Jan-Feb-Mar 2007
Volume 8, Issue 1

Targeting Trends

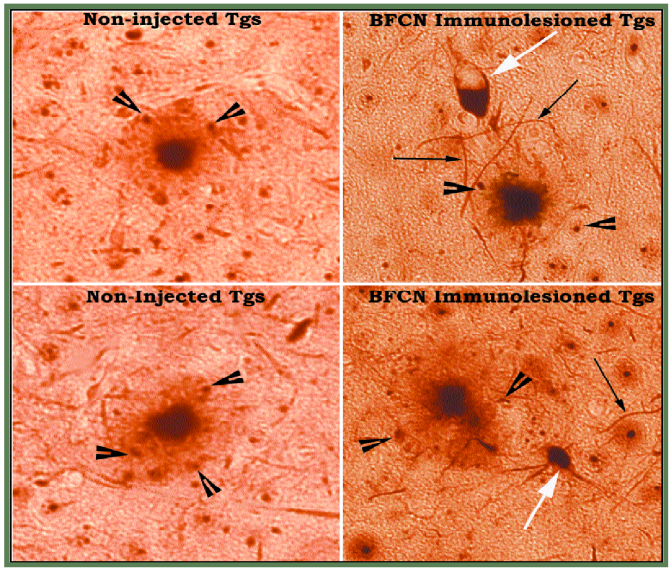
Reporting the latest news in Molecular Surgery

Cholinergic Immunolesioning Produced Tangle-like Inclusions in TgCRND8 Brain

Neelima Chauhan, Dept Anesthes/Anat & Cell Bio, Univ Illinois-Chicago; J. Brown VA Med Ctr Chicago, Chicago, IL and winner of the 2006 SfN Poster Award for work using ATS products.

Today's Alzheimer's disease (AD) research lacks a "complete" model that would represent both plaque and tangle pathology together with correlative memory deficits. The currently available transgenic model that includes APP/PS1/tau mutations does not truly represent AD because tangles observed in AD brain are independent of tau mutations. Subtly increased β-amyloid (Aβ) levels either due to familial mutations or sporadic causes, primarily signals pre-tangle cytopathology and degeneration of basal forebrain cholinergic neurons (BFCN) via deranged signaling of glygogen

(continued on page 6)

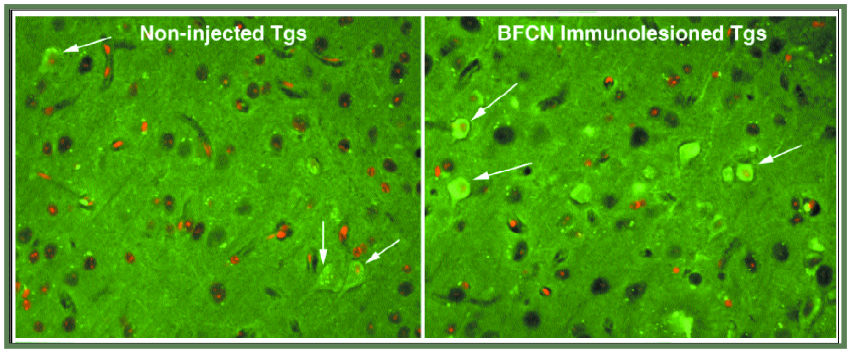


Development of a tangled neuron in plaque vicinity

Black arrowheads: plaque-associated hyperphosphorylated neurites; Black thin arrows: hyperphosphorylated neuropil threads in immunotoxin injected Tg brain (Right panel); White arrow: "Tangled" neuron in the vicinity of plaque showing intraneuronal phosphorylated tangle-like inclusion in immunotoxin injected Tg brain (Right panel). Note the absence of hyperphosphorylated neuropil threads and "Tangled" neuron in the vicinity of plaque in non-injected Tgs (Left panel).

Drastically increased AT8-positive neurons after BFCN immunolesioning

Left panel, white arrows: Cortical pyramidal neurons of non-injected Tg brain showing occasional punctate immunoreactivity for AT-8 (a marker protein for tangles) indicating "subtle" tau phosphorylation in untreated Tg brain.



Right panel, white arrows: Cortical pyramidal neurons of immunotoxin-injected Tg brain showing strong immunoreactivity for AT-8 (a marker protein for tangles) indicating the presence of "tangle-bearing" neurons in immunotoxin-injected Tg brain.

Inside this issue:

Newsletter Highlights

- Teaser Winners *(page 2)*
- SfN Award *(page 2)*
- Surf Contest *(page 2)*
- Featured Antibodies *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: VENTRAL
HYPOTHALAMUS
HORSERADISH
PATHOGENESIS
TRANSPORT

Answer: Straight from the ...
"HORSE'S MOUTH"

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

Winners: Shawn McClelland- California State University, Northridge CA * Vivian Yip- Tissuegene Inc, Gaithersburg MD * Seto Chice- SUNY HSC, Brooklyn NY * Mark Semenuk- Panacea Pharmaceuticals Inc, Gaithersburg MD * Thea Marlinga- Libertyville, IL * Jim Stetson- Dupont Institute, Wilmington DE * Sean Stocker- Univ Kentucky College of Medicine, Lexington KY * Rebecca Geevaratne- Georgetown University, Washington DC * Dr. Ruth Stornetta- Univ Virginia, Charlottesville VA

Surf Contest

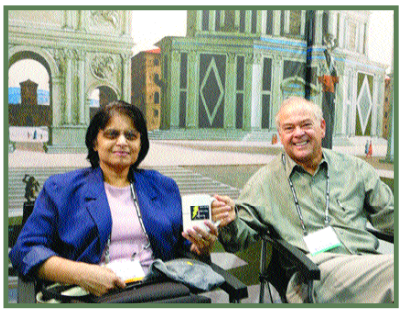
On September 23, ATS went to the Tony Mezzadri Surf Classic (tonycontest.org), held annually at the Ocean Beach, CA pier. This year was another thriller, with some enormous waves hitting the bottom of the pier. Tony was a teenager when he sustained a serious spinal cord injury while surfing near the pier, and the residents of Ocean Beach and the Sunset Cliffs Surfing Association stepped up with this contest to help Tony and his family. The Contest has grown such that now it is a fundraiser for spinal cord injury research for Mark Tuszynski's lab at UC, San Diego. And a fun day at the beach to watch great surfers surf great waves!

Upcoming Events

Spring Brain Conference
March 14-17, 2007
Radisson Poco Diablo Resort in Sedona, AZ

American Association for Cancer Research
April 14-18, 2007
Los Angeles, CA

2006 SfN Poster Award Winner



Every year we give a prize to the best poster presented at the *Society for Neuroscience* meeting that uses an ATS product. This year's winner is Dr. Neelima Chauhan of the University of Illinois-Chicago for her work combining a targeted toxin, murine p75-SAP (Cat #IT-16), which eliminates cholinergic neurons in the mouse basal forebrain, and a transgenic mouse that overexpresses a mutant form of APP. The increase in Alzheimer's Disease pathology makes this combination more closely model AD. This is an exciting advance and we congratulate Dr. Chauhan for her very interesting work.

The competition was indeed formidable, and we would like to cite two posters that were close contenders for the prestigious award. Arshad M. Khan's poster (with Kimberley Rapp and Alan Watts) on "Catecholaminergic afferents are required for hypothalamic parvicellular paraventricular neurons to transduce signals associated with hyploglycemia into p44/42 MAP kinase phosphorylation events" was an eloquent and beautifully presented poster that utilized anti-DBH-SAP (Cat # IT-03).

D. R. Sengelaub (with M. C. Osborne, C. M. Little, K. D. Huyck and T. Verhovshek) from Indiana University presented "Neuroprotective effects of testosterone in two models of spinal cord neurons" which detailed motoneuron support by testosterone and how it's achieved. They used cholera toxin B-SAP (CTB-SAP; Cat #IT-14). Congratulations to these and the many fine posters using ATS products.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

A putative flip-flop switch for control of REM sleep.
Lu J, Sherman D, Devor M, Saper CB
Nature 441(1):589-594, 2006.

The authors propose a REM sleep regulatory system that involves GABA-ergic and glutaminergic neurons in the mesopontine tegmentum. Two μl of 0.1% orexin-SAP (Cat. #IT-20) was injected into the medial medullary reticular formation of rats. The results suggest the sharp transitions into and out of REM sleep are controlled by recipro-cal interactions between GABAergic REM-off and REM-on neuronal populations.

Antisocial and seizure susceptibility phenotypes in an animal model of epilepsy are normalized by impairment of brain corticotropin-releasing factor.
Turner LH, Lim CE, Heinrichs SC
Epilepsy Behav [Epub Oct 3], 2006.

There appears to be an inverse relationship between seizure susceptibility and social interaction. This work examines the role that CRF may play in this system. 2.5 μg of CRF-SAP (Cat. #IT-13) was administered to the lateral ventricle of rats, and the lesioned animals were assessed in terms of social investigation times as well as handling-induced seizures. The results support the involvement of CRF systems in facilitating evoked seizures and suppression of social activity.

Effects of hypocretin-1 in 192-IgG-saporin-lesioned rats.
Blanco-Centurion CA, Shiromani A, Winston E, Shiromani PJ
Eur J Neurosci 24(7):2084-2088., 2006.

The basal forebrain is a major arousal center. Using 6 μg of 192-IgG-SAP (Cat. #IT-01) injected into the lateral ventricle of rats, the role of non-cholinergic neurons in the basal forebrain was investigated. Administration of orexin, also known as hypocretin, to lesioned animals produced sleep/wake patterns identical to non-lesioned animals. The results indicate that non-cholinergic neurons in the basal forebrain are sufficient to promote arousal in response to orexin.

Ablation of NK(1) receptor bearing neurons in the nucleus of the solitary tract blunts cardiovascular reflexes in awake rats.
Abdala AP, Schoorlemmer GH, Colombari E
Brain Res 1119(1):165-173, 2006.

Cardiovascular function is largely controlled by the nucleus of the tractus solitarius (NTS). This work focuses on the baroreflex, cardiopulmonary chemoreflex, and arterial chemoreflex. Rats were injected with either 20 nl of 2 μM SP-SAP (Cat. #IT-07) into the subpostremal NTS, or 200 nl into the subpostremal and commissural NTS. Saporin (Cat. #PR-01) was used as a control. It was established that NK-1 receptor-expressing neurons in the NTS are critical for these reflexes.

Selective depletion of cortical noradrenalin by anti-dopamine beta-hydroxylase-saporin impairs attentional function and enhances the effects of guanfacine in the rat.
Milstein JA, Lehmann O, Theobald DE, Dalley JW, Robbins TW
Psychopharmacology (Berl) 190(1):51-63, 2007.

The authors examined the effect of cortical noradrenalin depletion on a reaction time task. Rats received 0.2 μg-intracortical infusions of anti-DBH-SAP (Cat. #IT-03), then were trained in a reaction time task. The effect of guanfacine, a selective α-2 adrenergic agonist was also tested in these animals. Lesioned rats were not impaired on the baseline task, but were slower and less accurate during high rate conditions. Guanfacine only affected the lesioned animals.

Spinal NK-1 receptor expressing neurons mediate opioid-induced hyperalgesia and antinociceptive tolerance via activation of descending pathways.
Vera-Portocarrero LP, Zhang ET, King T, Ossipov MH, Vanderah TW, Lai J, Porreca F
Pain [Epub Nov 21], 2006.

Administration of opioids can induce hyperalgesia in humans and other mammals. In this work the authors examined the role of NK-1 receptor-expressing neurons in the spinal dorsal horn during a hyperalgesic condition not induced by tissue injury. Five μl of 10 μM SP-SAP (Cat. #IT-07) was injected into the intrathecal space of rats. Saporin (Cat. #PR-01) was used as a control. Data from the lesioned animals indicate that NK-1 receptor-expressing neurons play a critical role in this hyperalgesic circuit.

Neuronal nitric oxide synthase is upregulated in a subset of primary sensory afferents after nerve injury which are necessary for analgesia from alpha2-adrenoceptor stimulation.
Ma W, Eisenach JC
Brain Res [Epub Nov 16], 2006.

Peripheral nerve injury resulting in neuropathic pain often responds poorly to opioid treatment. α2-adrenoreceptor (AR) agonists, however, perform better after this type of injury. After a spinal nerve ligation, rats were treated with a 0.6 μg-intrathecal injection of 192-IgG-SAP (Cat. #IT-01). The increase of neuronal nitric oxide synthase (nNOS) caused by spinal ligation was abolished

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

in the lesioned animals. The data indicate that AR agonists may reduce sensitization by activating nNOS fibers in the superficial dorsal horn.

Noradrenergic inputs to the bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus underlie hypothalamic-pituitary-adrenal axis but not hypophagic or conditioned avoidance responses to systemic yohimbine.

Banihashemi L, Rinaman L
J Neurosci 26(44):11442-11453, 2006.

Yohimbine (YO) is an α2 adrenoceptor antagonist that increases transmitter release from adrenergic/noradrenergic (NA) neurons. The authors investigated whether NA inputs to the bed nucleus of the stria terminalis (BNST) were required for YO effects. After receiving 11 ng of anti-DBH-SAP (Cat. #IT-03) in the left and right BNST, rats displayed a marked decrease in the hypothalamic-pituitary-adrenal axis in response to YO administration.

Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release.

Marcos B, Gil-Bea FJ, Hirst WD, Garcia-Alloza M, Ramirez MJ
Eur J Neurosci 24(5):1299-1306, 2006.

The authors investigated a potential link between 5-HT_6 receptors, cholinergic activity, and learning. After 0.067 μg of 192-IgG-SAP (Cat. #IT-01) was injected into each hemisphere of the nucleus basalis magnocellularis in the basal forebrain of rats, 5-HT_6 receptor mRNA and protein expression were measured. Results demonstrate the involvement of multiple neurotransmitter systems in neurochemical actions following 5-HT_6 receptor blockade.

Please visit www.ATSbio.com to see a complete list of references.

Long-term effects of immunotoxic cholinergic lesions in the septum on acquisition of the cone-field task and noncognitive measures in rats.

van der Staay FJ, Bouger P, Lehmann O, Lazarus C, Cosquer B, Koenig J, Stump V, Cassel JC
Hippocampus 16(12):1061-1079, 2006.

192-IgG-SAP (Cat. #IT-01) has been used to make extremely specific lesions in the septohippocampal cholinergic system of the brain. The specificity of these lesions is allowing researchers to more accurately map the involvement of the septohippocampal cholinergic system in spatial learning and memory. Here, rats received 0.8 μg of 192-IgG-SAP in the medial septum and the vertical limb of diagonal band of Broca. Lesioned animals only exhibited deficits in attentional learning.

