Targeting Trends
2019 Review

Targeted hippocampal GABA neuron ablation *produces hippocampal sclerosis, epilepsy, and dissociable effects*

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Targeted ablation with SSP-SAP

Targeted hippocampal GABA neuron ablation produces hippocampal sclerosis, epilepsy, and dissociable effects on the Morris Water Maze and Object-Place Paired Association tasks

by Argyle V. Bumanglag,[1] Leah M. Truckenbrod,[1] Eugene Chun,[2] Abbi R. Hernandez,[1] Quinten P. Federico,[1] Andrew P. Maurer,[1] Robert S. Sloviter,[3,4] Sara N. Burke[1]

Temporal lobe epilepsy with hippocampal sclerosis is a common neurological disorder characterized by seizures that arise from the hippocampus and related structures, often as a consequence of brain injury.[1] Perforant path stimulation-induced hippocampal injury in rats is associated with immediate dentate granule cell hyperexcitability and a clinically subtle, focal epileptic brain state without significant delay.[2] Thus, an immediate disruption in inhibition from injury-induced neuron loss or GABA neuron dysfunction may be a sufficient cause of temporal lobe epilepsy with hippocampal sclerosis.[3-4] We recently tested this hypothesis by determining whether the selective ablation of hippocampal GABA neurons along a longitudinal expanse of the hippocampus, in the absence of any other induced brain injury, is sufficient to initiate hippocampal epileptogenesis.[5] The results of this experiment suggested that targeted hippocampal GABA neuron ablation by Stable Substance P-Saporin (SSP-SAP; Cat. #IT-11) initiated a subclinical state of non-convulsive status epilepticus, which produced hippocampal sclerosis and dentate granule cell-onset epilepsy, without involving convulsive status epilepticus or any lethality.[5] If correct, GABA neuron loss or GABAergic dysfunction alone may be a primary epileptogenic mechanism.[2-5]

In this study, we determined whether selective hippocampal GABA inhibitory interneuron loss produced a chronic epileptic state, and assessed cognitive function in chronically epileptic SSP-SAP-treated rats and vehicle-injected controls to identify behavioral co-morbidities associated with GABA neuron ablation. Male Sprague Dawley rats (350-450 g) were injected bilaterally with SSP-SAP (0.4 ng/10 nL) or PBS (vehicle control) into 4 sites along the longitudinal axis of each hippocampus. Receptor-mediated lesioning with SSP-SAP is highly selective because the neurotoxin Saporin enters GABA neurons via the NK-1 receptor, which all hippocampal GABA neurons constitutively and selectively express.[6] Cognitive function was assessed in chronically epileptic SSP-SAP-treated rats and their vehicle-injected controls ~8 months post-injection, when treated rats were observed to exhibit spontaneous clinical focal motor seizures.

Spatial learning and memory were initially assessed with the Morris Water Maze.[7] No significant differences were detected in Morris Water Maze performance between SSP-SAP-treated rats and the control animals. Controls and epileptic rats with dorsal hippocampal sclerosis were able to learn the location of the hidden platform in the Morris Water Maze task, suggesting that entorhinal regions and associated areas involved in spatial navigation may have retained their functional integrity (Fig. 2). The same animals were subsequently tested with the object-place paired association (OPPA) task, which requires animals to integrate spatial location memory with a

(continued on page 4)

[1]*Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL,* [2]*Graduate Education Biomedical Sciences Program, Morehouse SOM, Atlanta, G,* [3]*Neurobiology, Morehouse SOMe, Atlanta, GA,* [4]*Pharmacology and Toxicology, Morehouse SOM, Atlanta, GA.*

Targeted ablation with SSP-SAP

(continued from page 3)

correct object choice, and is a more sensitive measure of dysfunction within hippocampal-cortical circuits than the Morris Water Maze.[8-10] SSP-SAP-treated rats exhibited significantly impaired performance on the OPPA task, and made significantly more errors before reaching criterion compared to control animals (Fig. 3A). Thus, cognitive functions that require functional connectivity between the hippocampus and cortical areas may be selectively affected by hippocampal GABA neuron ablation. No significant differences were detected on a control simple object discrimination task (Fig. 3C), suggesting that observed impairments on the OPPA task were not simply due to differences in motivation, procedural or sensorimotor deficits. Histological analysis was performed ~12 months post-injection to determine the extent of hippocampal sclerosis in chronically epileptic rats. Targeted hippocampal GABA neuron ablation via SSP-SAP consistently caused hippocampal sclerosis (Fig. 1, see Page 1). Varying extents of neuron loss were observed in different rostrocaudal locations along the same hippocampus. Rats corresponding to panels 2-4 in Fig. 3 were observed to exhibit a minimum of two clinically-obvious seizures that were detected by intermittent observation. Clinically-obvious seizures were observed during feeding and cage changes, or on behavioral task trials, indicating that animals continue to be epileptic for more than a year after SSP-SAP microinjection. These data suggest that, similar to humans with mesial temporal lobe epilepsy, hippocampal sclerosis and epilepsy in this model do not result in global cognitive decline. Rather, cognitive functions that require functional connectivity between the hippocampus and cortical areas are selectively affected. Future studies will examine the effects of selective GABA neuron ablation on hippocampal network dynamics across the lifespan, and identify cognitive co-morbidities associated with temporal lobe epilepsy and aging. The relationship between different extents of hippocampal neuron loss and performance on cognitive tasks also remains to be determined.

