Jan-Feb-Mar 2004
Volume 5, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Cholinergic Deafferentation of the Entorhinal Cortex Impairs Working Memory for Novel, but not Familiar Stimuli in a Delayed Non-Match to Sample (DNMS) Task.

Contributed by ATS's 2003 Society for Neuroscience Poster of the Year Award Winner: Dr. Jill McGaughy, Boston University, 64 Cummington St., Boston, MA 02215 (see companion story on page 2)

Muscarinic cholinergic receptor activation in entorhinal cortex (EC) activates intrinsic depolarizing membrane currents, which cause self-sustained spiking activity in single neurons.[1] This effect may underlie delay activity and match-dependent activity changes in delayed match to sample tasks[2] and could allow accurate maintenance of novel information without dependence on synaptic modification associated with previous exposure (familiarization). Consistent with this, research in human subjects suggests that the medial temporal lobes are specifically activated during working memory for novel but not familiar stimuli,[3] and cholinergic deafferentation of the rhinal cortex in non-human primates has been shown to impair memory for trial-unique (novel) stimuli.[4] The current study tests the hypothesis that cholinergic deafferentation of the EC produces impairments in working memory for novel, but not familiar stimuli.

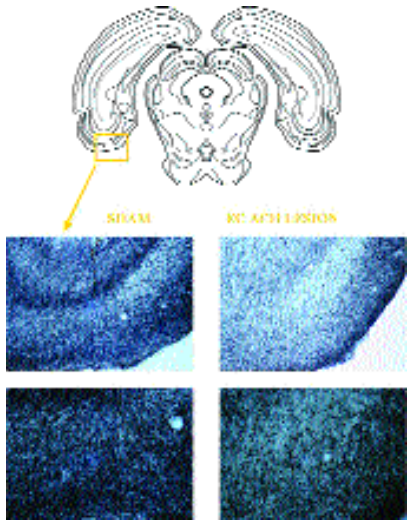


Figure 1. Acetylcholinesterase-positive fibers in the area of the EC (Bregma –7.3) for SHAM (A, C) and EC lesioned rats (B, D) at low and high magnification. The lesion produced a loss of approximately 60% of AChE fibers throughout the EC with damage centered on lateral EC.

(continued on page 6)

ATS Gets New Facility for SP-SAP Production

Progress continues to be made in moving SP-SAP closer to clinical trials as a treatment for chronic pain. The latest development is the leasing of a 3700-sq ft facility adjacent to the current ATS facility in San Diego.

This facility will be converted to GMP (Good Manufacturing Practice) space suitable for production of SP-SAP for use in Phase I/II human clinical trials. The site plan includes space for Class 10,000 clean rooms and quality control laboratories.

Early in March, the first of two GLP (Good Laboratory Practice) toxicology studies will begin. Dr. Jeff Allen will be leading this study at the University of California facility in San Diego. The UCSD toxicology studies are under the supervision of Dr. Tony Yaksh, who has expertise in the study of the effects of intrathecal delivery of pain medications.

Track progress on SP-SAP drug development on our website: www.ATSbio.com.

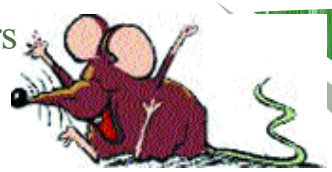
Accomplished Scientist. . . Talented Artist - Rosalind Ridley

Rosalind Ridley

Duomo in Prato (watercolor)

Rosalind Ridley is an internationally recognized expert on prion diseases and learning and memory in primates; the latter is a reason that we have interacted with her often. We discovered that we have in common a fascination with artwork and architecture of Renaissance Italy. Dr. Ridley has been drawing some of the beautiful places of that country, and we are extremely pleased to be able to present them to you over the next few quarters in *Targeting Trends*. This quarter, we have Dr. Ridley's watercolor of the wonderful pulpit on the façade of the Duomo in Prato. The pulpit was designed by Donatello and constructed by him and Pagno di Lapo and Michelozzo. It took ten years to construct and was finished in 1438. Please enjoy this serene view from Tuscany.

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: ANEMIA EXONS MAGNETIC FOSSIL POLYMER

Answer: What the creature thought upon closer self-examination — What a FINE SPECIMEN!