Inhibition within the nucleus tractus solitarius (NTS) ameliorates environmental exploration deficits due to cerebellum lesions in an animal model for autism.

Walker BR, Diefenbach KS, Parikh TN
Behav Brain Res [Epub Sep 11], 2006.

In this work the authors use environmental exploration deficits in rats as a model for autism. Animals received 2 μg of either OX7-SAP (Cat. #IT-02) or 192-IgG-SAP (Cat. #IT-01) into each ventricle. Only the OX7-SAP-treated rats displayed a reduction in exploration behavior, and the anticonvulsant muscimol restored exploration behavior to control levels. This system may have use in controlling behavior deficits seen in autism.

Up-regulation of cation-independent mannose 6-phosphate receptor and endosomal-lysosomal markers in surviving neurons after 192-IgG-saporin administrations into the adult rat brain.

Hawkes C, Kabogo D, Amritraj A, Kar S
Am J Pathol 169(4):1140-1154, 2006.

The cation-independent mannose 6-phosphate receptor (CI-MPR) plays a major role in the endosomal-lysosomal (EL) system. One of the tasks carried out by the EL system is clearance of abnormal proteins after injury. By administering 2.0 μg bilateral injections of 192-IgG-SAP (Cat. #IT-01) to rats, the researchers were able to increase CI-MPR expression levels, as well as other EL markers in response to the lesion. The up-regulation of EL components suggests that the EL system may be able to repair neuronal abnormalities induced by injury.

Basal forebrain cholinergic lesions reduce heat shock protein 72 response but not pathology induced by the NMDA antagonist MK-801 in the rat cingulate cortex.

Willis CL, Ray DE, Marshall H, Elliot G, Evans JG, Kind CN
Neurosci Lett 407(2):112-117, 2006.

The NMDA receptor antagonist MK-801 may have use in establishing a model for schizophrenia. The mechanism by which cortical neurons are damaged by these antagonists is unknown. The authors tested the theory that cholinergic hyperstimulation of cingulate neurons is involved by administering 80 ng of 192-IgG-SAP (Cat. #IT-01) unilaterally to rats. The results indicate that although cholinergic neurons are involved in the heat shock response to MK-801, the pathological effects follow a different pathway.

(continued on page 6)

Targeting Talk: *Targeted Toxin Controls*

by Dr. Douglas Lappi

Q: Your targeted toxin kits come with different controls. I'm not sure of the best way to use them. For example, with the anti-SERT-SAP kit there is included unconjugated antibody, unconjugated saporin, and a control conjugate, mouse IgG-SAP. Should I use them all in the same experiment or for different purposes?

A: Yes, perhaps we do have a few too many options for controls; better too many than too few. For anti-SERT-SAP, the ideal control is mouse IgG-SAP. Anti-SERT-SAP is made from saporin conjugated to a mouse monoclonal IgG that has SERT as its antigen. So, mouse IgG-SAP--that is, saporin conjugated to mouse IgG that has no specific antigen for targeting--would be the best control, in my mind. For years, the unconjugated antibody and unconjugated saporin mixed together was the best control available (until we came out with the "irrelevant" control immunotoxins), and still might be considered a second good control, or useful in cases where down-regulation by the antibody is a concern.

Other related articles:

Lappi, DA (2005) Featured Products: Control Immunotoxins. *Targeting Trends* 6(2):7.

Lappi, DA (2002) Control Conjugates: The Perfect Companion for Targeted Toxins. *Targeting Trends* 3(1):1,6.

Lappi, DA (2001) Featured Products: Controls for Immunotoxins. *Targeting Trends* 2(2):7.

Q: What about for the peptide toxins like orexin-SAP or SP-SAP — what controls are available for those?

A: We have produced Blank-SAP as a control for the peptide ligand toxins. Blank-SAP (Cat. #IT-21) is a peptide that has the usual common amino acids that are found in peptide neurotransmitters, but arranged in a sequence that is random and not detected in homology searches. So, it's like shooting blanks; it should never find an amenable receptor. This is quite an important control; the peptide ligand toxins are often delivered directly to tissue, and there are cases in which there will be no toxicity or non-specific toxicity. The best use we have seen for Blank-SAP has been in Bugarith K, Dinh TT, Li AJ, Speth RC, Ritter S (2005) Basomedial hypothalamic injections of neuropeptide Y conjugated to saporin selectively disrupt hypothalamic controls of food intake. *Endocrinology* 146(3):1179-1191.

As any journal reviewer will tell you, it's very important to document the specificity, and with Blank-SAP as a control, you can definitively show that toxicity is due to proper targeting, rather than non-specific cytotoxicity. This should provide the information needed so the reviewer doesn't have to make you go back and document specificity with further experimental work!

Blank-SAP, Cat. #IT-21

control for use with peptide conjugates

CCK-SAP (Cat. #IT-31)
CRF-SAP (Cat. #IT-13)
Dermorphin-SAP (Cat. #IT-12)
Galanin-SAP (Cat. #IT-34)
NPY-SAP (Cat. #IT-28)
Orexin-SAP (Cat. #IT-20)
SP-SAP (Cat. #IT-07)
SSP-SAP (Cat. #IT-11)

Goat IgG-SAP, Cat. #IT-19

control for use with second immunotoxins

Anti-M-ZAP (Cat. #IT-30)
Hum-ZAP (Cat. #IT-22)
Mab-ZAP (Cat. #IT-04)
Rab-ZAP (Cat. #IT-05)
Rat-ZAP (Cat. #IT-26)

Rabbit IgG-SAP, Cat. #IT-35

control for immunotoxins that use a rabbit polyclonal

mu p75-SAP (Cat. #IT-15)

Rat IgG-SAP, Cat. #IT-17

control for immunotoxins that use a rat monoclonal

Anti-CD25-SAP (Cat. #IT-29)
Anti-DAT-SAP (Cat. #IT-25)
Mac-1-SAP mouse/human (Cat. #IT-06)

Mouse IgG-SAP, Cat. #IT-18

control for immunotoxins that use a mouse monoclonal

192-IgG-SAP (Cat. #IT-01)
OX7-SAP (Cat. #IT-02)
Anti-DBH-SAP (Cat. #IT-03)
ME20.4-SAP (Cat. #IT-15)
Anti-SERT-SAP (Cat. #IT-23)
Anti-CD25-SAP (Cat. #IT-24)
Mac-1-SAP rat (Cat. #IT-33)

Questions about an ATS product or Molecular Surgery technique?
Write to us at ats@ATSbio.com

Cholinergic Immunolesioning Produced Tangle-like Inclusions *(continued from page 1)*

synthase kinase 3-beta (GSK3β)-, protein kinase A (PKA)-, and extracellular signal-regulated kinase (ERK2) of the ERK-mitogen-activated protein kinase (MAPK) cascade. This leads to reduced phosphorylation of cAMP responsive element binding protein (CREB) that results in synaptic and memory deficits much earlier than the emergence of classic AD pathology. Thus, subtly elevated Aβ, together with BFCN deficits resulting from Aβ-induced deranged signaling, set up a vicious feedback loop to produce characteristic plaque and tangle pathology observed in AD.

Based on these facts, we wished to test if selective lesioning of BFCN during the early stages of amyloid build-up exacerbate tau phosphorylation and produce tangle-like inclusions in transgenic mice with APP mutations. We produced selective immunotoxic lesions of BFCN by injecting the BFCN-specific cholinergic immunotoxin, mu p75-SAP (Cat. #IT-16) which is known to specifically target p75-expressing BFCN. This specific targeting agent was administered intracerebroventricularly in TgCRND8 mice harboring Swedish (KM670/671NL) and Indiana (V717F) mutations. The resulting model exhibited tangle-like inclusions, provoked already existing plaque pathology, and worsened impaired behavioral deficits. The combination of the transgenic mice treated with the immunotoxin provides a powerful tool for understanding AD progression.

Targeting Topics: Recent Scientific References

(continued from page 4)

Long-term effects of neonatal basal forebrain cholinergic lesions on radial maze learning and impulsivity in rats.
Scattoni ML, Adriani W, Calamandrei G, Laviola G, Ricceri L
Behav Pharmacol 17(5-6):517-524, 2006.

Work in the last decade has focused on clarifying the role of cholinergic dysfunction in Alzheimer's disease. Seven-day-old rats received 0.21 μg of 192-IgG-SAP (Cat. #IT-01) administered to the third ventricle, and were tested at 5 months of age in delay tolerance and radial maze. Test results suggest that prolonged basal forebrain cholinergic hypofunction is detectable only during highly complex tasks.

An activity-dependent assay for ricin and related RNA N-glycosidases based on electrochemiluminescence.
Keener WK, Rivera VR, Young CC, Poli MA
Anal Biochem 357(2):200-207, 2006.

The authors use synthetic biotinylated RNA substrates to assay adenine-specific RNA N-glycosidases, one of which is saporin (Cat. #PR-01). The RNA substrates are annealed to a ruthenylated oligodeoxynucleotide, allowing the capture of ruthenium chelate on magnetic beads. The N-glycosidase activity can then be detected by enzyme-linked chemiluminescence. The developed assay provides a robust method for assessing threats involving N-glycosidases.

Neurotoxic lesions centered on the perifornical hypothalamus abolish the cardiovascular and behavioral responses of conditioned fear to context but not of restraint.
Furlong T, Carrive P
Brain Res [Epub Nov 24], 2006.

This work examined the role of orexin-containing neurons in the perifornical hypothalamus (PeF) during stress response. Orexin-SAP (Cat. #IT-20) or the control conjugate Blank-SAP (Cat. #IT-21) was injected into the PeF of pre-conditioned rats. Tests measuring restraint and conditioned fear to context were then performed on the lesioned animals. While the lesioning was not specific enough to connect results to orexin-containing neurons, the data indicate that the PeF is critical for some forms of stress, but not others.

Role of catecholaminergic neurons of the caudal ventrolateral medulla in cardiovascular responses induced by acute changes in circulating volume in rats.
Pedrino GR, Maurino I, de Almeida Colombari DS, Cravo SL
Exp. Physiol 91(6):995-1005, 2006.

Catecholaminergic neurons in the caudal ventrolateral medulla (CVLM) are thought to help regulate body fluid homeostasis and cardiovascular response due to changes in circulating volume. The authors injected 6.3 ng of anti-DBH-SAP (Cat. #IT-03) into the CVLM of rats, and measured several physiological parameters following an injection of hypertonic or isotonic saline. Data from the lesioned rats indicate that catecholaminergic neurons mediate the cardiovascular response to volume expansion or increases in sodium levels.

Effect of selective cholinergic denervation on the serotonergic system: implications for learning and memory.
Garcia-Alloza M, Zaldua N, Diez-Ariza M, Marcos B, Lasheras B, Javier Gil-Bea F, Ramirez MJ
J Neuropathol Exp Neurol 65(11):1074-1081, 2006.

The authors compared two lesioning methods using 192-IgG-SAP (Cat. #IT-01) to examine the role of the serotonergic system in learning and memory. Administration of 0.067 μg of conjugate to each hemisphere of the nucleus basalis of Meynert reduced cholinergic markers in the frontal cortex. One μg of conjugate administered to the ventricle of each hemisphere reduced cholinergic markers in the frontal cortex and hippocampus. Both models reduced serotonin levels in the frontal cortex, but only the ICV injections modified learning.

Targeting Tools: Featured Antibodies

Check out Gangsta's new homepage at www.ATSbio.com/Gangsta/

*Anti-*Pseudomonas aeruginosa, *Cat. #AB-T057*

Pseudomonas aeruginosa is a Gram-negative bacterium that causes many infections, particularly in people with cystic fibrosis, burn victims, individuals with cancer and others who are immunosuppressed. This bacterium is studied by scientists not only because of its ability to cause disease and resist antibiotics, but also its metabolic capability and environmental versatility. **Anti-*Pseudomonas aeruginosa*** targets *pseudomonas aeruginosa* total proteins. Applications include ELISA and immunoblotting.

Anti-Conjugated Glutathione, Cat. #AB-T01

Glutathione is a small protein composed of three amino acids: cysteine, glutamic acid, and glycine. The primary biological function of glutathione is to act as a non-enzymatic reducing agent. Glutathione is also involved in detoxification. **Anti-Conjugated Glutathione** targets conjugated glutathione which is created by fixation in glutaraldehyde. Applications include ELISA and immunohistochemistry.

Anti-Conjugated Acetylcholine, Cat. #AB-T02

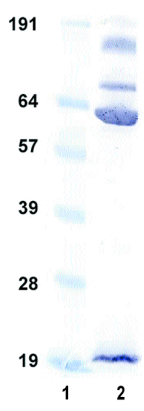
Acetylcholine is a neurotransmitter found in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans. **Anti-Conjugated Acetylcholine** targets conjugated choline-glutaric acid which is created by fixation in glutaraldehyde. Applications include ELISA and immunohistochemistry.

Anti-Conjugated Dopamine, Cat. #AB-T11

Dopamine is a neurotransmitter found in various areas of the central and peripheral nervous system. Dopamine and its agonists play an important role in cardiovascular, renal, hormonal, and central nervous system regulation. **Anti-Conjugated Dopamine** targets conjugated dopamine which is created by fixation in glutaraldehyde. Applications include ELISA, immunocytochemistry, immunohistochemistry, and immunoblotting.

Anti-CRH/CRF, Cat. #AB-02

Corticotropin-releasing hormone/factor (CRH/CRF) is the principal neuropeptide involved in regulating the stress response. It stimulates ACTH release from the pituitary gland. When centrally administered to animals it produces somatic changes analogous to those seen in both depression and anxiety. In humans, it is capable of reproducing the hormonal changes which are characteristically seen in depressed patients. **Anti-CRH/CRF** recognizes human and rat corticotropin-releasing hormone/factor. Applications include radioimmunoassay, affinity chromatography, immunoblotting, immunostaining and immunocytochemistry.



basic Fibroblast Growth Factor (FGF-2), Cat. #AB-07

Lane 1: Molecular weight standards (Invitrogen See-Blue)

Lane 2: 50 ng bFGF probed with AB-07 (Lot #30-160) at a 1:1000 dilution.

Anti-basic FGF (FGF-2), Cat. #AB-07, #AB-08

Basic Fibroblast Growth Factor (FGF-2) is a 155-amino-acid protein (154 amino acids in rat) that has wide-ranging effects in a variety of systems. FGF-2 (also known as basic FGF) can induce proliferation of fibroblasts, endothelial cells, chondrocytes, smooth muscle cells, melanocytes, and other cell types. FGF-2 also has the ability to cause adipocyte differentiation, stimulate astrocyte migration, and prolong neuron survival. Several isoforms of this protein exist, ranging from 16 to 24 kD in size. The FGFs, often because of proliferative activities, are now considered to play substantial roles in cellular development, tissue remodeling, hematopoiesis, and tumorigenesis. Anti-basic FGF recognizes FGF-2 in mammals (Cat. #AB-07) and in rat (Cat. #AB-08). Applications include immunoblotting and immunohistochemistry.

