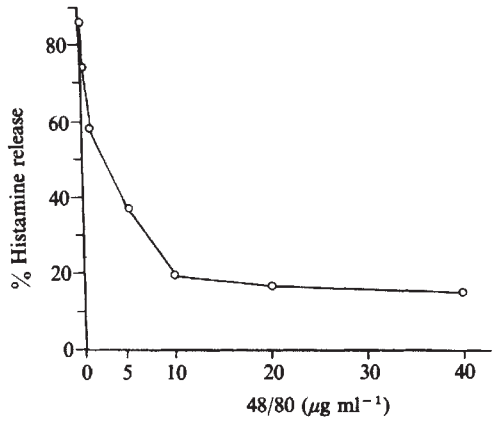

Fig. 2 Same culture as Fig. 1, but plates were incubated further for 3 d with the medium containing the various amounts of 48/80. These plates were then rechallenged with 15 μg 48/80 in 1 ml of medium. They were incubated for 20 min before processed for histamine assay.

determined immediately after plating in fresh medium and at different times thereafter. Results are shown in Fig. 3. At time zero histamine was not released, as compared to 10% in the control cultures; but the degranulation capacity gradually increased during the first 24 h, coinciding with the replenishment of the cellular histamine content until 48–72 h, when the response to 48/80 completely recovered. The study shows that without trypsinisation, restoration of the degranulation capacity was limited but virtually complete after such treatment. We presume that the refractoriness is induced by 48/80 which is bound to the cells during regeneration and detaches after trypsinisation.

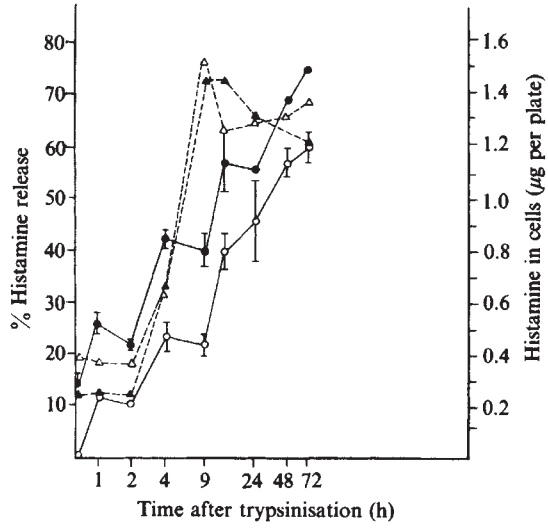

Fig. 3 Restoration of the degranulation capacity after passaging by trypsinisation and plating in 48/80-free medium. Culture was established by plating 10^7 lymph node cells prepared from 4-month-old BALB/c mice 14 d after last injection with horse serum. Culture was passaged 8 d later to make three new plates from each old plate. Seven days later, 40 μg 48/80 in 2 ml Dulbecco's medium were added to half the plates while the other half received fresh medium only. 65% of the histamine was released. The cultures were incubated further for 3 d. Then the medium was removed and trypsin solution (0.3% in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline) was added. After 20 min the cells were collected, spun down, resuspended in Dulbecco's medium and horse serum and plated in 35-mm Petri dishes to make 3.5 new plates from each old plate. At different intervals, the medium was removed and 1 ml medium containing 15 μg 48/80 was added. After 20 min the plates were processed for assay of histamine. % Histamine release: ○——○ 3 d with 48/80; ●——● 3 d without 48/80. Histamine in cells (μg): ▲----▲ 3 d with 48/80; △----△ 3 d without 48/80.

This work has revealed hitherto unknown properties of the mast cell. The new information is important in view of its role in allergy. It is clear that in the continuous presence of the histamine releaser 48/80, the cell is reversibly desensitised, an effect which possibly comes under the heading of tachyphylaxis—development of tolerance to second exposure to a drug or an agonist[6,7]. Obviously, it is still to be seen whether there is a similar response to reaginic antibodies and the corresponding allergens. Unfortunately, attempts to maintain mast-cell cultures in the continuous presence of mouse anti-horse serum plus horse serum as antigen, in dilutions optimal for total degranulation proved unsuccessful. This was probably because of the stimulation of a high metabolic rate with effects on the medium *p*H. A similar effect was obtained when such antibody–antigen complexes were added to fibroblast and macrophage cultures. This observation indicates recourse to classes and subclasses of purified immunoglobulin; thus, application of pure IgE, IgG_1, IgG_{2a} and IgG_{2b} to the different cells grown in our cultures would make it possible to assign the *in vitro* manifestation to its cause. This is important, as while both IgE and IgG_1 degranulate mast cells, they neither fix complement nor opsonise macrophages; by contrast, IgG_{2a} fixes complement and attaches to the Fc receptor on the macrophage[8].

