Is it possible to find several distinct NI in one given nucleus and, if so, are the NI the same or a different type?

Methods. After a short induction of anesthesia with fluothane, an adult male cat was given 0.25 mg/kg sodium pentobarbital solution i.v. The left celiac ganglion was removed, cut into small fragments, fixed in a 2.5% glutaraldehyde solution (pH 7.4) for 1 h, postfixed in a 2% osmium tetroxyde solution (pH 7.4) for 1 h and embedded in Epon. We have taken at random 4 blocks of ganglion from ten. White sections were made on an LKB ultramicrotome and taken at random on grids (mesh 200). One grid was examined for each block, then, counted 80 sections of nuclei on each grid obtaining 320 sections by ganglion. For serial sections, white-coloured serial sections obtained from one of these blocks have all been collected on one-hole grids (copper grids with a rectangular $2 \times 0{,}6$ mm hole, Janning) covered with 0.25% formwar membrane. The sections were stained with uranyl acetate and lead citrate then examined in a Siemens Elmiskop 101 with a goniometer stage.

Results. For 320 sections of neuronal nuclei we found a total number of 68 sections of NI, accordingly an 0.20 'apparent' frequency, as below:

- 40 sections of type I inclusions (roughly 59% of all the inclusions observed),
- 10 sections of type II (15%),
- 16 sections of type III (24%),
- 2 sections of type IV and V (2%).

In order to determine the 'actual' frequency of NI, we studied 400 serial sections of the same ganglion from a zone which we could identify by capillaries (figure 1). These capillaries were taken as reference-marks and were again discovered on the whole thickness of the examined tissue (30 µm). In this area we completed a study of 5 neurons designated as ABCDE (figure 1). All of the 5 nuclei were examined at a 40,000 magnification. We observed 19 NI clearly identified with the help of a goniometer stage (figures 2–4). It must be pointed out that no crystalloid inclusion in these serial sections were found. The table summarizes the repartition and the frequency of these NI in the 5 neuronal nuclei that were studied.

Discussion. Several remarks can be made about these results. For an 0.20 'apparent' frequency, all the examined neurons contain NI. This result still includes too few a number of cells to be extrapoled to the whole neuronal population. Indeed, one can think that the presence of NI is a specific feature of a group of adjacent neurons such as those that have been studied. However this hypothesis is unlikely as our previous studies carried out on sections taken at random on the ganglion have shown that NI occur in all parts of a sympathetic ganglion[2,5,6].

The most striking fact is that up to 5 different NI may occur in a single nucleus, a possibility which did not appear clearly by observing sections taken at random. Indeed out of 68 sections of inclusions counted by this means only one case showing simultaneously 2 sections of NI in the same nucleus occurred. It appears clearly that one same nucleus may contain different types of NI (see table).

However a difference appears in this work between the 'apparent' and the 'actual' frequency of the type III (see table). This difference is explained by our previous studies. If one considers the 3-dimensional pattern of these NI: this kind of NI is the longest (up to 10 µm) when the type II never exceed 4 µm[4]. The probability for the level of a section to cut through type III NI is consequently much greater and explains the difference between the 'apparent' and 'actual' frequency.

Conclusion. Serial sections show clearly that the 'actual' frequency of tubulo-filamentous NI in sympathetic neurons is more higher than previously claimed since a single nucleus may contain up to 5 different inclusions. The present observations confirm our previous ones according to such structures are normal components of the healthy nucleus[2,6].

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Prefrontal cortex of the cat: Evidence for an additional area

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Summary. Direct projections from the mediodorsal nucleus of the thalamus to ventral parts of the insular region of the cat's cortex were demonstrated by using the horseradish peroxidase technique.

The extent of the prefrontal cortex of the cat has been defined differentially in various behavioral[1] and anatomical[2–6] studies. However, in nearly all studies, there is conformity with respect to at least 2 basic positions: 1. The extent of the cat's prefrontal cortex is defined as the projection area of the mediodorsal nucleus of the thalamus (MD). 2. The extent of the cat's prefrontal cortex consistently is limited to the frontal pole[7,8].

