was first mentioned by Ramon y Cajal in 1894. He observed such a system of fibres in sections of mouse brain stained by Golgi method.

Investigations carried out on neurosecretory material stained by the Gomori method (Ban 1964, Diepen 1962, Schreiber 1963) showed that the

7*

fibres which lead to the pituitary gland from the paraventricular and supraoptic nuclei of the hypothalamus conduct substances regarded as neurosecretory. Since the studies of these authors do not indicate the occurrence of neurosecretion within the ventromedial nucleus (which nucleus also has a connection with the pituitary gland), it may be inferred that it transmits nerve impulses in the form of action potentials to the pituitary gland. The travelling of action potentials along the hypothalamo-hypophyseal tract has been confirmed by Schreiber (1963).

In literature two tracts leading from the hypothalamus to the pituitary gland are often distinguished, i.e., the supraoptico-hypophyseal and paraventriculo-hypophyseal tracts (Ban 1964, Bleier 1961, Diepen 1962, Schreiber 1963). These two tracts join in the infundibulum and their distinction within the pituitary gland is impossible. They have a similar course in the brain of the dog.

Dorsal supraoptic commissure (Fig. 1, CSOD)

A characteristic system of commissural fibres, which belongs to the dorsal supraoptic commisure, can be seen above the optic chiasm in a frontal section of the dog brain. The fibres of the commissure are about 5 μ in diameter and they stain readily by the Weigert-Wolters method.

Dorsally to the optic chiasm they pass to the other side of the hypothalamus in its ventral part, and then run laterocaudally. The division of this originally homogenous bundle into two components can be seen in further sections. Component I runs off laterally and component II dorsolaterally; both show a deviation to the rear.

Component I (ventral) of the dorsal supraoptic commissure corresponds to the pars ventralis commissurae supraopticae dorsalis described by Gurdjian (1927) in the rat. In the dog this branch reaches the area occupied by the posterior lateral nucleus and, passing through this nucleus, it loses more and more fibres. Next this component gets to the entopeduncular nucleus and the ventral portion of the internal capsule and here it escapes further observation.

Component II (dorsal) of the dorsal supraoptic commissure, which contains nearly two-thirds of its fibres, runs dorsocaudally, and comes to the region of the fornix, where some of its fibres vanish near the dorsal portion of the paraventricular nucleus. The remaining fibres turn laterad and eventually end in the entopeduncular nucleus, the ventral portion of the internal capsule and the zona incerta.

The dorsal supraoptic commissure runs in the hypothalamus of all the mammals examined. A number of papers confirm its presence in the dog brain. Kuhlenbeck (1954) described this commissure in the brain of man, assuming its termination in the globus pallidus and zona incerta.

Zyo et al. (1963) studied the course of the dorsal supraoptic commissure in the brain of the cat by degenerative methods. They described degeneration in the medial longitudinal fascicle running across Forel's area as well as in the lateral hypothalamic nucleus, the subthalamic nucleus and the zona incerta. These authors also traced the terminal parts of the dorsal commissure, leading to the entopeduncular nucleus and the ventral portion of the internal capsule.

These data, except for the connections with the medial longitudinal fascicle, agree with connections of this commissure here reported in the dog brain.

Ventral supraoptic commissure (Fig. 1, CSOV)

This is the second of the two supraoptic commissures. Its fibres have occasionally been described as the commissura Maynerti after the name of its discoverer. In addition, the name of Gudden is also connected with this bundle; he observed the ventral portion of the ventral supraoptic
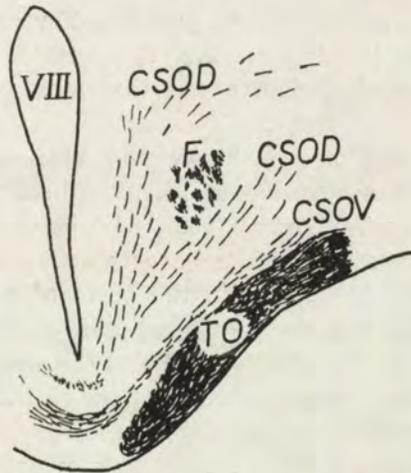
Fig. 1. Distribution of the supraoptic commissures in the dog's hypothalamus. Frontal section, Weigert-Wolters method

commissure after the destruction of the optic tract in the rabbit, and named it the commissura supraoptica ventralis, pars ventralis. In normal material this part cannot be distinguished from the optic tract. His investigations led to the description of the ventral portion of this commissure as a distinct unit, Gudden's commissure. My observations make me reject the distinction of Gudden's commissure and consider it together with the ventral supraoptic commissure.

