Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy

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Summary
This report describes the retrograde degeneration affecting olfactory sensory neurons of rats after severance of their axons and illustrates the reconstitution of new neurons originating from stem cells located at the base of the olfactory neuroepithelium.

Degeneration of the mature, axotomized neurons, signalled by an increased electron density of their cytoplasmic matrix and by the appearance of lipofuscin-like granules, can be detected in the neuroepithelium as early as 24 h after surgery and becomes conspicuous between the second and the third day. Degenerating neurons can be observed in decreasing number up to the tenth post-operative day. They are removed by macrophages which invade the epithelium. The reconstitution of new neurons begins to occur after eight days, when the stem cells undergo vigorous mitotic activity and differentiate into neurons. The morphology of the reconstituted neurons has been described in detail at different stages of their maturation. After 30 days, the olfactory epithelium appears similar to controls. On the basis of both morphological (in rats) and autoradiographic (in mice) observations, the basal cells have been recognized as stem cells of the olfactory neurons.

Introduction
Retrograde degeneration of the olfactory neurons following axotomy and/or removal of the olfactory bulb has been studied in a number of animal species by many authors, and the interpretation of the long-term effects of the experimental procedure has been controversial. Degeneration not followed by regeneration was reported in amphibians (Hoffmann, 1867; Exner, 1877; Nakamura, 1916; Takagi and Yajima, 1964, 1965) and mammals (Hoffmann, 1867; Baginski, 1894; Lustig, 1924; Takata, 1929; Sen Gupta, 1967). On the other hand, Nagahara (1940) described reconstitution of neurons in mice after axotomy. Partial degeneration of
the neural elements after bulbectomy was reported by Clark (1951, 1957). Recently Takagi (1971) has provided a comprehensive review of this subject.

Observations in our laboratory have shown that in frogs, pigeons and mice, severance of the olfactory axons results in complete degeneration of all the existing mature neurons, followed by reconstitution of new neural elements from stem cells which persist in the neuroepithelium of adult animals (Graziadei, 1973; Graziadei and DeHan, 1973; Oley et al., 1975; Harding et al., 1977; Graziadei and Monti Graziadei, 1978a, b; Graziadei and Okano, 1979).

In a previous report (Graziadei and Monti Graziadei, 1979) we have illustrated the morphological characteristics of stages in the maturation of olfactory sensory neurons in normal mice and rats. It is the purpose of this paper to describe in detail the retrograde degeneration affecting the perikarya after axotomy and the reconstitution of new neurons from stem cells located at the base of the neuroepithelium.

The term reconstitution will be used throughout the paper to define the regeneration of the entire olfactory neuron. We propose this term to avoid confusion with the term regeneration which is used to indicate the regrowth of the axon from the proximal stump after transection (Guth, 1975; Kerr, 1975).

The olfactory neuroepithelium of rats varies in thickness from 60-90 μm, with an average value of 75 μm. The supporting cells, whose nucleus is contained in the bulk of their cytoplasm, occupy the distal third of the epithelium and extend laminar processes among the neuron cell bodies to reach the basal lamina. The neural elements are located in the lower two-thirds of the epithelium; their arrangement has been described in detail in a previous report (Graziadei and Monti Graziadei, 1979). We need to stress here only that the neuroepithelium is a mosaic of areas, transitory in time, in which the mature and immature neural elements are found in different proportions. We have previously described these areas as quiescent and active. In the former, most elements are mature and functional, extending their axons to the olfactory bulb; consequently they are affected by axotomy. In the active zones, however, there is a preponderance of stem and immature cells which are not affected by axotomy because their growing axons do not yet extend to the lamina cribrosa. In our description we will refer primarily to the time sequence of events as they occur in the quiescent zones and we will briefly discuss at the end the events occurring in the active zones.

Materials and methods

Adult Sprague–Dawley male rats, varying in weight from 450–550 g and aged from 6–12 months, were used. Unilateral section of the fila olfactoria was performed intracranially at the level of the lamina cribrosa, after intra-peritoneal injection of Nembutal. The surgical procedure was identical to the one described for mice in a previous report (Harding et al., 1977). Control of the lesion was made in ten animals chosen at random and sacrificed immediately after surgery. Histological sections of the entire decalcified skull demonstrated that between 85% and 95% of the fibres were cut. The remaining animals, in groups of six, were sacrificed at different intervals of time after axotomy, from 15 h up to 60 days. No changes, however, were observed after 33 days. For this