History of Neuroscience Online Projects

Wachstum und Koerpergroesse - a Slide story

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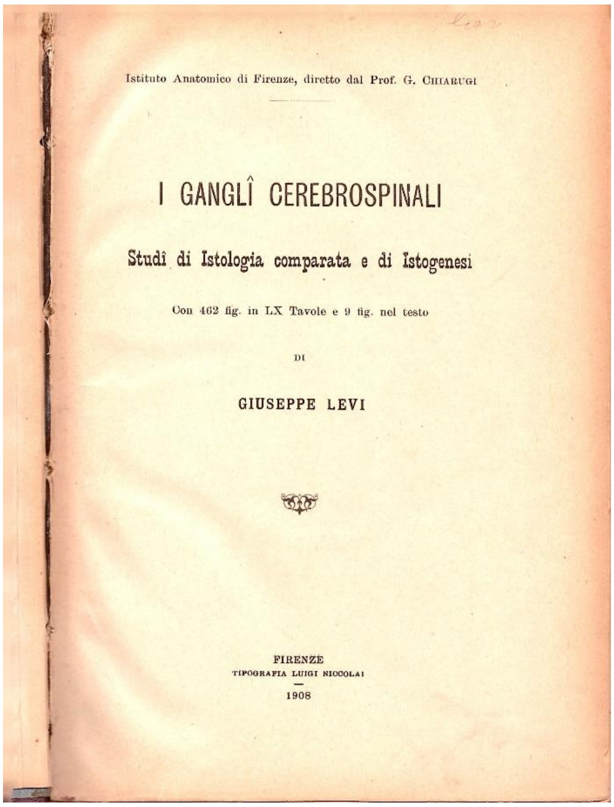
The Italian anatomist and neuroscientist Giuseppe Levi (1872 – 1965) is best remembered for having been the mentor of three Nobel laureates: Renato Dulbecco, Rita Levi-Montalcini and Salvador Luria (Amprino, 1967; Bentivoglio et al., 2006; Grignolio and De Sio, 2009; Ribatti, 2018). In fact, Levi was one of the most influential Italian biologists of his times, distinguished by the breadth of his scientific interests and his pioneering work with cell cultures and microcinematography (Cantino, 2018).

One of the enquiries that were at the center of Levi's interests throughout his long scientific career was the relationship between the number and size of nerve cells and body mass in different animals. This study was started in 1905 (Levi, 1906) and culminated three years later in a vast article on cerebrospinal ganglia, based on histological data obtained in 56 species of vertebrates, including human (Levi, 1908). This publication of nearly 400 pages contains 471 figures, almost all of which are original drawings (Fig. 1).

Through this astonishing research, Levi formulated the concept that sensory neurons grow larger in bulkier animals, their size being proportional to the innervation territory. This simple relationship, which is often indicated as Levi's law, has been validated in recent investigations (see for example Haberberger et al., 2019; Kudo et al., 2021). In subsequent years, Levi further extended the examination of the anatomical and developmental bases of body size, as well as the problem of cell growth and senescence (Levi, 1925).



Istituto Anatomico di Firenze, diretto dal Prof. G. CHIARUGI

I GANGLÎ CEREBROSPINALI

Studî di Istologia comparata e di Istogenesi

Con 462 fig. in LX Tavole e 9 fig. nel testo

DI

GIUSEPPE LEVI

FIRENZE
TIPOGRAFIA LUIGI NICCOLAI
1908

Fig. 1 - The heading of Levi's 1908 article on cerebrospinal ganglia.

Levi's work on neuronal size originated from two main considerations. The first is that a nerve fiber is the extension of an individual nerve cell, implying that the size of the cell body must be proportional to the mass of the cytoplasm contained in all the prolongations in order to sustain trophic and metabolic activities. This exceptional cell geometry is particularly challenging in large vertebrates, in which neurons have extensive innervation territories and the axonal volume may exceed over a thousand times that of the cell body (Wang et al., 2008; Smith, 2009). The second issue that was central in Levi's conception is that neurons cannot replicate themselves, as their numbers are fixed during relatively early phases of development. It follows that the size of neurons must increase progressively as peripheric innervation territories extend during somatic growth. It is no surprise, therefore, that the 1908 treatise contains observations made in large animals that are not commonly the object of histological analyses, including a shark, an iguana, and large mammals such as horse and ox. During a trip to the Norwegian Spitzbergen island, Levi even entered the skull of a whale to search for cranial nerve ganglia, without substantial success. This heroic episode is delightfully narrated in "*Lessico famigliare*", an authobiographic narrative by Natalia Ginzburg, Levi's daughter and a leading figure of the Italian literature of twentieth century.