The ventral supraoptic commissure of the dog deccusates caudally, behind the optic chiasm. Within the hypothalamus the commissural fibres run close to the posterior supraoptic nucleus, to which they send off their branches. It is possible that some of these fibres connect the posterior supraoptic nuclei of both sides, for having passed these nuclei the commissure shows a decreased number of fibres. Next it turns laterocaudad and its further course is hard to trace by anatomical methods, as it fuses with the optic tract, with which it runs on.

In the literature there are many data concerning the investigations of this commissure using degenerative methods. The course of the commissural fibres close to the optic tract hinders these investigations very much. In many cases the opinions of different authors are contradictory.

Studies conducted by Ban (1964) on the rabbit brain showed very extensive connections of the ventral supraoptic commissure, and so for the dorsal portion he describes the termination of its fibres in the diffuse supraoptic nucleus, entopeduncular nucleus, ventral part of the lateral geniculate body, colliculus superior, subthalamic nucleus, reticular nucleus of the thalamus and globus pallidus. For the ventral portion of this commissure Ban (1964) mentions the terminations in the medial geniculate bodies, lateral nucleus of the thalamus, pretectal nucleus and colliculus superior.

Probably the connections of the ventral supraoptic commissure are not, however, so numerous, since other authors give much smaller numbers. According to Wahren's (1959), who does not divide this commissure into parts, it connects the basal nucleus, corpus Luysi, tuber cinereum and pretectal structures in the brain of man. Still fewer connections are given by Diepen (1962), who is of the opinion that this commissure connects only the optic tract and the entopeduncular nucleus.

The present studies show the connection of the ventral supraoptic commissure with the posterior supraoptic nucleus in the dog hypothalamus. I failed to distinguish other connections of this commissure in this structure.

Medial forebrain bundle (Fig. 2, 3, 4)

The medial forebrain bundle (fasciculus medialis prosencephali) in the dog is a tract composed of many components. It reaches the lateral hypothalamic nucleus. These components take origin, or terminate in the many telencephalic, diencephalic and mesencephalic areas.

The posterior lateral hypothalamic nucleus contains all components of the medial forebrain bundle. Its course is well seen, especially in

horizontal sections. The fibres which is lying in front of the area pre-
optica and lateral hypothalamic nucleus have been described as anterior
components and another as the posterior components. Many fibres of the
medial forebrain bundle end in the lateral hypothalamic nucleus.

A. Anterior components

The sequence of description of each tract corresponds with its distribu-
tion in horizontal section (from the medial to the lateral side).

1. Fibres from the lamina terminalis (Fig. 2, LT) go out laterocaudally
and consist only of some scores of fibres. After coming through the
lateral preoptic area the fibres join the medial forebrain bundle. Few of
theme have a commissural character. They reach the medial forebrain
bundle on the opposite side via the lamina terminalis.
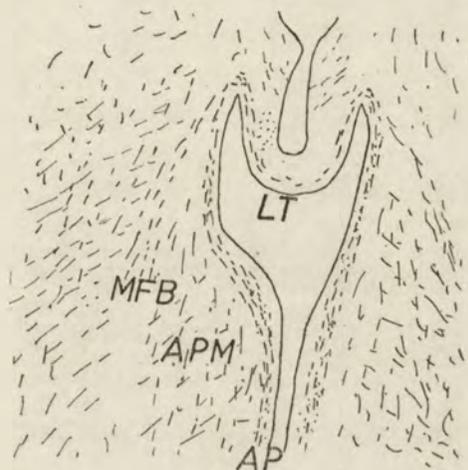
Fig. 2. Fibres from the lamina terminalis. Horizontal section, Weigert-Wolters stain

2. Laterocaudally thin, slightly myelinated fibres also leave the lateral
preoptic area. They enter the anterior lateral hypothalamic nucleus,
laterally to the first tract.

