Acta Neuropathol (1992) 83: 504 – 509

Acta Neuropathologica

Formation of "dark" (argyrophilic) neurons of various origin proceeds with a common mechanism of biophysical nature (a novel hypothesis)

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Received March 6, 1991/Revised Oct 17, 1991, accepted October 19, 1991

Summary. Based on recent findings described in accompanying reports as well as on relevant observations in the literature we hypothesize that: (1) the fundamental elements in the mechanism of the formation of "dark" (argyrophilic) neurons are independent of the causative conditions including post-mortem or in vivo mechanical injuries and various in vivo pathometabolic processes such as blood recirculation following ischemia; (2) the causative conditions, each in its own mechanical or metabolic way, induce the same morphopathological damage at one point only within each affected neuron; (3) this damage spreads throughout the respective somato-dendritic or axonal domain and entails type III argyrophilia; (4) the intraneuronal spread of the morphopathological damage consumes mechanical energy stored by the neurofilaments in the form of a metastable inner structure, and (5) is propagated by a process working, in certain structural and energetical respects, on the domino principle; and (6) the primary neuronal damage caused in the above manner might be secondarily modified in different directions by different post-causation conditions.

Key words: "Dark" neuron – Type III argyrophilia – Neurofilaments – Structural changes – Stored mechanical energy

The reason for finding a common mechanism for the formation of "dark" (argyphilic) neurons of various origin

In animal experiments post-mortem mechanical injury of the unfixed brain [32] as well as a large number of different pathological conditions brought about before perfusion fixation and delayed autopsy have been found to result in the same set of "exclusive" light microscopic changes in neurons. The pathological conditions mentioned include blood recirculation following cerebral ischemia, insulin hypoglycemia, status epilepticus, deafferentation, poisoning with various agents, as well as laceration, compression and concussion of the brain [9–12, 32]. The light microscopic changes in question are as follows: (1) considerable increase in stainability of the soma with basic dyes; (2) marked shrinkage of the soma; (3) pycnosis of the nucleus, (4) corkscrew-like deformation or tortuosity of numerous dendritic segments; (5) "all or nothing" type III argyrophilia of the somato-dendritic domain; and (6) co-existence of damaged and normal neurons next to each other, frequently within an otherwise normal parenchymal environment (Fig. 1). To designate neurons having these morphopathological features several synonyms have been used by various authors, such as "dark", "hyperchromatic", "chromophilic", "contracted", "retracted", "shrunken homogeneous" or "specialized" [3] and such cells have recently been called "type Ia and Ib injured neurons" [1], neurons showing "type III and IV neuronal response" [18] or "neurons with argyrophilic damage" [9].

The startling morphopathological similarity between the "dark" (argyrophilic) neurons of various origin strongly suggests a close similarity in their respective mechanisms of formation.

The argyrophilic neuronal damage spreads throughout the somato-dendritic domain after focal induction

The argyrophilic neuronal damage appears to be an "all or nothing" phenomenon with the following restrictions: (*i*) if the soma of a neuron is affected, a continuous but not necessarily complete network of its dendrites is also affected; (*ii*) the dendritic arborizations of unaffected somata also remain unaffected; (*iii*) somal argyrophilia does not necessarily entail axonal argyrophilia; and (*iv*) axonal argyrophilia does not necessarily entail somato-dendritic argyrophilia [9, 10].

Argyrophilic and non-argyrophilic neuronal somata can often be found next to each other. Consequently,

