REVIEW

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# Microglial voltage-gated sodium channels modulate cellular response in Alzheimer's disease – a new perspective on an old problem

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**Abstract**

Alzheimer's disease (AD) determines gradual loss of cognition and memory function, eventually leading to clinical manifest dementia. The pathogenic mechanisms of AD remain elusive and treatment options unsatisfactory, targeting only symptoms like memory loss, behavior changes, sleep disorders and seizures. These therapies are not stopping the disease's progression, at their best they can only delay it. Accumulating evidence suggests that AD is associated with a microglial dysfunction. Microglia are resident immune cells that provide continuous surveillance within the brain. When excessively activated, microglial response can also have detrimental effects *via* the exacerbation of inflammatory processes and release of neurotoxic substances. Recently, it was recognized that microglia express voltage-gated ion channels, in particularly voltage-gated sodium channels (VGSC). Pharmacological block of VGSC has been attempted symptomatically in AD to control the epileptic features often associated with AD, as well as to relieve detrimental behavioral and psychological symptoms of dementia. The success of VGSC treatment in AD was unexpectedly variable, ranging from very beneficial to plain detrimental. This variability could not be satisfactorily explained solely by the neuronal effects. This article will try to discuss possible implication of microglial VGSC dysfunction in AD according to available data, own personal experience of the authors and propose a new way to investigate its possible implications.

***Keywords***: voltage-gated sodium channels, microglia, Alzheimer's disease.

## Background of the problem

The worlds average life span has increased in the last decade in developing and developed countries as such a direct consequence is that the world's population is getting older. As a result, new health problems concerning older populations arise. One major health concerns in this regard is dementia [1].

Dementia is seen as a general term used by medical personnel to describe a number of diseases including Alzheimer's disease (AD) dementia [2]. At the moment, it is estimated that 35.6 million people worldwide are diagnosed with dementia and it is predicted that in approximately 20 years this number will double and triple in the next 40 years [3]. Alzheimer's disease is a progressive neurodegenerative disorder of the brain accounting for more than 60% of cases of dementia [2]. The pathogenic mechanisms in AD remain elusive and treatment options unsatisfactory, targeting symptoms like memory loss, behavior changes, sleep disorders and seizures. These therapies do not stop the disease's progression, at their best they can only delay it.

Research in the molecular and cellular mechanisms of AD have made a strong association between the onset of AD and central nervous glial cell dysfunction, particularly microglia [4]. Microglia are resident immune cells that provide continuous surveillance within the brain. When excessively activated, microglial response can also have detrimental effects *via* the exacerbation of inflammatory processes and release of neurotoxic substances.

Two main theories regarding microglia cells involvement in Alzheimer's disease exist. First one is the amyloid cascade/neuroinflammation hypothesis. It is centered on the assumption that amyloid activates microglia, that became involved in causing the neurodegeneration and leading to AD dementia [5]. The second hypothesis is the microglia dysfunction one. It states that microglia have a decreased clearance of beta Amyloid (Aβ), diminished trophic support and increased neurotoxin production [6]. The most important aspect of this theory is that it can explain both pathologic hallmarks that characterize AD: (*i*) plaques, tangles formation and

there clearance; (*ii*) increased neurotoxin production by senescent microglia cells [5, 6]. Evidence showing that microglia in aged brain are slower that in young one seems also to support this theory [7].

Regardless of the way in which microglia become dysfunction there is an accumulating evidence suggests microglial involvement in AD. It is, therefore, of great interest to develop strategies specifically aiming to limit the effects of microglial activation, although a suitable pharmacologic target has yet to be identified.

## Microglia sodium voltage-gated ion channels

Voltage-gated sodium channels are responsible for transforming the receptor potential into action potential and, then, for its propagation in excitable cells, like nerve, muscle, and neuroendocrine cells. This kind of channels is present also in non-excitable cells, but in a lower quantity and with an unclear role [8].

Sodium voltage channels (NaV) are formed of one pore-alpha-subunit associated with one/more beta-subunits. The alpha-subunit acts as the "voltage sensor" being activated by changes in membrane potential [8]. The roles of the beta-subunits are multiples, from modulating channel gating and regulating channel expression, to interacting with the cytoskeleton and the extracellular matrix, as cell adhesion molecules [9].

In the last years, several reports, as well as unpublished work carried out by our grope, have linked microglia dysfunctions to AD, by showing microglial motility impairment in AD mice models [4]. The microglial motility impairment coinciding to Aβ accumulation in the brain is, however, not explained by any of the two most known AD theories. There is however a less know theory called the channel hypothesis of AD proposing that Aβ damage is done by forming generally large, voltage-independent ion channels within the neuronal membrane. These channels are relatively poorly selective amongst physiologic ions, transporting a variability of ions from $Ca^{2+}$, $Na^{+}$, $K^{+}$, $Cs^{+}$, $Li^{+}$ to possibly $Cl^{-}$ and possess distinct physiologic characteristics that would be consistent with toxic properties [10]. This is particularly interesting because although there is grooving evidence that implicates Aβ peptides in the physiopathology of AD, it is the only one that can explained the mechanism of Aβ toxicity in AD.