Targeting Technology

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

Choose an ANTIBODY[§] specific to your cell type.

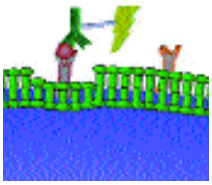
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

[§]or growth factor, peptide, ligand, or cytokine

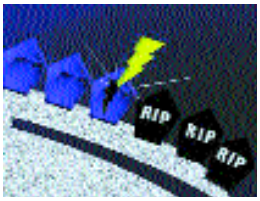
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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, one letter to each block, to solve the puzzle.

IFROOMS

NICEGRANTS

ATIMOUNT

GANDERED

SCOUF

How the bacteriologist said the secret formula was designed.

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

Answer: It all began with a...

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by February 28, 2007.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

PRSRT STD
UNITED STATES
POSTAGE PAID
SAN DIEGO, CA
PERMIT #1235

Apr-May-Jun 2007
Volume 8, Issue 2

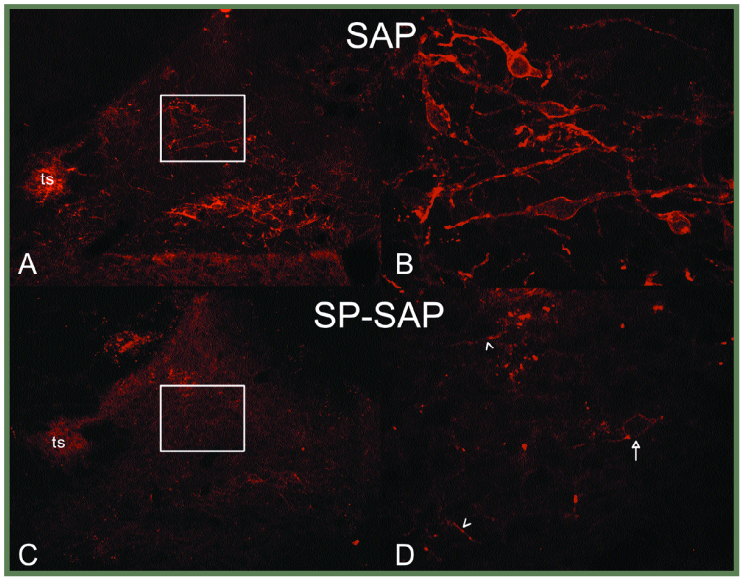
Targeting Trends

Reporting the latest news in Molecular Surgery

Nucleus tractus solitarii injections of substance P-conjugated saporin selectively disrupts baroflex-dependent pathways.

Contributed by Jeffrey T. Potts, Ph.D., Department of Biomedical Science, College of Veterinary Medicine, Dalton Cardiovascular Research Center, Columbia, MO 65211

The nucleus tractus solitarii (NTS) receives afferent input from visceral, somatic and cardiopulmonary structures and is the first central site for the processing of these sensory autonomic signals. Modulation of sensory neurotransmission has been well documented in the NTS. However, the specific role of the neuropeptide substance P (SP) in the sensory neurotransmission in the NTS remains debated. Previous studies have reported that stimulation of somatic and baroreceptor afferents release SP in the NTS and that neural feedback from skeletal muscle depresses arterial baroreflex function by activating local GABAergic NTS circuits. Since baroreflex depression can be prevented by a pharmacology blockade of neurokinin-1 (NK1) receptors, sensory feedback from skeletal muscle may target a local population of NK1 receptor-expressing GABA neurons that selectively inhibit barosensitive NTS neurons.



To address this question, we injected the neurotoxin substance P-saporin (SP-SAP, Cat. #IT-07), that selectively lesions cells expressing NK1 receptors, into the NTS. SP-SAP (1-3 ng in 100 nl) was bilaterally microinjected medial to the solitary tract at the level of

(continued on page 6)

Figure 1 – NTS lesioning of NK1 receptor-expressing cells.

Panel A and C: Cross-sectional image of rat hindbrain containing the NTS at low magnification (4x). NK1 immunoreactivity was highly expressed on cell soma and fibers in regions medial to the solitary tract (ts) of rats treated with non-targeted SAP toxin. In addition, neurons in the dorsal motor nucleus of the vagus also expressed intense NK1 immunoreactivity. In contrast, NK1 immunoreactivity in the NTS was virtually undetectable in rats receiving SP-SAP.

Panel B and D: High magnification (40X) of NTS region medial of the solitary tract (region of white boxes). The number of NK1-positive soma and fibers are clearly reduced by SP-SAP. Arrow indicates a surviving NK1 receptor-expressing cell and arrowheads indicated spared NK1 fibers.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

SP-SAP Drug Development Update

Substance P-Saporin (SP-SAP) is a drug candidate that eliminates the spinal cord neurons that send the chronic pain signal to the brain. SP-SAP has been tested in a variety of animal models and proven effective in permanently eliminating the perception of chronic pain. ATS has completed extensive preclinical studies, a GLP toxicology study, and a safety study. SP-SAP is currently being used in a veterinary trial in companion dogs who are suffering from bone cancer. We are hopeful that treatment with SP-SAP will allow for the reduction of opioid administration and restore a better quality of life to these beloved pets.

The ultimate goal is to have SP-SAP approved as a chronic pain drug for humans. ATS plans to meet with the FDA in the next few months to see what final steps are necessary to begin clinical trials. This meeting will determine any additional testing that is needed and will outline the parameters of patient participation and monitoring in a clinical trial. The FDA has recommended that the first population of patients be terminal cancer patients who are non-responsive to opioid treatment. These needy patients currently have no options for pain relief and are being sedated as the only recourse. It is our deepest hope that SP-SAP will allow these patients to be restored to a better quality of life in their last few months to share precious moments with their loved ones. We will continue to keep our website updated on this drug development process.

Upcoming Events

American Association for Cancer Research
April 14-18, 2007
Los Angeles, CA
Booth #1553

Experimental Biology
April 28 - May 2, 2007
Washington, DC
Booth #215

New Targets: Insula Receptors

In the January 26 issue of *Science* there was an article by Naqvi *et al.* about patients with brain damage to the insula losing their desire to smoke. A podcast interview with Dr. Bechara, one of the authors of the study, ended with him stating that there are a number of known receptors in the brain that should be tested for their association in all kinds of addiction—smoking, drugs, alcohol. He further stated that he also believes this area of the brain may be responsible for eating disorders, such as anorexia and bulimia.

The ATS targeting technology has important applications in identifying potential therapeutic targets in the insula. By using targeted toxins to specifically eliminate cell types in the insula based on their specific receptors, researchers can dissect this complicated system and identify specific cell functions. For example, the insula is known to have mu-opioid receptors. ATS has developed a targeted toxin, dermorphin-SAP (Cat. #IT-12) that specifically eliminates neurons that have the mu opioid receptor.

It is possible that two other ATS products that target transporters will also be helpful in search for potential pharmacological targets. These targeted toxins are anti-DAT-SAP (Cat. #IT-25) and anti-SERT-SAP (Cat. #IT-23).

Targeting Teaser Winners

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

The solution to the puzzle was:

Jumbles: ISOFORM
TRANSGENIC
MUTATION
DERANGED
FOCUS

Answer: It all began with a ...
"GERM OF AN IDEA"

Winners: Maria Montero- Institute of Medical Biology, Department of Anatomy and Neurobiology, Odense DENMARK * Darlene Martineau- UCSD, Whittier Institute, La Jolla CA * Shawn McClelland- California State University, Northridge CA * Mark Damico- Panacea Pharmaceuticals Inc, Gaithersburg MD * Robert Speth- University of Mississippi, School of Pharmacy, University MS

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Cholinergic modulation of sensory interference in rat primary somatosensory cortical neurons

Alenda A, Nunez A
Brain Res 1133(1):158-167, 2007.

One critical feature of cognition is the ability to focus on selected sensory inputs while ignoring irrelevant inputs. In this work the authors examine basal forebrain participation in sensory interference effects. Following 0.15 μg bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the basal forebrain of rats, the ability of primary somatosensory cortical neurons to respond in the presence of sensory interference was assessed. A decrease in the number of neurons showed sensory interference in lesioned animals.

Substance P-saporin down-regulates substance P receptor immunoreactive sensory dorsal root ganglion neurons innervating the lumbar intervertebral discs in rats

Ohtori S, Inoue G, Koshi T, Ito T, Doya H, Moriya H, Takahashi K
Spine 31(26):2987-2991, 2006.

Neurokinin-1 (NK-1) receptor expressing neurons that innervate lumbar intervertebral discs may be involved in lower back pain. Here the authors investigate the basic effect of SP-SAP (Cat. #IT-07) on neurons innervating the L5/6 intervertebral disc. Rats were injected with 175 ng of SP-SAP. The number of NK-1 receptor expressing neurons was reduced by over 75% in the treated animals, demonstrating SP-SAP as a useful tool to investigate the mechanism of discogenic low back pain, particulary for investigating behavioral impacts.

Selective lesions of the nucleus basalis magnocellularis impair cognitive flexibility

Cabrera SM, Chavez CM, Corley SR, Kitto MR, Butt AE
Behav Neurosci 120(2):298-306, 2006.

In humans, one aspect of cognitive flexibility is being able to shift attention under a variety of pressures. Here the authors suggest that lesions to the cholinergic nucleus basalis magnocellularis (NBM) will impair cognitive flexibility. The NBM of rats was lesioned with 0.08 μg of 192-IgG-SAP (Cat. #IT-01). Both lesioned and controlled animals displayed a similar ability to learn a discrimination task, but lesioned animals displayed perseveration - the uncontrollable repetition of a previously correct response - indicating a loss of cognitive flexibility.

Extensive training in a maze task reduces neurogenesis in the adult rat dentate gyrus probably as a result of stress

Aztiria E, Capodieci G, Arancio L, Leanza G
Neurosci Lett Feb 3, 2007 [ePub].

Ascending cholinergic inputs from the basal forebrain modulate hippocampal neurogenesis, although it is not clear if the modulation is direct or indirect. In this study rats experienced extended training in a spatial navigation task following 192-IgG-SAP (Cat. #IT-01) lesions. 192-IgG-SAP was injected into the basal forebrain nuclei and the cerebellar cortex. Although the lesioned animals displayed an 80% reduction in neuron proliferation in the dentate gyrus, extended training and learning did not affect proliferation.

Guanidinylated-Neomycin delivers large, bioactive cargo into cells through a heparan sulfate dependent pathway

Elson-Schwab L, Garner OB, Schuksz M, Esko JD, Tor Y
J Biol Chem Feb 20, 2007 [ePub].

The uptake of high molecular weight drugs into cells is a stumbling block for some potential therapeutics. Using a neomycin derivative in which guanidinium groups have replaced the ammonium groups, the authors show heparan sulfate-dependent uptake of large molecules. The guanidine-neomycin was biotinylated, and incubated with streptavidin-ZAP (Cat #IT-27). This complex was effective in killing CHO cells *in vitro*, but was no more effective than streptavidin-ZAP alone on cells lacking heparan sulfate expression, demonstrating specificity.

Targeted deletion of neurokinin-1 receptor expressing nucleus tractus solitarii neurons precludes somatosensory depression of arterial baroreceptor-heart rate reflex

Potts JT, Fong AY, Anguelov PI, Lee S, McGovern D, Grias I
Neuroscience Feb 8, 2007 [ePub].

Previous work by these authors examined the role of substance P in arterial baroreflex. Here, 1.5 ng bilateral injections of SP-SAP (Cat. #IT-07) into the caudal nucleus tractus solitarii of rats were used to further elucidate the fundamental role of substance P in this system. The depressive effect of somatosensory input by neurokinin-1 receptor-expressing neurons on arterial baroreceptor-heart rate reflex was abolished in lesioned animals. (see Cover Story.)

Selective cholinergic depletion of the hippocampus spares both behaviorally induced Arc transcription and spatial learning and memory

Fletcher BR, Baxter MG, Guzowski JF, Shapiro ML, Rapp PR
Hippocampus 17 (3):227-234, 2007.

The immediate early gene Arc is required for long-term synaptic changes and memory consolidation. The authors lesioned the fornix to examine cholinergic contributions of the medial septum and the vertical diagonal band to spatial learning impairments and behavioral induction of Arc transcription. 0.24-0.36 μg of 192-IgG-SAP (Cat. #IT-01) was delivered to the fornix of rats. Results from various water-maze tasks indicate that spatial learning deficits and impaired Arc transcription associated with lesions of the fornix are not caused by cholinergic deafferentation.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

From anxiety to autism: spectrum of abnormal social behaviors modeled by progressive disruption of inhibitory neuronal function in the basolateral amygdala in Wistar rats

Truitt WA, Sajdyk TJ, Dietrich AD, Oberlin B, McDougle CJ, Shekhar A
Psychopharm (Berl) Feb 3, 2007 [ePub].

The amygdala has been identified as being involved in social behaviors. Six 4 ng injections of SSP-SAP (Cat. #IT-11) were administered bilaterally into the basolateral nucleus (BLA) of the amygdala of rats. Blank-SAP (Cat. #IT-21) was used as a control. Results of a social interaction paradigm suggest that in normal animals social inhibition can be overcome by habituation. In lesioned animals, however, social inhibition is not reversed by habituation, indicating that NK-1 receptor-expressing GABAergic interneurons in the BLA are important in this system.

Astrocytic reaction to a lesion, under hormonal deprivation

Martinez L, de Lacalle S
Neurosci Lett Jan 12, 2007 [ePub].

One effect of estradiol on astrocytes is the mediation of neuronal sprouting. Astrocytes express glial fibrillary acidic protein (GFAP) in response to injury, but estradiol has been shown to repress GFAP expression. Ovariectomized female rats received 15 ng of 192-IgG-SAP (Cat. #IT-01) into the horizontal limb of the diagonal band of Broca, followed by long-term estrogen treatment. The results suggest that estradiol deprivation may exacerbate the effects of a cholinergic lesion, and administration of estradiol may aid the recovery of lesioned cholinergic neurons by blocking GFAP expression.

Targeted Delivery and Enhanced Cytotoxicity of Cetuximab-Saporin by Photochemical Internalization in EGFR-Positive Cancer Cells

Yip WL, Weyergang A, Berg K, Tonnesen HH, Selbo PK
Mol Pharm Jan 31, 2007 [ePub].