3. The main tract of the medial forebrain bundle inside the posterior
lateral nucleus forms the fibres of the diagonal band of Broca which join
in this nucleus to the remaining tracts of the medial forebrain bundle.
These fibres are well myelinized and clearly visible in the Weigert sec-
tions. They connect the lateral and medial septal nuclei, the nucleus ac-
cumbens and the fornix precommissuralis. Within the dog medial fore-
brain bundle fibres are most abundant in this connections.

4. Fibre tract from the preseptal area. This comes from the gyrus
subproreus (area subprorea II, Kreiner 1966) and gyrus subcallosus (area

subcallosa II, Kreiner 1966). After leaving the cortex these fibres pass in a laterocaudal direction. In the next sections the tract is traced laterally to the diagonal band sinking into the posterior lateral nucleus.

5. Olfacto-hypothalamic fibres are traced from the tuberculum olfactorium (Fig. 3) and the olfactory nucleus. This tract runs caudally and enters the main trunk of the medial forebrain bundle inside the posterior lateral nucleus. It is the most prominent tract in the dog's medial forebrain bundle, beyond the diagonal band. It consists of well myelinated fibres, about 5 μ in diameter. In frontal sections they run ventrally and ventrolaterally to the fornix inside the posterior lateral nucleus.

Fig. 3. Olfacto-hypothalamic tract. Weigert-Wolters stain, horizontal section

6. The next tract leads the fibres from the pyriform cortex the prepyriform cortex, the claustrum and the capsula interna. At first the fibres run medially, then passing through the substantia innominata they turn caudally and sink into the posterior lateral nucleus, mingling with other fibres of the medial forebrain bundle.

7. A tract of fibres from the amygdaloid complex. This connection contains the fibres coming orally from the basal and medial amygdaloid nuclei. They run anteromedially, then, entering the substantia innominata, meet the tract 6, then they both turn caudally and join the main tract of the medial forebrain bundle.

B. Posterior components

Some of the fibres of the medial forebrain bundle scatter inside the lateral hypothalamic nucleus. The rest of them, changing their direction, terminate in the adjacent diencephalic and mesencephalic nuclei. A small number of fibres from the medial forebrain bundle sink frontally into the anterior and posterior supraoptic nuclei. This is only a poor connection.

Fig. 4. Commissural component of the medial forebrain bundle. Weigert-Wolters stain, horizontal section

Caudally the tract of medial forebrain bundle fibres leaves the posterior lateral hypothalamic nucleus. The ventral part of this tract enters the lateral mammillary nucleus and tuberomammillary nucleus (lying more laterally). The fibres of the dorsal part pass by the lateral mammillary nucleus and pass caudally terminating in the substantia nigra, the interpeduncular nucleus, the tegmentum, the red nucleus and the corpus subthalamicus Luysi. A few fibres of the dorsal part of the tract passing above the mammillary bodies, turn medially and along with the fibres of the supramammillary commissure system (Fig. 4) reach the opposite side, then turning orally enter the posterior lateral hypothalamic nucleus.

C. Discussion

The lateral hypothalamic nucleus, where the medial forebrain bundle is traced is sometimes called a "bed nucleus of the medial forebrain bundle" (Valverde 1963). As the result of myeloarchitectonical analysis the lateral hypothalamic nucleus can be divided into two distinct parts: anterior and posterior. This division is confirmed by the fact that the

anterior lateral nucleus comprises only the two anterior components of the medial forebrain bundle, but the rest of them appear inside the posterior lateral nucleus. This proves the distinctness of the two nuclei.

The structure of the medial forebrain bundle is phylogenetically old. The first vertebrates, Petromyzons (Kappers et al. 1965) already have the olfacto-hypothalamic tract (the first component of the medial forebrain bundle). In Ganoidea and Teleostei two components appear: the medial and lateral olfacto-hypothalamic tracts. They are also described by Kappers et al. (1965) as the medial forebrain bundle.