Recently, it was recognized that microglia express voltage-gated ion channels, in particularly, voltage-gated $Na^{+}$ channels isoforms (VGSC): Nav1.1, Nav1.5 and Nav1.6 [11]. Unrelated to microglial VGSC, pharmacological block of this channels has been attempted as a symptomatic treatment of epileptic features often associated with AD, as well as to relieve detrimental behavioral and psychological symptoms of dementia.

Microglia dysfunction seems to affect all aspects of its physiology from interleukin and TNF-alpha release, microglia–neuron interaction, to microglial migration. Given the implication of ion channels to microglia physiology it could be possible that Aβ directly by/or indirectly could influence microglia functions, thus explaining why microglia dysfunctions coincide to Aβ accumulation in the brain [4] (Figure 1).

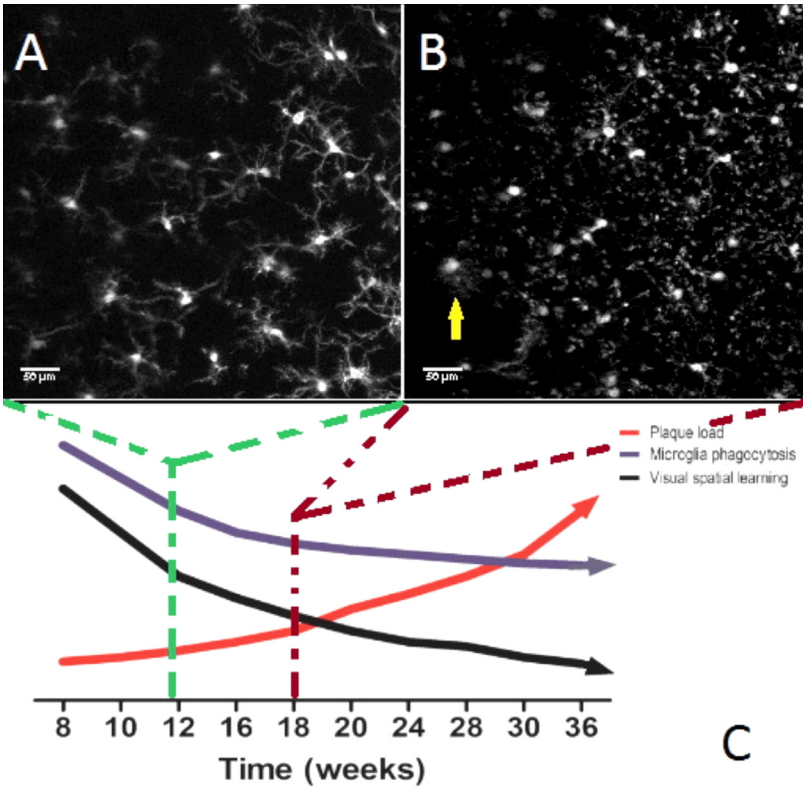


**Figure 1** – ***Transcranial two-photon laser scanning imaging reveals a rapid and dynamic microglia morphology with in just a couple of weeks. In 8-week-old mice, the morphology of microglial cells is almost normal (A), while in 16-week-old (B) microglia seem to be more activated, with rounder cell body and less ramified. This is despite the fact that just a few detectable amyloid plaques can be seen in the cortex (arrow). In the same time, window learning gets impaired compare to wile type, same age, animals (C). Data adapted from results showed in [4, 12] and illustrated by own images.***

A notable finding was that both AD mouse models and AD patients auxiliary subunits of voltage-gated sodium channels (β2 and β4) are shown to be cleaved by [β-site amyloid precursor protein (APP) cleaving enzyme 1] and γ-secretase that are increased around the Aβ plaques [12, 13]. This process seems to compromise normal trafficking of the α-subunit voltage-gated sodium channel (VGNC), resulting in reduced sodium current density

[13]. In an AD mouse model that was overexpressing human APP (hAPP carrying the Swedish and Indiana mutations in C57BL/6J background), it has been shown that animals experiencing non-convulsive seizures were expressing in the parietal cortex lower levels of Nav1.1 and 1.6 [14]. Recent data, that analyzed CA1 pyramidal neurons excitability in a mouse model of AD, found altered intrinsic neuronal excitability and reduced $Na^+$ currents in animals older than nine months but not in younger animals, with the same genetic background [15].

Even more surprisingly, the Na-channel deficiency was similar to some human idiopathic epilepsy caused by sodium channelopathy in GABAergic neurons [16]. This could raise an interesting debate about the safety of sodium channel blockers in the treatment of seizures in AD [15, 18].

Although the function of VGSC in microglia cells is still being debated, it has recently been shown that ion channels, including voltage-gated sodium channels, can actively transform external stimuli to intracellular stimuli [19]. Furthermore, many experimental works found that microglia motility [11, 20] and microglial phagocytosis [11] was directly affected by VGNC modulation. In summary, this dysfunction seems to affect all microglia physiology from interleukin and TNF-alpha release, microglia–neuron interaction, to microglial migration. Given the effects of ion channels on microglia physiology, it is possible that Aβ directly or indirectly influences microglia functions, thus explaining why microglia dysfunctions coincide with Aβ accumulation in the brain.

