Cholinergic interneurons (ChIs) are sparsely distributed within the striatum, a nucleus that plays important roles in voluntary motor control, associated learning, procedural memory, execution of movement, action selection, and planning.1

ChIs comprise 1-3% of all striatal neurons, are the main source of striatal acetylcholine, and have long been associated with deficits in Parkinson’s disease. A striatal imbalance between dopamine and acetylcholine has been suggested as one of the causes of parkinsonism.2

To selectively deplete ChIs in the dorsolateral striatum of 21-day-old male mice (C57BL/6J), we used the saporin immunotoxin that targets choline acetyltransferase (Anti-ChAT-SAP, Cat. #IT-42). Experimental animals received a stereotaxic unilateral infusion of the targeted toxin (0.3 μl/3 min) and sham controls received the same volume of sterile saline (sham control). Our histological analysis encompassed Weeks 2-6 postsurgery performed at 2-week intervals (Fig. 1). The loss of cells reached a stable ~70% level by 4 to 6 weeks with the additional surprising finding that axon terminals stained with a vesicular acetylcholine transporter antibody were more numerous two weeks after the injection, returning to control levels by six weeks.

From a functional point of view, it will be important to find out if despite the cell loss, axon terminals sprout to invade the Anti-ChAT-SAP injected area from ~30% surviving ChIs or from ChIs in the surrounding tissue. To begin the study of dorsolateral striatal function following Anti-ChAT-SAP-induced ChI loss, we followed the same procedures as before3 and observed the animal’s performance in a reach-to-grasp task (Fig. 2). Mice were divided in two control groups (intact and sham) and one experimental Anti-ChAT-SAP-injected group. Training started one week postsurgery during the animal’s active circadian cycle and following 12 hours of food deprivation.

(continued on page 6)
The 2018 Poster of the Year Award Goes To . . .

Nilupaer Abudukeyoumu! Her poster was presented at the 2018 Society for Neuroscience meeting that was held in San Diego, CA:

Impaired reach-to-grasp responses in mice depleted of striatal cholinergic interneurons

Authors on the winning poster are pictured at right L. to R.: Nilupaer Abudukeyoumu, Marianela Garcia-Munoz, Yoko Nakano, and Gordon W. Arbuthnott.

Nilupaer Abudukeyoumu is a PhD student in Dr. Arbuthnott’s laboratory in the Brain Mechanism for Behavior Unit, Okinawa Institute of Science and Technology Graduate University, Japan.

See Cover article for more details about their exciting work.

Congratulations!

Product Managers

LEONARDO ANCHETA
Leonardo has worked as a scientist at Advanced Targeting Systems for over 15 years and was recently promoted to Vice President. His expertise in conjugations, flow cytometry, and research assay development are a major reason for the company’s success.

Leonardo is also Scientific Director of ATS’s partner organization, CytoLogistics. He oversees all the 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.

MIGUEL GALVAN
Miguel is a Product Manager in his second year at ATS. He is responsible for the production and handling of one of our most important products: Saporin. His skills in the laboratory and attention to detail make him a valuable asset to the team. He continues to be involved in Research and Development and is currently in the final stages of a new product line of transfection kits that will greatly improve efficiency and success in cell transfections.

Miguel’s other products include the recombinant IB4 products, the AB-T conjugated antibodies, and the essential line of Saporin Control Conjugates.

PATRICK SHRANNM
Patrick just completed his 7th year at ATS. He is a Product Manager for the Melanopsin product line and several of our targeted toxins. Patrick also developed the ATS pHast line of products and is currently working on the latest addition: Streptavidin-pHast (Strep-pHast).

Patrick’s molecular biology expertise makes him a valuable contributor to new product development. His scientific research on a CRISPR line of targeting tools keeps him busy in the laboratory when he’s not answering customer questions or working on product conjugations.

RASCHEL BOUJARAM
Raschel, our newest Product Manager, has been on the ATS team for over a year now. She has great expertise in the important assays needed to provide quality control for our conjugates and antibodies. Raschel produced one of our new products: Recombinant MOA (Marasmius oreades agglutinin). Unavailable from any other commercial source, her success in expressing and purifying the material was much needed. She is also responsible for the Saporin conjugate version: MOA-SAP. This targeted toxin recognizes blood group B antigens and has a high affinity to alpha gal, a carbohydrate found on B blood type cells. Raschel is also responsible for the Angiotensin antibodies and is currently working on a mouse-specific version.
Targeting Topics: 2018 Scientific References

TARGETED TOXINS

Targeting agent attached to Saporin

IT-01 192-IgG-SAP

Neuropharmacology of attention.