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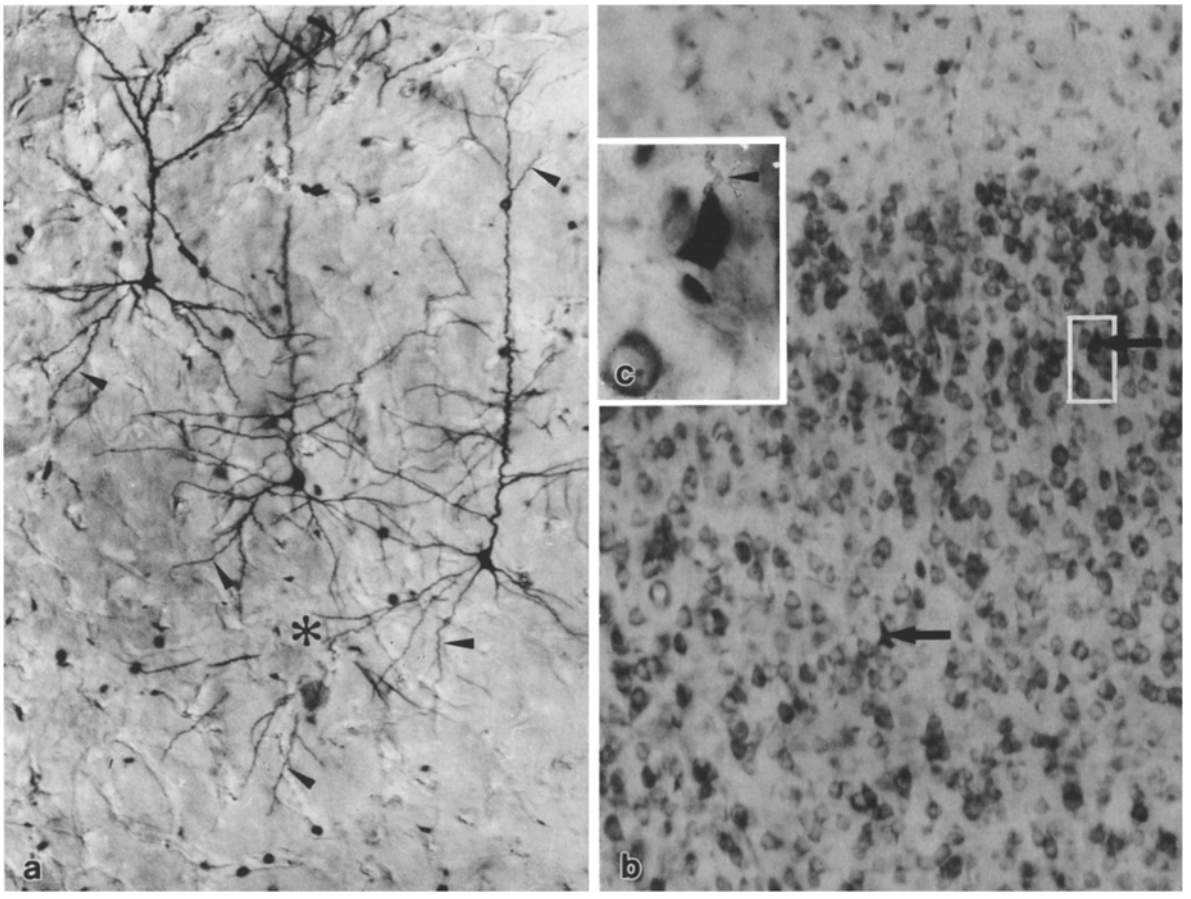

Fig. 1a–c. "Dark" (argyrophilic) neurons produced in the neocortex by 2-h blood recirculation following 30-min severe ischemia (cf [10]). Adjacent sections (60 μm thick) were stained with a new silver method demonstrating "dark" neurons together with their dendritic arborizations (**a**), and toluidine blue (**b**). **c** Enlargement of the area framed in **b**. *Arrows* point to "dark" neurons, *arrowheads* to corkscrew-like or tortuous dendritic segments; the *asterisk in* **a** indicate argyrophilic dendrites cut off from their parent argyrophilic soma which is situated in the adjacent section stained with toluidine blue. **a, b** ×160; **c** ×850

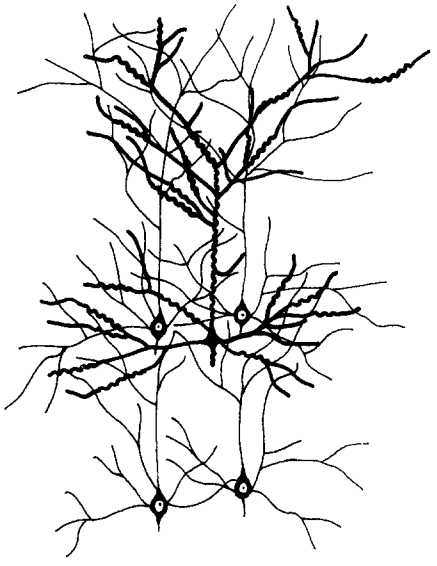

Fig. 2. Spatial arrangement of argyrophilic and non-argyrophilic dendritic arborizations of argyrophilic and non-argyrophilic neuronal somata existing in close vicinity of each other

