

**Commentary on:**

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Acquired and genetic channelopathies: *in vivo* assessment of axonal excitability

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## **ABSTRACT**

Neuronal or axonal ion channel function can be impaired or altered in a number of disorders, such as acquired (autoantibody-mediated, toxic, and metabolic) and genetic channelopathies, and even neurodegenerative (motor neuron disease) or inflammatory diseases (multiple sclerosis, immune-mediated neuropathies). When specific channels are affected, axonal/neuronal excitability primarily alters according to original function of the corresponding channels.

Separately, in the 1990's, axonal excitability testing was developed to assess ion channel function, membrane potential, and passive membrane properties non-invasively in human subjects. Using this technique, numerous papers on altered axonal excitability in a variety of disorders have been published since 2000. In a recent issue of *Experimental Neurology*, Park et al. demonstrated changes in peripheral axonal excitability in limbic encephalitis and acquired neuromyotonia with anti-voltage gated potassium channel antibodies.

Unexpectedly, the results were not consistent with those caused by simple potassium channel blockade, suggesting multiple other factors contribute to altered axonal excitability. In contrast it was reported that patients with episodic ataxia type 1 (genetic channelopathy with mutation of Kv1.1 channel gene) show prominent excitability changes exactly compatible with fast potassium channel blockade. This commentary aims to highlight findings of this study in a broader context, and provides possible explanations for the discrepancy of patterns of axonal excitability changes in acquired and genetic potassium channelopathies.

## Introduction

Ion channelopathies are caused by dysfunction of channels due to hereditary or acquired disorders. Channelopathies affect almost all areas of neurological practice, including epilepsy, movement disorders, migraine, **peripheral neuropathy, pain syndrome, and myopathy** (Kullmann and Waxman, 2010). Over the past 2 decades, the concept of ion channelopathy has been significantly expanded. In addition to genetic channelopathies, neuronal or axonal ion channel function can be altered in a number of conditions, such as acquired (autoantibody-mediated, toxic, and metabolic) and even neurodegenerative (motor neuron disease) or inflammatory diseases (multiple sclerosis, immune-mediated neuropathies) (Kuwabara and Misawa, 2004, 2008; Kishnan et al., 2009).

Furthermore, ionic conductances are largely affected by membrane potential and trans-axonal ionic concentration. For example, in chronic dialysis patients, axons are depolarized by hyperkalemia, resulting in increased **axonal** sodium and potassium conductances (Kiernan et al., 2002). Conversely under hypokalemia axonal membrane is hyperpolarized, and the ionic conductances are reduced (Kanai et al., 2002). Another example is diabetic neuropathy; under hyperglycemia, the activation of the polyol pathway leads to reduced  $\text{Na}^+/\text{K}^+$  pump activity, and resulting intra-axonal sodium accumulation decreases sodium currents due to decreased trans-axonal sodium gradient. In this regard, uremic or diabetic neuropathy is a type of channelopathy (Kitano et al., 2004; Misawa et al., 2006a,b). Therefore ionic conductances and axonal excitability are dependent on the environmental conditions, as well as channel function itself.

Separately, an exciting development has been the identification of neurological disorders that are associated with specific antibodies to ion channels. The most common CNS syndrome associated with voltage-gated potassium channel (VGKC) antibodies is a form of limbic encephalitis (Irani et al., 2010). Another example of anti-VGKC antibody-associated syndrome is acquired neuromyotonia, also termed as Isaacs syndrome and cramp-fasciculation syndrome, that is characterized by muscle cramp, myokymia, and fasciculations due to spontaneous repetitive firing of motor

axons. The motor axonal hyperexcitability are caused by suppression of fast potassium channels by anti-VGKC antibodies (Hart et al., 2002).

In a recent (or this) issue of *Experimental Neurology*, Park et al. reported changes in peripheral axonal excitability in patients with limbic encephalitis or acquired neuromyotonia, whose sera had high-titer of anti-VGKC antibodies (Park et al., 2014). Axonal excitability testing was performed at the wrist of the median nerve motor axons. Patients with limbic encephalitis demonstrated prominent abnormalities in peripheral axonal excitability during the acute phase, but the pattern of excitability property changes was not consistent with blockade of VGKC, and was possibly explained by reduced sodium currents because most of the patients had hyponatremia due to syndrome of inappropriate antidiuretic hormone secretion.

They also showed that patients with acquired neuromyotonia demonstrated no significant changes at the site of stimulation. The total findings suggest that serum anti-VGKC antibodies did not affect excitability properties at the site of stimulation (tested site), largely because the antibodies cannot assess the tested motor axons by the blood-nerve barrier (see below). The findings indicate that not only the effects of anti-VGKC antibodies, but also a complex interaction of multiple factors should be taken into consideration in clinical situation, and therefore this study is interesting and of clinical significance.

