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Targeting Trends

Reporting the latest news in Molecular Surgery

Striatal patch compartment lesions reduce cocaine-induced repetitive behaviors

by Kristen Ashley Horner and collaborators R. Murray and M.C. Logan
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Repeated exposure to psychostimulants, such as cocaine, can result in patterns of repetitive, inflexible behaviors, known as stereotypy.[1,2] These inflexible behaviors are thought to be similar to the type of behaviors observed with certain psychiatric disorders, such as Tourette syndrome and obsessive-compulsive disorder.[3] Stereotypic behavior has been associated with enhanced activation of the patch compartment of striatum, relative to the surrounding matrix compartment.[1,2] The striatum is a component of the basal ganglia that is important for the initiation of voluntary movement based on the appropriate environmental context. Enhanced activation of the limbic-associated patch compartment within the striatum may result in the perpetuation of behaviors that are driven by internal emotional states. This occurs at the expense of normal adaptive behavioral responses that may be mediated by the matrix compartment which surrounds the patch compartment.[1,3] The functional role of the patch compartment in the development of these types of behaviors has not been previously investigated. Thus, we sought to determine the contribution of the neurons of the patch compartment to the emergence of stereotypy induced by psychostimulant exposure, by lesioning the patch compartment with Dermorphin-SAP (Cat. #IT-12) prior to treatment with repeated, high doses of cocaine.

Mu opioid receptors are densely expressed by the neurons of the patch compartment, while the neurons of the matrix compartment contain relatively few mu opioid receptors.[4,5] Thus, internalization of the Dermorphin-Saporin complex ultimately leads to the destruction of the mu

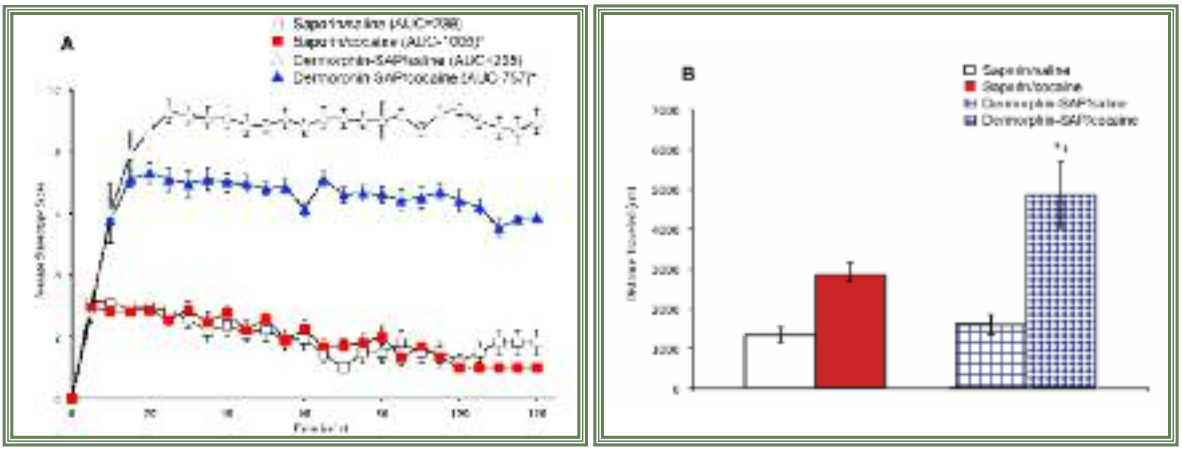

Fig. 1: Effects of intrastriatal infusion of Dermorphin-SAP (17 ng/µl) and repeated cocaine treatment (25 mg/kg), twice daily for one week followed by a week-long drug-free period, with a subsequent cocaine challenge (25 mg/kg) on stereotyped behavior (A) and locomotor activity (B). Values are expressed as the mean ±SEM. For stereotyped behavior the area under the curve (AUC values are in parentheses). Locomotor activity is expressed as the total distance traveled for the entire 2-hr obvservation period in centimeters.

**Significantly different from respective control group, p<0.005*
+Significantly different from saporin (vehicle)-pretreated cocaine-treated group, p<0.005.

(continued on page 6)

Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

New Beginnings

Denise Higgins, President

Welcome to Harrison James Russell!
Congratulations to proud parents: Candi and Brian Russell
Their bundle of joy arrived on December 7.

It's a new year. 2016. Another year passed and a brand new one to look forward to. After more than 21 years of serving the scientific community, Advanced Targeting Systems is making some changes and putting a 'fresh face' on things. Don't worry, we will still provide the same high level of service and expertise to help you move your research and discovery efforts forward.

One of the changes we have in store is a facelift for *Targeting Trends*, which Brian Russell (VP of Business Development) will be taking on as the new Editor in the next issue. This was going to happen with this first issue of the year, but as you can see by the beautiful picture here, he has had his hands full with the latest addition to his family. We are all so happy for him and Candi.

Besides his new role as Editor, Brian has also done a tremendous job with a redesign of the website -- executed skillfully and artfully by our webmaster and database guru, Kristen Hartman. We look forward to the exciting new business development directions Brian will be unfolding.

Those are some of the new things in store for us this year. But before I close, a quick look back. Our illustrious leader, former president, founder, and scientific genius, Doug Lappi, continues to guide our science team and is enjoying a much-deserved 'semi-retirement' with his wife, Darlene. He still comes in every week and meets with the scientists and often gives us a challenge at the ping-pong table! I want to take this opportunity to state very clearly for all to read (old customers, new customers, friends, cat lovers, chronic pain drug development followers -- everyone): Doug Lappi is the Sower of the Saporin seeds that bloomed into a successful company dedicated to providing quality targeting reagents for scientific research and pharmaceutical development. His contributions to science, and the research tools his work has provided, have not only made ATS a successful company, but have advanced the careers of scientists throughout the world. Thank you, Doug.

Douglas Lappi, Ph.D.

Upcoming Events

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San Diego, CA
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Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain.

Navratilova E, Xie JY, Meske D, Qu C, Morimura K, Okun A, Arakawa N, Ossipov M, Fields HL, Porreca F

J Neurosci 35(18): 7264-7271, 2015.

There are a number of neuronal circuits involved in the processing of pain, including those that control somatosensory, affective, and cognitive aspects of pain perception. Opioid signaling in the anterior cingulate cortex (ACC) plays a part in pain modulation - this area has also been implicated in the encoding of pain aversiveness. In order to examine the neuronal mechanisms of pain relief and the following reward, the authors of this paper administered 48 ng of Dermorphin-SAP (Cat. #IT-12) into the rostral ACC of rats. Saporin (Cat. #PR-01) was used as a control. The results illuminate the opioid pathway during pain treatment, and the dependence of nucleus accumbens dopaminergic transmission on upstream ACC opioid circuits during pain processing.

proBDNF and p75NTR Control Excitability and Persistent Firing of Cortical Pyramidal Neurons.

Gibon J, Buckley SM, Unsain N, Kaartinen V, Seguela P, Barker PA

J Neurosci 35(26): 9741-9753, 2015.

Principal neurons in the entorhinal cortex (EC) display persistent firing (PF) during working-memory tasks. Much of the communication between the hippocampus and the neocortex passes through the EC, and the EC also receives some cholinergic input from the medial septum and diagonal band of Broca. In this work the authors investigated the role of pro-brain-derived neurotrophic factor (proBDNF) and the p75 receptor in excitability and PF in the EC. The authors propose the proBDNF/p75 system as a regulator for pyramidal neuron excitability and PF in the EC, preventing runaway activity. Some of the western blot and current-clamp data was generated using Anti-p75 (Cat. #AB-N01; no concentration information provided).

New versions of Orexin-SAP are now in development and production. If you are interested in testing Orexin-SAP and/or orexin receptor antibodies, please contact us.

Repeated Mu-Opioid Exposure Induces a Novel Form of the Hyperalgesic Priming Model for Transition to Chronic Pain.

Araldi D, Ferrari LF, Levine JD

J Neurosci 35(36): 12502-12517, 2015.

Repeated administration of mu-opioid receptor agonists can lead to persistent mechanical hyperalgesia. One current hypothesis is that a form of hyperalgesic priming is triggered by the repeated activation of these receptors. Classic hyperalgesic priming is associated with signaling via protein kinase Cε (PKε), which is mediated by isolectin-B4+ (IB4) nociceptors. In this work the authors eliminated the IB4+ nociceptors with a 3.2 µg intrathecal injection of recombinant IB4-SAP (Cat. #IT-10). The authors found that hyperalgesic priming induced through the use of DAMGO was dependent on protein kinase A activation rather than activation of PKε. This work demonstrates a novel model for hyperalgesic priming transitioning to chronic pain.

Roles of isolectin B4-binding afferents in colorectal mechanical nociception.

La JH, Feng B, Kaji K, Schwartz ES, Gebhart GF

Pain. 2015 Oct 5. [Epub ahead of print]

Primary afferent neurons are often classified as peptidergic or non-peptidergic. One characteristic of the non-peptidergic neurons is that they bind isolectin-B4. In the spinal cord these neurons terminate mainly in inner lamina II. Non-peptidergic neurons in the spinal cord have been found to be involved in various aspects of pain response. In this work the authors examined the role of non-peptidergic neurons in the viscerosensory system. Rats received 1.5 µg of intrathecal recombinant IB4-SAP (Cat. #IT-10) between the L5 and L6 vertebrae. Saporin (Cat. #PR-01) was used as a control. While IHC demonstrated that a majority of viscerosensory L6 colon DRG neurons are IB4+, they do not play a significant role in colorectal mechano-nociception.

Phenotypic and functional characterization of Bst+/- mouse retina.

Riazifar H, Sun G, Wang X, Rupp A, Vemaraju S, Ross-Cisneros FN, Lang RA, Sadun AA, Hattar S, Guan MX, Huang T

Dis Model Mech 8(8): 969-976, 2015.

The belly spot and tail mutant mouse strain was first reported on in 1976. Among other phenotypic changes, it carries ocular mutations including retinal colobomas, reduced retinal ganglion cells (RGCs), and axon misrouting. In order to assess the use of this strain as a murine model for stem cell therapies of retinal degenerative diseases the authors performed a number of characterization experiments including electron microscopy, immunohistochemistry, testing of circadian rhythms, and morphological studies. Some of the immunohistochemistry was done using Anti-Melanopsin (Cat. #AB-N38) at a 1:5000 dilution.

Disrupting spinal noradrenergic activation delays recovery of acute incision induced hypersensitivity and increases spinal glial activation in the rat.

Arora V, Morado-Urbina CE, Aschenbrenner CA, Hayashida KI, Wang F, Martin TJ, Eisenach JC, Peters CM

J Pain. 2015 Nov 3. pii: S1526-5900(15)00924-4 [Epub ahead of print]

A significant percentage of patients who undergo surgery experience prolonged clinically impactful pain, reducing the quality of life and physical function. Disruption of the descending noradrenergic input has been hypothesized to be important to the generation of this type of pain state. Using an acute incision model, the authors administered 5 µg ofAnti-DBH-SAP (Cat. #IT-03) to the L5-L6 interspace of rats. Mouse IgG-SAP (Cat. #IT-18) was used as a control. Lesioned animals demonstrated a

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

significant increase in mechanical hypersensitivity, and a smaller increase in thermal hypersensitivity. This and other results suggest that spinally projecting noradrenergic pathways are necessary for normal recovery from surgical incision, and possibly other types of pain.

Denervation of the Lacrimal Gland Leads to Corneal Hypoalgesia in a Novel Rat Model of Aqueous Dry Eye Disease.

Aicher SA, Hermes SM, Hegarty DM

Invest Ophthalmol Vis Sci 56(11): 6981-6989, 2015.

One result of functional disruption of the tear gland is dry eye disease (DED), which represents a group of disorders rather than a singular one. DED manifests itself in altered responses to noxious corneal stimulation, but many of these patients do not actually have dry eyes or tear gland dysfunction. In order to investigate what circuits are involved in DED the authors created two models, one of which used the ablation of p75 receptor-expressing neurons innervating the extraorbital lacrimal gland. Rats received 2.5 µg of 192-IgG-SAP (Cat. #IT-01) directly into the left extraorbital lacrimal gland. Tear production in the lesioned animals was normal, and responses to noxious cold stimuli were impaired. This accompanied by unchanged fiber density indicates that the nociceptive signaling was affected on a molecular level.

Cholinergic deafferentation of the hippocampus causes non-temporally graded retrograde amnesia in an odor discrimination task.

Koppen JR, Stuebing SL, Sieg ML, Blackwell AA, Blankenship PA, Cheatwood JL, Wallace DG

Behav Brain Res 299: 97-104, 2015.

The memory impairments experienced in neurodegenerative disorders such as Alzheimer's disease have been well documented. One theory attributes these impairments to the loss of cholinergic basal forebrain neurons, a hallmark of Alzheimer's disease. Some patients experience a retrograde amnesia, in which older memories are relatively stable and more recent memories are frequently lost. The temporal relationship of memories to disease onset

has not been definitively established. In this work the authors administered either 150 ng or 200 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum of rats. Using a string-pulling task, a model for temporal learning was established. The results indicate that cholinergic projections originating in the medial septum are involved in long-term memory retrieval, and that loss of these neurons does not create a temporal type of amnesia.

Selective inhibition of dopamine-beta-hydroxylase enhances dopamine release from noradrenergic terminals in the medial prefrontal cortex.

Devoto P, Flore G, Saba P, Frau R, Gessa GL

Brain Behav 5(10): e00393, 2015.

Dopamine-beta-hydroxylase (DBH) is a neuronal enzyme that is a potential target for the treatment of cocaine abuse, alcohol dependence, and eating disorders. Here the authors administered 5 µg of icv Anti-DBH-SAP (Cat. #IT-03) to rats, and assessed the effect of the dopaminergic lesion on levels of extracellular dopamine. Mouse IgG-SAP (Cat. #IT-18) and saporin (Cat. #PR-01) were used as controls. Extracellular levels of dopamine were significantly increased in both lesioned animals and those treated with the DBH inhibitor nepicastat. Clonadine could reverse the nepicastat effect, but not the effect ofAnti-DBH-SAP treatement. The data demonstrate a mechanism for the synergistic effect of cocaine on nepicastat-induced dopamine release.