Photochemical internalization (PCI) releases macromolecules from endocytic vesicles using photosensitizer activation by light. This technique allows the release of endocytosed molecules before degradation occurs in the lysosome. The authors demonstrate the proof-of-concept for this technique by combining biotinylated cetuximab (a chimeric monoclonal antibody to the EGFr) with streptavidin-ZAP (Cat. #IT-27). The conjugate was applied to three different human cancer cell lines, demonstrating enhanced specificity and toxicity against cells expressing the EGFr.

Lesions to the nucleus basalis magnocellularis lower performance but do not block the retention of a previously acquired learning set

Bailey AM, Lee JM
Brain Res 1136(1):110-121, 2007.

A major source of cholinergic innervation to several cortices is the nucleus basalis magnocellularis (NBM). Rats were trained to acquire an olfactory discrimination learning set, then were lesioned with 192-IgG-SAP (Cat. #IT-01) or quisqualic acid. 0.075 μg of 192-IgG-SAP was administered in 2 sets of bilateral infusions. While treated animals performed poorly following the surgery, performance improved to better than expected by chance during the second trial. The authors discuss the role of the NBM in cognitive flexibility.

Noncholinergic lesions of the medial septum impair sequential learning of different spatial locations

Dwyer TA, Servatius RJ, Pang KC
J Neurosci 27(2):299-303, 2007.

The medial septum and the vertical limb of the diagonal band of Broca (MSDB) have extensive connections to the hippocampus. In general, impairments due to loss of cholinergic neurons in this area have been smaller than those due to the loss of noncholinergic neurons. The authors treated rats with either 192-IgG-SAP (Cat. #IT-01) or kainic acid into each hemisphere of the medial septum. Behavioral testing following surgery demonstrated that the animals with noncholinergic lesions had impaired performance, even when compared to the animals with cholinergic lesions.

Extracellular signal-regulated kinase-regulated microglia-neuron signaling by prostaglandin E2 contributes to pain after spinal cord injury

Zhao P, Waxman SG, Hains BC
J Neurosci 27(9):2357-2368, 2007.

Spinal cord injury frequently leads to the development of long-term chronic pain. Recent data has shown that activated microglia are involved in the maintenance of this pain state. Following a spinal cord contusion injury rats were treated with a 36-μg injection of Mac-1-SAP (Cat. #IT-06) into the lumbar enlargement. Treated animals were found to have reduced microglial staining, reduction in prostaglandin E2 levels, and fewer pain-related behaviors.

Decreased vesicular acetylcholine transporter and alpha(4)beta(2) nicotinic receptor density in the rat brain following 192 IgG-saporin immunolesioning

Quinlivan M, Chalon S, Vergote J, Henderson J, Katsifis A, Kassiou M, Guilloteau D
Neurosci Lett Mar 3, 2007 [ePub].

The vesicular acetylcholine transporter (VAChT) is a useful imaging target to assess Alzheimer's disease, since this transporter is expressed on cholinergic cells that are lost as the disease progresses. Through the use of 192-IgG-SAP (Cat. #IT-01) the authors demonstrate the use of two radioligands, one that binds VAChTs, the other which binds nicotinic acetylcholine receptors (nAChRs). The results show the efficacy of each radioligand, as well as the loss of nAChRs on cholinergic neurons after treatment with 192-IgG-SAP.

Please visit www.ATSbio.com to see a complete list of references.

Targeting Talk: *Agonist Effects*

by Dr. Douglas Lappi

Q: Do conjugated toxins (Dermorphin-saporin in particular) exhibit agonist effects? I've generated behavioral and tissue time course effects but have not established agonist effects for this conjugated toxin.

A: The peptide ligand toxins should exhibit agonist effects. They are constructed purposely to retain complete agonist activity, including for us the most important: internalization. So, for instance, SP-SAP (Cat. #IT-07) causes receptor internalization similar to SP, as reported in Mantyh *et al*. (Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279, 1997).

As to dermorphin-SAP (Cat. #IT-12) specifically, it has agonist activity very much like dermorphin. This is reported in Porreca *et al*. (Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the mu-opioid receptor. *J Neurosci* 21:5281-5288, 2001) in which it's stated:

The bilateral microinjection of 3 pmol of dermorphin or of dermorphin-saporin directly into the RVM produced a robust antinociceptive effect in the 52°C hot-water tail-flick test. The peak antinociceptive effect of dermorphin, 78 ± 13.2% MPE, was not significantly different from that of the dermorphin-saporin conjugate, which was 59 ± 4.7% MPE ($p > 0.5$, Student's t test).

Usually the amount needed to give a response is lower than the amount needed to kill a cell. Depending on what your system is; it may be a peculiarity of that system, but I would be a little concerned about not seeing an agonist effect. On the other hand, if you have demonstration of specific toxicity, it may not be all that crucial.

SP-SAP Research Replacement

SP-SAP (formerly Cat. #IT-07) is being replaced by SSP-SAP (Cat. #IT-11). Scientific advisors have given counsel for this replacement because SSP-SAP is a superior lesioning agent in many situations, due to its targeting vehicle, a protease-resistant form of substance P. An excellent example is the paper by Martin and Sloviter, *J Comp Neurol* 436:127-152 (2001), in which after ineffective intraparenchymal injection of SP-SAP in the hippocampus, SSP-SAP was used with tremendous efficacy. In almost all applications we expect SSP-SAP will be used at a lower dose than SP-SAP.

We will not compromise the work of those that are midstream in experiments with SP-SAP. Orders from those who wish to continue with SP-SAP will be honored. This will be a gradual phase-out since SP-SAP is being developed as a chronic pain therapeutic (see update on Page 2). Please rest assured that your work is the backbone of our company and all questions or comments will be carefully considered.

Questions about an ATS product or Molecular Surgery technique? Write to us at ats@ATSbio.com

SP-SAP Selectively Disrupts Baroflex-dependent Pathways

(continued from page 1)

the caudal pole of area postrema and rats recovered 5-7 days post injection. Lesions produced a substantial reduction in the number of NK1 receptor-expressing cells in the NTS compared to animals that received either phosphate buffered saline or non-targeted recombinant saporin (SAP) vehicle injections (Fig 1). Importantly, lesions were restricted to the medial and commissural subdivisions of the NTS and extended rostrocaudally from the caudal to the rostral pole of area postrema. The restricted loss of NK1 receptor-expressing cells may have resulted from the relatively low amount of toxin injected combined with its relatively high molecular weight (33kDa). Loss of NK1 cells was accompanied by a significant reduction in the number of GABA neurons in the same region of the NTS (Fig 2). Cellular toxicity was not responsible for cell loss since no group differences were found in the total number of NTS cells when tissue was stained using Neutral Red.

SP-SAP injections reduced baseline baroreflex function by 70% in treated animals. Despite the reduction in baroreflex sensitivity, SP-SAP also abolished the depressive effect of somatic afferent activation on arterial baroreflex function (Fig 3). In sharp contrast, arterial chemoreflex responses (that are transmitted by chemosensitive carotid body afferents of the glossopharyngeal nerve to similar regions of the NTS) were virtually unaltered by SP-SAP treatment.

Taken together, our results suggest that a restricted population of NK1 receptor-expressing GABA neurons participate in arterial baroreceptor signaling in the NTS. The ability of the SP-SAP toxin to selectively target and lesion NK1 receptor-expressing cells within a very limited region has provided a means to further investigate the role of SP on sensory transmission in the NTS.

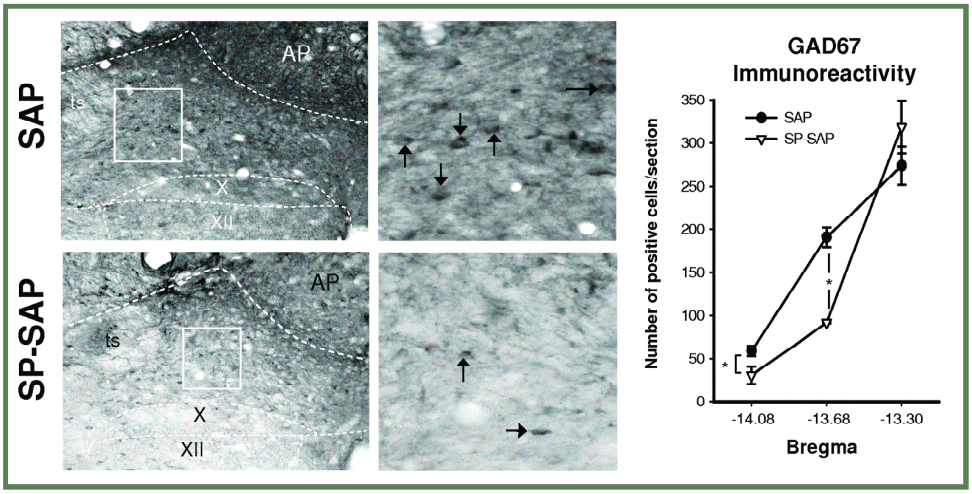


Figure 2 – Immunohistochemical labeling of GAD67 in the NTS using monoclonal mouse anti-GAD67 (MAB5406, Chemicon) and visualized using nickel-enhanced diaminobenzidine.

Upper panels show low and high magnification of GAD67 immunoreactivity medial to the solitary tract (ts) in a SAP-treated rat. Lower panels show similar images obtained from a SP-SAP-treated rat. Note: reduction in GAD67 positive soma (arrows) between SAP- and SP-SAP-treated animals.

Right panel shows group data (n=3 per group) illustrating a significant reduction in GAD67 immunoreactivity following SP-SAP treatment.

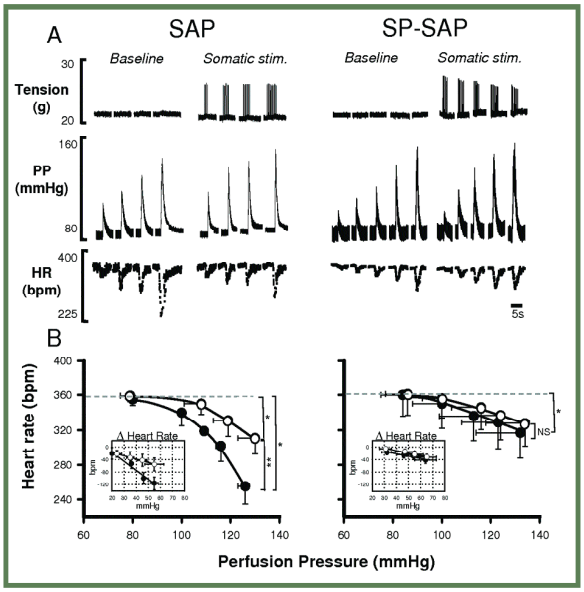


Figure 3 – Effect of SP-SAP on somatosensory-evoked depression of arterial baroreflex control of heart rate.

Panel A: Graded arterial baroreflex bradycardia produced before (baseline) and during activation of somatic afferents (somatic stim). Somatic stimulation blunted the peak bradycardic response in SAP, but not SP-SAP, treated animals.

Panel B: Group data showing that somatic stimulation failed to blunt the reflex bradycardia in SP-SAP treated animals.

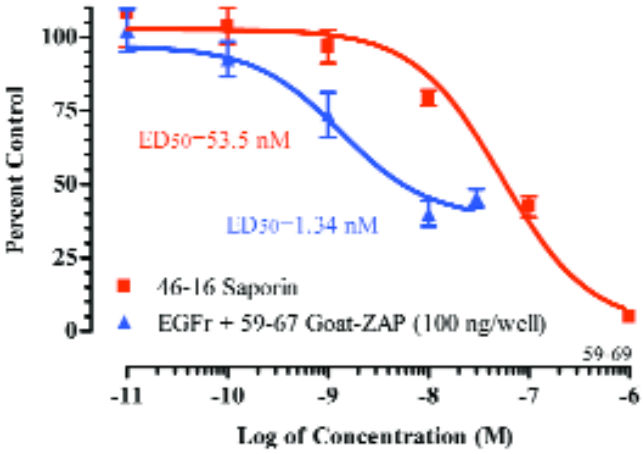
Acknowledgements – NK1 images were obtained by David McGovern, Research Assistant in the Potts Lab using an Olympus BX51-DSU microscope equipped with 4X and 40X objectives and a spinning disk confocal unit. Each image was acquired using Neurolucida software (confocal module) and a Z-axis encoded Ludl stage. Final images are maximal intensity Z-projections created using Image J software (NIH) and visualized using Cy3 secondary antibody. Potts lab is supported by NIH HL059167.

Targeting Tools: New Products

Secondary Conjugate: Goat-ZAP

Goat-ZAP (Cat. #IT-36) is a new secondary conjugate of affinity-purified rabbit anti-goat IgG and the ribosome-inactivating protein, saporin. This product uses affinity-purified rabbit anti-goat IgG to "piggyback" onto YOUR primary goat antibody in order to evaluate the ability of your primary antibody to internalize. Once the conjugate is internalized, saporin breaks away from the targeting agent and inactivates the ribosomes, which causes protein inhibition and, ultimately, cell death.

This product is excellent for determining the specificity of your antibody. When the *in vitro* results confirm the desired specificity, it is recommended that you order a custom conjugation of your antibody to saporin.



3T3 cells were plated at 1000 cells/90 µl/well in a 96-well plate and incubated overnight. Saporin dilutions were made in cell media, and 10 µl was added to each well. EGFr antibody was diluted in cell media containing, at a final concentration, 100 ng/10 µl of Goat-ZAP, and 10 µl was added to each well. The plates were incubated 72 hours and developed with MTS/PMS. Data was analyzed by Prism (GraphPad).

New Antibodies

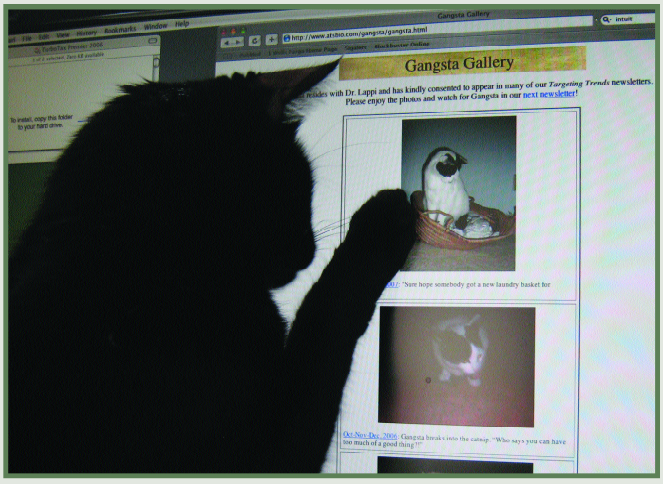
ATS is pleased to announce several new antibodies. For a complete list of antibodies and antigens, visit our website (www.ATSbio.com).

