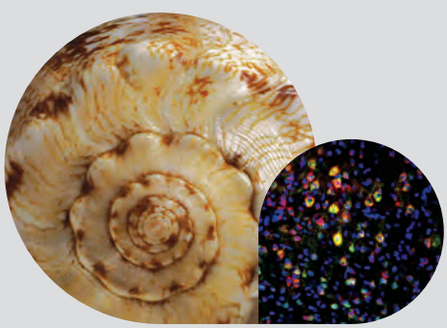
Modulator | 2019 | Issue No.27



About the cover:
Left: **Conantokin G** (#STC-666) is a peptide toxin originally isolated from *Conus geographus* venom. The toxin acts as a selective antagonist of GluN2B, a subunit of NMDA receptors. Right: Immunohistochemical staining of perfusion-fixed frozen rat parietal cortex sections using **Anti-PSD-95 Antibody** (#APZ-009) and **Anti-NMDAR2B (GluN2B) (extracellular)-ATTO-594 Antibody** (#AGC-003-AR). See "NMDA Receptor Dynamics Dictate Neuronal Plasticity and Function" for insights on the important role of these receptors.

About Us
Alomone Labs is a leading developer, producer and supplier of reliable research tools for membrane proteins. By leveraging 30 years of experience and scientific excellence, we produce tried, tested and trusted products.

Learn more about us:

About the Modulator
The *Modulator* is our in-house magazine dedicated to the ion channel research community. It is about the endless potential of science, and the power of reliable partnerships. In each issue we highlight notable research conducted in labs across the globe.

Modulator No. 27 online version:

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alomone labs
empowering the spirit of science

Greetings from the CEO

For the past three decades, Alomone Labs has been providing the international life science community with innovative and reliable research tools. Committed to delivering the highest quality of products, we are passionate about offering you unique products and beneficial insights to reinforce your scientific research and empower positive results.

Always connected to the pulse and pace of our industry, we make sure to foresee where the world of life science is heading. Our success has been made possible thanks to our highly dedicated and qualified scientific staff and to our distinguished customers, who throughout the years have come to trust the Alomone Labs brand. We are proud of the solid relationships we've created and sustained with numerous partners and customers across the globe – including you. You are part of our mission to achieve extraordinarily challenging goals, while continuously raising the standards of research.

The *Modulator* magazine is our contribution to the scientific community. Each article focuses on recent studies in ion channel research describing the unique ways our reagents are being used. We are grateful to you for trusting us by using our products in your experiments, for publishing significant results, and for continuing to propel science forward. Thank you for your contribution to this vision, and for sharing your scientific spirit with all of us.

Niko Bajayo, CEO

Letter from the Editor

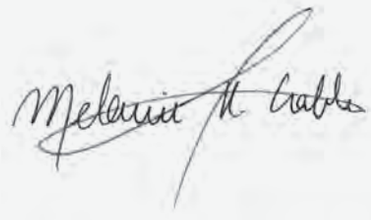
Dear researchers,

I am proud to present this new edition of the *Modulator*, our in-house magazine dedicated to the ion channel research community. This issue is special to us since we celebrate our 30th anniversary. The *Modulator* is a tribute to our dedicated research collaborators and partners, who have grown to become part of the extended Alomone Labs family. It is about the endless potential of science, and the power of reliable partnerships. We are excited to share the achievements of scientists across the globe; those who may just be embarking on their path or are already veterans in their field. Each story we present, is a celebration of scientific creativity and the positive impact quality research has on the advancement of research.

We are excited about the chance to highlight some of the notable research being conducted in labs across the globe, while providing a source of motivation to others. We hope this magazine provides you with valuable insights and inspiration - igniting ideas that enrich your mind, while reinforcing the collective mission we are all on together. For us, this is yet another opportunity to continue empowering the spirit of science.

In this 27th *Modulator* issue we celebrate 30th years and cover Na_v channels and pain, followed by a short piece on the research potential of new T-type Ca_v channel blockers. We continue our journey talking about the ever so important NMDA receptors and their role in neuronal plasticity, and finish off this celebration with K_v4 channels and their role in aging (yes, we are aging, let's embrace it!).

We hope you enjoy reading this issue as much as we did!



Melanie Grably, Ph.D.
Content editor

Alomone Labs Turns 30

30 years of innovation, 30 years of reliability, 30 years of reproducibility

1989 — Alomone Labs launched first toxins for ion channels research

1990's — Antibodies to Ion Channels, NGF from mouse submaxillary glands

2000+ — Recombinant toxins, Recombinant neurotrophins, Antibodies to GPCRs

2010+ — Synthetic peptides/peptide toxins, Small molecules/organic compounds, Guinea pig polyclonal antibodies.

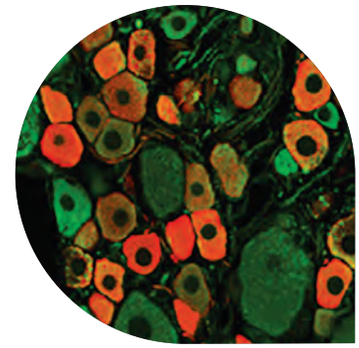
2018 — Leading supplier of research tools for: Neuroscience, Cardiovascular, Stem Cells, Cancer, Development, Immunology, Metabolism. Cited in over 15K papers.

2019 — Alomone Labs turns 30!

Na_V Channels and Pain

Melanie R. Grably, Ph.D. and Lior Zornitzki, M.D.

The role of Na_V Channels in several types of pain opens a gateway for the development of specific sodium-channel inhibiting agents for the treatment of chronic pain. In this short piece we demonstrate the use of Alomone Labs Na_V-related products in pain research.



Right: DRGs play a pivotal role in nociception.

Introduction

Pain sensation is an extremely common pathophysiological condition. The voltage-gated sodium (Na_V) channel isoforms $Na_V1.7$, 1.8 and 1.9 are selectively expressed in pain-conducting pathways of the peripheral nervous system (PNS). These isoforms are mostly localized to peripheral sensory ganglia, including dorsal root ganglia (DRG) and trigeminal ganglia (TG). $Na_V1.7$ is specifically associated with pain pathways since it is highly expressed in both unmyelinated C-fibers that contain the neuropeptide substance P and in free nerve endings in nociceptive receptor fields.

The association between Na_V channels and pain is further demonstrated by individuals who carry $Na_V1.7$ mutations. Some mutations, completely abolish nociception and these individuals do not experience pain.

Na_V channels have also been implicated in inflammatory pain states. Multiple studies have shown changes in sodium channels in experimental animals with inflammatory lesions. Inflammation typically causes increased expression of the $Na_V1.3$, 1.7, 1.8, and 1.9 isoforms in sensory neuronal cell bodies.

The role of Na_V channels in several types of pain opens a gateway for the development of specific sodium-channel inhibiting agents for the treatment of chronic pain[2].

Na_V Channels and Pain

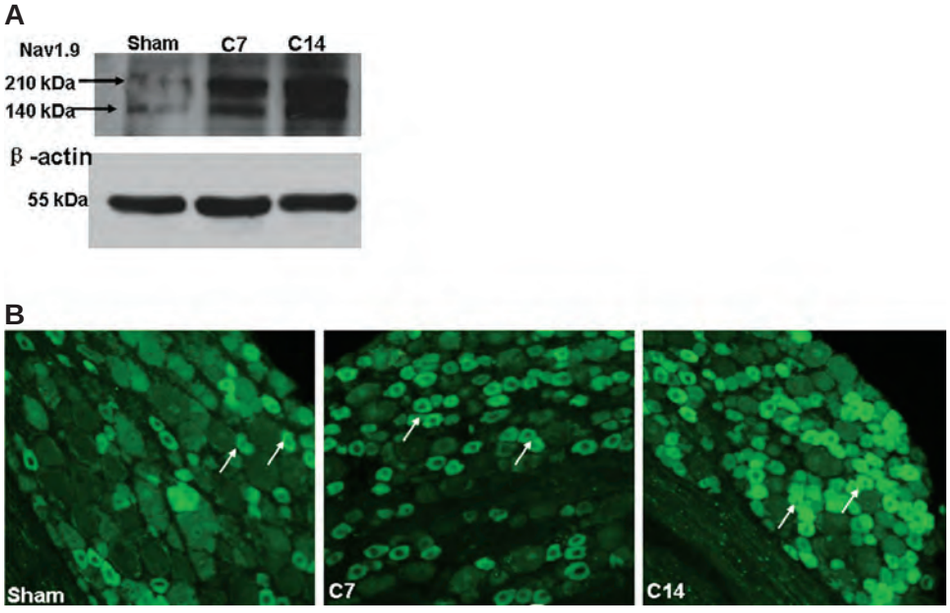
Resurgent Na^+ currents are currents activated during repolarization, and are the driving force for the generation of additional action potentials and thus contribute to repetitive firing of neurons. TTX-sensitive (TTX-s) resurgent currents are detected in cerebellar and dorsal root ganglia (DRG) neurons, and are achieved in a $Na_V1.6$-dependent manner[8]. A novel TTX-resistant (TTX-r) resurgent Na^+ current was isolated in rat DRGs[8]. In many aspects this current is similar to the TTX-s resurgent current in that it requires the presence of $Na_V\beta4$ subunit. However, TTX-r resurgent currents exhibit slower kinetics and occur at more depolarized voltages. They are also sensitive to a $Na_V1.8$ specific blocker. The involvement of $Na_V1.8$ in resurgent currents is further strengthened by immunoprecipitation studies using **Anti-Na_V1.8 (SCN10A) Antibody** (#ASC-016) in rat DRG lysates. $Na_V1.8$ was shown to interact with $Na_V\beta4$. These TTX-r resurgent currents may contribute to the membrane excitability of nociceptive DRG neurons under normal conditions. The increase in both types of resurgent currents by inflammatory mediators could contribute to neuronal hyperexcitability associated with inflammatory pain[8].

Pain-related bone cancer is caused in part by increased excitability of DRG neurons. Western blot analysis and electrophysiological recordings of rat DRGs show that $Na_V1.8$ channel expression and activity are upregulated in DRG neurons, and contribute to the development of cancer-induced bone pain[3]. Similar data is observed for $Na_V1.9$, for which changes in protein levels are significant, as observed in western blot analysis and immunohistochemistry using **Anti-SCN11A (Na_V1.9) Antibody** (#ASC-017) (Figure 1)[4].

Painful diabetic neuropathy is a complication from which diabetic patients can suffer from. It is believed to originate in the peripheral system. A study showed that $Na_V1.7$ and $Na_V1.8$ channels are both upregulated in small DRGs and in peripheral nociceptive C-fibers in both immunohistochemistry and western blot analyses using Anti-Na_V1.8 (SCN10A) Antibody and **Anti-Na_V1.7 (SCN9A) Antibody** (#ASC-008) (Figure 2). The increased excitability in small DRGs from diabetic rats might underlie the decreased conduction in the diabetic high-firing-frequency polymodal C-fibers, thus uncovering a novel mechanism for hyperalgesia associated with diabetes[7].

In a study, nociceptive sensory neurons were generated from HUES6

Figure 1. $Na_V1.9$ Expression Increases in DRGs in a Rat Model of Bone Cancer Pain



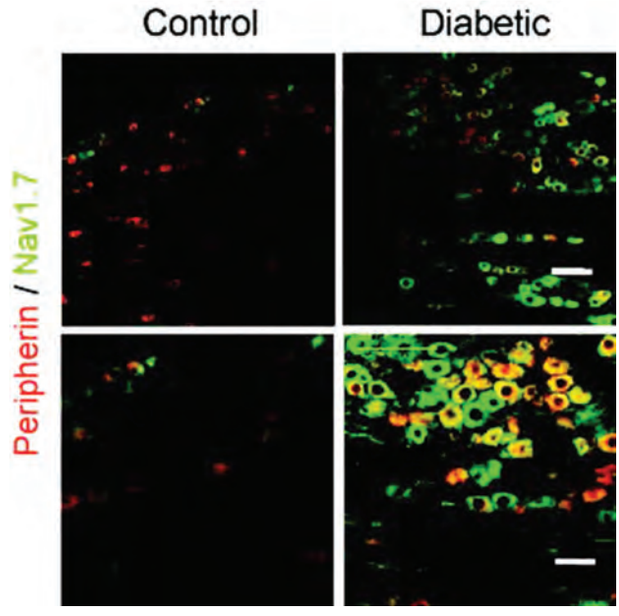
A. Western blot analysis of rat DRG lysates using **Anti-SCN11A ($Na_V1.9$) Antibody** (#ASC-017). B. Immunohistochemical staining of rat DRG sections using same antibody as in A. Both, western blot and immunostaining show that $Na_V1.9$ expression significantly increases in DRGs 14 days following bone cancer induction (C14), compared to sham-treated rats.
Adapted from reference 4 with permission of Elsevier.

embryonic stem cells. Characterization of the cells was achieved in part by immunocytochemical staining of the derived nociceptors using **Anti-TRPV1 (VR1) Antibody** (#ACC-030) and Anti-SCN11A ($Na_V1.9$) Antibody. These cells also express cardiac specific $Na_V1.5$ channels, stained using **Anti-$Na_V1.5$ (SCN5A) (1978-2016) Antibody** (#ASC-013), reinforcing its role during development. This work demonstrates that nociceptors can be derived from human pluripotent stem cells (hPSCs) and furthermore establishes a platform for studying developmental processes in nociceptive neurons and the possibility of developing targeted pharmacology[1].

Studies show that inflammatory cytokines are elevated in neuropathic pain. Furthermore, administration of inflammatory cytokines induces neuropathic pain which is also accompanied by an increase in pain-related Na_V channel expression. IL-10, an anti-inflammatory cytokine was shown to reverse the effects of inflammatory cytokines, and furthermore decrease the expression of $Na_V1.3$, $Na_V1.6$ and $Na_V1.8$ channels as shown in immunocytochemistry and western blot analyses of rat DRGs using Alomone Labs **Anti-SCN3A ($Na_V1.3$) Antibody** (#ASC-004), **Anti-$Na_V1.6$ (SCN8A) Antibody** (#ASC-009) and Anti-$Na_V1.8$ (SCN10A) Antibody. Results suggest that down-regulating Na_V channels might contribute to the effects of IL-10 in neuropathic pain[5].

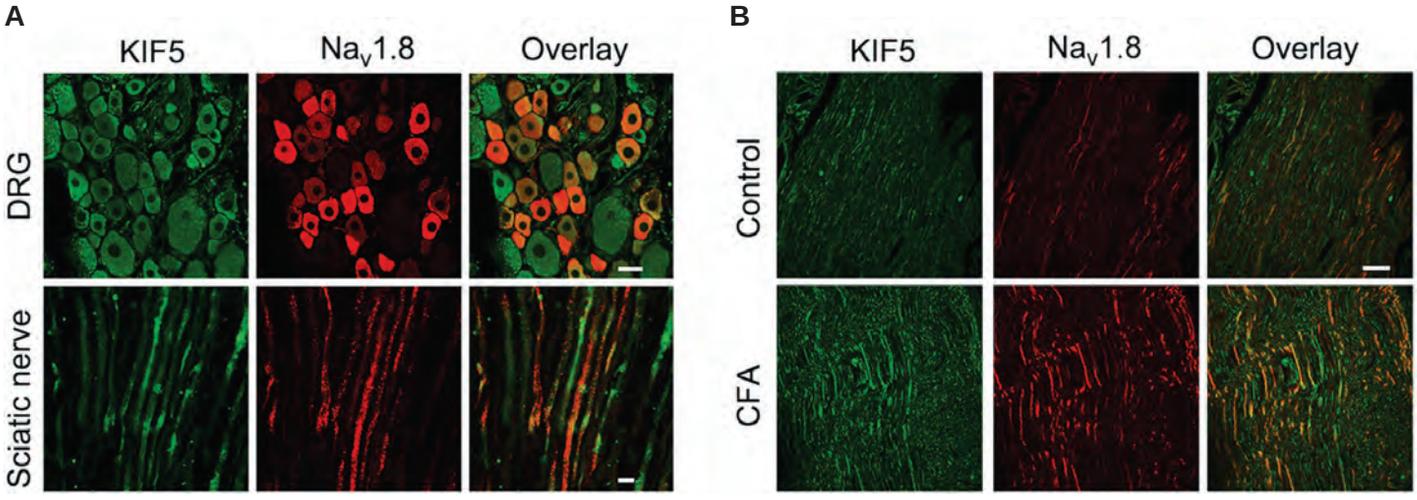
KIF5, a kinesin, is responsible for mediating the plus end-directed, microtubule-dependent transport of cargo proteins. It also plays an important role in transporting ion channels across long distances in axons. Following peripheral inflammation or nerve injury, $Na_V1.8$ accumulates in peripheral nerves. KIF5 was found to be responsible for transporting $Na_V1.8$ to the plasma membrane and axons in dorsal root ganglion (DRG) neurons. $Na_V1.8$ co-immunoprecipitates with KIF5 and both proteins immuno-colocalize in DRG and in the sciatic nerve as shown in immunohistochemical staining using Anti-$Na_V1.8$ (SCN10A) Antibody (Figure 3). This study provides a mechanism for $Na_V1.8$ accumulation following inflammation[6].