WINNERS: V.S. Ananth, McMaster University * Zvezdana Sever-Chroneos, Univ Texas Health Ctr • Teresa Milner, Cornell Univ
Bob Speth, Univ Mississippi * Eva Rose Dwyer, Rockefeller Univ * Douglas J. Taatjes, Univ Vermont

ATS's 2003 SfN Poster Award

This year, the Advanced Targeting Systems prize for the best poster at the Society for Neuroscience meeting that utilizes a saporin agent went to Jill McGaughy for her excellent presentation: **Cholinergic Deafferentation of the Entorhinal Cortex in Rats Impairs Encoding of Novel but Not Familiar Stimuli in a Delayed Non-Match to Sample Task (DNMS),** co-authors M. Jindal, H.B. Eichenbaum, M.E. Hasselmo. This is the fifth year we have given this award, which goes to the scientist that actually stands and presents the material. In the words of one of the judges, Dr. McGaughy's work "presents a hypothesis, tests the hypothesis and the data prove the hypothesis. It's just plain how science should be done." Please see our cover story in which the work is described. She is pictured here receiving her prizes from ATS president, Doug Lappi.

Competition was fierce again this year, with some excellent posters presented. Runner-up honors go to two scientists that used SP-SAP. M. Woods from Purdue Pharma presented his excellent **Ablation of a Population of NK-1 Expressing Neurons in the Dorsal Horn of the Spinal Cord Does Not Induce Alpha-Beta Sprouting into Lamina II**. E.E. Carstens from UC Davis presented **Reduced Scratching In Rats Receiving Intracisternal Substance P-Saporin to Ablate Cervical Superficial Dorsal Horn Neurons that Express NK-1 Receptors**.

We congratulate all the scientists that presented abstracts utilizing saporin agents this year, and we look forward to seeing the presentations at the 2004 meeting in San Diego.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neonatal cholinergic lesions and development of exploration upon administration of the GABAa receptor agonist muscimol in preweaning rats.

Scattoni ML, Calamandrei G, Ricceri L
Pharmacol Biochem Behav 76(2):213-221, 2003

The authors investigated GABAergic development in young rats lesioned with two 0.42-ng injections of 192-Saporin (Cat. #IT-01) into the third ventricle. The rats were then treated with the GABA agonist muscimol chloride and observed during locomotor and exploration tests. No change was noted in GABAergic agonist reactivity in lesioned animals.

Immunotoxic destruction of distinct catecholaminergic neuron populations disrupts the reproductive response to glucoprivation in female rats.

I'Anson H, Sundling LA, Roland SM, Ritter S
Endocrinology 144(10):4325-4331, 2003

The authors hypothesized that hindbrain catcholamine neurons suppressed estrous cycles during chronic glucoprivation as an extension of their role in glucoprivic feeding. 42-ng bilateral injections of anti-DBH-SAP (Cat. #IT-03) were made into the paraventricular nucleus of female rats. Lesioned rats demonstrated inhibition of reproductive function during chronic glucose deficit, but not when a normal amount of glucose was available.

Effects of hippocampal cholinergic deafferentation on learning strategy selection in a visible platform version of the water maze.

Bizon JL, Han JS, Hudon C, Gallagher M
Hippocampus 13(6):676-684, 2003

To investigate the effect of depleted acetylcholine (Ach) levels in the hippocampus on learning strategies that are thought to utilize the hippocampus, the authors lesioned the medial septum/vertical limb of the diagonal band of Broca in rats with two injections of 75 and 50 ng of 192-Saporin (Cat. #IT-01). The unexpected result was that a hippocampal place strategy was promoted in the absence of Ach.

Neurokinin-1 receptor-expressing neurons in the amygdala modulate morphine reward and anxiety behaviors in the mouse.

Gadd CA, Murtra P, De Felipe C, Hunt SP
J Neurosci 23(23):8271-8280, 2003

Previous work has demonstrated that mice lacking the neurokinin-1 receptor do not show some of the behaviors associated with morphine reward. Bilateral 1.0-μl injections of 1.0 μM SP-SAP (Cat. #IT-07) were made into either the nucleus accumbens or the amygdala of mice. Animals with lesions of the amygdala displayed a reduction of morphine reward behavior and an increase in anxiety-like behavior in an elevated maze test.

Enhanced evoked excitatory transmitter release in experimental neuropathy requires descending facilitation.