Treatment Efficacy of NGF Nanoparticles Combining Neural Stem Cell Transplantation on Alzheimer's Disease Model Rats.

Chen Y, Pan C, Xuan A, Xu L, Bao G, Liu F, Fang J, Long D

Med Sci Monit 21: 3608-3615, 2015.

NSC (neural stem cell) transplants into animals have been shown to compensate for the loss of cholinergic cells in the basal forebrain, a hallmark of Alzheimer's disease. One hurdle to overcome is the actuation of NSC differentiation into the specific replacement cells needed. In this work the authors administered 5 µL of icv 192-IgG-SAP (Cat. #IT-01) to rats, followed by a graft of NSCs in the presence of NGF nanoparticles with a polymer coating. Rats receiving both NSCs and NGF nanoparticles showed significantly improved memory and learning functions as compared to control animals.

Basal Forebrain Cholinergic Deficits Reduce Glucose Metabolism and Function of Cholinergic and GABAergic Systems in the Cingulate Cortex.

Jeong da U, Oh JH, Lee JE, Lee J, Cho ZH, Chang JW, Chang WS

Yonsei Med J Jan;57(1):165-72, 2016.

A common result of cholinergic neuron loss in the hippocampus and cortical regions due to Alzheimer's disease is a reduction in glucose metabolism. The authors examine the interaction between the cell loss and metabolic changes. Rats received 5-µg bilateral cortical injections of 192-IgG-SAP (Cat. #IT-01), were subject to water maze testing, and analyzed by 18F-2-fluoro-2-deoxyglucose positron emission tomography. Lesioned animals displayed decreased learning performance and reduced metabolic activity in the cingulate cortex.

Method for Confirming Cytoplasmic Delivery of RNA Aptamers.

Dickey DD, Thomas GS, Dassie JP, Giangrande PH

Methods Mol Biol 1364: 209-217, 2016.

In this work the authors describe a protocol involving combining biotinylated aptamers and Streptavidin-ZAP (Cat. #IT-27) at a 4:1 molar ratio, then testing the conjugates in an *in vitro* cytotoxicity assay. FGF-SAP (Cat. IT-38) was used as a control. This is a method to confirm delivery of a payload by RNA aptamers to the cytoplasm of cells.

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Targeting Talk: Product Q&A

Q: Our QA group wants to know about the safety of the toxin in your conjugates? What precautions should we take in handling saporin products?

A: Saporin is a Type 1 ribosome-inactivating protein (RIP), due to its N-glycosidase activity, from the seeds of *Saponaria officinalis*. It was first described by Fiorenzo Stirpe and his colleagues in 1983 in an article that illustrated the unusual stability of the protein.[1] Among the RIPs are some of the most toxic molecules known, including ricin and abrin (the latter is the poison preferred by the characters in movie *The Blue Lagoon*). These toxins contain a second protein strand that inserts the RIP into a cell, making it able to enzymatically inactivate the ribosomes, shutting down protein synthesis and resulting in cell death, and eventually causing death of the victim.

Saporin does not possess a cell-binding chain[2] and has no method of internalization without a targeting agent to escort it into a cell. It is this fact that also adds to the safety of its use in the lab. Autoclaving or exposure to 0.2 M NaOH is sufficient to decontaminate material that has been in contact with Saporin and its conjugates. The LD_{50} for Saporin in mice is 4-8 mg/kg;[3] this dosage amount would be insignificant in humans. Hundreds of articles in the scientific literature (search "Saporin" in Pub Med) have demonstrated tremendous specificity in targeting neuronal cells with many different Saporin conjugates and by many different scientists.

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Striatal patch compartment lesions reduce cocaine-induced repetitive behaviors

(continued from page 1)

opioid receptor-containing neurons of the patch compartment of striatum, while leaving non-mu opioid receptor-expressing neurons surrounding the patch compartment intact (Fig. 2). Animals were bilaterally-infused in the striatum with Dermorphin-SAP (17 ng/µl[6]) and allowed to recover for eight days. The animals were given daily injections of cocaine (25 mg/kg) or saline for one week, followed by a weeklong drug-free period.[7] Rats were then given a challenge dose of cocaine (25 mg/kg), placed in plexiglass chambers and the locomotor behavior was observed for 2h, followed by sacrifice. Stereotypic behaviors were rated on a scale from 1-10, with 10 representing the highest degree of the response 7-9. Stereotypy scores were generated by averaging the scores from four behavioral dimensions: repetitiveness/flexibility (the number of alternative motor responses emitted), frequency (the number of responses per unit time), duration [the percentage of time spent performing the most dominant response(s)] and the spatial distribution of the motor response.[7,9] Horizontal activity was defined as the number of quadrants the animal crossed on a 4 x 4 grid, using AnyMaze software (Stoelting, Wood Dale, IL, USA) and converted into centimeters.[9] The degree of activation in the patch and matrix compartments was determined using c-Fos immunohistochemistry, which is considered a ubiquitous indicator of neuronal activation, and was coupled with calbindin immunohistochemistry to delineate the patch and matrix compartments.

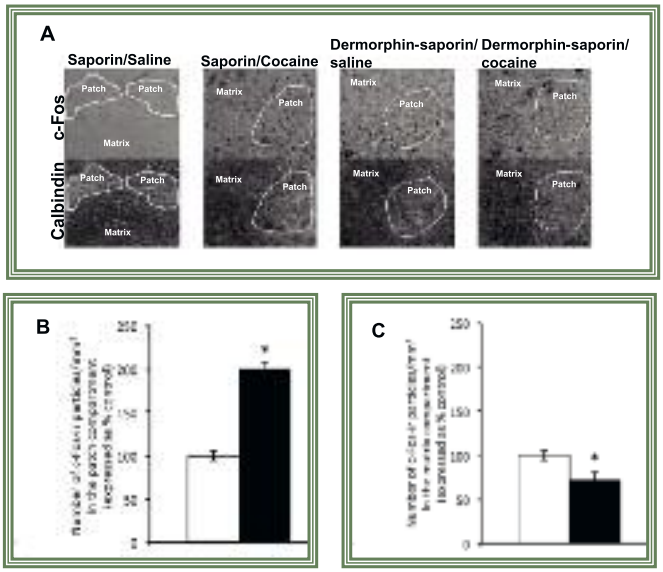

Fig. 2: Effects of Dermorphin-SAP pretreatment on cocaine-induced immunoreactivity in the patch and matrix compartments of striatum. Photomicrographs showing calbindin immunoreactivity and c-Fos immunoreactivity in adjacent sections of the striatum, superimposed over the calbindin-labeled sections (A). Quantitative analysis of c-Fos immunoreactivity in the patch (B) and matrix (C) compartments of rats intrastriatally-infused with Saporin alone or Dermorphin-SAP (17 ng/µl), prior to repeated treatment with cocaine. Data are presented as the percent control of the number of c-Fos immunoreactive particles/mm^2 in the patch and matrix compartments.
**p<0 .05 vs. saporin-pretreated control animals*
+p<0.05 vs. saporin-pretreated cocaine-treated animals.

RESULTS: Dermorphin-SAP pretreatment significantly reduced the intensity of cocaine-induced stereotypy. Stereotypic behavior is also accompanied by a reduction in locomotor activity, and increased locomotor activity was seen in cocaine-treated animals that were pretreated with Dermorphin-SAP (Fig. 1). This pretreatment attenuated cocaine-induced c-Fos expression in the patch compartment, while enhancing cocaine-induced c-Fos expression in the matrix compartment (Fig. 2). The patch compartment is thought to mediate emotional and motivational aspects of behavior while the surrounding matrix compartment is important for processing externally-based sensorimotor information.[7] The current data show that animals that underwent patch compartment lesions prior to repeated cocaine exposure exhibited increased locomotor activity and diminished stereotypy and enhanced relative activation of matrix compartment. When the patch compartment is fully intact, a subsequent dose of cocaine may result in relatively greater activation of the patch-based circuits thereby masking the effects of the activation of matrix-based pathways, leading to greater levels of stereotypy as compared to locomotor activity. On the other hand, when the patch compartment is not fully functional, a subsequent dose of cocaine may tip the balance of activity in favor of the matrix compartment, leading to a greater degree of activation of the direct pathway, and increased locomotor activity, as compared to stereotypy. Together, these data indicate that that the patch compartment of striatum is necessary for repetitive behavior and is an important component of the basal ganglia circuitry that mediates repetitive behaviors, and suggest that when the activity of this region is enhanced as a result of repeated psychostimulant exposure, internally-driven motivational states may overrule ongoing adaptive behaviors, leading to focused stereotypy and perhaps, maladaptive habitual behaviors.

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Apr-May-Jun 2016 Vol. 17, Iss. 2

Reporting the latest news in Molecular Surgery

Cerebral cholinergic lesion reduces operant responses to unpleasant thermal stimuli

by Ronald G. Wiley, M.D., Ph.D., Departments of Neurology and Pharmacology, Vanderbilt University, Nashville, TN and C. J. Vierck, Department of Neuroscience, McKnight Brain Institute, University of Florida College of Medicine, Gainesville, FL, USA

Degeneration of the cholinergic basal forebrain (CBF: medial septum, diagonal band of Broca, nucleus basalis of Meynert/substantia innominata) is a prominent feature of Alzheimer's disease (AD). The CBF supplies cholinergic input to most of the cerebral cortex and hippocampus including somatosensory areas and anterior cingulate cortex that are involved in pain perception and experiencing discomfort, respectively. Clinical literature suggests that patients with AD either feel less pain or express discomfort less than comparable patients without dementia. As a result, AD patients receive less analgesics, but there is concern that AD only impairs communicating discomfort. Rats with extensive CBF lesions show impairment in a wide range of learning tasks and ability to sustain selective arousal/attention, but it is not known what role the CBF plays in central pain processing.

The present study sought to assess the impact of CBF lesions on behavioral responses to nociceptive stimuli in rats. Rats were trained on a thermal escape task where they chose whether to spend time in a dark chamber with the floor temperature at 10° C or 44.5° C (both mildly unpleasant), or move to a connected room temperature chamber with bright lighting. After establishing baseline performance on the operant task, selective CBF lesions were produced by intracerebroventricular injection of 192-saporin (192-IgG-SAP, Cat. #IT-01; Fig. 1). This immunotoxin selectively destroys neurons expressing p75NTR, the low affinity neurotrophin receptor that is uniquely expressed by CBF neurons in the forebrain. The rats were retested repeatedly over 19 weeks. On several occasions, the rats were subjected to sound stress prior to escape testing, and

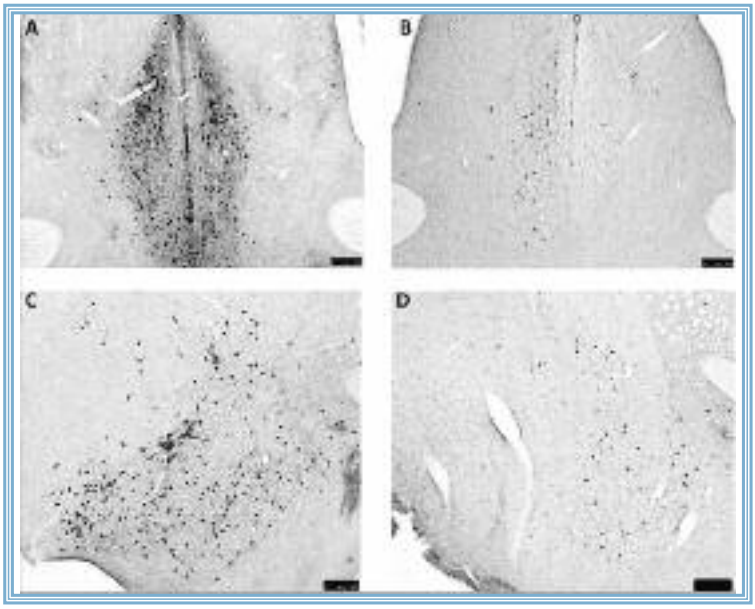


Fig. 1: Representative coronal (frontal) sections from control rats (A, C) and 192-sap-treated rats (B, D) showing loss of CBF cholinergic neurons in the 192-sap rats. (A, B) Show the medial septal nucleus. (C, D) Show the nucleus basalis/substantia innominate regions. Sections were stained for demonstration of choline acetyltransferase using the immunoperoxidase technique (see text). The magnification bars in lower right corners indicate 250 lm (1).

(continued on page 6)

A Sigh is (Not) Just a Sigh . . .

Denise Higgins - President

You must remember this
A kiss is just a kiss,
A sigh is just a sigh.
The fundamental things apply
As time goes by.

Casablanca theme song, that prompted the famous line, "Play it again, Sam." If you haven't seen this classic 1944 Academy Award winner, I highly recommend it!

The fundamental things apply. "The Peptidergic Control Circuit for Sighing," recently published in the prestigious journal *Nature*, has made us rethink our fundamental belief that sighs are only "long, deep breaths expressing sadness, relief or exhaustion." Often prompting someone to say, "What's wrong?" As it turns out, sighs "also occur spontaneously every few minutes to reinflate alveoli, and sighing increases under hypoxia, stress, and certain psychiatric conditions." Thanks to the clever researchers led by Dr. Jack Feldman at UCLA, and their collaborators at Stanford University School of Medicine, we now know a lot more about this process (see Fig. 1 below and Reference Summary on Pg. 4).

The Bötzinger Complex plays an important role in controlling breathing and was named by UCLA Professor Jack Feldman in 1978. Feldman named this area after a bottle of white wine named Botzinger present at his table (perhaps he was allowing it to breathe) during a scientific meeting in Hirschhorn, Germany, that year. Jack Feldman named the most rostral portion of the ventral respiratory group and continues to pave the way for important respiratory research. It's a song we all need to hear, so:

Play it again, . . . Jack!