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Orderly compression of the retinotectal projection following partial tectal ablation in the newborn hamster

MANY hypotheses have been proposed to account for the development of the exquisitely specific systems of neuronal connections in the brain. Axons might read chemical labels on their target cells, attaching only to cells with a matching label[1]; axon and target cell populations might arrange a match without cell-to-cell specificity, using such mechanisms as differential advantage in competition for terminal space[2,3]; or some feature of the development and maturation of an axon and target system might produce ordered patterns of connectivity. The principal model system used to test these hypotheses has been the retinotectal projection of animals with nervous systems which are capable of regeneration, although it is not known whether this system in fact behaves like a developing nervous system. After removal of half the tectum in goldfish or frog, for example, an entire retina will compress its representation uniformly onto the remaining half tectum[4,5]. We have repeated this experiment in the developing mammalian brain, and have found that following a partial tectal ablation in a neonatal hamster, the projection from the retina to the superior colliculus will terminate in an

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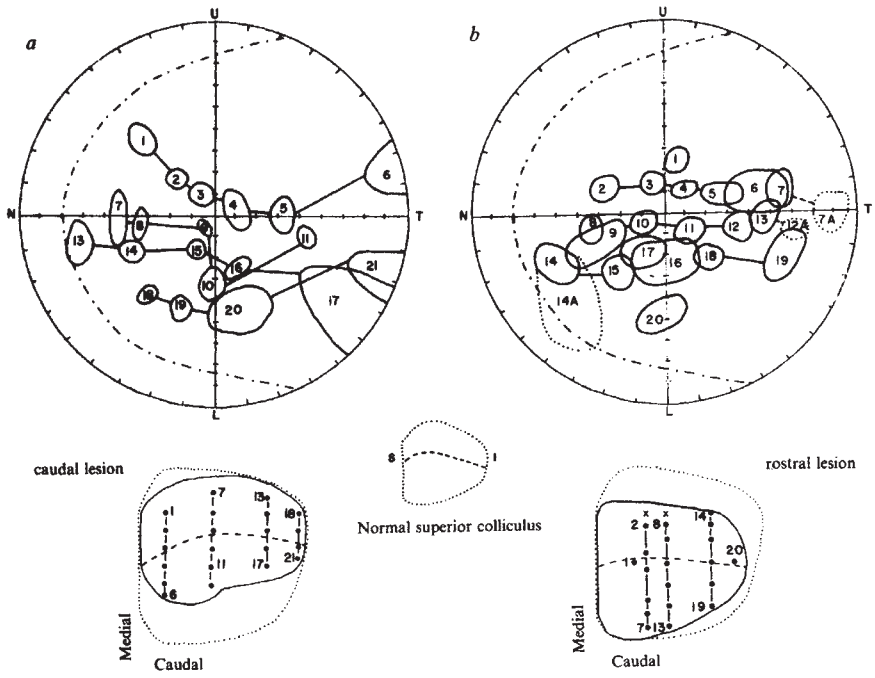

Fig. 1 Colliculi with reconstructed electrode penetrations and associated visual fields from a hamster with a neonatal lesion of the right caudal colliculus (*a*) and of the right rostral colliculus (*b*). The colliculi are shown in dorsal view; solid outlines indicate the remaining superficial gray layer in each hamster and dotted lines the normal extent of the superior colliculus. Electrode penetrations associated with receptive fields with defined borders are represented by black dots, and their corresponding numbered visual receptive fields are shown in the visual field diagram above. For visual convenience rostral to caudal series of penetrations and corresponding nasal-to-temporal visual receptive field are connected with lines. Medial colliculus, where upper visual field is represented, was not explored because of occlusion by the vein of Galen. The two penetrations marked by crosses were associated with diffuse receptive fields in the upper nasal quadrant including, but not limited to, the receptive field with the appropriate topographical position. The map of the visual field is centred about the projection of the optic disc. The broken line (– · – · –) indicates the perimeter of the visual field in normal hamsters. The nasal (N) to temporal (T) axis is determined by the projections of the attachments of the medial and lateral rectus muscles, and is not the same as the horizontal plane defined by gravity. The orthogonal upper (U) to lower (L) axis is defined by the insertions of the superior and inferior rectus muscles. This superior–inferior axis, which divides the visual field into nasal and temporal halves, is shown projected on the surface of a normal superior colliculus (centre inset) and both damaged colliculi as a dashed line (– – –). Dotted receptive fields indicate fields found deep to the surface of the superior colliculus.

orderly way in a smaller than normal tectal volume, as in goldfish and frog[1,4]. However, we have found preferential representation of nasal visual field which has not been reported in studies of non-mammalian animals in which the optic tract axons can regenerate. This may suggest some fundamental differences in developing and regenerating systems; in any case, it poses new problems for theories of neuronal specificity in developmental neurobiology.

