Synaptic Ultrastructure in a Marsupial, *Setonix brachyurus*

Although the detailed ultrastructure of the vertebrate synaptic region had been extensively investigated by the mid 1960's[1–6], the use of glutaraldehyde fixation and ethanolic phosphotungstic acid (E-PTA) staining[7–9] led to a greater understaining of its ultrastructure, particularly of the paramembranous components which are selectively stained by E-PTA. According to BLOOM and AGHAJANIAN[8] the organized dense synaptic material consists of: presynaptic dense projections separated from each other by electrontranslucent areas, a discontinous intrasynaptic line within the cleft, a postsynaptic band and postsynaptic whorls. With this technique synaptic membranes appear as electrontranslucent bands separating the presynaptic dense projections and the postsynaptic thickening from the intrasynaptic line. Synaptic contacts such as the above have been found in different vertebrate species and in different areas of the central nervous system. In addition, the E-PTA method and the related bismuth iodide uranyl lead (BIUL) technique of AKERT et al.[10] have thrown new light on the structural organization of the presynaptic grid and network as well as its relationship to the presynaptic dense projections.

Using the same techniques on fractionated rat cerebral cortex, JONES[9] described 2 types of synaptosome. In Type A, the synaptic membrane specializations are discontinuous and show presynaptic dense projections, postsynaptic densities and an intermediate band within the cleft which is intermittently thickened to form cleft densities. Type B exhibits continous thickenings of both synaptic membranes along their length of apposition, with each thickening forming projections into the adjacent cytoplasm. The intracleft material is in the form of 2 longitudinally running bands joined by transverse bars.

By contrast the contact region of octopus synaptosomes displays membranes having a triple layered appearance consisting of electronopaque internal and external coats separated by an electrontranslucent band[11]. An interesting feature, markedly different from that of vertebrates, is the presence at specialized synaptic contact regions of a prominent electonopaque synaptic plate thought to be formed by coalescence of the external coats of the pre- and postsynaptic membranes.

Preliminary unpublished light microscope observations on the dorsal lateral geniculate nucleus of the quokka (*Setonix brachyurus*), a diprotodont marsupial of the superfamily Phalangeroidea, indicate that it possesses at least 3 distinct cellular laminae. The only other non-primate mammals known to have more than 3 laminae are the phalanger (*Trichosurus vulpecula*)[12] and the sugar glider (*Petaurus breviceps*)[13], both members of the superfamily Phalangeroidea.

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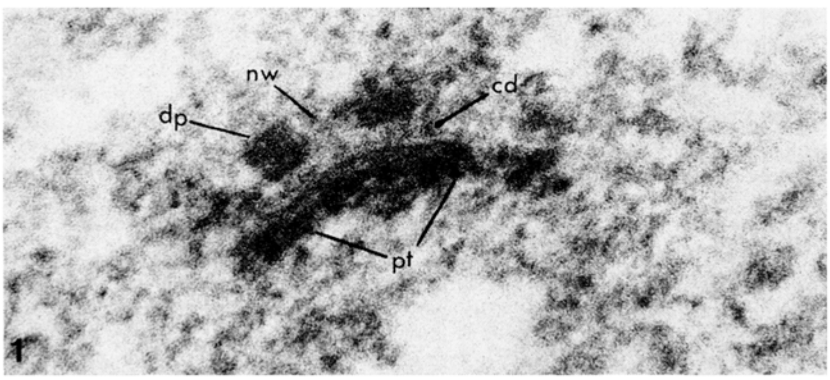

Fig. 1. The 'discontinous-continuous' pattern of the synaptic junction is clearly demonstrated. The dense projections (dp) are separated from each other, although a strand of the presynaptic network (nw) runs between them. Cleft densities (cd) and a postsynaptic thickening (pt) are also present. ×153,000.

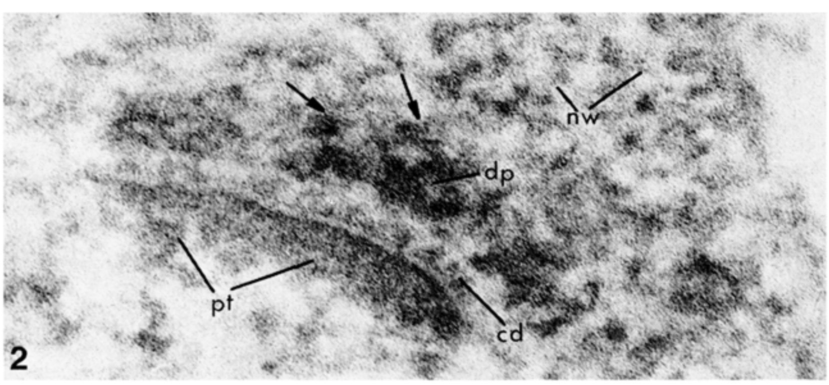

Fig. 2. The dense projections (dp) are irregularly arranged, and are continuous at a number of points (arrows) with the strands of the presynaptic network (nw). These strands also penetrate as far as the presynaptic network (between the projections). Cleft densities (cd) and a postsynaptic thickening (pt) are visible. ×235,000.

