Radioautographic identification of serotonergic neurones in *Aplysia*

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Summary. Radioautography shows that ^{3}H 5-HT specifically labels metacerebral and 2 other serotonergic cell bodies in *Aplysia* cerebroid ganglia as well as 5-HT terminals in close contact with giant neurone membrane in buccal ganglia.

Metacerebral serotonergic neurones of *Aplysia* have been thoroughly studied using electrophysiological, biochemical and pharmacological techniques[2-6]. However, owing to the difficulties of the cytochemical approach, little morphological information is available about these neurones and other possibly serotonergic cells whose existence is suggested by biochemical studies[7]. Reaping profit from the specific reuptake capacity of serotonin (5 HT) neurones for their neurotransmitter[8,9], we tried to detect and identify these cells in *Aplysia punctata* ganglia. Our results demonstrate that a radioautographic study of 5 HT neurones in such invertebrate models is possible[10]. Using this approach, metacerebral cells could be recognized together with another pair of 5 HT sequestering cells in the cerebroid ganglia, while labelled fibres were seen in close contact with buccal neurons. Control experiments strongly suggested that labelled neurones are serotonergic.

Materials and methods. After 30 min incubation in sea water containing 5.10^{-7} M tritiated serotonin (^{3}H-5 HT Amersham, 9 Ci/mM), dissected ganglia were fixed by glutaraldehyde and either embedded in paraffin, or post-fixed in osmium tetroxide and embedded in epon. Thick epon or paraffin sections were coated with Ilford K5 and thin sections with L4 nuclear emulsion by dipping. They were developed in D 19 B or microdol X Kodak[11].

Results and discussion. Histological preparations displayed 3 types of radioautographic reactions. A diffuse and non-selective scattering of silver grains was found up to the centre of the ganglia without any peculiar predilection for given structures. By contrast, in the cerebroid ganglia, preferential accumulations were observed upon cell bodies which corresponded to metacerebral C_1 cells, and to 2 smaller elongated more posterior and central cells which will be called N_2. Finally, dense clusters of silver grains were observed in both ganglia outside the cells, which were sparse in the cerebroïd ganglia and more numerous in the buccal ganglia where they surrounded non-labelled cells.

At the electron microscope level, the labelling of bilateral C_1 and N_2 cells was ubiquiteous upon cytoplasm and to a lesser extent upon the nucleus. Some silver grains overlaid mitochondria, but 100 nm dense core vesicles which were scarce in the cytoplasm (although more numerous in the Golgi area) did not display any preferential labelling. Some dense aggregations of silver grains were occasionally found upon the cytoplasm of glial cells. Labelled fibres in the cerebroid, as well as in the buccal ganglia, displayed silver grains upon their whole length with distinct accumulations at the level of varicosities. They contained neurotubules, mitochondria, and scarce 100 nm dense core vesicles, which were more numerous in dilated parts together with 40–70 nm clear vesicles. In buccal ganglia labelled fibres invaginated in the cytoplasm of perikarya were in close contact with the neuronal membrane. In these axonal sections dense core vesicles and aggregates of clear ones were not particularly labelled and no distinct differentiation could be detected on either of both adjacent membranes.

Specificity tests were performed by adding 2.10^{-6} M fluoxetine (specific uptake inhibitor of 5 HT by serotonergic neurones) to the 5.10^{-7} M ^{3}H-5 HT incubation medium, or