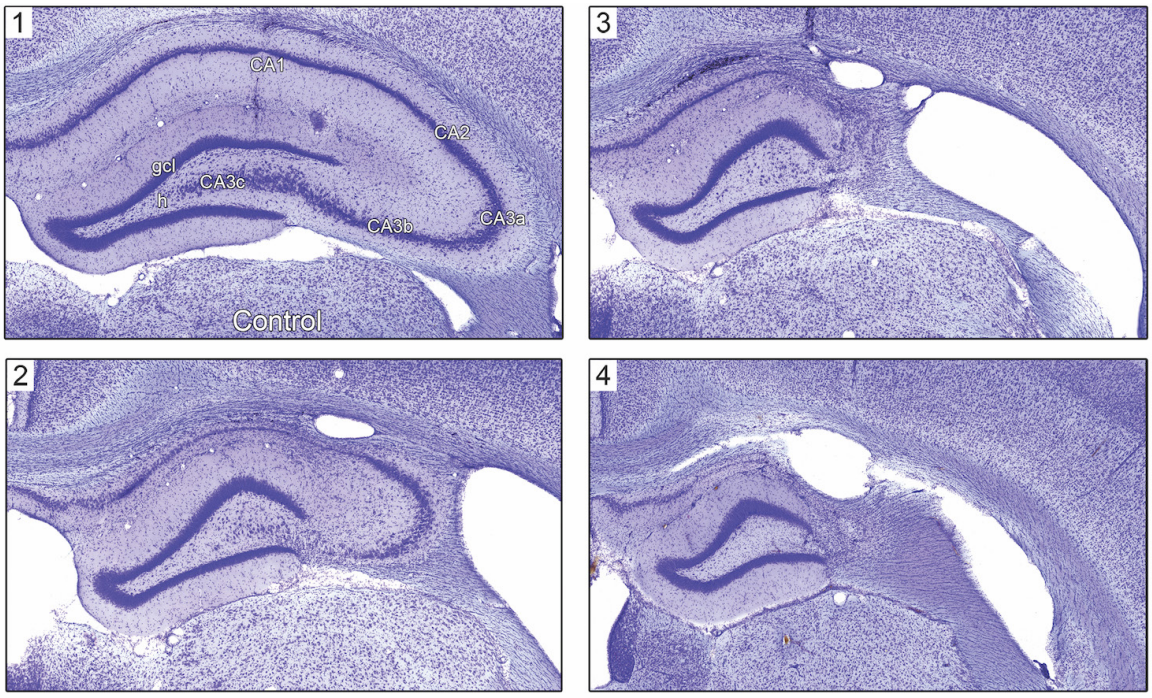

Fig. 1. Hippocampal pathology ~1 year after SSP-SAP injection. Nissl-stained hippocampi from treated rats (panels 2-4) showing variable hippocampal sclerosis pathology compared to a PBS vehicle-injected control animal (panel 1). Note the loss of neurons in the hilus (h) of the dentate gyrus, CA3, and CA1.

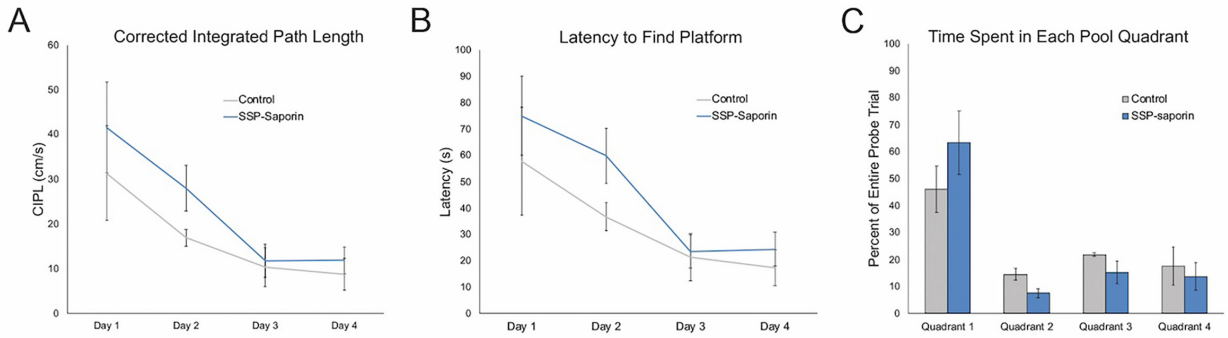

Fig. 2. Morris Water Maze performance in chronically-epileptic rats ~8 months after SSP-SAP injection. A) Average corrected integrated path length of each group across days of water maze testing. No significant differences were observed between experimental and control animals. B) Latency to find hidden platform within the water maze pool over days of water maze testing. No significant differences were found between the SSP-SAP treated rats and their control counterparts. C) Percent time spent in each pool quadrant during the probe trial. Both control and SSP-SAP treated rats spent more time in the quadrant that previously contained the hidden platform. There were no significant differences between treatment groups.

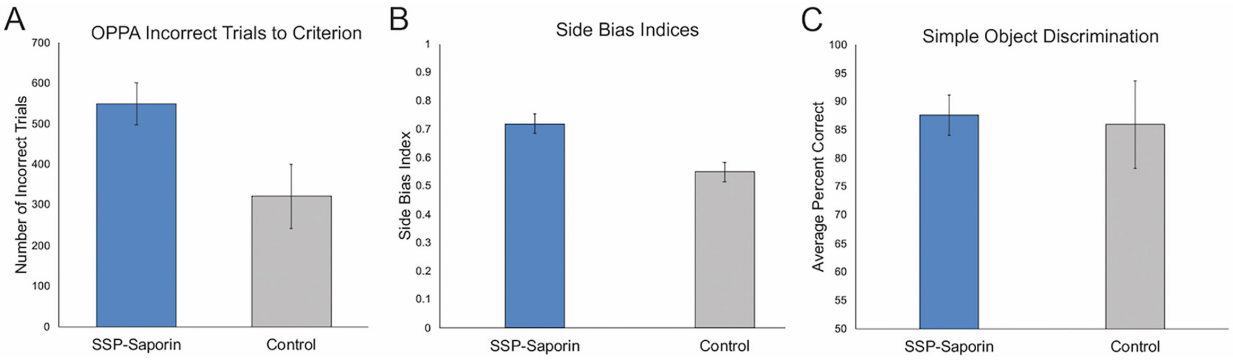

Fig. 3. Object-Place Paired Association task performance in SSP-SAP treated animals compared to control. A) Number of incorrect trials to reach criterion performance on the OPPA task. Treated rats performed greater numbers of incorrect trials (p=0.04) to reach criterion performance on the OPPA task. B) Average side bias index on the OPPA task. A value of zero indicates no bias towards either the left or right possible choices. SSP-SAP-treated rats had a trend towards greater bias to select a well on one side compared to control animals (p=0.07), indicating these rats persevered to one well side more frequently than their control counterparts. C) Average percent of correct object choices on a control simple object discrimination task. Controls and SSP-SAP treated rats could acquire a simple pair-wise object discrimination and showed no significant differences in performance (p=0.83).

Kermit looks good on red!

References

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2019 Scientific References

Targeted Toxins

Targeting agent attached to Saporin

IT-01 192-IgG-SAP

Co-treatment with rivastigmine and idalopirdine reduces the propensity for falls in a rat model of falls in Parkinson's disease (2019) Koshy Cherian A, *et al. Psychopharmacol (Berl)*

Basal forebrain chemogenetic inhibition disrupts the superior complex movement control of goal-tracking rats (2019) Kucinski A, *et al. Behav Neurosci* 133: (1):121-134.

Partial depletion of septohippocampal cholinergic cells reduces seizure susceptibility, but does not mitigate hippocampal neurodegeneration in the kainate model of epilepsy (2019) Soares JI, *et al. Brain Res* 1717: 235-246

Disruption of medial septum and diagonal bands of Broca cholinergic projections to the ventral hippocampus disrupt auditory fear. memory (2018) Staib JM, *et al. Neurobiol Learn Mem* 152: 71-79.

Are some animal models more equal than others? A case study on the translational value of animal models of efficacy for Alzheimer's disease (2019) Veening-Griffioen DH, *et al. Eur J Pharmacol* 859: 172524.

Astroglia in Alzheimer's Disease (2019) Verkhratsky A, *et al. Neuroglia in Neurodegenerative Diseases*. 273-324.

Cholinergic Deficit Induced by Central Administration of 192IgG-Saporin Is Associated With Activation of Microglia and Cell Loss in the Dorsal Hippocampus of Rats (2019) Dobryakova YV, *et al. Front Neurosci* 13: 146.