On the day of birth, the brain of the hamster is quite immature. The first fibres from the retina have grown to the superior colliculus, entering the structure at its rostral border[6]. Although some axonal end arbors may already have begun to form near the tectal surface, major arborisation seems to begin around day 3, with the caudal, medial and lateral margins lagging behind rostral and central colliculus. An adult-like pattern and density is found by the age of 12–14 d. Partial lesions made in the colliculus on the day of birth thus intercept the initial stage of tectal innervation, and major reorganisations of retinal projection patterns do occur[7–9].

The superior colliculus is a prominent structure in the newborn hamster, and its superficial layers may be destroyed directly through the thin cranium by local application of heat[7–9]. In 15 newborn hamsters partial lesions of the colliculus were inflicted on the day of birth by this method. After surgery, the animals were returned to the mother and allowed to mature normally. When these animals had reached the age of at least 2 months, the topography of the retinotectal projection was assessed electrophysiologically[10,11], and the extent of the neonatal lesion was determined thereafter by conventional histological reconstruction[7–11]. In the 15 animals studied, the surface area of the remaining intact superior colliculus ranged from 40% to 80% normal, and tissue loss was confined principally to the superficial gray layer, as determined from sectioned material.

The receptive fields associated with a series of electrode penetrations of the surface of the superior colliculus in a hamster with a neonatal lesion of the caudal part of the superficial layer are shown in Fig. 1*a*. The entire visual field was represented at the tectal surface. Multiunit receptive field sizes reflected this compression of the visual field onto a smaller tectum. The average receptive field size in a normal hamster is $8.6 \pm 0.3°$ (s.e. mean); in animals with caudal lesions average field size was $12.4 \pm 0.4°$. The topography was generally orderly, although localised mislocations or inversions were observed in this animal, as in every other. One striking anomaly was present in this and similarly prepared cases: the representation of the nasal visual field on the collicular surface was proportionately much greater than the representation of the temporal visual field (compare the positions of the superior–inferior meridian in the normal with the caudal lesion case in Fig. 1). The rostral-to-caudal extent of the nasal 70° of visual field was close to normal (75% normal length, as measured along the naso–temporal meridian). For the temporal 90° of visual field, the representation along the same meridian was only 48% of normal. This disparity in the rostro-caudal extent allotted to the nasal as opposed to the temporal visual field was present in all eight animals with caudal lesions. In three of the animals, in addition to the general compression, there was loss of 10–30° of visual field in the temporal periphery.

Lesions of rostral colliculus were made in a further seven newborn hamsters. The consequences of this lesion for axons entering the colliculus are somewhat different than the caudal lesion: axons entering the tectum must traverse an area of damaged tissue before innervating the remaining superficial gray layer. The mechanical disruption caused by this procedure may have contributed to a pattern of disorder in retinotopy observed in regions on the rostral border of the remaining superficial gray in four of the seven cases, an anomaly which has also been observed neuroanatomically[7–9]. Receptive fields of units at the rostral border were large, encompassing whole quadrants or hemifields and were topographically disorderly, both in relation to neighbouring penetrations and throughout the depth of any particular penetration.

The compressed representation of the visual field observed after day-of-birth lesions of the rostral tectum was considerably more like normal in representation of nasal and temporal visual field than was observed in cases with lesions of caudal tectum. Nevertheless, when asymmetries occurred (three of the seven cases exceeded a 5% difference in representation of nasal visual field versus temporal visual field when compared to normal), nasal visual field was again preferentially represented. In Fig. 1*b*, the rostrocaudal extent of colliculus devoted to the remaining nasal field was 95% of normal; the extent devoted to temporal visual field was 70% of normal. The preferential representation

was never so extreme as that observed after caudal lesions. In no case, however, was there marked compression of the representation of nasal field relative to temporal field, even though the normal tectal terminal area for axons of retinal cells representing nasal visual field had been removed at birth.