Effect of Placenta-Derived Mesenchymal Stem Cells in a Dementia Rat Model via Microglial Mediation: a Comparison between Stem Cell Transplant Methods.

Intracerebroventricular Administration of 192IgG-Saporin Alters Expression of Microglia-Associated Genes in the Dorsal But Not Ventral Hippocampus.

Lacrimal Gland Denervation Alters Tear Protein Composition and Impairs Ipsilateral Eye Closures and Corneal Nociception.

Neuronal Activity-Dependent Control of Postnatal Neurogenesis and Gliogenesis.

Impact of Chronic Stress on the Spatial Learning and GR-PKAc-NF-κB Signaling in the Hippocampus and Cortex in Rats Following Cholinergic Depletion.

Cholinergic basal forebrain structures are not essential for mediation of the arousing action of glutamate.

Cholinergic modulation of frontoparietal cortical network dynamics supporting supramodal attention.

The hot ‘n’ cold of cue-induced drug relapse.

The role of the supramammillary area of the hypothalamus in cognitive functions.

Disruption of medial septum and diagonal bands of Broca cholinergic projections to the ventral hippocampus disrupt auditory fear memory.


Cholinergic modulation of spatial learning, memory and navigation.

IT-03 Anti-DBH-SAP

Mo1548 - How Does the Extrinsic Nervous System (ENS) Connect with the Intrinsic Nervous System (INS) of the Esophagus and Stomach?

Modelling the dopamine and noradrenergic cell loss that occurs in Parkinson’s disease and the impact on hippocampal neurogenesis.

Noradrenergic Hypothesis Linking Neurodegeneration-Based Cognitive Decline and Astrogila.

Brainstem catecholaminergic neurones and breathing control during postnatal development in male and female rats.

Essential role of hippocampal noradrenaline in the regulation of spatial working memory and TDP-43 tissue pathology.

Why I can’t say “no” to hindbrain catecholamine neurons.

Mo1545 - Vagal Nerve Modulates the Effects of Esophageal Acid on the Periaqueductal Gray Functional Connectivity in a Rat Model.

Mo1547 - Enriched Environment Housing Alleviates Post-Colitis Pain, Visceral Hypersensitivity and Mood Disorders in Rats.

(continued on page 4)
Targeting Topics: 2018 Scientific References

(continued from page 3)

Mo1546 - Neonatal Colon Inflammation-Induced Increases in Pituitary Adenylate Cyclase Activating Peptide Expression in the Parabrachial Nucleus Contributes to Reduced Meal Consumption, to Increased Meal-Induced Aversive and Anxiety-Like Behaviors in Adult Rats. Winston J. Gastroenterology 154: (6, Supplement 1):S-748, 2018.


IT-06 Mac-1-SAP (mouse)


Summary: Depletion of spinal microglia with Mac-1-SAP was able to prevent and reverse neuropathic pain behavior.


IT-10 IB4-SAP


Summary: Results indicate that a combination of the CRISPR/Cas9 system and targeted toxin technology using IB4-SAP allows efficient enrichment of genome-edited clones, particularly bi-allelic KO clones.

Dose: Cells were trypsinized 3 days after transfection and approximately 80% were incubated for 30 min at 37°C in a solution (25 mcL) containing 0.5–1.0 mcg IB4-SAP.


IT-11 SSP-SAP
Role of GPCR (mu-opioid)-receptor tyrosine kinase (epidermal growth factor) crosstalk in opioid-induced hyperalgesic priming (type II). Araldi D, et al. Pain Epub 16 Feb, 2018


IT-12 Dermorphin (MOR)-SAP

IT-14 CTB-SAP
Targeting Topics: 2018 Scientific References

Targeted Ablation of Distal Cerebrospinal Fluid-Contacting Nucleus Alleviates Renal Fibrosis in Chronic Kidney Disease.

The Establishment of a CSF-Contacting Nucleus “Knockout” Model Animal.

Targeted ablation of cardiac sympathetic neurons improves ventricular electrical remodelling in a canine model of chronic myocardial infarction.

