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Somatostatin: Electron Microscope Immunohistochemical Localization in Secretory Neurons of Rat Hypothalamus

Abstract. *Somatostatin, the hypothalamic hormone which inhibits the secretion of growth hormone by the pituitary, has been localized in the rat hypothalamus by using electron microscope immunohistochemistry. Somatostatin occurs in secretory granules (80 to 110 nm in diameter) of a few neurons located in the hypothalamic periventricular nucleus. These observations establish the identity of neurosecretory neurons involved in the regulation of adenohypophyseal secretion.*

Since the isolation and characterization of a hypothalamic tetradecapeptide named somatostatin, which inhibits the release of immunoreactive growth hormone (*1*), many reports have been published on the distribution in the brain of this inhibiting hormone. Immunohistochemical techniques at the light microscopic (*2*) and ultrastructural levels (*3*), have shown that somatostatin is mainly concentrated in nerve endings of the median eminence.

Somatostatin-containing cell bodies have recently been described as occurring mainly in the rat preoptic and anterior hypothalamus (*4*). To establish that these somatostatin-containing cells are really secretory neurons, we have conducted an immunohistochemical study of somatostatin in the rat hypothalamus using the electron microscope. We report here that somatostatin is contained within secretory granules present in the perikarya of a few secretory neurons located in the hypothalamic periventricular nucleus.

Brains of seven adult male Sprague-Dawley rats were fixed by perfusion with 4 percent paraformaldehyde or 1 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer (*p*H 7.3). For light microscopic studies, the whole brain was fixed by perfusion for 1 to 3 days and embedded in paraffin. For immunoelectron microscopy, the hypothalami were dissected immediately after the perfusion and embedded in Araldite. Two hypothalami were subsequently fixed in 1 percent osmium tetroxide for conventional electron microscopy.

Immunohistochemical staining of somatostatin for both light and electron microscopy was performed with the peroxidase-antiperoxidase technique of Sternberger (*5*). Rabbits were injected either with synthetic somatostatin conjugated to human α-globulin by means of glutaraldehyde, or with synthetic somatostatin conjugated to bovine thyroglobulin by the glutaraldehyde method (*6*). The antiserums thus obtained were used at a dilution of 1:1000 to 1:10,000. Specificity of the staining was verified on adjacent paraffin sections by using antiserums which had been previously absorbed with an excess of synthetic somatostatin ($10^{-4}M$).

The studies with the light microscope showed that positively stained cell bodies occurred in the preoptic and anterior hypothalamus, mostly in areas adjacent to the ependyma of the third ventricle. Both antiserums produced the same results. Adjacent control sections stained with previously adsorbed antiserums confirmed the localization of somatostatin (Fig. 1).

Ultrathin sections from the periventricular nucleus were also stained for electron microscopy. A positive reaction, as indicated by the accumulation of molecules of the peroxidase-antiperoxidase complex, was observed in the secretory granules within the perikarya of a few neurons located in the proximity of ependymal cells (Fig. 2a). Most of the secretory granules, but none of the other

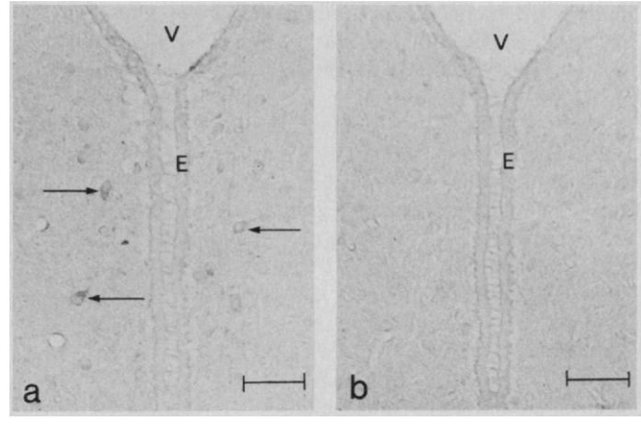

Fig. 1. (a) Frontal section from the periventricular nucleus of a male rat, stained with antiserum to somatostatin serum (diluted 1/1000). Immunostained cell bodies (→) are located adjacent to the ependyma (*E*); *V* indicates the third ventricle. (b) Control section adjacent to that shown in (a). Absorption with excess somatostatin has completely prevented the staining. Scale bars, 50 μm.

