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Commentary

Astroglial Ca^{2+} signals trigger pathological behaviour in optogenetic mouse*

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> "We thus obtain at the very outset the important criterion for the interpretation of morbid processes, that the affections of the brain and spinal marrow may sometimes be rather interstitial, at others rather parenchymatous, and experience shows us that this very interstitial tissue of the brain and spinal marrow (*i.e.* Neuroglia - AV) is one of the most frequent seats of morbid change…"

Rudolf Virchow, (1860). Cellular Pathology 1 st English translation; Robert M De Witt, New York, p. 317

Until recently neuropathology has been grossly neurono-centric. Neurones are universally acknowledged as the origin of the pathology and consequently neuropharmacology is focused on molecules interacting with neuronal channels, receptors, transporters and enzymes. The integrity and functional stability of the nervous system are however maintained not by neurones but by an extended class of neuroglial cells broadly responsible for tissue homeostasis and defence. The pathological potential of neuroglia was very much considered by neuroanatomists and pathologists of 19th - early 20th centuries (such as, for example Alois Alzheimer, Franz Nissl, Pio Del Rio-Hortega and William Lloyd Andriezen), who frequently characterised pathologically modified glial cells in the context of various neurological disorders. The recent decade had witnessed a renaissance of neurogliopathology, and it is now firmly established that various types of neuroglia contribute to pathological evolution of most, in not all, neurological diseases [4,7]. The role of glial cells in neuropathology is complex and disease-specific; glial changes can be primary and drive neuropathological changes; the glial reaction can be secondary in response to lesion or neuropathology, the combination of both in the most likely case.

Astrocytes are primary homeostatic cells of the CNS; they populate both grey and white matter and they assume full responsibility for supporting nervous tissue at all level of organisation, from molecular to organ [12]. Astrogliopatholgy is generally classified into (i) astroglial pathological remodelling, (ii) astrogliosis, (iii) astroglial degeneration with atrophy and loss of function and (iv) astroglial reactivity [13]. These fundamental pathological changes occur in sequence or in combination and thus may define progression and outcome of neurological disease. The study of Baljit Khakh and his colleagues, published recently in *Cell* [6] extended this classification by introducing a new type of astrogliopathy: according to their study the gain of normal physiological function of astrocyte emerged as a primary mechanism responsible for aberrant excitability of neuronal networks potentialy linked to a behavioural disorder known as Attention Deficit-Hyperactivity Disorder (ADHD).

Pathophysiology of ADHD remains virtually unknown; however imaging patients brains revealed malfunction of the striatum, the largest basal ganglia. Khakh and colleagues discovered that hyperactivity of striatal neurones is driven by astroglia-derived synaptogenic factor trombospondin-1 (TSP1). The signalling cascade leading to the release of astroglial TSP1 starts from enhanced Ca^{2+} activity in astrocytes induced by depolarisation of GABAergic striatal medial spiny neurones (MSN). The authors hypothesized that Ca^{2+} activity in astrocytes is triggered by dendritic GABA release. To test this hypothesis, they bath-applied 300 μM GABA on striatal astrocytes to stimulate their Ca^{2+} activity. Of note, extracellular GABA concentration is estimated in the low micromolar range, while the concentration used in this study is closer to the GABA concentration present in the synaptic cleft [5]. Therefore, it is unlikely that such concentration can be achieved through ectopic GABA release by the dendrites as suggested by the authors. Nonetheless, GABA-mediated potentiation of spontaneous Ca^{2+} activity in astrocytes has been recently reported [2]. GABA uptake is associated with Na^+ entry to the cells and subsequent Na^+ exchange for Ca^{2+}. The authors did not test this possibility, instead hypothesized that GABA activates astrocytic $GABA_B$ receptors. $GABA_B$ are G_i-coupled receptors and their role in astocytic Ca^{2+} activity was not hitherto described. Surprisingly, an agonist of $GABA_B$ receptors increased astrocytic Ca^{2+} activity.

To probe for the behavioural effects of astroglial Ca^{2+} activity *in vivo* Khakh and colleagues expressed human M4 muscarinic G_i-coupled