The medial forebrain bundle has many connections. Besides the components mentioned above Zyo et al. (1963) describes the connections of the medial forebrain bundle with the indusium griseum, the striae Lancisii, the anterior hippocampus, the cingulum, the habenula (via stria medullaris) and the thalamus opticus (anterior nuclei) in the rabbit brain.

In the dog the connections of the medial forebrain bundle with the cingulum, the indusium griseum, the anterior hippocampus and the striae Lancisii as well as the connections with the habenula (Zyo et al. 1963) have not been found. This types of connections are possible in the dog brain, but they are invisible in the Weigert-Wolters section. The stria medullaris thalami passes through the anterior lateral nucleus (Śmiałowski 1966) but it does not take part in the medial forebrain bundle system.

There is a similar connection in between the medial forebrain bundle and the stria terminalis described by Diepen (1962). The anterior lateral nucleus is entered by a system of fibres from the nucleus interstitialis of the stria terminalis (Śmiałowski 1966). Inside the nucleus the fibres disperse but they do not form a component of the medial forebrain bundle.

Diepen (1962) distinguishes in the medial forebrain bundle the fibres coming from the fornix. In dog hypothalamus the fornix extends in the dorsomedial region of the posterior lateral nucleus. Along its course fibres directed ventrocaudally leave the fornix but it has not been found whether they form a part of the medial forebrain bundle or not. According to our observations the fibres of the fornix terminate inside the posterior lateral nucleus.

Diepen (1962) and Zyo et al (1963) found that some of the medial forebrain bundle fibres pass to the opposite side of the brain, together with the supramammillary commissure fibres. This system is easily visible in the horizontal sections of the dog brain. These authors also mentioned the connection of the lanticular ansa with the medial forebrain bundle. Fibres from the lenticular ansa in the dog enter the posterior

lateral nucleus from the lateral side, these they disperse and their further course is hard to trace by our methods.

According to Zeman and Innes (1963) and A. Miodoński (1967) part of these fibres from the diagonal band have a connection with the amygdaloid complex. Our observations showed the course of the diagonal band only inside the lateral part of the hypothalamus (where it combined with the rest of the medial forebrain bundle components) and the medial part of the substantia innominata. These observations confirmed the degeneration studies carried out by Zyo et al. (1963). After the lesion in the diagonal band of the rabbit brain, degenerated fibres exist only in the preoptic area in the lateral hypothalamic area.

Klinger and Gloor (1960) reported the connection between the medial forebrain bundle and the amygdaloid complex as passing through the substantia innominata and described it as the ventral amygdaloid projection system. They affirm that in the human brain this tract is bigger than the stria terminalis. This tract is also distinguished (via the substantia innominata) by Diepen (1962), Valverde (1963), Zeman and Innes (1963) and R. Miodoński (1967).

Some authors report a connection of the medial forebrain bundle with the neocortex. De Vito and Smith (1964) found degenerations in the medial forebrain bundle tract after lesion of the prefrontal lobe in *Macacca nemestrina*. These degenerations exist at the level of the lateral preoptic area and lateral hypothalamic nucleus.

Maršala (1963) also found rich connections between the cat's hypothalamus and the cortex of the brain through the channel of the medial forebrain bundle. After the lesion in the medial area of the prefrontal granular cortex the degenerations are located in the septal region, the lateral preoptic area, the lateral hypothalamic area, the rostral part of the ventromedial nucleus and in the region of the posterior hypothalamus. The degenerated fibres do not enter the nuclei of the mammillary complex in the cat.

Wolf and Suttin (1966) reported the connection between the lateral hypothalamic area (via medial forebrain bundle) and the medial part of cingulum. These fibres pass through the medial septal nucleus and curves around the genu of the corpus callosum.

According to the very interesting studies carried out by Okinaka and Kuroiwo (1952) cutting the vagus nerve effects degenerations in the medial forebrain bundle of the dog. The degenerations are located at the level of the posterior lateral nucleus on the operated side.

