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

(continued on page 6)
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:

**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**


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

ATS is pleased to present a new product line specific for the vesicular GABA transporter (vGAT) protein. vGAT mediates both the accumulation 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-N44 AP ), 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 CHO-p75 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, CHO-p75 cells are standard CHO (Chinese Hamster Ovary) cells that stably express the rat low affinity nerve growth factor, p75 (p75NTR). Expression of p75NTR in CHO-p75 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.
The peptidergic control circuit for sighing.
Li P, Janczewski WA, Yackle K, Kam K, Pagliardini S, Krasnow MA, Feldman JL.
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 retrotangential 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.
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 rostral medial tegmental nucleus increases ethanol consumption and regulates ethanol-related behaviors.
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 rostralmedial tegmental 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
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.
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. #T-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 RL, Kilgard MP.

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.

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.

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.
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-immunotoxin. 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 Tuberonemammillary Nuclei Ablations Attenuate Hypocretin/Orexin Antagonist-Mediated REM Sleep.
Schwartz MD, Nguyen AT, Warrier DR, Palmerston JB, Thomas AM, Morarity SR, Neylan TC, Kilduff TS.

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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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
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.

Looking more closely at the study by French \textit{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
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*Patrick Shramm*

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*Chelsea Friedman*