Nerves and taste-buds degeneration in the cat-fish *Ictalurus melas*

J.-C. Desgranges

Université P. et M. Curie, UER de Zoologie et Laboratoire de Microscopie Electronique, 105 Bvd Raspail, F-75006 Paris (France), 9 January 1978

Summary. After nerve transection, external taste-buds remain apparently normal for more than 3 weeks, become round, then disappear. Cryonecrosis of the cranial ganglia V and VII induces a loss of polarity of the buds, which, though keeping up their form for 50 and even 150 days, are progressively deprived of sensory cells. This shows a dissociation of the actions of nerve factors.

According to previous works, denervation of barbels leads rapidly to degeneration and disappearance of external taste-buds in cat-fish[1-3]. Classically, degeneration takes place between 11 and 13 days after nerve section, at 20 °C. Nevertheless, some published results do not agree with the former ones: the denervated buds underwent a constriction of their neck and the cells shrunk without bud disappearance (at least till the 24th day, at 16 °C)[4]. Such a dedifferentiation included a loss of contact with the surface.

In order to determine the relations between buds and nerve cells, the following experiments were performed: 1. Section of the mandibular branch of the facial nerve. At 20 °C, spherical buds were quite visible in mental barbs 14 and 22 days after nerve section. This confirms the previous results[4]. Such a long persistence of the buds may be related to the greater length of the nerve fibres in contact with the bud cells[3]. The trophic action of nerve seems to be proportional to its tissue mass. The persistence is longer than was expected.

2. Section of the roots of the Vth and VIIth nerves. As shown by optic microscopy, sensory innervation alone is able to maintain taste-buds in cat-fish[5]. Effectively, the

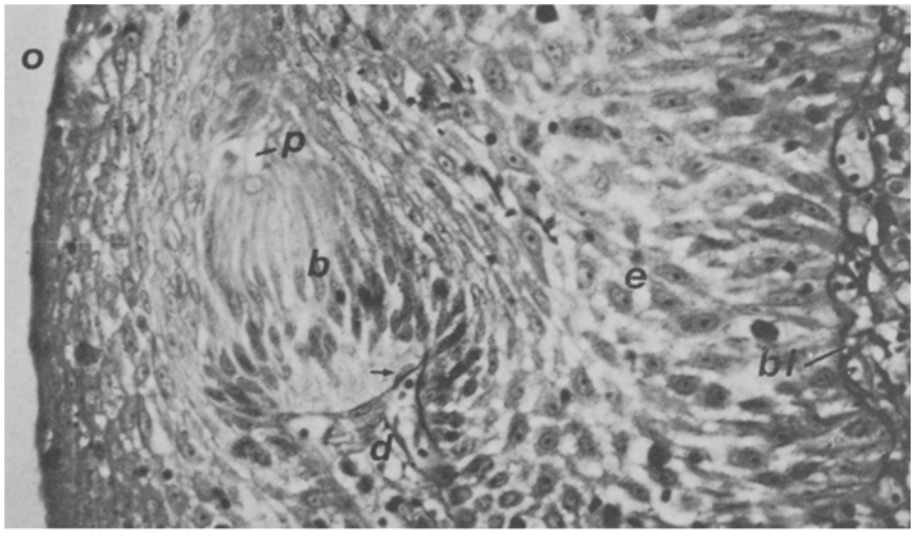

Fig. 1. Transverse section of a mental barb of cat-fish (×450). Result of Vth and VIIth ganglia ipsilateral freezing plus nerve-roots section 50 days later: loss of contact between the taste-pit and the outside, loss of functional polarity of the taste-bud, which becomes parallel to the basal lamina. b, taste-bud; bl, basal lamina; d, dermal papilla; e, epidermis; o, outside; p, taste-pit; arrow: basal cell.