These connections have been shown by degenerative methods. It should be expected that further degenerative studies will be also show this type of connections in the dog.

Our observations confirmed the connections of the medial forebrain bundle with the preseptal cortex. The rest of these connections have not been examined.

Fibres from the area H2 of Forel and the lenticular ansa

This is a system of fibres connecting the hypothalamus with the cerebral cortex, the capsula interna, the entopeduncular nucleus, the caudate nucleus.

The first component of these fibres is the cortico-hypothalamic tract. This pathway conducts cortical impulses to the hypothalamus. In frontal sections of the dog brain the fibres of this tract can be seen as a bundle which emerges from the ventral portion of the internal capsule. Next they extend in the medial direction and reach, across Forel's area H2, several hypothalamic nuclei (dorsal, ventrolateral, ventromedial and posterior lateral nuclei), which finding has been mentioned in the description of these nuclei (Śmiałowski 1968).

The second component is the lenticular ansa fibres from the entopeduncular nucleus and caudate nucleus. From the entopeduncular nucleus the ansa lenticularis passes dorso-medially and unites with the fibres from the area H2 of Forel in the vicinity of Forel's area H2.

It should also be mentioned that this component receives some fibres of more complex courses. These fibres arise in the caudate nucleus, leave it ventrally and travel through the internal capsule. Then they reach the entopeduncular nucleus, bend medial and having passed again through the internal capsule, mingle with the fibres of the area H2 of Forel and past this tract turns ventrally towards the ventromedial and posterior lateral nuclei.

The course of the cortico-hypothalamic tract has been confirmed by the investigations of Maršala (1963), who observed on his degenerative material after the destruction of the motor, premotor and prefrontal cortex in the cat that degeneration occurred in the basal regions of the internal capsule and mainly in the posterior lateral nucleus. Less intense degeneration was found in the ventromedial and dorsomedial nuclei.

Among the cortico-hypothalamic fibres of man Droogleveer Fortuyn (1953) found another connection i.e. that with the orbital cortex and area 6 in man.

The component of fibres from the caudate nucleus was not mentioned at all in other available papers.

SUMMARY

This paper presents a description of the connections of the intermediate part of the dog's hypothalamus, based mainly on series from the

dog brain stained by the Weigert-Wolters and Klüver-Barrera methods. In the intermediate (tuberal) part of the hypothalamus the following tracts have been distinguished: the hypothalamo-hypophyseal tract, the dorsal supraoptic commissure, the ventral supraoptic commissure, the medial forebrain bundle (fasciculus medialis prosencephali), the fibres from the area H2 of Forel and the lenticular ansa.

ABREVIATIONS

AP,	periventricular area	LT,	lamina terminalis
APM,	medial preoptic area	MC,	mecencephalic component of
CSOD,	dorsal supraoptic commissure		the medial forebrain bundle
CSOV,	ventral supraoptic commissure	MFB,	medial forebrain bundle
CSMD,	dorsal supramammillary com-	TO,	tractus opticus
	missure	TOL,	tuberculum olfactorium
F,	fornix	V III,	third ventricle
INT,	intercalate nucleus		

REFERENCES

BAN, T. 1964. The hypothalamus, especially on its fiber connections and the septo-hypothalamic system. Med. J. Osaka Univ. 15: 1—83.

BLEIER, R. 1961. The hypothalamus of the cat. Johns Hopkins Press, Baltimore, p. 9—34.

DE VITO, J. L. and SMITH, O. A. 1964. Subcortical projections of the prefrontal lobe of the monkey. J. Comp. Neurol. 123: 413—423.

DIEPEN, R. 1962. Handbuch der mikroskopischen Anatomie des Menschen. Der Hypothalamus. Springer Verlag, Berlin.

DROOGLEVEER FORTUYN, J. 1953. Anatomical basis of cortico-subcortical inter-relationships. Proc. III Int. EEG Congr. p. 149—158.

GURDJIAN, E. S. 1927. The diencephalon of the albino rat. J. Comp. Neurol. 43: 1—80.

