

## EEG and behavioral effects of the biogenic amines in the chick

Substance	Dose mg/kg	Injection route	EEG pattern	Behavior	Remarks
5-Hydroxytryptophan	50–280	i.p.	Sleep	Sleep-like	Latency to onset (25–50 min)
5-Hydroxytryptamine	0.1–20	s.c.	Sleep	Sleep-like	Rapid onset (2 min)
Dihydroxyphenylalanine	50–300	i.p.	Alert	Alert but akinetic	Latency to onset (10–20 min)
Dihydroxyphenylalanine + Iproniazid*	50–100	i.p.	Alert	Alert but akinetic	Latency to onset (20–50 min). Duration 3–4 × DOPA alone
Dihydroxyphenylalanine + Iproniazid*	200–400	i.p.	Alert followed by sleep	Alert followed by sleep-like	Latency to onset (15–20 min). Sleep-like state 30 min after onset of alert EEG
Dopamine	75–100	i.p.	Alert followed by sleep	Alert followed by sleep-like	Rapid onset of arousal followed by sleep-like state after 30 min
Norepinephrine	0.05–4.0	s.c.	Sleep	Sleep-like	Rapid onset (2 min)

\* Iproniazid PO<sub>4</sub>, 100 mg/kg i.p., 15 h pre-experiment.

the CNS<sup>9</sup>. The present and more direct evidence outlined in this study does not support this concept, but rather indicates that chlorpromazine exerts CNS effects by mimicking or potentiating the action of NE and perhaps other biogenic amines. This is substantiated in part by the close resemblance of the chick response to chlorpromazine and the NE response, or the late effects observed after DA.

The action of reserpine has been proposed to be mediated by the blockade of monamine storage sites<sup>10</sup>. The present results suggest that the reserpine depression may be caused by newly synthesized and unbound NE or 5-HT overflowing on receptor sites, since systemic injection of NE or 5-HT alone, or in combination, produces a state of depression that is similar to the reserpine induced state.

In summary, this report presents pharmacological evidence that: (1) 5-HT, DA and NE appear to enter the CNS of the young chick; (2) 5-HT and NE produce EEG and behavioral patterns of sleep; (3) DA or its precursor DOPA produce an initial akinesia, plus EEG and behavioral signs of arousal. These direct data are discussed in terms of a re-evaluation of several mechanisms of action of the psychotropic drugs whose actions are believed to be mediated via an interaction with the biogenic amines<sup>11</sup>.

*Zusammenfassung.* Pharmakologische Untersuchungen im Hinblick auf eine eventuelle Wechselwirkung von psychotropen Pharmaka und biogenen Aminen ergaben: (1) offenkundiges Eindringen von 5-HT, DA und NE in das Zentralnervensystem junger Hühner. (2) Schlafcharakteristisches Verhalten von EEG und Verhaltensweise nach Verabreichung von 5-HT und NE. (3) Auftreten der «arousal»-Charakteristika mit anfänglicher Akinese im EEG und Verhalten nach Dopamin- oder Dopazufuhr.

C. E. SPOONER and W. D. WINTERS

*Brain Research Institute and Department of Pharmacology, University of California School of Medicine, Los Angeles (California USA), January 4, 1965.*

<sup>9</sup> B. B. BRODIE, F. SULSER, and E. COSTA, in *Extrapyramidal System and Neuroleptics* (Ed.: J. BRODELEAU, Editions Psychiatriques, Montreal 1961), p. 183.

<sup>10</sup> J. M. ROBSON and R. S. STACEY, *Recent Advances in Pharmacology* (Little, Brown and Co., Boston 1962), p. 49.

<sup>11</sup> Supported by U.S.P.H.S. Grant No. 5 TI MH-6415.

## On the Presence of Mucopolysaccharide Material in the Labyrinthine Epithelium of Chick Embryo in the First Moments of Morphogenesis

The morphogenesis of the cupula ampullaris and of the otolithic membrane is still a somewhat obscure problem in the development of the membranous labyrinth<sup>1–5</sup>. Already during the course of previous studies, we have had occasion to classify the mucopolysaccharides that enter into the formation of these structures from the histo-

chemical point of view. Our investigations<sup>6</sup> brought to light the non-uniform nature of the mucopolysaccharides

<sup>1</sup> W. KOLMER, Arch. Ohrenheilk. 116, 10 (1926).

<sup>2</sup> K. WITTMACK, Arch. Ohr-, Nas- u. Kehlk.-Heilk. 114, 278 (1926).

<sup>3</sup> K. WITTMACK, Acta oto-laryng. 24, 424 (1937).

<sup>4</sup> B. FARKAS, Acta oto-laryng. 24, 53 (1936).

<sup>5</sup> T. VILSTRUP, Ann. Otol. Rhinol. Lar. 59, 19 (1950).

<sup>6</sup> M. DE VINCENTIIS, F. MARMO, and G. MATERAZZI, Riv. Istochim. norm. pat., in press.

of the membranous labyrinth of the chick embryo, noting the presence of sulphated mucopolysaccharides at the level of the cupula, and acid and not sulphated ones at the level of the tectorial and otolithic membranes.