MMP-1 (Cat. #AB-29)
MMP-2 (Cat. #AB-30)
MMP-3 (Cat. #AB-31)
MMP-9 (Cat. #AB-32)
actMMP-9 (Cat. #AB-28)
NSE (Cat. #AB-33)
TIMP-1 (Cat. #AB-34)
TIMP-2 (Cat. #AB-35)
Cytochrome P450
(Cat. #AB-22, AB-23, AB-24, AB-25, AB-26, AB-27)

Custom Services

You can have a fluorescent conjugate or primary immunotoxin custom made for your specific needs.

For more information, visit www.ATSbio.com. Click on Catalog, then check out Custom Services.



Kristen's cat, Piano, just loves the new Gangsta Gallery. Gangsta is his hero.

Check it out at www.ATSbio.com/Gangsta

Fluorescent Conjugates

ATS has added three new products to our fluorescent conjugates line.

Alexa488-labeled affinity purified NGFr* (Cat. #FL-09)
FITC-labeled Goat Anti-Rat IgG (Cat. #FL-10)
FITC-labeled Donkey Anti-Goat IgG (Cat. #FL-11)
*(Cat. #AB-N01AP)

These conjugates are excellent tools to verify specific binding to an antigen. By first incubating cells with the primary antibody, and then binding the fluorescent conjugate to the primary antibody, a fluorescent marker is formed that can demonstrate expression of an antigen or affinity of an antibody for its receptor.

Other fluorescent conjugates include markers for 192 IgG, Anti-NGFr, Anti-SAP, Mouse IgG and Rabbit IgG. Custom conjugates can also be made.

Targeting Technology

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

Choose an ANTIBODY[§] specific to your cell type.

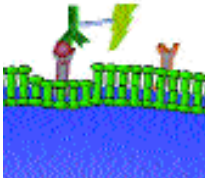
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

[§]or growth factor, peptide, ligand, or cytokine

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, one letter to each block, to solve the puzzle.

NICEJOINT

OXENINROUT

ITURCCI

STAGEINVITE

HEATSHOPP

How the scientist referred to his disappointing results.

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

Answer: It's just an. . .

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by May 31, 2007.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121 USA

PRSRT STD
UNITED STATES
POSTAGE PAID
SAN DIEGO, CA
PERMIT #1235

Jul-Aug-Sep 2007
Volume 8, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- New Targets: Claustrum *(page 2)*
- Teaser Winners *(page 2)*
- Flow Cytometry Services *(page 6)*
- Anti-CD22 and Anti-CD22-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

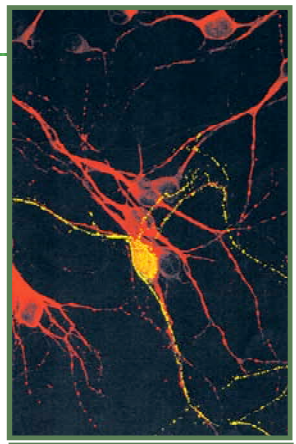
Targeted Therapy for Chronic Pain

In 1997, SP-SAP (a chemical conjugate of Substance P and the ribosome-inactivating protein saporin) was created to eliminate Substance P receptor (SPR) neurons that propagate the signal for chronic pain. ATS has successfully completed preclinical studies, safety tests in animal models and a GLP toxicology study in rat, funded both internally and by the National Institute for Mental Health. In 2000, ATS was granted patent protection for SP-SAP.

ATS is seeking an alliance to commercialize the therapeutic applications of Substance P-Saporin (SP-SAP), a large molecule, biochemical conjugate, that when delivered directly into the spinal fluid, seeks out and eliminates specific nerve cells that transmit chronic pain messages to the brain. Because of its size, this drug does not travel far from the site of administration. This innovative new treatment has created great excitement in the scientific and medical communities.

This is an opportunity for an experienced drug company to participate in the development of a therapeutic with (1) no drug discovery required, (2) patent protection, (3) successful preclinical data, and (4) a defined initial target market with accelerated development timeline.

SP-SAP preclinical data were the basis for two articles in *Science* and received international press coverage. The mechanism of SP-SAP is well understood. It enters SPR-positive neurons by internalization of the ligand-receptor complex. SAP enters the cytoplasm, enzymatically inactivates the ribosomes and shuts down protein synthesis, resulting in cell death. Four key points from these results are: (i) SP-SAP greatly reduces neuropathic and inflammatory pain, (ii) normal pain transmission is completely retained, (iii) pain relief appears permanent with no collateral pain states, and (iv) pain that occurs after treatment with SP-SAP can still be treated with morphine.



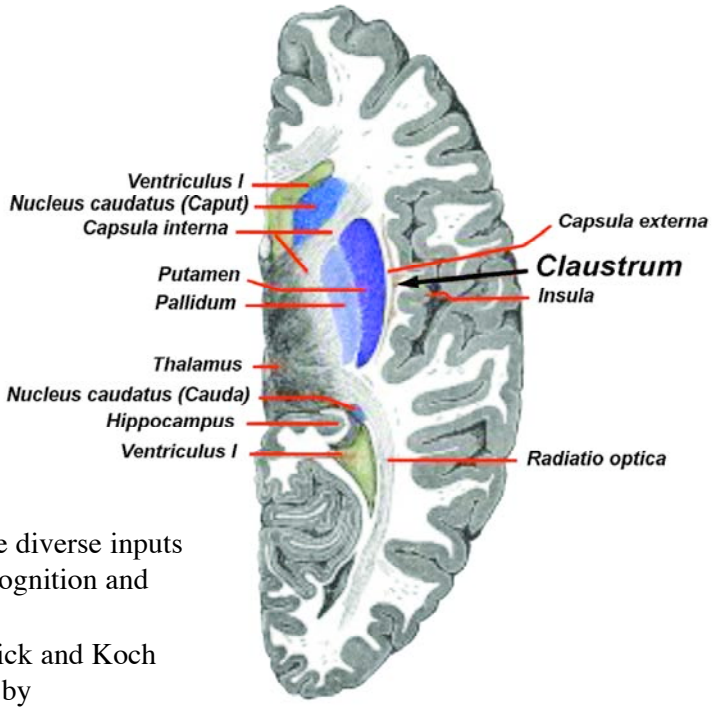
Yellow staining for saporin after internalization of SP-SAP by this single spinal cord neuron in primary culture.

The chronic pain population is one of the most pressing healthcare issues in the world, with an estimated size of 50-100 million persons in the U.S. alone. Chronic pain disables more people than cancer or heart disease and costs the American public more than both diseases combined—estimated at upwards of $40B in medical expenses annually.

Advanced Targeting Systems is seeking an alliance with an established pharmaceutical or biotechnology corporation in order to transfer SP-SAP into a working environment that can quickly and efficiently take the drug to human clinical trials and on to market. With the proper infrastructure SP-SAP could be in clinical trials in one year. Income from sales of the drug would be possible in three years. ATS is working with leading universities, has assembled a stellar advisory board, and industry-leading cancer clinicians are waiting to participate in clinical trials. The right alliance would be beneficial not only to the corporations involved, but to the millions who suffer from chronic pain.

For additional information, visit:
http://www.atsbio.com/chronicpain.htm

New Targets



What is the claustrum? The simple answer is that it's a brain structure that lies under the insular and piriform cortices. What it does is rather unclear, but one hypothesis has drawn considerable attention. The claustrum contains projections to the entire cortex, and this may indicate that it plays an important role in brain function. But that role has been elusive. The hypothesis that has attracted attention is from the late, great Francis Crick. In his article with Christoph Koch (Crick and Koch 2005), it is proposed that the claustrum acts like a conductor of an orchestra, bringing together the disparate pieces to make something other than the cacophony of the orchestra warming up. They propose that the claustrum integrates the diverse inputs to make the sense "that underlie(s) conscious perception, cognition and action." Perhaps this is the seat of consciousness?

Examination of the claustrum has not been easy. As Crick and Koch state: "It is very difficult to solely inactivate the claustrum by pharmacological substances or surgical ablation. Given its extended and sheet-like topography, ablating or otherwise shutting this structure down in a controlled manner—without interfering with fibres of passage or nearby regions—would require numerous, precisely targeted injections of the claustrum."

Or perhaps targeting at the molecular level with targeted toxins. What is needed is array analysis of the cell-surface proteins that are expressed in the claustrum, followed by administration of targeted toxins that would perform the suggested ablation. Koch, in his book, *The Quest for Consciousness*, proposes the identification of the "neuronal correlates of consciousness" (2004), and states, "knowing where the neurons that mediate a specific conscious percept are located, where they project to and receive input from, their firing pattern, their developmental pedigree from birth to adulthood, and so on, might provide a…breakthrough on the way to a complete theory of consciousness." Sounds like a job for targeted toxins.

And what happens if we see that the claustrum is the seat of consciousness? Well, insects have a claustrum, too. Do they have consciousness? There are lots of interesting questions in this project.

Crick, F. C. and C. Koch (2005). "What is the function of the claustrum?" *Philos Trans R Soc Lond B Biol Sci* 360(1458): 1271-9.
Koch, C. (2004). The Quest for Consciousness. Englewood CO, Roberts and Company.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: INJECTION
NEUROTOXIN
CIRCUIT
INVESTIGATE
PHOSPHATE

Answer: It's just an. . .INCONVENIENT TRUTH

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

WINNERS: John Downie- Dalhousie University, Pharmacology, Halifax Nova Scotia, CANADA * Shawn McClelland- California State University, Northridge CA * Tania Bedard- University of Texas Health Science Center, Pharmacology, San Antonio TX * Vivian Lee- Medical College of Wisconsin, Dept of Pediatrics, Milwaukee WI * Seto Chice- SUNY HSC at Brooklyn, Brooklyn NY * T. Michael Gill- UCSF, Dept of Neurology, San Francisco CA * Robert Speth- University of Mississippi, School of Pharmacy, University MS

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Anticonvulsant effects of damage to structures involved in seizure induction in rats exposed to soman

Myhrer T, Enger S, Aas P
Neurotoxicology [Epub Apr 24] 2007.

Soman is a nerve agent that irreversibly inhibits acetylcholinesterase, resulting in respiratory dysfunction, seizures, convulsions, coma, and death. In this work the authors investigated whether elimination of cholinergic pathways in the medial septum (MS) or diagonal band nucleus (DBN) would affect the onset of convulsions. 0.3 μl of 0.5 μg/μl 192-IgG-SAP (Cat. #IT-01) was infused into the MS and/or DBN. Although aspiration lesions of the MS increased time to the onset of convulsions, lesioning with 192-IgG-SAP produced no anticonvulsant effects. This data suggest that the aspiration lesions disrupted more than the cholinergic system of the MS.

Superficial NK1 expressing spinal dorsal horn neurones modulate inhibitory neurotransmission mediated by spinal GABA(A) receptors

Rahman W, Sikander S, Suzuki R, Hunt SP, Dickenson AH
Neurosci Lett 419(3):278-283, 2007.

It has been shown that elimination of lamina 1 NK1 receptor-expressing neurons affects pain behaviors. The authors investigated whether eliminating these neurons would alter GABAergic spinal inhibitory systems. Rats received 10-μl injections of 10 μM SP-SAP (Cat. #IT-07) into the L4-5 regions. Data generated by electrical and mechanical stimuli suggest that although GABAergic transmission is dependent on NK1 receptor-expressing neurons, loss of these cells results in a decrease in spinal cord excitability.

Cholinergic modulation of spindle bursts in the neonatal rat visual cortex *in vivo*

Hanganu IL, Staiger JF, Ben-Ari Y, Khazipov R
J Neurosci 27(21):5694-5705, 2007.

The authors investigated the relationship between cholinergic drive and spindle burst oscillation driven by retinal waves. 0.5 μl of 0.2 μg/μl 192-IgG-SAP (Cat. #IT-01) was injected into both ventricles of rat pups. The lesioned animals displayed markedly decreased oscillatory activity. Since this activity may be used as a functional template for cortical networks and architecture, the results suggest a link between cholinergic activity and cortical development.

Immunotoxic cholinergic lesions in the basal forebrain reverse the effects of entorhinal cortex lesions on conditioned odor aversion in the rat

Ferry B, Herbeaux K, Cosquer B, Traissard N, Galani R, Cassel JC
Neurobiol Learn Mem 88(1):114-126, 2007.

The entorhinal cortex (EC) is intimately involved in olfactory learning. Lesioning of this structure produces septo-cholinergic sprouting. Rats that had previously received EC lesions were treated with 5-μg intracerebroventricular injections of 192-IgG-SAP (Cat. #IT-01). The results point to a role for hippocampal cholinergic neurons in the modulation of memory processes involved with conditioned odor aversion.

Cholinergic lesions produce task-selective effects on delayed matching to position and configural association learning related to response pattern and strategy

Gibbs RB, Johnson DA
Neurobiol Learn Mem 88(1):19-32, 2007.

It has been well established that the cholinergic system of the basal forebrain plays a critical role in many cognitive processes. This work utilized injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum, the nucleus basalis magnocellularis, or both to examine the lesioning effect on two cognitive tasks in rats. Injections consisted of 0.20-0.40 μg of the conjugate. The data indicate that cholinergic lesions produce learning deficits that are task specific, and that learning is affected without corresponding deficits in memory.

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

Wiley RG, Kline RHt, Vierck CJ, Jr.
Neuroscience 146(3):1333-1345, 2007.

While lumbar injections of SP-SAP (Cat. #IT-07) produce specific lesions, use of this targeted conjugate in the forebrain has been problematic. The authors investigated the use of SSP-SAP (Cat. #IT-11), a conjugate of saporin with a more stable analog of substance P. The greater stability of SSP-SAP resulted in increased potency as well as better specificity. SSP-SAP is shown to be a highly effective reagent for the removal of NK1 receptor-expressing neurons in the brain and spinal cord.

Olfactory neophobia and seizure susceptibility phenotypes in an animal model of epilepsy are normalized by impairment of brain corticotropin releasing factor

Pascual J, Heinrichs SC
Epilepsia 48(4):827-833, 2007.

Olfactory recognition has been linked to epilepsy in behavioral phenotype models. This work examines the role brain stress neuropeptides play in the manifestation of neurological perturbations. Mice were injected with 2 μg/5 μl of CRF-SAP (Cat. #IT-13) into the lateral ventricle. Saporin (Cat. #PR-01) was used as a control. The lesioned mice displayed a temporary reduction in seizure susceptibility, and the reversal of olfactory deficits towards the detection of food.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Selective deletion of antigen-specific CD8+ T cells by MHC class I tetramers coupled to the type I ribosome-inactivating protein saporin

Hess PR, Barnes C, Woolard MD, Johnson MD, Cullen JM, Collins EJ, Frelinger JA
Blood 109(8):3300-3307, 2007.