Figure 2. Increased $Na_V1.7$ Expression in DRGs from Diabetic Rats



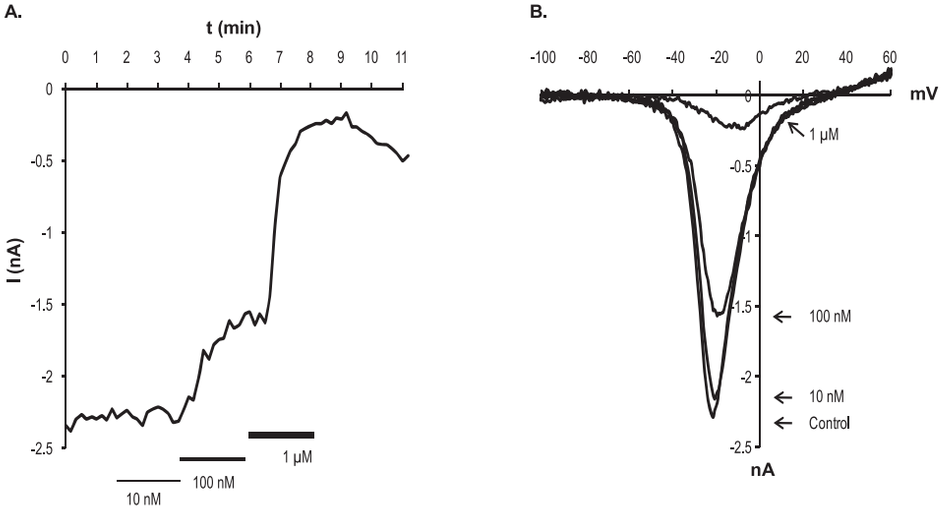
Immunohistochemical staining of rat DRG sections using **Anti-$Na_V1.7$ (SCN9A) Antibody** (#ASC-008). $Na_V1.7$ expression (green) is higher in DRGs from diabetic rats (right panels) compared to control rats (left). C-fibre marker peripherin is labeled in red. Top: x20 magnification. Bottom: x40 magnification.
Adapted from reference 7 with permission of Oxford University Press.

Figure 3. Immuno-Colocalization of KIF5 and $Na_V1.8$ in Rat DRG and Sciatic Nerve



Immunohistochemical staining of rat DRG and sciatic nerve using **Anti-$Na_V1.8$ (SCN10A) Antibody** (#ASC-016). A. $Na_V1.8$ (red) and KIF5B (green) co-localize in DRG and sciatic nerve. B. $Na_V1.8$ and KIF5B expression increases following inflammation induction.
Adapted from reference 6 with permission of the Society for Neuroscience.

Alomone Labs A-803467 Inhibits $Na_V1.8$ Channels Stably Transfected in ND7/23 Cells



Currents were elicited by 50 ms voltage ramp from the holding potential of -100 mV to +60 mV, applied every 10 sec, using whole-cell voltage clamp technique. A. Time course of $Na_V1.8$ current amplitude changes as a result of **A-803467** (#A-105) application at increasing concentrations, as indicated by the horizontal bars. B. Superimposed traces of $Na_V1.8$ currents under control conditions and after 100 sec perfusion with 10-8-10-6 M A-803467, as indicated.

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5. Shen, K.F. *et al.* (2013) *Exp. Neurol.* **247,** 466.
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7. Sun, W. *et al.* (2012) *Brain* **135,** 359.
8. Tan, Z.Y. *et al.* (2014) *J. Neurosci.* **34,** 7190.

Related Products

Product name	Cat. #
Rabbit Polyclonals	
Anti-SCN1A ($Na_V1.1$) Antibody	ASC-001
Anti-SCN3A ($Na_V1.3$) Antibody	ASC-004
Anti-SCN3A ($Na_V1.3$) (extracellular) Antibody	ASC-023
Anti-$Na_V1.5$ (SCN5A) (1978-2016) Antibody	ASC-013
Anti-$Na_V1.6$ (SCN8A) Antibody	ASC-009
Anti-$Na_V1.7$ (SCN9A) Antibody	ASC-008
Anti-$Na_V1.7$ (SCN9A) (extracellular) Antibody	ASC-027
Anti-$Na_V1.8$ (SCN10A) Antibody	ASC-016
Anti-$Na_V1.8$ (SCN10A) (extracellular) Antibody	ASC-028
Anti-SCN11A ($Na_V1.9$) Antibody	ASC-017
Anti-TRPV1 (VR1) Antibody	ACC-030
Conjugated Rabbit Polyclonals	
Anti-SCN1A ($Na_V1.1$)-ATTO-594 Antibody	ASC-001-AR
Anti-$Na_V1.7$ (SCN9A)-ATTO-633 Antibody	ASC-008-FR
Anti-$Na_V1.8$ (SCN10A)-ATTO-594 Antibody	ASC-016-AR
Guinea Pig Polyclonals	
Guinea pig Anti-SCN1A ($Na_V1.1$) Antibody	AGP-043
Guinea pig Anti-$Na_V1.8$ (SCN10A) Antibody	AGP-029
Guinea pig Anti-$Na_V1.7$ (SCN9A) Antibody	AGP-057
Guinea pig Anti-SCN11A ($Na_V1.9$) Antibody	AGP-030
Pain-Related Na_V Channel Activators	
Aconitine	A-150
Anthopleurin-C	A-400
ATX-II	STA-700
Jingzhaotoxin-II	STJ-150
1Kα-Pompilidotoxin	P-172
3Rα-Pompilidotoxin	P-174
3R12Rα-Pompilidotoxin	P-176
α-Pompilidotoxin	P-170
1Kβ-Pompilidotoxin	P-182
β-Pompilidotoxin	P-180
Veratridine	V-110
Pain-Related Na_V Channel Blockers	
4,9-Anhydrotetrodotoxin	T-560
A-803467	A-105
Ambroxol hydrochloride	A-145
α-Asarone	A-260
Carbamazepine	C-105
Ceratotoxin-1	STC-680
Ceratotoxin-2	STC-100
Co 102862	C-160
μ-Conotoxin BuIIIB	STC-661
μ-Conotoxin CnIIIC	STC-640
μ-Conotoxin KIIIA	C-280
μ-Conotoxin PIIIA	STC-400
μ-Conotoxin SIIIA	STC-450
GsMTx-4	STG-100
GTx1-15	STT-300
Hainantoxin-III	STH-120
Hainantoxin-IV	STH-130
Huwentoxin-I	STH-050
Huwentoxin-IV	STH-100
mHuwentoxin-IV	STH-101
ICA 121431	I-170
Jingzhaotoxin-V	STJ-050
KC 12291 hydrochloride	K-105
Lidocaine	L-105
Lidocaine hydrochloride	L-145
Lorcainide HCl	L-135
Mepivacaine hydrochloride	M-110
Mexiletine hydrochloride	M-115
$Na_V1.7$-Compound 36	CMN-003
N-Me-aminopyrimidinone 9	N-310
Oxcarbazepine	O-105
Orphenadrine hydrochloride	O-101
PF-04856264	P-265
PF-05186462	P-365
PF-05241328	P-345
Phenytoin	P-235
Pilsicainide hydrochloride	P-145
Pyrrolopyrimidine 48	P-275
ProTx-I	STP-400
ProTx-II	STP-100
ProTx-III	STP-150
Tetrodotoxin citrate free	T-500
Tetrodotoxin citrate	T-550
VSTX3	STT-350
XEN907	X-105
Explorer Kits	
Classical Ion Channel Blockers for Pain Research Explorer Kit	EK-355
Ion Channel Activators for Pain Research Explorer Kit	EK-395
Na_V Channel Antibodies for Pain Research Explorer Kit	AK-365
Na_V Channel Blockers for Pain Research Explorer Kit	EK-375
Pain-Related DRG Marker Antibody Kit	AK-550
Research Packs	
$Na_V1.7$ Channel Basic Research Pack	ESB-001
$Na_V1.7$ Channel Premium Research Pack	ESP-001
$Na_V1.7$ Channel Deluxe Research Pack	ESD-001
$Na_V1.8$ Channel Basic Research Pack	ESB-003
$Na_V1.8$ Channel Premium Research Pack	ESP-003
$Na_V1.8$ Channel Deluxe Research Pack	ESD-003

Na_V Channel product listing

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- Disease/indication (e.g. Parkinson's disease, pain, LongQT syndrome).

For more information on Alomone Labs Kits and Packs:

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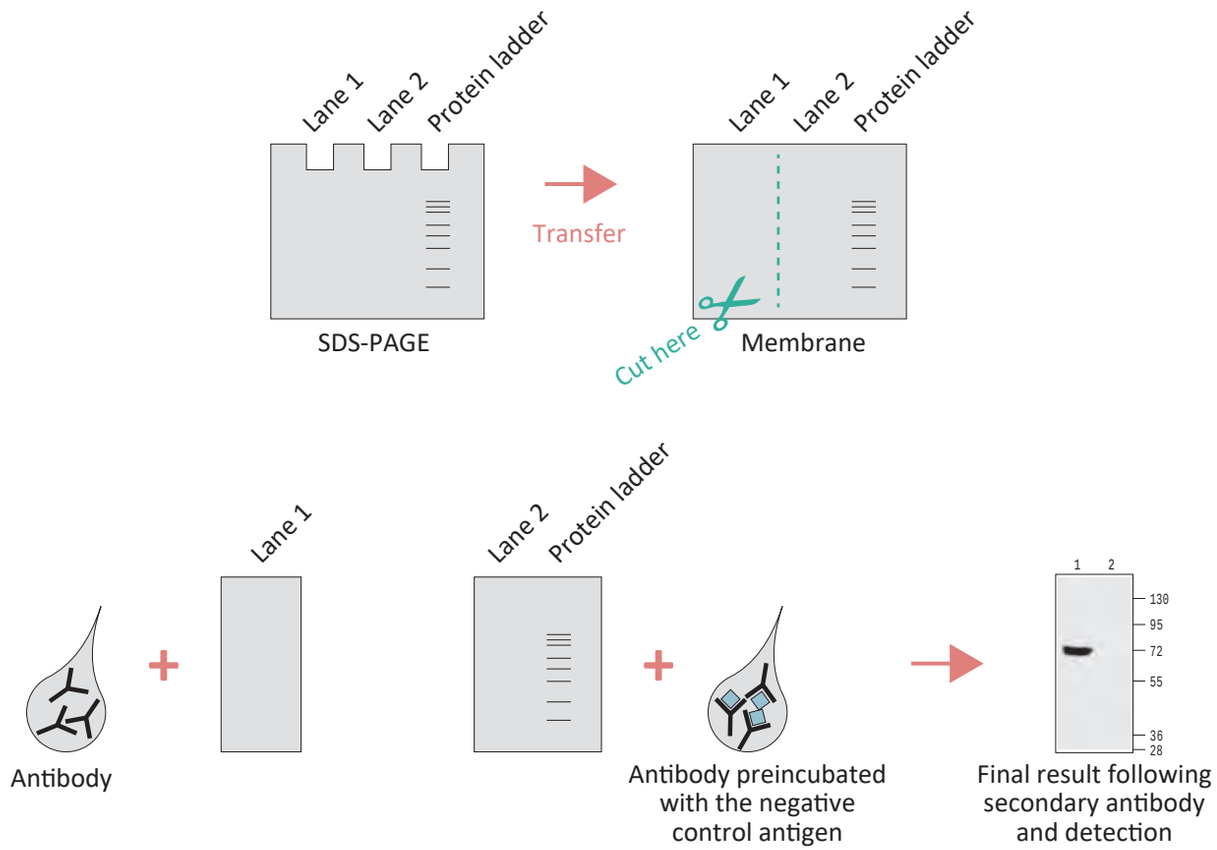
Negative Control Antigen

Specificity Validation. Simple Verification.



What is the negative control antigen?

The negative control antigen is the peptide used to immunize the animal to produce the antibody. Delivered in a clear vial with your antibody purchase, the negative control antigen is included with every polyclonal antibody.

What are the benefits?

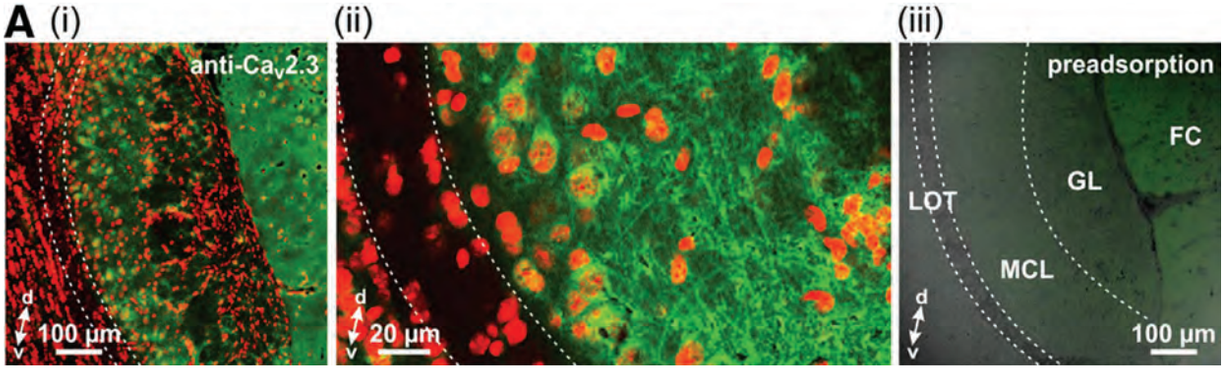
Prior to releasing new antibody lots, our dedicated in-house staff performs appropriate experiments using the negative control antigen, to ascertain that the new antibody lot is as specific as the previous one. Since you may have a different experimental setup, the negative control antigen we provide lets you validate the specificity of the antibody in your research as well.

How do I use the negative control antigen?

Using the negative control antigen is very easy. After calibrating the conditions under which the antibody works, simply run a side-by-side experiment – antibody alone vs antibody pre-incubated with the negative control antigen.