KAPPERS, C. V. A., HUBER, G. C. and CROSBY, E. C. 1965. The comparative anatomy of the nervous system of vertebrates, including man. Hafner Publ. Co. New York, p. 865—1186.

KLINGER, J. and GLOOR, P. 1960. The connections of the amygdala and the anterior temporal cortex in human brain. J. Comp. Neurol. 115: 333—369.

KREINER, J. 1966. Reconstruction of neocortical lesions within the dog's brain: Instructions. Acta Biol. Exp. 26: 121—143.

KUHLENBECK, H. 1954. The human diencephalon, a summary, development, structure and pathology. Conf. Neurolog., Suppl. 14: 1—200.

MARŠALA, J. 1963. Fronto-hypothalamic connections of the cat's brain (in Čech). Československ. Morf. 11: 266—277.

MIODOŃSKI, A. 1967. Myeloarchitectonics of the septum in the brain of the dog. Acta Biol. Exp. 27: 11—59.

MIODOŃSKI, R. 1967. Myeloarchitectonics and connections of substantia innominata in the dog brain. Acta Biol. Exp. 27: 61—84.

OKINAKA, S. and KUROIWO, Y. 1952. A contribution to the study of histological relationship between the hypothalamus and periferal autonomic nervous system. Fol. Psychiat. Neurol. Jap. 6: 45—57.

RAMON y CAJAL, S. 1894. Algunas contributiones al conocimiento de los ganglios del cerebro. III Hipofisis. Anal. Soc. Esp. Hist. Natural. ser. II.

SCHREIBER, V. 1963. The hypothalamo-hypophysial system. Publ. House Čechoslov. Acad. Sci., Prague.

ŚMIAŁOWSKI, A. 1966. The myeloarchitectonics of the hypothalamus of the dog. I. The anterior nuclei. Acta Biol. Exp. 26: 99—122.

ŚMIAŁOWSKI, A. 1968. The myeloarchitectonics of the hypothalamus of the dog. II. The intermediate (tuberal) nuclei. Acta Biol. Exp. 28: 121—144.

VALVERDE, F. 1963. Amygdaloid projection field. Progr. Brain Res. 3: 20—31.

WAHREN, W. 1959. Anatomy of the hypothalamus. In G. Schaltenbrandt and P. Bailey (ed.), Introduction to stereotaxis with atlas of the human brain. G. Thieme Verl. Stutgart. I: 119—151.

WOLF, G. and SUTTIN, J. 1966. Fiber degeneration after lateral hypothalamic lesions in the rat. J. Comp. Neurol. 127: 137—156.

ZEMAN, W. and INNES, J. R. 1963. Craigie's neuroanatomy of the rat. Acad. Press. New York, p. 115—136.

ZYO, K. OKI, T. and BAN, T. 1963. Experimental studies on the medial forebrain bundle, medial longitudinal fasciculus and supraoptic decussations in the rabbit. Med. J. Osaka Univ. 13: 193—239.

Miscellanea

THE SIXTH GAGRA CONFERENCE 13—25 JANUARY 1969
"ON THE PROBLEM OF MEMORY"

According to a tradition lasting already for two decades every few years a scientific conference is organized in Gagra concerning one of the essential problems of nervous activity. Such meetings are called "Gagra talks" (gagrskiie besedy) and are arranged by a special Committee whose permanent President is Academician I. S. Beritashvili. They take place in a beautiful health resort in Abkhasia on the Black Sea.

Only a limited number of scientists directly concerned with a given field is invited. Each day not more than two or three papers are presented, and extensive discussion follows each paper.

The present conference was devoted to the mechanisms of memory. Altogether 19 papers were delivered which covered the major aspects of this difficult and intriguing problem.

The conference was opened with two leading lectures. I. S. Beritashvili presented "Basic factual and theoretical statements concerning memory" which were developed in his recent book entitled: *Memory, its characteristics and origin in Vertebrates* (Tbilisi 1968). J. Konorski presented a paper: "The problem of memory in its physiological aspects". In this paper the author summarized some ideas which were presented in his recent book *Integrative activity of the brain* (Chicago 1968).