Deservedly, Jack Feldman's findings went viral. Here are just a few of the links to interviews and commentary:

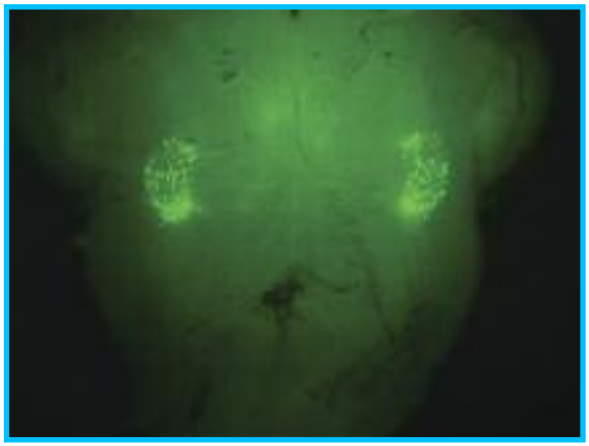


Fig. 1 On each side of the brain stem, a florescent-green marker illuminates the 200 neurons that control the sighing reflex. To determine if Neuromedin B receptor (NMBR)- and Gastric Releasing Peptide receptor (GRPR)-expressing neurons function specifically in sigh control, they were removed using Bombesin-SAP (Cat. #IT-40); Bombesin binds both receptors.

Photo Credit: Stanford/Krasnow lab

NEWS ARTICLES

The Washington Post: "Scientists uncover the brain mechanism that makes you sigh."

The LA Times: "Scientists locate the part of the brain where sighs are made."

NPR: "Sorry, Bogie, a Sigh Is Not Just A Sigh."

WSJ: "Scientists Pinpoint Brain Chemical Linked to the Sigh."

THE GUARDIAN: "A sigh's not just a sigh -- it's a fundamental life-sustaining reflex."

THE ONION: "Sighing a Life-Sustaining Reflex."

RADIO INTERVIEWS BBC: "How the brain's sighing reflex was named."

NZ Radio: "Sigh science."

TELEVISION COVERAGE CBS This Morning: "More than just a sigh."

Bötzinger Complex References Using ATS Products

Gray PA, Janczewski WA, Mellen N, McCrimmon DR, Feldman JL. (2001) Normal breathing requires preBotzinger complex neurokinin-1 receptor-expressing neurons. *Nat Neurosci* 4(9):927-930 (SP-SAP, Cat. #IT-07).

Feldman JL, Mitchell GS, Nattie EE (2003) BREATHING: Rhythmicity, Plasticity, Chemosensitivity. *Annu Rev Neurosci* 26:239-266 (SERT-SAP, Cat. #IT-23; SP-SAP, Cat. #IT-07*).

Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah T, Davis S, Forster HV (2004) Small reduction of neurokinin-1 receptor-expressing neurons in the pre-Botzinger complex area induces abnormal breathing periods in awake goats. J *Appl Physiol* 97(5):1620-1628 (SP-SAP, Cat. #IT-07*).

Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah TR, Davis S, Forster HV (2004) Large lesions in the pre-Botzinger complex area eliminate eupneic respiratory rhythm in awake goats. *J Appl Physiol* 97(5):1629-1636 (SP-SAP, Cat. #IT-07*).

McKay LC, Janczewski WA, Feldman JL (2005) Sleep-disordered breathing after targeted ablation of preBotzinger complex neurons. *Nat Neurosci* 8(9):1142-1144 (SP-SAP, Cat. #IT-07*).

McKay LC, Feldman JL (2008) Unilateral Ablation of preBotzinger Complex Disrupts Breathing During Sleep but not Wakefulness. A*m J Respir Crit Care Med* 178(1):89-95 (SP-SAP, Cat. #IT-07*).

Montandon G, Qin W, Liu H, Ren J, Greer JJ, Horner RL. (2011) PreBotzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression. *J Neurosci* 31(4):1292-1301 (anti-NK1r Cat. #AB-N04**).

Gray PA, Hayes JA, Ling GY, Llona I, Tupal S, Picardo MCD, Ross SE, Hirata T, Corbin JG, Eugenin J, Del Negro CA (2010) Developmental origin of preBotzinger Complex respiratory neurons. *J Neurosci* 30(44):14883-14895 (anti-NK1r Cat. #AB-N04**).

**See alternate product: SSP-SAP (Cat. #IT-11); **See alternate product: NK-1r affinity purified antibody (Cat. #AB-N33AP)*

ATS Toolbox - New Products

vGAT Products

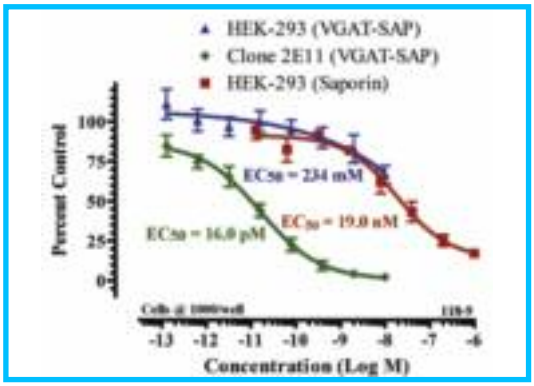


Fig. 1: Cells were plated at 1000 cells/90 µl/well in a 96 well plate and incubated overnight. Anti-vGAT-SAP was added in 10 µl volumes and the plates were incubated for 72 hours. The plates were developed with SRB and read at 564 nm in a plate reader. Data analysis was done by PRISM (GraphPad, San Diego).

ATS is pleased to present a new product line specific for the vesicular GABA transporter (vGAT) protein. vGAT mediates both the accumulaXon of GABA into synaptic vesicles and its release from nerve terminals. vGAT is expressed in nerve endings of GABAergic neurons throughout the CNS. The GABAergic system is crucial to the development and functional maturation of the nervous system, as well as the maintenance of balance between excitation and inhibition required for normal neural circuit function. Dysfunction of GABAergic neurons underlies aspects of clinical symptoms found in several diseases such as epilepsy, Down Syndrome, Fragile X Syndrome, Schizophrenia, and Autism among others.

Anti-vGAT-SAP (Cat. #IT-71) is highly specific for cells that express vGAT. Instead of spending precious time and money producing a vGAT knockout animal, you can use Anti-vGAT-SAP to specifically eliminate cells that express vGAT. Anti-vGAT-SAP also allows you to study the behavioral effects before and after treatment and subsequent elimination of vGAT expressing cells. We have demonstrated the specificity of our Anti-vGAT-SAP by cytotoxicity assays (Fig. 1).

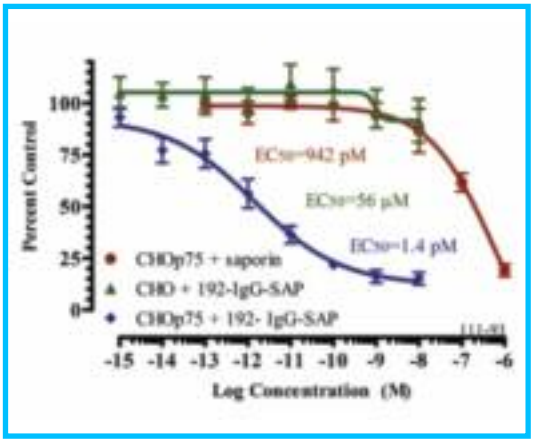
In addition to the targeted toxin, we are offering other vGAT products for your ELISA, flow cytometry and immunoblotting needs. We currently offer rabbit polyclonal vGAT antibody serum (AB-N44), affinity-purified antibody (AB-N44AP), biotinylated antibody (BT-N44), and Alexa-488 conjugated antibody (FL-N44). Keep an eye out for our transfected vGAT cell line, coming soon!

Stable Transfected Cell Lines

Advanced Targeting Systems is proud to announce that we are now making available to the public our proprietary line of stably transfected cell lines, used for years in-house to validate ATS targeted toxins:

192 IgG-SAP was the first ATS product and lesioning use of the product has been published 400+ times over 20+ years, so it is only appropriate that we launch this new product line with CHOp75 cells, now available for purchase. Used for some time now as the preferred cell line for QC verification of each new lot of 192 IgG-SAP, CHOp75 cells are standard CHO (Chinese Hamster Ovary) cells that stably express the rat low affinity nerve growth factor, p75 (p75NTR). Expression of p75NTR in CHOp75 cells has been verified by flow cytometry with 192-IgG-Alexa488 (Cat. #FL-03) and cytotoxicity assay with 192-IgG-SAP (Cat. #IT-01).

ATS is on the verge of releasing several other transfected cell lines, including those expressing VGAT and the orexin-2 receptor. Transfected cell lines are valuable tools that can be used for investigating the function of the transfected molecules. They are also highly useful for screening research and therapeutic reagents that target the transfected gene product. Visit the ATS website for updates and pricing on this new product offering.



CHO and CHOp75 cells were plated at 1000 cells/90 µl/well and incubated overnight. 192-IgG-SAP and saporin were added in 10 µl volumes, and the plates incubated for 72 hours. The cells were fixed with 10% TCA, then stained with 0.4% sulfarhodamine B/1% acetic acid. The plates were read at 564 nm. Data analysis done by Prism software (GraphPad, San Diego).

Recent Publications & References

Reviewed by Matthew Kohls

The peptidergic control circuit for sighing.

Li P, Janczewski WA, Yackle K, Kam K, Pagliardini S, Krasnow MA, Feldman JL.

Nature. 530(7590):293-297, 2016.

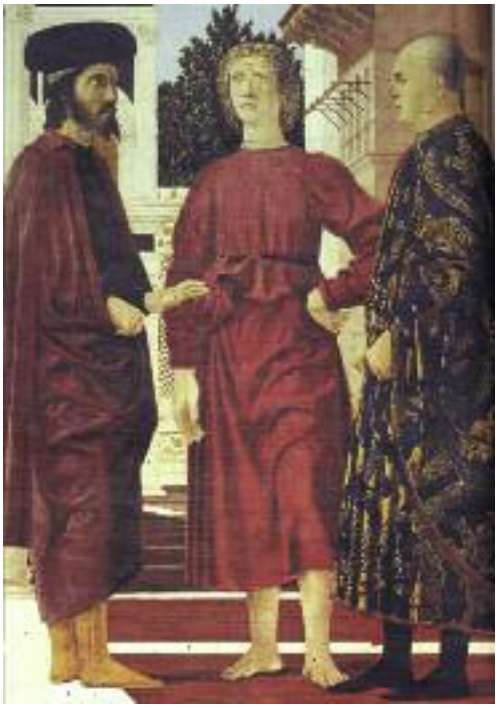
Sighs are often associated with relief or sadness, but rodents sigh spontaneously dozens of times per hour. There are physiological benefits to sighing, including enhancement of gas exchange and preservation of lung integrity. The authors identify a peptidergic sigh control circuit in the retrotrapezoid nucleus/parafacial respiratory group of the mouse brain that projects to the pre-Bötzinger complex. Mice received bilateral 6.2-ng injections of Bombesin-SAP (Cat. #IT-40) into the pre-Bötzinger complex. Blank-SAP (Cat. #IT-21) was used as control. Elimination of the bombesin receptor-expressing neurons or inhibition of neuromedin B receptor-expressing neurons suppressed sighing. Interfering with the activity of both receptors abolished sigh activity while leaving normal breathing intact. The data suggest that overlapping peptidergic pathways are the core of a sigh control circuit. (See article on Pg 2).

Pain sensitivity following loss of cholinergic basal forebrain (CBF) neurons in the rat.

Vierck CJ, Yezierski RP, Wiley RG.

Neuroscience 319:23-34, 2016.

There is a large amount of research on the involvement of cholinergic mechanisms on spinal transmission of pain signals, indicating that cholinergic agonists can attenuate this kind of pain. In contrast, some studies have shown affective reactions to pain are suppressed by cholinergic antagonists. The authors investigated the disagreement between reflexive and affective reactions with a 4-µg 192-IgG-SAP (Cat. #IT-01) injection into the left lateral ventricle of rats. Animals were tested in temperature escape and sound stress models. Lesioned rats displayed decreased escape from thermal stimulation, as well as loss of the normal hyperalgesic effect of sound



stress. Results indicate that the basal forebrain cholinergic system plays a role in central processing of pain. (See Pg 1.)

Ablation of mu opioid receptor-expressing GABA neurons in rostromedial tegmental nucleus increases ethanol consumption and regulates ethanol-related behaviors.

Fu R, Chen X, Zuo W, Li J, Kang S, Zhou LH, Siegel A, Bekker A, Ye JH.

Neuropharmacology 2016.

In this work the authors investigated cellular mechanisms underlying the aversive effects of alcohol that limit its intake. Previous work has linked synaptic inhibition of dopamine neurons in the ventral tegmental area to this aversion. Rats conditioned to ingest ethanol received bilateral injections totaling 3 pmol of Dermorphin-SAP (Cat. #IT-12) into the rostromedial tegemental nucleus (RTMg). Blank-SAP (Cat. #IT-21) was used as a control. Lesioned animals displayed significantly increased preference for, and intake of ethanol, while showing no change in the desire for sucrose. The results indicate that mu opioid expressing GABAergic neurons in the RTMg are highly involved in the regulation of ethanol consumption.

Substituting mouse transcription factor Pou4f2 with a sea urchin orthologue restores retinal ganglion cell development

Mao C-A, Agca C, Mocko-Strand JA, Wang J, Ullrich-Lüter E, Pan P, Wang SW, Arnone MI, Frishman LJ, Klein WH.

Proc Royal Society London B DOI: 10.1098/rspb.2015.2978

Although the regulatory genes for eye development are highly conserved, there is a vision is widely diversified between species. Little is known about how gene networks vary to produce the variety of structures and functions seen across organisms. The authors investigated photoreception in echinoderms, adult sea urchins. Urchins have no structures resembling vertebrate eyes, but recent work has demonstrated the presence of photoreceptor neurons. In this work the authors transferred the urchin version of a transcription factor involved in retinal ganglion cell development into mice lacking the mouse version of that gene. The urchin gene was able to restore function in the mouse, indicating the depth of conservation for eye development gene networks. Some of the immunohistochemical staining was done with anti-melanopsin (Cat. #AB-N39) at a 1:1000 dilution.

Current and Future Issues in the development of spinal agents for the management of pain.