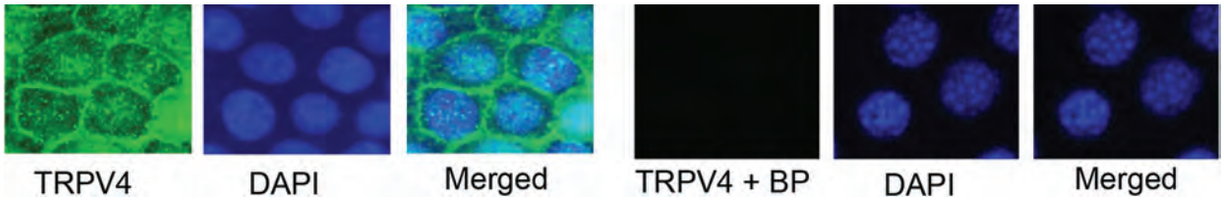
Examples of How You Use the Control Antigen

Expression of $Ca_V2.3$ Channel in Mouse Accessory Olfactory Bulb



Immunohistochemical staining of mouse accessory olfactory bulb sections using **Anti-$Ca_V2.3$ (CACNA1E) Antibody** (#ACC-006). A(i). $Ca_V2.3$ staining (green) is detected in mitral cells. A(ii). High-magnification of (i). A(iii). Note the absence of immunofluorescent staining when the antibody is preincubated with the control antigen.
Adapted from Gorin, M. *et al.* (2016) *J. Neurosci.* **36,** 3127 with permission of the Society for Neuroscience.

Expression of TRPV4 in Mouse Kidney Cell Line



Immunocytochemical staining of mouse mCCDcl1 kidney cells using **Anti-TRPV4 Antibody** (#ACC-034), (green). TRPV4 staining is completely abolished when the antibody is preincubated with the control antigen (right panels).
Adapted from Li, Y. *et al.* (2016) *PLoS ONE* **11,** e0155006. with permission of PLoS.

For a detailed protocol on how to use the negative control antigen:

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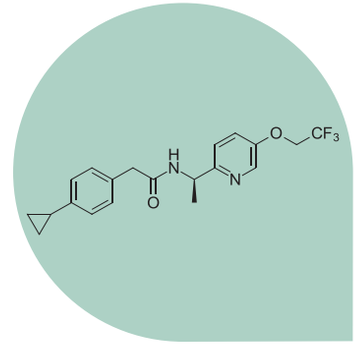
TTA-P2 and TTA-A2: Specific and Potent Ca_V3 Channel Blockers

Melanie R. Grably, Ph.D.

Genes encoding T-type Ca_V channels yield three pore-forming subunits: $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ which give rise to currents that are pharmacologically and electrophysiologically different from the high voltage Ca_V1 and Ca_V2 channel currents. Due to their important physiological roles, and to the increasing number of channelopathies associated with T-type channels, the need of specific and potent pharmacology is rising. In this piece, we highlight the use of TTA-A2 and TTA-P2, two specific and potent T-type channel blockers exclusively available at Alomone Labs.



Right: TTA-A2 is a selective and potent T-type Ca_V channel blocker.

Introduction

T-type Ca_V channels regulate neuronal excitability, hormone secretion, and neurotransmitter release. They also play important roles in the circadian cycle, cardiovascular and rennin-angiotensin systems.

Knockout of $Ca_V3.1$, $Ca_V3.2$ or $Ca_V3.3$ channels in mice yields viable phenotypes, albeit each with various problems. Channelopathies associated with T-type channels include autism, epilepsy, hypertension, hyperaldosteronism, chronic pain, and neuropathic pain. Due to their important physiological roles, and to the increasing number of channelopathies associated with the channels, the need of specific and potent pharmacology is rising[9].

TTA-P2

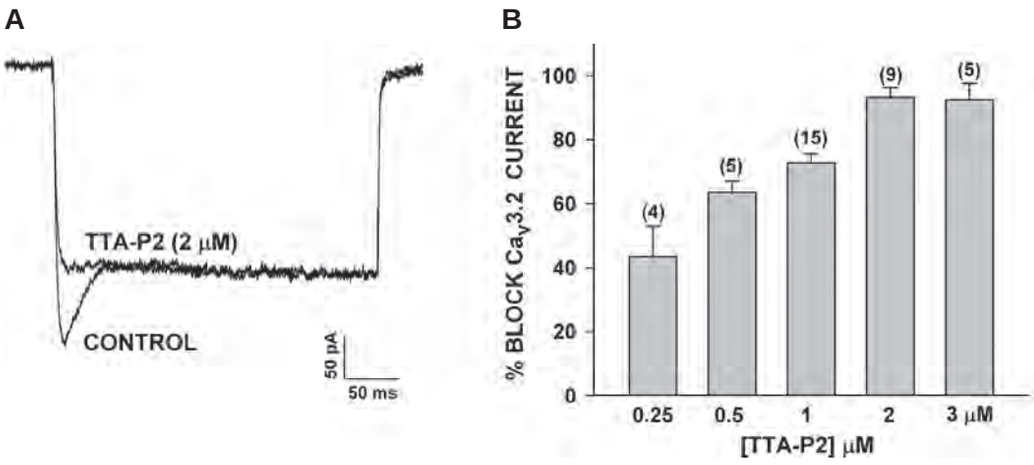
TTA-P2, a derivative of 4-aminomethyl-4-fluoropiperidine, was first discovered to potently and reversibly block T-type currents from rat acutely dissociated DRGs, with an IC_{50} of 100 nM and stabilize T-type channels in their inactivated states. L-type Ca_V channels and Na_V channels are 100-1000 fold less sensitive to the compound's blocking actions[1].

TTA-P2 (#T-155) was used to gain more insight on the specific role and identity of Ca_V channels expressed in adrenal fasciculate cells. Indeed, TTA-P2 inhibited $Ca_V3.2$ currents expressed in the cells (Figure 1) and inhibited adrenocorticotropic hormone (ACTH) and Angiotensin II-stimulated cortisol secretion[2]. Under hypoxia, catecholamine secretion by the adrenal gland increases in a BDNF/TrkB-dependent manner. TrkB receptor is highly expressed in the medulla as shown in immunohistochemical staining using Alomone Labs **Anti-TrkB (extracellular) Antibody** (#ANT-019), and its expression increased under hypoxic conditions. In addition to catecholamine secretion, activation of TrkB also lead to an increase in $[Ca^{2+}]_i$ caused by T-type Ca_V currents which was inhibited by the application of TTA-P2, thus showing a linkage between BDNF/TrkB signaling and T-type currents[7].

The transmission of pain begins at the spinal dorsal horn which then transmits the information onward. Rebound depolarization following hyperpolarization in dorsal horn neurons is an important feature in those cells and was characterized in part by the use of TTA-P2. Data show that rebound depolarization and firing by T-type Ca_V channels and their currents are important for integrating somatosensory information in the spinal cord[6].

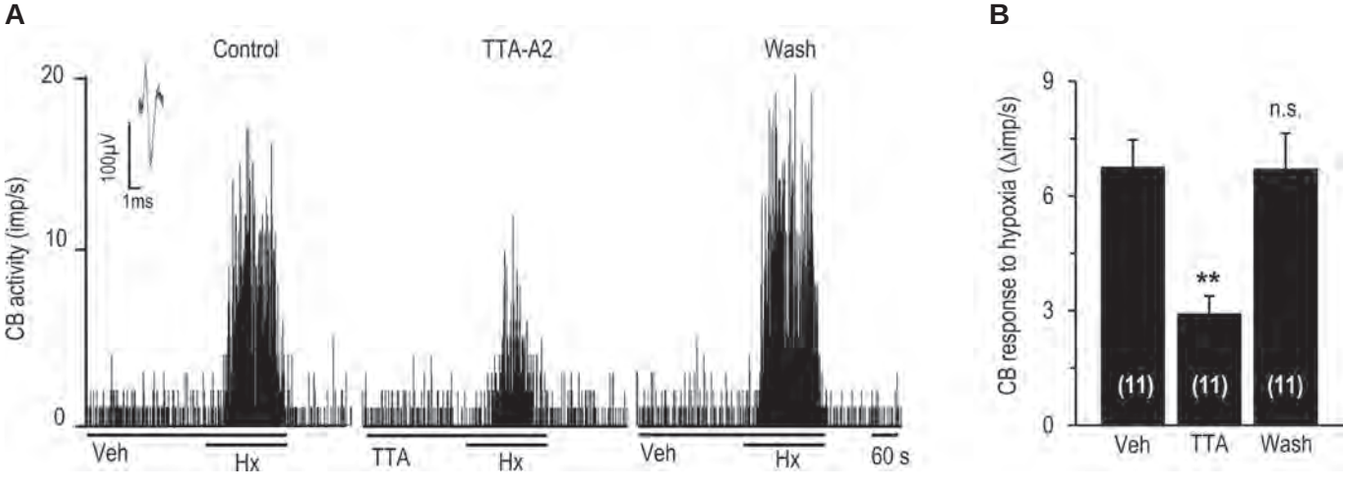
The thalamus plays an important role in integrating inputs from the cortex. Once the thalamus receives these inputs, it sends them back to the cortex creating a cortico-thalamo-cortical loop. These synapses and their contribution to higher brain functions were studied. Barrel cortex layer 5B neurons and those of the posteriomedial nucleus (POm) were used to study the thalamo-cortical synapse formation. Using conditional knockout of GluA4, shRNA targeting $Ca_V3.1$ channel and the T-type specific blocker TTA-P2, data show that GluA4 and $Ca_V3.1$ control important aspects of the synaptic transmission at L5-POm synapse[8].

Figure 1. T-Type Ca_V Channel Inhibition by TTA-P2 in AZF Cells



A. Bovine adrenal zona fasciculate (AZF) whole cell recordings. Ca^{2+} currents were recorded in 10 mM Ba^{2+} in response to voltage steps to -5 mV, applied from a holding potential of -80 mV, before and after superfusion of the cell with 2 μM **TTA-P2** (#T-155). B. Concentration-dependent inhibition of $Ca_V3.2$ currents by TTA-P2. Values are means ± SE for number of determinations shown in parentheses.
Adapted from reference 2 with permission of the American Physiological Society.

Figure 2. T-Type Ca_V Channel Response to Hypoxia Is Inhibited by TTA-A2



A. Representative example of sensory nerve response (impulses (imp)/s) to hypoxia in the presence of vehicle or 25 μM **TTA-A2** (#T-140), and 5 min after washout. B. Effect of TTA-A2 on sensory nerve response to hypoxia.
Adapted from reference 5 with permission of the American Physiological Society.

TTA-A2

TTA-A2 was discovered in an effort to identify T-type specific and potent blockers. The compound was characterized on transfected HEK 293 cells and on native T-type currents. Data show that TTA-A2 has an IC_{50} value of 100 nM, and like TTA-P2, it binds preferentially to Ca_V3 channels in their inactive state. TTA-A2 is 300-fold selective for Ca_V3 channels. *in vivo*, TTA-A2 administration suppresses active wake and promotes slow-wave sleep in wild-type but not in mice lacking $Ca_V3.1$ and $Ca_V3.3$[4].

Oxygen levels in the blood are detected by carotid bodies. Voltage-gated Ca^{2+} channels play an important role in detecting O_2 levels. $Ca_V3.2$ channel is the major T-type Ca_V channel expressed in glomus cells, the cells important for sensing O_2. Indeed, RT-PCR and immunohistochemistry using **Anti-$Ca_V3.2$ (CACNA1H) Antibody** (#ACC-025) shows that $Ca_V3.2$ is highly expressed in the rat carotid body. Western blot analysis of rat DRG lysates using the same antibody shows that the Ca^{2+} channel is also expressed in DRGs. Importantly, use of the control peptide antigen completely obliterated the signal obtained with the antibody. In addition, $Ca_V3.2$ was found to be involved in mediating the carotid body's response to hypoxia, an effect inhibited by the application of **TTA-A2** (#T-140) (Figure 2)[5].

The contribution of T-type channels in myogenic reactivity of retinal arterial vessels was studied in arterioles of the rat retinal microcirculation. Immunohistochemical staining of rat retinal arterioles using **Anti-CACNA1G ($Ca_V3.1$) Antibody** (#ACC-021) and Anti-$Ca_V3.2$ (CACNA1H) Antibody showed that while $Ca_V3.2$ was not detected, strong $Ca_V3.1$ staining was observed on the plasma membrane of retinal arteriole smooth muscle cells. $Ca_V3.2$ was detected on glial cell end-feet surrounding the vessels. Application of TTA-A2 or **ML 218** (#M-165) dilated isolated, myogenically active retinal arterioles, demonstrating that $Ca_V3.1$ channels are functionally expressed on arteriole smooth muscle cells or retinal arterioles and play an important role in myogenic signaling[3].

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Related Products

Product name	Cat. #
T-Type Ca_V Channel Antibodies	
Anti-CACNA1G ($Ca_V3.1$) Antibody	ACC-021
Anti-CACNA1G ($Ca_V3.1$)-ATTO-594 Antibody	ACC-021-AR
Anti-$Ca_V3.2$ (CACNA1H) Antibody	ACC-025
Anti-$Ca_V3.3$ (CACNA1I) Antibody	ACC-009
T-Type Ca_V Channel Blockers	
Amlodipine	A-110
Amlodipine besylate	A-120
Benidipine hydrochloride	B-120
Flunarizine dihydrochloride	F-110
GTx1-15	STT-300
L-Ascorbic acid	L-140
Mibefradil dihydrochloride hydrate	M-150
ML 218	M-165
ML 218 hydrochloride	M-166
Nicardipine HCl	N-125
NNC 55-0396 dihydrochloride	N-205
NNC 55-0396 dihydrochloride hydrate	N-206
Penfluridol	P-135
ProTx-I	STP-400
ProTx-II	STP-100
Roscovitine	R-300
SKF 96365 hydrochloride	S-175
TTA-A2	T-140
TTA-P2	T-155
TTA-P2 (S-enantiomer)	T-210
TrkB Antibody	
Anti-TrkB (extracellular) Antibody	ANT-019
Explorer Kits	
Cardiac Ca_V Channel Antibody Explorer Kit	AK-310
Cardiac Channel Blocker Explorer Kit	EK-345
Ca_V Channel Antibodies for Pain Research Explorer Kit	AK-360
Classical Ion Channel Blockers for Pain Research Explorer Kit	EK-355
Non-L-Type Ca_V Channel Antibody Explorer Kit	AK-216
T-Type Ca_V Channel Blocker Explorer Kit	EK-111
Research Packs	
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T-Type Ca_V Channel Premium Research Pack	ESP-101
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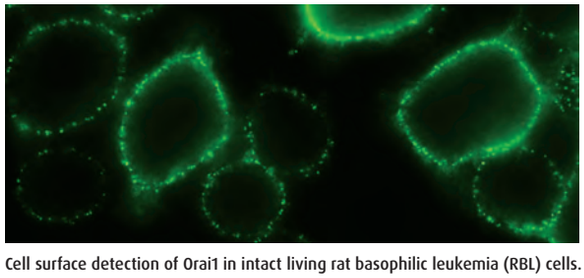

Cell surface detection of Orai1 in intact living rat basophilic leukemia (RBL) cells.
Extracellular staining of cells using **Anti-Orai1 (extracellular) Antibody** (#ACC-062).

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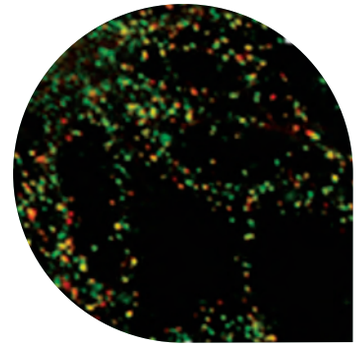
NMDA Receptor Dynamics Dictate Neuronal Plasticity and Function

Tommy Weiss Sadan, Ph.D. and Melanie R. Grably, Ph.D.

N-Methyl-*D*-Aspartate Receptor (NMDAR) are ubiquitously expressed along the central nervous system and are instrumental to various physiological processes such as synaptic plasticity and learning. Nevertheless, several mental disabilities including schizophrenia and Alzheimer's disease are all related to NMDAR dysfunction. Here, we review many aspects of NMDAR function and regulation and describe their involvement in pathophysiological states using Alomone Labs products.