Objective: To evaluate the cardiac electrophysiologic effects of targeted ablation of cardiac sympathetic neurons (TACSN) in a canine model of chronic myocardial infarction (MI).
Summary: Targeted ablation of cardiac sympathetic neuron attenuates sympathetic remodelling and improves ventricular electrical remodelling in the chronic phase of MI. These data suggest that TACSN may be a novel approach to treating ventricular arrhythmias.
Dose: 20 μl of CTB-SAP (1.2 mg/ml) was mixed with 4 μl of 3% Evans blue dye to make it visible (CTB-SAP is colorless), ensuring localization within the ganglia. The CTB-SAP/Evans blue dye solution was slowly and intermittently injected into the left stellate ganglia using a glass micropipette.

Targeted ablation of cardiac sympathetic neurons attenuates adverse post-infarction remodeling and left ventricle dysfunction.

IT-16 mu p75-SAP
Real-time electrochemical monitoring of choline during systemic inflammation in the freely-moving mouse.

Removal of p75 Neurotrophin Receptor Expression from Cholinergic Basal Forebrain Neurons Reduces Amyloid-β Plaque Deposition and Cognitive Impairment in Aged APP/PS1 Mice.

Objective: To lesion CBF neurons, a single infusion of mu p75-SAP or control Rabbit IgG-SAP (0.4 mg/ml) was stereotaxically-injected into the basal forebrain.

Acute Down-regulation of BDNF Signaling Does Not Replicate Exacerbated Amyloid-β Levels and Cognitive Impairment Induced by Cholinergic Basal Forebrain Lesion.

Cholinergic modulation targeting medial prefrontal cortex leads to behavior deficit in interval timing task.

IT-20 Orexin-SAP
Depletion of Hypocretin/Orexin Neurons Increases Cell Proliferation in the Adult Subventricular Zone.

Orexinergic neurons are involved in the chemosensory control of breathing during the dark phase in a Parkinson's disease model.

IT-23 Anti-SERT-SAP
Serotonin-specific lesions of the dorsal raphe disrupt maternal aggression and caregiving in postpartum rats.
Raphe Pallidus is Not Important to Central Chemoreception in a Rat Model of Parkinson's Disease.

IT-25 Anti-DAT-SAP
Macrophage migration inhibitory factor mediates peripheral nerve injury-induced hypersensitivity by curbing dopaminergic descending inhibition.

IT-31 CCK-SAP
A Neural Circuit for Gut-Induced Reward.

IT-33 Mac-1-SAP (rat)
Early CALP2 expression and microglial activation are potential inducers of spinal IL-6 upregulation and bilateral pain following motor nerve injury.
Microglial pannexin-1 channel activation is a spinal determinant of joint pain.

IT-40 Bombesin-SAP
Tac1-Expressing Neurons in the Periaqueductal Gray Facilitate the Itch-Scratching Cycle via Descending Regulation.
Spinal Mechanisms of Itch Transmission.

(continued on page 7)
Impaired reach-to-grasp responses in mice depleted of striatal cholinergic interneurons

(continued from page 1)

Once the animals passed the initial acquisition phase, the successful performance in the reach-to-grasp task -- expressed as mean ± SD percentage is shown in Fig. 3.

**Controls:** 51.11 ± 3.83; n = 25 [intact], 48.79 ± 4.6; n = 9 [sham]

**Treated:** 26.28 ± 3.74; n = 13

The significantly-impaired performance of the experimental group compared to controls was present even when the animals were pretrained. The loss of ChIs impairs the performance of striatally-mediated motor tasks, which suggests that cholinergic synaptic function is more important than non-synaptic communication in this situation. A non-synaptic cholinergic tone may be important for setting functional striatal states in other circumstances, however, these specific lesions of ChI cells suggest that performance of a learned forelimb task requires that the cholinergic synaptic circuits of the striatum are intact.

References


Other References Using Anti-ChAT-SAP


IT-44  Melanopsin-SAP

DSCAM promotes self-avoidance in the developing mouse retina by masking the functions of cadherin superfamily members.

Summary: Focus on DSCAM (Down syndrome cell adhesion molecule 1) self-avoidance function in the mouse retina. DSCAM and members of the cadherin superfamily have also emerged as key contributors to a variety of neurodevelopmental disorders, including autism, schizophrenia, bipolar disease, Down syndrome and intellectual disability.

Immunotoxin-Induced Ablation of the Intrinsically Photosensitive Retinal Ganglion Cells in Rhesus Monkeys.

Immunolesion of melanopsin neurons causes gonadal regression in Pekin drakes (Anas platyrhynchos domesticus).

