ments and finger flexion (Figure 1, B). The heart beat, evidenced by the EKG, was regular and with a frequency ot 42/sec.

B) During slow sleep. The EEG showed slow waves and spindles similar to those described in other mammals (Figure 2, C and E). The EMG activity of the posterior limb muscles varied greatly according to the environmental temperature. When it was below 28 °C, an intensive tremor of many muscles of the body was observed. This tremor was particularly noticeable in the limbs. This was the reason why, at temperature below 28 °C, a high degree of EMG activity of the flexor muscles was seen (Figure 2, D). When the environmental temperature was above 28 °C, the tremor disappeared completely and there was complete absence of EMG activity, in the flexor digitorum (Figure 2, F). The EMG activity of the neck muscles did not show significant variations in relation to the temperature.

C) During REM sleep. The EEG showed an activity very similar to that observed during wakefulness (Figure 3, G). The EMG of the flexor digitorum did not show different patterns, in relation to the environmental temperature. Both above and below 28 °C, there was complete absence of EMG activity (Figure 3, H and J). The EMG activity of the neck muscles was similar to that of slow sleep. No tremor was observed during this phase. Only the classic muscular twitches already described in mammals were seen.

Discussion. From the above mentioned results, we can conclude that the EMG activity of the flexor muscles of the hind limbs is extremely useful, below 28 °C, for the diagnosis or REM sleep, since the disappearance of the EMG activity is an unequivocal sign. The EMG activity or the neck muscles is obviously not useful. It is very probable that the exploration of other muscular groups will also reveal equally useful signs. All the above results, including the critical temperature of 28 °C for the disappearance of the tremor, are comparable to those found by AFFANNI et al.[1] in the small edentate *Chaetophractus villosus*.

Résumé. Les auteurs mettent en évidence l'existence d'un sommeil R.E.M chez le *Priodontes giganteus*, le plus grand Tatou actuellement vivant. A une température ambiante inférieure à 28 °C, on observe un tremblement intense au cours du sommeil lent, qui disparaît complètement lors du sommeil R.E.M.

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Induction of Ingestive Responses by cAMP Applied into the Rat Hypothalamus

It is very likely adenosine-3′, 5′monophosphate (cAMP) is an important factor in nervous system function[1]. Recently it has been reported that dibutyryl-cAMP (Db-cAMP) injected at high doses into some cerebral areas of several animal species[2,3] may induce overt behavioral effects. In acute experiments[2] Db-cAMP produced on rats motor hyperactivity, catatony and convulsions, whilst in chronic experiments[3] hyperphagia, hyperthermia and prolongation of the oestral cycle.

This note reports the results obtained with stereotaxic microapplications of Db-cAMP into some areas of rat diencephalon, where Acetylcholine and Nor-epinephrine are known to modify feeding behaviour[4–9].

Materials and methods. A double-cannula was implanted stereotactically into the brain of 23 male Wistar rats (230–260 g of body weight) by the method described by GROSSMAN[8]. The sites implanted (Figure 1) were the lateral hypothalamic area (LHA), the ventromedial hypothalamus (VMH) and the reticular formation (RF), following the stereotaxic coordinates of DE GROOT's atlas[10].

After implantation, the rats were housed individually in a temperature, humidity and lighting controlled room, and had food and water ad libitum. One week later, when the rats had regained their preoperative weight, the inner cannula was removed, washed in ethanol, dried and replaced without any substance added into it. The rats were then returned to their cages and after 1 h (control period) the amounts of food and water consumed were measured.

This procedure was repeated about 1 h later. However, at this time, the cannula was replaced after tapping into the tip 5–10 µg of one of the following substances: Db-cAMP, cAMP, Adenosine-5′-monophosphate (AMP), Adenosine triphosphate (ATP), Carbamoycholine (Carb), N-isopropyl-nor-epinephrine (NIE), Na-butyrate and NaCl[11]. Food and water consumed during the next 1 h (test period) were again measured.