The overall compromised sodium channel function, both in patients with AD and mice models of AD, provides a strong basis for a hypothesis that sodium channels blockers used as antiepileptic drugs for AD are ineffective and could have unfavorable effects. Regardless of the mechanism that could interfere with VGNC (be it altered expression, trafficking, or subunit cleavage), there is growing evidence that excitability is altered in the brain of AD patients [18].

As such, the success of VGSC treatment in AD was unexpectedly variable, ranging from very beneficial to somewhat detrimental [21–23], mainly because of the complex and intricate nature of VGSC membrane expression in both neurons and glial cells throughout AD progression. This variability could not be satisfactorily explained solely by the neuronal effects. This combined with the recently discovered voltage-gated ion channels that are expressed in microglia and in particular, voltage-gated $Na^+$ channels isoforms could explain the variability of sodium channel blockers treatment outcome in AD patients.

## Clinical consequence

### Neurodegeneration

Numerous reports have suggested that AD could be caused by an early neurodegeneration due to defective regulation or malfunction of the sodium and potassium adenylpyrophosphatase (Na,K-ATPase) system in brain [24, 25]. It appears that such malfunction of the Na pump is caused by some defects in the auto-regulation of the self-regulating 170-kDa cytosolic endogenous activator molecule rather than defects in the Na,K-ATPase molecule itself [26].

The most important aspect of Aβ channel forming is the possibility that it can induce the primary dysfunction in this mechanism. This is because sodium and potassium levels are the ones that regulates the Na,K-ATPase pump. As such an aberrant, nonselective Aβ channel can easily change sodium and/or potassium concentrations this inducing, eventually cell death by either direct neuronal signaling and/or microglial dysfunction.

### Seizures

Several studies tried to explain this increase of epileptic activity in AD patients. Some of them have been able to show that Aβ 1-42 and Aβ 25-35 (proto) fibrils (but not oligomers) can induce membrane depolarization of pyramidal cells and increase the activity of excitatory cell populations [27], thus explaining epileptogenesis in AD. Furthermore, other studies have shown that both intracellular or extracellular applied Aβ peptide and its fragments can modulate the function of ion channels [28–32].

Epileptic symptomatology varies in AD patients from complex partial seizures to non-convulsive status epilepticus. These pathologies are difficult to diagnose in AD patients and has made some groups conclude that undiagnosed seizures could contribute to daily cognitive fluctuations in individuals suffering from AD [33], and consequently, proper control of seizures using antiepileptic drugs (AEDs) would improve cognitive performance of these patients [23]. Interestingly, some groups working with animal models of AD reported spontaneous seizures and interictal discharges in animals with increased Aβ production [27, 23–35].

As such, the unknown link between AD and the increase number of seizures in these patients can have the same implication. The imbalance in currents outside and inside of the cell could destabilize the overall membrane current flow determining aberrant discharge patterns. This will also explain the variability of seizures in AD patients.

## New perspectives

Taking in consideration all effects of VGSC modulation in AD could reflect, at least in part, the modulation of microglia activation. As such, the timing of the VGSC block in relation to inflammatory response becomes essential for its effectiveness. This implies that a detailed, step-by-step and multi-approach investigation of microglia function within an animal model of AD treated with sodium channel blockers is needed to establish the beneficial and detrimental time-window of treatment.

This implies developments of new research methods to directly explore the effect of VGSC modulation on microglia activity *in vivo* such as long-term, imaging models using state-of-the art *in vivo* two-photon laser-scanning microscopy to investigate microglial physiology in rodents [36–38]. These techniques can allow real time cell tracking within the central nervous system over a period of months, thus providing direct feedback of microglial activity with Aβ accumulation and treatment administration [39].

Direct visualization of cell activity will not be enough because sodium channels are so variable in there structure and sensibility within the human body and its varying expression in AD. A more stable structure will be needed also to evaluate sodium channel activity as an entity. Recently, a simple, noninvasive method was adapted to animal experimenting for determining the level of pharmacologic VGSC block *in vivo*. This method uses an electrophysiological technique based on peripheral axon excitability threshold tracking [40]. If successful, these mixed morphological and functional studies could establish a suitable time-window for effective neuroprotection using VGSC blockers in AD, which could be readily translated to clinical setting.

All of these aspects raise the question of efficiency and safety of VGNC blockers in at least some AD patients if not in all of them. An interesting debate is if sodium channel activators could be used in specific time windows and for a limited amount of time to altered physiological properties of dysfunctional neuronal networks in AD patients [41]. It could also be possible that small amounts of NaV activators could just be enough to compensate microglial dysfunctions such as Aβ clearance and motility that coincide with Aβ overexpression, thus microglia will not need to up regulate sodium channels in a chronic inflammation.

**Conflict of interests**

The authors declare that they have no conflict of interests.

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**Author contribution**

All authors have an equal contribution.

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