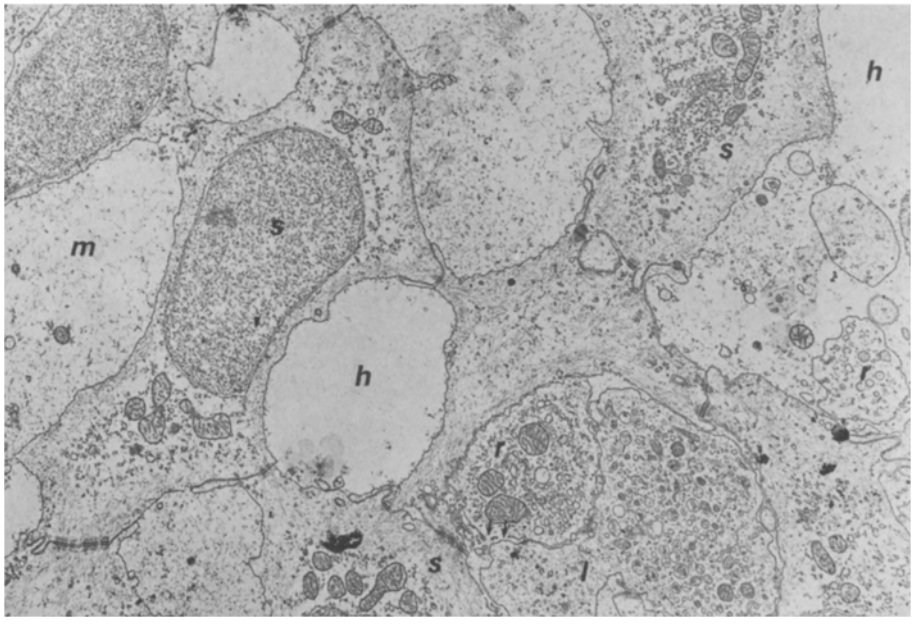

Fig. 2. Detail of an external taste-bud undergoing degeneration (transverse section, ×200). Same experimental conditions as in figure 1: most of the sensory (gustatory) cells have disappeared, the sustentacular cells enduring a slower degeneration; h, hole pointing out the place of a disappeared sensory cell; l, leucocyte; m, extracellular material filling a hole; r, degenerating remnants of sensory cells; s, sustentacular cell; arrows: openings of the plasmic membrane of a degenerating sensory cell.

buds were quite normal 14, 28 and 42 days after section. Electron microscopy revealed a consecutive disappearance of type II junctions (with postsynaptical cisternae), assigned to efferent innervation[6].

3. Freezing of the Vth and VIIth cranial ganglia. Cryonecrosis was performed by consecutively repeated applications of an ophthalmological cryode, frozen by liquid nitrogen (−180 °C, 3–8 times, 30 sec each). As was pointed out on rat sciatic nerve, local freezing of axon blocked conduction for 10 days but maintained untouched the basal lamina during degenerative phase, thus allowing a perfect reinnervation[7,8].

In the present experiment, such a restauration of efferent innervation, passing along the ganglia, was expected. The bodies of the sensory neurons underwent chromatolysis, but were still present 50 days after freezing. The buds were normal at 14 and 28 days. After 50 days, numerous buds were spherical and had no more taste pore, others were elongated, some of these being oblique or parallel to the surface of the epithelium, or even aiming towards the basal lamina.

4. Freezing of the Vth and VIIth ganglia plus section of the nerve roots. After this double intervention, the buds seemed quite normal for 28 days. As with single freezing, all buds were spherical or parallel to the surface after 50 days (figure 1). Basal cells were present. Some buds did not possess any dermal papilla. Examination with electron microscope revealed large areas of the buds where holes pointed out the site of disappeared sensory cells between the sustentacular cells. These holes, ultimately filled with light extracellular material, might confine cellular remnants and leucocytes involved in phagocytosis. The form of the sustentacular cells was well maintained, whereas the cytoplasmic feature was poorly preserved: vesicles and cisternae became rarefied, whilst mitochondriae and fibrillae remained (figure 2). However, some sectors of the buds seemed quite normal: the sensory cells even showed a dense, highly osmiophilic, perinuclear formation, located between endoplasmic reticulum profiles. This formation, not yet described, is well developed in springtime and normally undergoes seasonal changes. The difference in degenerative response may proceed by partial denervation, some neurons having escaped from freeze injury. Such a quantitative action of a partial denervation has already been shown on rat taste buds[9]. Besides, chromatolyzed neurons may possess, a trophic influence, as in the rat[10].

