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Feline dysautonomia: an ultrastructural study of neurones in the XII nucleus*

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Summary. A feline dysautonomia of unknown aetiology has been reported in numerous cats in the United Kingdom since 1981. The consistent histological lesion is a chromatolytic-type change within the neurones of the autonomic nervous system, which is also found less frequently in non-autonomic regions, such as the XII nucleus. This study describes the ultrastructural changes in the XII nucleus within the first 2 weeks of clinical disease. In the abnormal neurones there is a dispersion of the Nissl substance, progressing to dilation of individual cisternae by an electron-dense floccular material. Such cisternae have lost the majority of their ribosomes. Normal Golgi complexes can be seen in neurones where there is only slight dispersion of the Nissl substance, but no Golgi complexes, either normal or abnormal, can be identified in any cell in which the Nissl substance is markedly disrupted. There is proliferation of smooth endoplasmic reticulum in several neurones, and there may also be an increased number of morphologically normal mitochondria. The nuclei of affected neurones are eccentric with crenations of the nuclear envelope, and in some cases nucleolar changes are also observed. Autophagic vacuoles are present in small numbers. Other organelles appear normal. These findings compare closely to those for the autonomic neurones, suggesting that the primary effect of the causal agent(s) is on the protein synthetic pathway of specific neurones.

Key words: Dysautonomia – Protein biosynthesis – Rough endoplasmic reticulum – Golgi apparatus

Feline dysautonomia (the Key-Gaskell syndrome), first described by Key and Gaskell (1982) is a primary dysautonomia of unknown aetiology affecting predominantly young adult animals. The majority of cases occur within Great Britain, but isolated cases have been reported in other countries, particularly Scandinavia (Gaskell and Edney 1985).

The clinical appearance of this syndrome is well documented (Griffiths et al. 1982; Sharp et al. 1984; Pollin 1985). Most cats present with many, or all, of the following signs: dilated, non-responsive pupils; protruding membrana nictitans; decreased tear production; dry nose and mouth; megaoesophagus frequently resulting in regurgitation; constipation; an abnormal anal reflex and bradycardia. A much smaller percentage of cases also show urinary or faecal incontinence, and a few cats have mild proprioceptive defects which are found only on specific examination.

Gross pathological findings are inconsistent and non-specific, but chromatolytic change is seen at the light microscopic level in every case. Neurones in all autonomic ganglia are consistently involved to approximately the same degree within any given animal, a finding considered confirmatory of the clinical diagnosis. In more severe cases, damaged neurones may also be seen at a lower frequency in certain other areas (Table 1).

The chromatolytic changes in all these areas appear similar when studied under the light microscope. There is loss of the normal granular appearance of the Nissl substance and the cytoplasm becomes homogenously pale-staining in haematoxylin-eosin sections. Some neurones also demonstrate vacuolation of their cytoplasm. Affected perikarya are rounded and moderately swollen in the early stages, but later become shrunken and irregular, while the nuclei are commonly pyknotic. In cases of longer than 2 weeks duration, chromatolytic neurones are not seen. In the CNS, small neuronophagic nodules may be the only indication of degenerating neurones.

The clinical and pathological findings in this disease closely resemble those of grass sickness in horses

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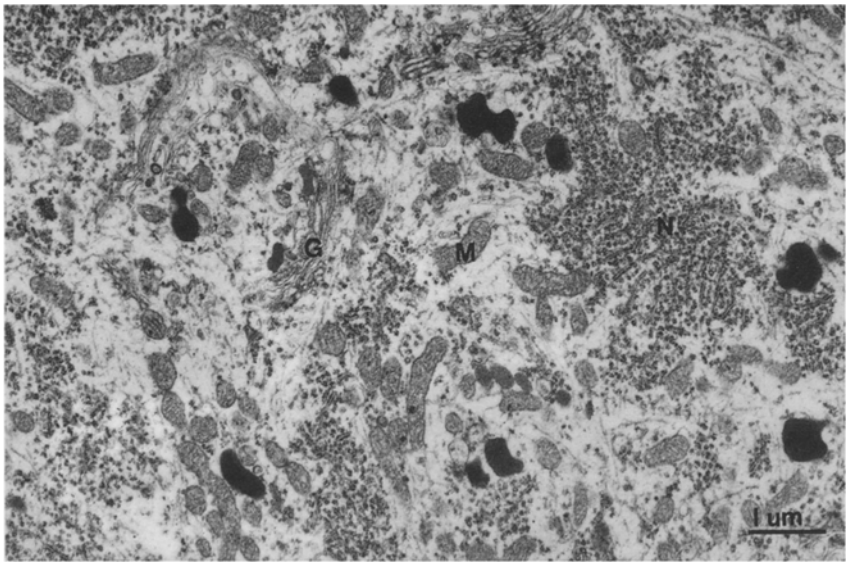