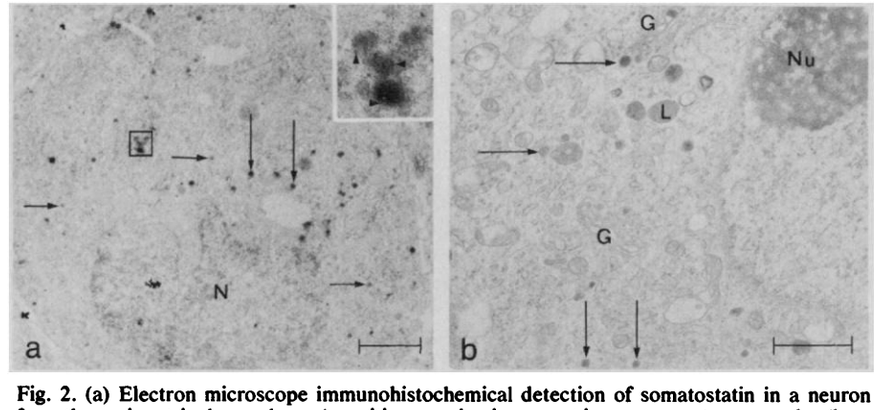

Fig. 2. (a) Electron microscope immunohistochemical detection of somatostatin in a neuron from the periventricular nucleus. A positive reaction is present in most secretory granules (long arrows) whereas a few granules are unstained (short arrows). In the inset, the higher magnification (× 44,600) indicates that the reaction is due to the accumulation of the molecules of the peroxidase-antiperoxidase complex (arrowheads). (b) Section of a neuron treated for conventional ultrastructural studies. This neuron, presumably containing somatostatin, is characterized by the presence of a well-developed Golgi apparatus (*G*), secretory granules (arrows), and lysosomes (*L*). The nucleolus (*Nu*) is prominent. Scale bars, 1 μm.

organelles, in a positive perikaryon were labeled. A few granules were consistently weakly positive or negative. The diameter of the somatostatin-containing granules was 80 to 110 nm. Even if the tissue was fixed in aldehyde only, it was possible to identify the rough endoplasmic reticulum, the mitochondria, and the plasma membrane delineating the somatostatin-containing neurons. However, fixation with aldehyde did not permit an extensive ultrastructural study of the positive secretory neurons. Axons containing positive secretory granules were routinely found throughout the sections. Other secretory neurons containing negative granules of smaller size (40 to 70 nm) were observed in the same area. On the basis of the distribution of the somatostatin cells as well as the diameter of their granules, it is relatively easy to identify this cell type in the tissue fixed in osmium. This cell has all the characteristics of a secretory neuron (Fig. 2b). Its rough endoplasmic reticulum and Golgi apparatus are well developed and the secretory granules as well as the lysosomes are relatively abundant.

The distribution of somatostatin-containing cell bodies observed in this study is in agreement with previous immunohistochemical studies with the light microscope (*4*). The absence of reaction in a small percentage of granules in the somatostatin-producing neurons remains to be explained. Since the diameter of the somatostatin-containing granules is the same in the perikarya and nerve endings (*3*), we suggest that somatostatin is stored in cytoplasmic granules before being transported into the vicinity of the fenestrated capillaries of the pituitary portal plexus. Thus, the somatostatin system appears to be very similar to the magnocellular system involved in the production of vasopressin and oxytocin (*7*).