Topologically therefore, the cat's prefrontal cortex is similar in position to the respective delineation of the monkey's prefrontal region, but differs from that of the rat: Both the monkey's and cat's prefrontal cortices are situated around the frontal pole, whereas the rat's is divided into 2 areas of which none extends to the frontal pole[9,10].

Our experiments show evidence that also the cat's prefrontal cortex topologically consists of 2 separable regions. After having detected horseradish peroxidase (HRP) labelled cells within the cat's insular region after injections into the frontal pole[11], we injected HRP in different parts of the insular cortex and of neighboring regions, using Mesulam's modification of the HRP technique[12,13]. In 11 cats, small amounts of HRP (Boehringer, Grade I) were injected into parts of the orbital, sylvian and ectosylvian gyri, as is schematized in figure 1. 8 of the cats received unilateral

injections only. For 8 cats the enzyme was delivered through a glass micropipette, which had been connected to a microsyringe by a short length of polyethylene tubing. The other 3 cats received injections iontophoretically[14,15]. After postoperative survival times of 40–65 h, each animal was perfused with at least 4 l Karnovsky solution. Removed blocks of stereotaxically cut brains were stored in cold 30% sucrose-phosphate buffer (pH 7.6) solution for up to 3 days; then serial coronal sections of 40 µm were cut and every 5th was processed according to procedure 8 of Mesulam[12]. Sections were mounted on alum-gelatin coated glass slides, counterstained with neutral red, and studied microscopically.

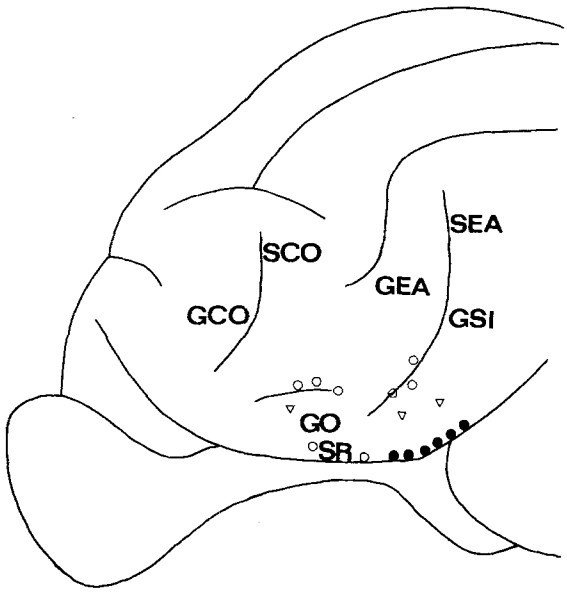

Fig. 1. Schematic view of the cat brain showing loci of HRP injections (dots and triangles). Open signs denote injection sites, not resulting in labelling of mediodorsal thalamic cells; triangles refer to loci already published[16]. Abbreviations: GCO: gyrus coronalis; GEA: gyrus ectosylvius anterior; GO: gyrus orbitalis; GSI: gyrus sylvius anterior; SCO: sulcus coronalis; SEA: sulcus ectosylvius anterior; SR: sulcus rhinalis.

All 14 injection sites led to retrogradely labelled cells in various thalamic nuclei. Most consistently, neurons of the suprageniculate and medial geniculate nuclei were labelled. However, 6 injection loci (black dots in figure 1) also led to labelled cells in MD (usually overlapping with an additional projection from the suprageniculate-medial geniculate complex; figure 2). At all these injection sites, labelled cells were detected in the posterior part of MD, caudal to about anterior-posterior level +9.0 mm. The area of labelled cells in MD was confined to the lateral and ventral borders of MD; only for the most posterior end of MD also dorsally situated cells were observed.