Nitric oxide donor molsidomine promotes retrieval of object recognition memory in a model of cognitive deficit induced by 192 IgG-saporin (2019) Hernández-Melesio MA, *et al. Behav Brain Res* 366: 108-117.

Focused ultrasound-induced blood-brain barrier opening improves adult hippocampal neurogenesis and cognitive function in a cholinergic degeneration dementia rat model (2019) Shin J, *et al. Alzheimer's Res Ther* 11: (1):110.

IT-02 OX7-SAP

RGS14414 treatment induces memory enhancement and rescues episodic memory deficits (2019) Masmudi-Martín M, *et al. FASEB J* fj.201900429RR.

IT-03 Anti-DBH-SAP

BDNF Downregulates Adrenergic β-Receptor-Mediated Hypotensive Mechanisms in the Paraventricular Nucleus of the Hypothalamus (2019) Thorsdottir D, *et al. Ameri J Physiol Heart Circulatory Physiol* 317:6.

Brainstem pre-sympathetic neurons contribute to irregular breathing patterns in volume overload heart failure (2019) Toledo C, *et al. FASEB J* 33: (1_supplement):lb630-lb630.

Central Glucagon-like Peptide-1 Receptor Signaling via Brainstem Catecholamine Neurons Counteracts Hypertension in Spontaneously Hypertensive Rats (2019) Katsurada K, *et al. Sci Rep* 9: (1):12986.

Hindbrain glucoregulatory mechanisms: Critical role of catecholamine neurons in the ventrolateral medulla (2019) Ritter S, *et al. Physiol Behav* 208: 112568.

Ablation of brainstem C1 neurons improves cardiac function in volume overload heart failure (2019) Andrade DC, *et al. Clin Sci* CS20180589.

Spinal α2-adrenoceptors and neuropathic pain modulation; therapeutic target (2019) Bahari Z, *et al. Brit J Pharmacol* 176: (14):2366-2381.

Rostral ventrolateral medullary catecholaminergic neurones mediate irregular breathing pattern in volume overload heart failure rats (2019) Toledo C, *et al. J Physiol* 597(24):5799-5820. doi: 10.1113/JP278845. Epub 2019 Nov 28.

Morphological analysis for neuronal pathway from the hindbrain ependymocytes to the hypothalamic kisspeptin neurons (2019) Deura C, *et al. J Reprod Dev* 65: (2):129-137.

IT-06 Mac-1-SAP (mouse)

Contribution of microglial reaction to increased nociceptive responses in high-fat-diet (HFD)-induced obesity in male mice (2019) Liang Y-J, *et al. Brain Behav Immun* 80: 777-792.

Inflammatory macrophages in the sciatic nerves facilitate neuropathic pain associated with type 2 diabetes mellitus (2019) Saika F, *et al. J Pharmacol Exp Ther* 368: (3):535-544.

IT-10 IB4-SAP

Role of Nociceptor Toll-like Receptor 4 (TLR4) in Opioid-Induced Hyperalgesia and Hyperalgesic Priming (2019) Araldi D, *et al. J Neurosci* 39: (33):6414-6424.

IT-11 SSP-SAP

Episodic stimulation of central chemoreflex elicits long-term breathing disorders and autonomic imbalance in heart failure rats (2019) Díaz HS, *et al. Eur Respir J* 54: (suppl 63):OA4936.

LEONARDO ANCHETA

Leonardo has been serving the Biotech industry for 17 years and as Vice President of ATS, his knowledge of saporin conjugates and the ZAP line of products for antibody screening have been invaluable to ATS customers. Along with his duties at ATS, Leonardo is the Director of Custom Conjugations & Cell-Based Assays for ATS's partner organization, CytoLogistics, where he oversees all the product manufacturing for ATS and contract laboratory services (e.g. compound screening, cell-based assays, custom conjugations). Streptavidin-ZAP and many other secondary conjugates are under his watchful eye and he is ready to discuss your custom conjugation needs.

2019 Scientific References

Episodic stimulation of central chemoreceptor neurons elicits disordered breathing and autonomic dysfunction in volume overload heart failure (2019) Diaz HS, *et al. Am J Physiol Lung Cell Mol Physiol* 318(1):L27-L40. doi: 10.1152/ajplung.00007.2019. Epub 2019 Oct 16.

Targeted hippocampal GABA neuron ablation by Stable Substance P–saporin causes hippocampal sclerosis and chronic epilepsy in rats (2019) Chun E, *et al. Epilepsia* 60: (5):e52-e57.

Objective: Hippocampal GABA neurons were targeted for selective elimination to determine whether a focal hippocampal GABAergic defect in an otherwise normal brain can initiate cryptogenic temporal lobe epilepsy with hippocampal sclerosis..

Summary: Hippocampal GABAergic dysfunction is epileptogenic and can produce the defining features of cryptogenic temporal lobe epilepsy.

Dose: Intrahippocampal injections of SSP-SAP (0.4 ng/10 nL) were performed using a 0.5-µL Neuros Syringe lowered into four hippocampal sites along both the transverse and longitudinal hippocampal axes bilaterally.

The Retrotrapezoid Nucleus: Central Chemoreceptor and Regulator of Breathing Automaticity (2019) Guyenet PG, *et al. Trends Neurosci* 42(11):807-824. doi: 10.1016/j.tins.2019.09.002. Epub 2019 Oct 18.

Contribution of retrotrapezoid nucleus and carotid bodies to asphyxia-induced arousal in rats (2019) Souza G, *et al. FASEB J* 33: (1_supplement):733.6-733.6.

Spinal cord projection neurons: a superficial, and also deep, analysis (2019) Wercberger R, *et al. Curr Opin Physiol* 11:109-115. https://doi.org/10.1016/j.cophys.2019.10.002

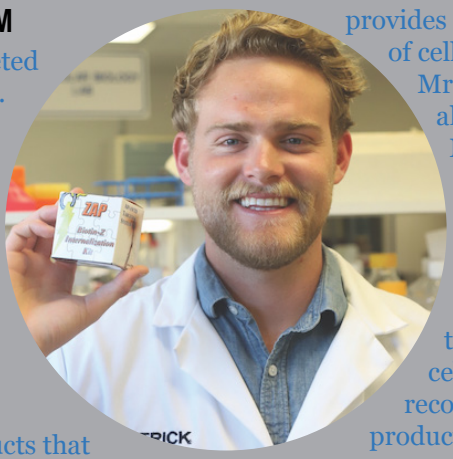
Contribution of the retrotrapezoid nucleus and carotid bodies to arousal from sleep elicited by hypercapnia and hypoxia (2019) Souza G, *et al. J Neurosci* 39(49):9725-9737. doi: 10.1523/JNEUROSCI.1268-19.2019

IT-12 Dermorphin (MOR)-SAP

Lesions of the Patch Compartment of Dorsolateral Striatum Disrupt Stimulus–Response Learning (2019) Jenrette TA, *et al. Neuroscience* 415: 161-172.