The above procedure was repeated on 5 consecutive days and followed by 2 days rest until the end of the experiments. In order to check the cannulae placement (Figure 1) all rats were killed and their brain perfused and fixed in 10% formalin solution. After freezing, 25 µm sections were cut and stained with Luxol blue and Cresyl violet[12].

Results. Stereotactical application of Db-cAMP into diencephalon of satiated rats significantly increased

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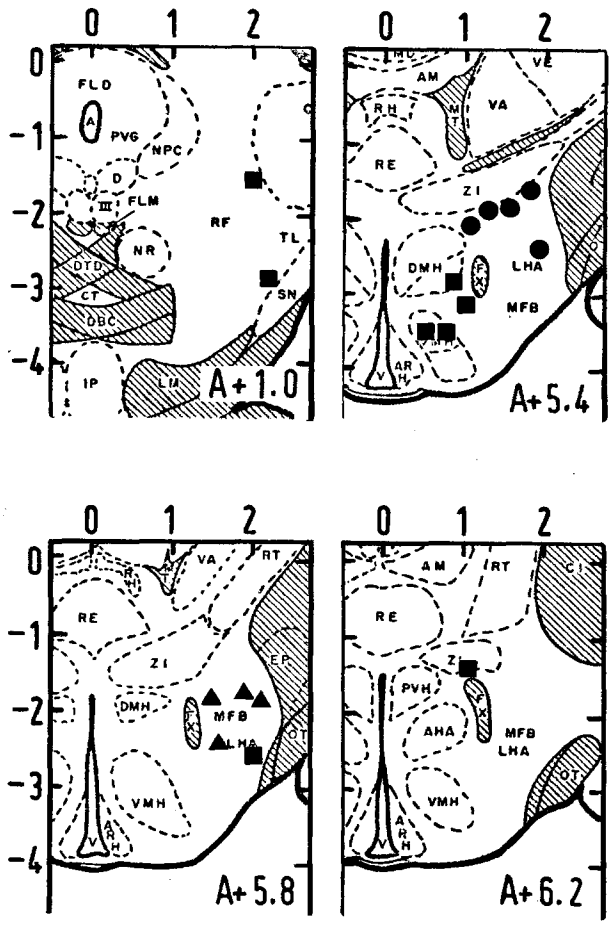

Fig. 1. Anatomical maps of: ●, very effective; ▲, moderately effective; and ■, ineffective sites of rat diencephalon where Db-cAMP elicited drinking. Each symbol represents 1 rat. For details see text and [8].