Right: Cell surface detection of GluN2B in rat hippocampal neurons.

Introduction

Glutamate is a key neuro-transmitter in the central nervous system and acts on a variety of cell surface receptors, collectively termed ionotropic glutamate receptors (iGluRs)[15]. The N-Methyl-*D*-Aspartate receptors (NMDAR) are members of the iGluR superfamily and are pivotal to many physiological processes such as the formation of long term memory, synaptic plasticity and many other cognitive functions. Therefore, it is not surprising that several mental disorders including schizophrenia, epileptic aphasia and other debilitating neurodegenerative diseases such as Alzheimer's are all related to NMDAR dysfunction[21].

Structure

Most NMDAR proteins form a functional heterotetramer protein-complexe by incorporating the obligatory NR1 (GluN1) subunit with different types of the four NR2 subunits, termed NR2A-D (GluN2A-D) or two NR3 isoforms (GluN3A,B)[6, 19]. Furthermore, this heterogenic complexity is further increased by the fact that NR1 is predisposed to alternative splicing events and can give rise to additional eight proteins[19]. Eventually, this diversity of protein assemblies brings about a variety of NMDAR receptors with different biophysical properties and expression patterns throughout the nervous system[15].

Each of these NMDAR protein complexes contain an extracellular N-terminal domain and a ligand binding domain for glycine on GluN1 and glutamate binding domain on GluN2 and GluN3 subunits[6]. In addition, the intracellular carboxy tail is another important domain as it has an impact on receptor trafficking, anchoring and mediates various interactions with intracellular signaling proteins[15].

Mechanism of Action

NMDAR activation depends on sequential conformational changes to relieve the magnesium blockade which is achieved by rapid membrane depolarization and binding of both glycine and glutamate ligands[6, 21]. This in turn removes the inhibitory electrostatic forces of magnesium and enables calcium influx and transmission of long lasting signals (i.e. long-term potentiation), a key mechanism to learning and memory formation[10].

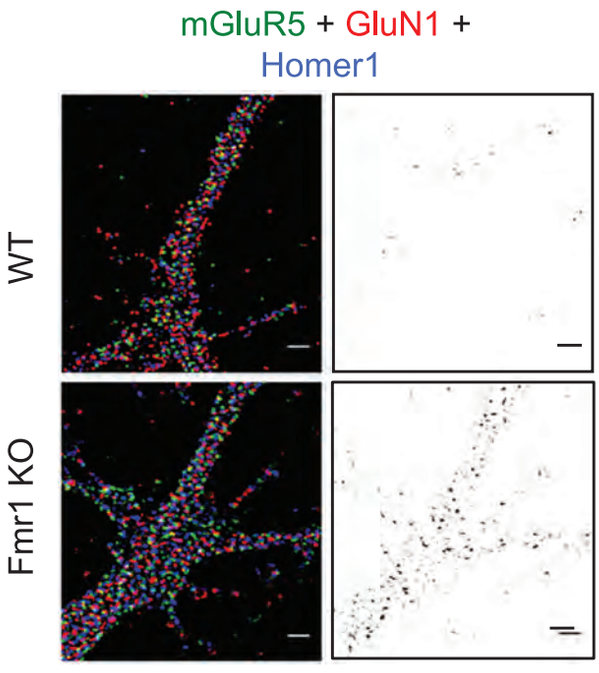
Regulation

Given their large diversity and wide-spread expression across the nervous system and the fact that NMDARs engage in essential physiological processes related to cognitive performance, it is not surprising that over the last decade numerous efforts were invested in understanding the regulation and function of these receptors.

NMDAR Function and Turnover by Scaffold Proteins

Neuronal communication occurs through synaptic connections where a presynaptic dendrite transmits a signal towards the postsynaptic body of another neuron. NMDARs are critical for these neuronal circuits and are tethered in the post synaptic density (PSD) areas by forming numerous connections with scaffold proteins and other cell-signaling mediators[10]. Homer proteins are an example of PSD scaffold proteins that mediate the connection between group-I metabotropic glutamate receptor subtype 5 (mGluR5) and NMDAR. Recently, Aloisi *et al.*, investigated the consequences

Figure 1. Increased Synaptic GluN1/mGluR5/Homer1 in *Fmr1*$^{-/-}$ Mice



Immunocytochemical staining of mouse hippocampal neurons using **Anti-NMDAR1 (GluN1) (extracellular) Antibody** (#AGC-001), (red). Triple immunostaining experiment indicates increased synaptic clustering of GluN1, mGluR5 and Homer1 in *Fmr1* knockout mice. Adapted from reference 1 with permission of SPRINGER NATURE.

of Homer disruption in the context of fragile x syndrome, where intellectual disabilities are frequently associated with abnormal mGluR5 and NMDAR functions[1]. Using **Anti-NMDAR1 (GluN1) (extracellular) Antibody** (#AGC-001), the authors uncovered a novel mechanism through which mGluR5 suppresses NMDAR activity in hippocampal neurons obtained from *Fmr1* knockout (KO) mice. Quantum dot tracing demonstrated that GluN1 and mGluR5 cluster in the synaptic area, suggesting a possible existence of a physical interaction[1]. Indeed, confocal microscopy showed co-localization of GluN1 with mGluR5, which increased in *Fmr1* KO neurons[1] (Figure 1). Lastly, the authors demonstrated that the mGluR5-NMDAR interaction attenuates NMDAR signal transduction as measured by NMDAR-dependent excitatory post-synaptic currents (ESPC).

PSD-95 is another important scaffold protein that mediates NMDAR anchoring at the PSD membranes of postsynaptic neurons[19]. PSD-95 activity is regulated by different mechanisms including phosphorylation on serine/threonine residues by the peptidyl-prolyl cis-/trans isomerase, Pin1[2]. This phosphorylation event induces conformational changes in PSD-95 and determines its substrate specificity[2]. The effect of PSD-95 interaction with NMDAR via its phosphorylation by Pin1 was investigated[2]. **Anti-NMDAR2B (GluN2B) (extracellular) Antibody** (#AGC-003) was used to probe GluN2B expression after immuno-purification of PSD-95 from brain extracts of *Pin1* KO mice or their wild type littermates[2]. GluN2B expression was significantly increased in brain lysates from *Pin1* KO compared to wild type[2]. Similarly, over-expression of GluN2B was also observed in hippocampal protein extracts of *Pin1* KO mice by western blotting[2]. Taken together, these data place Pin1 as a negative modulator of PSD-95-NMDAR interaction.

In light of the prominent role of scaffold proteins for neuronal cell function, Scribble1 (Scrib1) was found to be a critical modulator of NMDARs spatial distribution[16]. In particular, Scribble1-NMDAR interaction protects NDMARs from lysosomal degradation and thus, increases their synaptic cell-surface concentration. Investigation into the mechanisms that underpins Scribble1-NMDAR interactions, zeroed in on the PDZ domain of Scribble1 as the site of protein interaction[16]. Green fluorescent protein (GFP), Scribble1-GFP and a Scribble-mutant isoform, lacking two PDZ domains were overexpressed in primary hippocampal neurons and cell-surface levels of NMDAR were determined by fluorescent microscopy, using Anti-NMDAR2B (GluN2B) (extracellular) Antibody[16]. For quality control purposes, **Anti-GABA(A) α1 Receptor (extracellular) Antibody** (#AGA-001) was used to show that ectopic expression of Scrib1 did not alter the nature of these neurons. Thus, increased cell-surface levels of NMDAR due to Scribble interaction are specific and depend on PDZ2 and PDZ3 domains[16].

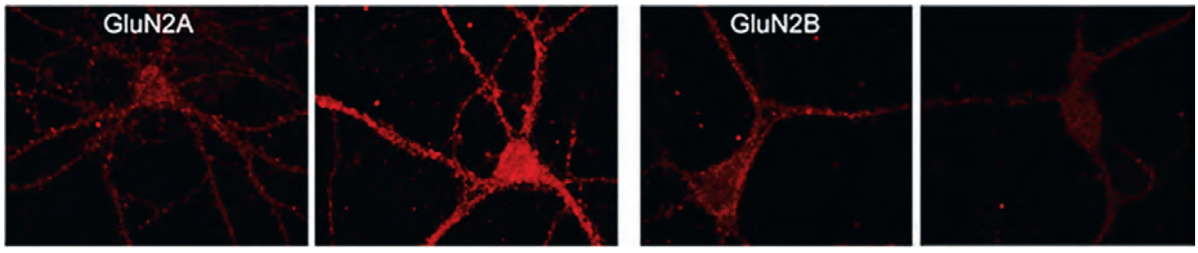
Regulation of Synaptic NMDAR Composition by Protein Degradation

Synaptic NMDAR composition is a highly dynamic process that has a substantial impact on signal transduction and developmental processes[15]. Synaptic composition is regulated by different mechanisms including transcriptional networks, protein trafficking systems but also through active proteolytic degradation[10, 19]. Accordingly, the ubiquitin ligase, F-box Only Protein 2 (Fbxo2) regulates synaptic expression of GluN1-GluN2A subunits by means of selective subunit degradation. Given the fact that Fbxo2 promotes GluN1 degradation is established, an *in vitro* model of non-neuronal cells was used to compare the effect of Fbxo2 on GluN2A and GluN2B using the specific **Anti-NMDAR2A (GluN2A) (extracellular) Antibody** (#AGC-002) and Anti-NMDAR2B (GluN2B) (extracellular) Antibody[3]. Intriguingly, a negligible effect of Fbxo2 on GluN2B degradation was observed as evident by western blotting. Likewise, brain lysates from *Fbxo2* KO mice displayed higher levels of GluN2A, while GluN2B expression remained unchanged[3]. To examine these observations from a different angle, Atkin *et al*., used the above-mentioned antibodies in immunocytochemical staining of primary hippocampal neurons. As anticipated, GluN2A expression was greater in *Fbxo2* KO neurons compared to wild type neurons, while GluN2B levels seemed to be down-regulated (Figure 2)[3]. Finally, the authors devised an ELISA assay, using Anti-NMDAR1 (GluN1) (extracellular) Antibody from Alomone Labs to verify whether increased synaptic expression of GluN1-GluN2A is due to decreased protein internalization. Indeed, cell surface expression of GluN1 was higher in *Fbxo2* KO neurons compared to control after treatment with bicuculline (to stimulate NMDAR internalization)[3]. Overall, these data shed light on a new model for neuronal plasticity, in which synaptic NMDAR composition is regulated by selective protein degradation.

Synaptic NMDAR Trafficking and Subunit Dynamics

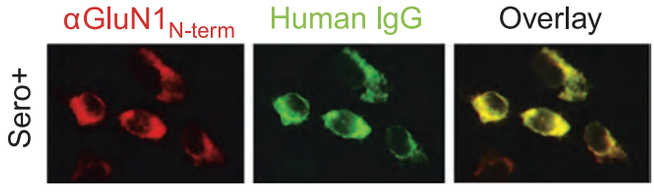
Lateral mobility shifts of NMDARs across the plasma membrane, are important for enabling neurons to rapidly respond and adapt to various environmental stimuli. In particular, enrichment of synaptic GluN2A over GluN2B containing NMDAR can have a large impact on synaptic long-term potentiation (LTP) however, the mechanisms underlying this remodeling process are not fully understood. Dupuis *et al*., have recently studied

Figure 2. Increased GluN2A Immunoreactivity in *Fbxo2*$^{-/-}$ Mice



Immunocytochemical staining of mouse hippocampal neurons using **Anti-NMDAR2A (GluN2A) (extracellular) Antibody** (#AGC-002) and **Anti-NMDAR2B (GluN2B) (extracellular) Antibody** (#AGC-003). GluN2A staining (left) increases in *Fbxo2*$^{-/-}$ neurons while GluN2B staining (right) decreases.
Adapted from reference 3 with permission of the Society for Neuroscience.

Figure 3. NMDAR Antibody IgGs in Human Serum



Immunocytochemical staining of live intact transfected HEK293 cells. Extracellular staining of cells with **Anti-NMDA Receptor 1 (GluN1) (extracellular) Antibody** (#AGC-001), (red), (left panel). Purified Human NMDAR IgGs (green) stain the transfected cells, (middle panel). Merge panel (right) shows complete overlap between the two stainings.
Adapted from reference 7 with permission of SPRINGER NATURE.

this process by tracing GluN2A and GluN2B mobility via single molecule quantum dots assay[4]. To do so, the authors used Anti-NMDAR2A (GluN2A) (extracellular) and Anti-NMDAR2B (GluN2B) (extracellular) antibodies in primary hippocampal neurons followed by chemical stimuli to generate neuronal LTP. GluN2B rapidly diffused to the peri-synaptic area, while GluN2A remained relatively stable in the PSD region[4]. In an antibody cross-linking experiment to immobilize GluN2B using the Alomone Labs GluN2B or GluN1 antibodies in cultured neurons, the authors noted that LTP signals were markedly suppressed[4]. Moreover, these observations were fully recapitulated in rat hippocampal brain slices following GluN1 cross-linking. Together these data imply on a causal relationship between NMDAR dynamics and perpetuated signal transmission. To delve deeper into the mechanism that underlies NMDAR dynamics, the authors tested several inhibitors and monitored GluN2B dynamics by using Anti-NMDAR2B (GluN2B) (extracellular) antibody coupled to quantum dot particles[4]. Data show that chemical inhibition or genetic manipulation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), largely reduced GluN2B-NMDAR membrane dynamics[4]. Altogether, Dupuis *et al.,* provide a mechanism through which CaMKII-GluN2B interaction reshapes synaptic NMDAR in response to stimuli.

Unlike CaMKII, which seems to support learning and memory, the protease tissue type plasminogen activator (tPA) seems to do the opposite. Lesept *et al*., provide fresh insights into the mechanism through which, tPA-NMDAR might be related to cognitive decline and neurotoxicity[11]. Using Anti-NMDAR1 (GluN1) (extracellular) Antibody in quantum dot application, they show that exogenous tPA increased NMDAR trafficking in extra-synaptic regions of cultured hippocampal neurons[11]. Furthermore, this diffusion pattern was mediated by tPA, as mutated tPA-protein did not replicate this mobility pattern[11]. Since tPA-NMDAR interaction increased calcium influxes in cultured neurons, the authors set out to investigate the influence of NMDAR mobility-shifts on intracellular calcium fluxes[11]. For this purpose, they decreased NMDAR surface mobility by antibody cross-linking. Consequently, receptor cross-linking generated extra-synaptic NMDAR clusters and increased intracellular calcium influx in response to stimuli[11], suggesting that tPA increased extra-synaptic calcium signaling owing to NMDAR clusters[11]. Using quantum dot tracing, the authors showed that tPA-NMDAR interaction stimulated NMDAR mobility in extra-synaptic regions and provided evidence for this mechanism to be involved in neurotoxicity and cell death.

In addition to protein-protein interactions, synaptic plasticity is also regulated by external factors such as hormones. Stress hormones like corticosteroids are rapidly released in response to environmental stress and regulate synaptic plasticity through various mechanisms including transcriptional regulation, but not limited to[12]. Mikasova *et al*., discovered a non-genomic mechanism by which corticosteroids trigger full-blown excitatory signals by regulating synaptic NMDAR dynamics[12]. Specifically, using Anti-NMDAR1 (GluN1) (extracellular) Antibody to block NMDAR mobility in cultured hippocampal neurons, the authors uncovered that synaptic signal potentiation due corticosterone necessitates synaptic remodeling of NMDAR[12].