Gardell LR, Vanderah TW, Gardell SE, Wang R, Ossipov MH, Lai J, Porreca F
J Neurosci 23(23):8370-8379, 2003

The authors investigate whether the effects of nerve injury-induced afferent discharge and central changes associated with experimental neuropathic pain might intersect at the spinal level. 1.5 pmol of dermorphin-SAP (Cat. #IT-12) was injected into each side of the rostral ventromedial medulla of rats. The data indicate that increased afferent input is a driving force of neuropathic pain, and that some aspects of nerve injury-induced hyperesthesias may occur through the convergence of descending modulation, spinal plasticity, and afferent drive.

Changes in cortical acetyl-CoA metabolism after selective basal forebrain cholinergic degeneration by 192IgG-saporin.

Tomaszewicz M, Rossner S, Schliebs R, Cwikowska J, Szutowicz A
J Neurochem 87(2):318-324, 2003

Alzheimer's disease subjects often show deficits in cerebral glucose metabolism. To investigate whether cortical cholinergic input affects acetyl-CoA metabolism in cholinoceptive cortical target regions, rats received 4 μg 192-Saporin (Cat. #IT-01) into the left lateral ventricle. The data show evidence of differential distribution of acetyl-CoA in subcellular compartments of cholinergic and non-cholinergic nerve terminals.

Rostral ventrolateral medulla C1 neurons and cardiovascular regulation.

Madden CJ, Sved AF
Cell Mol Neurobiol 23(4-5):739-749, 2003

The authors review the use of anti-DBH-SAP (Cat. #IT-03) to study the role of C1 neurons within the rostral ventromedial medulla in cardiovascular regulation. This immunotoxin specifically removes C1 neurons containing dopamine beta-hydroxylase.

Destruction of midbrain dopaminergic neurons by using immunotoxin to dopamine transporter.

Wiley RG, Harrison MB, Levey AI, Lappi DA
Cell Mol Neurobiol 23(4-5):839-850, 2003

The authors demonstrate the effective and specific removal of neurons expressing the dopamine transporter in the substantia nigra pars compacta and

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

the ventral tegmental area with anti-DAT-SAP (Cat. #IT-25). A 21-μg icv injection produced a highly significant loss of midbrain dopaminergic neurons, creating a useful model for Parkinson's disease.

Cholinergic activity enhances hippocampal long-term potentiation in CA1 during walking in rats.

Leung LS, Shen B, Rajakumar N, Ma J
J Neurosci 23(28):9297-9304, 2003

To investigate the role of the cholinergic system in long term potentiation (LTP) the authors lesioned the left and right medial septum of rats with 0.14 μg of 192-Saporin (Cat. #IT-01). LTP induced in lesioned walking animals is less robust than in control animals.

Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection.

Dommergues MA, Plaisant F, Verney C, Gressens P
Neuroscience 121(3):619-628, 2003

Brain lesions that mimic damage from cerebral palsy in mice are characterized by microglial activation within 24 hours of insult. Using intraperitoneal injections of Mac-1-SAP (90 μg/kg, Cat. #IT-06), a reduction in the density of resident microglial and blood-derived monocytes was obtained.

Hindbrain noradrenergic lesions attenuate anorexia and alter central cFos expression in rats after gastric viscerosensory stimulation.

Rinaman L
J Neurosci 23(31):10084-10092, 2003

Using 5-ng injections of anti-DBH-SAP (Cat. #IT-03) into hindbrain nucleus of the solitary tract in rats, the author investigated the role of DBH-positive neurons in the mediation of anorexigenic and central nervous system activation effects due to exogenous CCK.

Differential effects of cholinergic lesions on dendritic spines in frontal cortex of young adult and aging rats.

Harmon KM, Wellman CL
Brain Res 992:60-68, 2003

The authors used 0.15 μg of 192-Saporin (Cat. #IT-01) injected into the nucleus basalis magnocellularis of rats to study whether dendritic spine density is altered by cholinergic deafferentation. While the spine density decreased in young rats, middle-aged and aged animals did not display a density significantly different than controls.

A combinatorial network of evolutionarily conserved myelin basic protein regulatory sequences confers distinct glial-specific phenotypes.

Farhadi HF, Lepage P, Forghani R, Friedman HC, Orfali W, Jasmin L, Miller W, Hudson TJ, Peterson AC
J Neurosci 23(32):10214-10223, 2003

The authors used intrathecal injections of 0.3 μg CTB-SAP (Cat. #IT-14) to induce spinal cord demyelination for the purpose of defining the regulatory network controlling myelin basic protein transcription in mice.

