The growth of non-pyramidal neurons in the primary motor cortex of man: a Golgi study

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Summary. In Golgi-Cox-impregnated motor cortex (regio precentralis) of six children (36 weeks of gestation to 13 month after birth) four types of interneurons of laminae II-VI have been investigated qualitatively and quantitatively and finally, compared with interneurons of a child (3 month of age) with Down's Syndrome. All cell measurements have been made by using a computerized digital morphometric system. In order to study the developmental stage of interneurons in our cases we statistically compared analogue neuron types in the brains. As parameters for characterizing non-pyramidal neurons served the pattern of dendritic branching, dendritic length for all dendritic orders within single dendritic fields, and of the whole neuron. The quantitative morphological study of dendritic arborizations revealed that bitufted and large multipolar interneurons had significantly more branching material in the postnatal brains than their prenatal counterparts. These differences were due to the increase of both length and number of branches during perinatal period. Such constant increment in dendritic span and branching provides larger receptive areas and that should improve the development of connections in functional intracortical columns. The quantitative parameter area of dendritic fields and number of branching points of the brain with Down's syndrome revealed in comparison with normal brain of the same age differences such as lower dendritic areas and a higher amount of branching points.

Key words: Motor cortex, Interneurons, Man, Golgi technique

Introduction

The development, maturation, and growth of cerebral cortical interneurons (non-pyramidal neurons) is of interest to the developmental neurobiologist and to the clinician as well. Therefore, serial morphological studies

of the developing cerebral cortex around birth are necessary for the appreciation of both physiological function and structural changes seen in diseases.

The study of the function of single cells, synapto-genesis, and electrogenesis has initiated a renaissance of the Golgi impregnation methods for demonstrating the surface morphology of neurons (Takashima et al., 1980; Schulz and Scholz, 1992; Fernandez et al., 1993). Although the heterogeneous population of cortical non-pyramidal neurons has recently received some attention (Peters and Regidor, 1981; Fairen et al., 1984; Meyer et al., 1989; Meyer, 1987; Marin-Padilla, 1990), the majority of the developmental studies have concentrated predominantly on pyramidal cells. Till now, systematic surveys on the Golgi architecture of the motor cortex have rarely been carried out in human material (Marin-Padilla, 1970, 1976, 1990; Meyer, 1987). For that reason, there was a remarkable lack of sufficient quantitative examinations of Golgi-stained cortical neurons in human due to the complicated availability of this material. Another reason may be that the primary motor cortex was generally regarded as being pyramidal, the classical agranular cortex, in which the interneurons have only little interest.

In order to improve our understanding of the synaptic relationship of interneurons in the motor cortex, it was essential to obtain a clearcut picture of their morphology and distribution pattern. Therefore, the purpose of our quantitative and qualitative study of the developing motor cortex (area 4 of Brodman, 1909) was to provide such an account. We observed perinatal changes in the dendritic arborization of interneurons in the early differentiated human motor cortex in order to evaluate the order and the sequence of neuron growth during the perinatal period.

As the term "non-pyramidal cells" implies, the cells examined in this study were defined by exclusion. They consisted of those neurons which did not meet two primary criteria of classical pyramidal neurons of the motor cortex, the origin of the axon for the basal region of the perikaryon and the possession of an apical dendrite.

The present work is part of a continuing attempt to quantify normal development of interneurons in the

cerebral cortex.

Materials and methods

The precentral gyrus of 2 fetuses (gestational age 36 and 38 weeks) and of 4 term born infants (postnatal age 3days, 1month, 3months, 13months) were studied. The 2 fetuses were born prematurely and died soon after delivery because of immatury and heart failure. The 4 infants studied died of cardio-respiratory failure.

The autopsies of these infants were carried out between 1 and 3h after death. The brains were carefully removed and immersed in 4% formalin. Frontal sections of tissue slices were stained according to Golgi-Cox method.

