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Volume 12, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeted lesion of caudal brainstem catecholamine neurons reveals their role in symptoms of fatigue.

Contributed by Lisa E. Goehler and Ronald P.A. Gaykema, Center for the Study of Complementary and Alternative Therapies, University of Virginia School of Nursing, Charlottesville, VA 22908

Fatigue, experienced as reduced motivation and motor activity, is common in acute and chronic disease, including heart disease, cancer and cancer chemotherapy. Fatigue is part of a constellation of symptoms referred to as "sickness behavior," which is mediated by the brain and results from physiological and psychological challenges, including inflammation and stress.[1,9] It contributes to poor quality of life, but as yet there are no effective treatments.[9,10] A major hurdle to developing effective treatments for fatigue is the lack of knowledge regarding the mediating brain mechanisms that produce the symptoms. However, we recently determined that catecholamine neurons provide a connection between brain regions that respond directly to signals generated in the body (such as inflammation) and other regions involved in modulating brain activity related to behavior, that likely contribute to the neurocircuitry of fatigue.[2] We were able to make this determination by using targeted lesions of catecholamine neurons with saporin-conjugated anti-dopamine beta hydroxylase (anti-DBH-SAP; Cat. #IT-03).

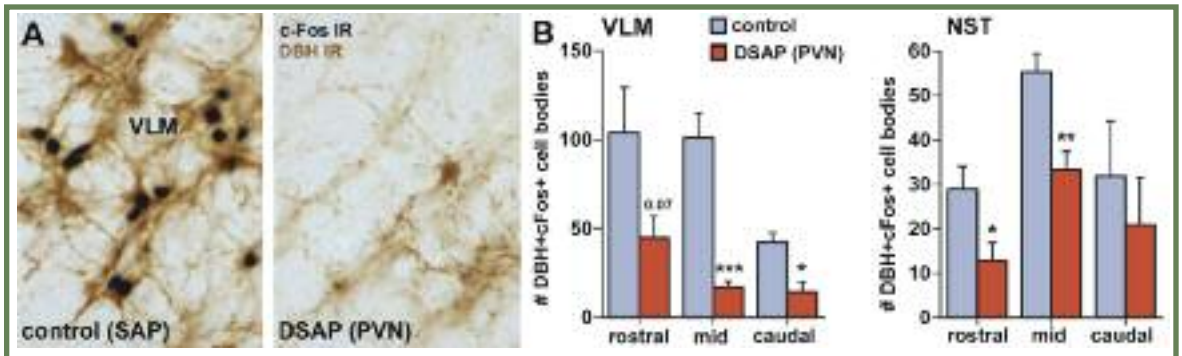


Fig. 1. Caudal brainstem catecholaminergic neurons (brown cells) in the VLM and NST responsive to immune challenge (black c-Fos stained cell nuclei) are specifically vulnerable to anti-DBH-SAP (DSAP) injected into the PVN (compare photomicrograhs in A). Graphs in B depict the loss of DBH and c-Fos double-labeled cells in the VLM and NST of immune-challenged rats across the rostro-caudal axis of the medulla oblongata.

Our previous studies[3-8] indicated that suppression of activity of brain regions in the hypothalamus and midbrain that modulate behavioral arousal contributes to fatigue symptoms associated with immune challenge that induces sickness. These brain regions receive input from catecholamine neurons, located in the nucleus of the solitary tract (NST) and ventrolateral medulla (VLM) of the caudal brainstem, that respond to stress and inflammation (Fig. 1A), suggesting that these neurons may serve as a link between physiological challenge and fatigue.

To determine whether catecholaminergic neurons in the caudal brainstem contribute to symptoms of fatigue, we assessed exploratory motor behavior[11] in immune-challenged animals that had previously received injection of anti-DBH-SAP into the paraventricular nucleus of the hypothalamus. This region receives extensive input from the catecholamine neurons of the NST and VLM, and pilot studies showed that injections dramatically reduce numbers of these neurons

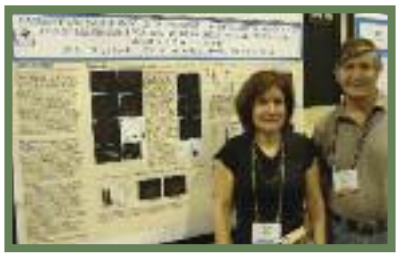
(continued on page 6)

SFN Poster of the Year Award

Poster of the Year Award:

Functional cholinergic neurons from human embryonic stem cells. *Y. Liu, R. Krencik, H. Liu, L. Ma, X. Zhang, S.-C. Zhang, Univ Wisconsin.*

Yan Liu presented this year's winning abstract describing work on transplantation of human embryonic stem cells into the hippocampus of mice that had cholinergic neurons of the medial septum eliminated by mu p75-SAP (Cat. #IT-16). The human stem cells demonstrated activity in their new home and apparently resulted in large human cholinergic neurons. Data concerning improvement in learning and memory deficit were also presented. Because these are controversial and even unexpected results, the data were extremely interesting and, as the abstract states, "indicate that the human stem cell-generated cholinergic neurons are functional, thus providing a new source for drug discovery and cell therapy for neurological disorders that affect cholinergic neurons."



Runner-up:

Carageenan evoked P-Akt in deep dorsal horn neurons is prevented by loss of neurokinin1 positive neurons in superficial dorsal horn. *L.S. Sorkin, J.-I. Choi, F.J. Koehrn, Univ Calif San Diego.*

Linda Sorkin, in a beautiful poster, discussed the effects of removal of NK1r-expressing neurons in the rat spinal cord and showed a potent effect on descending pain pathways. Selective ablation of NK1r+ neurons in superficial dorsal horn (DH) blocked peripheral inflammation-induced increase of p-Akt expression in both superficial and deep DH neurons. This is another clear demonstration that disruption of the spinothalamic tract by elimination of the small number of NK1r+ neurons (in this case by SSP-SAP, Cat. #IT-11) can have effects on deeper elements of pain pathways after they pass through to the thalamus and descend. These data add to the explanation of, e.g., the diminution of central sensitization that has been reported by removal of these neurons.

Runner-up:

Early post-natal cholinergic lesion impairs normal development and maturation of the motor cortex in rats. *D. Ramanathan, J.M. Conner, A.A. Anilkumar, M.H. Tuszynski, Univ Calif San Diego.*

Dhakshin Ramanathan discussed behaviorally-driven forms of cortical map plasticity in the rat, that is, how neurons in the cortex develop as a result of experience from youth to adulthood. Specifically, they looked at the cholinergic neurons of the basal forebrain by deleting them using 192-IgG-SAP (Cat. #IT-01). This resulted in a lesser area to a previously-mapped region, which was accompanied by long-term impairments in skilled motor learning. The cortical mapping data was especially interesting and the result for the studied area, the distal forelimb, was different from the effect of cholinergic neurons on wound plasticity in adults, which they had previously reported.

Upcoming Events

American Association for Cancer Research
April 2-6, 2011
Orlando, FL
Booth #2278

Experimental Biology
April 9-13, 2011
Washington, DC

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: SYMPATHETIC
BLADDER
SEGMENT
FIBERS
EPISODIC

Answer: He had an . . . INFLATED EGO!

Congratulations to the puzzle solvers. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Seto Chice, SUNY-HSC, Brooklyn, NY * Roger Guillemin, La Jolla, CA * Thea Marlinga, Libertyville, IL * April Price, UCSF, San Francisco, CA * Amalia Dingman, Norman, OK * Saurabh Joshi, UCSD, La Jolla, CA * Jason Kato, UC Davis, Sacramento, CA * Khalid Touzani, Brooklyn College of CUNY, Brooklyn, NY * Diana Halim, AnGes Inc, Gaithersburg, MD * Jason Moreau, Univ Western Ontario, London, ON * Barbara Attardi, Bioqual Inc, Rockville, MD * Bob Speth, Nova Southeastern Univ, Fort Lauderdale, FL

Solve the Teaser online at: www.ATSbio.com/news/11q1_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

The cell surface structure of Tumor Endothelial Marker 8 (TEM8) is regulated by the actin cytoskeleton

Yang MY, Chaudhary A, Seaman S, Dunty J, Stevens J, Elzarrad MK, Frankel AE, St Croix B
Biochim Biophys Acta 1813(1):39-49, 2011.

Tumor endothelial marker 8 (TEM8) is a cell surface protein that is up-regulated on tumor blood vessels. Overexpression of this protein, however, produces a form that is not recognized by the SB5 monoclonal antibody used to bind TEM8. While cells expressing normal levels of TEM8 were killed by an application of biotinylated SB5 plus either 1 nM or 10 nM streptavidin-ZAP (Cat. #IT-27), cells overexpressing the protein did not bind the immunotoxin. Understanding the structural differences between the two forms of TEM8 will help in the design of therapeutic antibodies against these tumor cells.

Intrinsically photosensitive retinal ganglion cells

Do MTH, Yau K
Physiol Rev 90(4):1547-1581, 2010.

This review presents recent data that has established the importance of intrinsically photosensitive retinal ganglion cells (ipRPG) in nonimage visual functions. The use of melanopsin-SAP (Cat. #IT-44) in both mice and rats is discussed. It is of note that depletion of ipRPG's using melanopsin-SAP resulted in deficits in communication to nonimage regions of the brain, but image vision appeared normal.

Neuropeptide Y conjugated to saporin alters anxiety-like behavior when injected into the central nucleus of the amygdala or basomedial hypothalamus in BALB/cJ mice

Lyons AM, Thiele TE
Peptides 31(12):2193-2199, 2010.

Neuropeptide Y (NPY) in the hypothalamus is known to modulate feeding behavior. In this work the authors used bilateral 48-ng injections of NPY-SAP (Cat. #IT-28) into the central amygdala or basomedial hypothalamus (BMH) of rats to investigate the role of NPY in anxiety. Blank-SAP (Cat. #IT-21) was used as a control. Injections into the amygdala increased anxiety-like behavior, while injections into the BMH reduced anxiety-like behavior. BMH injections also initiated an increase of NPY-1 receptor expression in the basolateral nuclei of the amygdala.

Targeted delivery of saporin toxin by monoclonal antibody to the transcobalamin receptor, TCblR/CD320

Quadros EV, Nakayama Y, Sequeira JM
Mol Cancer Ther 9(11):3033-3040, 2010.

Vitamin B12 is necessary for cell proliferation. Cancer cells display an increased expression of TCb1R, the receptor that facilitates the intake of B12. In order to evaluate the potential of using immunotoxins to eliminate cancer cells expressing TCb1R the authors performed a series of *in vitro* experiments using their monoclonal antibody plus Mab-ZAP (Cat. #IT-04) in varying concentrations. The results indicate that this is a viable therapeutic model that causes minimal peripheral damage.

Locus ceruleus and anterior cingulate cortex sustain wakefulness in a novel environment

Gompf HS, Mathai C, Fuller PM, Wood DA, Pedersen NP, Saper CB, Lu J
J Neurosci 30(43):14543-14551, 2010.

Locus ceruleus (LC) activity is strongly associated with wakefulness. In this work the authors examined arousal due to environmental stimuli after lesioning of the LC. Injections of 0.25-1 µg of Anti-DBH-SAP (Cat. #IT-03) were administered into the lateral ventricle of rats. Lesioned animals did not show sustained neurobehavioral and EEG arousal in response to a novel environment. The data suggest sustained attention requires an interaction between the LC and the anterior cingulate cortex.

Developmental origin of preBötzinger Complex respiratory neurons

Gray PA, Hayes JA, Ling GY, Llona I, Tupal S, Picardo MCD, Ross SE, Hirata T, Corbin JG, Eugenín J, Del Negro CA
J Neurosci 30(44):14883-14895, 2010.

Breathing in adult rats requires a subset of neurons in the preBötzinger Complex (preBötC) that express the neurokinin-1 receptor and the peptide somatostatin. In this work the authors investigate the developmental origins of these cells. Using various techniques, including immunohistochemistry with a neurokinin-1 receptor antibody (Cat. #AB-N04; product in re-development), it is demonstrated that neurons derived from Dbx-1-expressing progenitor cells are important in the generation of respiratory behavior.

Cerebellar modules: individual or composite entities?

Cerminara NL
J Neurosci 30(48):16065-16067, 2010.

This short review discusses the compartmentalization of cerebellar modules. Much research has been done to associate particular motor control functions with particular modules. Chemical lesioning is an inadequate technique because the lesion is non-specific. The use of CTB-SAP (Cat. #IT-14) to affect the function of a single module is discussed.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

The effects of neonatal forebrain cholinergic lesion on adult hippocampal neurogenesis

Rennie K, Fréchette M, Pappas BA
Brain Res Epub, 2010.

Intraventricular injections of 192-IgG-SAP (Cat. #IT-01) have been shown to reduce the number of cells expressing a marker for immature neuroblasts in the dentate gyrus, as well as possibly impairing the response to environmental enrichment. This study looked to expand on those observations. Infusion of 300 ng of 192-IgG-SAP was delivered into each ventricle of post-natal, Day 7 rats. The data suggest that the lesion accelerates the death of new cells, but does not affect survival rate or phenotypic differentiation.

Acetylcholine and attention

Klinkenberg I, Sambeth A, Blökland A
Behav Brain Res Epub, 2010.

This review article summarizes studies investigating the role of acetylcholine in attention and cognition. The roles of 192-IgG-SAP (Cat. #IT-01) and mu p75-SAP (Cat. #IT-16) in these experiments are discussed. Acetylcholine is thought to play a top-down role in the prefrontal, parietal, and somatosensory regions; playing an important role in the control of attentional-orienting and stimulus discrimination.

Spatial memory alterations by activation of septal 5HT(1A) receptors: no implication of cholinergic septohippocampal neurons

Koenig J, Lecourtier L, Cosquer B, Pereira PM, Cassel J
Psychopharmacology (Berl) Epub, 2010.

These experiments examined what effect damaged cholinergic neurons would have on memory deficits induced by the 5-HT1A/5-HT7 receptor agonist 8-OH-DPAT. Rats received 0.4-µg injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum, delivered through an infusion device. Through use of a water maze test, the authors show that several neuronal populations are involved in processing hippocampal information, and non-cholinergic neurons in this region may be more important than the cholinergic ones for memory processing.

Lesions of orexin neurons block conditioned place preference for sexual behavior in male rats

Di Sebastiano AR, Wilson-Pérez HE, Lehman MN, Coolen LM
Horm Behav Epub, 2010.