Does the release of acetylcholine in septal slices originate from intrinsic cholinergic neurons bearing p75^{NTR} receptors? A study using 192 IgG-saporin lesions in rats.

Birthelmer A, Lazaris A, Riegert C, Marques Pereira P, Koenig J, Jeltsch H, Jackisch R, Cassel JC
Neuroscience 122(4):1059-1071, 2003

The authors used 0.8 μg injections of 192-Saporin (Cat. #IT-01) into the medial septum and diagonal band of Broca to investigate whether release of acetylcholine was due to neurons expressing the p75 ^{NTR}.

Cytochrome oxidase activity in the monkey globus pallidus and subthalamic nucleus after ablation of striatal interneurons expressing substance P receptors.

Chiken S, Hatanaka N, Tokuno H
Neurosci Lett 353(2):103-106, 2003

1-6 μl of 15-20 ng/μl SP-SAP (Cat. #IT-07) was injected into the forelimb representation of the putamen. Animals were examined for the loss of interneurons as well as regional metabolic changes. The results indicate that substance P receptor-expressing neurons do not modulate inhibitory influences on the GP.

Macrophage-derived IL-18 targeting for the treatment of Crohn's disease.

Kanai T, Uraushihara K, Okazawa A, Hibi T, Watanabe M
Curr Drug Targets Inflamm Allergy 2(2):131-136, 2003

A single intravenous injection of Mac-1-SAP (Cat. #IT-06) significantly reduced the amount of intestinal inflammation in a 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis model.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: Not the Right Stuff

Are all lots of 192-Saporin (192-IgG-SAP) the same?

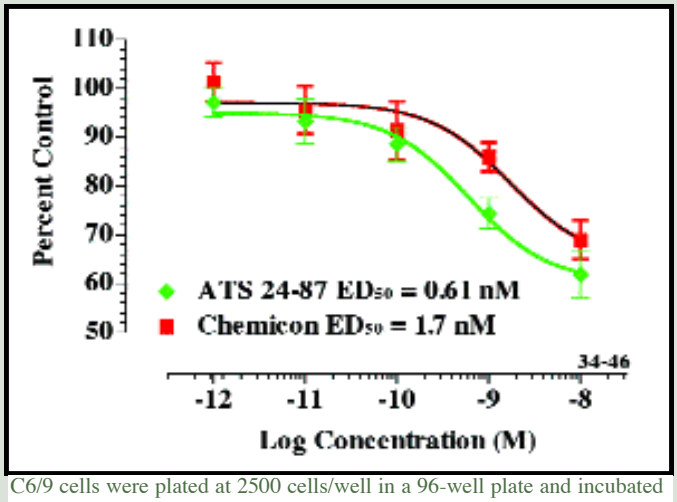
There are variances from lot to lot, and Advanced Targeting Systems includes a cytotoxicity graph on the data sheet with each product comparing the current lot with previous lots. Chemicon International also sells immunotoxins, and used to distribute ATS products. However, since early 2002 (according to Chemicon) they have been outsourcing their 192-Saporin from an un-named laboratory. A lot obtained from Chemicon was tested under ATS quality control conditions (see graph at right) and was found to be significantly less active than any of the ATS lots.

How do I find out the optimal dosage?

For each new lot and each new application of immunotoxin, it is recommended that the end user perform preliminary tests to ascertain the proper dosage. The material used and the method of administration are important aspects of each experiment that should be carefully considered prior to beginning a full-blown project.

What happens if I use too much immunotoxin?

Customers who had used the Chemicon material and then ordered 192-Saporin from ATS have reported that they needed to reduce the dosage



C6/9 cells were plated at 2500 cells/well in a 96-well plate and incubated overnight. 192-Saporin was added in 10-microliter volumes and the plates were incubated for 72 hrs. The plates were developed with PMS/MTS for 1-2 hrs, then read at 492 nm in a plate reader. Data analysis was done by PRISM (GraphPad, San Diego).

level with ATS product. The Chemicon material was not as potent. Higher doses of 192-Saporin, (as described in Leanza *et al*. *Eur J Neurosci*, 1995, 329-343) cause deficits in hindlimb coordination and support, and ataxia. So it is important to use less if you've switched from Chemicon's material to the ATS 192-Saporin.