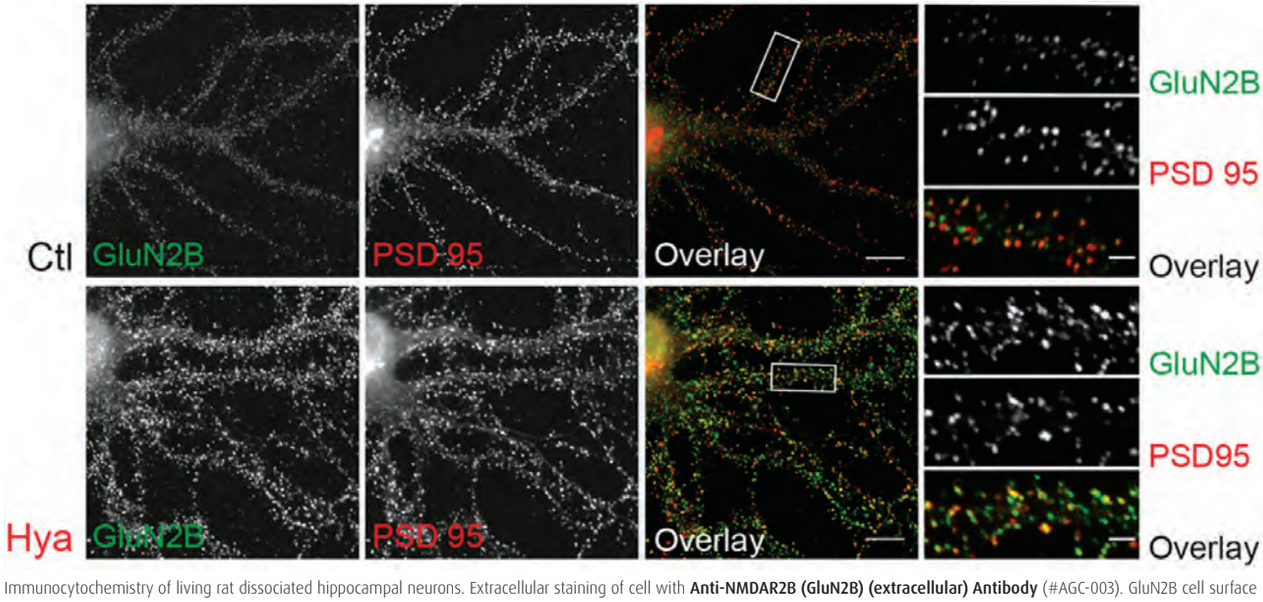
Given that NMDAR dynamics is key to LTP signals and cognitive performance, it is likely that several brain disorders such as schizophrenia (SCZ) and encephalitis are related to impaired synaptic NMDAR remodeling. Jézéquel *et al.*, demonstrated the presence of autoantibodies against NMDAR in patients with schizophrenia, but also in a small subgroup of healthy population by co-immunostainings of GluN1 expressed in embryonic kidney cells (HEK-293T) using the Alomone Labs antibody and IgGs purified from different donors (Figure 3)[7]. In addition, they also used brain hippocampal slices to validate their *in vitro* findings[7]. To reaffirm that patient-derived IgGs target NMDAR, the single nanoparticle quantum dots method was used to assess NMDAR membrane dynamics, given that different membrane proteins can be distinguished owing to different biophysical properties[7]. To demonstrate this, the authors labeled GluN1 using Anti-NMDAR1 (GluN1) (extracellular) Antibody as a positive control, patient-derived IgGs and an Anti-K_v1.3 Antibody from Alomone Labs, as a negative control and monitored their diffusion rates using quantum dots in live hippocampal neurons[7]. As anticipated, all targets displayed the same diffusion pattern, except for the potassium channel[7]. This confirms the presence of GluN1 autoantibodies in patients with SCZ as well as in a minor group of healthy population. To get a better understanding on how NMDAR-directed autoantibodies affect synaptic remodeling, the authors quantified the content of synaptic NMDAR in hippocampal neurons by confocal microscopy. Collectively, these data highlight the inflammatory basis of SCZ and encephalitis and suggest a mechanism by which autoantibody production affects neuronal synapses [7].

Given the inflammatory nature of several psychiatric disorders and the presence of autoantibodies for NMDAR in these patients, the provocative speculation for a possible immunotherapy to improve the outcomes in these patients was raised[8]. However, current diagnostic tools suffer from a lack of sensitivity when encountering patients with low antibody titers[8]. To work around this limitation, Jézéquel *et al.*, devised a sensitive bioassay based on quantum dot tracing of NMDAR using the GluN1 antibody from Alomone Labs in hippocampal neurons. In particular, the authors demonstrated that diffusion trajectories from patients with low titer antibodies are similar to those from patients with high titer antibodies[8]. Thus, quantum dot tracing outperforms the standard diagnostic assays as these patients would most probably be erroneously classified as false negatives.

NMDARs in Development and Developmental Disorders

Cortical neurons display a remarkable decline in functional plasticity during adulthood which is associated with two main events: the formation of hyaluronic acid-based extracellular matrix (ECM) and the preferential expression of GluN2A over GluN2B containing NMDAR[17]. Whether these two biological processes are related remains currently unknown[17]. A study by Schweitzer *et al.*, examined the influence of hyaluronic acid-based ECM on GluN2A and GluN2B expression in cortical neurons using Anti-

Figure 4. ECM Removal Leads to Increased GluN2B in Rat Hippocampal Neurons



Immunocytochemistry of living rat dissociated hippocampal neurons. Extracellular staining of cell with **Anti-NMDAR2B (GluN2B) (extracellular) Antibody** (#AGC-003). GluN2B cell surface expression (green) increases following extracellular matrix (ECM) removal (lower panels). GluN2B expression coincides with PSD-95 synaptic marker.
Adapted from reference 17. with permission of SPRINGER NATURE.

NMDAR2A (GluN2A) (extracellular) Antibody and Anti-NMDAR2B (GluN2B) (extracellular) Antibody. Surprisingly, when cultured hippocampal neurons were treated with Hyaluronidase (Hya) to remove hyaluronic acid-based ECM, no difference in GluN2B expression was observed as evident by western blot[17]. However, confocal microscopy studies on living hippocampal neurons showed that surface expression of GluN2B increased following Hya treatment (Figure 4)[17]. Furthermore, this phenomenon was not confined to the synapses but was also evident in extra-synaptic regions implying that this reflects a global effect rather than local synaptic plasticity. The fact that surface expression of GluN2B was changed without showing a notable difference in total GluN2B expression, raised the possibility that the ECM may regulate GluN2B dynamics. To test this possibility cortical neurons were stained with Anti-GluN2B antibody and the receptor's dynamics were tracked over time. As anticipated, lack of ECM due to Hya treatment decreased GluN2B endocytosis, thus confirming the hypothesis that hyaluronic acid-based ECM regulates GluN2B dynamics through endocytosis.

The prefrontal cortex (PFC) is a special cortical region that regulates many behavioral skills including the ability of decision making and moderating impulsive behaviors[9]. This brain region often matures later than other cortical areas and therefore, many impulsive and risky behaviors that are associated in adolescence, are thought to be attributed to this characteristic. Differences in GluN2A protein levels between premature and mature PFC of young and adult mice were observed in western blot analysis using the respective Alomone Labs antibody; no notable difference in GluN2B was observed regardless of age and brain region (Figure 5)[9]. Hence, the authors conclude that this characteristic could partially explain the impulsive behavior in adolescence[9].

Noonan syndrome is a genetic disorder associated with developmental and learning disabilities[14]. A recent study examined how a point mutation in SHP protein (associated with Noonan syndrome) may contribute to cognitive dysfunction. In early developed cultured hippocampal neurons increased MAPK signaling due to SHP mutation increased the number and size of NMDAR receptors as evident by immunocytochemical staining of rat premature hippocampal dissociated neurons using Anti-NMDAR1 (GluN1) (extracellular) Antibody[14]. In midstage neurons (div12), MAPK signaling favored the expression of GluA1 as evident by immunostaining of cultured hippocampal neurons using **Anti-GluR1 (GluA1) (extracellular) Antibody** (#AGC-004)[14]. In mature neurons (div18) SHP mutations increased both the size and number of GluA1 receptors[14]. Overall these data suggest that MAPK signaling caused by SHP mutations can alter neuronal development and cause cognitive impairments.

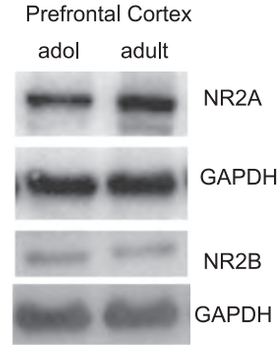
NMDAR Expression in Brain Disorders

Ischemic brain injury can inflict serious brain damage which results in various types of disabilities due neuronal cell death[13]. Despite the large interface and support of glial cells to neuronal function and synaptic plasticity, it is now clear that glial cells are involved in several pathologies related to brain ischemia[5, 13]. Glial cells also express NMDARs, however, remodeling processes related to these ion channels are not fully understood[5]. NMDAR remodeling in response to ischemic brain injury in glial cells was investigated in part by immunohistochemical staining of mouse brain sections using **Anti-NMDAR2C (GRIN2C) (extracellular) Antibody** (#AGC-018) and **Anti-NMDAR2D (GRIN2D) (extracellular) Antibody** (#AGC-020). Staining showed that ischemic injury induced their expression in cortical brain slices[5]. Next, the authors tested whether this expression pattern can influence calcium transitions in cortical glial cells. Indeed, they noticed a persistent elevation in calcium concentration in response to NMDA receptor agonist application. Blocking sodium Na_v channels with **Tetrodotoxin** (#T-500 or #T-550) did not influence calcium fluxes compared to specific NMDAR blockers, suggesting a causative link between NMDAR expression and function[5].

Epilepsy and its related diseases such as epileptic aphasia have been associated with NMDAR dysfunction and GluN2A mutations[18]. In a paper by Sibarov *et al.* the relationship between several point mutations in GluN2A protein and surface expression was explored by means of immunofluorescent microscopy in HEK-293T cells[18]. Mutated GuN2A variants were coupled to mCherry reporter and co-expressed with GluN1-GFP. To quantify surface expression of wild-type GluN2A, HEK-293T cells were stained using Anti-GluN2A antibody from Alomone Labs under non-permeabilizing conditions and quantified the overlap between mCherry (mutant) and antibody (WT) staining[18]. Mutants with low cell surface expression displayed similar low electrophysiological recordings[18].

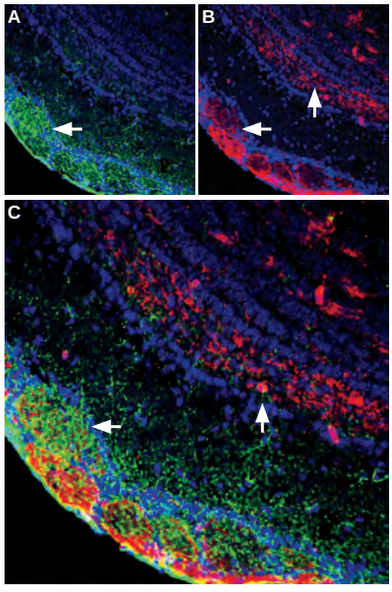
Alzheimer's disease is an age-related brain-disorder where amyloid beta aggregates are associated with cognitive decline and neurotoxicity. In Chinese medicine, Rhynchophylline (RIN) an herbal bioactive compound, is thought to have neuroprotective properties, but the mechanism remains elusive[20]. Using Anti-NMDA Receptor 2B antibody the authors found that RIN decreased extra-synaptic GluN2B expression when Dentate Gyrus (DG) neurons were exposed to amyloid beta protein[20]. Thus, given that extra-synaptic NMDARs mediate cytotoxic calcium cell-singling, inhibition of this pathway may partially explain RIN's neuro-protective effects.

Figure 5. Expression of NMDA Receptors in Adult and Adolescent Mouse PFC



Western blot analysis of mouse brain prefrontal cortex (PFC) lysates using **Anti-NMDAR2A (GluN2A) (extracellular) Antibody** (#AGC-002) and **Anti-NMDAR2B (GluN2B) (extracellular) Antibody** (#AGC-003). GluN2A expression (upper panel) appears to increase with age, while that of GluN2B (lower panel) does not change. Adapted from reference 9 with permission of The American Physiological Society.

Immuno-Colocalization of GluN1 and Plexin-A1 in Rat Olfactory Bulb



Immunohistochemical staining of immersion-fixed, free floating rat brain frozen sections using **Guinea pig Anti-NMDAR1 (GluN1) (extracellular) Antibody** (#AGP-046), (1:600) and rabbit **Anti-Plexin-A1 (extracellular) Antibody** (#APR-081), (1:400). A. NMDAR1 (green) is expressed in the glomeruli (arrow). B. Plexin A1 staining (red) in the same section, shows expression in the glomeruli (horizontal arrow). C. Merge of the two images show cases co-localization in the glomeruli. Cell nuclei are stained with DAPI (blue).

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Product name	Cat. #
Rabbit Polyclonal Antibodies	
Anti-NMDAR1 (GluN1) (extracellular) Antibody	AGC-001
Anti-NMDAR2A (GluN2A) (extracellular) Antibody	AGC-002
Anti-NMDAR2B (GluN2B) (extracellular) Antibody	AGC-003
Anti-NMDAR2C (GRIN2C) (extracellular) Antibody	AGC-018
Anti-NMDAR2D (GRIN2D) (extracellular) Antibody	AGC-020
Anti-NMDAR3A (GRIN3A) (extracellular) Antibody	AGC-030
Anti-NMDAR3B (GRIN3B) (extracellular) Antibody	AGC-031
Conjugated Rabbit Polyclonal Antibodies	
Anti-NMDAR2A (GluN2A) (extracellular)-ATTO-488 Antibody	AGC-002-AG
Anti-NMDAR2B (GluN2B) (extracellular)-ATTO-594 Antibody	AGC-003-AR
Guinea Pig Polyclonal Antibody	
Guinea pig Anti-NMDAR1 (GluN1) (extracellular) Antibody	AGP-046
Agonists	
cis-ACBD	A-281
trans-ACBD	A-280
(RS)-(Tetrazol-5-yl)glycine	T-205
NMDA	N-170
Antagonists	
5,7-Dichlorokynurenic acid	D-190
7-Chlorokynurenic acid sodium salt	C-286
CGP-37849	C-325
CGS 19755	C-310
CNS-1102	C-305
D-AP5	D-145
DL-AP5	D-140
Kynurenic acid	K-110
L-689,560	L-225
(+)-MK 801 maleate	M-230
PEAQX tetrasodium hydrate	P-255
Explorer Kits	
NMDA Receptor Antibody Explorer Kit	AK-214
NMDA Receptor Antagonist Explorer Kit	EK-350
NMDA Receptor Interacting Protein Antibody Explorer Kit	AK-615
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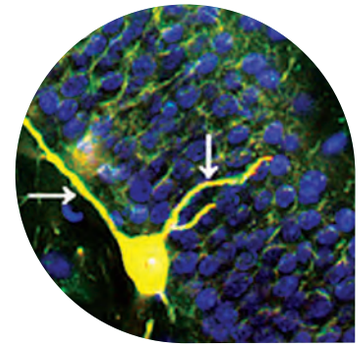
K_V4 Channels Link Cognitive Decline and Cardiac Dysfunction During Aging

Tommy Weiss Sadan, Ph.D. and Melanie R. Grably, Ph.D.

Voltage-dependent potassium channels (K_V4) are expressed in the brain and the heart where they regulate cognitive function and cardiac output. Aging is associated with increased tendency for cardiovascular disease and cognitive decline but the link between those debilitating diseases is missing. Here we show that K_V4 channels could possibly tie cardiovascular diseases and neuronal dysfunction and demonstrate a collection of functional tools from Alomone Labs to investigate this possibility.