The neuropeptide orexin is important in the feedback mechanisms of food intake and drugs of abuse. This work investigates the role of orexin in sexual reward in male rats. Two 200-ng bilateral hypothalamic injections of orexin-SAP (Cat. #IT-20; discontinued) were made into each hemisphere. Blank-SAP (Cat. #IT-21) was used as a control. Although it was shown orexin neurons are activated by sexual reward cues, the data suggest that orexin is not essential for sexual performance and motivation.

Cholinergic hypofunction impairs memory acquisition possibly through hippocampal Arc and BDNF downregulation

Gil-Bea FJ, Solas M, Mateos L, Winblad B, Ramírez MJ, Cedazo-Mínguez A
Hippocampus Epub, 2010.

The authors investigated the role of activity-regulated cytoskeleton associated protein (Arc) and brain-derived neurotrophic factor (BDNF) in cholinergic-induced memory formation. Rats received 67-ng bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the third ventricle after behavioral training. Lesioned animals had decreased protein and mRNA for both Arc and BDNF. Memory acquisition and recovery of acquisition were both affected. The data indicate that cholinergic denervation of the hippocampus affects the muscarinic facets of spatial memory acquisition.

Damage of GABAergic neurons in the medial septum impairs spatial working memory and extinction of active avoidance: Effects on proactive interference

Pang KCH, Jiao X, Sinha S, Beck KD, Servatius RJ
Hippocampus Epub, 2010.

Recent work implicates the medial septum (MS) and diagonal band (DB) in the control of proactive interference — forgetting older information when learning new information. Rats received GAT1-SAP (Cat. #IT-32) injections into the MS and the DB (162.5 ng and 130 ng, respectively; the DB injections were bilateral). The results parallel other studies using 192-IgG-SAP (Cat. #IT-01) and other toxins, reinforcing the indications that GABAergic MSDB neurons are an integral part of proactive interference control.

Cholinergic depletion in the nucleus accumbens: Effects on amphetamine response and sensorimotor gating

Laplante F, Lappi DA, Sullivan RM
Prog Neuropsychopharmacol Biol Psychiatry Epub, 2010.

Disruption of dopamine and acetylcholine balance in the striatum may play a role in conditions such as Parkinson's and schizophrenia. In this work the authors lesioned cholinergic neurons in the nucleus accumbens (N.Acc) with the novel toxin Anti-ChAT-SAP (Cat. #IT-42). Rats received 0.25-µg bilateral injections of the toxin into the N.Acc. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The results of this lesion produced responses that may parallel the loss of cholinergic neurons observed in schizophrenia.

Targeting Talk: Product Questions

by Dr. Douglas Lappi

GAT1-SAP

Q: I am interested in your product GAT1-SAP (Cat. #IT-32). Before ordering, I would like to know how long it takes until the toxin produces a lesion; Is seven days enough?

A: The usual time-course for saporin toxins is that behavioral effects start appearing at four days and usually plateau at seven days. However, keep in mind that it takes some time for dead cells to be cleared out, so histology on the animal should wait until at least two weeks after administration.

Q: After ordering, how long it will take to receive the toxin?

A: GAT1-SAP is in stock in both the U.S. and our warehouse in The Netherlands. Packages are shipped by overnight delivery; Monday-Thursday in the U.S. and Monday-Wednesday in Europe.

Q: Is there any saporin derivative available that selectively destroys all or some hippocampal neurons?

A: Assuming that you mean to eliminate cell bodies from the hippocampus, rather than just projections, a neat paper (Martin JL, Sloviter RS, *J Comp Neurol* 436(2):127-152) describes the use of SSP-SAP (Cat #IT-11) to do this. NPY-SAP (Cat. #IT-28) could also be interesting, but there are no publications to demonstrate that.

References ***(From Cover Article, pages 1 and 6)***

1. *Dantzer, R., Kelley, K.W., 2007.* Brain Behav Immun *21, 153.*
2. *Gaykema, R.P.A., Goehler, L.E. 2010.* Brain Behav Immun *doi:10.1016/j.bbi.2010.11.005*
3. *Gaykema, R.P.A., Goehler, L.E., 2009.* Brain Behav Immun *23, 926.*
4. *Gaykema, R.P.A., Chen, C.-C, Goehler, L.E., 2007.* Brain Res *1130, 130.*
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11. *Stone, E.A., Lehmann, M.L., Lin, Y., Quartermain, D., 2006.* Biol Psych *60:803.*

Preparing for a custom saporin conjugation

Q: When we contacted you to find out more about having a custom saporin conjugation performed with our primary antibody, you recommended that we use the ATS secondary conjugate system to determine that our antibody was specific to the population we want to eliminate. We looked more at the website, and it seems that we are supposed to start with Anti-M-ZAP, Cat. #IT-30 (our primary Ab is a mouse IgM), and use the Mouse IgM-SAP, Cat. #IT-41 for control. Is this correct?

We don't quite understand what the Mouse IgM-SAP control is or how it should be used. If it is a control for the Anti-M-ZAP, then it should consist of saporin coupled to a dummy antibody, and we should add it to our primary in our test. Is it also supposed to be used as a control for a directly conjugated primary+saporin?

A: If your primary antibody is a mouse IgM, then you are correct that Anti-M-ZAP (Cat# IT-30) is the appropriate secondary conjugate to use. As for control conjugates, the best control would be a secondary conjugate using an IgM isotype control mixed with Anti-M-ZAP. An alternative would be to use Goat IgG-SAP (Cat# IT-19) made with normal goat IgG and mimics Anti-M-ZAP without the specific affinity for mouse IgM.

Once you determine you need a direct conjugate made between your mouse IgM primary antibody and saporin, then you would want to use the Mouse IgM-SAP (Cat# IT-41) as a control toxin just as you use your direct conjugate. ATS currently offers a 50% discount on the purchase of control conjugates when you have a custom saporin conjugation service with your primary targeting agent.

Anti-ChAT-SAP, Cat. #IT-42

targets cells expressing
choline acetyltransferase (ChAT) in multiple species

available in these sizes:
25 micrograms, 100 micrograms, and 250 micrograms

Kit includes the following controls in equal amounts:
Anti-ChAT affinity-purified, Cat. #AB-N34AP
Saporin, Cat. #PR-01 • Blank-SAP, Cat. #IT-21

Targeted lesion of caudal brainstem catecholamine neurons reveals their role in symptoms of fatigue.

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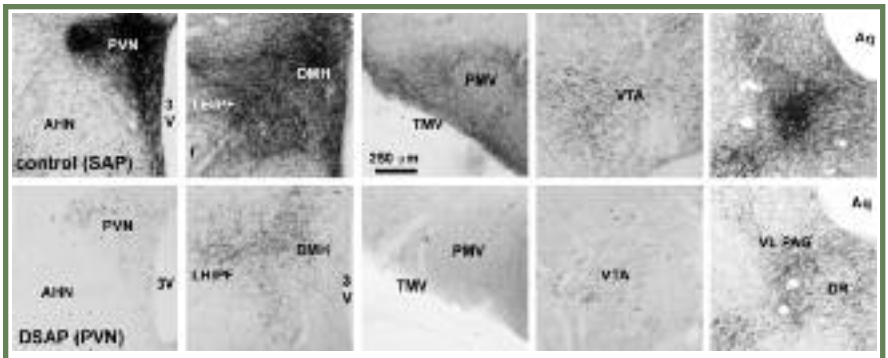

Fig. 2. Lesion with anti-DBH-SAP (DSAP) aimed at the PVN leads to dramatic loss of DBH-labeled innervation in the PVN, but also other parts of the hypothalamus (dorsomedial [DMH], lateral/perifornical [LH/PF], ventral tuberomammillary [TMV] portions), as well as the ventral tegmental area (VTA) and the ventrolateral periaqueductal grey (VLPAG) and dorsal raphe (DR). Telencephalic regions targeted by the locus coeruleus were not affected.

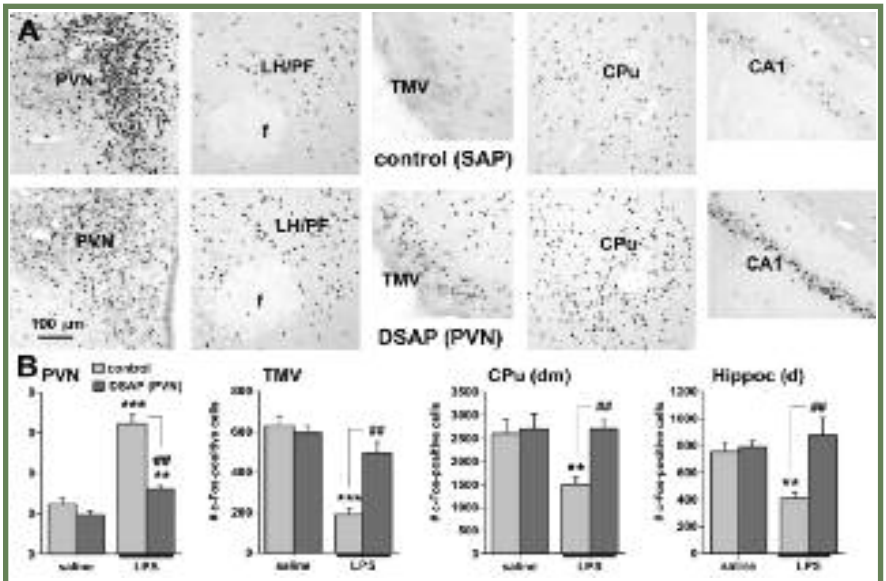

Fig. 3. Lesion with anti-DBH-SAP (DSAP) suppressed PVN c-Fos expression induced by immune challenge, but prevented immune-mediated suppression of c-Fos expression associated with behavior, e.g., the lateral/perifornical hypothalamus (LH/PF), the ventral tuberomammillary nucleus (TMV), the caudate putamen (CPu) and the hippocampus (CA1 region). Immune challenge was induced by ip injection of lipopolisaccharide (LPS). Control rats received saline injections prior to behavioral testing. ** $p < 0.01$,*** $p < 0.0001$ (LPS vs. saline); ## $p < 0.01$ DSAP vs. control.

from caudal brainstem sources (Fig. 1B), but have little effect on other neurons such as those in the locus coeruleus. Control animals were injected with Mouse IgG-SAP (Cat. #IT-18). To explore brain regions potentially influenced in the context of fatigue and by activity of the caudal brainstem catecholaminergic neurons, we assessed two contributing factors. 1) The loss of DBH-positive neurons and innervation (Fig. 2), and 2) The brain activation patterns (reflected by the induction of the cell activation marker c-Fos) normally evident during exploratory behavior during immune challenge, and how the lesion modifies the pattern (Fig. 3A).

Exploratory behavior was associated with activation in brain areas involved in behavior and motivation, including the hippocampus, dorsal striatum, and ventral tegmental area, and in hypothalamic neurons involved in arousal. Activation of these areas was greatly reduced after immune challenge, but targeted lesion of catecholaminergic projections with anti-DBH-SAP prevented reductions in exploratory behavior (Fig. 4), and completely prevented the suppressive effects of immune challenge on c-Fos expression (Fig. 3B). The findings support the idea that functional inhibition of neuronal populations associated with exploratory behavior, including the ventral tegmental area, dorsal striatum, hippocampus and the hypothalamus could contribute to the behavioral deficits occurring during illness due to inflammatory challenge. Caudal medullary catecholamine projections originating in the VLM and NST provide a link between immune-responsive brainstem regions and forebrain areas that control behavior (Fig. 5).

See page 5 for References.

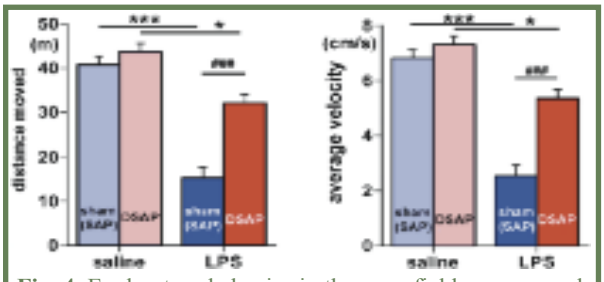

Fig. 4. Exploratory behavior in the open field as measured by total distance travelled and average locomotor speed was reduced by immune challenge (LPS injection). This reduction was largely mitigated in rats that received anti-DBH-SAP (DSAP) injections. * $p < 0.05$; ***, $p < 0.001$, LPS vs. saline; ### $p < 0.0001$. DSAP vs. control.

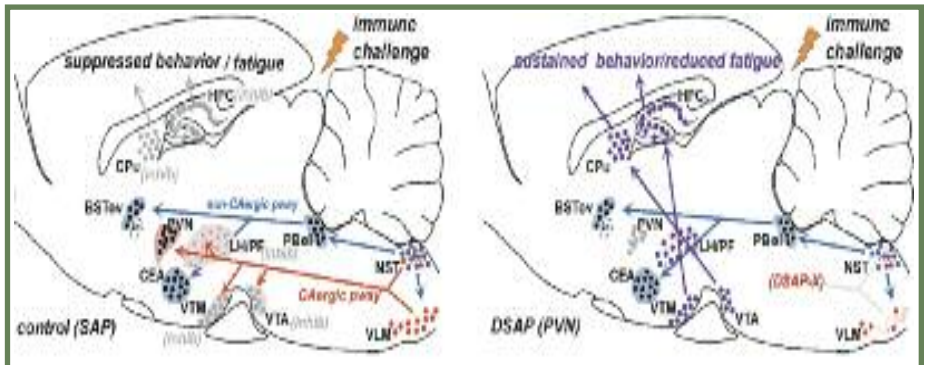

Fig. 5. Model diagram depicting the effects of catecholaminergic projections from the VLM and NST on brain activity -- and ultimately behavior -- as a result of immune challenge (A), which can be prevented by specific lesion of this pathway (B).

Targeting Tools: Featured Products

Anti-DBH-SAP targets dopamine beta-hydroxylase

Anti-dopamine beta-hydroxylase-saporin (anti-DBH-SAP) is a highly specific noradrenergic lesioning agent. It specifically targets rat cells that express dopamine beta-hydroxylase and is also reported to react with mouse protein.[1] This vesicular enzyme is exposed to the exterior milieu upon release of noradrenaline and thus allows these cells to be targeted with saporin.

The specificity of Anti-DBH-SAP correlates well with uptake of the antibody when injected intraventricularly. After systemic administration, animals have a massive reduction in plasma norepinephrine levels, indicating efficient targeting and sympathectomy. Unlike other lesioning methods, this molecular lesioning agent assures definitive ablation of the target neurons.

Permanent and selective removal of cerebral noradrenergic innervations provides an important animal model for the study of drug effects, behavior, plasticity of other systems in response to loss, and primary autonomic failure.