Among the animal models studied by Levi, one species that particularly attracted his interest was the huge ocean sunfish *Orthagoriscus mola* (Linnaeus 1758, presently accepted as *Mola mola*). The sunfish, which is also found in the Mediterranean sea, is one of the largest bony fishes, with adults reaching a weight of several hundred kilograms. Levi was particularly fascinated by the fact that the sunfish seems to be genetically programmed for being a small fish, with a relatively short spinal cord. As the animal grows, the relatively low number of neurons must extend their fibers considerably, to keep the pace with the expansion of the innervation territories (Fig. 2). Moreover, as other animal species investigated by Levi, the sunfish never stops growing, but continues to increase its size throughout life, albeit at a progressively reduced rate.

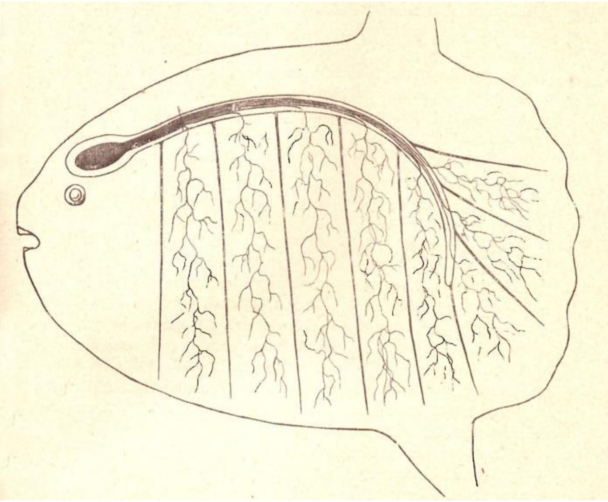


Fig. 2 - One of the drawings used by Levi to illustrate the relationship between the nervous system of *Orthagoriscus mola* and innervation territories (Levi, 1908). The spinal cord occupies only a limited part of the vertebral canal implying that the *cauda equina* has a huge extension. The sunfish used for the 1908 study had a rostral-caudal length of 77 cm and a weight of 20 kg.

Levi had initially studied relatively small specimens of sunfish (a juvenile of 3 kg and two larger individuals of 18 and 20 kg) during the period he spent at the Stazione zoologica in Naples (1905-1906). Eight years later, Levi moved to Palermo, where he remained until 1919, when he finally became director of the Institute of Normal Anatomy at the University of Torino (Filogamo, 2018). In Palermo, he could examine two other specimens of sunfish, of 40 and 80 kg, both "in excellent state of conservation" (Levi, 1919). As detailed by Levi himself, the two fish had been donated to him by "Cav. Dentici", who was director of the tuna fishery in Trabía, a coastal town located 30 km East of Palermo (see also Piccolino et al., 2020).

One of the slides that Levi produced in his laboratory in Palermo has survived to the present day and has been made available to the authors of this brief essay (Fig. 3). Most likely, the slide was carried by Levi himself to Torino when he moved from Palermo in 1919. The writing on the label leaves little doubt on the identification of the slide, as it seems obvious that "O." stands for *Orthagoriscus*, "80 k" identifies the weight of the animal, "G spin O" stands for Spinal ganglion of *Orthagoriscus*, and "2°" likely distinguishes the ganglion level. Therefore, the slide most likely contains sections of a dorsal root ganglion obtained from the largest sunfish that Levi had received from Cav. Dentici in Palermo. Comparison of Levi's original drawings (see below) with micrographs obtained with a modern microscope also revelas striking similarities (Fig. 4, 5). One can also appreciate the high level of accuracy and attention to detail that characterizes Levi's histological labor, and the scientific and artistic quality of his *camera lucida* illustrations.

Fig. 3 - The slide containing 12 sections of a spinal ganglion of *Orthagoriscus* (a) and a tiled reconstruction of one of the sections (b). Scale bar = 1 mm.