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Birth Order and Intellectual Development: The Confluence Model in the Light of Cross-Cultural Evidence

Abstract. *For Israeli eighth-grade students of Asian-African origin, achievement decreases as a function of birth order in small families and increases as a function of birth order in large families. This finding cannot be accounted for by differences in developmental rate or size of birth intervals. It can be accounted for by considering the effect of external influences, such as schooling, on intellectual development.*

Using test performance on Raven Progressive Matrices of Dutch army recruits presented by Belmont and Marolla (*1*), Zajonc and Markus (*2*) have formulated a model that relates intellectual development to birth order and family size. The model defines intellectual environment in the home as the average of the absolute intellectual levels (that is, mental age rather than intelligence quotient) of all the inhabitants. The intellectual development of each child is affected by the intellectual environment, and his or her increase in level, in turn, raises the average level of the home. According to the model, family size has an effect because in larger families a greater proportion of the inhabitants are at lower intellectual levels. The effects of birth order result from the growth of later-born children in an environment that reflects the relatively low intellectual levels of their older siblings. However, the decreasing performance for later-born children in large families can be reversed as older children mature and provide them with a richer environment.

We now present achievement test data of eighth-grade Israeli students as a function of birth order and family size and

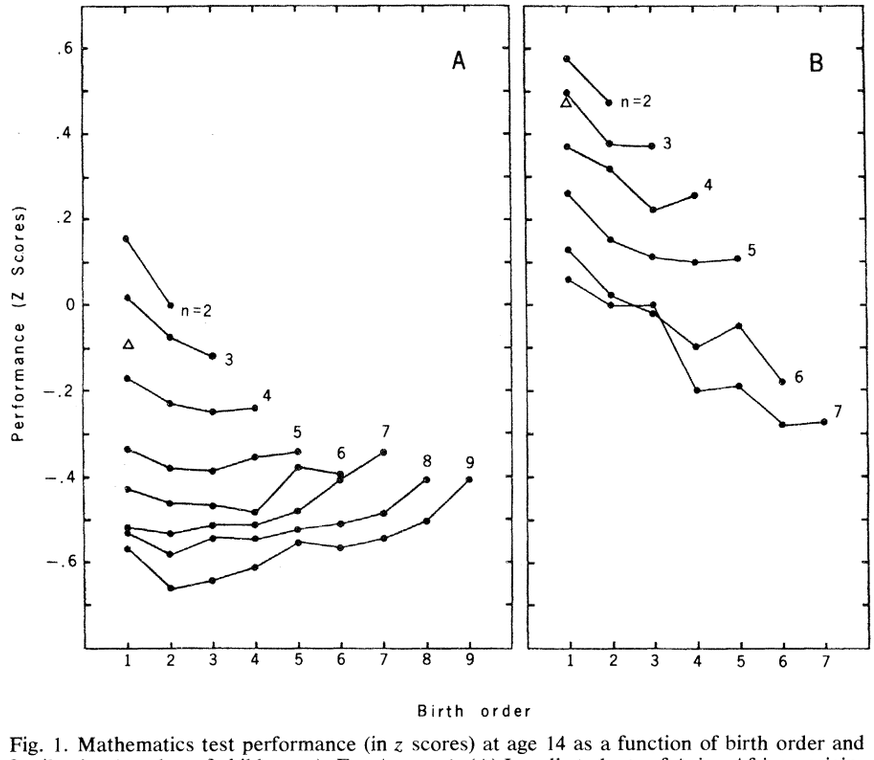

Fig. 1. Mathematics test performance (in z scores) at age 14 as a function of birth order and family size (number of children, n). For △, $n = 1$. (A) Israeli students of Asian-African origin, born between 1951 and 1956 (N = 109,304). (B) Israeli students of European-American origin, born between 1951 and 1956 (N = 82,689). (Because of very low frequencies of large families in this sample, the results for family sizes above 6 are grouped.)