Autoreactive T cells are involved in autoimmune diseases such as type 1 diabetes and multiple sclerosis. It is thought that selective depletion of pathogenic cytotoxic T lymphocytes would be an effective treatment. The authors coupled biotinylated major histocompatibility complex tetramers to streptavidin-ZAP (Cat. #IT-27) and were able to eliminate specific T-cells both *in vitro* and *in vivo*, while leaving control T-cells intact. This technique may prove to be a useful therapy for immune-mediated diseases.

Specificity and generality of the involvement of catecholaminergic afferents in hypothalamic responses to immune insults

Schiltz JC, Sawchenko PE
J Comp Neurol 502(3):455-467, 2007.

Interleukin-1 (IL-1) is one of the cytokines that mediates interactions between the immune system and the central nervous system. 380-ng injections of anti-DBH-SAP (Cat. #IT-03) were made into the paraventricular nucleus (PVH) of rats. Saporin (Cat. #PR-01) and mouse IgG-SAP (Cat. #IT-18) were used as controls. Lesioned animals demonstrated reduced responses to administration of IL-1, but restraint stress responses were left intact. The data suggest that ascending catecholaminergic projections mediate PVH response to IL-1.

Effect of the destruction of cells containing the serotonin reuptake transporter on urethrogenital reflexes

Gravitt K, Marson L
J Sex Med 4(2):322-330, 2007.

Using the fact that the urethrogenital (UG) reflex is an autonomic and somatic response, the authors developed a model for ejaculatory-like reflexes. Anti-SERT-SAP (Cat. #IT-23) was bilaterally injected into the ventrolateral medulla of rats. 80 nl of a 1-μM solution removed inhibition of the UG reflex after acute spinal cord transection, while this reflex could not be evoked in control animals. The data suggest that SERT-expressing neurons in the ventral medulla are involved with the inhibition of UG reflex.

Spinal NK-1 receptor expressing neurons mediate opioid-induced hyperalgesia and antinociceptive tolerance via activation of descending pathways

Vera-Portocarrero LP, Zhang ET, King T, Ossipov MH, Vanderah TW, Lai J, Porreca F
Pain 129(1-2):35-45, 2007.

Although used for treatment of pain, opioids can induce hyperalgesia. In this work the authors evaluated the role that NK-1 receptor-expressing neurons play in morphine-induced hyperalgesia and spinal antinociceptive tolerance. Rats received a 5-μl intrathecal injection of 10 μM SP-SAP (Cat. #IT-07). Saporin (Cat. #PR-01) was used as a control. The results indicate that NK-1 receptor-expressing neurons play a critical role in morphine-induced neuroplastic changes.

Scavenger receptor-A-targeted leukocyte depletion inhibits peritoneal ovarian tumor progression

Bak SP, Walters JJ, Takeya M, Conejo-Garcia JR, Berwin BL
Cancer Res 67(10):4783-4789, 2007.

Vascular leukocytes (VLC) are immunosuppressive cells that facilitate tumor progression in ovarian cancer. One potential tumor therapy is to eliminate these cells. The authors determined that scavenger receptor-A is specifically expressed on VLCs. Mice were injected with tumor cells, as well as an anti-scavenger receptor-A antibody combined with Rat-ZAP (Cat. #IT-26). This was followed by additional treatment with the antibody-Rat-ZAP complex. Treatment with the immunotoxin eliminated VLCs, inhibited peritoneal tumor burden, and reduced ascites accumulation.

Combined damage to entorhinal cortex and cholinergic basal forebrain neurons, two early neurodegenerative features accompanying Alzheimer's disease: effects on locomotor activity and memory functions in rats

Traissard N, Herbeaux K, Cosquer B, Jeltsch H, Ferry B, Galani R, Pernon A, Majchrzak M, Cassel JC
Neuropsychopharmacol 32(4):851-871, 2007.

Cognitive decline in Alzheimer's disease is linked with the cholinergic system of the basal forebrain (BF), but damage is also found in the entorhinal cortex (EC). This work describes the use of 192-IgG-SAP (Cat. #IT-01) and L-N-methyl-D-aspartate to eliminate neurons in the BF and EC. 5 μg of 192-IgG-SAP was injected into the ventricle of rats. OX7-SAP (Cat. #IT-02) was used as a control. The combination of BF and EC lesions resulted in larger permanent deficits in learning and memory than either lesion alone.

Please visit www.ATSbio.com to see a complete list of references.

Targeting Talk

by Dr. Douglas Lappi

Q *Your targeted toxin data sheet gives the following instruction for disposal: "Care in disposal is mandatory; autoclaving or exposure to 1 M sodium hydroxide will inactivate the material. All labware that comes into contact with this material should be likewise treated." I am wondering if I can deactivate saporin by using 10% bleach or if everything has to be autoclaved?*

A Yes, you can use bleach to deactivate saporin prior to disposal or reuse of labware. If you are using nanogram quantities, these are too low to be toxic, so you can discard as you do your other non-hazardous laboratory materials without fear.

Q *I was wondering if there is any indication that intrastriatal administration of 192-IgG-SAP (Cat. #IT-01) will lesion the cholinergic neurons of the striatum. My sense from reviewing the literature is that these cholinergic neurons are not susceptible to the toxin, but I thought I'd ask to see if you had any information / experience regarding this point.*

A No, I don't think it will work because the target of 192-IgG-SAP is p75, LNGFr, which is only expressed on the rat basal forebrain cholinergic neurons. Those striatal neurons don't express p75 in the adult. The NK1r is often expressed in the striatum, and you can use SSP-SAP for them, but that's the best we can do right now.

Q *I have a few questions about the Alexa488-labeled affinity purified NGFr antibody (Cat. #FL-03).*
Is it specific to extracellular p75?
Can you use it on live cells? Does it work on fixed cells?
Does it cause activation of the p75 receptor (i.e., result in apoptosis or changes in axon outgrowth in neuronal cells)?

A This product does recognize extracellular p75 in both live and fixed cells. As for the activation, that's an interesting question. There is no evidence of 192-IgG either causing apoptosis or neurite outgrowth as far as I can see. Chandler *et al.* (1984) report that the antibody "partially inhibits the regeneration of neurites from primed PC12 cells," and it enhances NGF binding. But that's about it, despite several studies being done with PC12 cells and *in vivo*. We assume all this holds upon treatment with 192-IgG-SAP — until the cell dies from saporin poisoning.

Chandler CE, Parsons LM, Hosang M, Shooter EM (1984) A monoclonal antibody modulates the interaction of nerve growth factor with PC12 cells. *J Biol Chem* 259(11):6882-6889.

Q *I purchased your secondary conjugate, Mab-ZAP (Cat. #IT-04). I am preparing to do a cytotoxicity assay and I'm wondering if my primary antibody should be sterilized prior to combining with Mab-ZAP?*

A Depending on the conditions of your lab in which you are using your antibody, it is possible that within a 72-hour period, you may see bacterial growth in your plates if the antibody was accidentally exposed to bacteria. It is recommended that, if you feel comfortable with the antibody, you can just go ahead and try it without sterilizing it, and if you do see bacterial growth, you can certainly filter sterilize the material through a 0.2 micron filter before using.

Society for Neuroscience
November 3-7, 2007
San Diego, CA

American Society for Cell Biology
December 1-5, 2007
Washington, DC

Cytometry Research, LLC

www.CytometryRes.com

Cytometry Research, LLC — Your Flow Cytometry Core Facility

What is Flow Cytometry?

Flow cytometry is a laser-based technology that is used to measure characteristics of biological particles.

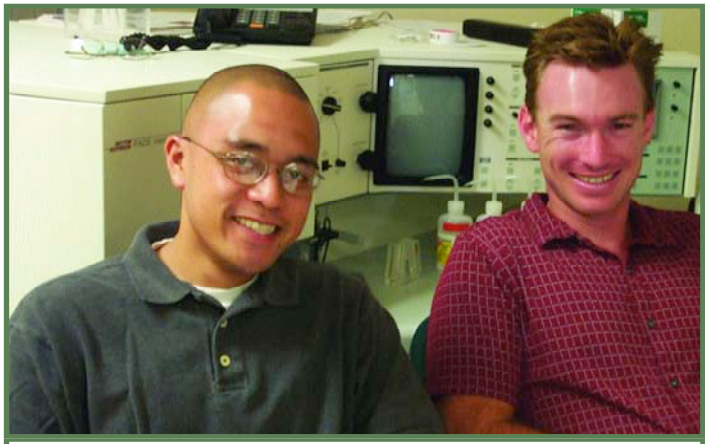
Flow cytometers can be considered as specialized fluorescence microscopes. Flow cytometers have the added capability of being able to sort or separate cells or particles based on the chemical characteristic of interest.

Why choose Cytometry Research?

Cytometry Research has been meeting customer's needs on time for 15 years. We have experienced technicians, meticulously-maintained equipment, and competitive pricing.

Quality you can count on!

- Standard Operating Procedures for equipment and processes to ensure consistently reliable results.
- Full documentation of samples, data, and testing results provided with each analysis.



Leonardo Ancheta and Brian Russell are the lead flow cytometry technicians for Cytometry Research.

Cytometry Research is located in San Diego, California and provides service to scientists throughout the United States, Canada, and Mexico.

Ship your samples to us using an overnight service.

Provide a protocol for cell preparation if you want us to stain, fix, or otherwise process your samples.

Your data will be sent to you via email within one business day of receipt of samples.

Download a Sample Shipping Checklist at:
http://www.cytometryres.com/forms/shipping.html

Cytometry Research was established to serve the scientific community as a contract flow cytometry service.

- *Do you want to enhance your research with cutting edge technology to sort, separate, and analyze your cells?*
- **Cytometry Research has been providing state-of-the-art flow cytometry services to Research, Biotechnology and Pharmaceutical scientists since 1993.**
- *Call now or visit us online to schedule an appointment.*

(858) 642-1988 telephone
(877) 889-2288 tollfree
(858) 642-1989 fax
www.CytometryRes.com

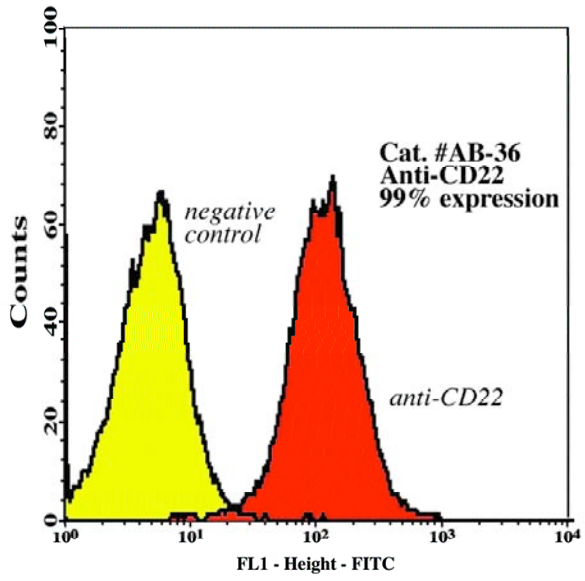
Targeting Tools: Featured Products

Targeting B-Cell Leukemias and Lymphomas

Anti-CD22 (UV22-2) Mouse Monoclonal

This IgG1k antibody (Cat. #AB-36) recognizes the group A epitope of the human B-cell antigen, CD22. Clone UV22-2 reacts exclusively with cells of human B-cell lineage.

The fluorescent conjugate mouse IgG-FITC (Cat. #FL-07) is available as a research tool to use with this and other mouse monoclonal antibodies.



Daudi cells, a Burkitt lymphoma cell line, were used in flow cytometry with a mouse monoclonal antibody to CD22 (Clone UV22-2). Cells were treated with 0.5 μg per million cells of Anti-CD22 mouse monoclonal and subsequently with anti-murine IgG-FITC (Cat. #FL-07). A 99% shift is seen as compared to the secondary alone.

Visit www.ATSbio.com to see a complete list of products.

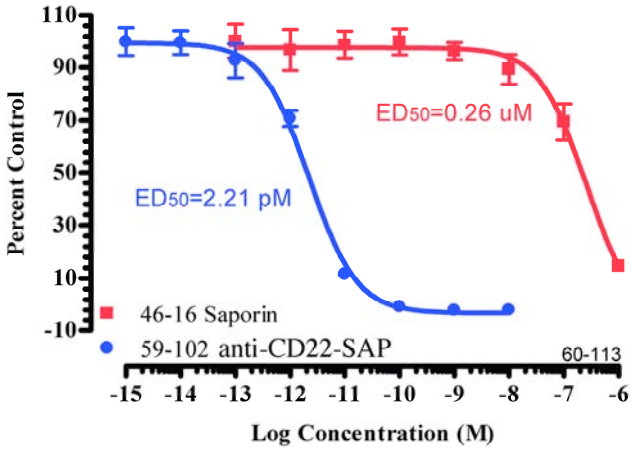
Ahh. Nothing like a little snooze while watching one of the great old movies. I'm sure Doug will tell me how it turns out.

$100 product credit for anyone who can identify the movie!

Anti-CD22-SAP Immunotoxin

This immunotoxin (Cat. #IT-37) will specifically eliminate cells that possess the group A epitope of the human B-cell antigen, CD22. Cells that do not express CD22 will be unaffected.

This immunotoxin is also available in a kit (Cat. #KIT-37) that includes equal amounts of immunotoxin, unconjugated saporin, unconjugated antibody, and a control immunotoxin, Mouse IgG-SAP.



Daudi cells, a Burkitt lymphoma cell line, were plated at 3000 cells per well and were allowed to acclimate. Samples were added at the indicated concentrations and cells were incubated for 72 hours. MTS (Promega) was added and, after color development, wells were read with a Molecular Dynamics SpectraMax 340. ED50's of each compound are color-coded. Data analysis is by PRISM (GraphPad).

Targeting Technology

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

Choose an ANTIBODY[§] specific to your cell type.

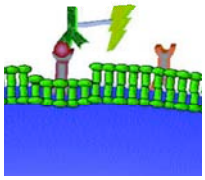
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

[§]or growth factor, peptide, ligand, or cytokine

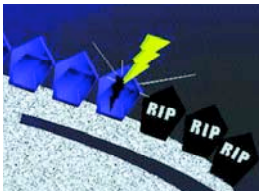
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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, one letter to each block, to solve the puzzle.

