Methods. The experiments were performed in chronic cats. In 1st operation, the animals have electrodes implanted in the lateral rectus muscle of the eye and in the 6th nucleus of the pons. Cortical and neck electrodes complete the polygraphic study of the sleep-wakefulness cycle. PS episodes were monitored in an 8-channel EEG grass model 7A Polygraph. In a 2nd operation, the animals had either a total ablation of the cerebellum or an ablation of the frontal lobes by removing all the brain tissue in front of a plane passing through the bregma. After these two types of operation, PS episodes were monitored in the same way as in Control experiments.

Results and discussion. After cerebellectomy, the most striking result is the increase in amplitude of PGO waves (Figure 1). This increase in amplitude lasts all the time in all the animals studied (up to 2 weeks after cerebellectomy). It appears in isolated as well as in the PGO discharging in bursts and it affects the positive as well as the negative part of the PGO waves. It is noteworthy that not all of the PGO in the paradoxical sleep episode increase their amplitude. On the contrary, the most striking effect produced by frontal lobe lesions is the change in the pattern of discharges of the PGO, without a noticeable change in their amplitude (Figure 2). The change in pattern of the PGO is very marked in the first 2–3 days following the operation. After 5–6 days there is a new change in the pattern of PGO. It is modified in the sense of a great complexification of the PGO discharges related to control experiments and the bursts of PGO contain a greater number of waves.

It appears from our results that the cerebellum controls mainly the amplitude of PGO waves and that the frontal lobes act upon the pattern of PGO discharge. Recently the cerebellum has been proposed as being a very important relay relation in the control of rapid eye movements[6]. On the other hand, the output of the cerebellum has mainly an inhibitory character[5]. It was already proposed that the Purkinje cell discharges bore some relationship to eye movements[7]. The increase in amplitude of PGO waves clearly demonstrated here indicates that the cerebellum has an inhibitory action upon the phasic events of paradoxical sleep. This effect could be exerted through a cerebellum-locus coeruleus pathway recently discorvered[8], and the influence that this latter structure has upon paradoxical sleep[9] is well known. On the other hand, this inhibitory cerebellar influence could be exerted through anatomically demonstrated direct connections from the cerebellum with oculomotor neurons[10] or through the connections with reticular formation structures[11]. That the frontal lobes have an important influence upon the oculomotor apparatus is well known[4]. This action could also be exerted through their connections with the reticular formation[11]. Our results confirm the findings of other authors[12] that show an increase in the density of oculomotor activity of paradoxical sleep episodes and other oculomotor alterations[13] after frontal lobe lesions. Moreover we demonstrated that the frontal lobes control the pattern of PGO discharge. These early changes could be more easily explained through the connections of the frontal lobes with the reticular formation and the oculomotor neurons[4,11] than through retrograde degeneration of the pathway that project from the locus coeruleus to the frontal lobes[14]. Therefore it is concluded from our studies that the oculomotor neurons of the brain stem and/or the reticular formation surrounding them are, during paradoxical sleep, under the influence of several structures. During this phase of sleep-waking cycle, one of them, the cerebellum, controls mainly the amplitude of phasic PGO activity, while the other one (frontal lobes) controls mainly the pattern of their sequential discharges. There is conclusive anatomical data to support our functional findings.

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Weak Neuronal Accumulation of Octopamine in Dopaminergic Neurons of the Rabbit Retina

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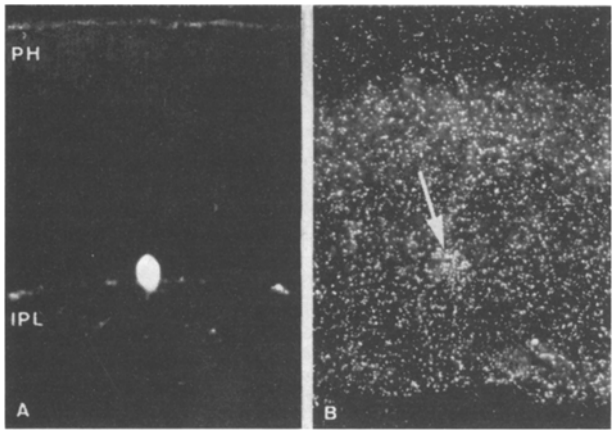
Summary. Dopaminergic retinal neurons become weakly radioactive after the injection of tritiated octopamine into the vitreous, but no other neurons do so. This indicates the absence of any effective mechanism for the accumulation of octopamine in rabbit retina.