A magnification of x390 was used for the cell measurements by light microscopy (Axioplan, Carl-Zeiss Oberkochen, Germany). The slice thickness of 150μm was the best for the evaluation of whole dendritic arborization (Leontovich, 1975), and axonal pattern was mostly absent, due to the nature of the Golgi-Cox method, which does not stain axons. All cell measurements were made by using a computerized digital morphometric system (VIDAS-system). All visible interneurons that fulfilled the following criteria were measured: adequate impregnation of soma and dendrites, approximately symmetrical and complete dendritic tree and minimum degree of overlapping with neighbouring neurons. This situation allowed us to establish valid statistical comparisons between data obtained for the different aged brains.

As parameters for evaluation of interneuron growth served the shape and size of perikaryon, the pattern of dendritic branching, the number of branching points, the dendritic length for all dendritic orders within single dendritic fields, and of the whole neuron. The centrifugal ordering method was used to assess the numbers and length of dendritic branches situated at varying distances for the soma (Uylings et al., 1975; Parnavelas and Uylings, 1980). According to this method, all primary dendrites arising for the cell body were considered as first-order segments and the order of dendrites increased by one beyond each bifurcation, with the two segments arising for a common segment always being in the same order.

Results

The following 4 types of cells were observed in our material: 1) large multipolar interneurons with radiating and mostly smooth dendrites (Fig. 1a); 2) small multipolar interneurons with thin dendrites (Fig. 1b); and finally, two types of polarized interneurons: 3) bitufted neurons with a radially elongated dendritic arbor (Fig. 1c); and 4) bipolar interneurons with long, thin dendrites (Fig. 1d).

After birth the non-pyramidal neurons of the multipolar variety were mostly present in superficial layers, but also occasionally in deeper layers of human area 4 (Table 1). The dendrites of small multipolar interneurons were always thin and delicate, with few μm in diameter and poorly branched. They usually divided less than three times before tapering to the end. The shape of the dendritic field was irregularly stellate, but the slenderness of the dendrites implied that their contribution to the neuropil was somewhat less dominant than that of large multipolar interneurons. There were no statistically significant changes (according to Mann-Whitney-test) in used quantitative parameters of minute multipolar interneurons around birth (Figs. 2-4). One cause for this fact could be the small number of bipolar cells measured at each age, preventing an accurate description of their growth rate.

The architecture of the larger multipolar interneurons recapitulated the pattern described above but in expanded form. The primary differences were the more developed, larger and more numerous dendrites and the bigger soma. These cells tended to have perikarya with a mean diameter of approximately 13-19 μm (in comparison with the average 11-14μm soma diameter of small multipolar interneurons), which were relatively constant over the stages of age. From four to nine main dendrites were visible. These could originate for any point on the perikaryon and radiated outward in any direction. The length of the dendritic branches according to their order revealed maximum values in the 3rd order in large multipolar interneurons (Fig. 3). The total dendritic length exhibited no significant changes over the observed period. However, this quantitative parameter increased more or less steadily between week 36 of gestation and 13 months after birth with a

Table 1. Number and distribution of interneurons in superficial (layers I-III) and deep (V, VI) layers.