HINT: All words are used in Targeting Topics

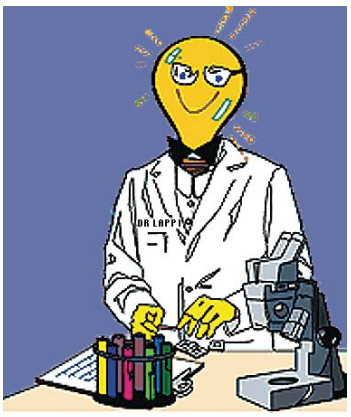
CLAYFORTO

SECTAIS

WAPATHY

TULIPMEL

INTRAREST



How the brilliant scientist greeted his visitor.

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

Answer: ____' __ __?!

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by August 31, 2007.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 2.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

PRSRT STD
UNITED STATES
POSTAGE PAID
SAN DIEGO, CA
PERMIT #1235

Oct-Nov-Dec 2007
Volume 8, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Summer Fun *(page 2)*
- Flow Cytometry Services *(page 2)*
- Teaser Winners *(page 5)*
- FGF-SAP, Anti-AChR, and SP-CTA *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Inducing Central Sensitization with a Substance P/ Cholera Toxin Conjugate

Contributed by Robert M. Caudle, Ph.D.
University of Florida College of Dentistry, Gainesville, FL 32610

A substantial amount of work with Advanced Targeting Systems' substance P/saporin conjugate (SP-SAP) has demonstrated that neurokinin-1 (NK1) receptor-expressing neurons in the spinal cord and brain stem are necessary for the full expression of central sensitization following a peripheral injury [1-7]. Eliminating these neurons with SP-SAP suppresses the hyperalgesia (an enhanced sensation of pain) and allodynia (pain to normally non-painful stimuli) associated with the injury, yet, amazingly, leaves normal pain sensation intact. These findings indicate that the NK1 receptor marks an important set of pain processing neurons in the central nervous system and that alterations in their function lead to an enhancement of the pain sensations experienced by an individual.

Studies on the molecular properties of central neurons during chronic pain conditions suggest that activation of several kinases through either enhanced calcium entry into the cells, enhanced internal calcium release or through the stimulation of cAMP production leads to the sensitization of these neurons [8-22]. These studies provide valuable information on the function of the NK1 receptor-expressing neurons, but they require an injury to produce the sensitization, which involves the activation of a large number of

(continued on page 6)

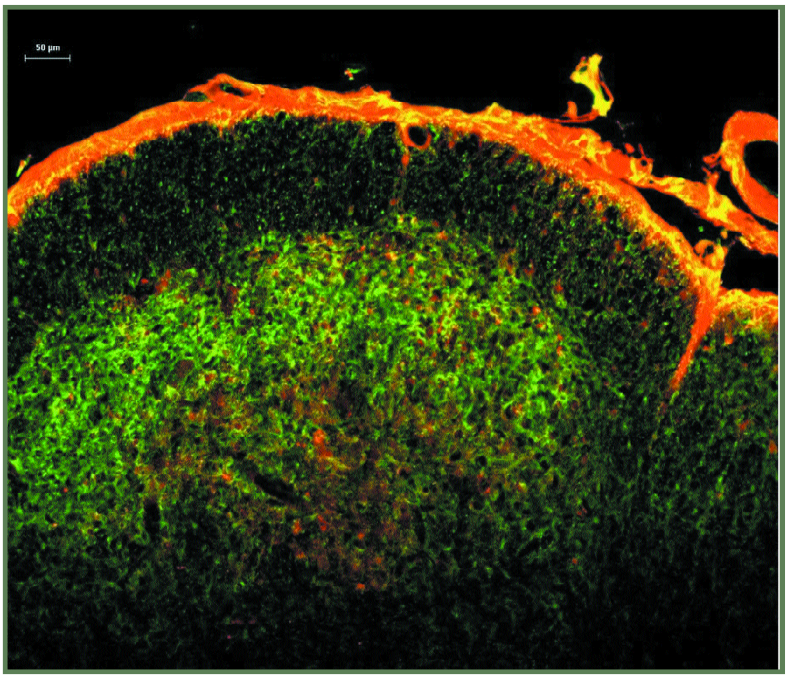
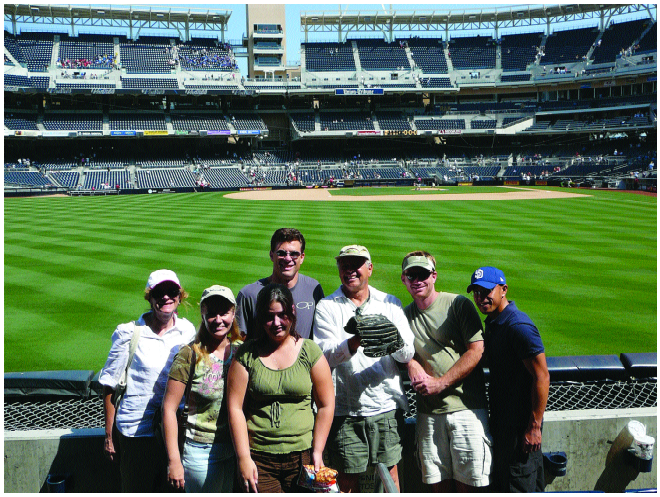


Figure 1. Immunohistochemical localization of SP-CTA uptake in the dorsal horn of the cervical spinal cord. SP-CTA (10 µg) was injected intracisternally into rats via a percutaneous puncture under isoflurane anesthesia. One hour later the animals were euthanized and sections (20 µ) of the brain stem and cervical spinal cord were prepared for immunohistochemistry. Immunofluorescence co-labeling for the NK1 receptor (Green) and for the A subunit of cholera toxin (Red) was performed. The cholera toxin subunit was found only in neurons co-labeled with NK1 receptor. Note that at this time point a significant amount of the cholera toxin subunit is still on the surface of the cord.

Summer Fun



The ATS gang at a Padres game.
(left to right: Denise, Kristen, Amalia, Matt, Doug, Brian, & Leonardo)

August 2, ATS enjoyed their annual outing to a Padres game. The Diamondbacks were soundly defeated 11-0 and a good time was had by all. At least, by all the Padres' fans!

The other regular summer event for ATS is the Tony Mezzadri Surf Contest. On August 19, ATS went to the Tony Mezzadri Surf Classic (tonycontest.org), held annually at the Ocean Beach, CA pier. This year the waves gave the surfers the opportunity to show off some fancy footwork (see photos).

Tony was a teenager when he sustained a serious spinal cord injury while surfing near the pier, and the residents of Ocean Beach and the Sunset Cliffs Surfing Association stepped up with this contest to help Tony and his family. The Contest has grown such that now it is a fundraiser for spinal cord injury research for Mark Tuszynski's lab at UC, San Diego. And a fun day at the beach to watch great surfers surf great waves!

Advanced Targeting Systems employees and loved ones enjoy the surf contest at Ocean Beach (left to right: James, Amalia, Kate, Leonardo, Doug, Darlene, Denise, Jessica, and Brian)

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Cortical cholinergic deficiency enhances amphetamine-induced dopamine release in the accumbens but not in the striatum

Mattsson A, Olson L, Svensson TH, Schilstrom B
Exp Neurol [Epub Jul 24], 2007.

Previous data have implicated cholinergic dysfunction in the pathogenesis of schizophrenia. Here the authors investigated whether increased amphetamine-induced release of dopamine was a response to cortical cholinergic denervation. Rats received bilateral 0.067 μg-injections of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis, and dopamine release was monitored in the nucleus accumbens and striatum. Surprisingly, the increased dopamine release was not linked to loss of cholinergic neurons, but to blocking of muscarinic receptors.

Neonatal basal forebrain cholinergic hypofunction affects ultrasonic vocalizations and fear conditioning responses in preweaning rats

Ricceri L, Cutuli D, Venerosi A, Scattoni ML, Calamandrei G
Behav Brain Res 183(1):111-117, 2007.

In order to expand on previous work investigating the effect of early cholinergic lesions on processing of aversive stimuli the authors administered 0.21 μg of 192-IgG-SAP (Cat. #IT-01) into the third ventricle of 7 day-old rat pups. One unexpected result in lesioned animals was the enhancement of fear-conditioned responses that are dependent on the hippocampus. The authors discuss several theories addressing the implications of these data.

Ketanserin-induced baroreflex enhancement in spontaneously hypertensive rats depends on central 5-HT(2A) receptors

Shen FM, Wang J, Ni CR, Yu JG, Wang WZ, Su DF
Clin Exp Pharmacol Physiol 34(8):702-707, 2007.

Ketanserin is an antihypertensive drug that effectively lowers blood pressure, decreases blood pressure variability, and enhances blood pressure response in spontaneously hypertensive rats. Using the fact that ketanserin is a selective 5-HT2A antagonist, the authors investigated which of these effects utilized the 5-HT2A receptor. Following a 5-nmol ventricular injection of anti-SERT-SAP (Cat. #IT-23) the blood pressure parameters modified by ketanserin were monitored. The data suggest that the baroreflex sensitivity-enhancing effects of ketanserin use the 5-HT2A pathway, but antihypertensive effects follow a different route.

Experimental dissociation of neural circuits underlying conditioned avoidance and hypophagic responses to lithium chloride

Rinaman L, Dzmura V
Am J Physiol Regul Integr Comp Physiol [Epub Aug 1], 2007.

Lithium chloride (LiCl) is frequently used to study neural attributes of "sickness behavior." Previous work by these authors showed that noradrenergic neurons in the nucleus of the solitary tract (NST) are involved in the inhibition of food uptake by cholecystokinin. Here, 20 ng total of anti-DBH-SAP (Cat. #IT-03) was injected into the NST of rats. Lesioned animals demonstrated significantly reduced inhibition of food intake in response to LiCl, but conditioned flavor avoidance was left intact.

Psst. The film noir classic Gangsta napped through in last issue is...***Out of the Past*** **with Robert Mitchum and Jane Greer**

Selective hippocampal cholinergic deafferentation impairs self-movement cue use during a food hoarding task

Martin MM, Wallace DG
Behav Brain Res 183(1):78-86, 2007.

There are conflicting data surrounding the role of the septohippocampal system in spatial orientation. The authors suggest that the presence of spatial clues during some of these tests may skew those results. Rats were injected with a total of 0.35 μg of 192-IgG-SAP (Cat. #IT-01) into the medial septum. Lesioned animals had more difficulty navigating by self-movement cues, but the ability to use environmental cues was left intact. These experiments demonstrate that rats can use environmental information to compensate for loss of circuits that analyze self-movement.

Behavioral and immunohistological effects of cholinergic damage in immunolesioned rats: Alteration of c-Fos and polysialylated neural cell adhesion molecule expression

Chambon C, Paban V, Manrique C, Alescio-Lautier B
Neuroscience 147(4):893-905, 2007.

In this work the authors looked to expand the knowledge of molecular events and brain structure changes following cholinergic immunolesion.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Rats were treated with bilateral injections of 192-IgG-SAP (Cat. #IT-01); 37.5 ng per side into the medial septum, and 75 ng per side into the nucleus basalis magnocellularis. One month after treatment behavioral deficits were drastic and cholinergic neurons had completely disappeared. Elevated levels of polysialylated neural cell adhesion molecule were temporarily able to compensate for the loss of cholinergic neurons.

Estradiol enhances DMP acquisition via a mechanism not mediated by turning strategy but which requires intact basal forebrain cholinergic projections

Gibbs RB
Horm Behav 52(3):352-359, 2007.

Estradiol appears to enhance cholinergic projections to the hippocampus and frontal cortex as shown by tests of response patterns and strategy in rats. The author tested whether this effect was involved with turning strategy, defined as which arm was chosen first in a T-maze. 0.22 μg injections of 192-IgG-SAP (Cat. #IT-01) were made into the medial septum of rats. Lesioned animals utilized a persistent turning strategy; they always chose the same arm of the maze first, even after the administration of estradiol. These data suggest that although the effects of estradiol are not linked to turning strategy, estradiol does interact with the cholinergic system.

Have you published using an ATS product?

Send us an email to let us know: ats@ATSbio.com

Sensory experience determines enrichment-induced plasticity in rat auditory cortex

Percaccio CR, Pruette AL, Mistry ST, Chen YH, Kilgard MP
Brain Res [Epub Aug 9], 2007.

Animals housed in enriched environments display numerous signs of good neural health. In this work the authors examined the role acetylcholine plays in this plasticity. 2.6 μg of 192-IgG-SAP (Cat. #IT-01) was injected into the left lateral ventricle of rats. Auditory-evoked responses were used to assess the effect of lesioning cholinergic neurons. Response strength was not reduced in lesioned animals, indicating that cholinergic deficits do not affect this system.

Induction and survival of binucleated Purkinje neurons by selective damage and aging

Magrassi L, Grimaldi P, Ibatici A, Corselli M, Ciardelli L, Castello S, Podesta M, Frassoni F, Rossi F
J Neurosci 27(37):9885-9892, 2007.

Donor bone marrow-derived cells are thought to fuse with host Purkinje cells in small numbers to create binucleated cells. These fusions have been found to persist within the recipient for long periods of time. The authors injected 2.2 μg of 192-IgG-SAP (Cat. #IT-01) into the right lateral ventricle of rats to examine whether the damage of host Purkinje cells is a method to increase the numbers of binucleated cells. The data suggest an alternate method is present for the creation of these cells.

Raphe Magnus Nucleus is involved in ventilatory but not hypothermic response to CO2

Dias MB, Nucci TB, Margatho LO, Antunes-Rodrigues J, Gargaglioni LH, Branco LG
J Appl Physiol [Epub Sep 9], 2007.

In this work the authors investigated the role that serotonergic neurons in the Raphe Magnus Nucleus (RMg) play in ventilatory and thermal responses to hypercapnia. 0.1 μl of 1 μM anti-SERT-SAP (Cat. #IT-23) was injected into the RMg of rats. Mouse-IgG-SAP (Cat. #IT-18) was used as a control. Lesioned animals had a decreased ventilatory response to CO_2, but hypercapnia-induced hypothermia was not affected. The data indicate that RMg serotonergic neurons contribute to CO_2 ventilatory response but not to maintenance of ventilation.

A limited role for microglia in antibody mediated plaque clearance in APP mice

Garcia-Alloza M, Ferrara BJ, Dodwell SA, Hickey GA, Hyman BT, Bacskai BJ
Neurobiol Dis [Epub Jul 28], 2007.

Microglia are thought to play a key role in the clearance of amyloid-beta (Abeta) in Alzheimer's disease. To examine this role the authors applied 30 μl of 0.5 mg/ml Mac-1-SAP (Cat. #IT-06) to the brain surface of mice for 20 minutes. The number of microglia and plaques was determined by counting of immunohistochemical samples. Results indicate that microglia play a minor role in clearing Abeta plaques, although the interaction of microglia-mediated inflammation and anti-Abeta antibodies appears to be vital in this process.