1. Harle P, Pongratz G, Albrecht J, Tarner IH, Straub RH (2008) An early sympathetic nervous system influence exacerbates collagen-induced arthritis via CD4+CD25+ cells. Arthritis Rheum 58:2347-2355.

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Northrop LE, Polston EK, Erskine MS (2010) Noradrenergic nuclei that receive sensory input during mating and project to the ventromedial hypothalamus play a role in mating-induced pseudopregnancy in the female rat. J *Neuroendocrinol* 22(10):1061-71.

Anti-DBH-SAP, Cat. #IT-03

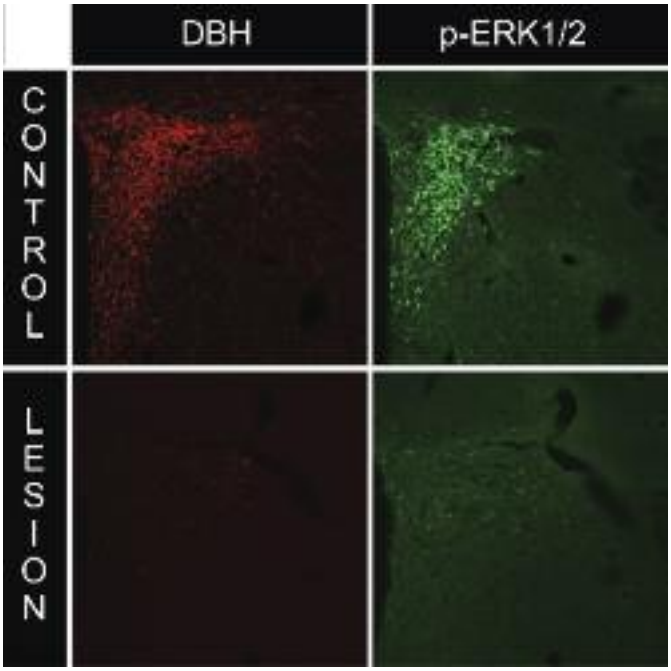
available in these sizes:

25 micrograms, 100 micrograms, and 250 micrograms

Kit includes the following controls in equal amounts:

Saporin, Cat. #PR-01

Mouse IgG-SAP, Cat. #IT-18



Rats receiving PVH injections of anti-DBH-SAP show a marked loss of catecholaminergic inputs, as seen from the drastic reductions in DBH immuno-staining (red signal, left). This loss was accompanied by loss of insulin-induced phospho-ERK1/2 signaling (green signal, right column). Khan *et al. Targeting Trends* 10(2):1,6, 2009.

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, Lutz TA (2010) Noradrenergic neurons of the area postrema mediate amylin's hypophagic action. *Am J Physiol Regul Integr Comp Physiol* 299(2):R623-31.

Vianna DML, Carrive P (2010) Cardiovascular and behavioural responses to conditioned fear and restraint are not affected by retrograde lesions of A5 and C1 bulbospinal neurons. *Neuroscience* 166(4):1210-8.

Worlicek M, Knebel K, Linde HJ, Moleda L, Schölmerich J, Straub RH, Wiest R (2010) Splanchnic sympathectomy prevents translocation and spreading of E coli but not S aureus in liver cirrhosis. *Gut* 59(8):1127-34.

For a complete listing of references, go to:

http://www.atsbio.com/reference/it03.html

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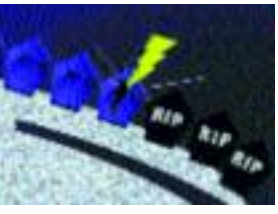

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

GATEFIU

POLARTROXEY

BASTERMIN

DACULA

TARPTEN

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER: It was a . . .

‘ ‘

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Apr-May-Jun 2011
Volume 12, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

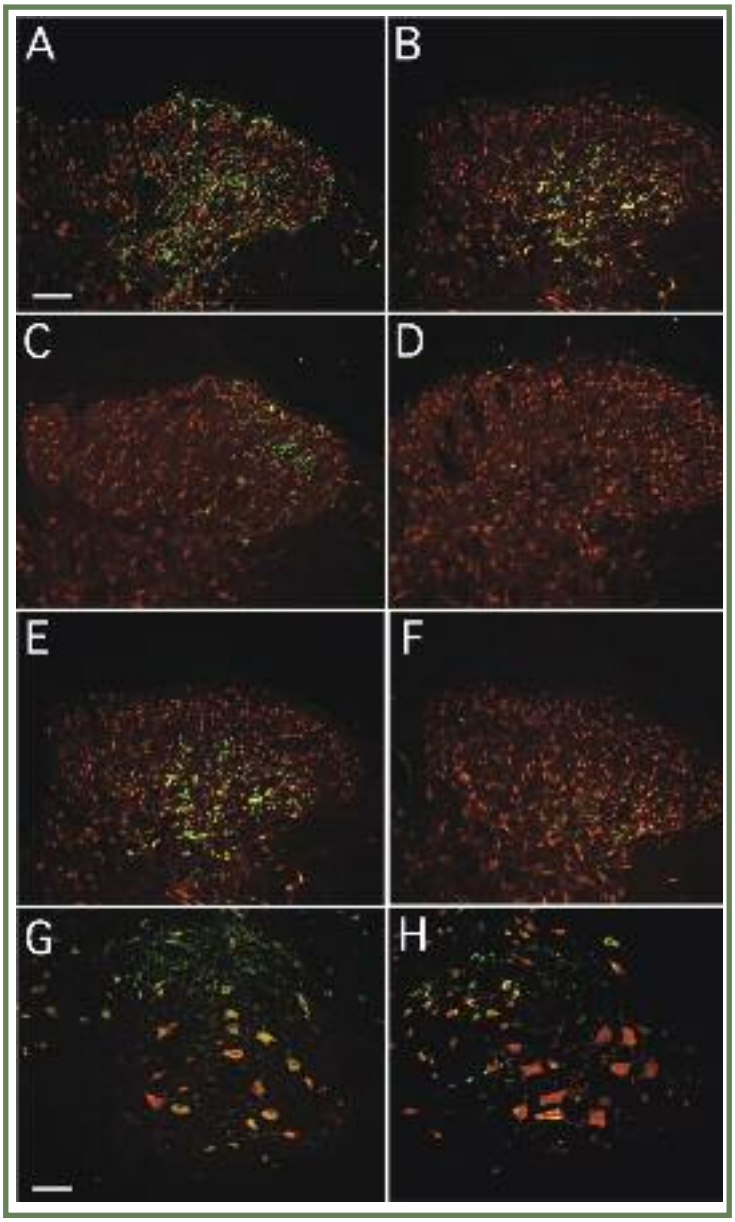
Carrageenan evoked P-Akt in deep dorsal horn neurons is prevented by loss of neurokinin1 positive neurons in superficial dorsal horn

Contributed by L.S. Sorkin, J.-I. Choi, and F.J. Koehrn, Univ. California San Diego, La Jolla, CA

Paw inflammation with carrageenan elicits phosphorylation of Akt in spinal cord neurons with an unusual time course (Choi *et al*., 2010). Activation of Akt is seen first in neurons of the superficial dorsal horn and in α-motor neurons, followed by its appearance as much as 2 hrs after injection in large, deep (lamina V) dorsal horn neurons. P-Akt is thought to be an indicator of neuronal activity and a marker of sensitization. In the present study, we determined whether carrageenan-induced expression of P-Akt in the deeper lamina of the dorsal horn and in ventral horn α-motor neurons required a linkage through neurons in the superficial laminae (I-III).

Rats were given a spinal injection of 100 ng of either non-

(continued on page 6)



Panels A, C, E and G are spinal cord sections taken from SAP-treated animals; B, D, F and H are from SSP-SAP-treated animals.

Panel A vs. B shows loss of NK1 receptor (green) from the superficial, but not deep, dorsal horn, with SSP-SAP treatment.

Panels C/D (dorsal horn) and G/H (ventral horn) were obtained 45 min after paw carrageenan. In SAP, but not SSP-SAP animals, carrageenan induces an increase in P-Akt (green- for C-H) compared to BSA.

The same pattern of carrageenan-induced P-Akt prevented by SSP-SAP pretreatment was seen in deeper laminae of the dorsal horn.

Bar in A applies to Panels A-F = 100 mm; Bar in G applies to G-H = 50 mm; Red staining = NeuN.

Inside this issue:

Newsletter Highlights

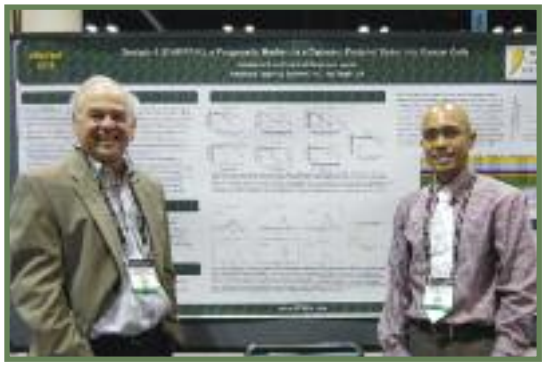
Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Basigin-2 (EMMPRIN), a Prognostic Marker, is a Dynamic Portal of Entry into Cancer Cells

by Leonardo R. Ancheta and Douglas A. Lappi,
American Association of Cancer Research abstract #5218

Basigin-2 (EMMPRIN) is a marker for prognosis of several tumor types. With the identification of the internalization of basigin-2 in response to antibody binding, we now further explore the possibility of its use as a dynamic portal of entry for molecules into cancer cells. We also wish to identify a correlation between number of basigin-2 on the cell surface and half maximal effective concentration (EC_{50}) of a targeted toxin by examining eleven tumor cell lines which encompass various tumor types such as breast (MCF-7, BT549), leukemia (K562, HL-60), multiple myeloma (RPMI-8226), neuroblastoma (SH-SY5Y), non-small cell lung (A549, NCI-H23, NCI-H522), and prostate (PC3). In this investigation, we employed the use of flow cytometry to characterize each cell line according to mean fluorescence as well as to determine the number of basigin-2 molecules expressed on the cell surface through a surface quantification assay. This was visualized by staining with a monoclonal antibody directed to basigin-2 ECD (Ref. 1; Cat. #AB-42) with comparison of results against a standard with known Antibody Binding Capacity (ABC) and standardizing the results into Molecules of Equivalent Soluble Fluorochromes (MESF; Bangs Labs).



Doug Lappi and Leonardo Ancheta
AACR meeting in Orlando, FL

Last year at this meeting we showed the ability of the antibody to basigin-2 ECD to cause internalization of secondary targeted toxins to bring about cell death. We now report on the activity of a primary toxin: the ribosome-inactivating protein saporin attached directly to an antibody to basigin-2 (Cat. #IT-54). Cytotoxicity assays yielded an EC_{50} range of 3.5-420 pM while surface quantification assays resulted in expression that ranged from 1-4 million basigin-2 molecules per cell. Cell lines showing potent EC_{50} values were characterized to have higher surface expression of basigin-2 when compared to cell lines with less potent EC_{50} values. Preliminary results suggest a correlation between EC_{50} and basigin-2 surface expression such that more basigin-2 available on the cell surface would facilitate greater opportunity for a primary toxin to bind and enter into the cell and cause cell death. These data suggest the use of basigin-2 as an entry system for molecules into cancer cells.

1. Belton, RJ Jr, Chen L, Mesquita FS and Nowak RA (2008) Basigin-2 is a cell surface receptor for soluble basigin ligand. J Biol Chem 283:17805-17814.

Upcoming Events

Amer Assoc of Immunologists
May 13-17, 2011
San Francisco, CA
Booth #426

American Pain Society
May 18-21, 2011 • Austin, TX
Booth #229

International Brain Res Org
July 14-18, 2011 • Florence, Italy
Booth #12

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: FATIGUE
EXPLORATORY
BRAINSTEM
CAUDAL
PATTERN

Answer: It was a . . . 'PURR'FECT EXPERIMENT

Congratulations to the puzzle solvers. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Majeeb & Ryoko, Cal State Univ Northridge, CA * Seto Chice, SUNY-HSC at Brooklyn, NY * Kevin Oakley, Uniformed Serv Univ Health Sci, Bethesda, MD * Barbara Attardi, Bioqual Inc, Rockville, MD * Shikha Gaur, City of Hope, Duarte, CA * Shelle Malkmus, Univ Cal San Diego, La Jolla, CA * Bob Speth, Nova Southeastern Univ, Fort Lauderdale, FL * Susan Fischer, Univ Texas MD Anderson Cancer Center, Smithville, TX * Glenn H. Kageyama, Cal State Polytechnic Univ Pomona, CA * Ruth Stornetta, Univ Virginia, Charlottesville, VA

Solve the Teaser online at: www.ATSbio.com/news/11q2_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Anti-amnesic and neuroprotective actions of the sigma-1 receptor agonist (-)-MR22 in rats with selective cholinergic lesion and amyloid infusion.

Antonini V, Marrazzo A, Kleiner G, Coradazzi M, Ronsisvalle S, Prezzavento O, Ronsisvalle G, Leanza G.
J Alzheimers Dis, Epub Feb, 2011.

Sigma-1 receptor agonists such as (-)-MR22 are potential therapeutic drugs for the treatment of cognitive and affective disorders. To model a cognitive disorder, rats received 81-ng bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum/vertical limb of the diagonal band of Broca, and 130-ng bilateral injections into the nucleus basalis magnocellularis. Lesioned animals also were treated with pre-aggregated amyloid peptide. Pretreatment with (-)-MR22 reversed cognitive impairments in the double-lesioned animals, indicating the potential use of sigma-1 receptor agonists as protective agents.

Donepezil plus estradiol treatment enhances learning and delay-dependent memory performance by young ovariectomized rats with partial loss of septal cholinergic neurons.

Gibbs RB, Chipman AM, Nelson D.
Horm Behav Epub Feb, 2011.

Among the beneficial effects of estrogen on the brain are improved cognitive performance and prevention of age-related cognitive decline. These positive effects diminish over time following loss of ovarian function. To investigate the role of cholinergic neurons in this process, rats received 96-250 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septal nucleus followed by a cholinergic enhancer and estradiol therapy. The dual therapy had a positive effect on partially lesioned animals, but did not improve the performance of animals with severe lesions.

Neurotrophin/receptor expression in urinary bladder of mice with overexpression of NGF in urothelium.

Girard BM, Malley SE, Vizzard MA.
Am J Physiol Renal Physiol 300(2):F345-55, 2011.