Fig. 1. Cytoplasm from a normal cat. The Nissl granules (*N*) and Golgi complexes (*G*) are prominent, and there are frequent mitochondria (*M*)

Table 1. Distribution of neuronal damage in feline dysautonomia

Area affected	% Cats affected[a]	% Cells affected[a,b]
Autonomic ganglia	100	30–100
Non-autonomic ganglia	50	<1– 10
Spinal cord (ventral horn cells)	62	5– 30
Brain stem nuclei: XII	66	5– 90
Dorsal nucleus vagus	69	10– 90
VII	51	5– 75
V	47	5– 50
III (incl. Edinger-Westphal)	25	5– 50
Nucleus ambiguus	40	5– 40

[a] Based on data in: Sharp et al. (1984); Pollin (1985)
[b] Semiquantitative visual assessment

(Obel 1955; Barlow 1959; Mahaffey 1959) and a similar condition has recently been described in dogs (Rochlitz and Bennett 1982; Pollin and Sullivan 1986). There is no pathologically documented referable condition in man, although one case in a young girl, who survived, showed many clinical similarities (Inamdar 1982).

This paper is concerned with the ultrastructure of the perikarya of chromatolytic neurones in the XII nucleus, as representative of the non-autonomic CNS lesion.

Materials and methods

This study examined five dysautonomic cats which had been ill for less than 14 days. Three non-affected cats were also studied.

Each cat was heparinised, induced by intravenous injection of thiopentone and anaesthesia maintained by O_2 and N_2O and halothane administered by respiratory pump via an endotracheal tube.

The aorta was cannulated and the cats were killed by perfusion with mixed aldehydes in 0.08 M sodium cacodylate buffer as follows: 500 ml containing 1% paraformaldehyde and 1.25% glutaraldehyde (final concentrations) and 250 mg $CaCl_2$, which was followed by 1500 ml containing 4% paraformaldehyde and 5% glutaraldehyde (final concentrations) and 750 mg $CaCl_2$.

The brain was removed entire, and further fixed by immersion in 2.5% glutaraldehyde in 0.025 M sodium cacodylate buffer for a variable period of not less than 48 h.

One millimetre sections of the brain stem in the region of the XII nucleus were post fixed for 1–2 h in 1% OSO_4, and processed through graded alcohols and propylene oxide on a 48-h schedule prior to embedding in an Araldite-based resin.

One micrometre sections, stained with methylene blue, were used to select the best areas for ultrastructural examination. Thin sections of 60–80 nm were then cut, supported on 200 mesh copper grids, stained with Reynold's lead citrate and uranyl acetate and examined in a Philips 301 electron microscope.

Results

Normal

The perikarya of neurones within the normal XII nucleus are large and multipolar, with organelles well dispersed throughout the cytoplasm (Fig. 1). They have a prominent round or oval nucleus, with pale-staining karyoplasm containing a variety of randomly distributed granules. The nucleolus is usually round and has a typical "honeycomb" appearance.

The Nissl granules – discrete stacks of 3–12 flattened, approximately parallel cisternae of rough endoplasmic reticulum (RER) – are distributed throughout the cytoplasm, and smooth endoplasmic reticulum (SER) may also be present. This tends to occur as isolated single cisternae not always associated with the RER.