All injection loci which labelled MD neurons, were situated in the anterior sylvian gyrus above the rhinal sulcus. Their anterior-posterior extent varied from about A +17 to A +11 mm. Dorsally, they were restricted to the ventral half of the anterior sylvian gyrus. Within these boundaries, there is a rather precise congruence between that region of the insula receiving MD afferents and that projecting to the cat's traditional prefrontal area (situated around the frontal pole)[11,16].

These results emphasize that - on the basis of Rose's and Woolsey's definition[5] - a further area of the cat's cortex has to be named prefrontal. This area, situated in a region which has traditionally been named insula[17,18], is known as an integration zone containing polysensory units[19,20]. Siegel et al.[21] describe a direct connection from the cat's insular region to exactly that postero-lateral part of MD, reported herein to project to the insula. Furthermore, the insula of both rat[22] and tree shrew[23] projects to MD; and, as stated earlier, one of the subregions of the prefrontal cortex of the

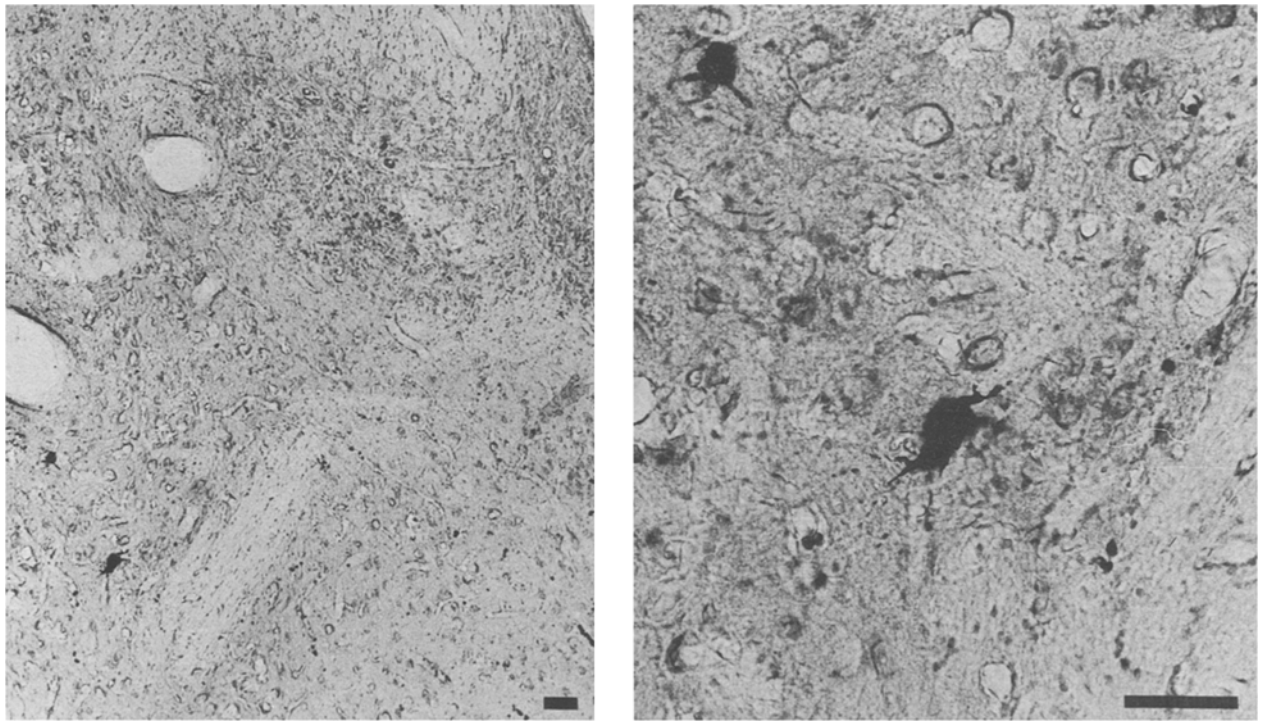

Fig. 2. Left: Example of labelled neurons in the mediodorsal nucleus of the thalamus after HRP injections in the ventral part of the anterior sylvian gyrus (anterior-posterior level is about +8 mm). Right: same neurons under higher magnification. Scales: 0.1 mm.

rat is situated at a topologically similar locus as the presently found area.

