## Chapter 3

# The Neuronal Porosome Complex in Mammalian Brain: A Study Using Electron Microscopic Study

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Porosomes are the universal secretory machinery in cells where membrane-bound secretory vesicles dock and transiently fuse (kiss-and-run) to release intravesicular contents to the outside of the cell during secretion. Earlier it was shown that in rat neurons 12–17 nm cup-shaped lipoprotein porosomes, possessing a central plug are present at the presynaptic membrane sometimes with 35–50 nm in diameter docked synaptic vesicles. In the current study, neuronal porosome structures following hypokinetic stress were evaluated using electron microscopy. Experiments were carried out to identify and evaluate the porosome structure at the presynaptic membrane in the rat and cat brain in control and experimental

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animals and to demonstrate the presence of porosomes in the dog brain. The results reveal for the first time the presence of neuronal porosomes in dog brain and further confirm their existence at the presynaptic membrane in rat and cat brain. Furthermore, the results demonstrate neuronal porosomes to possess a cup-shaped morphology in all the three mammalian species examined, i.e., the rat, cat, and dog. The next series of experiments were designed to evaluate morphological changes in the porosome structure as a consequence of pathological condition—chronic hypokinetic stress. This condition is known to produce structural alterations in the synapses, including the presynaptic regions of limbic region. The depth and diameter of porosome in the central nucleus of amygdale of normal rat and rat subjected to 90 day hypokinetic stress were measured. Morphometric analysis point out the heterogeneity of porosome dimensions that remain unchanged in pathological states. These studies demonstrate for the first time that despite alterations in the presynaptic terminal structure and synaptic transmission provoked by chronic hypokinetic stress in the limbic region, the gross morphology of porosome is unaffected. These results do not, however, rule out possible changes in the composition of the porosome complex following stress. Furthermore, longer period of stress may elicit changes in the neuronal porosome complex, which remains to be established.

## 3.1 Introduction

In all cells, cellular cargo destined for secretion is packaged and stored within membranous vesicles that transiently dock and establish continuity at the base of cup or flask-shaped plasma membrane structures called "porosomes"[1–5] and neurons are no exception.[6–16] Therefore, "porosomes" are the universal secretory machinery in cells where vesicles transiently dock and fuse to release intravesicular contents to the outside of the cell during secretion.

It is suggested that in each type of secretory cell special content of secretory vesicles, different speed of release and different volume of content release dictates specific size of porosomes. In neurons and astrocytes, representing fast secretory cells, porosomes range in size from 10 to 17 nm. In an earlier study, using the atomic force microscope (AFM) and the electron microscope (EM), it was

demonstrated that in the neurons 40–50 nm synaptic vesicles are docked at roughly 10 nm in diameter neuronal porosomes.[4] Recent EM 3D tomography in rat brain also reveals the presence of 12–17 nm permanent presynaptic densities to which 35–50 nm synaptic vesicles are found docked.[15] Moreover, the inside-out ultrahigh-resolution AFM study of presynaptic membrane preparations of isolated synaptosomes clearly displays the presence of the inverted cup-shaped 10–17 nm neuronal porosomes. In contrast, in slow secretory cells, such as in acinar cells of the exocrine pancreas, secretory granules measuring approximately 1000 nm in diameter, expel their content following transient fusion at the porosome base measuring 100–180 nm.[1,6] Furthermore, the results from more recent studies using AFM, EM, electron density, and 3D contour mapping provided additional nanoscale information on the structure and assembly of proteins within the neuronal porosome complex.[8] Particularly, it has become clear that neuronal porosomes possess a central plug that is absent in porosomes in other kinds of secretory cells. This central plug interacts with proteins at the periphery of the structure, conforming to an eightfold symmetry; each of them is connected with spoke-like elements to the central plug that is involved in the rapid opening and closing of the neuronal porosome to the outside.[9] The neuronal central plug has been further examined at various conformational states, providing its gatekeeping role in neurotransmitter release during neurotransmission. Thus, the central plug at various conformations–fully pushed outward, halfway retracted, and completely retracted into porosome cup–has been elegantly demonstrated.[17] Although it is easy to observe porosomes in intact synaptosomes and in inside-out synaptosome preparations using the AFM, in fixed cells it becomes difficult, owing to artifacts due to fixation, dehydration, and tissue processing for EM, and compounded with the fact that the presynaptic membrane contain a high density of plasma membrane proteins resulting in heavy metal staining, rendering it difficult to separate porosomes from the other structures. Nonetheless, porosomes are clearly identifiable in electron micrographs in numerous reported studies.[4,7–9,11,13–15]

In the present research, we continue our EM studies of the porosome morphology in healthy and disease conditions. Two main goals are identified: (1) to further evaluate the porosome structure at the presynaptic membrane in the rat and cat brains and