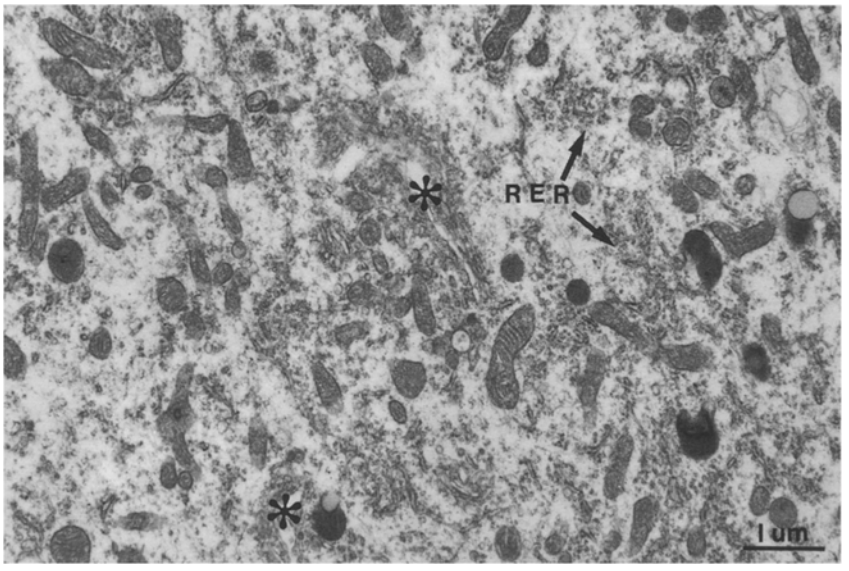

Fig. 2. Cytoplasm from a case of 3-day duration. The Golgi complexes (*asterisk*) appear normal. The rough endoplasmic reticulum (*RER*) is partially dispersed throughout the cytoplasm and is not present in discrete Nissl granules. The mitochondria appear normal

The prominent Golgi complex is concentric with the nucleus, though not closely related to it or the cell membrane, and is associated with many coated and non-coated vesicles, particularly on the "trans" side.

Numerous mitochondria of fairly uniform appearance are evenly distributed throughout the cytoplasm, being found most frequently in those areas not occupied by the other large organelles. They are, however, occasionally seen within an area of RER.

Several "dense bodies" and occasional multivesicular bodies may be found, and the cytoplasm also contains numerous neurotubules and filaments which are orientated approximately along the long axes of the cells' dendrites.

In the dendrites, neurotubules and neurofilaments are the most prominent organelles, although all other organelles may be found proximally, mitochondria occurring with the greatest frequency.

Affected

A proportion of the neurones in the XII nucleus remain ultrastructurally normal in all affected cats (Table 1). Abnormal neurones also have large multipolar perikarya with prominent nuclei, most of which appear normal, although some show slight crenations of the nuclear envelope and ring nucleoli are occasionally seen.

The Nissl substance shows a great variety of changes. In the earliest case examined (3 days after onset) the only abnormality seen in some neurones is an unusual distortion of the RER (Fig. 2). Individual cisternae with ribosomes attached are no longer arranged into discrete "granules", but are more dispersed throughout the cytoplasm, surrounding other organelles, and not distinct at low magnifications.

In slightly later cases (i.e. 3–6 days duration) the changes in the RER become much more pronounced, with no normal cisternae visible. Instead, the cytoplasm is filled with numerous distended cisternae containing an electron-dense floccular material (Fig. 3). The presence of occasional ribosomes adherent to the membranes of these cisternae indicate their origins in the RER.

Other neurones in these cases show no evidence of any RER, but have a great increase in the number of SER profiles. This was very pronounced in a case of 2-week clinical duration, where some neurones and dendrites are packed with proliferated SER, with slender mitochondria, multivesicular bodies and lysosomes the only other recognisable organelles (Fig. 4). Degenerating "ghost cells" contain only smooth membranous profiles, and vacuoles, with occasional lysosomes and mitochondria.

The Golgi complex is also very severely affected. It appears normal in some cells in which the only abnormality is a dispersion of otherwise unchanged RER, but in all other affected cells no Golgi complexes, either normal or abnormal, can be recognised (Figs. 3, 4).

Mitochondrial numbers are perhaps increased in early cases and definitely increased in lesions of longer duration, where individual mitochondria appear to be more slender than those in normal neurones.

With the exception of the earliest case, there is an increased number of lysosomes (many of which show secondary activity) in most affected neurones. Multivesicular bodies and autophagic vacuoles are also seen (Fig. 4). Neurofilaments and neurotubules appear normal in all the cases examined.