their respective argyrophilic and non-argyrophilic dendrites run in the close vicinity of each other at a large number of sites (Fig. 2). For this reason, it is inconceivable that the pathological conditions in question (e.g., concussive head injury) should damage every point of the dendritic arborizations of the argyrophilic somata and should, at the same time, spare all points of the dendritic arborizations of the neighboring non-argyrophilic somata. To explain this phenomenon, we advance the idea that each silver-stained soma-dendrite complex is affected initially by the causative condition at one point only, and some intraneuronal focal disturbance caused thereby spreads throughout the soma and its dendritic tree, resulting in generalized morphopathological damage entailing argyrophilia. This idea implies that the probability of inflicting the intraneuronal focal disturbance is extremely low compared to the rate of the intraneuronal spread of the argyrophilic damage.

After head injury the occurrence of argyrophilic axonal segments coursing within a fasciculus parallel with and next to non-argyrophilic ones can be explained by the same train of thoughts. In the case of argyrophilic axons belonging to non-argyrophilic somata the focus of the primary disturbance initiating the spread of the argyrophilic damage must be within the affected axonal segment itself. Thus, neither the initiation nor the spread of

the argyrophilic damage needs assistance from the cell nucleus or the perikaryal metabolic machinery.

To prevent misinterpretation, the following difference between Golgi-staining and type III argyrophilia should be realized: in the Golgi method the coloring substance (silver dichromate), starting from a single crystallization nucleus, grows into the whole somal dendritic domain of the respective neuron during staining [2, 6]. In contrast, the formation of metallic silver during staining with methods demonstrating type III argyrophilia is simultaneously initiated at a multitude of sites within each affected soma-dendrite complex [7, 8, 32]; even in dendrites separated from their parent argyrophilic soma during sectioning (Fig. 1a; cf. [9]). Consequently, the intraneuronal spread of the type III argyrophila is a pre-fixation phenomenon.

The intraneuronal spread of the argyrophilic damage induced by post-mortem concussive head injury is independent of metabolic processes

Argyrophilic neuronal damage showing all morphopathological features enumerated in the first section of this report can also be brought about by concussive head injuries inflicted at the 30th minute of transcardial perfusion of chilled (<3 °C) physiological saline or at the 30th min of transcardial perfusion of a buffered formaldehyde-glutaraldehyde fixative [12]. If the assumption described in the previous passage (focally initiated intraneuronal spread of some morphological damage entailing argyrophilia) also applies to the "cold" and "half-fixed" conditions, two major problems arise: (i) by what biological machinery is the argyrophilic damage driven forward, and (ii) what kind of energy is consumed by it.

Considering point (i), there are two neurophysiological processes capable of transporting a "disturbing" signal which trigger a pathometabolic process damaging the cell structure and inducing argyrophilia, from the intracellular focus of the traumatic damage throughout the whole affected neuronal domain: the fast intraneuronal transport and the bioelectrical activity of the plasma membrane. However, both of these are known to stop functioning in mammals at about 10 °C and under anoxid condition [16, 26, 27]. Similarly, any other as yet unknown cytobiological process depending on metabolism (chemical energy), which would be capable of transporting a triggering signal would at the same time be slowed down dramatically in the "cold" and "half-fixed" conditions. Intracellular diffusion of any signalling substance which might be focally released by the trauma in the affected neuronal elements can also be disregarded because of the low rate of diffusion and the progressive dilution of such a substance as it moved forward.

Considering point (ii), as regards the energy supply, even if a triggering signal could cover long distances, no notable structural damage (somal shrinkage, dendritic tortuosity) would be caused by any pathometabolic process during the relatively short time available, both because of the serious shortage of easily consumable chemical energy due to the anoxic condition [17] and because of the massive decrease in the rate of the enzymatic consumption of chemical energy due to the "cold" [30] and "half-fixed" conditions.