Targeting Talk

by Dr. Douglas Lappi

Q: Can you tell me how I would calculate the amount of a SAP-type targeted toxin that I might want to buy based on a desire to bilaterally kill cells of interest within a typical diffusion zone around an injection site in a rodent brain? Use CRF-SAP (Cat. #IT-13) as an example. Are we talking a few hundred dollars to run 40 animals or thousands of dollars?

A: For intraparenchymal application, usually 100 nanograms per injection site is too much; on the order of 5-10 nanograms is appropriate (see, for example, Lappi DA, Wiley RG, Entering through the doors of perception: Characterization of a highly selective Substance P receptor-targeted toxin. *Neuropeptides* 34(5):323-328, 2000.)

So for CRF-SAP: at 10 nanograms per site, 20 nanograms per animal times 30 animals is 600 nanograms. Actual end price will depend on whether you purchase direct or from a distributor. However, the average cost for this experiment using one vial of CRF-SAP in the 25-microgram size will run $8-$10 to treat all 30 animals. The CRF-SAP kit includes unconjugated saporin and a control immunotoxin; if you purchase that, the cost for the experiment described with 30 animals will be $12-$15.

Your question about how many injections are needed depends on the diffusion at the site of injection of the agent. Usually, peptide ligand toxins do not diffuse very far and in some cases (such as SSP-SAP, Cat. #IT-11) more than one injection is required. For the antibody targeted toxins, the antibody targeting agent is more resistant to tissue proteases and so there is a greater diffusion, though many researchers like to use several injections to completely cover an area. We're not sure how big of an area you need or want to deplete; that would, of course, require a dosing regimen to determine.

Q: Will one injection permanently and reliably accomplish the deed?

A: One injection might or might not deplete the entire area that you are interested in; again, it depends on diffusion distance of the agent. However, those cells that are eliminated are eliminated permanently and will not come back. Plasticity to replace them is usually not seen.

Q: What are the typical criticisms (e.g., perhaps related to non-specific effects or interpretive difficulties) that the use of such products engender?

A: The typical reviewer requests demonstration of specificity and making a determination that, in fact, the proper cell type is hit and non-target cells are left intact is important.

Upcoming Events

Society for Neuroscience
November 3-7, 2007
San Diego, CA
Booth #1022

Amer Society for Cell Biology
December 1-5, 2007
Washington, DC
Booth #602

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: OLFACTORY
ASCITES
PATHWAY
MULTIPLE
RESTRAINT

Answer: WATT'S UP?!

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

WINNERS: Ruth Stornetta- Univ of Virginia, Dept of Pharmacology, Charlottesville VA * Seto Chice- SUNY HSC at Brooklyn, Brooklyn NY * Robert Speth- University of Mississippi, School of Pharmacy, University MS

Inducing Central Sensitization with SP-CTA

(continued from page 1)

molecular pathways simultaneously. To selectively stimulate the cAMP pathway we chose to use cholera toxin. Cholera toxin stimulates cAMP production by ADP-ribosylating Gs, which then stimulates adenylate cyclase [23]. By chemically coupling substance P to the C-terminus of the A subunit (catalytic subunit) of cholera toxin (SP-CTA) we were able to target the CTA specifically to NK1 receptor-expressing cells [24]. Figure 1 illustrates the uptake of SP-CTA into rat cervical dorsal horn neurons following an intracisternal injection. The section is co-labeled with antibodies to NK1 receptors demonstrating co-localization of the toxin and receptors. The conjugate was not observed in any neurons or cells that did not express NK1 receptors.

In a series of experiments we tested SP-CTA on behavior following intrathecal administration to rats. Doses from 0.3 to 3 μg produced significant sensitization to thermal stimuli on the hind paws of rats 24 hours following the injections. Interestingly, with higher doses the sensitization appeared to be suppressed [24]. These findings suggested that descending inhibitory pathways were activated by the SP-CTA-induced sensitization. Preliminary data indicate that the opioid antagonist naloxone can suppress the descending inhibitory control induced by SP-CTA, exposing profound hypersensitivity to thermal stimuli (data not shown). These findings support the hypothesis that descending control systems are activated by the actions of SP-CTA on spinal cord and brain stem NK1 receptor-expressing neurons.

In summary, SP-CTA is an exciting new tool to examine the function of neurons that possess neurokinin receptors in the central nervous system. The agent selectively enters these neurons through the NK1 receptors and stimulates the production of cAMP for up to three days [24]. As revealed by our studies novel cell functions can be found through this form of selective neuronal stimulation.

References

1. Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA (2002) Spinal neurons that possess the substance P receptor are required for the development of central sensitization. *J Neurosci* 22(20):9086-9098.
2. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.
3. Simons CT, Gogineni AG, Iodi Carstens M, Carstens E (2002) Reduced aversion to oral capsaicin following neurotoxic destruction of superficial medullary neurons expressing NK-1 receptors. *Brain Res* 945:139-143.
4. Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH (2002) Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 5(12):1319-1326.
5. Vierck CJ, Kline RH, Wiley RG (2003) Intrathecal substance p-saporin attenuates operant escape from nociceptive thermal stimuli. *Neuroscience* 119(1):223-232.
6. Wiley RG, Lappi DA (1997) Destruction of neurokinin-1 receptor expressing cells *in vitro* and *in vivo* using substance P-saporin. *Neurosci Lett* 230:97-100.
7. Wiley RG, Lappi DA (1999) Targeting neurokinin-1 receptor-expressing neurons with [Sar9, Met(O2)11] substance P-saporin. *Neurosci Lett* 277(1):1-4.
8. Brenner GJ, Ji RR, Shaffer S, Woolf CJ (2004) Peripheral noxious stimulation induces phosphorylation of the NMDA receptor NR1 subunit at the PKC-dependent site, serine-896, in spinal cord dorsal horn neurons. *Eur J Neurosci* 20(2):375-384.
9. Caudle RM, Perez FM, Del Valle-Pinero AY, Iadarola MJ (2005) Spinal cord NR1 serine phosphorylation and NR2B subunit suppression following peripheral inflammation. *Mol Pain* 1:25.
10. Chen L, Huang LY (1992) Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. *Nature* 356(6369):521-523.
11. Gao X, Kim HK, Chung JM, Chung K (2005) Enhancement of NMDA receptor phosphorylation of the spinal dorsal horn and nucleus gracilis neurons in neuropathic rats. *Pain* 116(1-2):62-72.
12. Guo W, Zou S, Guan Y, Ikeda T, Tal M, Dubner R, Ren K (2002) Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia. *J Neurosci* 22(14):6208-6217.
13. Ji RR, Woolf CJ (2001) Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiol Dis* 8(1):1-10.
14. Lin Q, Peng YB, Willis WD (1996) Possible role of protein kinase C in the sensitization of primate spinothalamic tract neurons. *J Neurosci* 16(9):3026-3034.
15. Ren K, Dubner R (1999) Central nervous system plasticity and persistent pain. *J Orofac Pain* 13(3):155-163.
16. Riedel W, Neeck G (2001) Nociception, pain, and antinociception: current concepts. *Z Rheumatol* 60(6):404-415.
17. Sluka KA, Rees H, Chen PS, Tsuruoka M, Willis WD (1997) Capsaicin-induced sensitization of primate spinothalamic tract cells is prevented by a protein kinase C inhibitor. *Brain Res* 772(1-2):82-86.
18. Woolf CJ, Thompson SW (1991) The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* 44(3):293-299.
19. Woolf CJ (1996) Windup and central sensitization are not equivalent. *Pain* 66(2-3):105-108.
20. Woolf CJ (1993) The pathophysiology of peripheral neuropathic pain--abnormal peripheral input and abnormal central processing. *Acta Neurochir Suppl (Wien)* 58:125-130.
21. Woolf CJ, Thompson SW, King AE (1988) Prolonged primary afferent induced alterations in dorsal horn neurones, an intracellular analysis *in vivo* and *in vitro*. *J Physiol (Paris)* 83(3):255-266.
22. Zou X, Lin Q, Willis WD (2002) Role of protein kinase A in phosphorylation of NMDA receptor 1 subunits in dorsal horn and spinothalamic tract neurons after intradermal injection of capsaicin in rats. *Neuroscience* 115(3):775-786.
23. Ganguly NK, Kaur T (1996) Mechanism of action of cholera toxin & other toxins. *Indian J Med Res* 104:28-37.
24. Caudle RM, Mannes AJ, Keller J, Perez FM, Suckow SK, Neubert JK (2007) Sensitization of spinal cord nociceptive neurons with a conjugate of substance P and cholera toxin. *BMC Neurosci* 8:30.

Targeting Tools: Featured Products

Targeting FGF Receptors

FGF-2, or basic fibroblast growth factor, binds all of the FGF receptors with high affinity. We have used this molecule to produce FGF-SAP (Cat. #IT-38), which has a healthy experimental publication record ("FGF" and "saporin" in PubMed: 25 hits). It has been used to clean primary cultures of fibroblasts.[1] It was important in determining the role of smooth muscle cells in restenosis of damaged vasculature.[2] It was widely used *in vivo* for the elimination of FGF receptor-expressing cells, including neuronal cell types,[3] cancer cells,[4] and lens epithelial cells.[5] Figure 1 shows the cytotoxicity of ATS' rat FGF-SAP conjugate to mouse NIH3T3 cells, with a potent ED50 of 68 pM. This conjugate will be useful for the study of systems biology.

References

1. Beattie GM, Lappi DA, Baird A, Hayek A (1990) *Diabetes* 39:1002.
2. Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA (1991) *Circulation Res* 68:106.
3. Gonzalez AM, Lappi DA, Buscaglia ML, Carman LS, Gage FH, Baird A (1991) *Ann NY Acad Sci* 638:442.
4. Beitz JG, Davol-Lewis P, Clark JW, Kato J, Medina M, Frackelton AR, Lappi DA, Baird A, Calabresi P (1992) *Cancer Res* 52:227.
5. David T, Tassin J, Lappi DA, Baird A, Courtois Y (1992) *J Cell Physiol* 153:483.

Anti-AChR (mAb 35) Rat Monoclonal

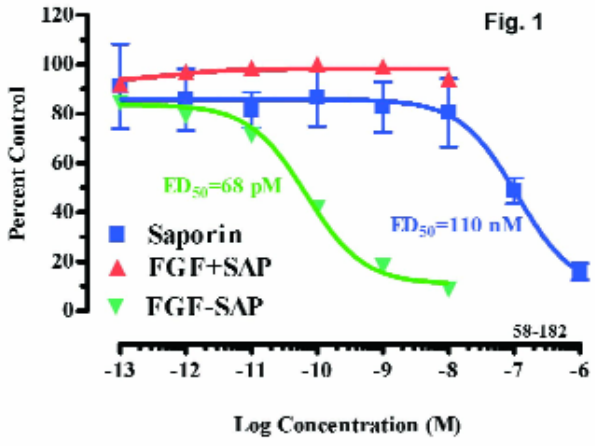
Species Reactivity: Human, Rat, Mouse, Rabbit

Anti-AChR (Cat. #AB-N36) binds with high affinity to the AChR subtype with 59 kDa ACh-binding subunits, but with considerably lower affinity to the AChR subtype with 75 kDa subunits. Anti-AChR binds to the main immunogenic region on α1 subunits of muscle-type AChRs.[1]

Reference

1. Schoepfer R, Halvorsen SW, Conroy WG, Whiting P, Lindstrom J. *FEBS Lett*. 1989 Nov 6;257(2):393-9.

Visit www.ATSbio.com to see a complete list of products.



SP-CTA (Substance P - Cholera Toxin)

SP-CTA (Cat. #IT-39) is an exciting new tool for use in the research of neurokinin (NK-1) receptor-expressing cells of the central nervous system. A conjugate of the substance P molecule and the catalytic subunit of Cholera toxin, this product can be used very effectively *in vivo* for increasing sensitization of these neuronal cells. Selectively targeting the NK-1r-expressing cells with the substance P moiety allows the researcher to stimulate only the cells of interest by amplifying their cAMP production with the CTA, without altering the neighboring cells. This effect lasts for a few days, and gives the researcher an opportunity to study behaviors such as those related to the perception of pain or the control of breathing.

Targeting Technology

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

Choose an ANTIBODY[§] specific to your cell type.

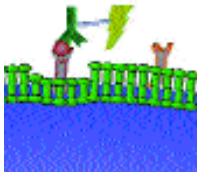
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

[§]or growth factor, peptide, ligand, or cytokine

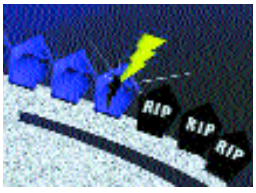
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

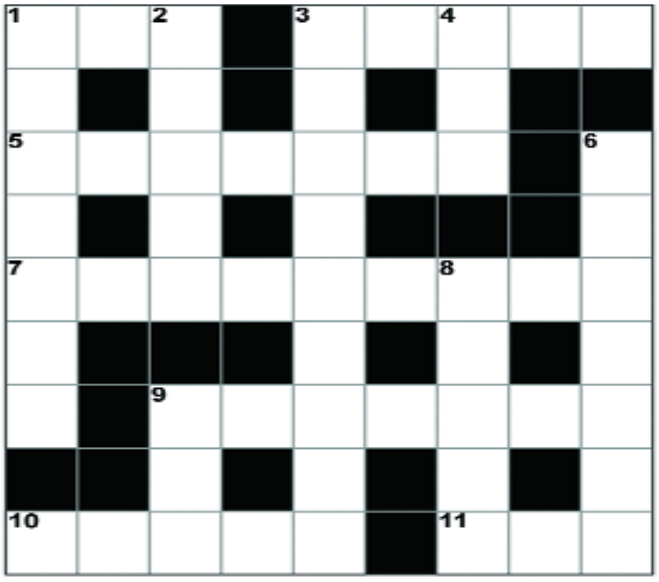
The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser



Across

1. Saporin: Abbr
3. Shared 1962 Nobel Prize for work in Genetics
5. Fatty substance from wool
7. Wood trim at bottom of wall
9. Stretchy material in waistband
10. Moulin ____
11. Phenol ____ (pH indicator)

Down

1. Easily dissolved
2. Small squares of glass
3. Adjust to standard (e.g. equipment)
4. ____ -exchange chromatography
6. Influenced or started protein expression
8 Change
9. Largest bird native to Australia

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by November 30, 2007.
3. Win $100 credit toward your next purchase.

Limit one entry per laboratory.

See last quarter's winners, page 5.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

PRSRT STD
UNITED STATES
POSTAGE PAID
SAN DIEGO, CA
PERMIT #1235