Right: $K_V4.3$ is expressed in GABAergic neurons and co-localizes with parvalbumin.

Introduction

Structure

Potassium channels are a large and diverse protein superfamily that are typically grouped based on their structure and mode of activation[23]. The voltage-gated potassium (K_V) channels encompass a large portion of this protein superfamily and are further divided into 12 subfamilies based on sequence homology to their *Drosophila* orthologs (*Shaker, Shab, Shal* and *Shaw)*[18,23]. The *Shal*-type potassium channels also known as $K_V4.1$, $K_V4.2$ and $K_V4.3$ (in mammals) are highly expressed in the brain and the heart where they regulate numerous physiological functions including neuronal excitability and cardiac pacemaking respectively[2]. In the context of aging and its related diseases this article will mainly focus on these types of potassium channels.

The *Shal*-type potassium channels, share a prototypical structure with other potassium channels such as the cytoplasmic carboxy terminal, T1 assembly domain, a six α-helical transmembrane domain (S1-S6) and the pore loop (P-Loop), which is selective for potassium ions[2]. The helical S4 segment is unique among other S subunits as it is considered to be the voltage sensor due to dense clusters of positively charged arginine and lysine residues[2]. Upon membrane depolarization, the S4 domain mediates protein conformational change and opens the channel for potassium ion flux[18].

The regulation over K_V channel activity is a complex and multi-step process that depends on many factors including protein-protein interactions, cell signaling events, transcriptional and post translational activities as will be demonstrated in detail below.

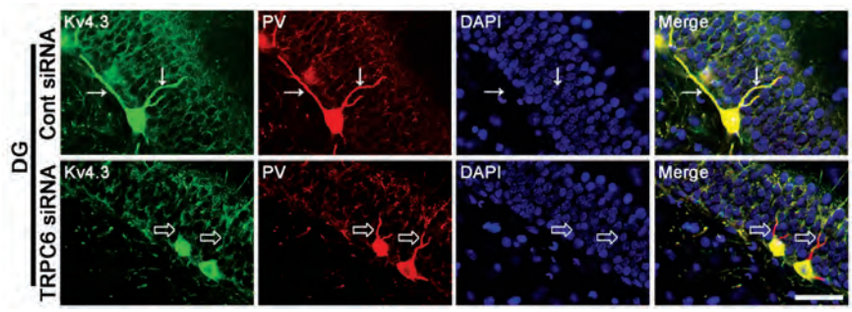
Transcriptional regulation

K_V4 expression is influenced by numerous factors including changes in transcriptional activity. Argenziano *et al.* found that testosterone controls $K_V4.3$ expression in the heart[1]. Using **Anti-$K_V4.3$ Antibody** (#APC-017), the authors showed that Finasteride and Flutamide (drugs preventing androgen signaling through different mechanisms) decrease $K_V4.3$ expression in the right ventricle of rat heart, suggesting a role for testosterone and androgen signaling in potassium channel expression[1].

Micro RNA (miRNA) miR-223-3p is highly expressed/upregulated in a rat model of acute myocardial ischemia (AMI)[12]. Its expression was shown to be inversely proportional to that of $K_V4.2$ as determined by western blot analysis using **Anti-$K_V4.2$ Antibody** (#APC-023). The possibility that miR-223-3p negatively regulates $K_V4.2$ expression was tested and validated by expressing the miRNA in primary neonatal rat ventricular myocytes. Indeed, $K_V4.2$ suppression by miR-223-3p was abolished by the administration of Antagomir to silence miR-223-3p[12]. The effect of miR-223-3p seemed to be specific to $K_V4.2$ as protein levels of other K_V channels, namely $K_V4.3$, were not altered by the miRNA[12].

In the nervous system cleaved GLP-1 peptide stimulates long term potentiation (LTP) signal in hippocampal nerves[5]. Western blot analysis of hippocampal lysates using Anti-$K_V4.2$ Antibody showed decreased expression

Figure 1. Effect of TRPC6 siRNA on K_V4.3 Localization



Immunohistochemical staining of rat brain sections using **Anti-K_V4.3 Antibody** (#APC-017). K_V4.3 staining (green) under control conditions (upper panels) is strongly expressed in GABAergic neurons and co-localizes with parvalbumin immunostaining (red). In response to TRPC6 siRNA administration (lower panels), K_V4.3 distribution in dendrites significantly decreases.
Adapted from reference 9 with permission of Frontiers.

of the protein following chronic administration of GLP-1 (9-36) in mice which was associated with increased LTP signals[5].

Subcellular Trafficking and Localization

The spatial distribution of potassium channels is important for proper cellular functions[10]. This is especially true for cells with complex structures such as neurons[10]. The possible role of TRPC6 channel in regulating K_V4 channel distribution was recently addressed. TRPC6 knockdown resulted in a decrease of cell membrane associated K_V4.3 and an increase in cytosolic abundance of the protein[9] as evident by western blot of cytosolic/membrane fractions of hippocampal tissue homogenates using Anti-K_V4.3 Antibody[9]. In addition, immunohistochemical staining of rat brain sections using Anti-K_V4.3 Antibody showed that rats administered with TRPC6 siRNA displayed reduced K_V4.3 clusters in dentate gyrus cells and parvalbumin (PV) positive GABAergic interneurons (Figure 1)[9], supporting the possible role of TRPC6 in regulating K_V4.3 subcellular distribution.

A novel interaction between the glycoprotein Nectin-2α and K_V4.2 was shown to tune K_V4.2 localization to specialized plasma membrane regions of adjacent somata of cholinergic neurons. This interaction was demonstrated by high resolution electron microscopy using Anti-K_V4.2 Antibody[16]. Furthermore, genetic ablation of Nectin-2α in mice reduced K_V4.2 fluorescent signal at the apical membrane of cholinergic neurons, confirming that Nectin-2α-K_V4.2 interaction is important for K_V4.2 localization.

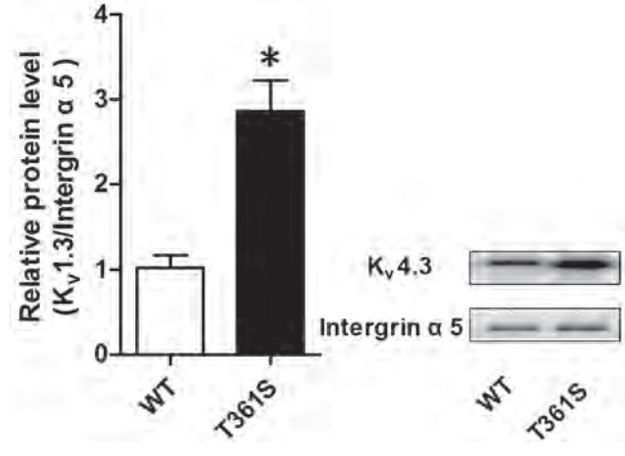
K_V4 Regulation by Post-Translational Modifications

Glycosylation is a common post translational modification important for numerous biological processes including protein folding in the endoplasmic reticulum (ER) and protein trafficking to the plasma membrane[2]. Recently, Endie *et al.*, investigated the effects of syalic acid (a negatively charged sugar) modifications on K_V channels in mouse ventricular myocytes[6]. Using transgenic mice lacking the sialyltransferase ST3Gal4, the authors demonstrated a delayed potassium outward (I_{to}) current that consequently delayed ventricular repolarization period as evident by prolonged QT intervals[6]. To investigate potential mechanisms, the authors used Anti-K_V4.2 Antibody and **Anti-K_V1.5 (KCNA5) Antibody** (#APC-004) to compare protein expression by western blot. Whereas, no change in protein expression was evident, the authors suggest that sialic acid modification of K_V channels is a key step in finetuning K_V channels activity[6].

K_V4 Interacting Proteins

The diversity in K_V channel activity can be influenced by protein-protein interactions[2]. For example, Turnow *et al.*, demonstrate that K_V4.3-DPP10a interaction regulates transient potassium currents (I_{to})[21]. Using immunofluorescence, they provide evidence for K_V4.3-DPP10a co-localization using Anti-K_V4.3 Antibody in human atrial myocytes and Chinese Hamster Ovaries (CHO)[21]. Furthermore, using functional assays they demonstrate this interaction is physiologically relevant since potassium currents were not visualized in the absence of DPP10a in CHO[21].

Figure 2. Cell-Surface Expression of K_V4.3 Bearing a Missense Mutation



Western blot analysis of HEK 293 cells transfected with wildtype (WT) K_V4.3 or with K_V4.3 (T361S) mutant. Immunodetection of cell surface K_V4.3 with **Anti-K_V4.3 Antibody** (#APC-017) shows that the mutant displays increased cell-surface expression compared to WT. Integrin α5 is used as a loading control.
Adapted from reference 8 with permission of Impact Journals.

Similarly, Wang *et al.*, demonstrated the interaction between K_V4.2-KChIP3-DPP10a in rat neocortical brain sections and olfactory bulbs using Anti-K_V4.2 Antibody and suggest this protein complex mediates sub-threshold potassium currents *in vivo*[22].

K_V4 Channels in Cardiovascular Diseases

Potassium channels regulate the electrical driving force for normal cardiac function[7]. In particular, they act to restore membrane polarization and counterbalance depolarizing ions such as sodium and calcium[7].

Impaired cardiac electrical activity is frequently observed following myocardial hypertrophy, which is an adaptive response to cardiac dysfunction. M3 muscarinic receptors are members of cholinergic receptors that innervate cardiac cells and control their electrical function. Chen *et al.*, examined whether M3 over-expression could alleviate the adverse electrical signal after cardiac hypertrophy[3]. To test this hypothesis the authors generated transgenic mice, over-expressing M3 muscarinic receptors. Using **Anti-M3 Muscarinic Receptor Antibody** (#AMR-006), the authors confirmed the over-expression of M3 receptors by western blot and observed that that cardiac electrical activity was comparable to sham controls following transverse aortic constriction model[3]. To identify a potential mechanism by which M3 over-expression restores cardiac function, the authors examined the expression of various channels, which control potassium outflow and hence could potentially re-establish normal cardiac pace[3]. They found that M3 muscarinic receptors elevated K_{ir}2.1 expression but no change was observed in K_V4.3, as determined using Anti-K_V4.3 Antibody[3].

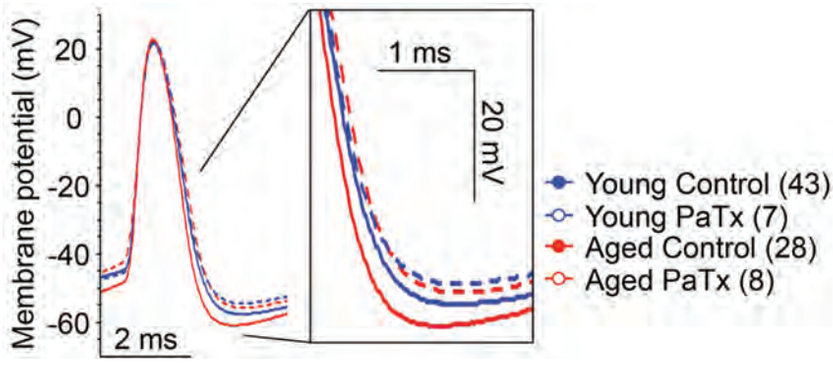
Recently, a genetic survey in a small cohort of Chinese population identified a missense mutation within K_V4.3 gene (KCND3) associated with atrial fibrillation (AF)[8]. This mutation leads to Thr 361 to Ser (Thr→Ser) replacement in K_V4.3 protein. To delve deeper into the mechanisms leading to atrial fibrillation, Huang *et al.*, expressed wild type K_V4.3 or its mutated counterpart, together with KChIp2 in HEK293T cells. Using this system, they discovered that Thr→Ser mutation increases total K_V4.3 expression and increases its membrane localization, as determined by western blot with Anti-K_V4.3 Antibody (Figure 2). In addition, the authors measured potassium currents and discovered that Thr→Ser is associated with K_V4.3 gain of function[8].

In contrast, Cheng *et al.*, demonstrated that K_V4.3 over-expression in cardiac myocytes protected mice from heart failure[4]. Specifically, using Anti-K_V4.3 Antibody, the authors showed the association between elevated K_V4.3 expression and decreased phosphorylation of calmodulin-dependent protein kinase (CaMKII) and suggested this protective effect to be due to calcium homeostasis[4].

Recent studies have uncovered novel mutations within K_V interacting proteins that can have debilitating effects on cardiac function such as the Brugada syndrome, which causes irregular heart beats[7]. Accordingly, Portero *et al.*, identified a mutation in $K_V\beta$2 associated with the syndrome[14]. Using an *in vitro* expression system, the authors were able to confirm that Arg to Gln replacement does not affect K_V4.3 expression as seen by western blot using Alomone Labs' respective antibody, but rather affects cardiac electrophysiology in a manner that has yet to be uncovered.

Similarly, Tsai *et al.*, identified KChIP1 copy number variants to be a strong genetic predictor for AF in Taiwanese population[20]. To investigate how KChIP1 is involved in AF, the authors undertook protein-protein interaction studies in adult rat hearts using **Anti-KChIP1 (KCNIP1) Antibody** (#APC-141) in pull-down assays and re-probing with antibodies against the potassium channels K_V4.2 and K_V4.3 or the calcium channel with **Anti-Ca_V1.2 (CACNA1C) Antibody** (#ACC-003)[20]. Unexpectedly, none of these proteins were detected, indicating a different mechanism by which KChIP1 regulates cardiac performance. The authors tried a genetic approach in which they silenced KChIP1 in atrial cell line, HL-1 and found a significant change in potassium currents and membrane depolarization, suggesting that KChIP1 itself regulates potassium outward currents[20].

Figure 3. Inhibition of K_V4.2 and K_V4.3 by Phrixotoxin-1 Reverses Aging-Related Fast Afterhyperpolarization



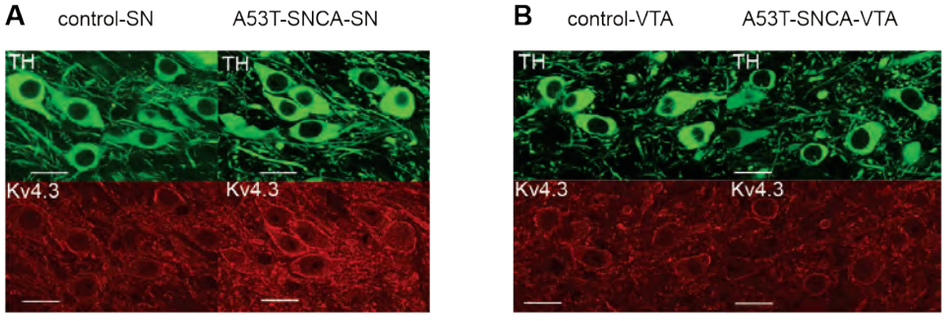
Representative traces of single orthodromically elicited action potentials from aged (red) and young (blue) CA3 neurons with (dashed line) and without (solid line) 1 µM **Phrixotoxin-1** (#STP-700), (PaTx) in recording pipette (from AP threshold). PaTx treatment reduced the fast afterhyperpolarization (fAHP) of aged CA3 neurons to young-like values. Adapted from reference 17 with permission of the Society for Neuroscience.

K_V4 Channels in Neuronal Dysfunction

Cognitive decline due to aging is associated with CA1 and CA3 pyramidal neuron malfunction in the hippocampus[17]. Simkin *et al.*, speculated that the increased firing in CA3 pyramidal neurons contributing to cognitive decline in aged brains may be caused by K_V channels[17]. Administration of **Phrixotoxin-1** (#STP-700), a selective and potent blocker of $K_V4.2$ and $K_V4.3$ channels significantly improved the electrophysiological activity of CA3 neurons in old rats (Figure 3). Additional results indicated that $K_V4.2$ inhibition could possibly slow down neurodegenerative processes during aging[17].