Yaksh TL, Fisher C, Hockman T, Wiese A.

Curr Neuropharmacol 2016 Mar 7 [Epub]

Although conscious pain experience is driven by signals mediated supraspinally, the more high intensity pain generated by strong stimuli, tissue injury, and nerve injury is encoded at the spinal dorsal horn level. The control of pain signals at the spinal dorsal horn level is a tempting target for targeted pain therapy. This review discusses the potential targets for pain therapeutics in the spinal dorsal horn, and some of the spinal agents used to modulate pain transmission through that location. The use of SSP-SAP (Cat. #IT-11) is mentioned as a neurokinin-1 targeted

(continued on page 5)

Recent Publications & References

(continued from page 4)

molecule that can block some pain transmission.

Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation.

Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RLn, Kilgard MP.

Brain Stimul 9(2):174-181, 2016.

Recent work has suggested that vagus nerve stimulation (VNS) can enhance neuroplasticity, and coupled with other training can drive motor cortex reorganization. These findings highlight the potential of VNS to support recovery from neurological disease. Pretrained rats received bilateral injections totaling 3.75 µg of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis (NB). Mouse-IgG-SAP (Cat. #IT-18) was used as control. Control animals displayed a substantial increase in proximal limb representation, lesion of the NB prevented this increase. Motor performance was similar between lesion and control groups, indicating that the difference in representation was not due to altered limb function.

The effect of nucleus basalis magnocellularis deep brain stimulation on memory function in a rat model of dementia.

Lee JE, Jeong da U, Lee J, Chang WS, Chang JW.

BMC Neurol 16(1):6, 2016.

Deep brain stimulation (DBS) is the application of electrical impulses to specific parts of the brain for treating disorders such as Parkinson's disease, chronic pain, and obsessive-compulsive disorder. It has been theorized that stimulation of brain structures associated with memory can enhance cognitive function. The authors lesioned the basal forebrain of rats through bilateral injections totaling 5 µg of 192-IgG-SAP (Cat. #IT-01) into the lateral ventricle. Animals then received DBS to the nucleus basalis magnocellularis and were tested in a Morris water maze task. Results indicate that DBS has beneficial effects on consolidation and retrieval of visuospatial memory.

Neuroplasticity and Repair in Rodent Neurotoxic Models of Spinal Motoneuron Disease.

Gulino R.

Neural Plast 2016:2769735, 2016.

TDP-43 (Transactive response DNA-binding protein) is a highly conserved nuclear protein that binds both DNA and RNA. It has been found in cytoplasmic protein aggregates of patients with conditions such as amyotrophic lateral sclerosis and Alzheimer's disease. In this work the authors examine the role of TDP-43 in spinal cord plasticity. Mice received bilateral 3-µg injections of CTB-SAP (Cat. #IT-14) into the lateral and medial gastrocnemius muscles. The results indicate that motor performance is dependent on expression of synapsin-I, which in turn may be dependent on TDP-43.

Aminopeptidase N (APN/CD13) as a target molecule for scirrhous gastric cancer.

Nohara S, Kato K, Fujiwara D, Sakuragi N, Yanagihara K, Iwanuma Y, Kajiyama Y.

Clin Res Hepatol Gastroenterol 2016 Jan 13 [Epub ahead of print]

Scirrhous gastric cancer has the worst prognosis of gastric carcinoma, and treatment with standard cancer therapies has had minimal success. In this work the authors target CD13 as a marker for scirrhous gastric cancer. A gastric cancer cell line was challenged with a CD13 antibody coupled to Mab-ZAP (Cat. #IT-04) in an in vitro cytotoxicity assay. The anti-CD13 complex was more cytotoxic than an anti-EpCAM-immmunotoxin. These data, combined with flow cytometry analysis and enzyme activity assays, demonstrate the expression of CD13 as a marker for scirrhous gastric cancer.

Perinatal 192 IgG-Saporin as Neuroteratogen.

Petrosini L, De Bartolo P, Cutuli D, Gelfo F.

Curr Top Behav Neurosci 2015 Dec 23. [Epub ahead of print].

The authors discuss the effects of perinatal administration of 192-IgG-SAP (Cat. #IT-01) and areas of research that have been investigated through the use of these lesions. The chapter covers a description of 192-IgG-SAP, lesioning methods, and outlines the short- and long-term biochemical, structural, behavioral, and cognitive effects of 192-IgG-SAP administration.

Neuroteratology and Animal Modeling of Brain Disorders.

Archer T, Kostrzewa RM.

Curr Top Behav Neurosci 2016 Feb 9. [Epub ahead of print].

This work covers development and use of the neurotoxins that are most commonly used as neuroteratologic agents - producing permanent, lifelong destruction of specific groups of neurons. Saporin conjugates are discussed, in terms of animal models of human neurodegenerative, neuropsychiatric, and neurological conditions.

Locus Coeruleus and Tuberomammillary Nuclei Ablations Attenuate Hypocretin/Orexin Antagonist-Mediated REM Sleep.

Schwartz MD, Nguyen AT, Warrier DR, Palmerston JB, Thomas AM, Morairty SR, Neylan TC, Kilduff TS.

eNeuro. 2016 Mar 21;3(2). pii: ENEURO.0018-16.2016.

To examine the mechanism by which the Orexin 1r/Orexin 2r antagonist almorexant decreases wakefulness and increases NREM and REM sleep the authors utilized Anti-DBH-SAP (Cat. #IT-03) and Orexin-SAP (Cat. #BETA-031). Rats received 3-µg injections of Anti-DBH-SAP into the LC, or bilateral 57-80 ng injections of Orexin-SAP into the TMN. Both conjugates attenuated the increased REM sleep seen upon administration of almorexant without altering almorexant-induced changes in NREM sleep.

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Cerebral cholinergic lesion reduces operant responses to unpleasant thermal stimuli

(continued from page 1)

the rats were also tested on the thermal plate (hot/cold plate) without an escape option to measure lick guard (reflex) responses with and without preceding stress.

Compared to controls, 192-IgG-SAP injected rats showed highly significant ($p<0.001$) loss of neurons from all subdivisions of the CBF based on post mortem brain sections stained for choline acetyltransferase. The CBF-lesioned rats escaped less than controls after 192-IgG-SAP injection (i.e. less motivated to get away from the aversively hot or cold stimuli). Reflex lick/guard responses, which are mediated at the spinal level, were not affected. The usual hyperalgesic effect of stress on the operant thermal escape task was absent in the CBF-lesioned rats. These results indicate a role for the CBF in modulating central pain processing. The loss of stress effect on thermal escape responses is consistent with loss of the arousal/attention function(s) of the CBF. These results also demonstrate the usefulness of 192-IgG-SAP for studies of the role of central (cerebral) cholinergic mechanisms in pain processing and are consistent with the idea that AD patients experience less discomfort for a given painful condition.

Reference

1. Pain sensitivity following loss of cholinergic basal forebrain (CBF) neurons in the rat. Vierck CJ, Yezierski RP, Wiley RG. *Neuroscience* 319:23-34, 2016.

Where to See Us:

Am. Assoc. of Cancer Research
New Orleans, April 17-20

Am. Assoc. of Immunology
Seattle, May 13-17

American Pain Society
Austin, May 11-14

Targeting Membrane Proteins
Boston, June 16-17

For years ATS fans have been Gangsta and Kermit fans, and now it is time to show your love by adding a caption to Kermit's picture! Each quarter we will post a picture of Kermit up to his usual hijinks and ask our readership to offer their most creative captions.

Email your captions to:

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Subject line: Kermit Caption.

Winning Caption Goes Here!!!

The winning caption will be posted in the next newsletter and the author will receive a
$350 product credit
runner-up will receive
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Talking about Targeting

Saporin Safety

Over the years, ATS has frequently been confronted with questions about Saporin's safety for use in the lab as well as when used clinically. Residual awareness of alternate Ribosome-Inactivating Proteins (RIPs) and 'toxins' such as Ricin have poisoned the belief that Saporin is safe. As a Type I RIP, Saporin has no binding chain and consequently no means of entering the physiological space necessary for the protein to act as a toxin. As such, in response to specific concerns about safety from casual users, reviewers of work with saporin, and potential 3rd-party manufacturers of saporin and SP-SAP, the following is a review of safety in handling and potential toxicity within the human body for systemic events not related to the predicted therapeutic application of SP-SAP.

The acute LD50 for saporin in mice (25 g) is 6.8 mg/kg;[1] that would translate in humans (75 kg) to 510 mg! A concentration of about 100 nM is the threshold to see even a vague hint of saporin toxicity. In human blood, that would correspond to 24 mg injected systemically into a person. The fermentation process to produce recombinant saporin has a titer of 2 mg/L meaning that the production broth itself contains no more than 67 nM concentration of saporin. Furthermore, the final protein concentrations from production batches of recombinant Saporin used in our drug are 4 mg/ml, meaning 6 mL of final material would need to accidentally end up in a human before the 'hint of toxicity' threshold would potentially be met.

The toxicology studies of SP-SAP contained within ATS's IND prior to the current human Phase I clinical trial evaluated effects related to the intended method of administration, intrathecal local injection. SP-SAP is not expected to ever be a self-administered therapy, so the effects of gross off-target events, such as accidental auto-injection, swallowing, spillage, or immersion were not considered.

The table below[2] highlights antibody-saporin conjugates approved by the FDA for Phase I/II clinical trials in humans. The therapeutics listed below were administered intravenously and imply what the FDA accepted as non-toxic levels of saporin-based conjugates in these studies.

Table 2. Clinical trials in patients with SAP containing ITs.

Antibody	Antigen	Disease	Total Dose	PR	SD/MR	No. patients	Ref.
$F(ab')_2$ BsAb	CD22	NHL	5 mg	-	1	1	[32]
4KB128 + HD6	CD22	B-cell lymphoma	5–20 mg	-	4	4	[33]
$F(ab')_2$ BsAb	CD22	NHL	5–20 mg	-	5	5	[34]
Ber-H2	CD30	HD	0.8 mg/kg	3 (75%)	1 (25%)	4	[35]
Ber-H2	CD30	HD	0.2–0.8 mg/kg	5 (40%)	3 (25%)	12	[36]

HD: Hodgkin's disease; NHL: non-Hodgkin's lymphoma; PR: partial remission; SD/MR: stable disease/minor response.

Looking more closely at the study by French *et al.*,[3] several milligrams of antibody conjugate were repeatedly injected into human patients under a FDA regulated clinical trial and peak serum levels tested, demonstrating rapid clearing of saporin from the system.

As a company that specializes in Saporin, our two-plus decades of experience working with the protein in research, preclinical, and clinical environments has taught us that with minimal standard laboratory precautions users are not at any real risk of toxic effects. Even our CSO, after 30+ years of working with Saporin exhibits undetectable levels of Saporin antibodies in his blood!

References

1. Thorpe, P.E.; Brown, A.N.; Bremner, J.A., Jr.; Foxwell, B.M.; Stirpe, F. An immunotoxin composed of monoclonal anti-Thy 1.1 antibody and a ribosome-inactivating protein from Saponaria officinalis: Potent antitumor effects *in vitro* and *in vivo. J. Natl. Cancer Inst.* 1985, 75, 151–159.
2. Polito L, Bortolotti M, Pedrazzi M, Bolognesi A. Immunotoxins and other conjugates containing saporin-s6 for cancer therapy. *Toxins* (Basel). 2011 Jun;3(6):697-720.
3. French, R.R.; Bell, A.J.; Hamblin, T.J.; Tutt, A.L.; Glennie, M.J. Response of B-cell lymphoma to a combination of bispecific antibodies and SAP. *Leuk. Res*. 1996, 20, 607–617.

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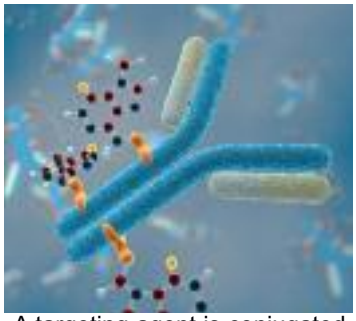
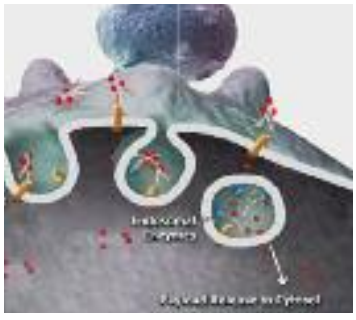


A targeting agent is conjugated to a payload. here the conjugate is an antibody linked to a small molecule.

The targeted conjugate binds only the cells that are specific to the antibody causing the receptor to internalize.



The internalized conjugate is digested by enzymes releasing the payload into the cytosol. The payload is then free to alter the cell.

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Kit UPgrade
Purchase a 250-mcg size Targeted Conjugate and receive a FREE upgrade to a KIT that includes all the specific controls necessary to validate your assay.
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Brian Russell

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CHOp75 cells stably express the rat low affinity nerve growth factor, p75 (p75NTR). Order this cell line and receive 1 mg of positive control antibody FREE until June 30, 2016.

Matt Kohls

Save on ZAP products
Let ATS help you screen your antibodies with our secondary conjugate product line. Choose your size: 100-mcg for 75-mcg price; 400-mcg for 250-mcg price; 1000-mcg for 500-mcg price.
Use Discount Code ZAPQ216.
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Leonardo Ancheta

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Patrick Shramm

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Chelsea Friedman

Jul-Aug-Sep 2016 Vol. 17, Iss. 3

Reporting the latest news in Molecular Surgery

Targeted depletion of hematopoietic stem cells promises safer transplantation

By Rahul Palchaudhuri, Ph.D., Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, USA

Hematopoietic stem cell transplantation (HSCT) has been clinically used for 58 years and offers life-saving therapies for a variety of malignant and non-malignant blood disorders. Currently 50,000 transplants are performed globally per year with 90% of these for the treatment of malignancies.