Examining the sections treated with the Cajal-De Castro reduced silver method, Levi described, and depicted through drawings, the astonishing shape that characterizes sensitive neurons in the larger specimens of *Orthagoriscus*. Levi directed his attention to the unique polymorphic appendages that arise from the cell body, giving rise to "an intricated and extensive fenestrated apparatus" (Fig. 4, 5).

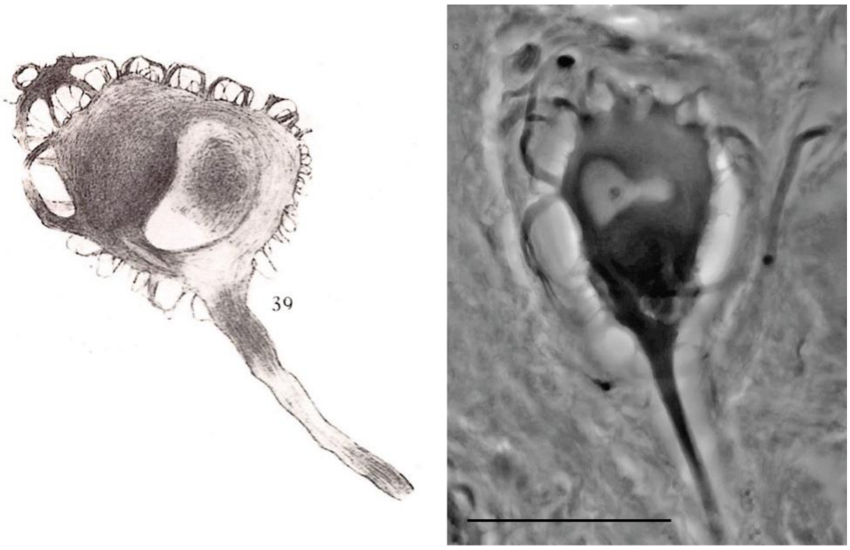


Fig. 4 - A drawing from the 1908 essay showing a "type 1" fenestrated cell with protoplasmic arcades emerging from the cell body and a light photomicrograph taken from one of the slide sections showing similar appendages. The photomicrograph is a maximum-intensity projection of a stack of 8 optical sections obtained with a motorized microscope. Scale bar = 100 µm.

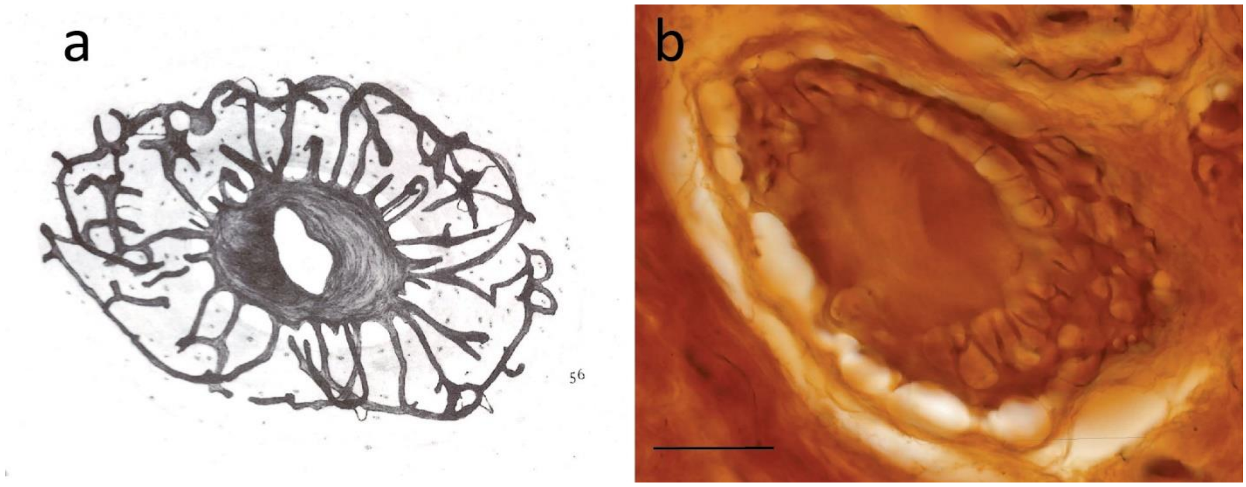


Fig. 5 - A drawing from the 1908 essay showing a "type 8" fenestrated cell with distinct protoplasmic arcades emerging from the cell body and a light photomicrograph of a ganglion neuron with similar morphology. Scale bar = 50 µm.