Chronic overexpression of nerve growth factor (NGF) in the urinary bladder results in neuronal sprouting, increased voiding frequency, and referred somatic hypersensitivity. The authors investigated several growth factor and receptor responses to chronic overexpression of NGF. A p75 antibody (Cat. #AB-N01) was used to obtain the immunohistochemistry data. The data suggest that changes due to NGF overexpression may be a compensatory attempt to reduce voiding frequency.

TrkB (tropomyosin-related kinase B) controls the assembly and maintenance of GABAergic synapses in the cerebellar cortex.

Chen AI, Nguyen CN, Copenhagen DR, Badurek S, Minichiello L, Ranscht B, Reichardt LF.
J Neurosci 31(8):2769-2780, 2011.

In this work the authors investigated the role of TrkB in GABAergic inhibitory synapses in the mouse cerebellar cortex. Using a variety of techniques, including immunohistochemistry utilizing an mGluR2 antibody (Cat. #AB-N32), it was shown that TrkB is required for both assembly and maintenance of these synapses. The primary role of TrkB appears to be regulating the localization of synaptic constituents.

Molsidomine modulates the cNOS activity in an experimental model of cholinergic damage induced by 192-IgG saporin.

Hernandez-Melesio MA, Gonzalez-Esquivel D, Ortiz-Plata A, Sanchez-Mendoza A, Sanchez-Garcia A, Alcaraz-Zubeldia M, Rios C, Perez-Severiano F.
Neurosci Lett 491(2):133-137, 2011.

Nitric oxide (NO) is required for the survival of cholinergic neurons in the basal forebrain. Delivery of nerve growth factor (NGF) is related to the modulation of NO – as excessive NO can lead to excitotoxicity. The authors administered molsidomine to rats that had previously received 220 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum. Molsidomine is a NO donator, and produced a significant recovery of NO activity in lesioned animals, indicating a potential therapeutic pathway.

Histaminergic regulation of seasonal metabolic rhythms in Siberian hamsters.

I'anson H, Jethwa PH, Warner A, Ebling FJ.
Physiol Behav Epub Feb, 2011.

The role of central histaminergic mechanisms on seasonal catabolic state was investigated in hamsters. Siberian hamsters received bilateral 3.8-ng injections of orexin-SAP (Cat. #IT-20; discontinued) into the tuberomammillary posterior hypothalamic region. Saporin (Cat. #PR-01) was used as a control. During long days, lesioned animals displayed higher locomotor activity, greater oxygen intake, and no net weight gain. During shorter days (hibernation) with less activity, lesioned animals did not lose weight. The data indicate that histaminergic neurons are involved in body weight regulation.

Lesion of cholinergic neurons in nucleus basalis enhances response to general anesthetics.

Leung LS, Petropoulos S, Shen B, Luo T, Herrick I, Rajakumar N, Ma J.
Exp Neurol Epub Feb, 2011.

Consciousness and response to general anesthesia have been linked to acetylcholine in the brain. The authors treated rats with 150-ng bilateral injections of 192-IgG-SAP

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

(Cat. #IT-01) into the nucleus basalis magnocellularis to examine this connection. Lesioned animals were more affected by propofol and phenobarbitol than control animals. Some effects of halothane were also increased. The data indicate a role for acetylcholine in the brain in the response to general anesthesia.

PreBötzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression.

Montandon G, Qin W, Liu H, Ren J, Greer JJ, Horner RL.
J Neurosci 31(4):1292-1301, 2011.

In order to identify the neurons involved with respiratory depression due to administration of opioids, some neurotransmission networks in the preBötzinger complex were locally manipulated. Among various techniques used to analyze the results was immunohistochemistry with anti-NK1r (Cat. #AB-N04, discontinued). Results show the preBötzinger complex is responsible for suppression of respiratory rate due to opioids.

Gene regulation in the rat prefrontal cortex after learning with or without cholinergic insult.

Paban V, Chambon C, Farioli F, Alescio-Lautier B.
Neurobiol Learn Mem Epub Feb, 2011.

Microarray technology was used to screen gene expression in a model of attention and memory deficit. Rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum and nucleus basalis magnocellularis (37.5 ng per side and 75 ng per side respectively). Gene expression in memory loss following the lesion was defined by one cluster related to cytoskeleton organization and proliferation, and glial and vascular remodeling. These are processes associated with brain repair after injury.

Human monoclonal antibodies to sialyl-Lewis^a (CA19.9) with potent CDC, ADCC, and antitumor activity.

Sawada R, Sun SM, Wu X, Hong F, Ragupathi G, Livingston PO, Scholz WW.
Clin Cancer Res 17(5):1024-1032, 2011.

In this work the authors investigated the use of a carbohydrate antigen, sialyl-Lewis^a (CA19.9), as a target for cancer therapeutics. Human monoclonal antibodies were generated against CA19.9 and characterized using ELISA and flow cytometry. To assess internalization one antibody, 5B1, was combined with Hum-ZAP (Cat. #IT-22) and applied to CA19.9-expressing BxPC3 cells. The cytotoxicity of the 5B1-Hum-ZAP complex indicates that CA19.9 may be a target for cancer therapy.

Corticotropin-releasing factor critical for zebrafish camouflage behavior is regulated by light and sensitive to ethanol.

Wagle M, Mathur P, Guo S.
J Neurosci 31(1):214-224, 2011.

The authors used the hardwired camouflage response of zebrafish to explore neural circuit assembly and function. Corticotropin-releasing factor is a critical component of this pathway. Immunostaining, done with a CRF antibody (Cat. #AB-02) was part of the data that showed how both light exposure and ethanol affect the camouflage response. Understanding this system could provide a tool to further investigate the effect of alcohol on neural circuits.

Sézary syndrome cells overexpress syndecan-4 bearing distinct heparan sulfate moieties that suppress T-cell activation by binding DC-HIL and trapping TGF-ß on the cell surface.

Chung JS, Shiue LH, Duvic M, Pandya A, Cruz PDJ, Ariizumi K.
Blood 117(12):3382-3390, 2011.

Syndecan-4 (SD-4) is a transmembrane heparan sulfate proteoglycan. The Sézary syndrome (SS) subset of cutaneous T-cell lymphoma overexpresses distinct heparan sulfate moieties, giving the authors a specific target for these cells. Biotinylated DC-HIL-Fc (the extracelluar domain of dendritic cell-associated heparan sulfate proteoglycan-integrin ligand fused to Fc of mouse IgG) was combined at a 1:1 molar ratio with streptavidin-ZAP (Cat. #IT-27). *In vitro*, this targeted toxin eliminated SS cells, preventing their proliferation and suggesting a method for SS treatment.

Selective depletion of Mac-1-expressing microglia in rat subventricular zone does not alter neurogenic response early after stroke.

Heldmann U, Mine Y, Kokaia Z, Ekdahl CT, Lindvall O.
Exp Neurol Epub Mar, 2011.

One result of ischemic stroke is migration of newly formed neuroblasts into the injured area from the subventricular zone (SVZ). The authors investigated the role of microglia, which also accumulate in the SVZ after stroke, in this process. Rats received 5-µg or 10-µg intracerebroventricular injections of Mac-1-SAP (Cat. #IT-33) with varying schedules as to injection and sacrifice. The data indicate that the presence of microglia after stroke does not affect the number or migration of neuroblasts from the SVZ.

Brain stem catecholamines circuitry: Activation by alcohol and role in the hypothalamic-pituitary-adrenal response to this drug.

Lee S, Craddock Z, Rivier C.
J Neuroendocrinol Epub Mar, 2011.

In this work the authors investigated mechanisms underlying the stimulatory effect of alcohol on the hypothalamic-pituitary-adrenal axis (HPA). One method used was 33-ng injections of anti-DBH-SAP (Cat. #IT-03) into the A2/C2/C3 and A1/C1 regions. The data generated show that catecholamines, especially in the brainstem, regulate the HPA response to alcohol. This regulation utilizes α1-adrenergic receptors. Administration of anti-DBH-SAP to the A1-A2/C1-C3 regions disrupted the catecholaminergic input to the paraventricular nucleus.

(continued on page 5)

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Regarding Custom Saporin Conjugations

Q: We recently spoke to you about performing a custom saporin conjugation using our antibody. Is 0.09% azide in PBS in the antibody stock acceptable?

A: There are a number of dialysis steps within the conjugation protocol that will ultimately remove the azide from your antibody solution. So as long as your antibody will be happy in PBS without azide during the procedure, sending the material in 0.09% azide is fine. The final conjugate will be returned to you in PBS, sterile-filtered, without azide.

Q: In general, how many saporin molecules are incorporated per antibody? Can we test this by HPLC?

A: We aim for 2-2.5 moles of saporin per mole of antibody. You should be able to see differences in HPLC between your antibody with one vs. two vs. three saporins attached, however we will provide you with a saporin molar ratio and a product that has had free saporin and free antibody removed from the final conjugate.

Regarding Quinolinic Acid Antibody

Q: We need to have quinolinic acid conjugated to BSA to coat our plates for our experiment. Do you have a protocol to perform this conjugation? We have already purchased your Quinolinic Acid Mouse Monoclonal (Cat. #AB-T170).

A: We can do better than that. You can purchase quinolinic acid pre-conjugated to BSA (Cat. #AG-033).

Regarding Assay Parameters for Mab-ZAP

Q: Using Mab-ZAP in a cytotoxicity assay, I obtained a nice dose-response curve up to around 10^{-9} M of antibody and then lost progressively the toxic effect of Mab-ZAP. What should I do to improve in my assay?

A: If the highest dose for which you got a good response was 10 nM and you lost effect when the primary concentration was increased beyond that, then that is the result we would expect. Often we have seen in cytotoxicity assays that when the primary antibody concentration is raised beyond a certain level — 10-100 nM frequently being that level — there is so much free primary antibody that it competes with the Mab-ZAP-bound antibody for binding sites, thereby reducing the toxic effect. We recommend that you pre-incubate your primary with Mab-ZAP before adding the solution to the wells.

Targeting Topics: Recent Scientific References

(continued from page 4)

Participation of hindbrain AMP-activated protein kinase in glucoprivic feeding.

Li AJ, Wang Q, Ritter S.
Diabetes 60(2):436-442, 2011.

Catecholamine neurons innervating the medial hypothalamus are involved in the control of glucoprivic feeding as well as other responses to glucose deficit. Rats received bilateral 82-ng injections of anti-DBH-SAP (Cat. #IT-03) into the paraventricular hypothalamic nucleus. Saporin (Cat. #PR-01) was used as a control. Lesioned animals did not respond to the administration of a competitive glucose inhibitor, nor did they display phosphorylation of pAMPKα, suggesting that AMPK may be part of a glucose-sensing mechanism.

The effects of intrathecal and systemic gabapentin on spinal substance p release.

Takasusuki T, Yaksh TL.
Anesth Analg 112(4):971-976, 2011.

Intrathecal or systemically-administered gabapentin is an antihyperalgesic. Given that gabapentin binds a voltage-sensitive calcium channel and that some of these channels regulate substance P (SP) release, the authors investigated whether gabapentin affects SP levels. Immunohistochemistry was done in rats following a gabapentin/formalin pain model. A neurokinin-1 receptor antibody (Cat. #AB-N04; discontinued) was used to quantitate NK1r, and therefore assess SP activity. It was found that both spinal and systemic gabapentin inhibit SP release from small, primary afferents.

Send a message on our website or email ats@ATSbio.com for answers to your targeting questions.

Carrageenan evoked P-Akt in deep dorsal horn neurons is prevented by loss of neurokinin1 positive neurons in superficial dorsal horn

(continued from page 1) targeted Saporin or [$Sar^{9}Met(O_2)^{11}$] substance P coupled to saporin (SSP-SAP, Cat. #IT-11). Injection was at the level of the thoraco-lumbar junction. It has been reported that this treatment results in loss of neurons with NK1 receptors (substance P receptors) in the superficial, but not the deep dorsal horn (Wiley *et al*., 2007). Two weeks post-injection, rats were tested for locomotor ability using a rotorod or for carrageenan-induced cutaneous sensitization to mechanical stimuli. Some animals with paw carrageenan were perfused at 45 min or 2 hrs post injection and their lumbar spinal cords processed and reacted for NK1 receptor, P-Akt and a variety of cell markers.

Immunohistological staining demonstrated that NK1 receptor was gone from lamina I-III ($p \leq 0.01$) of the dorsal horn with no loss in lamina V compared to SAP-pretreated animals. SSP-SAP animals had no carrageenan-associated induction of P-Akt in any spinal lamina at any time point. Behavioral testing indicated a significant loss in mechanical sensitization compared to the SAP animals ($p \leq 0.001$), with no loss of motor ability. Despite this, SSP-SAP animals still had substantial mechanical sensitization ($p \leq 0.001$), which peaked 2 hrs after paw injection. We interpret these data as meaning that loss of NK1 receptor bearing neurons in the superficial dorsal horn blocks a large component of spinal sensitization. It is likely that paw carrageenan-induced expression of P-Akt in motor neurons requires an excitatory interneuronal link, which is not required for normal locomotor activity. Inflammation-induced activation of P-Akt in lamina V also requires a superficial dorsal horn linkage (either via excitatory interneurons or the lamina I projection neurons). Allodynia seen in the SSP-SAP animals is probably due to peripheral sensitization of primary afferent fibers and a resultant heightened afferent drive, rather than to a significant spinal sensitization component.

References

1. Choi JI, Svensson CI, Koehrn FJ, Bhuskute A, Sorkin LS (2010) Peripheral inflammation induces tumor necrosis factor dependent AMPA receptor trafficking and Akt phosphorylation in spinal cord in addition to pain behavior. *Pain* 149:243-253.
2. Wiley RG, Kline RHt, Vierck CJ, Jr. (2007) Anti-nociceptive effects of selectively destroying substance P receptor-expressing dorsal horn neurons using [Sar^{9},$Met(O_2)^{11}$]-substance P-saporin: behavioral and anatomical analyses. *Neuroscience* 146:1333-1345.

Note: Dr. Sorkin is a runner-up for the 2010 SfN Poster of the Year Award. We are delighted she was able to provide this cover article.

ATS Welcomes Our Newest Members to the Targeting Team!

Khrysten Taylor and David Young
Providing support in the laboratory!

Miranda Trevathan and Brandon Preddy
Taking your orders with a smile!

It's an important part of life to enjoy your work and the people you work with. Here at ATS we are extremely fortunate and privileged to have a great group to work hard with and then to play ping pong with at Friday Happy Hours. The thing that drives and motivates us is serving our customers throughout the world. We are proud to have an innovative technology that can be put to such creative use by scientists in a variety of research fields.