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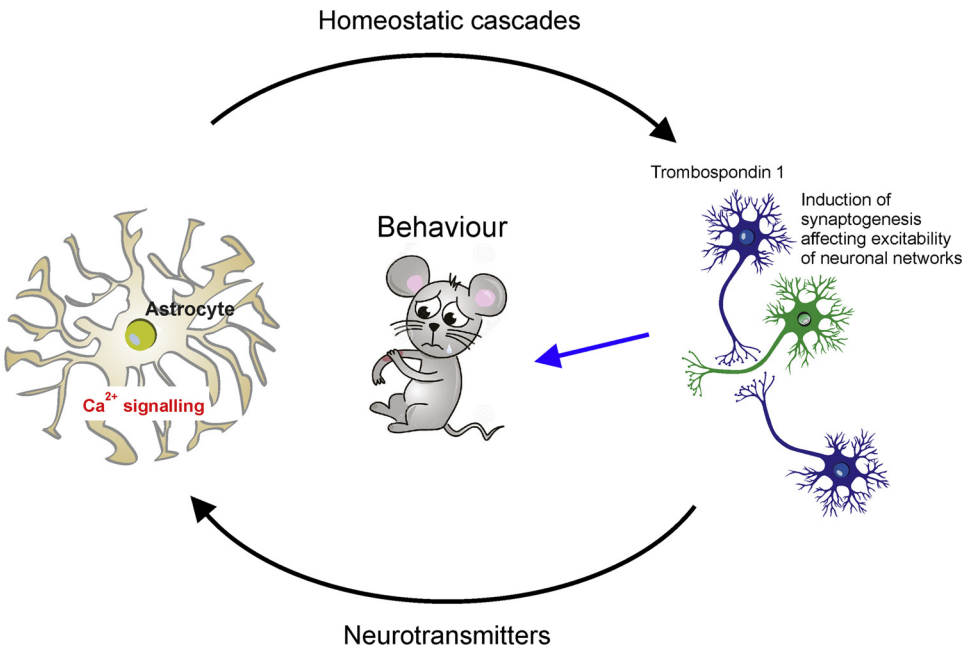

Fig. 1. Stimulation of astroglial receptors following neuronal activity activates astroglial homoeostatic cascades, which remodel neuronal network; for example release of astroglial synaptogenic factor trombospondin-1 (TSP1) increases synaptic denisty in striatal medial spiny neurones [6] that affects their excitability, firing patterns and ultimately behaviour. Emergence of positive feedbak may instigate pathological phenotypes.

receptor activated by designer drugs in striatal astrocytes. Activation of M4 receptors with clozapine-*N*-oxide evoked Ca^{2+} signals similar to those triggered by the agonist of $GABA_B$ receptors. Stimulation of M4 receptors in mice by intraperitoneal injection of clozapine-*N*-oxide substantially modified mice behaviour in the open field task and in the novel object recognition task: in contrast to control animals the M4 receptors expressing mice showed signs of hyperactivity and absence of attention; signs somewhat similar to manifestations of attention deficit hyperactivity disorder in humans. At the cellular level activation of astroglial Ca^{2+} signalling through M4 receptor pathway increased the density of dendritic spines and remarkably (˜50 times) increased expression of the *Thbs1* gene encoding TSP1. Inhibition of TSP1 pathway with gabapentin prevented the increase in the dendritic spine number and the synaptic drive to MSNs and reversed behavioural abnormalities induced by CNO in M4 bearing mice. To conclude, activation of Ca^{2+} signalling in striatal astrocytes up-regulates synthesis and subsequent release of TSP1, which in turn stimulates synaptogenesis in MSN neurones thus increasing network excitability and instigating hyperactivity with associated behavioural metamorphoses. This report suggests that targeting mechanisms regulating Ca^{2+} dynamics in astrocytes may represent a promising therapeutic target. Recent methodological advances, which allow to analise spatiotemporal properties of Ca^{2+} events in single astrocytes and astrocytic networks can further clarify which patterns of astrocytic activity that may be associated with their normal function and pathological malfunction [11].

Contribution of neuroglia, and particularly astrocytes in the information processing and regulation of behaviour has been discussed since the late 19th century with Carl-Ludwig Schleich and Santiago Ramon y Cajal postulating that astroglia may control synaptic transmission and induce complex behaviours such as sleep [8,10]. The notion of “thinking” glia has been brought to the extreme by Robert Galambos [3], who apprehended astrocytes as the primary seat of intelligence and humanity; the ideas of glyocentric brain has been resurfacing regularly since (see for example [9]). Aside from these eccentricities, astrocytes are considered to participate in transmission in neural networks. The underlying mechanism is usually assumed to be associated with the release of neurotransmitters (which are commonly named “glio”transmittres to highlight their origin) from astrocytes [1]; although the concept of such gliotransmission remains under debate. The study of Khakh and colleagues highlights a role for distinct and specifically astroglial pathway, associated with astroglial supportive function. In this paradigm, release of the astroglial synpatogenic factor is sufficient to remodel neuronal circuitry and increase the excitability of neuronal network resulting in a specific behaviour (Fig. 1). The gain-of-function of the astroglial physiological pathway may even cause pathological development instigating aberrant behaviour. The most puzzling part of this story is a positive feedback in neurone-astrocyte-neurone communication. Neuronal activation promotes Ca^{2+} activity in astrocytes, astrocytic Ca^{2+} activity enhances excitatory synaptic input to these neurones. This potentially can lead to uncontrolled overexcitation of the neuronal network and excitotoxicity. What is the mechanism that sustains the excitation in this loop, what is the physiological purpose of such unusual feedback and whether this mechanism has a pathological relevance in the context of human neuropathology, remains to be further explored.

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