These projections are described meticulously in the 1908 essay, in which Levi distinguished 8 distinct types of multipolar cells and 16 types of fenestrated cells through an astonishing (arguably excessive) level of histological detail. The main observations are conveyed more concisely in the 1919 paper, based on a comparison of the four sunfish of different ages and sizes (3, 20, 40 and 80 kg) that he investigated (Fig. 6). Levi noted that "as ganglion cells attain a certain size, the peripheral part of the cell body stops growing as a compact mass, but undergoes compicated transformations". These "transformations" consist in the outgrowth of lobulated and club-like projections ("paraphytes" by Nageotte) as well as in a process of "canalization" of the protoplasm of the ganglion cells, resulting in an intricated "network of fibers" connected to the cell body. In some of the largest cells, the fenestrated apparatus appears as a composite web of merged protoplasmic arcades (Fig. 5, 7).

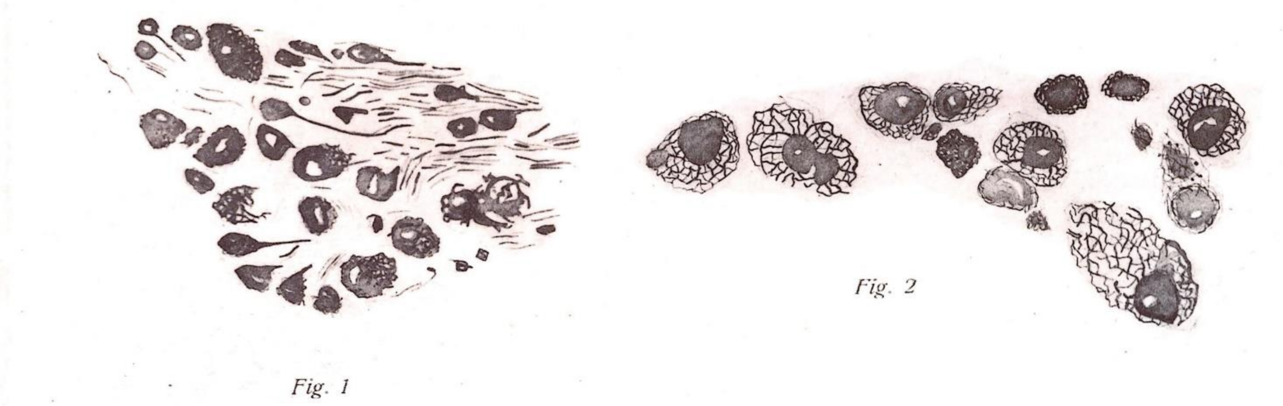


Fig. 6 - Drawings from Levi's 1919 article. *Fig. 1* depicts one section obatined from a spinal ganglion of an *Orthagoriscus* of 20 kg; *Fig. 2* shows a section from the specimen of 80 kg. Notice that the cell body size of sensory neurons is comparable in the two sections. However the "fenestrated apparatus" appears considerably more extended and elaborated in neurons of the larger specimen.