IT-50  Anti-CD103-SAP

Indoleamine 2,3-dioxygenase-dependent expansion of T-regulatory cells maintains mucosal healing in ulcerative colitis.

IT-69  Nppb-SAP

Circuit dissection of the role of somatostatin in itch and pain.

Objective: To investigate human NKp46 activity and its critical role in Natural Killer (NK) cell biology.
Summary: A unique anti-human NKp46 monoclonal antibody was developed and conjugated to Saporin. Targeted toxin inhibits growth of NKp46-positive cells; thus, exemplifying the potential as an immunotherapeutic drug to treat NKp46-dependent diseases, such as, type I diabetes and NK and T cell related malignancies.
Dose: Conjugation of the antibodies to Saporin, treatment of cells, and cell viability assay Biotin-Z Kit instructions.

Enhanced targeting of triple-negative breast carcinoma and malignant melanoma by photochemical internalization of CSPG4-targeting immunotoxins.

IT-22  Hum-ZAP

Conservation of oncofetal antigens on human embryonic stem cells enables discovery of monoclonal antibodies against cancer.

IT-27  Streptavidin-ZAP

Sequential Prodrug Strategy to Target and Eliminate ACPA-Selective Autoactive B cells.

Human anti-NKp46 antibody for studies of NKp46-dependent NK cell function and its applications for type 1 diabetes and cancer research.

Objective: Investigate the therapeutic potential of antibody to EPHA2 against melanoma in vitro.
Summary: Observations indicate a promising role for EPHA2 as a target in antibody treatments for melanoma, and demonstrate the potential therapeutic effects of an agonistic antibody to EPHA2.
Dose: A375 cells were plated into a flat-bottom, 96-well plate (2,000 cells per well) and incubated for 4 days at 37˚C. Cell suspension included different concentrations of Mab-ZAP, along with either anti-EPHA2 mAb (SHM16, SHM17, or SHM20 at 2 μg/ml final concentration), or a control IgG1 mAb (2 μg/ml final concentration).

Targeting of embryonic annexin A2 expressed on ovarian and breast cancer by the novel monoclonal antibody 2448.

Objective: To investigate human NKp46 activity and its critical role in Natural Killer (NK) cell biology.
Summary: A unique anti-human NKp46 monoclonal antibody was developed and conjugated to Saporin. Targeted toxin inhibits growth of NKp46-positive cells; thus, exemplifying the potential as an immunotherapeutic drug to treat NKp46-dependent diseases, such as, type I diabetes and NK and T cell related malignancies.
Dose: Conjugation of the antibodies to Saporin, treatment of cells, and cell viability assay Biotin-Z Kit instructions.

Enhanced targeting of triple-negative breast carcinoma and malignant melanoma by photochemical internalization of CSPG4-targeting immunotoxins.

Antibody Drug Conjugates Targeted to CD45 or CD117 Enable Allogeneic Hematopoietic Stem Cell Transplantation in Animal Models.
IT-27 Streptavidin-ZAP
Synergistic Cytotoxic Effect on Gastric 
Combine Phage Antibody Display 
Library Selection on Patient Tissue 
Specimens with Laser Capture 
Microdissection to Identify Novel 
Human Antibodies Targeting 
Clinically Relevant Tumor Antigens. 

Cancer Cells of an Immunotoxin 
Cocktail in Which Antibodies 
Recognize Different Epitopes on 
CDH17. 
Immunodiagn Immunother 37: (1):1-11, 
2018.

Characterization of the first fully 
human anti-TEM1 scFv in models of 
solid tumor imaging and 
immunotoxin-based therapy. 

Development and evaluation of T-Zap: 
a novel antibody-drug conjugate for 
the treatment of Her2 positive breast 
cancer. 

Interaction between the retrotrapezoid 
nucleus and the parafacial respiratory 
group to regulate active expiration and 
sympathetic activity in rats. 
Mol Physiol Epub 7 Nov, 2018.

IT-65 FabFc-ZAP human
Adalimumab:TNF complexes are cleared more efficiently by human 
esteolasts than those with etanercept 
through FCG-receptor binding and 
internalization. 
Harvey BP, et al. Ann Rheum Dis 77: 

SELECTED ANTIBODIES
AB-02 Anti-CRH
Immunohistochemical detection of 
prolactin-releasing peptide2 in the 
brain of the inshore bagfish Eptatretus 
burger. 
Amano M, et al. Gen Comp Endocrinol, 
2018.