Prior to receiving a transplant, a patient must be "conditioned" which serves to destroy resident hematopoietic stem cells in the marrow, in order to create niche vacancies for successful donor stem cell engraftment. Unfortunately, current conditioning strategies are non-targeted and genotoxic as they use DNA-damaging whole body irradiation and chemotherapy. As expected, these crude methods induce severe short-term and long-term conditioning-related toxicities that ultimately limit the application of hematopoietic stem cell transplantation, particularly in non-malignant conditions (e.g. sickle cell anemia, thalassemia, immunodeficiencies and autoimmune conditions).

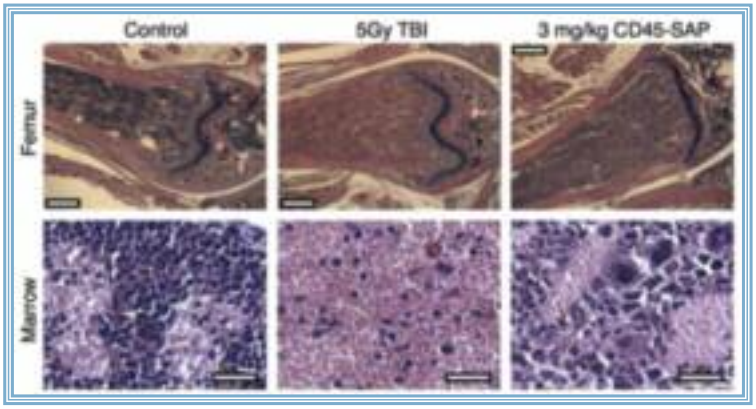

Fig. 1: Hematoxylin and eosin staining of femur marrow sections of non-treated control, 3 mg/kg CD45-SAP or 5Gy TBI conditioned C57BL/6 mice 2 days post-conditioning. Representative images from independent experiments (n = 2 mice/group) are shown. Scale bars in top and bottom images represent 500 µm and 20 µm, respectively.[1]

While antibodies are potentially an appealing alternative to current conditioning methods, previous antibody-based strategies relying on naked antibodies have been met with limited success in immunocompetent animals. We therefore explored antibody-based immunotoxins created using the ribosome-inactivating protein, saporin, as a means of depleting hematopoietic stem cells in immunocompetent mice. By combining various biotinylated monoclonal antibodies with streptavidin attached to saporin (Streptavidin-ZAP, Cat. #IT-27), we created immunotoxins and screened their ability to achieve stem cell depletion *in vivo*. From our screen, we identified CD45-SAP as a potent stem cell-depleting agent capable of depleting >98% of hematopoietic stem cells following a single-dose administration. Using CD45-SAP we demonstrated successful donor stem cell engraftment with long-term donor chimerism levels greater than 90%.

(continued on page 6)

What's ZAP?

Denise Higgins - President

Fig. 1 Saporin is obtained from the seeds of the Soapwort plant (*Saponaria officinalis*). Saporin is a plant enzyme with N-glycosidase activity that depurinates a specific nucleotide in the ribosomal RNA 28S, thus irreversibly blocking protein synthesis. It belongs to the well-characterized family of ribosome-inactivating proteins (RIPs).

Wuzzup? No. What's ZAP? Some of our products have SAP in the name, like 192-IgG-SAP (Cat. #IT-01). Some of our products have ZAP in the name, like Hum-ZAP (Cat. #IT-22).

First, what's the same about ZAP and SAP? They both mean Saporin. The payload that Advanced Targeting Systems made famous to specifically eliminate targeted cells. For those of you new to this techology, Saporin is a ribosome-inactivating protein (Fig. 1).

Now, what's different about ZAP and SAP? The difference is in what the conjugate can do. A SAP conjugate has two components: 1) Saporin and 2) A targeting agent that is recognized on the cell surface and internalized. A ZAP conjugate has two components: 1) Saporin and 2) A non-specific agent that is NOT recognized on the cell surface and internalized (e.g. a secondary antibody, nonspecific peptide, or streptavidin).

If you want to make a saporin conjugate with your cell surface targeting agent, check out our ZAP products: ZAP Internalization Kits (Z-Kits) and Streptavidin products (see Page 7 for more information).

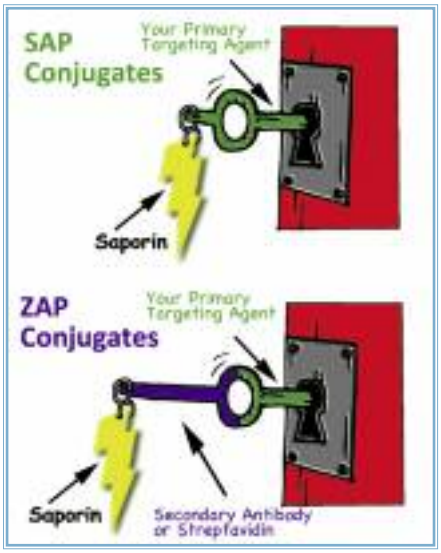

Fig. 2. The difference between SAP conjugates and ZAP conjugates. SAP conjugates target and eliminate specific cells. ZAP conjugates need a primary targeting agent to be internalized.

Presidential Promotions for this Quarter

Summer special on this year's HOT antibodies
Buy one, Get one free (equal or lower-priced item) — mix or match.
Enter coupon code HOT1 at checkout. Offer expires July 31, 2016.
Choose from these top 20 best sellers:

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Angiotensin II receptor (AT-1r) Rabbit Polyclonal, affinity-purified [AB-N27AP]
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Corticotropin Releasing Hormone Rabbit Polyclonal [AB-02]
Dopamine Rabbit Polyclonal, Conjugated [AB-T07]
Dopamine Transporter Rat Monoclonal (DAT-NT) [AB-N18]
Fibroblast Growth Factor Rabbit Polyclonal, mammalian [AB-07]
GABA Rabbit Polyclonal, Conjugated [AB-T10]
Histamine Rabbit Polyclonal, Conjugated [AB-T024]
Melatonin Polyclonal, Conjugated [AB-T177]
Metabotrophic Glutamate Receptor 2 (mGluR2) Mouse Monoclonal [AB-N32]
NGFr (mu p75) Rabbit Polyclonal, affinity-purified [AB-N01AP]
NGFr (mu p75) Rabbit Polyclonal [AB-N01]
NGFr (ME20.4, p75) Mouse Monoclonal [AB-N07]
NO-L-Cysteine Mouse Monoclonal, Conjugated [AB-T125]
Quinolinic Acid Rabbit Polyclonal, Conjugated [AB-T095]
RFP Mouse Monoclonal [AB-332]
Trans-hydroxyproline Rabbit Polyclonal, Conjugated [AB-T044]
Tri-methyl Lysine Rabbit Polyclonal [AB-265]
vGAT Rabbit Polyclonal, affinity-purified [AB-N44AP]

ATS Toolbox - Orexin Products

Orexin-B-SAP (Cat. #IT-20)

Fig. 1: Targeted toxins offer the ability to develop "knockouts" through cell surface-based targeting that has several advantages over the gene-based approach. The "knockout" has a slight but important difference: instead of knocking out a particular protein from a set of cells (or even the whole animal), you eliminate a particular cell type. And this happens at your convenience: you inject the animal, put it back in its cage and then usually four days later, behavioral differences begin to show. These usually become permanent after a week or so.

ATS is pleased to re-release a product and kit specific for the Orexin 2 receptor (OX2R). The Orexin 1 and Orexin 2 receptors are found in the perifornical area/latero-posterior hypothalamus, and projections from this area cover much of the brain. These receptors have been implicated in various neurophysiological and neuropsychological disorders such as narcolepsy, insomnia, drug addiction, anxiety, and migraine headaches. The Orexin-B-SAP conjugate consists of the rat/mouse-specific orexin-B peptide conjugated to Saporin. Orexin-B binds to OX2R with approximately 5X greater affinity than to OX1R (Fig. 2).

Orexin-B-SAP (Cat. #IT-20) is highly specific for cells that express OX2R. Instead of spending precious time and money producing an OX2R knockout animal, you can use Orexin-B-SAP to specifically eliminate cells that express OX2R (Fig. 1). Orexin-B-SAP also allows you to study the behavioral effects before and after treatment and subsequent elimination of OX2R expressing cells. The specificity and efficacy of Orexin-B-SAP has recently been reported in the published work of Schwartz *et al.*[1]

1. Schwartz MD, Nguyen AT, Warrier DR, Palmerston JB, Thomas AM, Morairty SR, Neylan TC, Kilduff TS. (2016) Locus Coeruleus and Tuberomammillary Nuclei Ablations Attenuate Hypocretin/Orexin Antagonist-Mediated REM Sleep. eNeuro 3(2) PMID: 27022631.

Orexin Receptor Antibody Conjugates (Cat. #BETA-007 and BETA-008)

Anti-OX1R-SAP (Cat. #BETA-007) eliminates mouse, rat, and guinea pig cells expressing the orexin 1 receptor. All other cells are left untouched.

Anti-OX2R-SAP (Cat. #BETA-008) eliminates rat cells expressing the orexin 2 receptor (OX2R). All other cells are left untouched.

These two antibodies are being offered as part of our Beta Testing program. They have not been characterized or reported in scientific literature but they are being offered at no charge for the product (S&H extra). The researcher who first publishes data* will receive a $500 credit for use on ATS products.

*Data submitted will be reviewed by the scientific team at ATS. If data are sufficient to prove specific activity of Beta material in either *in vivo* or *in vitro* conditions, the Beta Tester will be informed and product credit will be awarded to the first Beta Tester to publish.

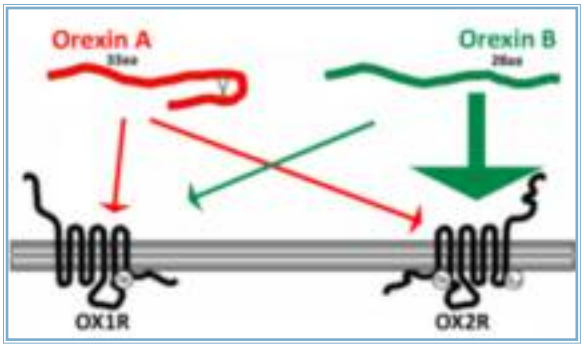


Fig. 2. The orexin receptor (also referred to as the hypocretin receptor) is a G-protein-coupled receptor that binds the neuropeptide orexin. There are two variants, OX1R and OX2R. There are two orexin peptides: Orexin A and Orexin B. These peptides bind to both receptors, however, Orexin B shows a >5-fold selectivity for OX2R . Orexin A is equipotent at both receptors.

Recent Publications & References

Reviewed by Brian Russell

Involvement of nigral oxytocin in locomotor activity: A behavioral, immunohistochemical and lesion study in male rats.

Angioni L, Cocco C, Ferri GL, Argiolas A, Melis MR, Sanna F.

Horm Behav 83:23-38, 2016.

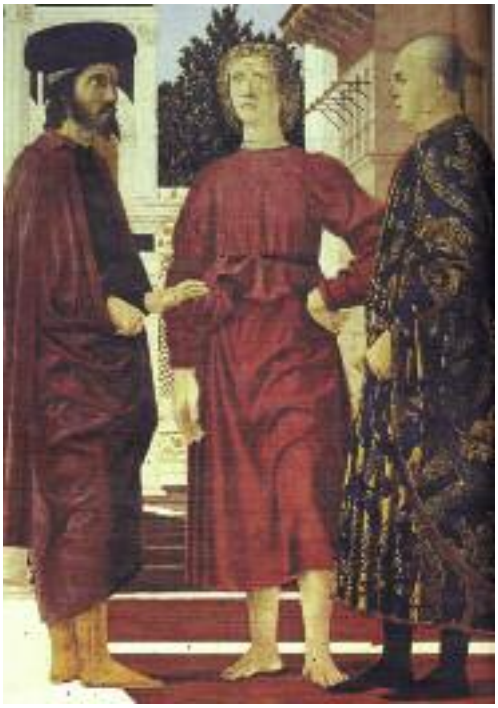
Oxytocin is well known for its hormonal role in lactation and parturition, but also exerts widespread actions in central nervous system. Previous experiments revealed the existence of a correlation between the changes in locomotor activity found in Oxytocin-SAP-treated rats and the extent of the changes in nigral TH and vesicular glutamate transporters immunoreactivity, provide support for a modulatory role of oxytocin on locomotor activity at the level of the substantia nigra. The day after a prior assessment of spontaneous locomotor activity, rats were randomly injected bilaterally with 0.3 µL of Oxytocin-SAP (Cat. #IT-46, 60 ng/µL/site), or with the same amount of Blank-SAP (Cat. #IT-21, 60 ng/µL/site) or with vehicle (0.3 µL/site of PBS, pH 7.4). Whether oxytocin may be considered as a target for controlling motor disturbances, as those occurring in Parkinson's disease and/or in other motor disturbances related to basal ganglia dysfunctions, remains to be evaluated.

Gi-Protein Coupled 5-HT1B/D Receptor Agonist Sumatriptan Induces Type I Hyperalgesic Priming.

Araldi D, Ferrari LF, Levine JD.

Pain 2016.

The present study explored the possibility that, like MOR and A1-adenosine receptor agonists, triptans would also induce type II hyperalgesic priming. In addition, they explored the 5-HT receptor subtypes at which triptans act (5-HT1B, 5-HT1D and 5-HT7) to induce priming. They report that while sumatriptan, a prototypical 5-HT1B/D receptor agonist induces hyperalgesic priming, this priming meets the criteria for type I rather than type II priming. Isolectin B4 (IB4)-saporin (Cat. #IT-10), was diluted in saline, and a dose of 3.2 µg, in a volume of 20 µL was administered intrathecally to rats. The neurotoxin [Sar9,Met(O2) 11]-substance



P-saporin (SSP-Saporin, Cat. #IT-11) was diluted in saline, and a dose of 100 ng, in a volume of 20 µL was administered intrathecally. In a model of pain chronification, sumatriptan induces both mechanical hyperalgesia at the site of injection and type I hyperalgesic priming, in nociceptors innervating the cutaneous injection site.

Retinal Waves Modulate an Intraretinal Circuit of Intrinsically Photosensitive Retinal Ganglion Cells.

Arroyo DA, Kirkby LA, Feller MB.

J Neurosci 36(26):6892-6905, 2016.