Thank you for allowing us to provide you with quality products and service. If there's anything we can do to help you further your research goals, please let us know. Our Product Managers are here to answer your technical questions: Matt Kohls, Brian Russell, and Leonardo Ancheta. Kristen Hartman keeps our website and databases running smoothly so we can process your orders accurately. Doug Lappi, as President and Chief Scientific Officer is always excited to hear your new targeting ideas. Stop by and visit Denise Higgins, Vice President and *Targeting Trends* editor, at one of our upcoming trade shows.

Targeting Tools: Featured Products

Basic Fibroblast Growth Factor Human Recombinant

Basic fibroblast growth factor (FGF-2) is a member of the fibroblast growth factor family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This protein functions as a modifier of endothelial cell migration and proliferation, as well as an angiogenic factor. It acts as a mitogen for a variety of mesoderm- and neuroectoderm-derived cells *in vitro*, thus is thought to be involved in organogenesis.

FGF-2 Human Recombinant, Cat. #PRP-218
available in these sizes:
10 micrograms, 50 micrograms, and 1 milligram

Related Products:
FGF-2 Rat Recombinant, Cat. #PR-09
Antibody to mammalian FGF-2, Cat. #AB-07
Antibody to rat FGF-2, Cat. #AB-08
mammalian FGF-SAP, Cat. #IT-38

Leptin Human Recombinant

Leptin is a 16-kDa peptide hormone secreted from white adipocytes and implicated in the regulation of food intake and energy balance. It provides the key afferent signal from fat cells in the feedback system that controls body fat stores.

Leptin Human Recombinant, Cat. #PRP-328
available in these sizes:
200 micrograms, 1 milligram, and 5 milligrams

Related Products:
Antibody to human Leptin, Cat. #AB-524
mouse Leptin-SAP, Cat. #IT-47

Gangsta has his own personal drinking fountain. Perched on marble, surrounded by orchids, that's the life!

Granulocyte Macrophage-Colony Stimulating Factor Human Recombinant

Granulocyte Macrophage Colony Stimulating Factor (GM CSF) is a cytokine that controls the production, differentiation, and function of granulocytes and macrophages. The active form of the protein is found extracellularly as a homodimer. This gene has been localized to a cluster of related genes at chromosome region 5q31, which is known to be associated with interstitial deletions in the 5q- syndrome and acute myelogenous leukemia. Other genes in the cluster include those encoding interleukins 4, 5, and 13. GM CSF stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes.

GM-CSF Human Recombinant, Cat. #PRP-221
available in these sizes:
2 micrograms, 10 micrograms, and 1 milligram

Related Products:
GM-CSF Human Recombinant - Pichia, Cat. #PRP-222
GM-CSF Human Recombinant - Sf9, Cat. #PRP-223
GM-CSF Human Recombinant - His tag, Cat. #PRP-224
Antibody to human GM-CSF, Cat. #AB-452

Growth Hormone Human Recombinant

Growth Hormone (GH) is a member of the somatotropin/prolactin family of hormones which play an important role in growth control. The gene, along with four other related genes, is located at the growth hormone locus on chromosome 17 where they are interspersed in the same transcriptional orientation; an arrangement which is thought to have evolved by a series of gene duplications. This particular family member is expressed in the pituitary but not in placental tissue as is the case for the other four genes in the growth hormone locus. Mutations in or deletions of the gene lead to growth hormone deficiency and short stature.

Growth Hormone
Human Recombinant, Cat. #PRP-202
available in these sizes:
100 micrograms, 500 micrograms, and 1 milligram

Related Products:
Antibody to Growth Hormone IgG_1, Cat. #AB-458
Antibody to Growth Hormone IgG_{2b}, Cat. #AB-457

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Targeting Technology

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

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ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

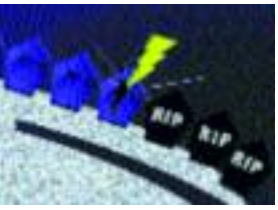
§ or anything recognized on the cell surface and internalized.

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

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

Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

FLIERSCUPIA

RINKNONEUI

FLINTMAMIONA

JOINTINEC

VISITOPE

What you have to be to figure out which hybrid is the greenest.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER: A . . .

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by June 15, 2011.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 2.

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Jul-Aug-Sep 2011
Volume 12, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Cell-specific targeting and removal: cheaper and quicker than knockouts with high impact results

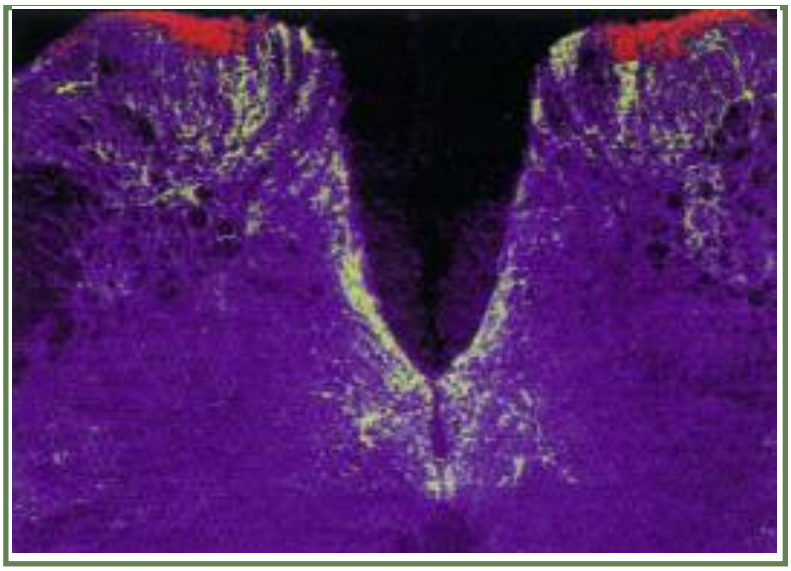
Contributed by Douglas A. Lappi, Ph.D., Advanced Targeting Systems, San Diego, CA USA

Knockout models are helpful tools in scientific research. They have been useful in studying and modeling in all sorts of research in biology; so much so that the pioneers--Smithies, Capecchi and Evans--won a well-deserved Nobel Prize for Physiology and Medicine in 2007. But there are some reasons not to go down that path:

1) About 15% of gene knockouts are developmentally lethal (from www.genome.gov).

2) According to information posted on a major university's core facility website, it will take a minimum of 40 weeks to produce a knockout mouse. The cost for this best-case scenario is at least $11,000. And that doesn't include the time and money spent for the molecular biology construction.

3) Statistics published by university core facilities range from 10% to 50% success rate in producing gene-based knockout models.



Internalization of Substance P receptor (SPR) after injection of SP-SAP into the cerebrospinal fluid. This pseudo-color figure shows SPR in red after concentration due to internalization. Lesser concentrated SPR, and also that which is still on the surface membrane is shown in yellow.

This figure illustrates how targeted toxins can be used to 'knockout' specific, cell surface-based targets.

4) You are pretty much limited to mice. Of course, if you want to spend more, you can try rats.

That's a lot of time and money for a grad student or post-doc to find out, "Gee, that didn't give me a high impact result."

Here's an alternative that has a high rate of success, can give high impact results, takes about 10 days to see behavioral results, and costs $200 to $700 to treat about 20 mice. Or you can use rats. Or ferrets (see IBRO abstract, page 2). Or many other species.

(continued on page 6)

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Advanced Targeting Systems Opens Incubator Labs - San Diego Biotechnology Center

by Denise Higgins

Last month, Advanced Targeting Systems expanded its space into Suite 200, a 6673-square foot space right next door to our current space. This facility will primarily be used as an incubator space for new biotechnology businesses to set up laboratories.

When ATS began 17 years ago, we were fortunate enough to have two other biotechnology businesses help us out with space and shared equipment/ services. It's our turn to pay it forward and help out other new companies. In 1994, what was then known as Chemicon International invited us to set up a laboratory space in their facility in Temecula, California, about 60 miles north of our current facility. It was a long commute for Dr. Lappi, but much appreciated. Converting someone's garage was about the only option our small company could afford.

In 1997, ATS subleased some laboratory space from what was then known as Invitrogen Corporation in Carlsbad, California, about 15 miles north of our current facility. The shorter commute allowed Dr. Lappi to spend more time in the laboratory and for ATS to hire its second scientist, Matthew Kohls.

San Diego Biotechnology Center occupies an adjoining suite next to the ATS facility.

The invaluable opportunities ATS had to share laboratory space with bigger, more established companies allowed us to learn the ins and outs of how an entire operation needs to function: ordering, facilities, shipping. Our biotech hosts shared their expertise, equipment, facilities, and supplies to teach ATS important lessons about becoming a successful company.

With that history, we moved into our own facility in 1999, and from the beginning opened our laboratory to sub-tenants to occupy a lab bench and get started on their journeys to becoming independent companies. ATS is pleased now to expand that service to offer three research spaces (500 to 3000 sq ft) to young biotech companies. There are shared conference room/kitchen space, cell culture, reception, restrooms, and storage areas. Each quarter ATS will host a meeting with the San Diego Biotechnology Center companies to share scientific ideas and business issues. Two of the lab spaces have been subleased and ATS plans to lease the third space in early 2012.

Abstracts at the 8th IBRO World Congress of Neuroscience Florence, Italy - July 14-18 2011

Congratulations to scientists with abstracts using ATS products. Listed below are excerpts from their submissions.

A central role for BDNF and Sonic Hedgehog in controlling synaptic plasticity in motoneuron-depleted spinal cord

R. GULINO & M. GULISANO, University of Catania, Department of Bio-Medical Sciences, Section of Physiology, Catania, Italy

Here, we measured the expression levels of several proteins involved in synaptic plasticity and motoneuronal function (ChAT, Synapsin-I, Shh, Notch-1, AMPA receptor subunits, NMDA receptor and BDNF) in a mouse SC lesion model obtained by intramuscular injection of Cholera toxin-B-saporin, which selectively kills motoneurons. (CTB-SAP, Cat. #IT-14)

Cholinergic denervation disrupts temporal learning in rodent visual cortex

E.B. ROACH & M.G. HUSSAIN SHULER, Johns Hopkins University School of Medicine, Neuroscience, Baltimore, United States

Local cholinergic terminals were removed using the selective neurotoxin 192 IgG-saporin between contingency reversal. This manipulation tested the necessity of cholinergic innervation in two key processes: expressing previously learned reward timing and shifting reward timing to new behaviorally relevant intervals. (192-IgG-SAP, Cat. #IT-01)

The role of cholinergic cortical modulation in visual and olfactory attention using the 5-Choice serial reaction time task

V. LJUBOJEVIC[1], P. LUU[2] & E. DE ROSA[1], [1]University of Toronto, Psychology, Toronto, Canada, [2]University of Toronto, Toronto, Canada

After successful acquisition of both visual and olfactory task, the rats were subjected to either a cholinergic immunotoxic or sham lesion surgery of the NBM. Cholinergic deafferentation of the neocortical mantle was induced by bilaterally infusing the cholinergic immunotoxin, 192 IgG-saporin, into the NBM (0.2 µl of 0.2 µg/µl per site; two sites per hemisphere). (192-IgG-SAP, Cat. #IT-01)

The cholinergic basal forebrain in the ferret and its inputs to the auditory cortex

V.M. BAJO LORENZANA, N.D. LEACH, P.M. CORDERY, F.R. NODAL & A.J. KING, Univ Oxford, Physiol, Anat, Genetics, Oxford, UK

Projections from the NB to the auditory cortex were investigated by injecting tracers into the NB itself (n=5), or by applying tracer deposits to the surface of the auditory cortex (n=4). Tracers included Rhodamine, Fluorescein, Cascade Blue, as well as the cholinergic immunotoxin ME20.4-SAP. Both ME20.4-SAP injections in the auditory cortex and epipial tracer deposits revealed that NB provides the main cholinergic input to the cortex, and that this projection is predominantly ipsilateral. (ME20.4-SAP, Cat. #IT-15)

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

BDNF concentrations are decreased in serum and parietal cortex in immunotoxin 192 IgG-Saporin rat model of cholinergic degeneration.

Angelucci F, Gelfo F, Bartolo PD, Caltagirone C, Petrosini L.
Neurochem Int Epub, 2011.

Brain-derived neurotrophic factor (BDNF) plays a role in neuronal function during the degeneration of neurons caused by pathological conditions such as Alzheimer's disease. In order to investigate the relationship between brain and serum BDNF levels the authors administered 2 µg of 192-IgG-SAP (Cat. #IT-01) into each lateral ventricle of rats and measured brain and serum BDNF levels by ELISA. It was found that BDNF levels dropped in lesioned animals, but not until 15 days post surgery.

Enhanced control of attention by stimulating mesolimbic-corticopetal cholinergic circuitry.

St Peters M, Demeter E, Lustig C, Bruno JP, Sarter M.
J Neurosci 31(26):9760-9771, 2011.

Motivation and attention interact to preserve cognitive performance under challenging conditions. In order to better define the circuitry connecting these two processes, the authors lesioned the prefrontal cortex (200 ng of 192-IgG-SAP, Cat. #IT-01) and the posterior parietal cortex (280 ng of 192-IgG-SAP). Mouse IgG-SAP (Cat. #IT-18) was used as a control. The data indicate that cholinergic projections to the cortex modulate detection of clues and filtering of distractors during attentional tasks, accentuating cognitive control.

Selective formation of covalent protein heterodimers with an unnatural amino acid.

Hutchins BM, Kazane SA, Staflin K, Forsyth JS, Felding-Habermann B, Smider VV, Schultz PG.
Chem Biol 18(3):299-303, 2011.

This work demonstrates the creation of a variety of constructs containing specific defined conjugation sites. One use for these molecules is to create homogenous antibody conjugates – meaning the properties of these conjugates can be quantitatively evaluated. Having greater control of such conjugations is essential if these types of constructs are to move toward use as therapeutics. The authors created an anti-Her2 Fab-saporin molecule and tested it *in vitro*. Analysis by western used anti-SAP-HRP (Cat. #AB-15-HRP) to detect the conjugated molecule.

A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response.

Radley JJ, Sawchenko PE.
J Neurosci 31(26):9683-9695, 2011.

In order to better understand how response to emotional stress is regulated, the authors injected 114 ng of GAT-1-SAP (Cat. #IT-32) into each side of the anterior bed nucleus of the stria terminalis. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The results suggest that medial prefrontal cortex and hippocampal formation influences on stress regulation use the same access to modulate emotional stress rather than having parallel networks.

Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity.

King T, Qu C, Okun A, Mercado R, Ren J, Brion T, Lai J, Porreca F.
Pain Epub, 2011.