In this note we report the results of our cytochemical research on the inner ear bud of the chick embryo with special reference to the earliest stages of morphogenesis.

The observations were made on chick embryos from the second to the 13th day of incubation. The embryos were fixed in Helly, Gendre, Carnoy and Bouin fluids, in formalin and in acetone. The various tests for the identification of glucide substances (PAS, Alcian-PAS<sup>7</sup>, chromotropic research with toluidine blue 1:5000 at various pH) were carried out, as well as extraction with acetone and with pyridine in hot medium for the lipids.

The data obtained from our observations can be summed up as follows:

(A) In embryos of the 5th, 6th and 7th day of development (the moment when the morphogenesis of the cristae with the cupulae begins and that of the maculae with the otolithic membrane), a characteristic localization of PAS-positive material could be noted in the cytoplasm of some cells of the bud of the crista ampullaris. This material, localized at the level of some cell groups situa-

ted in the central part of the bud, appears in the form of granules of a considerable size in coincidence with the cells of the basal part of the crista, while in the more superficial one (towards the lumen of the ampullar bud) it is seen as small granules. It is not chromotropic to toluidine blue or alcianophile, and is resistant to digestion with saliva (on material fixed in Gendre) and to extraction with acetone or pyridine under heat.

These findings make one think that it is a material formed of neutral mucopolysaccharides.

(B) As development progresses, that is to say on the 7th and 8th day of incubation (the period when the morphogenesis of the structures in question is almost complete), a decrease of the intracellular PAS positive material described above can be noted, a decrease which is even more accentuated with the further development. Around the 13th day of incubation, only small and scattered PAS-positive granules can be observed in the cytoplasm of support cells of the sensory epithelium of the crista.

A similar picture to that observed in the ampullar bud is also found in the buds of the otolithic maculae at approximately the same period of development. This picture, however, is less evident in the latter, the cells of which only show fine and scattered granules of PAS-positive material.

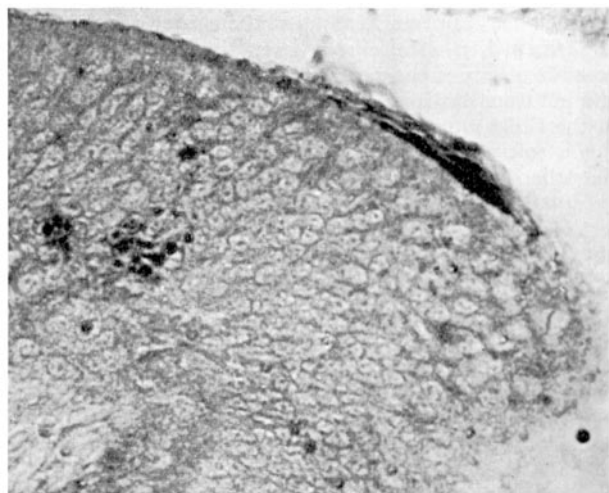
We wish to report the new finding of the appearance of cells with a neutral mucopolysaccharide content, in the epithelium of the buds in question, immediately preceding and concomitant with the early period of the morphogenesis of the cupula and the otolithic membrane. This finding might justify one in advancing the hypothesis that this material could present the precursor of the acid mucopolysaccharides that characterize the cupula ampullaris and the otolithic membrane.

**Riassunto.** Viene segnalata la presenza di materiale mucopolisaccaridico neutro in alcune cellule dell'epitelio del labirinto membranoso dell'embrione di pollo durante i primi periodi dello sviluppo. Viene avanzata l'ipotesi che tale materiale possa essere connesso con la genesi delle cupole ampollari e delle membrane otolitiche.

M. DE VINCENTIIS and F. MARMO

*Istituto di Biologica Generale e Genetica della Università di Napoli e Cattedra di Istologia ed Embriologia dell'Università di Camerino (Italy), November 25, 1964.*

<sup>7</sup> R. W. MOWRY, J. Histochem. Cytochem. 4, 407 (1956).



Crista ampullaris of chick embryo around the 6th day of incubation. PAS reaction ( $\times 404$ ). Note the presence of the PAS positive cupula and granules in some of the cells of the central part of the bud of the crista.

### Localization of Non-Specific Phosphatases in the Testes of *Meriones hurrianae* Jerdon, the Indian Desert Gerbil, and *Suncus murinus sindensis* Anderson, the House Shrew

*Meriones hurrianae*, inhabitant of the Rajasthan desert, and *Suncus murinus sindensis*, the common house shrew, were used to determine the localization and distribution of acid and alkaline phosphatases in the testes. The animals were killed by rapid exsanguination and the

testes quickly removed and chilled at 4°C before fixing in previously cooled absolute acetone for 24 h or 85% cold alcohol, and embedded in paraffin after rapid dehydration. Sections were incubated at 37°C using sodium  $\beta$ -glycerophosphate as the substrate. GOMORI's<sup>1</sup> metal precipitate techniques were used to demonstrate these

<sup>1</sup> G. W. GOMORI, *Microscopic Histochemistry: Principles and Practice* (University of Chicago Press, 1952).