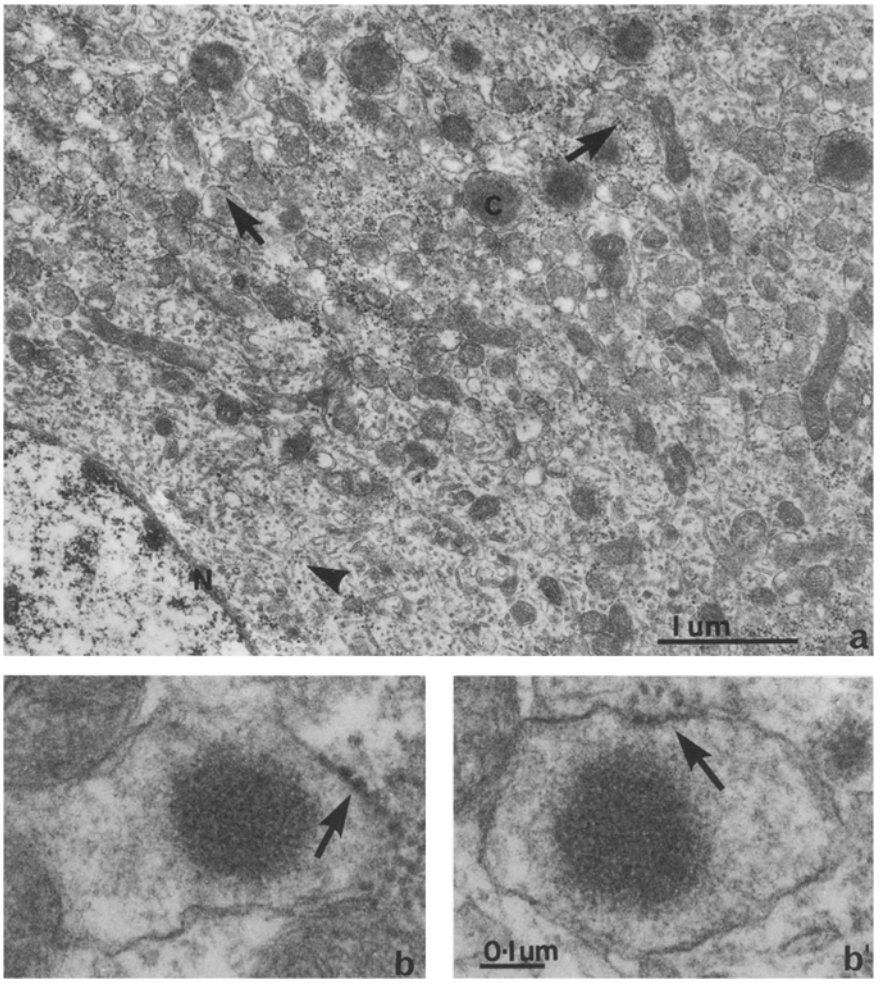

Fig. 3. a Cytoplasm from a case of 5-day duration. No Golgi complexes or normal RER can be seen. The cytoplasm is filled with cisternae distended with a electron-dense floccular material (*C*) and numerous profiles of smooth endoplasmic reticulum (*SER, arrowhead*). Some of these cisternae still have ribosomes attached (*arrows*) indicating their origin in RER. There is no distension of the nuclear envelope (*N*). **b** and **b¹** The typical appearance of the distended cisternae with attached ribosomes (*arrows*), which contain condensed material

Discussion

Several reports confirm that the distribution of lesions is very specific in feline dysautonomia (Griffiths et al. 1982; Sharp et al. 1984; Pollin 1985). The clinical signs and pathology indicate a predominantly autonomic involvement. Lesions do not embrace the entire autonomic nervous system, and are also found within non-autonomic areas such as the XII nucleus, for which there are frequently no referable clinical effects.

The severity of lesions as assessed by light microscopy is very similar in any one cat, suggesting that the initiating agent damages the neurones within a short time span. This theory is supported by the fact that chromatolytic neurones are always seen within the first 2 weeks of disease and not subsequently. Greater variation between individual cells in each animal is found ultrastructurally with a proportion of neurones appearing normal.

The changes in both autonomic (Griffiths et al. 1985) and non-autonomic neurones are closely comparable, suggesting that the causal agent(s) acts in a similar way in these two cell populations. The one major difference is that the frequent, complex membranous stacks (Griffiths et al. 1985, see Fig. 5) evident in autonomic neurones have not been found in the XII nucleus. It is not known whether this is due to a difference in the severity of the initial damage to centrally and peripherally situated neurones, or whether there is a differing response to a similar insult in the two cell types. One typical membranous stack has been seen in a central autonomic neurone in the dorsal nucleus of the vagus (unpublished observation). This suggests that both the position and type of the neurone may be important in determining the degree of damage to that neurone.