Hypothesized mechanism of the intraneuronal spread of the argyrophilic damage induced by post-mortem concussive head injury

To solve the problem discussed in the previous passage, we re-raise the idea of Metuzals and Izzard [22] that the neurofilaments, elements of a continuous cytoskeletal network, are capable of storing mechanical (torsional) energy. In addition we put forward two complementary premises: (1) the mechanical energy is stored in the form of a metastable ("dischargable") inner structure of the individual neurofilaments and (2) the "discharge" of stored mechanical energy can spread throughout the neurofilamentous network, after focal initiation, in a manner similar to the falling of a row of dominoes when one is pushed (Fig. 3).

Point 1 tacitly recognizes the existence of another inner structure of neurofilaments, that has a lower level of mechanical energy. As an inherent quality of each metastable structure, the metastable inner structure of neurofilaments is maintained without consumption of any external or internal energy. Thus, it remains unchanged for hours or longer even after death. However, as another inherent quality of each metastable structure, the metastable inner structure of neurofilaments can be transformed into an activated form by an external mechanical force at the point of its application, if its parameters (direction, magnitude, duration, etc.) are "adequate". This activated structure spontaneously turns into the low-energy structure with local release of mechanical (kinetic) energy manifesting itself as a mechanical force.

Point 2 suggests that the parameters of this "internal" mechanical force are "adequate" for the transformation of the metastable inner structure of neurofilaments into the activated form at the next-neighboring site capable of the structural change described in the previous point.

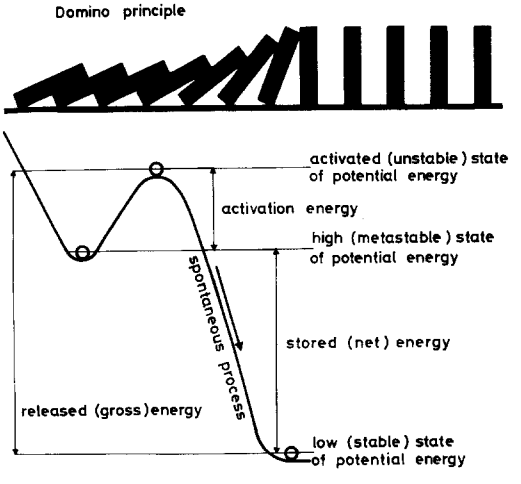

Fig. 3. The "energy hill" and the terminology of energy levels and their differences

In this way the change from the metastable structure into the low-energy structure of neurofilaments will spread throughout their entire network without consumption of chemical (metabolic) energy, if an external force initiates it at one site.

Neurofilaments have a complicated helical inner structure [14]. It can be easily imagined that all the structural and energetical features described in the above two points apply to any helical supramolecular structure if each turn of the helix is "wound up" enzymatically before being cross-linked to the next turn by an appropriate number of weak chemical (van der Waals) bonds. In such a structure splitting of a few of adjacent cross-linking bonds by some external mechanical force (or some pathometabolic process, see below) would initiate the spread of the splitting of all cross-linking bonds along the "backbone" of the helix, driven forward by the torsional energy stored.

Correlation with the light microscopic features of the argyrophilic neuronal damage induced by post-mortem concussive head injury

The stored (the net, see Fig. 3) mechanical energy released in the whole network of neurofilaments after focal initiation may also damage the structure of other cytoskeletal components interconnected with them as well as the structure of the plasma and nuclear membranes to which they are anchored [13, 23, 31]. Concomitant release of loosely bound proteins and sequestered K^+ ions [21] could result in the rapid shrinkage of the soma.

As an additional consequence of the supramolecular damage of cytoskeletal components a re-arrangement of negative and positive side groups of various macromolecules may occur, which could account both for the increase in stainability with basic dyes [15] and the induction of type III argyrophilia [7] without any subsequent metabolic (chemical) change.

Conversion of the supramolecular helical structure of individual neurofilaments from the metastable form to the low energy form could cause a change in their macro-helicity [24]. Co-ordinated change of the macro-helicity of all neurofilaments existing within a dendritic or axonal segment could result in its becoming coiled (its corkscrew-like deformation or tortuosity).