Take care to test each new lot and compare the cytotoxicity data to decide on the proper dose. If you have any questions, contact ATS.

Upcoming Events

Amer Assoc for Cancer Research
March 28-31, 2004
Orlando, FL
Booth 1040

Experimental Biology
April 17-21, 2004
Washington, DC
Booth 1030

Society for Neuroscience
October 23-27, 2004
San Diego, CA

Torrey Pines Golf Course

The 2004 SfN meeting will once again be in San Diego, home of one of the most beautiful golf courses . . . Torrey Pines. The name itself stirs the imagination of golf enthusiasts all over the world. Situated atop cliffs towering above the Pacific Ocean in San Diego, California, golfers marvel at the views of the coastline and deep ravines. Nature lovers can spot wildlife and the famous Torrey Pines trees that are indigenous to the area.

Each year, the PGA Buick Invitational is held at Torrey Pines Golf Course and in 2008, the US Open Championship will be held on the newly remodeled South Course. Saturday, October 23, 2004 is YOUR opportunity to play the South Course. Format will be either a shotgun beginning at 7am (if sufficient golfers enroll) or set tee times beginning at 7:30am. Green fees, including golf cart will be about $150 per person.

Limited space available! Contact ATS as your San Diego area hosts to reserve your tee time at www.ATSbio.com/golf.

Cholinergic Deafferentation of the Entorhinal Cortex

(continued from page 1)

Methods: Prior to surgery, Long Evans, male rats weighing between 300-350 grams were presented with 10 trials/day of an odor-based delay non-match to sample with a subset of odors chosen from a total of 30 odors until reaching criterion performance at a zero delay (8/10 correct for 2 consecutive days). Subjects were then subdivided into two groups. Half received infusions into the entorhinal cortex of 192 IgG-saporin, a targeted toxin that destroys cholinergic afferents and the other half received vehicle infusions. After a two-week recovery period, rats were tested to re-establish criterion performance at the zero delay. Tests of varying delays with these "familiar" odors (3,15,60,180 minutes) or with varying list lengths (3,5,10,15) were then given. These testing conditions were counterbalanced across subjects. After completing testing with familiar odors, rats were tested with novel odors (6 trials, 15-min delay). On day 2, a replication was run with an additional set of novel odors (6 trials, 15-min delay). Novel odors were then repeatedly trained over four additional days/list so that they became familiar.

Results: Lesioned rats showed a loss of ~60% of the cholinergic afferents in the area of the EC relative to sham-lesioned rats as assessed by fibers counts of the acetylcholinesterase positive (AchE+) fibers in the area (Fig 1 A, B, C, D). Damage was centered on the lateral EC.

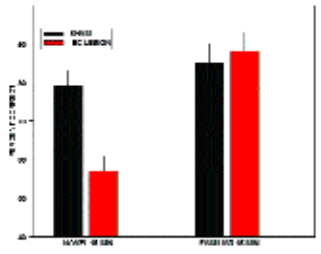


Figure 2. EC lesioned rats (red bars) show impaired recognition memory as indexed by percent correct (correct/correct + incorrect) on the DNMS with novel items relative to SHAMS (black bars). EC rats are also significantly impaired relative to their own performance on DNMS with familiar items (right side) at the same delay (15 min).

Rats with lesions of the EC were significantly impaired on tests with novel odors as shown in Fig 2. The initial impairment in the processing of novel information was highly correlated with the extent of damage to caudal portions of the lateral EC (Fig 3). The

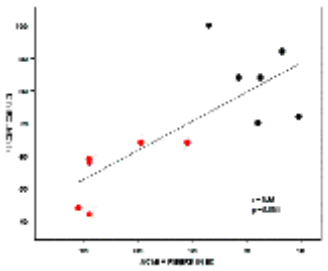
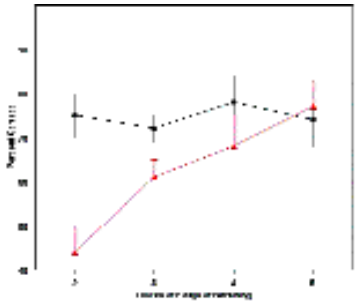


Figure 3. The number of AChE + fibers in the EC was strongly correlated with the accuracy on tests of DNMS with novel items. EC rats are shown in red with SHAM represented by black dots. The behavioral data shown here are the same data as shown in Fig 2.