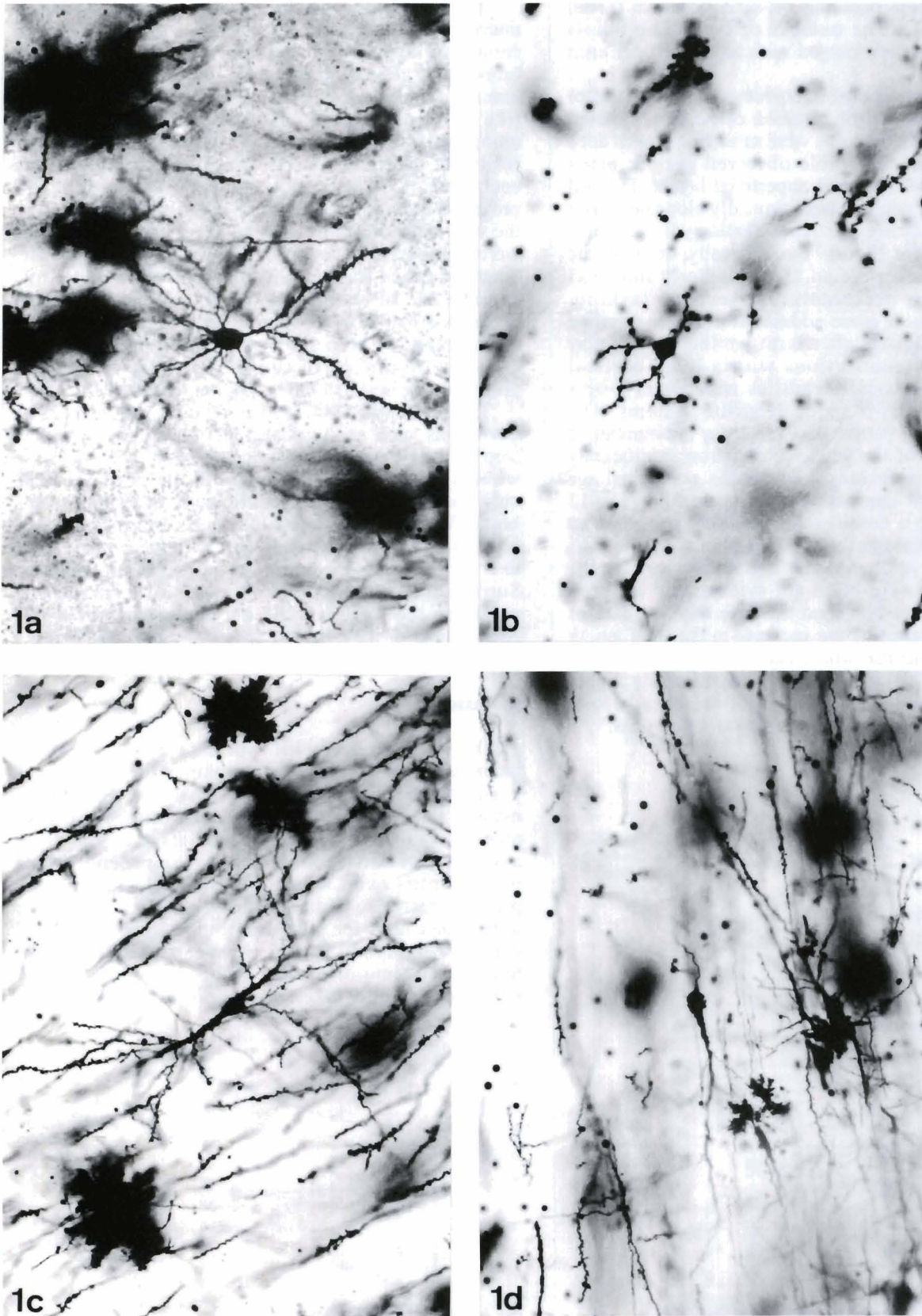| AGE	LARGE MULTIPOLAR INTERNEURON		SMALL MULTIPOLAR INTERNEURON		BITUFTED INTERNEURON		BIPOLAR INTERNEURON		Σ
	Superficial	Deep	Superficial	Deep	Superficial	Deep	Superficial	Deep	
36 weeks	0	2	2	0	1	2	0	0	7
28 weeks	0	0	0	0	0	10	0	4	14
3 days	0	4	1	0	0	16	0	5	26
26 days	3	11	4	3	4	28	2	5	60
3 months	6	5	8	3	4	25	1	1	53
3 months (Down)	4	1	7	0	1	39	0	2	54
13 months	2	9	0	0	2	55	2	0	70
Σ	47		28		187		22		284

Fig. 1. Photographs of four interneuron types found in the motor cortex: large multipolar interneurons with radiating and mostly smooth dendrites (a), small multipolar interneurons with thin dendrites (b), bitufted neurons with a radially elongated dendritic arbor (c), and bipolar interneurons with long, thin dendrites (d). x 160

pronounced increase occuring between postnatal month 3 and 13 (Fig. 2). The number of branching points remained relatively unchanged with around 10 per each neuron (Fig. 4).