Future research will have to show whether the zone of the cat's cortex described can also be termed prefrontal on the basis of its functional characteristics.

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Changes in the plasma membrane surface of lymphocytes stimulated in vivo with DNCB

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Summary. Stereological principles have been used to evaluate ultrastructural changes which accompany the transformation of lymphocytes stimulated in vivo with dinitrochlorobenzene (DNCB). Whereas unstimulated lymphocytes and blast cells have slightly more than the minimal containing plasmalemmal surface for their volume, stimulated (blast-derived) lymphocytes have an excess surface area in the order of 30%. This observation is discussed in the context of altered cell function and the biosynthesis of additional membrane components.

During cell-mediated immune responses, T-lymphocytes in the thymus-dependent paracortices of draining lymph nodes are stimulated to transform into blast cells which then divide to produce a new generation of lymphocytes[2,3]. These daughter lymphocytes are functionally well-defined and are classified as killer, helper, suppressor or memory cells according to their subsequent activity[4,5]. Recently, Dunlap et al.[6] have demonstrated biochemical differences between the plasmalemmae of unstimulated and stimulated lymphocytes. However, little or no difference between functionally distinct populations of lymphocytes is apparent on purely morphological inspection.

In this report, using conventional stereological techniques[7] we establish ultrastructural differences between unstimulated lymphocytes and cells stimulated in vivo with DNCB. In particular, there is a marked increase in the plasma membrane surface area of stimulated lymphocytes. The quantitative morphological changes probably reflect the altered functional status of stimulated cells.

Materials and methods. Adult male C57 Black mice were sensitized by painting 10% DNCB in acetone on to their shaved right flanks. 4 days after sensitization, when blast cells are maximal in the draining axillary lymph nodes[8], 1 group of mice was killed and their axillary nodes removed and processed for electron microscopy. Each mouse of a second group was injected with 1 µC of tritiated thymidine per g b.wt at 4 days after sensitization and sacrificed at 6 days. At this time after labelling, there is a maximal number of labelled (daughter, stimulated) lymphocytes in the nodes. A third group of mice was untreated; their nodes were removed to provide a source of unstimulated lymphocytes.

All tissue was processed in a standardized fashion[9]. Thin strips of lymph node tissue were fixed in 3% buffered glutaraldehyde at pH 7.3, postfixed in 2% aqueous osmium tetroxide and embedded in Araldite resin after dehydration. A Huxley (Cambridge) ultramicrotome was used for sectioning. Semithin (ca. 0.5 µm) sections were cut for light microscopic measurements and ultrathin (ca. 70 nm) sections for electron microscopy. Autoradiographic preparations were coated with Ilford L4 nuclear emulsion, exposed for 20 weeks and developed in Kodak D19 developer. Stimulated cells were identified as those lymphocytes having silver grains overlying the nucleus.

From toluidine blue-stained sections, light micrographs of paracortical areas were sampled randomly and printed at a final magnification of $\times 2450$. These prints were used for estimating mean nuclear volumes of unstimulated lymphocytes, immunoblasts and stimulated cells. About 112 micrographs were taken to represent each node. Nuclear profile sizes were evaluated by measuring their long (a) and short (b) axes. Each profile was then converted to an equivalent circle of diameter $d=(a \cdot b)^{1/2}$. Equivalent diameter histograms were corrected by the Schwartz-Saltikov regime[10] to obtain estimates of mean nuclear diameter. As a convenient approximation, mean nuclear volumes were computed for spheres of this diameter.

A systematic sampling of cell profiles was performed using an AEI-Corinth electron microscope. To ensure unambiguous recognition of cell types, only cell profiles containing a nucleus were recorded[11]. Final print magnification was $\times 19{,}650$ as determined from micrographs of a grating replica. Morphometric data were recorded with the aid of a coherent double lattice test system superposed on to each of