PATRICK SHRAMM

Patrick just completed his 8th year at ATS. He is a Product Manager for the Melanopsin product line and several of our targeted toxins and ZAP conjugates. Patrick also developed the ATS pHast line of products that provides a quick readout of cell internalization. Mr. Shramm is also the in-house Molecular Biology expert and consults on all the ATS and CytoLogistics projects from cell transfections to cell-based assays to recombinant protein production.



Inflammatory mediators of opioid tolerance: Implications for dependency and addiction (2019) Eidson LN, *et al. Peptides* 115: 51-58.

IT-14 CTB-SAP

A2A and 5-HT Receptors are Differentially Required for Respiratory Plasticity Over the Course of Motor Neuron Loss in Intrapleurally CTB-SAP Treated Rats (2019) Borkowski LF, *et al. FASEB J* 33: (1_supplement):843.3-843.3.

Neuroprotective Effects of Exercise on the Morphology of Somatic Motoneurons Following the Death of Neighboring Motoneurons (2019) Chew C, *et al. Neurorehabil Neural Repair* 33: (8):656-667.

Neuromuscular Plasticity in a Mouse Neurotoxic Model of Spinal Motoneuronal Loss (2019) Gulino R, *et al. Int J Mol Sci* 20: (6):doi: 10.3390/ijms20061500.

Objective: To use a neurotoxic model of spinal motoneuron depletion, induced by injection of CTB-SAP, to investigate the possible occurrence of compensatory changes in both the muscle and spinal cord.

Summary: Plastic changes in surviving motoneurons produce a functional restoration probably similar to the compensatory changes occurring in disease. These changes could be driven by glutamatergic signaling; astrocytes contacting surviving motoneurons may support this process.

Dose: Mice received 2 injections of CTB-SAP (3 mg in 2 mL PBS) into the medial and lateral left gastrocnemius muscle.

Adenosine 2A Receptor Inhibition Promotes Neuroprotection Following Toxic Insult to Phrenic Motor Neurons (2019) Sajjadi E, *et al. FASEB J* 33: (1_supplement):844.3-844.3.

Adenosine 2A receptor inhibition protects phrenic motor neurons from cell death induced by protein synthesis inhibition (2019) Seven YB, *et al. Exp Neurol* 323:113067. doi: 10.1016/j.expneurol.2019.113067. Epub 2019 Oct 17.

Renal denervation for treating congenital long QT syndrome: shortening the QT interval or modulating sympathetic tone? (2019) Kiuchi MG, *et al. EP Europace* 21: (11):1755-1756.

IT-16 mu p75-SAP

3D Reconstruction of the Neurovascular Unit Reveals Differential Loss of Cholinergic Innervation in the Cortex and Hippocampus of the Adult Mouse Brain (2019) Nizari S, *et al. Front Aging Neurosci* 11: (172)

Central cholinergic neuronal degeneration promotes the development of postoperative cognitive dysfunction (2019) Xu H, *et al. Lab Invest* 99: (7):1078-1088.

IT-20 Orexin-SAP

0054 SUVN-G3031, a Histamine H3 Receptor Inverse Agonist Produces Wake Promoting Effect in Orexin-2-saporin Lesioned Rats (2019) Benade V, *et al. Sleep* 42: (Supplement_1):A22-A23.

Cannabidiol partially blocks the sleepiness in hypocretin-deficient rats. Preliminary data (2019) Murillo-Rodriguez E, *et al. CNS Neurol Disord Drug Targets* 18: (9):705-712.

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Discovery and Development of N-[4-(l-Cyclobutylpiperidin-4-yloxy) phenyl]-2-(morpholin-4-yl) acetamide dihydrochloride (SUVN-G3031): A Novel, Potent, Selective and Orally Active Histamine H3 Receptor Inverse Agonist with Robust Wake-Promoting Activity (2019) Nirogi R, *et al. J Med Chem* 62: (3):1203-1217.

Objective: To discover and develop a therapeutic for human sleep disorders.

Summary: Histamine H3 Receptor Inverse Agonist demonstrated high receptor occupancy and marked wake promoting effects with decreased REM sleep in Orexin-B-SAP lesioned rats. This study supports its potential therapeutic utility in treating human sleep disorders.

Dose: Injections (490 ng/0.8 µl) were made bilaterally to the lateral hypothalamus.

IT-23 Anti-SERT-SAP

S38. Dissecting the Functional Heterogeneity of Serotonergic Systems That Regulate Fear and Panic (2019) Bernabe C, *et al. Biol Psychiatry* 85: (10):S311.

Serotonin and motherhood: From molecules to mood (2019) Pawluski JL, *et al. Front Neuroendocrinol* 53. 00742. doi: 10.1016/j.yfrne.2019.03.001. Epub 2019 Mar 13.

IT-28 NPY-SAP

Facilitation of neuropathic pain by the NPY Y1 receptor-expressing subpopulation of excitatory interneurons in the dorsal horn (2019) Nelson TS, *et al. Sci Rep* 9: (1):7248.

IT-31 CCK-SAP

OP11: Role of spinal cholecystokinin receptor 2 in alloknesis models (2019) Tominaga M, *et al. Itch* 4: 1-62.

IT-32: GAT1-SAP

The undeveloped properties of GABA neurons in the ventral tegmental area promote energy intake for growth in juvenile rats (2019) Maejima Y, *et al. Sci Rep* 9: (1):11848.

IT-40 Bombesin-SAP

Spinal Neuropeptide Y1 Receptor-Expressing Neurons Form an Essential Excitatory Pathway for Mechanical Itch (2019) Acton D, *et al. Cell Reports* 28: (3):625-639.e6.

Identification of a Spinal Circuit for Mechanical and Persistent Spontaneous Itch (2019) Pan H, *et al. Neuron* 103: 1-15.

Selective role of neurokinin B in IL-31–induced itch response in mice (2019) Sakata D, *et al. J Allergy Clin Immunol* 144: (4):1130-1133.

IT-42 Anti-ChAT-SAP

Cholinergic neural activity directs retinal layer-specific angiogenesis and blood retinal barrier formation (2019) Weiner GA, *et al. Nature Commun* 10: (1):2477.

Objective: To determine which neurons are responsible for angiogenesis and blood retinal barrier formation.

Summary: Anti-ChAT-SAP reduces SAC (starburst amacrine cell) number and inhibits deep-layer angiogenesis.

Dose: Anti-ChAT-SAP or control Rabbit-IgG-SAP was injected intravitreally at P3 and P11 (0.12 mg/mL in PBS).

IT-46: Oxytocin-SAP

Oxytocin attenuates phencyclidine hyperactivity and increases social interaction and nucleus accumben dopamine release in rats (2019) Kohli S, *et al. Neuropsychopharmacology* 44: (2):295-305.

IT-47: Leptin-SAP

Leptin receptor-expressing neurons in the ventromedial nucleus of the hypothalamus contribute to weight loss caused by fourth ventricle leptin infusions (2019) Seamon M, *et al. Am J Physiol Endocrinol Metab* 317: (4):E586-E596.