### **Nerve excitability testing**

Testing the excitability of axons can provide insights into the ionic mechanisms underlying the pathophysiology of axonal dysfunction in human. The technique of threshold tracking was developed in the 1990's, to non-invasively measure a number of axonal excitability indices, which depend on membrane potential and on the sodium and potassium conductances. By delivering conditioning stimulus, which alters membrane potential or activates specific ion channels, the current required to produce a target potential (threshold) will change. The techniques have been extensively applied to the study of the biophysical properties of human peripheral nerves *in vivo* and have provided important insights into axonal ion channel function in health and disease. This commentary focused on

assessment of potassium channel function (for the details on methodology and interpretations of nerve excitability testing, please refer to previous excellent review articles [Bostock et al., 1998; Krishnan et al., 2009]).

There are many types of potassium channels on axons (Reid et al., 1999), but it is convenient, for clinical purposes, to restrict discussion to two groups that are dependent on the membrane potential, those with fast kinetics (fast potassium channels) and those with slow kinetics (slow potassium channels). Fast potassium channels are located in the juxta-paranodal region, where they contribute to the resistance of the internodal membrane and limit the depolarizing afterpotential responsible for supernormality. Slow potassium channels have a density at the node 25 times that at the internode, but their kinetics are too slow to allow them to affect the action potential directly. They help to determine the resting membrane potential, contribute to accommodation to depolarizing stimuli, and are responsible for the late subnormality. In excitability testing, the S1d phase of threshold electrotonus and supernormality are limited by fast potassium conductance, and the S2 phase and subnormality are caused by slow potassium conductance. Therefore, patterns of combined findings of the threshold electrotonus and recovery cycle can provide information about fast and slow potassium conductances (Table 1; Figure 1).

Briefly, in the threshold electrotonus studies, the membrane potential was altered by use of DC polarizing currents that were 40% of the unconditioned threshold. Depolarizing and hyperpolarizing currents were used, each lasting 100ms, and their effects on the threshold current for the test motor responses were examined. The recovery cycle of axonal excitability after a single supramaximal stimulus was measured by delivering the test stimulus at different intervals after the conditioning stimulus. The interval between the conditioning and test stimulation were changed systematically from 2 to 200ms. When fast potassium channels are blocked, both the S1d phase and supernormality should increase (Bostock et al., 1998).

### **Genetic potassium channelopathy (episodic ataxia type 1) and excitability**

Detailed nerve excitability findings in episodic ataxia type 1, a representative

genetic potassium channelopathy, have been reported (Tomlinson et al., 2010); episodic ataxia type 1 is a neuronal channelopathy caused by mutations in the KCNA1 gene encoding the fast potassium channel subunit **Kv1.1**. The disorder presents with brief episodes of cerebellar dysfunction and persistent neuromyotonia, and is associated with an increased incidence of epilepsy. The S1d phase in threshold electrotonus, and supernormality in the recovery cycle were prominently greater than those in normal controls. The findings exactly show loss of function of fast potassium channels. Using these two parameters, the patients with episodic ataxia type 1 and controls could be clearly separated into two non-overlapping groups. The authors concluded that nerve excitability testing is useful in diagnosis, since it can differentiate patients with episodic ataxia type 1 from normal controls with high sensitivity and specificity.

In a study by Park et al. (2014), the authors presumably expected the same findings, but results of actual recordings with the same techniques in patients with anti-VGKC antibodies were very different from those in patients with episodic ataxia type 1. The S1d phase of threshold electrotonus, and supernormality in the recovery cycle were smaller than those in normal controls, the opposite patterns to those of episodic ataxia type 1 (see Figure 1 in their paper). The findings indicate that changes in axonal excitability in patients with anti-VGKC antibodies are not caused by potassium channel blocking, and other factors should have contributed to the altered excitability. The unexpected results are considered to be due to **multiple factors**, and inaccessibility of the antibodies to the site (at the wrist portion of the median nerve) by the blood-nerve barrier **is one of the factors**.

### **The blood-nerve barrier and autoantibodies**

Because of the blood-nerve barrier, large molecule substance such as immunoglobulin (antibodies) cannot access the nerve trunk. The internal microenvironment in the peripheral nerves is highly regulated. In humans, this regulation is facilitated by specialized tight junction-forming endoneurial microvascular endothelial cells. The endoneurial endothelial cells come in direct contact with circulating blood and, thus, can be considered the blood-nerve

barrier.

However, the blood-nerve barrier is anatomically deficient in the distal nerve terminals, nerve roots, and dorsal root ganglia (Olsson, 1990). In immune-mediated neuropathies, such as Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy, it is established that the distal nerve terminals and nerve roots, where the blood–nerve barrier is deficient, are preferentially affected (Brown and Snow, 1994; Kuwabara et al., 2002), and the blood-nerve barrier partly determines the distribution of demyelinating lesions. Motor nerve conduction studies frequently show prolonged distal latencies and temporal dispersion of distally evoked compound muscle action potential, suggesting demyelination in the distal nerve segments in Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy.

In anti-VGKC antibody-related disorders, the circulating antibodies can access the motor nerve terminals and roots, and fast potassium channels would be affected in these regions. Actually patients with acquired neuromyotonia in that study had motor nerve hyperexcitability evidenced by muscle cramp and fasciculations, and spontaneous motor unit activity on EMG examinations. Nerve excitability testing used in the study assess the excitability at the stimulus site (the wrist portion of the median nerve), where blood-nerve barrier functions, and therefore anti-VGKC antibodies did not affect the tested site (nerve trunk).