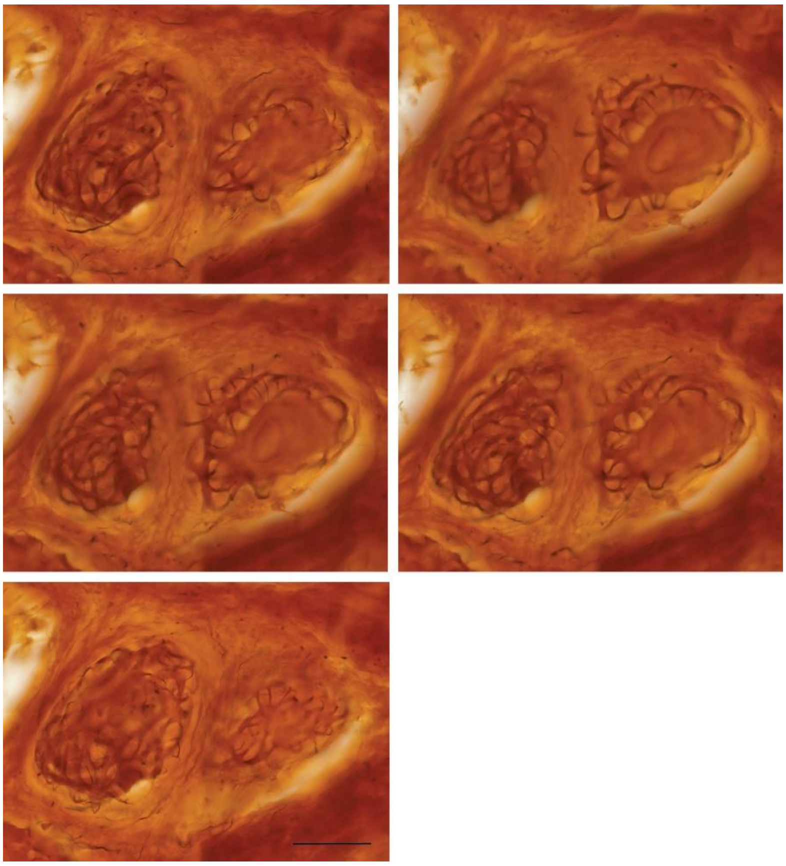


Fig. 7 - Consecutive optical sections of two ganglion neurons characterized by an extensive fenestrated apparatus. The presence of protoplasmic arcades rising from the cell body is evident in one of the neurons (right). The other neuron is "sectioned" more peripherally, revealing the complexity of the network of protoplasmic projections surrounding the cell body. Scale bar = 50 μm.

No doubt Levi was fascinated by these remarkable neuronal specializations, and in both the 1908 and 1919 treatises he elaborated on their possible significance. Levi realized that the impressive growth that the *Orthagoriscus* sensitive neurons undergo (the largest cells attain 500 micrometers in diameter!) entails structural and metabolic problems largely related to the changes in the cell surface to volume ratio. A rough estimation suggested that the volume of the largest cells in the 20 kg fish was 634 times greater than the volume of ganglionic neurons in the smaller fish of 3 kg. Such an extraordinary growth needs to be accompanied by changes in cell morphology. The "fenestrated apparatus" thus appears as an adaptation to increase the cell surface and cope with the challenges imposed by an increased neuronal size. It is worth mentioning that the presence of fenestrations in sensory ganglia neurons had been described, a few years earlier, by Ramon y Cajal, who introduced the expressions *células fenestradas* and *aparado fenestrado*. However, contrary to Levi, Cajal did not establish a relationship between the fenestrated cells and the concept of cell and somatic growth (for a detailed account see Piccolino et al., 2020).
Levi realized that neuronal size is not determined uniquely by body mass, but also depends on ontogenetic processes that intervene to set a limit for body growth in different species. He believed that in animals, like *Orthagoriscus*, characterized by a continuous growth, neurons undergo a persistent period of expansion and "differentiation", accompanied by the appearance of structural modifications. Obviously, this is due to the fact that neuroblasts stop dividing at relatively early stages of development, and the nervous system, contrary to other organs, cannot increase its volume by the proliferation of undifferentiated cells. In

agreement with this view, Levi reported that not only ganglion cells, but also spinal and brainstem neurons reach larger sizes in the bigger specimens of *Orthagoriscus* (Levi, 1919).

In the 1919 article, Levi also reflected on the fact that the nervous system of mammals, including human, is not susceptible to modifications in adulthood. Examining spinal ganglia of various adult individuals, he noted the absence of major modifications at the cellular level. However, when his attention shifted to cortical areas, he referred to the existence of a potential "morphological perfectivity" of neurons. Levi inferred that neurons involved in sustaining the most elaborated psychic functions are subject to receiving novel inputs, which may lead to an increase of their "neurofibrillary mass" (i.e. their prolongations), even after the end of the developmental period. This is a tremendously innovative view, which precedes by three decades the work of Donald Hebb about neuronal plasticity (Hebb, 1949). It seems that Levi derived this concept from his contemporaries rather than from his own cytological investigations. The work of Verworn (1906) appears to have been particularly influential in formulating these ideas. As quoted in the 1919 article, Verworn hypothesized that "repeated stimuli activate anabolism and leave physical traces in neuronal cells". Curiously, Levi predicted that histological work might reveal those physical traces, described by him as "an increased complexity of dendritic arborizations" and the "growth of new collaterals of neurites". However, he concluded, "it is doubtful that the present techniques may make manifest such subtle differences". Hence, Levi recognized that a thorough appreciation of the "morphological perfectivity" of neurons would have been possible only after the introduction of major technological advances in microscopic techniques. Despite this, Levi's colleague Ernesto Lugaro, in Italy, and Santiago Ramon y Cajal, in Spain, using basic microscopes, were laying the foundations of modern neurocytology and launching the concept of neural and synaptic plasticity (Berlucchi & Buchtel, 2009).