novel odors were then familiarized over days of training using a unique testing sequence for each day. The impairment in the working memory of novel odors persisted over the second day of training but dissipated by day 3 with lesioned rats' performance stabilizing by days 4 and 5 (Fig 4). Lesioned rats were not impaired at any delay (up to 180 min) when tested with familiar odors (Fig 5). Sham-lesioned animals showed comparable impairments in performance when tested with novel stimuli or long

Figure 4. Novel items were retested over subsequent days. The performance of EC-lesioned rats (red line) improved significantly over days of testing so that there was no difference in performance between the groups by day 3 of testing. Though two lists of novel items were tested, performance did not differ on these two tests so data are combined over the lists to form blocks of testing as indicated on the ordinate.



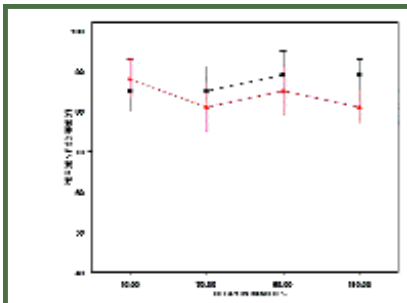


Figure 5. EC lesions (red line) did not impair memory for familiar items even when extremely long delays (180 min) were imposed between sample and choice. Accuracy is shown on the ordinate with delay in minutes on the abscissa.

lists of items to remember (Fig 6) suggesting these tasks were similarly difficult. Rats with EC lesions were only impaired in tests with novel items suggesting an increase in task difficulty alone was insufficient to explain these impairments. These data support the hypothesis that cholinergic afferents to the EC activate cellular mechanisms of sustained spiking activity necessary for maintenance of novel, but not familiar stimuli in a working memory task.

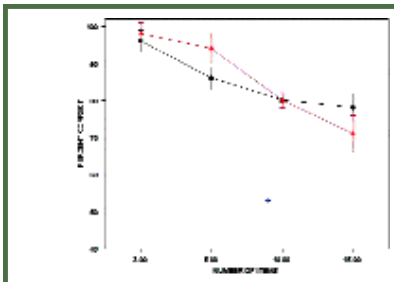


Figure 6. Lesions of the EC (red line) did not impair memory of familiar items even when task difficulty was manipulated by increasing the list of items to be remembered. Note: decrease in accuracy in SHAM rats under these conditions is similar to that produced by the introduction of novel test items so that the EC rats' impairment on tests of DNMS with novel items cannot be explained by an increased sensitivity to the difficulty of the task.

References

1. Klink R and Alonso A (1997). *J Neurophysiology* 77: 1813-1828.
2. Fransen E, Alonso A, Hasselmo ME (2002). *J Neurosci* 22: 1081-1097.
3. Stern CE, Sherman SJ, Kirchhoff BA, Hasselmo ME (2001). *Hippocampus* 11: 337-346.
4. Turchi JN, Saunders RC, Mishkin M (2002). *Soc Neurosci* Abstract #32.

Targeting Tools: Featured Products

Somatostatin receptor targeting

Advanced Targeting Systems is pleased to announce the availability of polyclonal antibodies to somatostatin receptor (SSTr) sub-types 1 and 4.

Antibody to SSTr1

SSTr1 is a 391 amino acid G-protein-coupled receptor that contains three glycosylation sites and that is expressed in several areas of the rat CNS.[1] It is strongly expressed in the amygdala and cortex, and less so in the hypothalamus, hippocampus, olfactory tubule, olfactory bulb and thalamus and spinal cord.[2] In the periphery, it is also expressed in stomach, colon and liver. We have created an antibody to an extracellular domain of SSTr1 and it is available both as antiserum and as the affinity purified antibody. Figure 1 shows western blotting of membrane extracts of MIA PaCa-2; both versions of the antibody reveal a single band of 53 kDa, consistent with a glycosylated GPCR. The affinity-purified also functions in FACS analysis with MIA PaCa-2 cells (Figure 2).

References

1. Selmer I, Schindler M, *et al*. (2000). Advances in understanding neuronal somatostatin receptors. *Regul Pept* 90(1-3):1-18.
2. Bruno JF, Xu Y, *et al*. (1993) Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. *Endocrinology* 133(6):2561-2567.

Antibody to SSTr4

Coming Soon!