The researchers explore the neural circuits underlying the ipRGC driven light responses of the developing retina and the mechanisms by which retinal waves regulate these circuits. They demonstrate that, even in the presence of cholinergic waves, ipRGC gap junction microcircuits propagate light-driven signals, thus strongly contributing to the overall light response of the developing retina. Following fixation, retinas were washed in PBS and remounted onto a new piece of filter paper. They were incubated in blocking buffer and then in primary immunoreaction solution, 1:2500 rabbit anti-melanopsin (Cat. #AB-N38). Results show that, during development, ipRGCs form extensive gap junction microcircuits that shape the early retinal light response. Retinal waves exert a far-reaching, neuromodulatory influence on these circuits via dopaminergic modulation of gap junctions, thus potentially impacting the processing of early visual input.

Brainstem opioidergic system is involved in early response to experimental SAH.

Cetas JS, McFarlane R, Kronfeld K, Smitasin P, Liu JJ, Raskin JS.

Transl Stroke Res 6(2):140-147, 2015.

Subarachnoid hemorrhage (SAH) is a particular type of stroke that has high morbidity and mortality. The damage due to SAH is manifested in numerous ways, including global hypoperfusion, neuronal death, infarcts, microhemorrhages, and cortical spreading depression - as well as other acute autonomic dysfunctions. In this work the authors investigated how some autonomic and sensorimotor systems in the rostral ventromedial medulla (RVM) are involved in the maintenance of cerebral blood flow in a SAH model. Rats received 1 pmol total of dermorphin-SAP (Cat. #IT-12) in bilateral injections to the RVM. Blank-SAP (Cat. #IT-21) was used as a control. The results indicate that µ-opioid receptor-expressing cells in the RVM are important in reducing mortality rates after SAH.

Role of the RVM in Descending Pain Regulation Originating from the Cerebrospinal Fluid-Contacting Nucleus.

Fei Y, Wang X, Chen S, Zhou Q, Zhang C, Li Y, Sun L, Zhang L.

Neurochem Res 41(7):1651-1661, 2016.

The researchers investigated whether the CSF-contacting nucleus contributed to descending pain modulation in normal and neuropathic rats, and detected the 5-HT expression changes in both RVM and spinal dorsal cord. They also detected the possible anatomical and function correlation between the CSF-contacting nucleus

(continued on page 5)

Recent Publications & References

(continued from page 4)

and the RVM. Targeted ablation of the CSF-contacting nucleus was performed using CTB-SAP (Cat. #IT-14; 500 ng/3 µl), which was administered i.c.v. to the normal rats and rats 7 days before the CCI procedure. Based on the findings of the present study, they believe that the CSF-contacting nucleus may act as a component of descending pain regulation system. RVM, which acts as an important brain nucleus, is involved in the relay of nociceptive information between the CSF-contacting nucleus and spinal cord. Moreover, RVM 5-HT system plays a critical role in descending pain inhibition originating from the CSF-contacting nucleus.

Functional characterization of a mouse model for central post-stroke pain.

Gritsch S, Bali KK, Kuner R, Vardeh D.
Molecular Pain 12:1744806916629049, 2016.

While clinical evidence has pointed toward central pain pathway dysfunction in central post-stroke pain (CPSP), the underlying mechanisms have not been defined. In this work the authors created a mouse model of CPSP through lesions of the thalamic ventral posterolateral nucleus. In order to examine the role of neurokinin-1 receptor-expressing (NK1R) neurons in lamina I/III of the spinal cord in the development and maintenance of CPSP the authors administered 1 µmol intrathecal injections of SSP-SAP (Cat. #IT-11). Saporin (Cat. #PR-01) was used as a control. While the NK1R+ neurons in the spinal cord were not involved in establishing CPSP, the data indicate that sensory changes in the mice are comparable to those observed in human patients with CPSP.

Possible Involvement of the Rat Hypothalamo-Neurohypophysial/-Spinal Oxytocinergic Pathways in Acute Nociceptive Responses.

Matsuura T, Kawasaki M, Hashimoto H, Yoshimura M, Motojima Y, Saito R, Ueno H, Maruyama T, Ishikura T, Sabanai K, Mori T, Ohnishi H, Onaka T, Sakai A, Ueta Y.
J Neuroendocrinol 28(6)2016.

It has been suggested that the amplification of GABAergic neurons in the inhibitory system induces the selective inhibition by Oxytocin (OXT) of excitability in the spinal cord, and the pain transmitted from the periphery to the dorsal horn of the spinal cord by this action may be attenuated at the spinal cord level. Rats were injected IT with Oxytocin-SAP (Cat. #IT-46) dissolved in saline (0.06 µg/µl), Blank-SAP (Cat. #IT-21) dissolved in saline (0.06 µg/µl), or saline. Formalin-induced acute nociception activated OXT-containing cells in both the magnocellular and parvocellular divisions of hypothalamus, and that the parvocellular division remains activated longer than the magnocellular division. Acute nociception-induced activation of the hypothalamo-neurohypophysial system caused elevation of plasma OXT levels. In addition, the OXTergic spinal pathway may be involved in pain modulation via OXTRs in the spinal cord.

Effects of central administration of oxytocin-saporin cytotoxin on chronic inflammation and feeding/drinking behaviors in adjuvant arthritic rats.

Matsuura T, Kawasaki M, Hashimoto H, Yoshimura M, Motojima Y, Saito R, Ueno H, Maruyama T, Sabanai K, Mori T, Ohnishi H, Sakai A, Ueta Y.
Neurosci Lett 621:104-110, 2016.

In the present study, Oxytocin-SAP, which chemically disrupts oxytocin (OXT signaling was administered centrally and an OXT receptor (OXTR) antagonist administered peripherally to determine whether central and peripheral OXT is involved in chronic inflammation and feeding/drinking behavior in adjuvant arthritis (AA) rats. Rats were injected i.t. with Oxytocin-SAP (Cat. #IT-46) or Blank-SAP (Cat. #IT-21) dissolved in saline (0.06 µg/µl). The results demonstrated that the arthritis index values were significantly enhanced and suppression of food intake was transiently attenuated in Oxytocin-SAP treated rats when AA developed, The arthritis index and food intake did not significantly change in the OXTR antagonist i.p.-injected rats. These results suggest that central oxytocinergic pathways may be involved in anti-inflammation at the spinal level and suppression of feeding behavior at the forebrain-brainstem level in AA rats.

Ablation of KNDy neurons results in hypogonadotropic hypogonadism and amplifies the steroid-induced LH surge in female rats.

Mittelman-Smith MA, Krajewski-Hall SJ, McMullen NT, Rance NE.
Endocrinology 157(5):2015-2027, 2016.

KNDy neurons are a subpopulation of neurons in the infundibular nucleus that coexpress estrogen receptor α, kisspeptin, and neurokinin B (NKB) mRNA. Previous work indicated that altered signaling from KNDy neurons may play a role in the low levels of circulating sex steroids found in hypogonadotropic hypogonadism. Rats received bilateral 10-ng injections of NK3-SAP (Cat. #IT-63) dorsal to the arcuate nucleus. Blank-SAP (Cat. #IT-21) was used as control. In animals with intact ovaries the NK3-SAP lesion resulted in hypogonadotropic hypogonadism. In contrast, the LH surge in lesioned ovariectomized rats was 3-fold higher, demonstrating that KNDy neurons are integral for the control of serum LH levels, estrous cyclicity, and may also have some control over the magnitude of the LH surge.

Locus coeruleus noradrenergic innervation of the amygdala facilitates alerting-induced constriction of the rat tail artery.

Mohammed M, Kulasekara K, Ootsuka Y, Blessing WW.
Am J Physiol Regul Integr Comp Physiol 310(11):R1109-19, 2016.

The researchers tested the hypothesis that release of noradrenaline within the amygdala is important for the occurrence of SCVARS (sympathetic cutaneous vasoconstrictor alerting responses). A long-shanked 5-µl glass micropipette calibrated in 100-nl steps, was filled with vehicle or Anti-DBH-SAP

(continued on page 6)

Cerebral cholinergic lesion reduces operant responses to unpleasant thermal stimuli

(continued from page 1)

As only hematopoietic cells express the CD45 receptor, CD45-SAP offered significant advantages with regard to toxicity compared to conventional whole body irradiation. Notably, CD45-SAP enabled quicker recovery of bone marrow cellularity, avoided damage to marrow blood vessels and other non-target marrow cells, and preserved the thymic function. Combined together, these features resulted in notably quicker recovery of B- and T-cells following CD45-SAP versus irradiation. In addition, CD45-SAP avoided neutropenia, preserving innate immunity and the ability to resist fungal infection.

To demonstrate correction of a clinically relevant disease, we employed CD45-SAP in a mouse model of sickle cell anemia and demonstrated our method achieved >90% donor cell chimerism, all mice in three groups (18/18), resulting in complete disease correction (red blood cell counts, hemoglobin levels, hematocrit levels and reticulocyte frequencies were returned to normal). Fig. 2 show hematopoietic stem cell (HSC) depletion. If these pre-clinical results can be successfully translated to the clinic, it would greatly reduce conditioning-related toxicities and expand the use of hematopoietic stem cell transplantation.

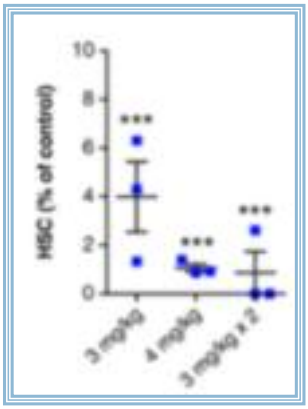


Fig. 2: HSC depletion in sickle disease mice 8 days post-administration of various doses of CD45-SAP. Data represent the mean ± SEM (n = 3 mice/dose, assayed individually).

Reference

1. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol*. 2016 Jun 6. doi: 10.1038/nbt.3584. [Epub ahead of print] PMID: 27272386.

Recent Publications & References

(continued from page 5)

(Cat. #IT-03). Anti-DBH-SAP (5 µg in 250 nl) or vehicle was injected into the amygdala during ~1 min, and the pipette was left in place for an additional The locus coeruleus has been implicated in many aspects of emotional arousal, so that functional inhibition of the extensive locus coeruleus-derived noradrenergic innervation of centers known to be important in emotional arousal, including the amygdala, is likely to contribute to the therapeutic actions of clonidine-like agents. The locus coeruleus also has major reciprocal connections with the orexin-synthesizing neurons in the hypothalamus, and rats with genetically lesioned orexin receptor neurons (alternatively, oen could lesion with Orexin-SAP, Cat. #IT-20) have reduced emotional arousal as reflected in reduced SCVAR responses to alerting stimuli.

Did we miss you?
If so, please send a PDF of your publication to admin@atsbio.com so we can feature your research in our next issue!

Dynamics of spinal microglia repopulation following an acute depletion.

Yao Y, Echeverry S, Shi XQ, Yang M, Yang QZ, Wang GY, Chambon J, Wu YC, Fu KY, De Koninck Y, Zhang J.
Sci Rep 6:22839, 2016.

This study confirms that similar to microglia in the brain, spinal microglia can repopulate rapidly following elimination, which is driven essentially by a self-renewal process. To deplete microglia in spinal cords, Mac-1-SAP (Cat. #IT-06) was injected i.t. (7 µl, 1.6 µg/µl) at the level of L4-L5 in mouse. The results support the concept that microglia repopulation, whether in the brain or in the spinal cord, is the consequence of onsite resident microglia proliferation. Newly generated microglia are fully functional and are able to respond to peripheral nerve injury and contribute to the development of neuropathic pain.

Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin.

Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT.
Nat Biotechnol 2016.

see cover article

Talking about Targeting

Streptavidin-ZAP Applications

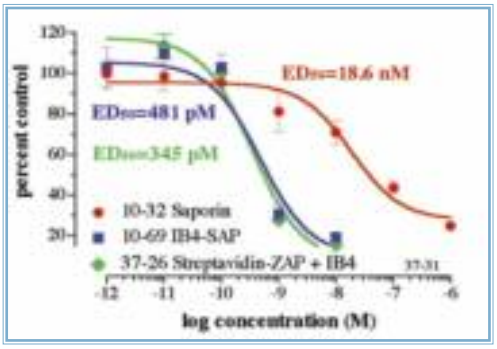
Q: I've been looking at your secondary conjugates and want to see if my targeting agent is specific to certain cells. Which secondary conjugate should I use?

A: It depends on two factors: 1) the type of assay you want to use, and 2) the kind of targeting agent you want to use.

For *in vitro* assays, in particular, internalization assays, you can use any of the ZAP Internalization Kits (Z-Kits that include all the materials necessary to test your targeting agent). For the Secondary Antibody Z-Kits, you use your primary antibody and select the appropriate secondary antibody species (e.g. for a human antibody, use Hum-ZAP (Cat. #KIT-22-Z), Fab-ZAP human (Cat. #KIT-51-Z), FabFC-Human (Cat. #KIT-65-Z), Hug-M-ZAP (Cat. #KIT-43-Z), or Fab-ZAP Hug-M (Cat. #KIT-78-Z), depending on the isotype of your primary antibody *(See page 8 Promos)*. Or you can biotinylate your antibody and use Streptavidin-ZAP (Cat. #KIT-27-Z).

For *in vivo* applications, it depends on the kind of targeting agent you want to use. Regardless of whether you use an antibody, peptide or ligand, you will need to biotinylate the material first. ATS offers a biotinylation service that is efficient and economical *(See page 8 Promos)*.

If you are using a biotinylated peptide, you will use a kit that includes the appropriate control -- Blank-Streptavidin-SAP. Order Cat. #KIT-27-B (25 mcg, 100 mcg, 250 mcg, or 1 mg.



KNRK cells are plated at 2500 cells/well and incubated overnight. Streptavidin-ZAP is premixed with Biotinylated-IB4 in equimolar concentrations. Saporin, IB4-SAP, and the Streptavidin-ZAP+ Biotinylated IB4 mixture are then added in 10- ml volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 15-30 minutes, then read at 490 nm.