Bibliography

Amprino R (1967) Giuseppe Levi (1872–1965). *Acta Anat*. 66: 1-44. doi: 10.1159/000142913

Bentivoglio M, Vercelli A, Filogamo G (2006) Giuseppe Levi: mentor of three Nobel laureates. *J. Hist. Neurosci.* 15: 358-368. doi: 10.1080/09647040600888974

Berlucchi G, Buchtel HA (2009) Neuronal plasticity: historical roots and evolution of meaning. *Exp. Brain Res.* 192: 307–319. doi: 10.1007/s00221-008-1611-6

Cantino D (2018) Le principali linee di ricerca di Giuseppe Levi. *Medicina nei Secoli Arte e Scienza: Journal of History of Medicine* 30/1: 359-380.

Filogamo G (2018) Giuseppe Levi. *Medicina nei Secoli Arte e Scienza: Journal of History of Medicine* 30/1: 383-402.

Grignolio A, De Sio F (2009) Uno sconosciuto illustre: Giuseppe Levi tra scienza, antifascismo e Premi Nobel. *Medicina nei Secoli Arte e Scienza: Journal of History of Medicine* 21/3: 847-914.

Haberberger RV, Barry C, Dominguez N, Matusica D (2019) Human dorsal root ganglia. *Front. Cell. Neurosci.* 13: 271. doi: 10.3389/fncel.2019.00271

Hebb DO (1949) The organization of behaviour. A neuropsychological theory. Wiley, New York. doi: 10.1016/s0361-9230(99)00182-3

Kudo M, Wupuer S, Kubota S, Seki K (2021) Distribution of Large and Small Dorsal Root Ganglion Neurons in Common Marmosets. *Front. Syst. Neurosci.* 15: 801492. doi: 10.3389/fnsys.2021.801492

Levi G (1906) Studi sulla grandezza delle cellule. 1. Ricerche comparative sulla grandezza delle cellule dei mammiferi. *Arch. Ital. Anat. Embriol.* 5: 291-35.

Levi G (1908) I gangli cerebrospinali. Studi di istologia comparata e di istogenesi. *Arch. Ital. Anat. Embriol*. Suppl. al vol. 7: 1–393.

Levi G (1919) Nuovi studi sull'accrescimento delle cellule nervose. Ricerche in *Orthagoriscus mola*. *Atti della Reale Accademia delle Scienze, Lettere e Arti di Palermo* 11: 3-11.

Levi G (1925) Wachstum und Körpergröße. Die strukturelle Grundlage der Körpergröße bei vollausgebildeten und im Wachstum begriffenen Tieren. Ergebn. *Anat. Entwickl.-Gesch.* 26: 187–342.

Piccolino M, Barasa A, con la collaborazione di Cantino D (2020) Giuseppe Levi, le balene, il pesce luna e i gangli cerebrospinali. Viaggio storico-scientifico tra le ricerche sulle cellule nervose di un grande biologo del Novecento. *Naturalmente. Fatti e trame delle scienze.* Gennaio 2020.

Ribatti D (2018) Il maestro dei Nobel. Giuseppe Levi, anatomista e istologo. Carocci Editore.

Smith DH (2009) Stretch growth of integrated axon tracts: extremes and exploitations. *Progr. Neurobiol.* 89: 231-239. doi:10.1016/j.pneurobio.2009.07.006

Verworn M (1906) Die cellularphysiologische Theorie des Gedächtnisses. *Zeitschr. F. allg. Physiol.*, Bd. 6.

Wang SS, Shultz JR, Burish MJ, Harrison KH, Hof PR, Towns LC, Wagers MW, Wyatt KD (2008) Functional trade-offs in white matter axonal scaling. *J. Neurosci.* 28: 4047-4056. doi: 10.1523/JNEUROSCI.5559-05.2008