IT-63 NK3-SAP

Glutamatergic Neurokinin 3 Receptor Neurons in the Median Preoptic Nucleus Modulate Heat-Defense Pathways in Female Mice (2019) Krajewski-Hall SJ, *et al. Endocrinology* 160: (4):803-816.

Evidence that the LH surge in ewes involves both neurokinin B-dependent and –independent actions of kisspeptin (2019) Goodman RL, *et al. Endocrinology* 160: (12):2990-3000.

2019 Scientific References

Secondary Conjugates

ZAP conjugates use your secondary agent conjugated to Saporin and makes a targeted toxin for use in vitro or in vivo. Use your biotinylated targeting agent with Streptavidin-ZAP.

IT-27 Streptavidin-ZAP

Targeting the niche: depleting haemopoietic stem cells with targeted therapy (2019)Abadir E, *et al. Bone Marrow Transplant* 54: (7):961-968.

Nongenotoxic antibody-drug conjugate conditioning enables safe and effective platelet gene therapy of hemophilia A mice (2019) Gao C, *et al. Blood Adv* 3: (18):2700-2711.

Hematopoietic chimerism and donor-specific skin allograft tolerance after non-genotoxic CD117 antibody-drug-conjugate conditioning in MHC-mismatched allotransplantation (2019) Li Z, *et al. Nature Commun* 10: (1):616.

Selective hematopoietic stem cell ablation using CD117-antibody-drug-conjugates enables safe and effective transplantation with immunity preservation (2019) Czechowicz A, *et al. Nat Commun* 10: (1):617-617.

Targeting prostate cancer: Prostate-specific membrane antigen based diagnosis and therapy (2019) Wüstemann T, *et al. Med Res Rev* 39: (1):40-69.

Summary: Conjugation to the antibody was achieved by reacting the biotinylated antibody with Streptavidin-ZAP . Binding potency of the conjugate was comparable to that of the naked antibody and in vivo experiments proved potent for selective tumor growth inhibition in mice bearing LNCaP tumors.

Identification of lineage-specific markers for therapeutic targeting of mast cells (2019) Plum T. *Journal Ph.D.* 112.

Selected Antibodies

AB-02 Anti-CRH

Immunohistochemical detection of prolactin-releasing peptide2 in the brain of the inshore hagfish Eptatretus burgeri. (2019) Amano M, *et al. Gen Comp Endocrinol*, 274:1-7.

Objective: Localization of PrRP2 in the brain of the inshore hagfish was examined.

Summary: Reciprocal connections are suggested between the PrRP2 and PQRFa neurons. IHC (1000-fold)

Disrupted compensatory response mediated by Wolfram syndrome 1 protein and corticotrophin-releasing hormone family peptides in early-onset intrahepatic cholestasis pregnancy. (2019) Xu T, *et al. Placenta*, 83 63-71.

Western (1:1000)

Effects of Shugan Hewei Granule on Depressive Behavior and Protein Expression Related to Visceral Sensitivity in a Rat Model of Nonerosive Reflux Disease. (2019) Wang Y, *et al. Evidence-based complementary and alternative medicine : eCAM*, 2019 12.

IF (1:200)

Light stimulation into dorsal raphe nucleus contributes to antidepressant effect for a stressed rat model. (2019) Li X. *bioRxiv821421*.

IHC (1:500)

Ro41-5253, a selective antagonist of retinoic acid receptor α, ameliorates chronic unpredictable mild stress-induced depressive-like behaviors in rats: Involvement of regulating HPA axis and improving hippocampal neuronal deficits. (2019) Ke Q, *et al. Brain Res Bull*, 146 302-309.

Histology

Conditioned Aversion and Neuroplasticity Induced by a Superagonist of Extrasynaptic GABAA Receptors: Correlation With Activation of the Oval BNST Neurons and CRF Mechanisms. (2019) de Miguel E. *et al. Front Mol Neurosci*, 12 130.

IHC (1:250)

Enteric apelin enhances the stress-induced stimulation of colonic motor functions. (2019) Bülbül M, *et al. Stress*, DOI: 10.1080/10253890.2019.1658739 1-12.

Histology (1:100)

AB-07 Anti-FGF-2

MALAT1 overexpression promotes the proliferation of human periodontal ligament stem cells by upregulating fibroblast growth factor 2. (2019) Chen P. *et al.. Exper Ther Med*, 18 (3):1627-1632. 07/08.

Western (1:1300)

Adipose-derived stem cells embedded in platelet-rich plasma scaffolds improve the texture of skin grafts in a rat full-thickness wound model. (2019) Gao Y, *et al. Burns*, DOI: 10.1016/j.burns.2019.07.041

Western (1:500)

Palladium based nanoparticles for the treatment of advanced melanoma. (2019) Elsey J, *et al. Sci Rep*, 9 (1):3255.

IHC (1:300)

A ZEB1/p53 signaling axis in stromal fibroblasts promotes mammary epithelial tumours. (2019) Fu R, *et al. Nature Commun*, 10 (1):3210.

IHC (1:100)

Cilostazol Promotes Angiogenesis and Increases Cell Proliferation After Myocardial Ischemia–Reperfusion Injury Through a cAMP-Dependent Mechanism. (2019) Li J, *et al. Cardiovasc Eng Technol*., 10 (4):638-647.

Western

YY1-mediated overexpression of long noncoding RNA MCM3AP-AS1 accelerates angiogenesis and progression in lung cancer by targeting miR-340-5p/KPNA4 axis. (2019) Li X, *et al. J Cell Biochem*, DOI: 10.1002/jcb.29448.

Western (1:1000)

Adipose tissue-derived stem cells boost vascularization in grafted ovarian tissue by growth factor secretion and differentiation into endothelial cell lineages. (2019) Manavella DD, *et al. Mol Hum Reprod*, 25 (4):184-193.

IHC (1:2000)

Effectiveness of topical administration of Anethum graveolens essential oil on MRSA-infected wounds. (2019) Manzuoerh R, *et al. Biomed Pharmacother*, 109 1650-1658.

IHC (1:500)

2019 Scientific References

Metalloprotease inhibitor TIMP proteins control FGF-2 bioavailability and regulate skeletal growth. (2019) Saw S, *et al. J Cell Biol*, 218 (9):3134-3152.

Immunostaining (1:100)

Downregulation of Linc-RNA activator of myogenesis LncRNA participates in FGF2 mediated-proliferation of human periodontal ligament stem cells. (2019) Wu X, *et al. J Periodontol*, DOI: 10.1002/JPER.19-0317

Western (1:1200)

AB-265 Anti-Tri-methyl Lysine

Human FAM173A is a mitochondrial lysine-specific methyltransferase that targets adenine nucleotide translocase and affects mitochondrial respiration. (2019) Małecki J, *et al. J Biol Chem* DOI: 10.1074/jbc. RA119.009045.