The third major class of interneurons in human motor cortex was composed of bitufted cells. Impregnated examples of bitufted neurons were most frequent in deep layers throughout the whole observed period; many fewer examples were seen in superficial layers. The cell bodies of bitufted neurons were typically elongated, with the major axis being vertical. Perikaryal diameter commonly range for 12 to 19 µm. Typically, one dendrite emerged for each pole of the cell body and was distributed in a preferentially vertical fashion. Occasionally, one or more additional dendrite ramified laterally and obliquely after birth, getting in this way more horizontal connections within local cortical circuits. But these small dendrites were never longer than either of the two major dendritic systems. The dendritic branching pattern as a result for the number of branching points of the whole interneuron significantly changed for 21.7 branching points at the gestational age of 36 to 31.2 branching points at the postnatal age of 13 month, indicating a considerable dendritic growth after birth (Fig. 4). Although the two principle dendritic tufts tended to extend approximately equal distances for the perikaryon, they were often not symmetrical in form, one tuft being more branched than the other. A rate of growth was displayed by the increase in the total length per bitufted interneuron which also reaches a peak at 13 month (Fig. 2). This peak value was significantly higher than the values obtained for each of the ages before, between which there were no significant differences.

The fourth and final type of observed non-pyramidal neuron was the bipolar one. These interneurons most frequently appeared in deep layers of immature fetal brains, and after birth they also occurred in superficial laminae (Table 1). The perikarya of bipolar interneurons, like those of bitufted cells, were usually vertically elongated and with an average diameter of 11-16 µm relatively constant over the investigated period. From each pole of the cell body one dendrite emerged and projected in a rigidly vertical fashion. In some instances, the two major dendritic systems of bipolar interneurons were approximately symmetrical. The overall dendritic length of pencil-shape dendritic arbor was subjected to a considerable increase for 486.6 µm (gestational age of 36 weeks) to 874.64 µm (postnatal age of 3 months). The number of branching points remained reletivaly constant around birth and only during the second postnatal year reached the maximum (Fig. 4).

The brain with Down's syndrome showed no cellular deviations such as atypical, inverted, hypo- or hyper-trophic interneurons if compared with control brain without chromosomal aberration at the same age. The only stastically significant difference (according to the Mann-Whitney-test) revealed large multipolar inter-neurons with 13.09 branching points in the Down´s brain to 8.45 branching points in the normal brain (Fig. 5). Surprisingly, this remarkable increase of branching points on the one hand did not cause a simultaneous enlargement of the receptive dendritic fields on the other (Fig. 6).