Releasable octopamine has been detected in high amounts in crustacean peripheral nerves[1,2], and the amounts present seemed to correlate with the number of perikarya present in nerve. The substance was suggested to have a modulating action on muscle contractions. It is also present in high amounts in certain other invertebrate neurons[3–5]. In insects, an adenylate cyclase system was described, which was more sensitive to octopamine than dopamine or 5-hydroxytryptamine[6]. In mammals, octopamine is accumulated by sympathetic nerves, from which it can be released by nerve stimulation, and it has been suggested as a kind of neurotransmitter[7,8]. It is also present in the rat brain, although in much lower concentrations than, for instance, the catecholamines[8,9].

Catecholamines are well known as CNS transmitters, and in the retina dopamine is the dominating one (see EHINGER[10]). The dopaminergic neurons as well as the ones presumably operating with glycine or GABA have an efficient mechanism for accumulating their transmitter and it seems that this ability is of importance for terminating the action of released transmitter. Octopamine is structurally related to dopamine, and it was therefore of interest to test to what extent it would be selectively accumulated by retinal neurons.

25 μCi ^{3}H-octopamine was injected intravitreally into albino rabbit eyes, which were then excized after 4 h and freeze-dried, fixed in formaldehyde vapor according to the FALCK and HILLARP method, embedded directly in epoxy resin (Durcupan, Fluka) in vacuo, sectioned, photographed in the fluorescence microscope and covered with autoradiographic stripping film (Kodak AR 10). Exposure times were 1 to 3 months.



Rabbit retina, 4 h after the injection of 25 μCi ^{3}H-octopamine intravitreally. A) fluorescence micrograph showing one dopamine-containing, strongly fluorescent cell body and several fluorescent terminals throughout the inner plexiform layer (IPL). The photoreceptors (PH) at the top are faintly autofluorescent, and the pigment cells above them are somewhat more autofluorescent. B) is a dark field micrograph of the autoradiogram of the same area. There is a diffuse distribution of silver grains all over the retina, with a slight increase in radioactivity in the dopamine-containing cell (arrow). × 340.

Fluorescence microscopy demonstrated the by now well-known dopaminergic junctional cells and the 3 sublayers of dopaminergic terminals in the inner plexiform layer (Figure A). The autoradiography showed mainly diffuse distribution of the radioactivity, but on close inspection it was observed that there was a slight increase in dopaminergic neurons (Figure B). The radioactivity of these cells was far less than that seen after the injection of tritiated catecholamines under comparable circumstances.

The experiments show that octopamine or a metabolite is only weakly accumulated by dopaminergic neurons and not at all in the recently detected indoleamine-accumulating retinal neurons[11]. They further show the absence in the rabbit retina of a selective, efficient neuronal accumulation of octopamine, but they do not show whether this is so because there are no neurons operating with octopamine, or because such hypothetical neurons have no efficient uptake and storage mechanism for their transmitter.

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Age-Dependent Increase of Thermal Stability of in situ Chromatin of Rat Liver and its Reversal after Hepatectomy

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Summary. An age-dependent increase of thermal stability of DNA in situ has been demonstrated in rat liver by means of microfluorimetry, which was reversed to a great extent in old regenerated liver.

Cellular ageing involves a progressive loss of the ability of the cell to maintain the homeostasis[2], which may be due to an alteration in the DNA-protein association determining the structure and physiological activity of chromatin. Numerous biochemical studies[3–7] showed an age-dependent increase of thermal stability of the extracted chromatin. It has also been revealed that the method of extraction, as well as the ionic strength of the medium used, have a strong influence on the results obtained. In order to avoid the problems of chromatin extraction[8], thermal denaturation experiments were performed on chromatin in situ of young and old rat hepatocytes. On the other hand, in order to reveal whether the age-dependent changes of thermal stability of chromatin are reversible, similar experiments were carried out also in regenerated liver of old animals. Some preliminary data of these experiments have been presented elsewhere[9].

Female Wistar rats of our own breed were used: A) 3 rats of 2 months of age (young group); B) 3 rats of 28 months of age (old group); C) 3 rats of 25 months of age

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