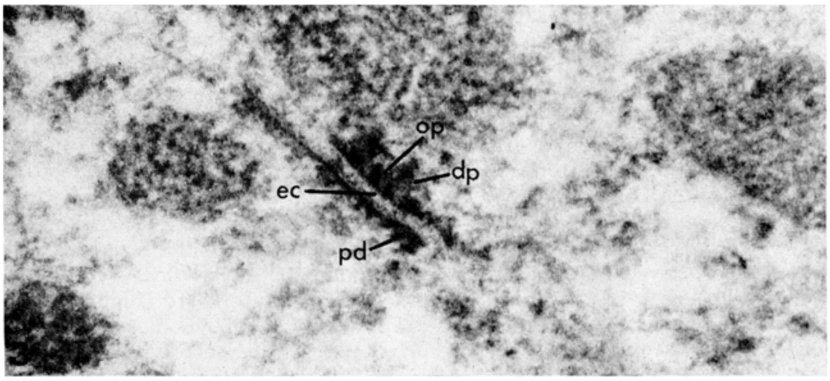

Fig. 3. Small triangular opacities (op) as well as dense projections (dp) are seen along the presynaptic membrane, which displays a thin barely visible external coat (ec). Postsynaptic focal densities (pd) are recognizable. × 102,000.

Because of its apparently unique phylogenetic position and the paucity of neurological studies on Australian marsupials, the present study was carried out to investigate the synaptic organization of the quokka lateral geniculate nucleus. It shows that the fine synaptic structure has a distinct pattern, differing markedly from the arrangement in an invertebrate (e.g. *Octopus*) but resembling in certain respects the mammalian pattern. There are, however, significant differences from the latter and these may throw new light on our understanding of the degree of localization possible at the synaptic region.

Samples of tissue from the dorsal lateral geniculate nucleus of the adult quokka (*Setonix brachyurus*) were fixed in 2.5% phosphate-buffered glutaraldehyde, pH 7.3, for 4 h. Following dehydration in ethanol the material was block stained with a 1% ethanol soution of PTA for 2 h[8,9]. After embedding in Araldite, thin sections were examined with a Philips 300 electron microscope.

Dense projections constitute a prominent feature of the synaptic junction, appearing either as discrete, fairly uniform structures (Figure 1) or as irregular prolongations of a presynaptic thickening (Figure 2). In almost all instances they are separated from neighbouring projections, although the distance between them varies from junction to junction and within the same junction. The projections are generally 40–70 nm high, with inter-projection distances ranging from 10 to 70 nm. In some junctions (Figure 3) typical dense projections have much smaller (15–25 nm high) triangular opacities associated with them. In all cases the dense projections are continuous with strands constituting the presynaptic network (e.g arrows in Figure 2)[9,14].

The material within the cleft is arranged in the form of an intermediate band parallel with and close to the postsynaptic thickening. From this band opaque areas, triangular or quadrilateral in outline, extend towards the presynaptic membrane and bear some resemblance either to the cleft densities of Type A synaptosomes or, in a small minority of cases, to the developing transverse bars of Type B[9,15] Breaks generally occur at one or more points along the band, while the presynaptic membrane sometimes exhibits an external coat (Figure 3).

The postsynaptic thickening is characterized by its continous nature, resembling Type B but unlike Type A. Focal densities are visible on its cytoplasmic aspect.

The majority of lateral geniculate synapses in the quokka, therefore, display a 'discontinuous-continuous' pattern, the presynaptic moieties being discontinous and the postsynaptic continous. This contrasts markedly with the predominantly discontinuous type A and continnuous type B patterns described by Jones[9] and Jones and Revell[15] in E-PTA stained rat synaptosomes. Although this arrangement does not differ as greatly from that described in E-PTA and BIUL studies of intact mammalian material[8,16,17], there are significant points of contrast. In quokka, dense projections are more variable in outline, sometimes resembling the branching presynaptic spicules of *Octopus* and giving the appearance of an incomplete network[11,14]. It is unlikely that this is a consequence of oblique sectioning as the corresponding cleft regions are sectioned transversely. The presence of triangular opacities resembling small dense projections (Figure 3) has not been previously described and warrants further investigation. The dense projections usually have an irregular border which is sometimes prolonged outwards to surround electrontranslucent spherical areas 20 nm across.

The variation in inter-projection distances raises the question of whether more than one vesicle can come into contact with the presynaptic membrane between neighbouring dense projections. This is probably the case in some instances, although the vesicles appear to be separated by strands of the presynaptic network, Nevertheless, this raises the possibility that more than one vesicle may discharge its contents simultaneously at some inter-projection sites, in contrast to the 'one vesicle to one dense projection' arrangement described in mammals[9,10,16,18].

Résumé. L'organisation synaptique du noyau latéral geniculé du quokka *Setonix brachyurus* fut examiné dans le tissu fixé en glutaraldéhyde et coloré avec de l'E-PTA. Les projections denses présynaptiques sont de contours irréguliers et l'intervalle, qui les sépare de la membrane postsynaptique est continue et large. Les densités de la fente sont quadrilatéraux ou ressemblent à une barre. Entre les projections denses adjacentes plus d'une vésicule touche la membrane présynaptique.

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