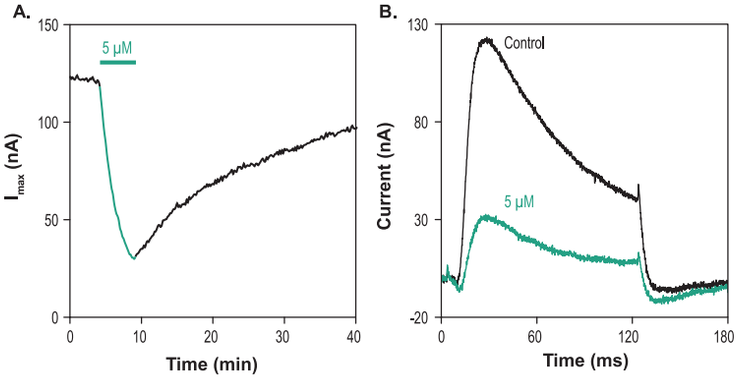
Parkinson's Disease is another example of progressive neurodegenerative process where α-synuclein accumulation can harm vulnerable neurons such as the substantia nigra (SN) dopaminergic neurons[19]. In genetically engineered mice, bearing A53T mutation in α-synuclein protein, selective high firing rates in SN dopaminergic neurons (DA) were observed[19]. Comparable with the *in vivo* model, isolated neurons from mutated α-synuclein DA neurons displayed increased firing rates in a pattern that suggested a shift in pacemaker currents. Voltage-gated K_V4 channels were previously shown to control dopaminergic neuron pacemaking. Application of **Phrixotoxin-2** (#STP-710), a specific K_V4 channel blocker completely hindered the difference in electrophysiological recordings between the

Figure 4. $K_V4.3$ Expression Increases in DA Neurons of α-Synuclein Mutant Mice



Immunohistochemical staining of mouse brain sections using **Anti-$K_V4.3$ Antibody** (#APC-017). A. Expression of $K_V4.3$ increases in DA substantia-nigra neurons of α-synuclein mutant mice (lower right panel). B. Expression of $K_V4.3$ does not increase in DA neurons of the ventral tegmental area in α-synuclein mutant mice (lower right panel).
Adapted from reference 19 with permission of the Society for Neuroscience.

Alomone Labs AmmTx3 Toxin Inhibits $K_V4.2$ Currents Heterologously Expressed in *Xenopus* Oocytes



A. Time course of **AmmTx3 Toxin** (#STA-305) blocking action on $K_V4.2$ currents. Maximum current amplitudes were plotted as a function of time. Membrane potential was held at -90 mV and cells were stimulated by a 120 ms voltage step to 0 mV. 5 µM AmmTx3 Toxin were perfused as indicated by the bar (green) during 280 sec application. B. Superimposed examples of $K_V4.2$ channel current in the absence (control) and presence (green) of 5 µM AmmTx3 Toxin (taken from the experiment in A).

control group and α-synuclein mutant neurons (Figure 4A)[19]. In addition, immunohistochemical staining of mouse brain sections using Anti-K_V4.3 Antibody showed that K_V4.3 expression is higher in substantia nigra of mice bearing the mutation in α-synuclein (Figure 4B). This suggests that altered K_V4 activity or expression underlies this phenotypic difference[19].

Different cell types respond differently to elevated α-synuclein levels[11]. For example, the dorsal motor nucleus of the vagus (DMV) nerve can tolerate high levels of α-synuclein levels and display milder apoptosis compared to the SN dopaminergic neurons[11]. Furthermore, α-synuclein accumulation alters electrical activity in SN dopaminergic neurons, but has no evident effect on DMV neurons[11]. In accordance, immunohistochemical staining of mouse brain sections bearing the A53T mutation in α-synuclein protein shows similar K_V4.3 levels and expression pattern when compared to wildtype[11].

Pharmacological Approaches to Studying K_V4 Channels

Over the past decade, the list of small molecule/peptide inhibitors that target K_V4 channels has grown substantially[23]. These tools, can now be exploited to discover novel functions and biological processes in which K_V4 channels are involved. For example, Phrixotoxin-1 and **AmmTx3 Toxin** (#STA-305) peptide toxin blockers for K_V4.3 and K_V4.2 were used to study how toxin transient potassium currents affect preBotzeiger type-1 neurons rhythmicity and their impact on breathing[13].

In a similar way **Heteropodatoxin-2** (#STH-340), a specific K_V4.2 blocker was used to uncover a novel mechanism of synaptic plasticity in tuft dendrites of layer 5 pyramidal neurons[15] where low frequency electrical stimulation in tuft dendrites induced long term potentiation activity.

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Related Products

Product name	Cat. #
Antibodies	
Anti-DPP6 (extracellular) Antibody	APC-146
Anti-DPP10 (extracellular) Antibody	APC-147
Anti-KChIP1 (KCNIP1) Antibody	APC-141
Anti-KChIP2 Antibody	APC-142
Anti-KChIP3 (CSEN, DREAM) Antibody	APC-143
Anti-K_V4.1 (KCND1) Antibody	APC-119
Anti-K_V4.2 Antibody	APC-023
Anti-K_V4.2-ATTO-633 Antibody	APC-023-FR
Guinea pig Anti-K_V4.2 Antibody	AGP-038
Anti-K_V4.3 Antibody	APC-017
Blockers	
4-Aminopyridine	A-115
AmmTx3 Toxin	STA-305
Dapoxetine hydrochloride	D-175
(S)-Duloxetine hydrochloride	D-170
Fluoxetine hydrochloride	F-155
Heteropodatoxin-2	STH-340
Jingzhaotoxin-V	STJ-050
Jingzhaotoxin-XII	STJ-100
Pandinotoxin Kα	STP-500
Phrixotoxin-1	STP-700
Phrixotoxin-2	STP-710
Rosiglitazone	R-125
SNX-482	RTS-500
Trifluoperazine dihydrochloride	T-175
Explorer Kits	
Shaker (K_V)-Related Channel Antibody Explorer Kit	AK-220
K_V4 Channel Blocker Explorer Kit	EK-130
Shaker (K_V)-Related Channel Blocker Explorer Kit	EK-108
Research Packs	
K_V4 Channel Basic Research Pack	ESB-502
K_V4 Channel Premium Research Pack	ESP-502
K_V4 Channel Deluxe Research Pack	ESD-502

K_V4 Channel product listing

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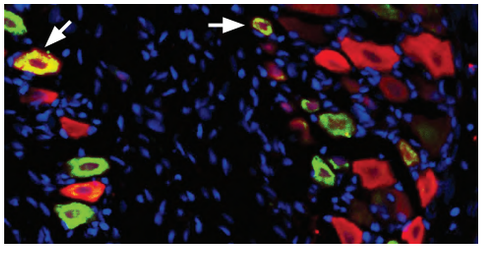
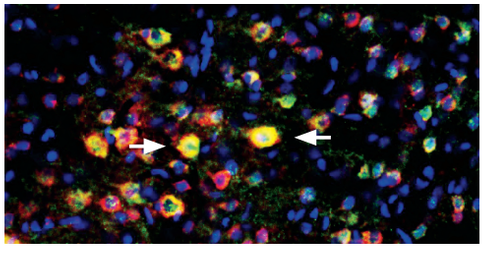


Immuno-colocalization of TRPV1 and $Na_V1.8$ in rat DRG using **Anti-Rat TRPV1 (VR1) (extracellular)-ATTO-488 Antibody** (#ACC-029-AG), (green) and **Anti-$Na_V1.8$ (SCN10A)-ATTO-594 Antibody** (#ASC-016-AR), (red).



Immuno-colocalization of GluN2B and PSD-95 in rat parietal cortex sections using **Anti-NMDA Receptor 2B (GluN2B) (extracellular)-ATTO-594 Antibody** (#AGC-003-AR), (red) and **Anti-PSD-95 Antibody** (#APZ-009) followed by goat-anti-rabbit-Alexa-488 secondary antibody (green).

For more information on ATTO dyes and ATTO-conjugated antibodies:

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IN FOCUS

5-HT3 Receptors

5-HT (5-hydroxytryptamine, serotonin) is one of the most versatile neurotransmitters. It signals in part through 5-HT3 receptors which belong to the super family of ligand-gated ion channels.

5-hydroxytryptamine type 3 (5-HT3) receptors are cation-selective Cys-loop receptors expressed in the central and peripheral nervous systems where they mediate fast excitatory neurotransmission[2]. Five receptor subunits have been identified thus far (5-HT3A to 5-HT3E) with 5-HT3A and 5-HT3B receptors being the best characterized among the different types. To form a functional receptor, five subunits assemble around a pore permeable to Na^+, K^+, and Ca^{2+} ions. The presence of one or more 5-HT3A receptor subunits is necessary and essential[3]. Each 5-HT3 receptor subunit has a large extracellular domain critical for ligand binding, four transmembrane domains important for pore formation, and an intracellular domain responsible for activity modulation, receptor trafficking and sorting[2].

5-HT3 receptors have become important therapeutic targets for irritable bowel syndrome (IBS), side effects resulting from chemotherapeutic treatment, schizophrenia and bipolar disorder[1,4].

Alomone Labs offers an extensive 5-HT3 receptor reagent portfolio. Our product range includes highly specific antibodies to the receptors, which enable detection of 5-HT3 receptors in western blot analysis and various immunostaining protocols.

We also offer a large array of pharmacological reagents:

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- All lots biologically tested
- Large size range available
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5-HT3 receptor Explorer Kits are Budget-Friendly priced screening packages. Each Explorer Kit contains a set of antibodies or a group of pharmacological reagents targeting 5-HT3 receptors.

References

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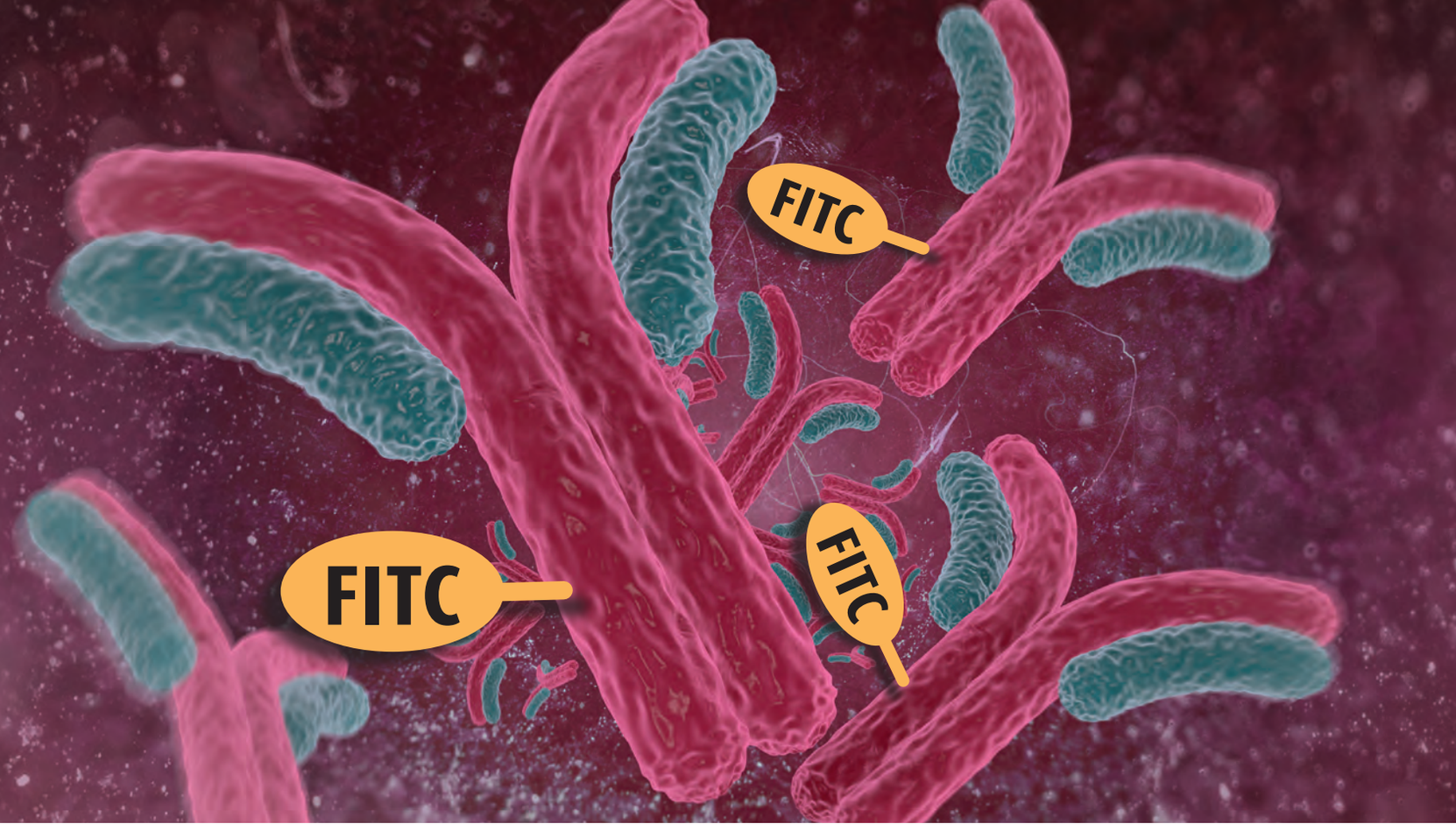
Related Products

Product name	Cat. #
Rabbit Polyclonal Antibodies	
Anti-5HT3A Receptor Antibody	ASR-031
Anti-5HT3B Receptor (extracellular) Antibody	ASR-032
Conjugated rabbit polyclonal antibody	
Anti-5HT3B Receptor (extracellular)-ATTO-488 Antibody	ASR-032-AG
Agonists	
1-Phenylbiguanide hydrochloride	P-190
2-Methyl-5-hydroxytryptamine hydrochloride	M-170
5-Chloroindole	C-260
5-Hydroxyindole	H-115
m-Chlorophenylbiguanide hydrochloride	M-160
RS 56812 hydrochloride	R-130
Serotonin hydrochloride	S-165
SR 57227A	S-155
Topotecan	T-161
Topotecan hydrochloride	T-160
Antagonists	
3-AQC	A-205
Alosetron	A-235
Alosetron hydrochloride	A-236
Azasetron	A-240
Azasetron hydrochloride	Y-100
Bilobalide	B-145
Etomidate	E-200
Ginkgolide B	G-170
Granisetron hydrochloride	G-105
Indisetron dihydrochloride	I-175
Lerisetron	L-175
LY278584	L-180
MCI-225	M-190
MDL 72222	M-155
Mirtazapine	M-130
Mosapride citrate	M-225
Ondansetron hydrochloride	O-110
Palonosetron	P-226
Palonosetron hydrochloride	P-225
PU02	P-165
Ramosetron	R-146
Ramosetron hydrochloride	R-145
Ricasetron	R-150
SDZ 205-557 hydrochloride	S-145
Explorer Kits	
5-HT3 Receptor Antagonist Explorer Kit	EK-208
5-HT3 Receptor Modulator Explorer Kit	EK-209
Serotonin Signaling Antibody Explorer Kit	AK-590

5-HT3 Receptor product listing



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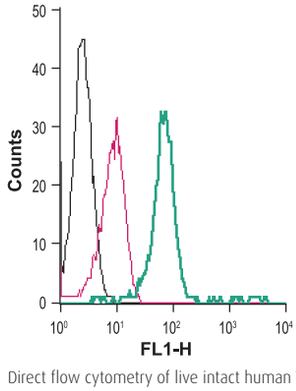

Direct flow cytometry of live intact human THP-1 monocytic leukemia cells using **Anti-P2X7 Receptor (extracellular)-FITC Antibody** (#APR-008-F).