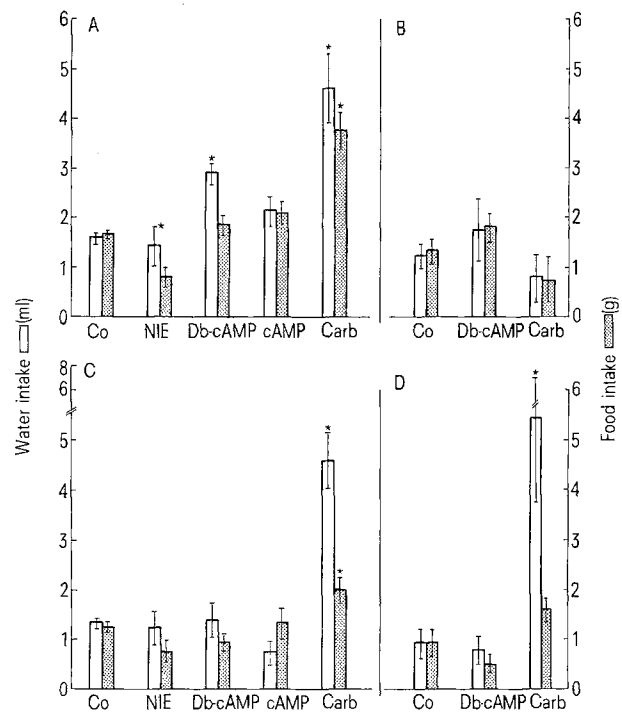

Fig. 2. Food and water intake determined during 1-h test period following stereotaxic application of some substances (NIE: N-isopropyl-nor-epinephrine; Db-cAMP; Dibutyryl-adenosine-3′, 5′-monophosphate; cAMP: Adenosine-3′, 5′-monophosphate; Carb: Carbamoylcholine) into rat diencephalon. Co is referred to food and water intake determined during 1-h control period previously the substance application. Means ± SE of 10–30 observations. (*) $P < 0.001$ (Student's t-test) as compared to control period. A) Lateral hypothalamic area, LHA: (A, + 5.4; H, − 2.0; L, ± 1.5). B) Reticular formation, RF: (A, + 1.8; H, − 2.0; L, ± 2.0). C) Lateral hypothalamic area, LHA: (A, + 5.8; H, − 2.5; L, ± 1.7). D) Ventromedial hypothalamus, VMH: (A, + 5.4; H, − 3.5; L, ± 0.5).

(+80%) water intake (Figure 2, A) only when Db-cAMP was applied in a restricted area of LHA in or just below the 'zona incerta' (ZI, Figure 1). On the contrary application of cAMP into LHA was practically ineffective (Figure 2, A and C).

Application of Carb into all the sites of LHA tested produced polydipsia and hyperphagia, the former more marked than the latter (Figure 2, A and C). When applied into the VMH, however, Carb increased only thirst (Figure 2, D). It had no effect when placed into the RF (Figure 2, B).

Applied into the same sites as Db-cAMP and Carb, NIE did not elicite any increase of food and water consumed (Figure 2, A and C); indeed it inhibited food intake particularly in LHA (Figure 2, A). Application of AMP, ATP, NaCl and Na-butyrate (this latter used in order to check some possible effect of butyric radical which is presumed [13] to be quickly discharged by Db-cAMP) never produced any significant modification of feeding and drinking responses.

Discussion. Experiments here reported show cAMP to be active only as dibutyryl derivate. Since Db-cAMP is more resistant to phosphodiesterase action and probably it can easily move across the cell membrane [14], it is quite possible that it could act inside the nervous cell. Since NaCl, Na-butyrate, ATP and AMP had no action, the molecular structure of Db-cAMP appears specific in eliciting the observed effect. Results obtained show no analogy between Db-cAMP and NIE, since in the sites where the former was active, the latter had no effect. A lack of action by Nor-epinephrine applied near the 'zona incerta' was previously reported by BOOTH [15], while other authors obtained different results [6, 8].

Qualitatively the increase of water intake produced by Db-cAMP was similar to that of Carb (Figure 2, A). Nevertheless Db-cAMP effect appeared later (10–15 min) and lasted longer (1 h) than that of Carb (1–5 min and 20–30 min, respectively). The finding that the sites where Carb was active did not strictly correspond to those affected by Db-cAMP, is under discussion. We suggest that it may depend either on a better diffusion pattern of Carb or on a tissue concentration of Db-cAMP insufficient to produce a significant response.

Results reported seem apparently in keeping with BRECKENRIDGE's suggestion [16, 17] that cAMP can act on the complex Acetylcholine storage or release mechanism occurring at the synaptic clefts. Although this effect has not been directly proved [18], it is strongly supported by the

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finding that Db-cAMP increases miniature end-plate potentials[19,20].

Studies are now in progress to define whether the Db-cAMP action we found could be eventually mediated by Acetylcholine.

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Riassunto. L'applicazione stereotassica di microdosi di dibutirril-adenosin-3′5′ monofosfato nell'area ipotalamica laterale ‹zona incerta› di ratti sazi causa un netto aumento nell'ingestione di acqua. L'aumento è molto simile a quello causato dalla carbamoilcolina posta nella stessa zona.