Discussion

Our study has attempted to depict the development of interneurons in the human motor cortex for 36 weeks of gestation to 13 months of postnatal age. In spite of random staining attained by the Golgi method, we were able to evaluate the developmental stage of cell types and their dendritic material. An impregnation of interneurons axon was not common enough in the present material to permit an analysis of their patterns or to provide a basis for cell classification, as in the work of Fairen et al. (1984). For this reason, we subdivided the interneurons into distinctive groups according to their soma shape and size, and their dimensions and
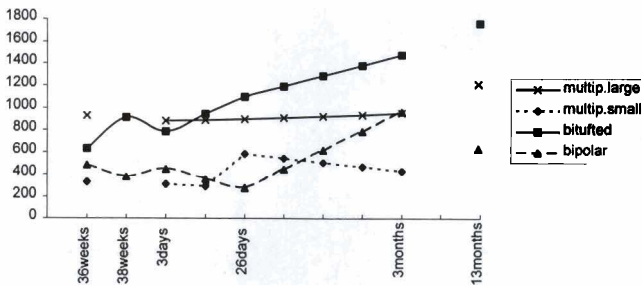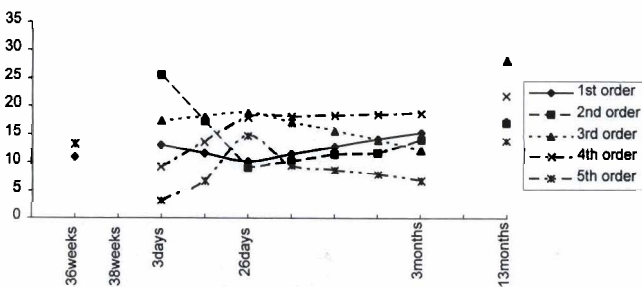

Fig. 2. Total dendritic length.

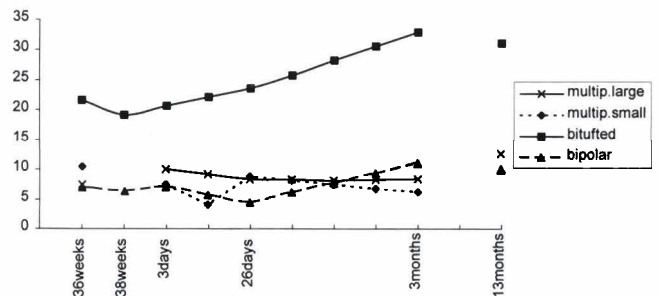**Fig. 3.** Length of dendritic segments according to their order.

Fig. 4. Number of branching points.

branching pattern of dendritic trees. Definition of dendritic patterns was made using the following criteria: shape and size of dendritic arbor, relation to the perikaryon; and their extent through one or more layer. The combination of any of these features produced the characteristic appearance of a dendritic pattern which constitutes an essential component of an interneuron type. This kind of classification had already been used by Feldman and Peters (1978), and Meyer (1987).

The number of identifiable non-pyramidal neurons increased remarkably during postnatal days and reached highest values by the end of the first postnatal month. The table further shows that some neuronal types impregnated very commonly in our material, while others where encountered only in a small number. Several reasons may account for this numerical difference. One reason for the preponderance of certain cell types was a kind of specifity with which different Golgi variants impregnate different neurons. Another cause could be a certain preference for more conspicuous interneurons by the subjective observer.

Except for bitufted interneurons the number of branching points indicating the structure of dendritic arbor seems already established at the date of birth. However, the fact that the basic geometry of the drendritic trees was settled by the moment of normal delivery does not necessarily imply that these interneurons reach final stages of maturation at this age. Existing studies indicate that maturation of these neurons as judged, in addition to dendritic geometry, the density of spines, and the complement of axo-somatic synapses, was achieved during the first postnatal weeks (Marin-Padilla, 1970, 1990).

The most frequently observed interneurons were bitufted cells followed by large multipolar neurons. The bitufted interneurons were characterized by a fusiform soma and a dendritic pattern that corresponded to the bitufted neurons desribed by Feldman and Peters (1978) in the cat. The quantitative morphological study of dendritic arborizations in the motor cortex revealed that bitufted and large multipolar interneurons had significantly more branching material in the postnatal brains than in their prenatal counterparts. These differences were due to an increase in both length and the number of branches during postnatal life. Such

simultaneous and constant increment in dendritic span and branching provides larger receptive areas and that could improve the development of connections within intracortical columns. Our results were consistent with a study performed by Meyer (1987). These studies have found a significant growth acceleration of bitufted and large multipolar interneurons of motor cortex around birth.