In vitro examination of microglia- 
neuron crosstalk with BV2 cells, and 
primary cultures of glia and 
hypothalamic neurons. 
Tao X, et al. Heliyon 4: (8):e00730-e00730, 
2018.

Histological and morphofunctional 
parameters of the hypothalamic- 
pituitary-adrenal system are sensitive 
to daidzein treatment in the adult rat. 
Trifunovic S, et al. Acta Histochem 120: 

AB-213 Anti-6His
Isolation of blood-brain barrier- 
crossing antibodies from a phage 
display library by competitive elution 
and their ability to penetrate the 
central nervous system. 

AB-N01 Anti-mu p75
Non-canonical Ret signaling augments 
p75-mediated cell death in developing 
sympathetic neurons. 
2018.

Nicotinamide Mononucleotide 
Adenylyltransferase 2 maintains 
n neuronal structural integrity through 
the maintenance of golgi structure. 
Pottorf T, et al. Neurochem Int 121: 86-97, 
2018.

Enteric Neurodegeneration is 
Mediated Through Independent 
Neuritic and Somal Mechanisms in 
Rotenone and MPP+ Toxicity. 
Virga DM, et al. Neurochem Res 43: 

Myoepithelial Cells of Submucosal 
Glands Can Function as Reserve Stem 
Cells to Regenerate Airways after 
Injury. 
2018.

AB-N01AP Anti-mu p75 
affinity-purified
Nitration and Glycation Turn Mature 
NGF into a Toxic Factor for Motor 
Neurons: A Role for p75NTR and 
RAGE Signaling in ALS. 
Kim MJ, et al. Antioxid Redox Signal 28: 

Cell-Specific Transcriptome Analysis 
Shows That Adult Pillar and Deiters' 
Cells Express Genes Encoding 
Machinery for Specializations of 
Cochlear Hair Cells. 
356, 2018.

Regenerative effects of human 
embryonic stem cell-derived neural 
crest cells for treatment of peripheral 
nerve injury. 
2018.
Targeting Trends

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

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

#3. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
  targets cells expressing rat p75NTR

#4. Anti-ChAT-SAP (Cat. #IT-42)
  targets cells expressing choline acetyltransferase

#5. Nppb-SAP (Neuropeptide natriuretic polypeptide B) (Cat. #IT-06)
  targets cells expressing Nppb or BNP receptor

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Targeting Tools: Top Twenty (Five Each in Four Categories)

**Top Five Targeted Toxins**

#1. Streptavidin-ZAP (Cat. #IT-27)
  Uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

#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 Five Antibodies**

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

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

#3. Melanopsin Rabbit Polyclonal (Cat. #AB-N38)

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

#5. Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N28AP)

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**Top Five in Number of Publications**

#1. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
  targets cells expressing rat p75NTR

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

#3. Streptavidin-ZAP (Cat. #IT-27)
  Uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

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

#5. Melanopsin Rabbit Polyclonal (Cat. #AB-N38)

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**AB-N39 Anti-Melanopsin affinity-purified**


**AB-N40 Anti-SERT Serotonin (5HT) Transporter**


**AB-N43 Anti-p75 (192 IgG)**


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Parental HEK-293 cells, and HEK-293 cells transfected with the p75 receptor, were plated in a 96-well plate overnight. Titrated 192-IgG antibody (Cat. #AB-N43) was incubated at RT with 50 nM of Fab-pHast Mouse (Cat. PH-02) for 20 min prior to addition to cells. Plates were incubated overnight to allow maximum internalization, but a few hours is sufficient for detection.
Targeting Technology

Advanced Targeting Systems’ technology - Molecular Surgery - is a modification of one of the most widely used techniques: surgical lesioning of a region and observation of the effect.

The targeting agent is administered to the cells (in vivo or in vitro).

Choose an ANTIBODY specific to your cell type.

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§ or anything recognized on the cell surface and internalized.

The antibody seeks out its target receptor on the cell surface.

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New Targets

Selected researchers will receive support for smaller scale or pilot research projects ($1,000 - $25,000) with the targeting tools and contract services needed for the identification and characterization of new cell-surface markers for specific delivery of a payload.

• Proposals are encouraged from scientists across a variety of behavior or disease-related fields.

• Successful candidates will be notified of award within 30 days of receipt of application.

• Dates for submission are 1 March 2019 through 31 September 2019

• Visit the ATS or CytoLogistics websites today for details.