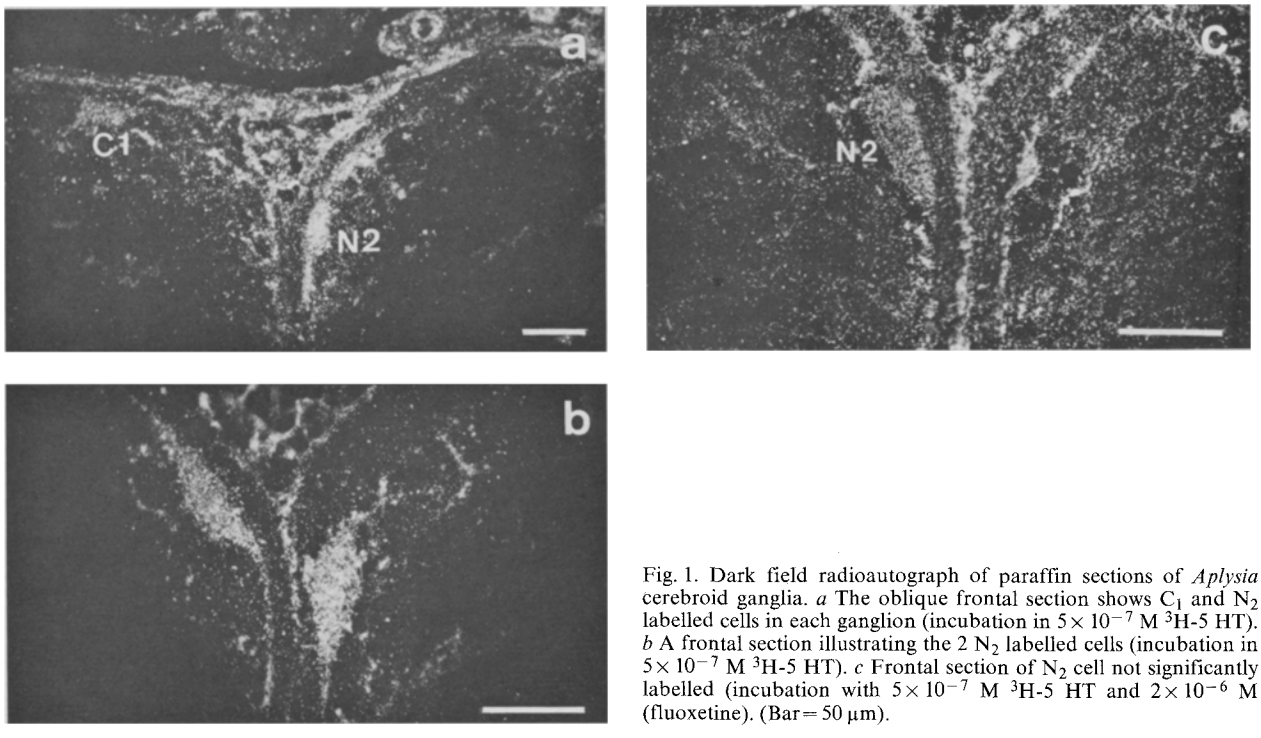

Fig. 1. Dark field radioautograph of paraffin sections of *Aplysia* cerebroid ganglia. *a* The oblique frontal section shows C_1 and N_2 labelled cells in each ganglion (incubation in 5×10^{-7} M ^{3}H-5 HT). *b* A frontal section illustrating the 2 N_2 labelled cells (incubation in 5×10^{-7} M ^{3}H-5 HT). *c* Frontal section of N_2 cell not significantly labelled (incubation with 5×10^{-7} M ^{3}H-5 HT and 2×10^{-6} M (fluoxetine). (Bar = 50 µm).

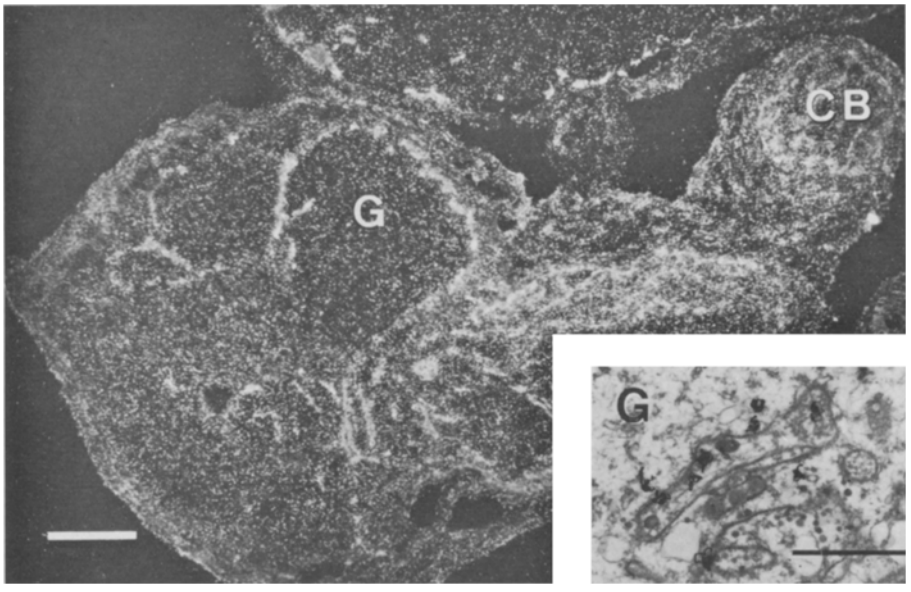

Fig. 2. Dark field radioautograph of paraffin section of *Aplysia punctata* buccal ganglia incubated in 5×10^{-7} M ^{3}H-5 HT. Intensely labelled fibres from cerebrobuccal connective (CB) in contact with a large neurone (G). Inset: Electron microscope radioautograph of a labelled fibre invaginated in the cytoplasm of G. (Bar = 50 µm); (Inset Bar = 2 µm).

by incubation in 5.10^{-7} M ^{3}H-NA. In both cases, the diffuse reaction persisted but preferential localizations on cell bodies and fibres disappeared. Thus these preferential reactions result from a selective uptake process, not excluding a possible labelling of 5-HT receptors. However, in a vertebrate model administration of ^{3}H-LSD results in a diffuse labelling[12].

Similar incubation experiments recently demonstrated that radioactivity observed in the ganglia may correspond to ^{3}H-5 HT and/or to 2 of its derivatives (sugar conjugates)[13]. Concerning the identification of labelled structures, the serotonergic nature of C_1 cells has been well established by electrophysiological[14] and biochemical methods[5], but N_2 cells had not yet been described as serotonergic. However, identical results of test control experiments for both types of cells, together with the previously demonstrated existence in other models of a specific in vitro uptake of 5 HT at low concentration, strongly suggest that labelled neurones are truly serotonergic[15]. Thus labelled 5 HT terminals described in buccal ganglia may originate from either of these cells. The contacts between 5 HT terminals and buccal neurones could provide the morphological basis for the transmitter action of 5 HT at this level.

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Cerebellar decussation of fibres from the nucleus reticularis tegmenti pontis in the brain of the albino rat[1]

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Summary. The existence of a cerebellar decussation of fibres from the medial portion of each nucleus reticularis tegmenti pontis (Rtp) of the albino rat is indicated. Definite cell loss in the medial aspect of the most rostral third of Rtp is detectable after cerebellar hemisection involving parts or the entire depth of sublobule VIb. Cell loss in the medial aspect of the caudal half of Rtp is evident as a consequence of experimental lesions which damage both sublobules IIb and III.

The nucleus reticularis tegmenti pontis (Rtp) of the mammalian brain is part of a group of nuclei within the brainstem reticular formation which is considered to project exclusively to the cerebellum[3]. The Rtp nucleus in particular is a relay station for impulses en route to the cerebellum from numerous regions including both the cerebral cortex and the spinal cord[3].

The distribution of projection fibres from Rtp to the cerebellum has been described as both contralateral and ipsilateral[4]. The effect of unilaterally sectioning the middle