Whether exaggerated pain response to a normally innocuous tactile stimulus should be defined as allodynia has been debated. Through the use of several techniques, one of which was intrathecal injection of SSP-SAP (Cat. #IT-11, 16.5 pg), the authors examined which pathways were utilized in this type of pain. Blank-SAP (Cat. #IT-21) was used as a control. The data indicate that tactile stimulation may reflect a different pain state than allodynia.

Synaptic plasticity and pain: role of ionotropic glutamate receptors.

Larsson M, Broman J.
Neuroscientist 17(3):256-273, 2011.

This review discusses the role of glutaminergic sensory synapses in pain hypersensitivity caused by tissue or nerve injury. The focus is on the roles of ionotrophic glutamate receptors, and how they are involved in dorsal horn synaptic plasticity. The role of substance P in such mechanisms is briefly discussed, as elucidated by the use of SP-SAP (alternative: SSP-SAP; Cat. #IT-11).

Involvement of Tuberomamillary Histaminergic Neurons in Isoflurane Anesthesia.

Luo T, Leung LS.
Anesthesiology Epub, 2011.

Although previous studies indicate that histaminergic neurotransmission may mediate reaction to general anesthesia, it is not clear whether the histominergic tuberomammilary nucleus (TMN) is involved. Rats received 250-ng infusions of orexin-SAP (discontinued) into the TMN after which the righting reflex was assessed for several anesthetics. Loss of histaminergic neurons in the TMN only altered the effect of isoflurane – suggesting that the neural circuits involved in isoflurane anesthesia are different than circuits affected by propofol, pentobarbital, and ketamine.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Signal peptide-regulated toxicity of a plant ribosome-inactivating protein during cell stress.

Marshall RS, D'Avila F, Di Cola A, Traini R, Spano L, Fabbrini MS, Ceriotti A.
Plant J 65(2):218-229, 2011.

Type I ribosome inactivating proteins (RIPs) are thought to have a role in defending plants against viral or fungal infections. Most type I RIPs have signal peptides for insertion into the endoplasmic reticulum, followed by transportation to a vacuole or the cell wall. The authors examined signal peptide regulation under stress in tobacco plants transfected with saporin. One method of analysis was western blots using anti-saporin (Cat. #AB-15).

Activation of immobility-related hippocampal theta by cholinergic septohippocampal neurons during vestibular stimulation.

Tai SK, Ma J, Ossenkopp KP, Leung LS.
Hippocampus Epub, 2011.

The vestibular system is highly involved with spatial navigation and memory. It is thought that modulation of hippocampal function by the vestibular system is mediated by a hippocampal theta rhythm. Rats received 140 ng of 192-IgG-SAP (Cat. #IT-01) infused into the medial septum, followed by measurement of hippocampal EEG's and evoked potentials. Theta was attenuated in rats receiving the lesion, as well as other changes that suggest the importance of septohippocampal cholinergic activity in sensorimotor processing and spatial memory.

Ventilatory Effects of Substance P-Saporin Lesions in the Nucleus Tractus Solitarii of Chronically Hypoxic Rats.

Wilkinson KA, Fu Z, Powell FL.
Am J Physiol Regul Integr Comp Physiol Epub, 2011.

Interaction of the multiple brainstem areas that have been established as CO_2-sensitive is not well understood. In order to investigate chemoreceptor roles in the nucleus tractus solitarii (NTS) the authors injected 2.6 ng of SP-SAP (alternative: SSP-SAP; Cat. #IT-11) into the caudal NTS of rats. Blank-SAP (Cat. #IT-21) was used as a control. The results indicate that neurokinin-1 receptor-expressing cells in the NTS contribute to plasticity during chronic hypoxia.

SIGLEC12: A human-specific segregating (PSEUDO) gene encodes a signaling molecule expressed in prostate carcinomas.

Mitra N, Banda K, Altheide T, Schaffer L, Johnson-Pais TL, Beuten J, Leach RJ, Angata T, Varki N, Varki A.
J Biol Chem Epub, 2011.

Siglec 12 (sialic acid-binding immunoglobulin-like lectin 12) is a sugar molecule that has mutated in humans to be inactive, but is active in other primates. The human version is found on some macrophages, various epithelial cell surfaces, and some human carcinoma cell lines. Using Mab-ZAP (Cat. #IT-04) and monoclonal antibodies against Siglec 12, the researchers demonstrated binding and internalization in a prostate cancer cell line, indicating that Siglec 12 may be a target for some cancer therapies.

Don't see your publication here? Send us a PDF at ats@ATSbio.com and we'll be sure to review it in our next issue of *Targeting Trends*.

Targeting Talk: Product Questions

Secondary Conjugates

Q: I would like to know if the secondary antibody used to prepare Mab-ZAP (Cat. #IT-04) reagent binds to heavy chain of mIgG's (only) or if it recognizes light chains as well?

A: Mab-ZAP will recognize whole IgG and will bind to both the heavy and light chain.

* * *

Q: We've been using Mab-ZAP to test our primary mouse monoclonal antibody in cytotoxicity assay. Relative to the Primary mAb-Mab-ZAP complex, the Mab-ZAP alone has quite a bit of activity (40-60% growth inhibition) at the recommended concentrations used (45 or 100 ng/well) or even half that dose. Even though we get a dose response, the higher concentrations of primary antibody give me less cytotoxic activity than the lower concentrations. Can you give your thoughts on this matter?

A: The effect you are seeing is something that is actually typical and indicates that you are using the material correctly. Described in Kohls MD, Lappi DA (2000) *BioTechniaues* 28(1):162-165, unbound primary antibody will compete with primary antibody bound to a secondary conjugate and may reduce cytotoxicity through competitive inhibition of the primary antibody-Mab-ZAP complex. This is especially noticeable with our Fab-ZAP line of secondary conjugates.

We still recommend that our customers try a 10 nM dose as a starting point, but always recommend adjusting the concentration to better accommodate their experiments. As a reference, our data sheets show a cytotoxicity curve with a starting concentration of 10 nM and an ending concentration of 1 fM.

* * *

Send a message on our website or email ats@ATSbio.com for answers to your targeting questions.

Secondary Conjugates

Anti-6 His-ZAP (IT-52)
Goat-ZAP (IT-36)
Anti-GFP-ZAP (IT-53)
Hug-M-ZAP (IT-43)
Anti-M-ZAP (IT-30)
Hum-ZAP (IT-22)
Fab-ZAP human (IT-51)
Mab-ZAP (IT-04)
Fab-ZAP mouse (IT-48)
Rab-ZAP (IT-05)
Fab-ZAP rabbit (IT-57)
Rat-ZAP (IT-26)
Fab-ZAP rat (IT-55)
Streptavidin-ZAP (IT-27)

Other Products

Q: I am using your Bombesin-SAP (Cat. #IT-40) to kill GRP-receptor in mouse brain. I have a plan to inject it by iontophoresis. Do you know which charge dose Bombesin-SAP and Blank-SAP (Cat. #IT-21) have; plus charge or negative charge?

A: Both of these products will have a negative charge, though you may have to look into the literature for any needed guidance on dosing with that type of delivery.

* * *

Q: We are setting up some experiments in rat where we'd use your 192-IgG-SAP (Cat. #IT-01) in cytotoxicity assays. How much material do we need to order?

A: You can find protocols for calculating the amount of material needed for a cytotoxicity assay and protocols for the assay and interpretation of results on our website. Just click on the PROTOCOLS link on our home page.

Other Products

Bombesin-SAP (IT-40)
Blank-SAP (IT-21)
192-IgG-SAP (IT-01)

Cell-specific targeting and removal: cheaper and quicker than knockouts with high impact results

(continued from page 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. So you don't have to wait 40 weeks to even start your experiments. People usually begin immunohistochemistry after a couple of weeks.

The Cost

Let's say you're using a rat. Well, there's the cost of the rat and its boarding. Often people use 100 ng of the targeted toxin. Since the price is usually $350 per 25 micrograms, for a single injection that would be $1.40 per animal. Then there's the 2-week wait, the cost of the behavioral experiment, and the IHC. That's less than $11,000. Much less. And then publish in *Science, Nature, Journal of Neuroscience, Diabetes, Cancer Research, Endocrinology, Journal of Immunology*, or many others.

The Animal Model

Using targeted toxins, you can begin with a fully mature, healthy animal. And the result is a fully mature, healthy animal, but with something missing which usually doesn't affect its overall health and the animal is ready to run through mazes.

Now you know you've got a choice.

International Brain Res Org
July 14-18, 2011
Florence, Italy
Booth #12

Upcoming Events

Society for Neuroscience
November 12-16, 2011
Washington, DC
Booth #616

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: SUPERFICIAL
NEUROKININ
INFLAMMATION
INJECTION
POSITIVE

Answer: A . . . ROCKET SCIENTIST

Congratulations to the puzzle solvers. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Kim Van Vliet, University of Florida, Gainesville, FL * Shikha Gaur, City of Hope, Duarte, CA * Glenn H. Kageyama, Cal Poly Pomona University, Pomona, CA * Seto Chice, SUNY Health Science Center, Brooklyn, NY * E. Polin-Purch, Montefiore Hospital, Bronx, NY

Solve the Teaser online at: www.ATSbio.com/news/11q3_teaser.html

Targeting Tools: Featured Products

Antibody Conjugates

NEW!

Angiotensin II, 50 micrograms

AB-N25AP: AngII AT-1A affinity purified rabbit pAb
FL-N25AP: Alexa488-conjugated
BT-N25AP: Biotin-conjugated
AB-N26AP: AngII AT-1B affinity purified pAb
FL-N26AP: Alexa488-conjugated
BT-N26AP: Biotin-conjugated
AB-N27 AP: AngII AT-1 affinity purified rabbit pAb
FL-N27AP: Alexa488-conjugated
BT-N27AP: Biotin-conjugated
AB-N28 AP: AngII AT-2 affinity purified rabbit pAb
FL-N28AP: Alexa488-conjugated
BT-N28AP: Biotin-conjugated

Dopamine Transporter, 100 micrograms

AB-N17: Anti-DAT-ECD rat mAb
FL-N17: Alexa488-conjugated
BT-N17: Biotin-conjugated
AB-N18: Anti-DAT-NT rat mAb
FL-N18: Alexa488-conjugated
BT-N18: Biotin-conjugated

Mac-1 (CD11b), 100 micrograms

AB-N05: Mac-1 rat mAb
FL-N05: Alexa488-conjugated
BT-N05: Biotin-conjugated
AB-N06: Mac-1 mouse mAb
FL-N06: Alexa488-conjugated
BT-N06: Biotin-conjugated

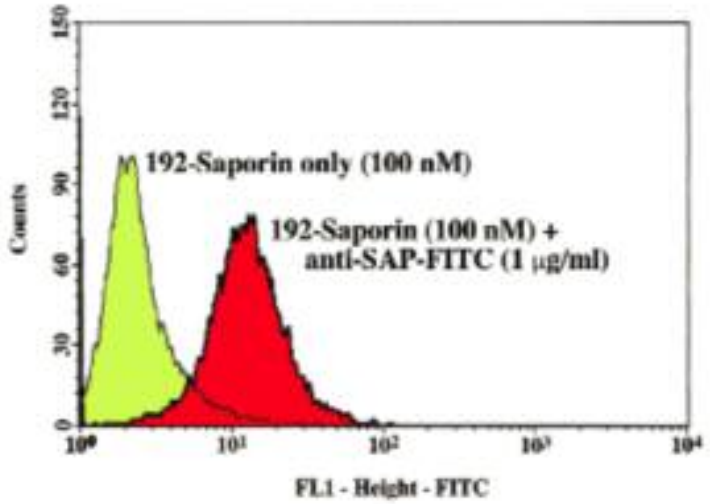
GAD65, 50 micrograms

AB-N12: GAD65 (B78) human mAb
FL-N12: Alexa488-conjugated
BT-N12: Biotin-conjugated
AB-N13: GAD65 (B96) human mAb
FL-N13: Alexa488-conjugated
BT-N13: Biotin-conjugated

Somatostatin Receptor, 50 micrograms

AB-N20AP: SSTr-1 affinity purified rabbit pAb
FL-N20AP: Alexa488-conjugated
BT-N20AP: Biotin-conjugated
AB-N21AP: SSTr-4 affinity purified rabbit pAb
FL-N21AP: Alexa488-conjugated
BT-N21AP: Biotin-conjugated

visit www.ATSbio.com
for a complete list of products



Flow Cytometry using Cat. #FL-02, Anti-Saporin-FITC

Other Antibodies
Now available as Alexa488 or Biotin Conjugates

AB-18: Anti human-CD25 mouse mAb
AB-19: Anti mouse-CD25 rat mAb
AB-N07: NGFr (ME20.4, p75) mouse mAb
AB-N08: OX7 (Anti-Thy 1.1) mouse mAb
AB-15 AP: Saporin affinity purified goat pAb
AB-17 AP: Saporin affinity purified chicken pAb
AB-42: Basigin-2 mouse mAb
AB-N03: TrkA, protein A purified rabbit pAb
AB-16AP: GHRH/GHRF affinity purified rabbit pAb

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Cells that do not have the receptor will not be affected.

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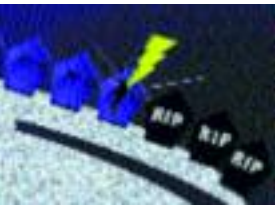

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

TOKUNKCO

☐☐☐◯☐☐☐☐

TELLAH

◯☐☐◯☐☐

CATTISISTS

☐☐◯☐☐◯☐◯☐☐

VIRALEHOAB

◯◯☐☐☐☐☐☐◯☐

FRIENDCEEF

☐☐◯☐☐☐◯☐☐☐

What Leonardo thought as he pondered the Vitruvian man in the hot summer sun.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER: ☐☐☐☐'☐ ☐ ☐☐☐☐☐

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by September 30, 2011.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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Oct-Nov-Dec 2011
Volume 12, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity

Contributed by Tamara King and Frank Porreca
Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ, USA

A common symptom of patients with neuropathic pain is spontaneous pain, often described as burning. Some patients also suffer from pain elicited by normally non-noxious stimuli, such as touch, referred to as allodynia. Preclinical studies have relied on enhanced withdrawal responses to normally innocuous tactile stimuli or noxious thermal stimulation. As such, we have learned much about mechanisms driving gain of function responses to evoked stimuli. Indeed, such measures are used as the main translation feature of experimental neuropathic pain models. An important criticism directed against animal models of pain is that behavioral assessment depends on reflexive evoked responses to innocuous or noxious external stimuli. However, the primary complaint from pain patients is that of ongoing pain, i.e., pain that is independent of an external stimulus. As such, the reliance of testing protocols on evoking a nocifensive response has been considered a significant barrier for development of new therapies for pain treatment.