Western

Evidence for the involvement of protein lysine methylation in the response of sensitive and tolerant Arabidopsis species to cadmium stress. (2019) Serre NBC, *et al. bioRxiv652651*.

Western

Protein lysine methylation contributes to modulating the response of sensitive and tolerant Arabidopsis species to cadmium stress. (2019) Serre NBC, *et al. Plant Cell Environ* DOI: 10.1111/pce.13692:

Western

AB-N01 Anti-mu p75

Direct Conversion of Mouse Embryonic Fibroblasts into Neural Crest Cells. (2019) Motohashi T, & Kunisada T. In K Turksen (Ed.), *Skin Stem Cells: Methods and Protocols* (pp. 307-321). New York, NY: Springer New York.

Flow Cytometry

Development of Intestinal Scaffolds that Mimic Native Mammalian Intestinal Tissue (2019) Ladd MR, *et al. Tissue Engineering* Part A 25: (17-18):1225-1241.

IHC (1:500)

AB-N01AP Anti-mu p75 ap

Knockdown of Fidgetin Improves Regeneration of Injured Axons by a Microtubule-Based Mechanism. (2019) Matamoros AJ, *et al. J Neurosci*, 39 (11):2011-2024. 01/15.

IHC

Combining naproxen and a dual amylin and calcitonin receptor agonist improves pain and structural outcomes in the collagen-induced arthritis rat model. (2019) Katri A, *et al. Arthritis Res Ther*, 21 (1):68.

Histology (1:4000)

Early Forebrain Neurons and Scaffold Fibers in Human Embryos.(2019) Qin J, *et al. Cereb Cortex*, DOI: 10.1093/cercor/bhz136

IF (1:600)

Upregulation of proBDNF in the Mesenteric Lymph Nodes in Septic Mice. (2019) Wang Z, *et al. Neurotox Res*, 36 (3):540-550.

Western (1:2000)

Brain-Derived Neurotrophic Factor Precursor in the Hippocampus Regulates Both Depressive and Anxiety-Like Behaviors in Rats.(2019) Zhong F, *et al. Front Psychiatr*, 9 776-776.

Western

AB-N03 Anti-trkA

The impact of bone cancer on the peripheral encoding of mechanical pressure stimuli.(2019) Kucharczyk MW, *et al. bioRxiv498980*.

IHC (1:400)

AB-N27AP Angiotensin IIR (AT1R)

The regulation effect of WNT-RAS signaling in hypothalamic paraventricular nucleus on renal fibrosis. (2019) Zhou G, *et al. J Nephrol*, https://doi.org/10.1007/s40620-019-00637-8

IHC and Western

Infrared fluorescence imaging of infarcted hearts with Ag2S nanodots. (2019) Ortgies DH, *et al. Nano Res*, 12 (4):749-757.

Western (1:1000)

Attenuation of Angiotensin II–Induced Hypertension in BubR1 Low Expression Mice Via Repression of Angiotensin II Receptor 1 Overexpression. (2019) Aoyagi Y, *et al. J Am Heart Assoc*, 8 (23):e011911

Western

Kangxianling decoction prevents renal fibrosis in rats with 5/6 nephrectomy and inhibits Ang II-induced ECM production in glomerular mesangial cells. (2019) Ji J, *et al. J Pharmacol Sci*, 139 (4):367-372.

Western (1:100)

Objective: To investigate effects of Kangxianling decoction on renal fibrosis in a ratmodel of 5/6 Nephrectomy (5/6 N) *in vivo* and in angiotensin II (Ang II)-treated rat glomerular mesangial cells (HBZY-1) in vitro.

Mixed Vehicle Emissions Induces Angiotensin II and Cerebral Microvascular Angiotensin Receptor Expression in C57Bl/6 Mice and Promotes Alterations in Integrity in a Blood-Brain Barrier Coculture Model. (2019) Suwannasual U, *et al. Toxicol Sci*, 170 (2):525-535.

AT1R Antibody was used in IF staining of cerebral tissue. Brain sections (10 mm) were prepared for AT1 receptor (1:1000).

RASCHEL BOUAJRAM

Raschel, our newest Product Manager, has been on the ATS team for 2 years. She has great expertise in the important assays needed to provide quality control for our conjugates and antibodies. Raschel is responsible for the Angiotensin antibodies and many more. She also presented a poster at the Neuroscience

Meeting in Germany about one of our newest products "FITC-SA-ZAP," which is a tool for efficient receptor-mediated delivery of a cytoxic payload with fluorescence validation for screening receptor-specific Targeting Agents. She is currently working on the production and purification of CTB (Cholera Toxin B).

2019 Scientific References

Losartan, an Angiotensin II Type 1 Receptor Antagonist, Alleviates Mechanical Hyperalgesia in a Rat Model of Chemotherapy-Induced Neuropathic Pain by Inhibiting Inflammatory Cytokines in the Dorsal Root Ganglia. (2019) Kim E, *et al. Molec Neurobio* l1-12.

Western (1:100)

Sulfisoxazole inhibits the secretion of small extracellular vesicles by targeting the endothelin receptor A. (2019) Im E-J, *et al. Nature Commun*, 10 (1):1387.

Western (1:1000)

Inhibition of Methamphetamine Self-Administration and Reinstatement by Central Blockade of Angiotensin II Receptor in Rats. (2019) Xu X, *et al. J Pharmacol ExpTher*, 369 (2):244. PMID: 30867225

Membranes were incubated at 4°C overnight (1:1000).

Cardiac hypertrophy in mice submitted to a swimming protocol: influence of training volume and intensity on myocardial renin-angiotensin system. (2019) Soares DdS, *et al. Am J Physiol Regul Integr Comp Physiol*, 316 (6):R776-R782.

Incubation at 4°C with anti-AT1R diluted in TTBS buffer with 5% non-fat 220 milk (1:800).

LncRNA AK094457 promotes AngII-mediated hypertension and endothelial dysfunction through suppressing of activation of PPARγ. (2019) Zhuo X, *et al.. Life Sciences*, 233 116745.

IHC

AB-N28AP Angiotensin IIR (AT2R)

Targeting AngII/AT1R signaling pathway by perindopril inhibits ongoing liver fibrosis in rat. (2019) Abd El-Rahman SS, & Fayed HM. J *Tissue Eng Regen Med*, 13 (12):2131-2141.

IHC (1:100)

Altered expression of renin-angiotensin system receptors throughout colorectal adenoma-adenocarcinoma sequence. (2019) Beitia M, *et al. Int J Mol Sci*, 16 (6):813-821.

IHC

Expression of the intrarenal angiotensin receptor and the role of renin-angiotensin system inhibitors in IgA nephropathy. (2019) Zhang Z, *et al. Mol Cell Biochem*, 453 (1):103-110.

IHC (1:500).

AB-N27AP and AB-N28AP

AT2R Activation Prevents Microglia Pro-inflammatory Activation in a NOX-Dependent Manner: Inhibition of PKC Activation and p47(phox) Phosphorylation by PP2A. (2019) Bhat SA, *et al. Mol Neurobiol*, 56 (4):3005-3023.