An additional deviation from the normal representation of the retina in the tectum was observed in 12 of the 15 animals studied: extreme temporal visual field was represented both at the tectal surface and also deep to the tectal surface, lying directly ventral to receptive fields representing more nasal areas. In some cases, extreme temporal field could be found represented only deep below the tectal surface (Fig. 1*b*, dotted fields). This corresponds to an anatomical pattern observed previously[7–9]; in adult hamsters which had suffered caudal tectal lesions at birth, lesions of the nasal retina elicited axon terminal degeneration in deep superficial gray and in the upper part of intermediate gray in the caudal part of the remaining colliculus. These observations indicate an orderly topographic representation of the retina perpendicular to the surface of the superior colliculus which is never found in normal animals[10,11]. Factors such as inappropriate angles of electrode penetration and reorganisation of collicular lamination, investigated thoroughly, could not account for these results[7–10].

We do not know if the nonlinear compression of the visual field in the hamsters represents the stable end point of a process of compression, or a fixation of the map by maturation before a stable end point has been reached. In either case, the priority of nasal field representation in these compressed maps is an interesting clue to the mechanisms of map formation. The two most likely sources of this priority are features of development: the direction of retinal fibre entrance to the tectum from the rostrolateral quadrant where nasal field is represented, and the sequence of maturation of retinal ganglion cells in hamster retina. We are presently investigating these two possibilities.

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A rich VIP nerve supply is characteristic of sphincters

VASOACTIVE INTESTINAL PEPTIDE (VIP), isolated from extracts of porcine duodenum by Said and Mutt[1], was at first thought to be a hormone[2]. Immunohistochemical studies have since revealed that VIP has a neuronal localisation. VIP-containing nerves occur throughout the body, being particularly

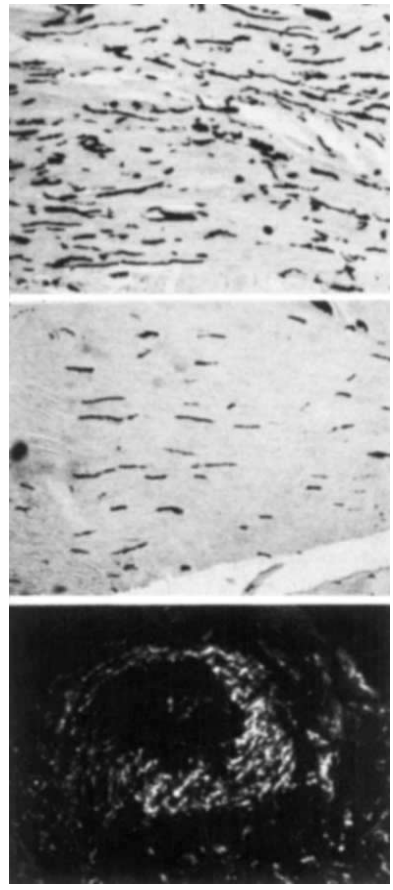

Fig. 1 VIP-immunoreactive nerves (PAP staining) in smooth muscle of the feline pyloric sphincter (top) and duodenum (middle). Note the aggregation of coarse nerves in the sphincter region compared with the much lower number of immunoreactive nerves in the duodenum (×250). Bottom, VIP nerves (immunofluorescence) around the opening of the pancreatic duct in the papilla of Vater (×150).

frequent in the digestive tract[3–7], the genitourinary tract[8–10] and the upper respiratory tract[11]. The nerves storing VIP or other neuropeptides (substance P, somatostatin and enkephalin) apparently represent additional types of autonomic nerves, distinct from the adrenergic and cholinergic ones. A great proportion or the peptidergic nerves seems to originate in nerve cell bodies located close to or within the innervated organ[5–9,11]. The physiological significance of these new types of nerves is a matter of speculation, but a knowledge of their precise anatomical distribution will assist in defining their targets. We have previously observed that structures believed to exert a sphincter function receive a particularly rich supply of such nerves. We have now examined several sphincters, recognised or anticipated, and have established the presence of VIP nerves in all of them.

For chemical analysis specimens were taken from the mid and lower portion of the oesophagus, the stomach (cardia, antrum and pylorus) and the upper duodenum of seven cats of either sex. The material was immediately frozen and stored at −20 °C until extraction in acidified ethanol and analysis by radioimmunoassay[12]. For histochemistry material was collected from the above mentioned locations and from the sphincter of Oddi and the papilla of Vater from three cats. In addition, we collected specimens from the mid and lower portion of the ureter, from the bladder–urethra connection and from the bladder wall. From three male cats specimens were taken from the prostate, urethra and urethral collicles. The specimens were frozen to the temperature of liquid nitrogen in a propane–propylene mixture and freeze-dried. They were then exposed to formaldehyde gas at +80 °C for 1 h (ref. 13) or to diethylpyrocarbonate vapour at

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