Axonal lesions are present in cases of feline dysautonomia (Griffiths et al. 1985), but the changes in affected perikarya are not thought to be secondary to these. In the 'classic' axonal reaction there is a dispersal of the RER (Torvik 1976; Aldskogius 1978), a proliferation of SER (Lieberman 1971; Aldskogius 1978), nuclear eccentricity and folding (Aldskogius

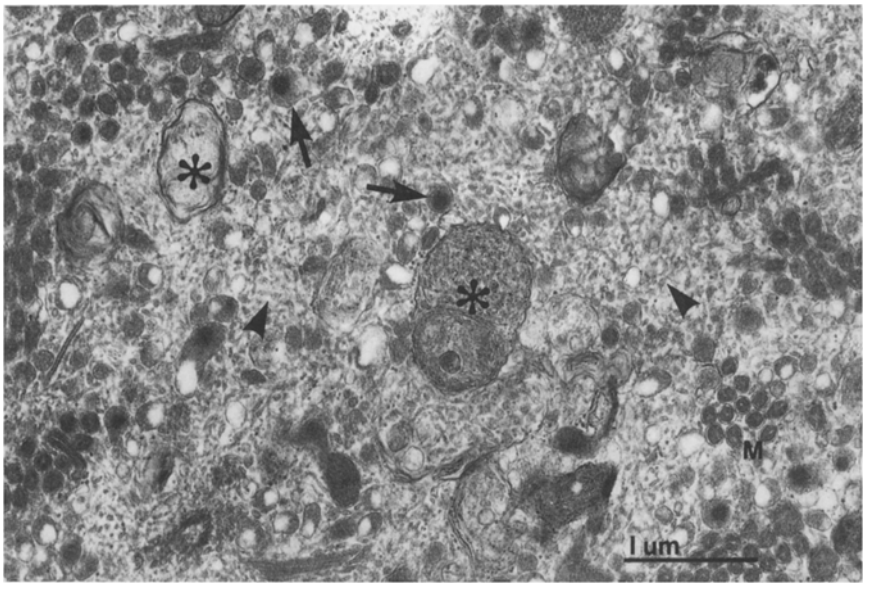

Fig. 4. Cytoplasm from a case of 14-day duration. No Golgi complexes or RER are present. The cytoplasm is filled with proliferated SER (*arrowheads*). There are several cisternae distended with condensed, electron-dense material (*arrows*). The numerous mitochondria are dense and slender, and tend to occur in clumps (*M*). Lyosomes and autophagic vacuoles are prominent (*asterisk*)

1978) and increased numbers of autophagic vacuoles and lysosomal structures. Aggregates of mitochondria (Barron et al. 1971), which may appear more elongated and slender than usual (Aldskogius 1978), may also be seen but degenerative changes are frequent. The Golgi complex, however, is relatively unchanged in all descriptions and, although it has been variously described as increased (Aldskogius 1978) and displaced (Barron et al. 1971), no circumstances have been reported where there is a complete absence of this organelle in a morphologically recognisable form. Also, the marked distension of the cisternae of RER is not usually found in the axonal reaction.

The ultrastructural findings suggest that the lesion primarily involves the protein biosynthetic pathway of specific neurones, since RER and Golgi complexes are consistently affected, with organelles such as mitochondria appearing relatively normal.

An infectious agent or a toxin is considered the most likely aetiology for this condition, and numerous toxins are known which have effects on the organelles associated with protein synthesis in neurones. For example *Shigella dysenteriae* cytotoxin disrupts neuronal RER (Wiley et al. 1985), isoniazid (Jacobs et al. 1979; Jones and Cavanagh 1981) and acrylamide (Prineas 1969; Sterman 1983) cause degranulation of RER and increased mitochondrial numbers and toxic substances, such as adriamycin and many others, may result in nucleolar abormalities (Bernhard and Granboulan 1968). There are no descriptions, however, of any toxins causing a comparable range of structural effects with a similar specificity of lesion distribution. Pathological changes have been produced in autonomic ganglia of experimental ponies using fractionated serum from animals with grass sickness (Gilmour and Mould 1977) suggesting the presence of some neurotoxic factor with a molecular weight of greater than 30,000. Clinical disease was not seen in these animals, however.

Feline dysautonomia is, therefore, one of a group of primary dysautonomias of domestic animals in Britain, in which there is damage to the protein synthetic pathway of certain neurones. The affected neurones are predominantly autonomic, but specific non-autonomic regions, such as the XII nucleus, the dorsal root ganglia and the ventral horn cells of the spinal cord, can be affected. The close correlations of clinical appearance and the distribution of lesions in the three species suggest that the conditions have a very similar, as yet undefined, aetiology.

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