We have recently developed an approach that is based on the knowledge that relief of pain is rewarding in humans. We hypothesized that chronic pain produces an aversive state providing persistent and strong behavioral motivation to seek relief that is rewarding. Reward achieved from removal of an aversive stimulus is denoted as "negative reinforcement" and is applicable to alleviation of the aversive state induced by chronic pain. We explored this concept using the well-characterized conditioned place preference (CPP) assay, in which pairing pain relief with a distinct context was demonstrated to result in increased time spent in that context.[1]

(continued on page 2)

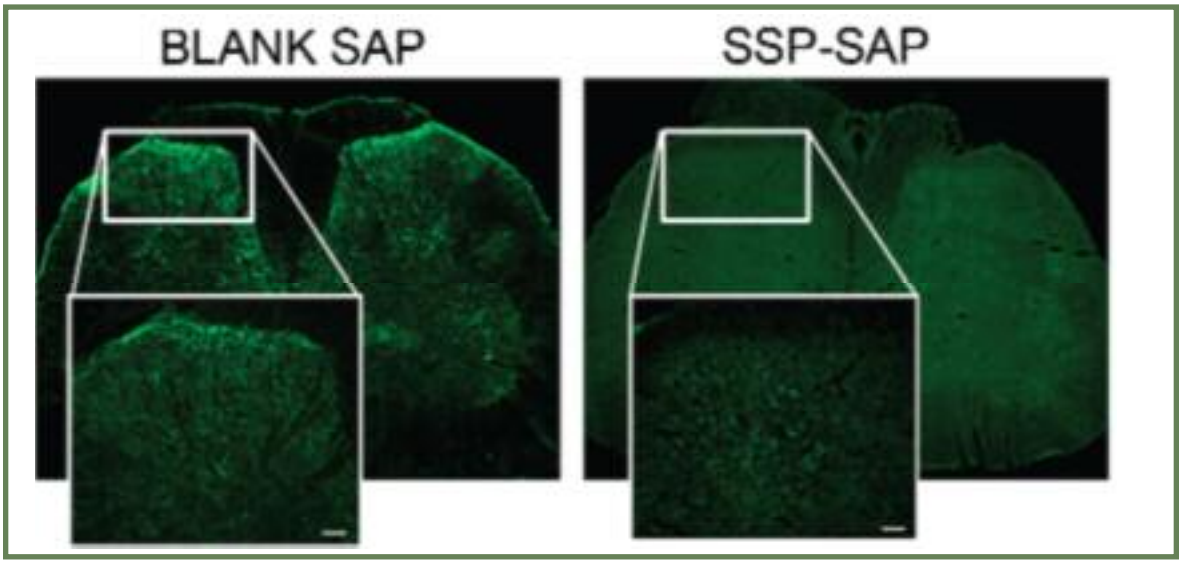

Figure 1. Representative image showing immunofluorescent staining of NK-1 receptors within the spinal dorsal horn 40 days after spinal administration of Sar-Substance P saporin (SSP-SAP) or control injection (Blank-SAP). SSP-SAP-treated rats showed greatly diminished NK-1 immunopositive staining. Animals that received the control injection (Blank-SAP) show clear labeling of the NK-1 receptor.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

SfN Abstracts Using ATS Products • Nov 12-16 Washington DC

SATURDAY, November 12

1-2pm #37.13/D4 M Parent *et al.*
IT-01 192-IgG-SAP

#88.05/TT17 S Ritter *et al.*
IT-03 DBH-SAP, PR-01 Saporin

2-3pm #37.06/C47 M Cyr *et al.*
IT-01 192-IgG-SAP

#47.02/N7 M Pacheco-Herrero *et al.*
IT-16 mu p75-SAP

#86.06/SS9 M A Holschbach *et al.*
IT-03 Anti-DBH-SAP

SUNDAY, November 13

8-9am #179.17/OO8 G F Corder *et al.*
IT-28 NPY-SAP

9-10am #199.22/WW70 S P Sinha *et al.*
IT-32 GAT1-SAP

11am-12 #179.16/OO7 R R Donahue *et al.*
IT-28 NPY-SAP

1-2pm #249.09/T3 C A Rossi *et al.*
IT-11 SSP-SAP

2-3pm #244.02/I6 C A Spuz *et al.*
IT-01 192-IgG-SAP

3-4pm #294.07/VV39 V Ljubojevic *et al.*
IT-01 192-IgG-SAP

#296.03/VV67 A T Bates *et al.*
IT-03 Anti-DBH-SAP

4-5pm #232.12/B50 DA Van Der List *et al.*
IT-44 Melanopsin-SAP

MONDAY, November 14

8-9am #345.09/I8 C L Taxini *et al.*
IT-03 Anti-DBH-SAP

10-11am #396.11/WW51 M F Wiater *et al.*
IT-47 Leptin-SAP, IT-21 Blank-SAP

#397.15/XX1 A V Kalinchuk *et al.*
IT-01 192-IgG-SAP

11am-12 #331.04/A52 S S Winter *et al.*
IT-01 192-IgG-SAP

1-2pm #513.09/YY37 A L Stewart *et al.*
IT-01 192-IgG-SAP,
IT-32 GAT1-SAP

2-3pm #513.10/YY38 J J Roland *et al.*
IT-32 GAT1-SAP

TUESDAY, November 15

8-9am #550.01/C19 B Clausen *et al.*
IT-01 192-IgG-SAP

#600.13/RR29 T T Dinh *et al.*
IT-47 Leptin-SAP

#600.17/RR33 A-J Li *et al.*
IT-03 Anti-DBH-SAP

11am-12 #608.16/VV54 E B Roach *et al.*
IT-01 192-IgG-SAP

#610.12/VV89 G Paolone *et al.*
IT-01 192-IgG-SAP

1-2pm #712.09/VV51 S J Krajewski *et al.*
Custom: NKB-SAP

2-3pm #664.14/I8 WT Talman *et al.*
IT-03 DBH-SAP, IT-11 SSP-SAP,
IT-18 Mouse IgG-SAP,
IT-21 Blank-SAP, PR-01 Saporin

#702.10/RR33 F Carr *et al.*
IT-12 Dermorphin-SAP

3-4pm #712.07/VV49 M A Smith *et al.*
Custom: NKB-SAP,
IT-21 Blank-SAP

WEDNESDAY, November 16

8-9am #790.01/FF8 S T Savage *et al.*
IT-01 192-IgG-SAP

#804.21/NN21 A W Saeed *et al.*
IT-10 IB4-SAP

9-10am #804.14/NN14 R G Wiley *et al.*
IT-56 Neurotensin-SAP,
IT-60 Neurotensin-CTA

10-11am #822.19/VV64 T Kozicz *et al.*
IT-47 Leptin-SAP, IT-21 Blank-SAP

1-2pm #901.09/JJ3 C Lee *et al.*
IT-13 CRF-SAP

2-3pm #878.10/N1 J Lee *et al.*
IT-01 192-IgG-SAP

4-5pm #878.08/M11 D Jeong *et al.*
IT-01 192-IgG-SAP

#883.20/Y19 N S Bhide *et al.*
IT-03 DBH-SAP

Stop by the ATS Booth (#616) for a printed itinerary.

Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity

(continued from page 1)

Importantly, CPP to a context paired with pain relief was only observed in nerve-injured rats, leading to the "unmasking" of spontaneous experimental neuropathic pain.[1-3] Validation of this model for detecting ongoing, or non-evoked, pain, was performed with rats across different models of experimental nerve injury (i.e., spinal nerve ligation or spared nerve injury) and treatments (e.g. spinal clonidine, ω-conotoxin) known to alleviate spontaneous neuropathic pain (pain at rest) in clinical reports.[1] This approach allows for investigation of mechanisms that may promote pain as well as those that may be associated with pain relief. For example, this approach demonstrated that blocking descending pain facilitatory pathways from the RVM produces robust CPP, indicating

(continued on page 6)

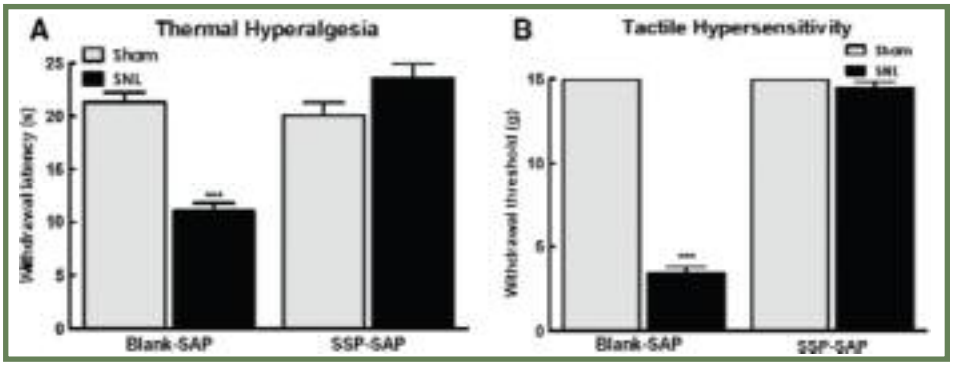

Figure 2. Spinal SSP-SAP ablation of NK-1 receptor-expressing cells blocks SNL-induced evoked pain. A) SNL induced thermal hyperalgesia within 7 days in rats that received the control saporin injection (spinal Blank-SAP) 28 days prior to the SNL surgery. In contrast, SNL rats treated with spinal SSP-SAP 28 days prior to SNL surgery failed to develop thermal hypersensitivity. SSP-SAP failed to alter paw withdrawal latencies of sham rats. B) SNL induced tactile hypersensitivity within 7 days in rats that received spinal Blank SAP 28 days prior to the SNL surgery. In contrast, SNL rats treated with spinal SSP-SAP 28 days prior to SNL surgery failed to develop tactile hypersensitivity. All graphs are mean ± SEM, ***indicates $p<0.001$ vs. pre-surgery values, n=12-18.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Galanin receptor-expressing dorsal horn neurons: Role in nociception.

Lemons LL, Wiley RG.
Neuropeptides Epub, August 2011.

This work examines the nociceptive role of galanin receptor-1-expressing neurons found in the superficial dorsal horn. 500 ng of galanin-SAP (Cat. #IT-34) was injected into the lumbar intrathecal space of rats; blank-SAP (Cat. #IT-21) was used as a control. The rats were then tested in a series of thermal nociception models. Lesioned animals were less sensitive to heat, suggesting that loss of the GalR1-expressing excitatory interneurons disrupted the pain transmission pathway.

Impaired social interaction and enhanced sensitivity to phencyclidine-induced deficits in novel object recognition in rats with cortical cholinergic denervation.

Savage S, Kehr J, Olson L, Mattsson A.
Neuroscience Epub, August 2011.

Forebrain cholinergic dysfunction is thought to be part of the pathophysiology of schizophrenia. The authors lesioned the cholinergic corticopetal projection of rats by infusing 0.081 µg of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis. The lesioned animals displayed a reduction in the duration of social interaction. When the lesioned animals were then given PCP (phencyclidine), they were no longer able to recognize a novel object. The data suggest a role of cholinergic hypofunction in the cognitive symptoms of schizophrenia.

Neural and hormonal control of food hoarding.

Bartness TJ, Keen-Rhinehart E, Dailey MJ, Teubner BJ.
Am J Physiol Regul Integr Comp Physiol 301(3):R641-55, 2011.

Hoarding of food is a commonly found behavior in humans and animals. This review discusses the neuronal and hormonal processes involved in the control of food hoarding. Several aspects of food hoarding are examined, including the role of food deprivation, environment, levels of hormones like leptin, ghrelin, and levels of peptides such as cholecystokinin. One experiment reviewed injected NPY-SAP (Cat. #IT-28) into the arcuate nucleus of rats, which changed food hoarding responses to deprivation.

Patterning of somatosympathetic reflexes reveals non-uniform organization of presympathetic drive from C1 and non-C1 RVLM neurons.

Burke PG, Neale J, Korim WS, McMullan S, Goodchild AK.
Am J Physiol Regul Integr Comp Physiol Epub, July 2011.

Some neurons in the rostral ventrolateral medulla are part of the circuitry that helps maintain blood pressure. This control is exerted through both feed-forward and reflex adjustment mechanisms. The authors used bilateral injections of anti-DBH-SAP (Cat. #IT-03, 24 ng per side) into the spinal cord of rats between T1 and T2 to better understand the organization of this circuitry. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The results suggest that myelinated neurons may control baseline tone, while stressor response uses unmyelinated neurons.

Circadian Integration of Sleep/Wake and Feeding Requires NPY-Receptor Expressing Neurons in the Mediobasal Hypothalamus.

Wiater MF, Mukherjee S, Li AJ, Dinh TT, Rooney EM, Simasko SM, Ritter S.
Am J Physiol Regul Integr Comp Physiol Epub, August 2011.

Feeding and sleep/wake states interact rhythmically across the circadian cycle. It is suspected that the mediobasal hypothalamic area (MBH) is the site where these rhythms are integrated. The authors administered bilateral 24-ng injections of NPY-SAP (Cat. #IT-28) into the arcuate nucleus in order to eliminate NPY receptor-expressing neurons in the MBH of rats. Blank-SAP (Cat. #IT-21) was used as a control. The results indicate that these neurons are required for the interaction of feeding and sleep/wake timing.

Itch signaling in the nervous system.

Jeffry J, Kim S, Chen ZF.
Physiology (Bethesda) 26(4):286-292, 2011.

This review examines recent work done to elucidate the molecular mechanisms behind the sensation of itch. The progress of mouse genetics has allowed the field to move beyond clinical and physiological studies, toward a better understanding of the signaling involved in nonhistaminergic itch. One study discussed the use of bombesin-SAP (Cat. #IT-40) to ablate GRPr-positive neurons in the dorsal horn of mice. This lesion

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

reduced scratching in response to pruritogens, but did not affect pain behavior – indicating that pain and itch use entirely different pathways.

The sympathetic nervous system stimulates anti-inflammatory B cells in collagen-type II-induced arthritis.

Pongratz G, Melzer M, Straub RH.
Ann Rheum Dis Epub, Sep 2011.

The sympathetic nervous system exerts anti-inflammatory effects on collagen-induced arthritis. To examine whether these effects are mediated by B-cells producing interleukin-10 (IL-10) the authors treated mice with 5-µg intraperitoneal injections of anti-DBH-SAP (Cat. #IT-03). The sympathectomy efficacy was assessed by analyzing norepinephrine levels in the spleen. The data suggest that increasing the number of IL-10 producing B cells can slow arthritis progression.