Immunoblotting: membranes were incubated with respective primary antibodies AT1R (1:1000), AT2R (1:500). ICH: AT1R (1:100), AT2R (1:100).

AB-N32 Anti-mGluR2

RGS4 Maintains Chronic Pain Symptoms in Rodent Models. (2019) Avrampou K, *et al. J Neurosci*, 39 (42):8291.

Western

Localization of group II and III metabotropic glutamate receptors at pre- and postsynaptic sites of inner hair cell ribbon synapses. (2019) Klotz L, *et al. FASEB J*, 33 (12):13734-13746.

IHC (1:150)

AB-N33AP Anti-NK-1R

Structural and functional defects of the respiratory neural system in the medulla and spinal cord of Pax6 mutant rats. (2019) Ikeda K, *et al. Brain Res Bull*, 152 107-116.

Immunofluorescence (1:800).

AB-N38 Anti-Melanopsin

Viability of Mouse Retinal Explant Cultures Assessed by Preservation of Functionality and Morphology. (2019) Alarautalahti V, *et al. Invest Ophthalmol Visual Sci*, 60 (6):1914-1927.

Immunostaining (1:2500)

Degeneration of ipRGCs in Mouse Models of Huntington's Disease Disrupts Non-Image-Forming Behaviors Before Motor Impairment. (2019) Lin M-S, *et al. J Neurosci*, 39 (8):1505.

Immunostaining (1:3000)

The M6 cell: A small-field bistratified photosensitive retinal ganglion cell. (2019) Quattrochi LE, *et al. J Comp Neurol*, 527 (1):297-311.

Immunofluorescence (1:10,000).

Expression of transcription factors divides retinal ganglion cells into distinct classes. (2019) Sweeney NT, *et al. J Comp Neurol, 527 (1):225-235.*

Immunostaining (1:1000).

pHast Conjugates

Fab-pHast products bind to your primary antibody via a secondary antibody cross-linked to a pH-dependent fluorescent reporter for quantitative testing of your primary antibody's specificity, binding, and internalization, providing results in 1 day.

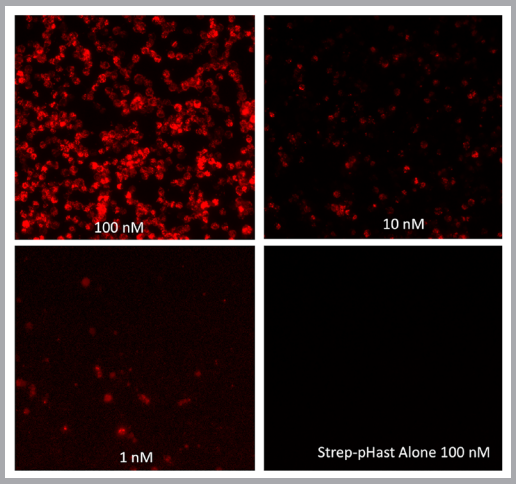
PH-01 Fab-pHast human

SLC46A3 as a Potential Predictive Biomarker for Antibody-Drug Conjugates Bearing Noncleavable Linked Maytansinoid and Pyrrolobenzodiazepine Warheads. (2018) Kinneer K, *et al. Clin Cancer Res*, 24 (24):6570-6582.

Objective: To develop biomarkers to uncover the underlying mechanism of resistance by certain cell lines for ADCs.

Summary: Loss of SLC46A3 expression was found to be a mechanism of innate and acquired resistance to ADCs bearing DM1 and SG3376.

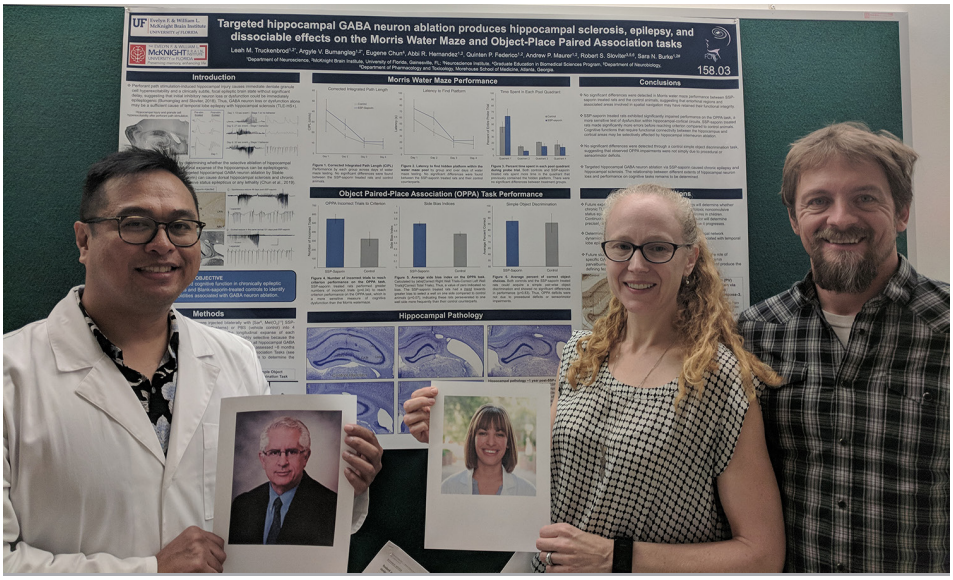
Dose: For Lysosomal trafficking, ADCs were labeled with Fab-pHast human. Cells were incubated with 3 mg/mL of labeled ADCs at 37°C for desired time points and fluorescence quantified by flow cytometry.



Immunocytochemistry images of KNRK cells with biotinylated IB4 conjugated and illuminated with Streptavidin-pHast.

2019 Poster of the Year Award

The 2019 Poster of the Year Award goes to … Argyle Bumanglag! His poster was presented at the 2019 Society for Neuroscience meeting and was titled: "Targeted hippocampal GABA neuron ablation produces hippocampal sclerosis, epilepsy, and dissociable effects on the Morris water maze and object-place paired association tasks. Poster authors are L. M. Truckenbrod, A. V. Bumanglag, E. Chun, A. Hernandez, Q. P. Federico, A. P. Maurer, R. S. Sloviter, S.N. Burke.
Congratulations to the team at University of Florida for the important work they presented using SSP-SAP (Cat. #IT-11) to create an animal model of temporal lobe epilepsy (TLE). Injections of SSP-SAP into the hippocampus of a rat cause acute hippocampal injury, permanent dentate granule cell-onset epilepsy, and hippocampal sclerosis that closely resembles the selective hippocampal pathology exhibited by patients diagnosed with TLE. The rats are chronically epileptic, with data going out one year for lesioned animals.



Argyle Bumanglag (holding portrait of Bob Sloviter), Sara Burke (holding portrait of Leah Truckenbrod), and Andrew Maurer. See the Featured article for more details about their exciting work.

2019 Scientific References

Custom Conjugates

Novel high molecular weight albumin-conjugated angiotensin II activates beta-arrestin and G-protein pathways.(2019) Pang *et al. Endocrine* 66(2):349-359.