SSTr4 has a higher affinity for somatostatin 28. It is a 388-amino acid glycosylated GPCR. It has a single glycosylation site, and so the molecular weight is lower (44 kDa) than that of SSTr1. This new antibody is also being prepared from a peptide from an extracellular part of the receptor. SSTr4 is widely distributed in the rat brain and its mRNA has been recorded in the heart, stomach, lung, kidney and other organs.

Advanced Targeting Systems is developing both polyclonal and monoclonal antibodies to extracellular domains of the SSTrs, primarily for use as targeting agents. These first antibodies will be very useful in research on the characterization of the role of these receptors in systems biology.

SSTR Antibodies

AB-N19	SSTr1, antiserum	100 µl, $150
AB-N20	SSTr1, affinity purified	50 µg, $200
AB-N21	SSTr4, affinity purified	50 µg, $200

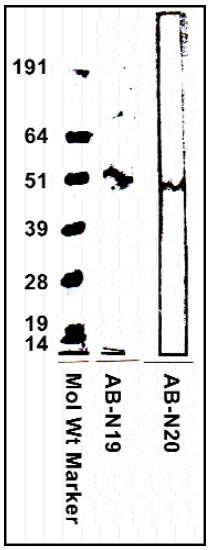


Figure 1

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

Lanes 2 and 3: 111 µg of MIA PaCa-2 (human pancreatic cells) cell membrane extract probed with Anti-SSTr1 (AB-N19, Lane 2) and Anti-SSTr1 affinity purified (AB-N20, Lane 3) at a 1:100 dilution.

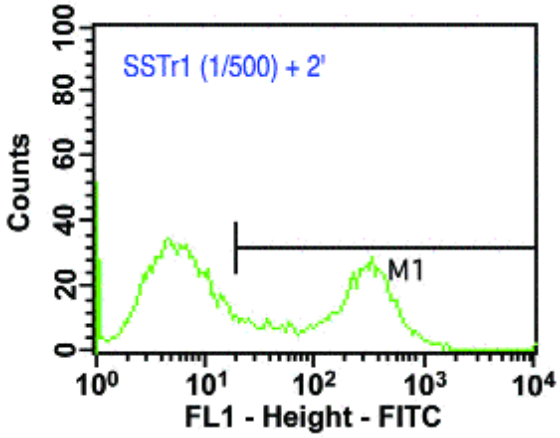


Figure 2. *MIA PaCa-2 cells, human pancreatic cells transfected with the SSTr1, were used for FACS analysis with the SSTr1 antibody. Cells were treated with affinity purified Anti-SSTr1 (AB-N20) at a 1:500 dilution and subsequently with goat-anti-rabbit-FITC.*

New Year's Resolution?

Gangsta is taking a bite out of life —

and savoring it!

Visit the ATS website for a complete list of products.

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.

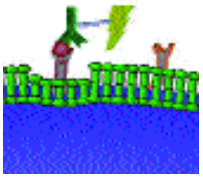
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

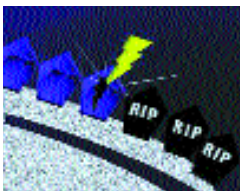
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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 form five words associated with this famous scientist.

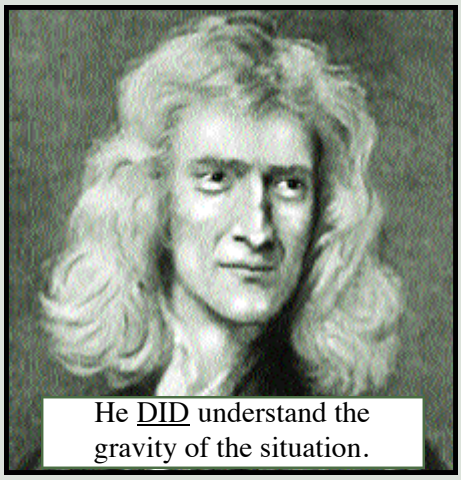
STENPAL

IPSTOC

SACCULLU

STEWMERSTIN

COVEYLIT



He DID understand the gravity of the situation.

Arrange the circled letters to form the name of this noted scientist, as suggested by the above clue.