According to the views of I. S. Beritashvili each memory trace begins with throwing into action reverberating circuits of neurons. The action of these circuits, which lasts no longer than some dozens of seconds, produces an increased excitability in synaptic structures due to accumulation of vesicles with the mediator in axon terminals. This second phase of short-term memory lasts many minutes and is responsible for the occurrence of delayed responses.

Further retention of memory traces of images, a retention which has a long-lasting character, is due to protein changes in the postsynaptic regions. "It is conjectured that in these regions a special active protein is formed with participation of ribonucleic acid of ribosomes; this protein acting on the postsynaptic membrane facilitates transmission of excitation to these regions". Finally, during the conditioned-reflex training, when the given response becomes completely automatized and does not require any images for its occurrence, the structural changes take place consisting in diminution of synaptic clefts.

In his paper on the mechanisms of memory J. Konorski brought forth a slightly different classification of memory, distinguishing between perceptual memory, when the subject learns to recognize a certain stimulus-pattern, and associative memory, when he learns to associate two or more stimulus-patterns when they coincide in time. Each of these memories can be either shortlasting or longlasting, depending on whether it is used only for direct short-term utilization, or whether

it broadens the extent of information possessed by a subject. Konorski emphasized that the Beritashvili hypothesis suggesting a second phase in short-term memory (accumulation of the mediator) successfully explains the new discovery of Baldwin and Sołtysik to the effect that full blocking of the blood supply to the brain in goats during the delay period fails to disrupt the short-term memory traces.

An interesting hypothesis concerning the intimate mechanism of long-term memory was proposed by A. I. Roitbak. On the basis of some electrophysiological findings connected with longlasting depolarization of neuroglia, the author suggests that concurrent activation of two anatomically linked centers leads to the myelinization of axon terminals connecting these centers; in this way the transmissibility of these terminals is greatly increased and the conditioned connection is formed.

Another important contribution to the mechanism of consolidation of memory traces was presented by O. S. Vinogradova who has shown with her co-workers that activation of hippocampal neurons by an external stimulus significantly outlasts the action of that stimulus. Beside this the multimodal character of the hippocampal and mammillary units was shown and their great selectivity in habituation was manifested.

Concerning the mechanism of habituation E. N. Sokolov has found that this process is not necessarily transsynaptic, since in molluscs intracellular electric stimulation of neurons leads to habituation.

Two authors — V. S. Rusinov and L. L. Voronin — dealt with the problem of modeling short-term memory traces by applying anodal polarisation to cortical neurons. A tempting hypothesis was proposed to the effect that anodal polarisation accumulates synaptic vesicles at the nerve endings and in this way enhances the transmissibility of synapses by increasing the amount of the mediator.

R. I. Kruglikov was concerned with blocking of consolidation processes by administering ECS or injecting amysil in rats. It is worth mentioning that according to the author's findings amysil administered before the learning session does not affect the course of training, but does affect its consolidation on the following day.

In the papers of E. A. Asratian and B. F. Sergeiev the problems of memory were dealt with on the basis of conditioning experiments. A. L. Mikeladze presented interesting anatomical data concerning the connections of the proreal region in dogs and cats. T. N. Oniani studied the changes in the hippocampal rhythmic activity in various periods of delayed responses and found that this activity increases during the action of preparatory stimulus, decreases during the delay period and again increases in the moment of release. T. G. Urmantcheieva studied in monkeys the effects of stimulation or ablations of the hippocampus on the conditioned reflexes, whereas N. A. Tushmalova explored the effects of drugs on this structure. E. A. Kostandov spoke on some mechanisms of memory in man and D. G. Smirnov in his interesting review discussed the structural plasticity of the brain in relation to memory. O. A. Krylov gave evidence on the role of macromolecules in the formation of conditioned reflexes.

It may be seen from this short summary that a number of important problems in the field of memory were raised in the papers delivered at the conference, and all of them were subjected to a thorough and manysided discussion. Both the papers delivered at the conference and discussion will be published in a special volume.

J. Konorski

CONTENTS