Conclusions. The persistence of the taste-buds after denervation continues longer than previously thought. The form of the bud is preserved for a time by the sustentacular cells, after gustatory cells degeneration. The longer persistence of sustentacular cells after the freezing experiments suggests a trophic influence by chromatolyzed neurons. The disappearance of most gustatory cells and the loss of polarity of the buds after freezing of the ganglia reveal a dysjunction of 2 factors of nervous origin, active on the 2 cellular types, or at least a differential sensitivity to a diminished flow.

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Effet du fractionnement de dose sur la survie de bourgeons axillaires et de cellules isolées de liseron irradiés aux rayons gamma

Split-dose recovery after gamma-irradiation of in vitro cultivated nodes and single cells of bindweed

J. Durand[1]

Biologie Cellulaire 5, Bâtiment 478, Université Paris-Sud, F-91405 Orsay (France), 7 décembre 1977

Summary. Split-dose recovery has been shown on bindweed (*Calystegia* = *Convolvulus sepium* [L.] R.Br.) nodes and isolated single cells in aseptic culture. Recovery from sublethal damage occurs within 2 h.

Depuis les premiers travaux de Elkind[2] sur cellules de mammifères, le fractionnement de dose s'est révélé d'un grand intérêt pour l'étude de la réaction des cellules vivantes aux irradiations. Le plus souvent, il n'y a pas additivité des effets de doses successives. C'est ce qui a été montré dans ce laboratoire sur des bourgeons de plusieurs plantes du genre *Nicotiana*[3,4]. Pour évaluer la généralité du phénomène, nous avons choisi une espèce très différente, *Calystegia* (= *Convolvulus*) *sepium*, le liseron commun, qui offre la possibilité de cultiver des cellules de mésophylle isolées[5] et donc de vérifier si la non-additivité se manifeste aussi au niveau cellulaire.

Matériel et méthodes. Un stock de tiges de liseron a été constitué par bouturage in vitro sur le milieu suivant: sels minéraux de Skoog[6], vitamines de Nitsch[7] sans glutamine, saccharose 20 g/l. acide indolyl butyrique 1 mg/l (communiqué par L. Rossini). Les bourgeons sont prélevés sur des tiges âgées d'un mois, débarrassés de la feuille dont il ne reste qu'un fragment de pétiole. Tous les bourgeons débourrés sont éliminés. On irradie 50 bourgeons par point. Ils sont ensuite repiqués sur le même milieu en boîtes de Pétri. Après un mois de culture à 24 °C et éclairage de 2000 lx, la fréquence de survie est évaluée: le critère en est l'apparition d'une petite tige à partir du bourgeon.

Culture de cellules isolées. Les feuilles de Liseron proviennent de plantes cultivées au Phytotron de Gif-sur-Yvette. Les cellules du parenchyme foliaire sont préparées et cultivées selon la technique de L. Rossini[5]. Après l'irradiation, les cellules sont rincées et la densité cellulaire est ajustée à 20000 cellules/ml pour tous les points. Au 7e jour de culture, le nombre de cellules vivantes et divisées plus d'une fois (micro-colonies d'au moins 3 cellules) est compté à l'hématimètre. Le rapport de ce nombre au nombre de cellules vivantes à la mise en culture définit la fréquence de survie. Chaque point est établi sur 3 boîtes, en faisant 1 prélèvement par boîte et en comptant de 200 à 800 cellules par prélèvement.

Irradiation. L'irradiation est effectuée avec la gamma-cell au ^{60}Co de l'Institut du Radium d'Orsay (débit: 1650 rad/min). Les 50 bourgeons correspondant à un point sont placés dans des petites boîtes de Pétri (⌀ 5 cm). Les cellules en suspension très dense sont en erlens stériles