If you are using a biotinylated antibody, you will use the Streptavidin-ZAP kit that includes the appropriate antibody species control:
BIgG-SAP Human, BIgG-SAP Mouse
BIgG-SAP Rabbit, BIgG-SAP Rat

Select the kit that matches the species of your biotinylated primary antibody. Each kit is available in 25, 100, 250 or 1000 mcg sizes.

KIT-27-Ahu - human antibody
KIT-27-Amu - mouse antibody
KIT-27-Arb - rabbit antibody
KIT-27-Art - rat antibody

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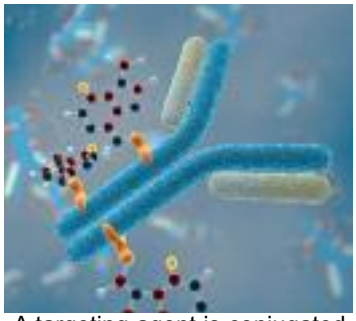

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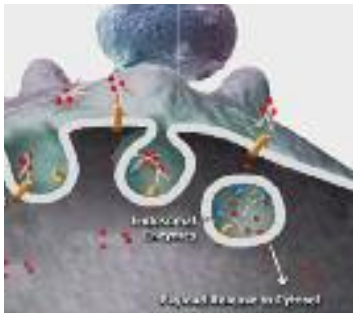

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Oct-Nov-Dec 2016 Vol. 17, Iss. 4

Reporting the latest news in Molecular Surgery

Targeted lesioning reveals role of nucleus incertus in the anxiogenic effect of buspirone

By Jigna Rajesh Kumar, [a, b, c, d] *Ramamoorthy Rajkumar,* [a, b, c] *Liying Corinne Lee,* [a, b, c] *Gavin S. Dawe* [a, b, c, d]

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The nucleus incertus (NI), located strategically at the prepontine brainstem, has widespread connections across the forebrain to various structures involved in arousal, behavioral regulation, anxiety, appetite, and cognition.[5, 6, 9, 11-12, 18-20] The NI expresses one of the highest density of corticotropin releasing factor receptor 1 (CRF1) in the brain. which raised interest in this structure and suggested its possible role in the extra-pituitary behavioral stress response.[16] The NI is the chief source of the neuropeptide relaxin-3, and the NI/relaxin-3 system is highly conserved phylogenetically, pointing to a critical functional role that is presently not well understood.[15, 22]

CRF-saporin (CRF-SAP; Cat. #IT-13) was stereotaxically injected into the NI to enable selective lesioning of the CRF1-expressing NI cells (Fig. 2, Page 6).[13] This procedure established at our laboratory was found to show significant reduction in the expression of CRF1, relaxin-3, GAD 65 as well as relaxin-3 in a representative target structure, the medial septum.[13] Based on the anxiogenic effect of CRF-SAP lesioning of the NI, as depicted by the significantly reduced time spent, and entries into the open arms of the elevated plus maze, it can be inferred that the NI may act to reduce anxiety physiologically (Fig. 1).

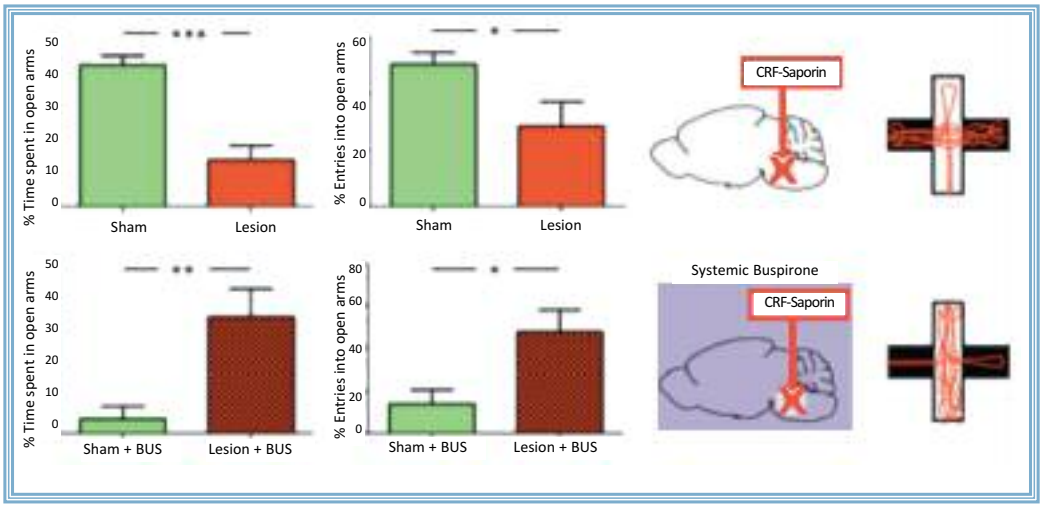


Fig. 1. Rats stereotaxically injected with CRF-SAP (Lesion) or Saporin (Cat. #PR-01; Sham) in the NI are tested in the elevated plus maze. Lesioning of the NI has an anxiogenic effect based on the reduced time spent and number of entries into the open arms. Systemic buspirone (3mg/kg; BUS) treatment reduces the anxiety levels of lesioned rats, increasing the time spent in, and number of entries into, the open arms.

(continued on page 6)

Strategic Partners

Denise Higgins - President

This issue of Targeting Trends brings an exciting report of new partners that will bring more products, more expertise, and more service to our customers around the world. The primary purpose for making this change is to better facilitate the various functions that serve you, our customer. This restructure will also challenge and reward our long-time employees with the responsibility of managing their own strategic unit.

Four partners with unique strengths combine to complete the Targeting Puzzle:

- Advanced Targeting Systems will continue to provide excellent Customer Service
- BioSyntheSys will provide quality conjugation services
- CytoLogistics will provide consistent, reliable antibody production, flow cytometry, and other laboratory services
- TLC Shipping & Storage will ensure all products are stored, packaged and shipped properly.

Brian Russell, celebrating his 16th anniversary at ATS on October 2nd, will be managing **BioSyntheSys**. His team will provide custom conjugation services: ADCs, saporin conjugates, biotinylations, fluorescent labeling, etc. Log on to ***BioSyntheSys.com*** to enter your contact information to discuss your next conjugation service.

Leonardo Ancheta, celebrated his 13th anniversary at ATS on September 2nd, and will be managing **CytoLogistics**' contract services division. Leonardo's team will provide flow cytometry services, cell culture, laboratory assays, antibody production, and GLP contract services. Log on to ***CytoLogistics.com*** to reserve your next service.

Doug Lappi (Founder and President Emeritus of ATS) will be heading up the Research Division of **CytoLogistics**. His team will work on product development and offer stellar laboratory expertise and consulting in Biochemistry, Molecular Biology, assay development, Cell biology and standard laboratory skills.

Tom Cobb and Chelsea Friedman will be co-managing **TLC Shipping & Storage**. Tom has extensive experience with shipping logistics and will ensure that all orders are packaged, shipped and tracked to their destinations worldwide. Chelsea is a scientist with an excellent background in storage and handling of research reagents (biologics, antibodies, etc.) and will manage the inventory tracking of multiple temperature storage units. Log on to ***TLCshipstorage.com*** to see the services they have to offer.

As for me, I will be continuing to manage **Advanced Targeting Systems** with an emphasis on administrative support for all of our partners. By consolidating all the administrative functions (customer orders, sales & marketing, purchasing, payroll, licensing, legal, etc.) for multiple entities, there is greater efficiency and organization in the processes. We are all excited about the prospects for each of our partners and for the greater service we can provide to our customers.

TLC Shipping & Storage is a San Diego-based service company experienced in logistics of managing research-grade laboratory supplies. Advanced Targeting Systems has partnered with TLC to provide:

- Inventory tracking
- Storage
- Packaging
- Shipping worldwide

TLC Shipping specializes in transportation and logistics for perishable commodities. TLC ships chilled, frozen, and shelf-stable goods to destinations around the world. Customers can rely on TLC to handle every aspect of packaging and shipping research reagents. TLC communicates each step of the process with visibility to track and trace every shipment.

TLC Storage provides short and long term temperature-controlled storage for non-hazardous research samples, production batches and products. Experienced associates manage your inventory from receipt through distribution TLC provides customers with peace of mind that inventory is well-protected, organized and secure through regular audited inventory reports and updates.

ATS Toolbox - New Partners

CytoLogistics is the Contract and Research Services partner to Advanced Targeting Systems. This new CRO (Contract Research Oorganization) used the foundation laid out by Cytometry Research LLC (established in 1993 to offer flow cytometry services to San Diego customers), and has expanded to help fulfill more encompassing Life Science and Preclinical Research needs.

CytoLogistics has a scientific staff with expertise to provide a wide range of services:

- Flow Cytometry (Design, Analysis, Sorting)
- Immunocytochemistry, immunohistochemistry
- *In vitro* laboratory services (cell culture, cell staining/preparation)
- *In vitro* cell–based assays (cytotoxicity, proliferation, compound/drug screening)
- Cell analysis and protein characterization (SDS-PAGE, Immunoblotting, ELISA, BCA, HPLC).
- Basic research (biochemistry, molecular biology)

Preclinical research often requires a higher level of service than other programs. To meet this need, **CytoLogistics** has GLP processes in place to ensure consistently reliable results, a quality assurance program to provide independent confirmation of procedures and results, and full documentation samples, data, and testing results provided with each service.

So, whether your laboratory needs extra hands for cell culture and basic research or you need professionally-designed and executed cell services, CytoLogistics delivers encompassing service and fluidity.

BioSyntheSys (BSS) is the preferred contracted partner of Advanced Targeting Systems for all crosslinking needs, including their well-established line of targeted Saporin conjugates. It is the mission of BioSyntheSys to provide academic and industry R&D clients with access to custom-crosslinking of proteins, peptides, and activated small molecules via boutique contract services. Their team of specialists leverage an extensive knowledge-base and catalog of strategies to make a complex process simple, allowing BSS clients to stay focused on what they do best.

- Antibody-drug conjugates
- Saporin and saporin-related conjugates
- Biotinylations
- Fluorescent labeling
- Linker design and consultation

If your research or drug development program needs a production partner or perhaps a company with specialized skills to outsource projects to, contact BioSyntheSys to discuss your next big discovery.

Recent Publications & References

Reviewed by Chelsea Friedman

Neural activity promotes long-distance, target-specific regeneration of adult retinal axons.

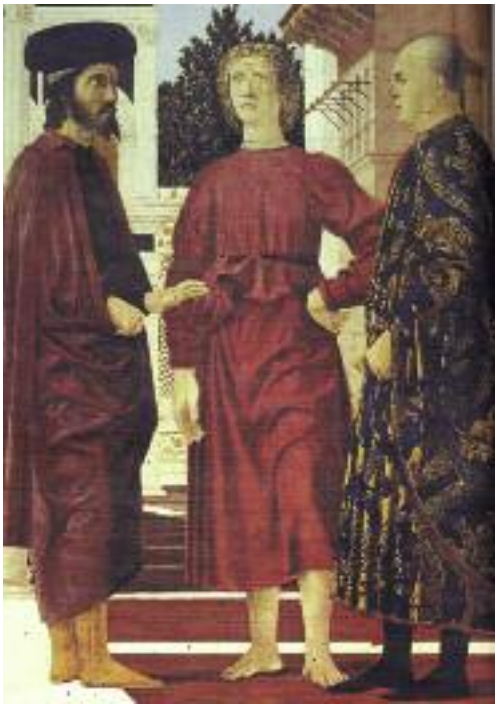
Lim JH, Stafford BK, Nguyen PL, Lien BV, Wang C, Zukor K, He Z, Huberman AD.
Nat Neurosci 19(8):1073-1084, 2016.

Axons in the CNS fail to regenerate after injury. Scientists sought to identify strategies that would allow retinal ganglion cell (RGC) axons to regenerate in the eye-to-brain pathway, and if that was possible, whether the axons could reconnect with their correct targets and restore visual function. It was previously shown that increasing mTOR signaling could trigger RGC axon regeneration. Several conditions were tested, but combining increased mTOR signaling and then exposing mice to high-contrast visual stimulation daily for 3 weeks after optic nerve crush resulted in long distance RGC axon regeneration, re-innervation of the brain, and partial recovery of a subset of visual behaviors. A 1:1000 dilution of Anti-Melanopsin (Cat. #AB-N38) was used for the immunohistochemical analysis of retinas, optic nerves, and brain tissue.

Effective antitumor therapy based on a novel antibody-drug conjugate targeting the Tn carbohydrate antigen.

Sedlik C, Heitzmann A, Viel S, Ait Sarkouh R, Batisse C, Schmidt F, De La Rochere P, Amzallag N, Osinaga E, Oppezzo P, Pritsch O, Sastre-Garau X, Hubert P, Amigorena S, Piaggio E.
Oncoimmunology 5(7):e1171434, 2016.

Scientists wanted to study the potential of Chi-Tn, a monoclonal antibody against a glycol-peptidic tumor-associated antigen, as an anticancer antibody-drug conjugate. They demonstrated that Chi-Tn specifically targeted tumor cells *in vivo*, using flow cytometry and deconvolution microscopy to show that Chi-Tn is rapidly internalized. Chi-Tn-SAP (ATS Custom Services) effectively killed Tn-positive cells, but had no effect on Tn-negative cells. Saporin (Cat. #PR-01) was used as a control. The cytotoxicity of the Chi-Tn-SAP correlated with the level of tumoral Tn expression.



Limiting glucocorticoid secretion increases the anorexigenic property of Exendin-4.

Lee SJ, Diener K, Kaufman S, Krieger JP, Pettersen KG, Jejelava N, Arnold M, Watts AG, Langhans W.
Mol Metab 5(7):552-565, 2016.