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On the Intraganglionic Neurohaemal Organs in the Ventral Nerve Cord of *Hydrophilus olivaceus* (Hydrophilidae. Coleoptera)

The existence of neurosecretory cells in the ganglia of the ventral nerve chain of insects is well-known[1]. Many recent investigators have presented cytological evidence to establish the neurosecretory character of the cells in the ventral ganglia[2–5]. These cells were associated with varied important physiological functions such as diapause[6–8], pigment migration[9], oogenesis[10,11] and production of anti-diuretic hormone[12]. The neurosecretory cells, which exhibit cyclical activity, remain relatively constant in their number and topographical situation[1,4].

In the Coleoptera, segmental neurohaemal organs in association with the ventral ganglionic chain have been described by some authors[13–15]. In our histological and histochemical investigations on the ventral nerve cord of the aquatic beetle *Hydrophilus*, certain neurosecretory patches have been found in the various ganglia of the ventral chain. The object of the present report is to give a brief description of these segmental intraganglionic patches and to examine their possible role as neuro-haemal organs.

The ventral nerve cord in this insect shows very little concentration of its constituent ganglia. All the ganglia are separate, excepting the first abdominal which is fused with the metathoracic and the last abdominal which is, as usual, a composite structure resulting from fusion of the posterior 4 or 5 ganglia.

The neurosecretory cells of the ventral ganglionic chain are distinguishable into 2 main types, namely the A and B types, on the basis of their staining properties. The A cells stain purple with paraldehyde-fuchsin (AF), violet with chrome haematoxylin-phloxin (CHP) and bluish-green with alcian blue-phloxin (ABP) techniques. The A type cells are further divisible into A_1 and A_2 types, according to the differences in their volume, number and topographical disposition. The A_1 cells are distinctly larger than the A_2 cells and 2 of them occur mediodorsally only in the suboesophageal ganglion. In all the other ganglia, only the A_2 cells are represented in varied numbers (Figure 1). The B type cells are distinctly phloxinophilic, both in the CHP and ABP techniques, while they are only poorly stainable with AF. They are also represented in all the ganglia in variable numbers. These cells are pear-shaped, often highly vacuolated and have long thick axons which are also strongly phloxinophilic. Both A_1 and A_2 cells contain large amounts of cystine, as revealed by performic acid-alcian blue technique. With Heidenhain's azan, however, all the cell types are stainable, though the B cells stain with lesser intensity than the A type. In all the ganglia the A_2 and B types of cells are located peripherally, surrounding the central neuropile that is composed of several fibre tracts (Figure 2).

Whole preparations, as well as sections of all the ganglia stained with AF, reveal the presence of a variable number of irregularly-shaped patch-like areas within the ganglia

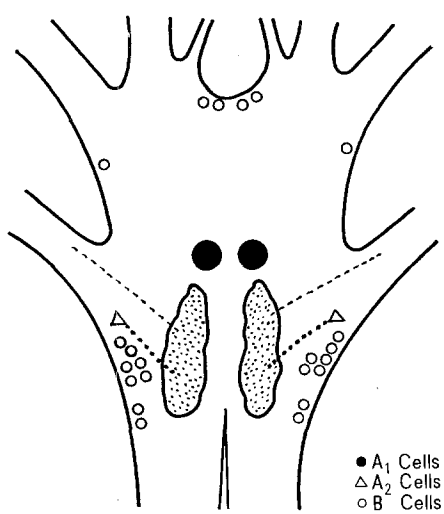

Fig. 1. Diagrammatic representation of the suboesophageal ganglion showing the disposition of the various cell types. Note that the axons of the 'A_2' cells (dotted line) lead into the irregularly-shaped AF-positive patches and some nerve fibres arising from these patches (broken line) join the fibre tracts of the lateral nerves.

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