Selective ablation of mu-opioid receptor expressing neurons in the rostral ventromedial medulla attenuates stress-induced mechanical hypersensitivity.

Reynolds J, Bilsky EJ, Meng ID.
Life Sci 89(9-10):313-319, 2011.

Animals have been shown to develop hyperalgesia in response to chronic stress. Recent data has implicated the rostral ventromedial medulla (RVM) in this process. In order to clarify what role mu-opioid receptor-expressing neurons in the RVM play in rat, the authors injected 1.8 pmol of dermorphin-SAP (Cat. #IT-12) into the RVM. The rats were then subjected to a model designed to produce hypersensitivity in the hind paw. Stress-induced behavior did not change in the lesioned animals, but mechanical hypersensitivity was reduced.

Decrease of GABAergic Markers and Arc Protein Expression in the Frontal Cortex by Intraventricular 192 IgG-Saporin.

Jeong DU, Chang WS, Hwang YS, Lee D, Chang JW.
Dement Geriatr Cogn Disord 32(1):70-78, 2011.

The authors examined the use of 192-IgG-SAP (Cat. #IT-01) to establish a standardized model for dementia. Rats received several different doses of toxin in bilateral intraventricular injections. This injection method resulted in reliable memory impairment in a behavioral test, decreased GABAergic activity in the frontal cortex affecting spatial memory, and no change in the hippocampus. Using this technique, 8 µg of 192-IgG-SAP produced the optimal memory impairment.

Effect of orexin-B-saporin-induced lesions of the lateral hypothalamus on performance on a progressive ratio schedule.

Olarte Sanchez CM, Valencia Torres L, Body S, Cassaday HJ, Bradshaw CM, Szabadi E.
J Psychopharmacol Epub, Sep 2011.

It has been suggested that orexigenic neurons in the hypothalamus consist of two anatomically distinct groups. The lateral hypothalamic area group (LHA), which modulates reinforcement mechanisms; and the dorsomedial hypothalamus and perifornical area group involved in regulation of stress and arousal. Rats received bilateral 15-ng injections of orexin-SAP (discontinued, new production under way) into the LHA. Results from a progressive ratio model indicate that the lesioned neurons control the motor component of food-reinforced responding.

Participation of brainstem monoaminergic nuclei in behavioral depression.

Lin Y, Sarfraz Y, Jensen A, Dunn AJ, Stone EA.
Pharmacol Biochem Behav Epub, Aug 2011.

While the classical model states reductions in central noradrenergic activity produce depression, more recent work has indicated that higher activity in this brain region directly correlates with depression. Using a dopamine-β-hydroxlase targeted toxin to lesion the locus coeruleus of mice, along with goat-IgG-SAP (Cat. #IT-19) as a control, the authors found that treated animals showed increased resistance to depressive behavior in several tests. The results suggest that monoaminergic lesions are greatly affected by mouse strain, lesion size, and involvement of other neuronal systems.

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Targeting Talk: Product Questions

Choosing the Correct Secondary Conjugate

Q: I have a mouse monoclonal antibody and a rabbit polyclonal antibody that I would like to test using your secondary conjugate system. Which products do I need to order?

A: For mouse monoclonals, you can use:
Mab-ZAP (Cat. #IT-04) -- Cells that internalize your mouse monoclonal antibody will be eliminated.
Or, Fab-ZAP mouse (Cat. #IT-48) -- Cells that internalize your mouse monoclonal IgG antibody will be eliminated.

For rabbit polyclonals, you can use:
Rab-ZAP (Cat. #IT-05) -- Cells that internalize your rabbit polyclonal antibody will be eliminated.
Or, Fab-ZAP rabbit (Cat. #IT-57) -- Cells that internalize your rabbit IgG antibody will be eliminated.

Q: What is the difference between the Fab-ZAP products and Mab-ZAP or Rab-ZAP?

A: The difference between Fab-ZAP products and other secondary conjugates is that Fab-ZAP is made with a monovalent secondary antibody which eliminates the possibility of cap formation as cross-linking of the Fab-ZAP molecules cannot occur. The Fab-ZAP products will still recognize the heavy and light chains of antibodies, and should be used in the same way and at the same molar concentrations as the original secondary conjugates (such as Mab-ZAP and Rab-ZAP). Cytotoxicity assays using Fab-ZAP (mouse) have demonstrated an improved EC_{50} when directly compared to Mab-ZAP.

Any of our secondary conjugates offers a very cost-effective diagnostic method for screening primary antibodies for *in vitro* or *in vivo* use.

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Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: KNOCKOUT
LETHAL
STATISTICS
BEHAVIORAL
DIFFERENCE

Answer: LIFE'S A BEACH

Congratulations to the puzzle solvers. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Anthony St. Leger, Univ Pittsburgh, Pittsburgh, PA * Glenn Kageyama, Cal State Pomona Univ, Pomona, CA * Cliff Berkman, Washington State Univ, Pullman, WA * Syd Johnson, MacroGenics Inc, Rockville, MD * Ashley Furr, Univ Texas Health Science Center, San Antonio, TX * Seto Chice, SUNY-HSC, Brooklyn, NY * Wayne Kuenzel, Univ Arkansas, Fayetteville, AR * Bob Speth, Nova Southeastern Univ, Davie FL * Thea Marlinga, Libertyville, IL * Saurabh Sen, Lucigen, Middleton, WI * Eric Wong, Univ California, San Diego, La Jolla, CA

Solve the Teaser online at: www.ATSbio.com/news/11q4_teaser.html

Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity

(continued from page 2) that descending pain facilitation from the RVM promotes nerve-injury induced spontaneous pain.[1-3]

With support provided by the National Institutes of Health, the role of TRPV1-positive sensory fibers and spinal NK-1 positive cells in driving the evoked and spontaneous components of neuropathic pain were determined.[2] At the level of the spinal dorsal horn, ablation of NK-1 receptor-expressing cells with the substituted SP analog, $Sar^{9}Met(O_2)^{11}$ substance P attached to saporin (SSP-SAP; Cat. #IT-11), blocked nerve-injury thermal and tactile hypersensitivity as well as ongoing pain. Immunhistochemical analysis of spinal NK-1 receptor expression following behavioral testing verified elimination of NK-1 receptors within the spinal dorsal horn at time of behavioral testing (Fig 1).

Baseline thermal and tactile sensory thresholds were determined followed by spinal nerve ligation (SNL) or sham (control surgeries). Analysis of sensory thresholds 14 days following surgery by an experimenter blinded to the treatment conditions demonstrated that rats that received blank-SAP (control; Cat. #IT-21) developed robust thermal and tactile hypersensitivity whereas rats that had been treated with SSP-SAP showed normal thermal and tactile sensory thresholds, equivalent to sham-treated rats (Fig 2A,B respectively). SNL-induced spontaneous pain was assessed using CPP to a context paired with pain relief induced by RVM lidocaine. Rats had RVM cannulas implanted followed by a 1-week recovery period followed by SNL or sham (control surgeries). Rats were placed in 3 chamber conditioning boxes with 2 pairing chambers distinguished by texture (rough vs. smooth floors) and visual cues (striped vs. solid gray walls). Preconditioning analysis of time spent in the conditioning chambers demonstrated no difference in time spent in the chambers, indicating no pre-conditioning chamber bias (Fig 3A). Conditioning day occurred 14 days post-SNL or sham surgery. Rats received RVM saline and were immediately confined to one of the pairing chambers for 30 min. Four hours later, rats received RVM lidocaine and were immediately confined to the opposite pairing chamber for 30 min. RVM lidocaine selectively induced CPP in SNL rats treated with blank-SAP (Fig 3A,B). In contrast, SNL rats that had been treated with SSP-SAP spent equivalent time in the RVM saline and lidocaine paired chambers (Fig 3A), indicating no increase in time spent in the RVM lidocaine paired chamber (Fig 3B). These findings indicate that elimination of the NK-1 receptor-expressing cells blocks development of nerve-injury induced spontaneous pain.

These data suggest that spontaneous neuropathic pain likely depends on spinal NK-1 positive ascending projections offering opportunities for exploration of therapeutic interventions.

References

1. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci* 2009;12(11):1364-1366.
2. King T, Qu C, Okun A, Mercado R, Ren J, Brion T, Lai J, Porreca F. Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity. *Pain* 2011.
3. Qu C, King T, Okun A, Lai J, Fields HL, Porreca F. Lesion of the rostral anterior cingulate cortex eliminates the aversiveness of spontaneous neuropathic pain following partial or complete axotomy *Pain* 2011;in press.

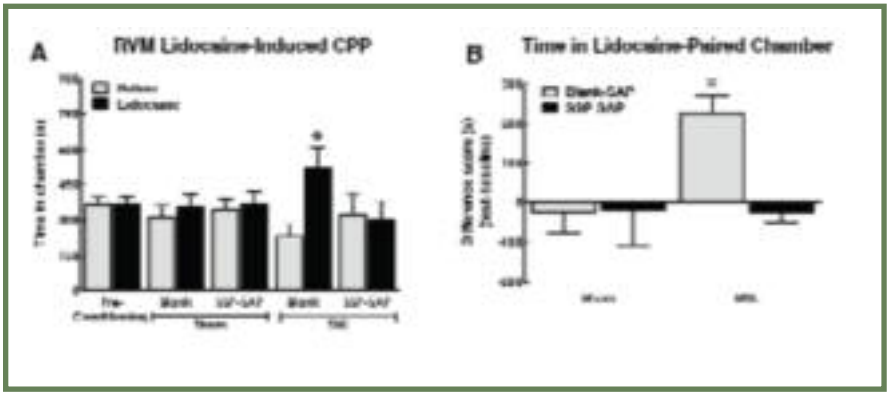

Figure 3. Spinal SSP-SAP ablation of NK-1 receptor-expressing cells blocks SNL-induced spontaneous pain. A) Pre-conditioning time spent in the conditioning chambers did not differ across treatment groups. RVM lidocaine did not produce CPP in sham-operated rats irrespective of treatment group. SNL rats that received spinal control injection (Blank-SAP) 28 days prior to SNL surgery showed increased time spent in the lidocaine-paired chamber, * indicates $p<0.05$ compared to pre-conditioning values. SNL-rats that received spinal SSP-SAP injection 28 days prior to SNL surgery failed to show CPP to the lidocaine-paired chamber. B) Difference scores confirm that only SNL rats that received spinal injection of the control (Blank-SAP) showed CPP to the lidocaine-paired chamber, * indicates $p<0.05$ vs. Sham-Blank control rats. All graphs are mean ± SEM, n = 6-8.

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Society for Neuroscience
November 12-16, 2011
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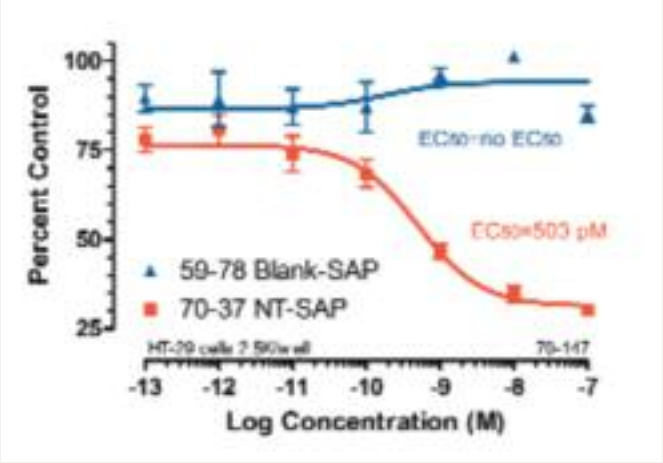
Targeting Tools: Featured Products

Neurotensin Conjugates

Neurotensin is a 13 amino acid peptide found in the brain and spinal cord (first characterized by Carraway and Leeman[1]). It affects pituitary hormone release, interacts with the dopaminergic system, and is involved in vasodilation and hypotension. It can also modulate pain perception.

Advanced Targeting Systems is pleased to announce two conjugates that will be valuable tools for researchers. **Neurotensin-SAP (Cat. #IT-56)** is a chemical conjugate of neurotensin and the ribosome-inactivating protein, saporin. Neurotensin-SAP will specifically *eliminate* cells that express neurotensin receptors. **Neurotensin-CTA (Cat. #IT-60)** is a chemical conjugate of neurotensin and the catalytic A subunit of cholera toxin. Neurotensin-CTA will specifically *stimulate* cells expressing neurotensin receptors.



Neurotensin-SAP specifically eliminates neurotensin receptor-expressing cells.

HT-29 cells, a human epithelial colorectal adenocarcinoma cell line, were plated at 2500 cells/90 µl/well in a 96-well plate and incubated overnight. Blank-SAP and Neurotensin-SAP were then added in 10-µl volumes to each well. The plates were incubated 72 hours. Plates were developed with sulforhodamine B then read at 564 nm. Data analysis was done by PRISM (GraphPad).

To Terminate or Stimulate?

That is the question. . . Here are the tools!

IT-56 Neurotensin-SAP
available individually or in a kit with saporin (Cat. #PR-01) and Blank-SAP (Cat. #IT-21)

IT-60 Neurotensin-CTA
available individually or in a kit with Blank-CTA (Cat. #IT-61)

When either of these targeted conjugates are administered to cells (*in vitro* or *in vivo*), the targeting agent seeks out and binds only to cells expressing neurotensin receptors. The conjugate is internalized.

To Terminate: When using Neurotensin-SAP, saporin breaks away from the targeting agent, and inactivates the ribosomes which causes protein synthesis inhibition and, ultimately, cell death. Cells which do not have the cell surface marker are not affected.

To Stimulate: When using Neurotensin-CTA, the catalytic A subunit of cholera toxin (CTA) breaks away from the targeting agent, and activates the cAMP pathway within the cells by ribosylating adenylate cyclase. Cells which do not have the cell surface marker are not affected.

Reference

1. Carraway R, Leeman S. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J Biol Chem* 1973;248(19):6854-6861.

As the seasons begin to change and the cooler weather creeps upon the land, Gangsta enjoys a brief sleep in the waning sunlight.

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§ or anything recognized on the cell surface and internalized.

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

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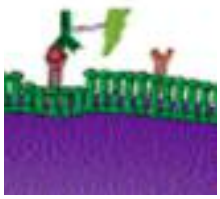

Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

MOMONC

PETCONC

SNIFIECOVEN

YRIPSINSEETHYVIT

SLODAR

What the scientist cooked up for the Halloween party.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER: ○○○○○○○ ○○○○!

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by January 1, 2012.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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