Glucagon-like peptide-1 (GLP-1) analogs lower blood surgar levels and cause a loss of appetite. Exendin-4 (Ex-4) is a GLP-1 receptor agonist, and also increases glucocorticoid secretion. Several tests were conducted to determine if the released glucocorticoids interact with Ex-4's anorexigenic effect. One method involved ablating hindbrain catecholaminergic neurons by stereotaxically injecting 42 ng of Anti-DBH-SAP (Cat. #IT-03) bilaterally into the paraventricular nucleus of the hypothalamus in rats. Animals were injected with equimolar concentrations of unconjugated Saporin (Cat. #PR-01) as a control. Anti-DBH-SAP lesions reduced the efficacy of Ex-4 to increase corticosterone secretion but increased the anorexigenic effect, indicating that Ex-4-dependent corticosterone secretion opposes Ex-4's actions. Anti-DBH-SAP lesions increased Ex-4's ability to reduce food intake and body weight.

Brain penetration, target engagement, and disposition of the blood-brain barrier-crossing bispecific antibody antagonist of metabotropic glutamate receptor type 1.

Webster CI, Caram-Salas N, Haqqani AS, Thom G, Brown L, Rennie K, Yogi A, Costain W, Brunette E, Stanimirovic DB.
FASEB J 30(5):1927-1940, 2016.

To generate a BBB-transmigrating antibody that could be reformatted to full IgG, scientists started with the BBB-crossing llama single domain antibody FC5. Standard phage display protocols were used to isolate single-chain variable fragments (scFv) from the FC5-scFv library. 6His Mouse Monoclonal antibody (Cat. #AB-213) was used to assess cell binding of scFvs of FC5 using fluorescence microvolume assay technology. An scFv that competed with FC5 binding was selected for further testing. An antibody antagonist of the metabotropic glutamate receptor-1 was fused with this scFv antibody fragment (BBB-mGluR1) and tested in an *in vitro* BBB model. The resulting bispecific antibody retained selective mGluR1 binding and saw a 20-fold enhanced rate of transcytosis across the BBB compared to fusion with control antibody fragment. Intravenous injection of BBB-mGluR1 had analgesic properties in a rat model of persistent inflammatory pain.

Lysophosphatidylcholine acyltransferase 1 protects against cytotoxicity induced by polyunsaturated fatty acids.

Akagi S, Kono N, Ariyama H, Shindou H, Shimizu T, Arai H.
FASEB J 30(5):2027-2039, 2016.

Dietary consumption of polyunsaturated fatty acids can influence the degree of fatty acid unsaturation in membrane phospholipids, and consequently membrane-associated functions. Scientists set out to investigate how mammalian cells change their membrane lipid composition in response to loading with excess polyunsaturated fatty acids (PUFAs). Lipidomic analysis showed that PUFA treatment induces production of dipalmitoylphosphatidylcholine (DPPC). By

(continued on page 5)

Recent Publications & References

(continued from page 4)

suppressing phospholipid metabolism-related genes by RNA interference, they found that Lysophosphatidylcholine acyltransferase 1 (LPCAT1) was involved in DPPC production. To reveal the role of DPPC produced by PUFA treatment, HeLa cells were transfected with an siRNA against LPCAT1 to reduce its protein expression. The cells were lysed after treatment with a PUFA and subjected to western blot analysis using a 1:1000 dilution of Anti-SCD-1 (Cat. #AB-259) as the primary. SCD-1 desaturates the substrate of LPCAT1 for producing DPPC. PUFAs significantly reduced both the protein and mRNA expression of SCD-1. They showed that inhibiting DPPC production by LPCAT1 knockdown enhanced apoptosis, suggesting that DPPC produced via LPCAT1 protects against PUFA-induced cytotoxicity.

Selective noradrenaline depletion impairs working memory and hippocampal neurogenesis.

Coradazzi M, Gulino R, Fieramosca F, Falzacappa LV, Riggi M, Leanza G.

Neurobiol Aging 48:93-102, 2016.

Neuronal loss in the locus coeruleus (LC) of Alzheimer's patients is well known, but the contribution of LC-derived noradrenergic afferents to learning and memory function is unknown. To model noradrenergic neuron degeneration in the LC, rats were bilaterally injected directly into the LC with 0.2 mcg of Anti-DBH-SAP (Cat. #IT-03). Lesioned and sham-lesioned animals were tested behaviorally and exhibited robust working memory deficits, yet lesioning did not affect reference memory. They concluded that ascending noradrenergic afferents might be involved in more complex aspects of working memory, possibly via newly generated progenitors in the hippocampus.

Nucleus incertus contributes to an anxiogenic effect of buspirone in rats: Involvement of 5-HT1A receptors.

Kumar JR, Rajkumar R, Lee LC, Dawe GS.

Neuropharmacology 110(Pt A):1-14, 2016.

To see if the NI is necessary for the anxiogenic effects of high doses of buspirone, rats were bilaterally injected with 86 ng of CRF-SAP (Cat. #IT-13) into the NI. Blank-SAP (Cat. #IT-21) was used as a control. (See Cover Article)

Substituting mouse transcription factor Pou4f2 with a sea urchin orthologue restores retinal ganglion cell development.

Mao CA, Agca C, Mocko-Strand JA, Wang J, Ullrich-Luter E, Pan P, Wang SW, Arnone MI, Frishman LJ, Klein WH.

Proc Biol Sci 283(1826):20152978, 2016.

The sea urchin genome contains SpPou4f1/2, a distant orthologue of Pou4f2, but they have no obvious eyes and their photoreceptors are located around their tube feet disc. Scientists replaced genomic Pou4f2 with an SpPou4f1/2 cDNA to see if SpPou4f1/2 could support RGC development in mice. Mice expressing SpPou4f1/2 developed retinas that looked like wild-type mice. Immunolabeling of retinas with a 1:1000 dilution of Anti-Melanopsin (Cat. #AB-N39) showed the presence of many well-bundled axons emanating from SpPou4f1/2-expressing RGCs. Electroretinogram recordings from these mice indicate that their RGCs are functionally active. These results suggest that there is a high degree of functional conservation between the two genes.

GABA-A receptor activity in the noradrenergic locus coeruleus drives trigeminal neuropathic pain in the rat; contribution of NAalpha1 receptors in the medial prefrontal cortex.

Kaushal R, Taylor BK, Jamal AB, Zhang L, Ma F, Donahue R, Westlund KN.

Neuroscience 334:148-159, 2016.

The goal of this study was to investigate the role of the locus coeruleus (LC) in a rat orofacial pain model of trigeminal neuropathy induced by chronic constrictive injury of the infraorbital nerve (CCI-ION). Noradrenergic (NA) neurons were lesioned with 5-mcg injections of Anti-DBH-SAP (Cat. #IT-03) into the left lateral ventricle. Mouse-IgG-SAP (Cat. #IT-18) was used as a control. After ablation of NA neurons there was a notable increase in the mechanical threshold of Von Frey filaments tested on whisker pads compared to control animals. Injecting a GABAA receptor antagonist into the LC after injury had an inhibitory effect on nerve injury induced hypersensitivity. They concluded that GABAA-mediated activation of NA neurons during CCI-ION can facilitate hypersensitivity through NAα1 receptors in the mPFC, and that the LC is a chronic pain generator.

A2 noradrenergic neurons regulate forced swim test immobility.

Nam H, Kerman IA.

Physiol Behav 165:339-349, 2016.

Researchers discovered relative hyperactivation in the locus coeruleus of Wistar-Kyoto (WKY) depression model rats compared to the genetically related Wistar rats when exposed to one- and two-day forced swim tests (FSTs). A2 noradrenergic neurons of Winstar rats were lesioned by injecting 2.2 mcg of Anti-DBH-SAP (Cat. #IT-03) into the nucleus tractus solitaris (NTS). Lesioned rats exhibited increased FST immobility on both days of the test, similar to natural WKY behavior in the same test.

Saponins from Saponaria officinalis L. Augment the Efficacy of a Rituximab-Immunotoxin.

Gilabert-Oriol R, Thakur M, Haussmann K, Niesler N, Bhargava C, Gorick C, Fuchs H, Weng A.

Planta Med 2016.

Investigators wanted to know if triterpenoidal saponins that come from *Saponaria officinalis* could increase the therapeutic affect of Rituximab-Saporin. In the presence of saponins, Rituximab-Saporin had a 700-fold increase in efficacy. Concentrations of 0.0001-1nM of Anti-CD22-SAP (Cat. #IT-37) and 0.001-10nM of Anti-CD25-SAP (Cat. #IT-24) were also tested *in vitro* with saponins for comparison. They saw a 170-fold and 25-fold increase in cytotoxicity, respectively.

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Targeted lesioning reveals role of nucleus incertus in the anxiogenic effect of buspirone

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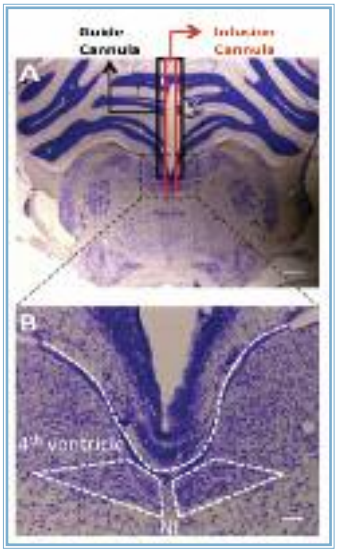

Fig. 2. Representative images showing cannula position in the NI at 2X (A; scale=1 mm) and 10X (B; scale = 200 μm).

We further utilized this technique to determine if the NI was involved in mediating the anxiety-modulating effects of buspirone, a clinically prescribed novel anxiolytic whose mechanism of action is not well understood.[3-4] Buspirone is a anxioselective drug that acts specifically on symptoms of anxiety without affecting cognition, motor ability, and reward pathways thus indicating that it likely acts on structures that regulate physiological anxiety. Buspirone is a 5-HT1A partial agonist and a D2 receptor antagonist;[(14)] both receptors are expressed in the NI.[13, 17] Buspirone tends to show anxiolytic effects at a narrow low dose range and anxiogenic effects at a wide high dose range, the latter effect being robust and reproducible.[1, 4, 7, 10, 21] The anxiolytic effects are widely thought to be mediated by the agonism of the 5-HT1A autoreceptors at the raphe nuclei, particularly the median raphe.[2, 8, 23] A 3 mg/kg intraperitoneal dose of buspirone was found to induce a strong anxiogenic effect on various anxiety paradigms, namely the elevated plus maze, the open field, and the light-dark box.[12] This dose was also found to robustly induce c-Fos expression and therefore activate the NI. The anxiogenic effect of systemic buspirone was attenuated when the NI was lesioned by CRF-SAP, thus indicating that the NI plays a role in the effects of buspirone (Fig. 1). Infusing buspirone (5 mcg) into the NI produced increased anxiety as well, suggesting that buspirone may be acting directly on the NI.[12] Pharmacological interaction studies conducted with a 5-HT1A antagonist, NAD 299, and D2/D3 agonist, quinpirole, indicated that these effects are mediated through the 5-HT1A receptors. Intra-NI infusion of NAD 299 attenuated the anxiogenic effects of systemic buspirone while intra-NI quinpirole did not have any effect.[12] Therefore, the NI is likely to be part of the physiological anxiety circuit and the 5-HT1A receptors may be particularly important in mediating this function.

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Talking about Targeting

New Additions

In this quarter's Targeting Talk, ATS is proud to talk about more than new products, a new Product Manager! Her name is Alena Bishop and although originally from the San Francisco Bay Area, she recently graduated from UCSD in June of this year. With a newly minted degree in Molecular Biology and ATS's need for a Research Associate with just such a pedigree, it has proved to be an excellent match. Since starting in July, Alena has taken quickly to the innovative work being done in the lab and appreciates participating in the development of products that play such an important role in the everyday research of so many scientists globally. Eager to dive into the customer-facing aspects of our products, she looks forward to engaging with each of you regarding any technical questions you may have.

She's here to tell you, in her own words, what she is working on and what products to look forward to:

"One of the projects I have been working on involves a novel use of specific strains of Clostridium botulinum *that inhibits cells by blocking the release of neurotransmitters. This effect is long-lasting but not permanent, and this attribute gives the toxin great potential as a new targeted payload. This toxic payload could be used to temporarily prevent cell function without actually killing the cells, and coupled with ATS's targeting technology this treatment could be administered specifically to cells of interest. Avoiding killing the cells completely would provide a life-like model to study the effects of temporary loss of cell function in organisms. After a certain period of time, the effects of the toxin would disappear, restoring normal neuronal cell function. My immediate goal is to put the finishing touches on an assay kit for quantifying the activity of Clostridium botulinum useful for a variety of strains. Look for this activity assay kit to be released in the coming quarter!"*

Where to See Us:

Pain Summit
Chicago, October 19-20

Society for Neuroscience
San Diego, November 12-16
Booth #2623

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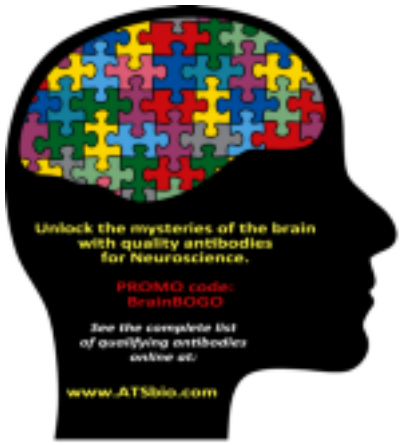

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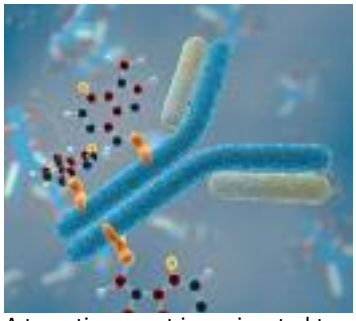
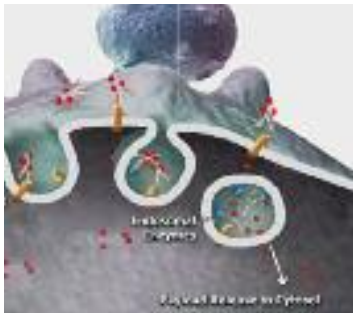


A targeting agent is conjugated to a payload. Here the conjugate is an antibody linked to a small molecule.

The targeted conjugate binds only the cells that are specific to the antibody causing the receptor to internalize.



The internalized conjugate is digested by enzymes releasing the payload into the cytosol. The payload is then free to alter the cell.

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