Only few neurons belonged to the class of small multipolar interneurons. They were particularly found in the elderly brains mostly in superficial layers II and III. The other neuron type observed in our material could clearly be distinguished on the basis of its smaller soma diameter and its poorly arborized ramified dendrites. These cells belonged to the bipolar type which is very typical for the human cortex but not specific (Cajal, 1911; O'Leary, 1941). All of the general cell types encountered have been described in the literature on interneurons, indicating the lack of any specific forms in the human motor cortex.

In our Golgi preparations, the perikarya of interneurons were distributed in all of the cellular laminae of the cortex. This distribution was not random, however, since approximately 70% of the impregnated cells in the present material were localized in the cortical layers III and VI. The remaining impregnated cells were about equally distributed in layers II and V. Only a few impregnated typical Cajal-Retzius-cells in layer I were encountered in this study. Despite the relatively small sample of interneurons described here, the laminar patterns of distribution were in surprising agreement with the pattern described in previous Golgi studies (Marin-Padilla, 1970, 1988; Meyer, 1987) and with extracellular injections of HRP (horseradish peroxidase, Anderson et al., 1993), which described the average connectivity pattern.

Although our cases do not represent complete development series of ontogenesis of the human motor cortex, some general ideas can be drawn for their analysis. The most significant observations that can be derived for this study were: 1) From the analysis of laminar cell types at different ages, it seems that except for cells of Cajal-Retzius, the interneurons appear and mature first in deep layers. This generally accepted
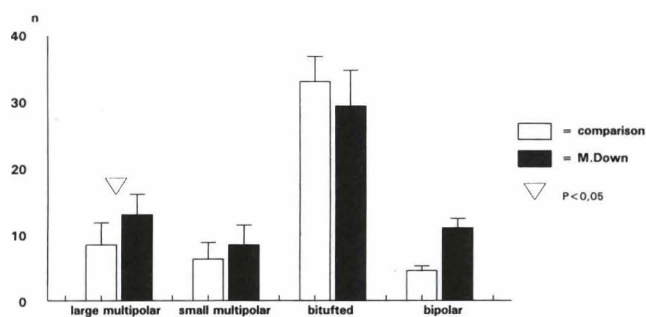

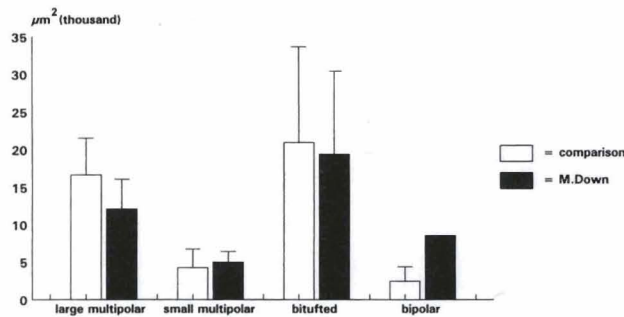**Fig. 5.** Number of branching points.

Fig. 6. Area of dendritic fields.

regularity of cortical development and maturation corresponds to the Marin-Padilla's (1988) ventro-dorsal gradient. According to these results we found a preference of interneurons in superficial layers II and III in the older postnatal brains. 2) The most important figure of development in cortical interneurons was a simultaneous and constant increase in dendritic length and number of branches whereas the size of perikaryon remained relatively constant. 3) The quantative parameter area of dendritic fields and number of branching points of the brain with Down's syndrome revealed only slight differences in comparison with normal brain of the same age, such as lower dendritic areas and a higher amount of branching points. For this reason, it is suggested that possible neuronal damage in Down's syndrome is probably visualized as a loss of dendritic length in the orders which could be compensated by some higher ramification and higher number of branches. This assumption can be supported by data indicating a dramatic cessation of dendritic growth during the first 5 years of life in Down's syndrome children preceded by an excessive early outgrowth of dendritic branches at 3-4 months of age (Wisniewski and Kida, 1994). The slight changes might be structural correlates of the psychomotoric retardation of patients suffering from Down's syndrome.

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