For a complete list of Alomone Labs live cell flow cytometry products:

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IN FOCUS

Piezo Channels

Mechanical stimulus is the driving force for numerous physiological processes including pain sensation, hearing, and blood pressure regulation[2].

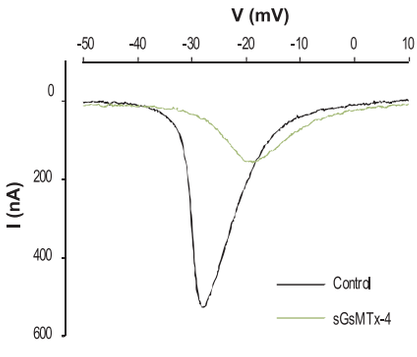
Piezo1 and Piezo2 were first characterized in a mouse neuroblastoma cell line, where shear force to the cell membrane induced mechanically activated currents[2].

Piezo channels are unusually large ion channels with 38 putative membrane spanning domains. They form trimeric structures that are shaped into a propeller form with three blades assembled around a central pore. Recent structural data reveal that Piezo1 bends the local lipid environment to form a "dome-like" structure. The dome structures are created by the blades in the inactive conformation. Mechanical pressure or tension on the plasma membrane causes a flattening of the dome shape, leading to the subsequent activation of the channel[1,3,8,10].

While the race to determining the structure of Piezo channels is at its peak, there are numerous studies on the functional expression and the various roles for Piezo channels. Piezo1 was found to be important for cell volume control of human red blood cells[4]. The expression and activation of Piezo1 was recently found to optimize human T-cell activation[5]. Piezo1 also is important for lineage choice in human neural stem cells[6] and mesenchymal stem cells[9].

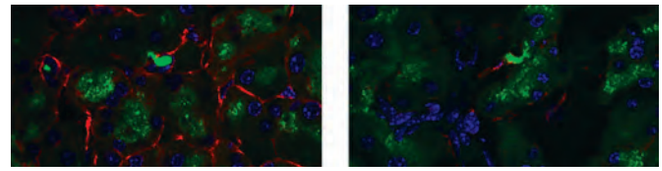
Alomone Labs **Anti-Piezo1 Antibody** (#APC-087) was recently used to determine in part the efficacy of conditional Piezo1 knockouts in pancreas acinar cells (Figure 1)[7]. In this paper, the authors elegantly show that Piezo1 mediates the development of pancreatitis[7].

The importance of Piezo channels, namely that of Piezo1, is only just becoming clear, and the best is surely yet to come. Alomone Labs, proudly offers **Anti-Piezo1 Antibody** (#APC-087) which has been tested in western blot and immunohistochemistry applications. With scarce pharmacology, we offer the known and well documented **GsMTx-4** (#STG-100), a peptide toxin originally isolated from a tarantula venom and a blocker of Piezo1. Free samples of both products are available!



Alomone Labs **GsMTx-4** (#STG-100) inhibits $Na_v1.7$ channel currents expressed in *Xenopus* oocytes.

Figure 1. Knockout Validation of Anti-Piezo1 Antibody in Mouse Pancreas



Immunohistochemical staining of mouse pancreas sections using **Anti-Piezo1 Antibody** (#APC-087). Piezo1 staining (red) is detected in acinar cells. $Piezo1^{\Delta ci}$ KO mice (right panel) do not express Piezo1. Trypsin staining is shown in green nuclei are stained with DAPI (blue). Adapted from Romac, J.M. *et al.* (2018) *Nat. Commun.* **9,** 1715. with permission of SPRINGER NATURE.

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Related Products

Product name	Cat. #
Rabbit Polyclonal Antibody	
Anti-Piezo1 Antibody	APC-087
Blocker	
GsMTx-4	STG-100

Mechanosensitive Channel product listing

Product Highlight

α-Bungarotoxin Conjugates

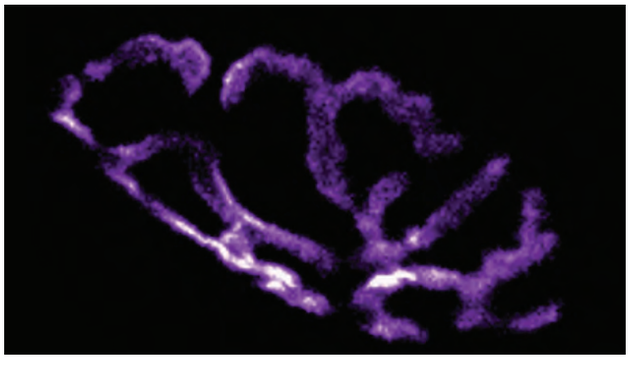
Conjugated to fluorescent dyes or biotinylated, labeled α-BTX enables sensitive detection of neuromuscular junctions, GABA(A) Receptors, nAChRs, and α-Bungarotoxin binding sites.
Interested in a different label? We gladly take on personal requests! Contact us: info@alomone.com

α-Bungarotoxin-ATTO-488 | CAT #: B-100-AG

α-Bungarotoxin-ATTO-633 | CAT #: B-100-FR

α-Bungarotoxin-FITC | CAT #: B-100-F

α-Bungarotoxin-Biotin | CAT #: B-100-B



Alomone Labs α-Bungarotoxin-ATTO-633 in whole mount staining of mice neuromuscular junction (NMJ).
Whole mount staining of mouse neuromuscular junction (NMJ) was stained with the NMJ marker **α-Bungarotoxin-ATTO-633** (#B-100-FR) (purple) at 1 µg/ml concentration. The image was taken using Nikon Epifluorescence microscopy at 60X magnification and is kindly provided by Dr. Eran Perlsson, Dept. of Physiology and Pharmacology, Tel-Aviv University, Tel-Aviv, Israel.

Novel Toxins, Novel Targets

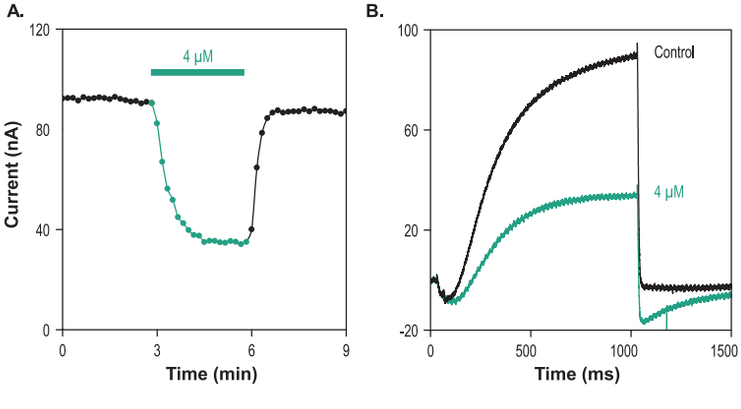
As a result of being popular potential therapeutics, the rate at which new toxins are being unraveled has increased. With state-of-the-art technology in our facilities and keeping pace with newly published work we strive to deliver novel and exclusive ion channel modulators. **SsTx Toxin** (#STS-700), originally isolated from *Scolopendra mutilans* (Chinese red-headed centipede), is the first toxin isolated from the centipede venom and found and acts as a KCNQ channel blocker. Coral snake venom is becoming a rich source of exciting new toxins such as **MmTx1 Toxin** (#STM-550), **MmTx2 Toxin** (#STM-600) modulators of GABA(A) receptors, and **alpha/beta MitTx** (#M-100), a novel ASIC1 channel activator. Scorpion venom never ceases to supply a rich source of ion channel modulators. Here, we focus on Na_v Channel activators which may be crucial in deciphering key roles for Na_v channels in various pathologies like epilepsy.

SsTx Toxin | Cat #: STS-700

A Blocker of KCNQ Channels

SsTx Toxin is a peptide toxin originally isolated from the Chinese red-headed centipede venom. It is the first peptide toxin found to potently and selectively block KCNQ (K_v7) channels, members of the voltage-gated potassium (K_v) channel family, with IC_{50} values of 2.5, 2.8, 2.7, and 2.7 µM for KCNQ4, KCNQ1, KCNQ2, and KCNQ5 respectively.

EVIKKDTPYKKRKFPYKSECLKACATSFTGGDESRIQEGKPGFFKCTCYFTTG-OH

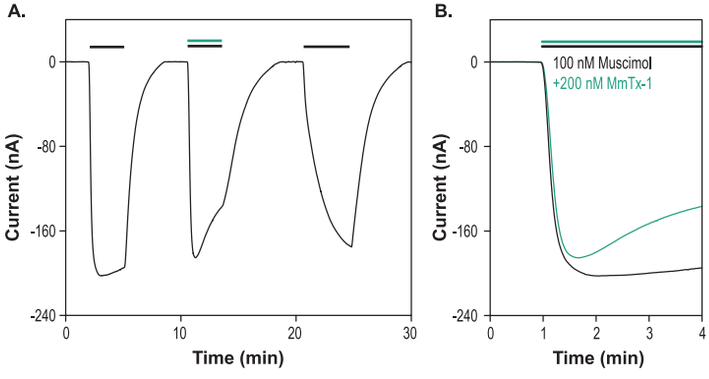


Alomone Labs SsTx Toxin inhibits the current of KCNQ2 channels expressed in *Xenopus* oocytes. A. Representative time course of KCNQ2 channel current inhibition by **SsTx Toxin** (#STS-700). Membrane potential was held at -80 mV, current was elicited by a 1000 ms voltage step to 0 mV every 10 sec, and reversibly inhibited by application of 4 µM SsTx Toxin (green). B. Superimposed traces of KCNQ2 current following the application of control (black) and of 4 µM SsTx Toxin (green), taken from the recording in A.

MmTx1 Toxin & MmTx2 Toxin

Potent Allosteric Modulators of GABA(A) Receptors

MmTx1 Toxin (#STM-550) and **MmTx2 Toxin** (#STM-600) (Micrurotoxin 1 & 2 respectively) peptide toxins, are two novel and highly potent allosteric modulators of GABA(A) receptors. They were originally identified and isolated from *Micrurus mipartitus* (Red-tailed coral snake) venom (Rosso, J.P. *et al.* (2015) *Proc. Natl. Acad. Sci. U.S.A.* **112,** E891.). They may be a priceless tool in evoking seizures for testing novel antiepileptic drugs or as lead molecules for designing therapeutics that modulate GABA(A) receptor activity (Rosso, J.P. *et al.* (2015) *Proc. Natl. Acad. Sci. U.S.A.* **112,** E891.).



Alomone Labs MmTx1 Toxin (200 nM) modulates GABA(A) receptors expressed in *Xenopus* oocytes.
A. Representative time course of GABA(A) α1/β2 current activated at a holding potential of -80 mV by 100 nM **Muscimol hydrobromide** (#M-240) applications (black bars), and modulated by co-application of 200 nM **MmTx1 Toxin** (#STM-550), as indicated (green bar). A significant modulation of receptor desensitization and reactivation rates is observed. B. Superimposed traces of GABA(A) receptor currents upon application of 100 nM Muscimol (black) or co-application of 100 nM Muscimol and 200 nM MmTx1 Toxin (green). Taken from the recording in A.

alpha/beta MitTx | Cat #: M-100

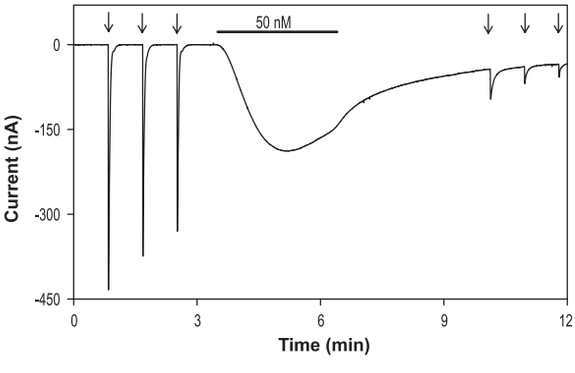
An Activator of ASIC1-Containing Channels

Courtesy of the Houston Zoo, Houston, Texas, USA

alpha/beta MitTx is a natural toxin isolated from the Texas Coral snake venom. The purified ASIC-activating component of the venom, MitTx, elicits robust nocifensive (Bohlen, C.J. and Julius, D. (2012) *Toxicon* **60,** 254.).

α-Subunit: QIRPAFCYEDPPFFQKCGAFVDSYYFNRSRITCVHFFYGQCDVNQNHF
TTMSECNRVCHG-OH

β-Subunit: NLNQFRLMIKCTNDRVWADFVDYGCYCVARDSNTPVDDLDRCCQAQKQ
CYDEAVKVHGCKPLVMFYSFECRYLASDLDCSGNNTKCRNFVCNCDRTATLCILTATY
NRNNHKIDPSRCQ-OH



Alomone Labs alpha/beta (1:1) alpha/beta MitTx activates ASIC1a channels expressed in *Xenopus* oocytes. Membrane potential was held at -80 mV. ASIC1a channels current was elicited every 50 sec by transient pH5.5 stimulation (arrows) or activated by a 3 min application of 50 nM **alpha/beta MitTx** (#M-100), as indicated.

Scorpion-Derived Na_V Channel Activators

Scorpion-Derived Toxins Continue to Supply a Plethora of Ion Channel Modulators

AaH1 Toxin | CAT #: STA-155

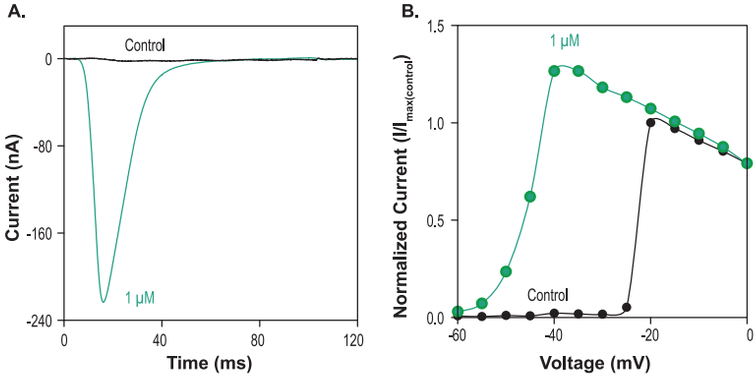
AaH1 Toxin is a peptide toxin originally isolated from *Androctonus australis* scorpion venom. It blocks the fast inactivation phase of voltage-gated Na^+ channels. We show the toxin's activity on $Na_V1.2$ channel currents expressed in *Xenopus* oocytes.

BmKI Toxin | CAT #: STB-100

BmKI Toxin (Alpha-like toxin BmK-M1) is a peptide toxin originally isolated from *Mesobuthus martensii* scorpion venom. It is a positive modulator of voltage-gated Na^+ channels by inhibiting the inactivation of activated Na_V channels. We demonstrated BmKI Toxin activity $Na_V1.6$ channels in *Xenopus* oocytes.

Tf2 Toxin | CAT #: STT-050

Tf2 Toxin is a β-scorpion peptide toxin originally isolated from the venom of the Brazilian scorpion *Tityus fasciolatus*. Tf2 Toxin acts as a specific $Na_V1.3$ channel opener. It shifts human $Na_V1.3$ channel voltage activation towards negative values and effectively opens the channel at resting membrane potentials.



Alomone Labs Tf2 Toxin affects the activation of human $Na_V1.3$ channels expressed in *Xenopus* oocytes. A. Representative traces of $Na_V1.3$ channel currents before (black) and after (green) the application of 1 µM **Tf2 Toxin** (#STT-050). Tf2 Toxin caused a significant current at a voltage that does not normally activate the channels. Membrane potential was held at -100 mV, and a voltage step to -30 mV was applied every 10 sec. B. Normalized I-V curve. 1 µM Tf2 Toxin (green) shifts the activation of $Na_V1.3$ channels to more negative voltages compared to control (black).