Objective: To study the ability of a novel bovine serum albumin-angiotensin II (BSA-Ang II) conjugate to effect responses of the AT1 angiotensin II receptor subtype mediated by the G-protein-coupled and the beta-arrestin pathways.

Summary: BSA-Ang II and Ang II stimulated water appetite equivalently but BSA-Ang II stimulated saline appetite more than Ang II. Both BSA-Ang II and Ang II were considerably more potent at causing calcium mobilization than β-arrestin binding.

Dose: Angiotensin II (Ang II) was conjugated with bovine serum albumin and compared with Ang II for competition binding to AT1 receptors, to stimulate aldosterone release from adrenocortical cells, to promote beta-arrestin binding to AT1 receptors, to promote calcium mobilization, and stimulate drinking of water and saline by rats.

SAT-421 Cell-Specific Ablation of GnRH Neurons Using Kisspeptin-Saporin in the Preoptic Area of Sheep, but Not Mice. (2019) Porter D, et al. Journal of the Endocrine Society, Volume 3, Issue Supplement_1, April-May 2019.

Integrin α10, a Novel Therapeutic Target in Glioblastoma, Regulates Cell Migration, Proliferation, and Survival. (2019) Munksgaard Thorén M, *et al. Cancers*, 11 (4):587.

Objective: To invesigate the potential of integrin α10β1 as a therapeutic target in glioblastomas (GBMs).

Summary: integrin α10β1 has a crucial role in the migration, proliferation, and survival of GBM cells and that an integrin α10β1 antibody–drug conjugate induced cell death of GBM cells both *in vitro* and *in vivo*.

Dose: Infusions of anti-10-SAP or Anti-ctrl-SAP were made icv (1 µg/2 L per infusion).

SERVICES FOR SELECTIVELY ALTERING AND ASSAYING CELLS

ATS has partnered with CytoLogistics (CYTO) to help with your specific targeting needs.

Custom Conjugations

Custom Conjugates are powerful tools that deliver specific payloads to targeted cell types. This technique has important research applications in determining the function or contribution of the targeted cells in a biological system or for the removal of interfering cell types. Let CytoLogistics synthesize your next custom conjugate to allow you to eliminate, stimulate or temporarily paralyze any cell type.

Flow Cytometry

Flow cytometry is a laser-based technology that is used to measure characteristics of biological particles. This is done as the particles travel in suspension one by one past a sensing point. Flow cytometers have the added capability of being able to sort, or physically separate, cells or particles based on the chemical characteristic of interest. CytoLogistics can design and assay your next flow cytometry project.

Cell and Plate-Based Assays

Screening large numbers of peptides or proteins such as antibodies can be prohibitively expensive in both cost and time. From antibody and compound internalization assays to ELISAs and Fluorescent Microscopy, CytoLogistics has a wide array of kits and services for all of your cell and plate-based assays

www.CytoLogistics.com

Top 20 Targeting Tools of 2019

Top 5 Targeted Toxins

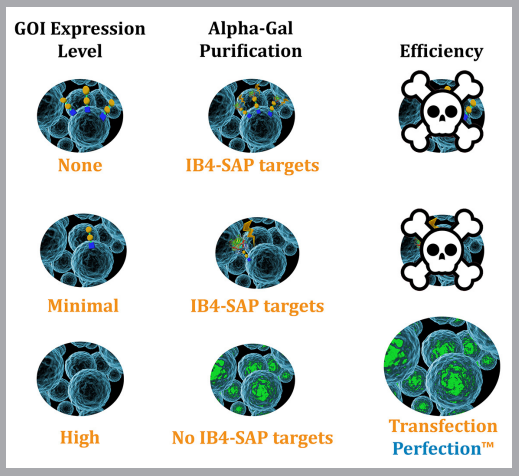
#1. CTB-SAP (Cat. #IT-14)
targets cells expressing GM1 receptor

#2. 192-IgG-SAP (Cat. #IT-01)
targets cells expressing rat p75NTR

#3. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase

#4. IB4-SAP (Cat. #IT-10)
targets cells expressing alpha-D-galactopyranoside residues

#5. CCK-SAP (Cat. #IT-06)
targets cells expressing sulfonated Cholecystokinin



Transfection Perfection™ utilizes IB4-SAP to purify transfected populations.

Top 5 in Number of Publications

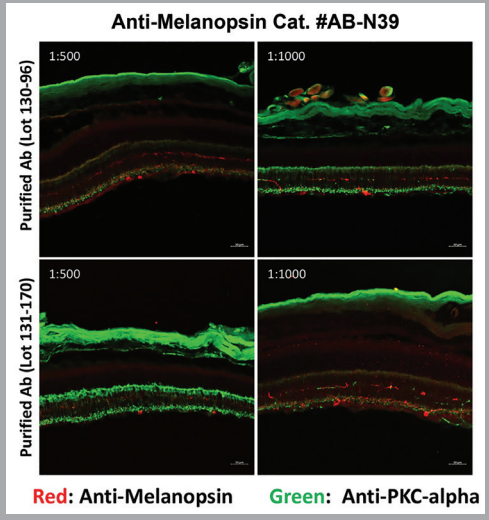
#1. Fibroblast Growth Factor (FGF-2) rabbit polyclonal, mammalian (Cat. #AB-07)
Usage: immunoblotting, immunohistochemistry

#2. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase

#3. SSP-SAP (Cat. #IT-11)
targets cells expressing substance P receptor (NK-1)

#4. Blank-SAP, (Cat. #IT-21)
a control for peptide-targeted lesioning agents

#5. 192-IgG-SAP (Cat. #IT-01)
targets cells expressing rat p75NTR



AB-N39 (Melanopsin Rabbit Polyclonal Antibody, affinity-purified). Staining of a cross-sectioned mouse retina. The counter-label in green is PKCalpha, a bipolar cell marker. AB-N39 staining is in red.
Images courtesy of Jingyi Gao, Xiaorong Liu, and Ignacio Provencio.

Top 5 ZAP Products

#1. Streptavidin-ZAP (Cat. #IT-27)
Uses your biotinylated material

#2. Fab-ZAP human (Cat. #IT-51)
Uses your primary human monoclonal antibody

#3. Fab-ZAP mouse (Cat. #IT-48)
Uses your primary mouse monoclonal antibody

#4. Mab-ZAP (Cat. #IT-04)
Uses your primary mouse monoclonal antibody

#5. Fab-ZAP rat (Cat. #IT-55)
Uses your primary rat monoclonal IgG antibody

Top 5 Antibodies

#1. NO-L-Cysteine Mouse Monoclonal, Conjugated, affinity-purified (Cat. #AB-T125)

#2. Angiotensin II receptor (AT-1r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N27AP)

#3. Melanopsin Rabbit Polyclonal, affinity-purified (Cat. #AB-N39)

#4. Trans-hydroxyproline Rabbit Polyclonal (Cat. #AB-T044)

#5. NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)