The "all or nothing" involvement of the affected neurons and co-existence of damaged and normal neurons are explained in earlier passages.

The stop of the supposed intraneuronal spread of the argyrophilic damage at the axon hillock could be accounted for by the differences in the composition and cytoskeletal arrangement of neurofilaments between the axonal and the somato-dendritic domains [29].

Correlation with earlier findings concerning the storage of mechanical energy

Using high-resolution electron microscopy Metuzals et al. [22–25] demonstrated that individual neurofilaments had a complicated helical inner structure and, in different regions of the same axon, two variants of their macro-helicity could occur. Their network had thereby two different states of structural order. They proposed that these structural states represented different levels of mechanical energy, and that, as a result of coiling and uncoiling of the individual neurofilaments, the transition from one structural state to the other was reversible. Thus, the neurofilamentous network was imagined as the working substance of a biological machine capable of converting chemical energy into mechanical energy.

Based on a study of the physical properties and morphological peculiarities of the axoplasm isolated from the Myxicola giant axon, Gilbert [14] concluded that these could be accounted for by the assumption that individual neurofilaments behaved like elastic rods identically twisted before being cross-linked with each other at numerous sites. In this way a large, relatively rigid structure is constructed of tiny, nonrigid elements. Although he did not mention the possibility that the stored elastic energy could be discharged, he supposed some relationship to several puzzling observations made on vertebrate nerve cells, such as the macro-helicity of neurofilaments, the spiral and ring forms of condensed bundles of neurofilaments (neurofibrils) as well as the corkscrew shape of axons near transsections or contrictions.

In biological structures other than neurofilaments a focally initiated spreading discharge of stored mechanical energy is a well-documented phenomenon (cf. association-induction theory of Ling [19]). For example, the negatively supercoiled DNA double-strand ring represents a twisted helical spring, which is enzymatically "wound up" with simultaneous splitting of ATP; relaxation of the supercoiled DNA proceeds without consuming chemical energy [4]. Similarly, during the centrifugal migration (dispersion) of pigment granules in erythrophores mechanical energy is stored in some cytoskeletal structure through the expenditure of chemical energy, while their centripetal migration (aggregation) is powered by the stored mechanical energy [20].

Correlation with the artifactual formation of "dark" neurons

The artifactual formation of "dark" neurons by unintentional compression of unfixed or improperly fixed brains during removal from the skull is a century-old mystery in both human and experimental neuropathology [5, 28]. This is not surprising, since artifactual "dark" neurons can be produced, even after careful perfusion-fixation, if autopsy is performed within a relatively long ("critical") period of time, which is 24 h for 10 % formaldehyde, 6 h for Bouin's fixative and 4 h for Heidenhain's Susa solution [3]. Based on the conclusion of the first section of this article we assume that the artifactual "dark" neurons are produced by intracellularly stored mechanical energy, the discharge of which is initiated by the unintentional compression of the brain before the

metastable inner structure of neurofilaments has been made stable by fixation.

Correlation with the in vivo formation of "dark" (argyrophilic) neurons

Concerning the in vivo-produced "dark" (argyrophilic) neurons we assume that the causative conditions enumerated in the first section of this article initiate, each in its own mechanical or pathometabolic way, the conversion of the metastable inner structure of neurofilaments into the low-energy structure at a single focus within each affected soma-dendrite complex. From this primary focus the same type of structural conversion spreads throughout the entire cell domain, driven forward by stored mechanical energy, as described in a previous passage. Finally, the morphopathological change thereby caused is secondarily modified in various directions by different pathometabolic conditions.

Correlation with human neuropathology

It goes without saying that the hypothesis presented also applies to human neuropathology. However, because perfusion fixation is impracticable, it is difficult, especially in acute cases, to differentiate between artifactual "dark" neurons and those actually produced by a disease or pathological condition.

Terminological proposal

We propose that the traditional term "dark" neuron and its synonyms including "argyrophilic" neuron should be replaced with the term "collapsed" neuron, which better expresses the essence of the underlying morphopathological process and reckons with the potential for recovery of the neurons affected [11].

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