### **Factor affecting axonal excitability**

As described in the Introduction, many factors can affect axonal ionic conductances and excitability. Ion channel dysfunction itself reasonably impairs the ionic currents, but many other factors, such as membrane potential, trans-axonal ionic gradient, exposure of channels by demyelination, and increased channels in axonal regeneration, can alter ionic conductances and thereby axonal excitability.

In a paper of Park et al. (2014), altered excitability properties in limbic encephalitis patients may be caused by hyponatremia due to syndrome of inappropriate antidiuretic hormone, and resulting reduced trans-axonal sodium gradient and reduced sodium currents. In patients with acquired neuromyotonia,

nerve excitability testing did not significantly affected by the lack of other factors influencing axonal excitability. In both cases, anti-VGKC antibodies did not affect the results. The results of this study are basically negative from a view of potassium channel blockade by the autoantibodies, but sufficiently educational, telling us that in clinical practice, the determinants of axonal excitability include many other factors, some of which are secondary or co-incident, and patients show a total effects of multiple factors.



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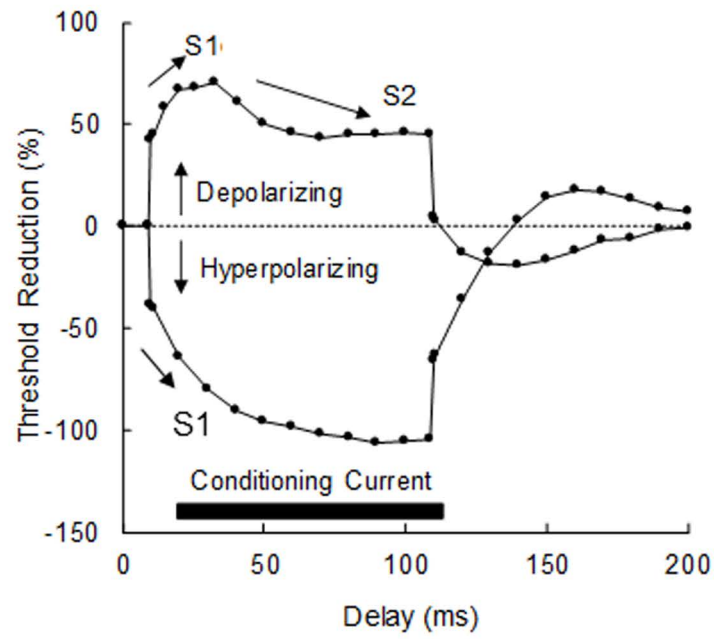
<b>Table 1. Axonal excitability indices and potassium channel conductance</b>			
Parameter		Ion channel	
Threshold electrotonus			
	S1d		(Limited by) fast potassium channel
	S2		Slow potassium channel
	S3		Inward rectifying channel
Recovery cycle			
	Refractoriness		Recovery from inactivation of sodium channel
	Supernormality		(Limited by) fast potassium channel
	Late subnormality		Slow potassium channel
S1d = peak of the slow phase in the depolarizing direction, see Figure 1.			

## Figure legends

### Figure 1.

Threshold electrotonus and recovery cycle measured in the median nerve at the wrist of a normal subject. A. Threshold electrotonus; changes in the threshold stimulus current for the target potential produced by subthreshold depolarizing or hyperpolarizing currents lasting 100 ms, with the intensity of the polarizing currents being 40% of the threshold. B. The recovery cycle of axonal excitability following a single supramaximal conditioning stimulus. At a short-conditioning test interval, the axons fall within the relatively refractory period, and the threshold current increases. At 3-4 ms, axons enter the supernormal period, and less current is required to activate them. Then axons enter the late subnormal period. The size of S1d and supernormality is limited by fast potassium currents, and therefore the size increase when potassium channels are impaired.

### A. Threshold Electrotonus



### B. Recovery Cycle

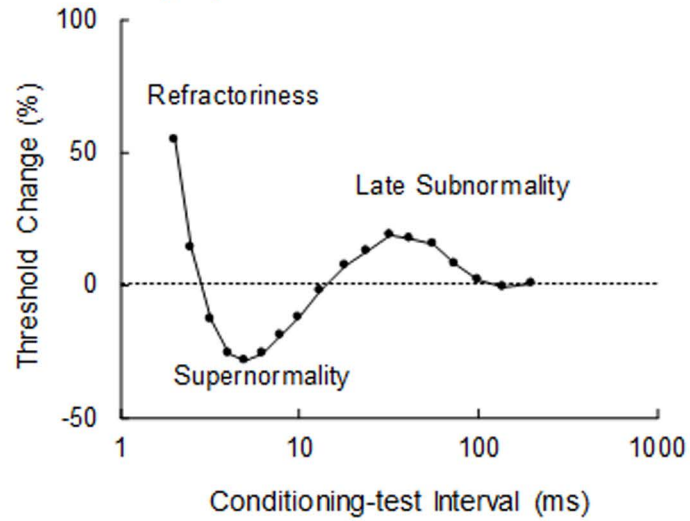


Figure 1