Answer:

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by February 29, 2004.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 2

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Apr-May-Jun 2004
Volume 5, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- 10th Anniversary *(page 2)*
- San Marco Watercolor *(page 2)*
- Streptavidin vs. Avidin *(page 5)*
- mu p75-SAP, anti-Tac-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

192 IgG-Saporin-Induced Lesions Identify an Inhibitory Role in Cocaine Reward for Cholinergic Neuronal Systems in the Diagonal Band and Ventral Pallidum

Contributed by Dr. James Smith, Wake Forest University School of Medicine, Department of Physiology and Pharmacology, Medical Center Blvd, Winston-Salem NC 27157

Recent data implicate cholinergic neurons in the brain processes that underlie reinforcement. The involvement of cholinergic neurons in cocaine self-administration has been recently demonstrated using muscarinic and nicotinic agonists and antagonists, microdialysis, assessment of choline acetyltransferase activity and acetylcholine (ACh) turnover rates. The ACh turnover rate study implicated specific cholinergic neuronal systems in cocaine self-administration. The present experiment was initiated to identify subsets of cholinergic neurons involved in the brain processes that underlie cocaine self-administration by lesioning discrete populations with a selective neurotoxin. Rats were trained to self-administer cocaine, and the immunotoxin 192 IgG-saporin or vehicle was then bilaterally administered into the posterior nucleus accumbens (NAcc) - ventral pallidum (VP) forebrain regions (Figure 1). The effects of the lesion or sham treatment on cocaine self-administration were assessed followed by real time RT-PCR to evaluate the extent and specificity of the lesion by assessing p75 (the protein on the low affinity nerve growth factor receptor that is the target for 192 IgG-saporin) and choline acetyltransferase (ChAT) gene expression in four brain regions.

192 IgG-saporin-induced lesions of the posterior NAcc-VP-MS-DB (medium septum-diagonal band) region shifted the cocaine self-administration dose-intake relationship to the left compared to sham-treated controls. Real time RT-PCR showed significant reductions in

(continued on page 6)

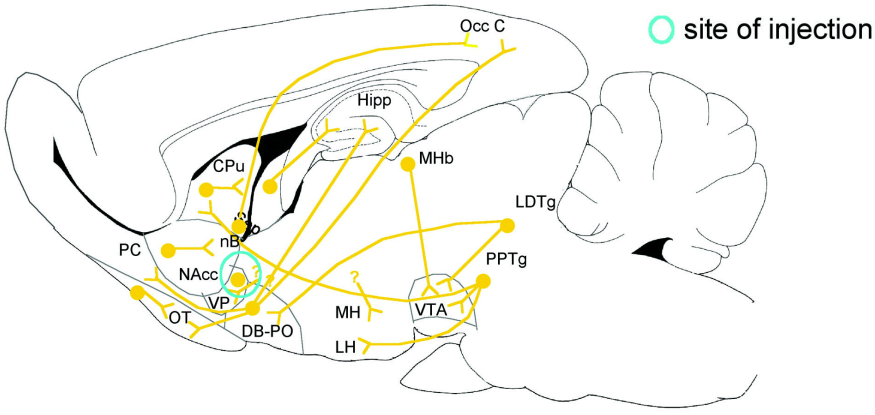


Figure 1. Site of injection and neuronal pathways indicated in cocaine self-administration. Abbreviations used: C Pu, caudate putamen; DB-PO, diagonal band of Broca, pre-optic area; Hipp, hippocampus; LDTg, lateral dorsal tegmental nucleus; LH lateral hypothalamus; MH medial hypothalamus; MHb, medial habenula; NAcc, nucleus accumbens; Occ C, occipital cortex; OT, olfactory tubercle; PC pyriform cortex; PPTg, pedunculo pontine tegmental nucleus; Sep, septum; VP, ventral pallidum; VTA, ventral tegmental area. Question marks next to symbols indicate unknown origin of the innervations. The site of injection is shown in a blue circle.

Ten Years and Growing Strong

On April 22, 2004 ATS celebrates ten years in business!

As this issue goes to press, the first of two toxicology studies for the chronic pain therapeutic, SP-SAP, has begun. Each of the studies will last for six months and will provide valuable information on whether the drug will have any undesirable side effects.

ATS is expanding its facilities to be able to construct GMP (good manufacturing practices) space for the production of the drug for use in human clinical trials. Most importantly, we have added new talent to our growing team to expand our capabilities and improve our processes.

Our Scientific team: Douglas Lappi, Matthew Kohls, Brian Russell, Leonardo Ancheta, Stephen Abreu, David Dunbar, Majid Siadat-Pajouh, Courtney Gosch.

Our Administrative team: Thea Marlinga, Nicholas Jolson, Kristen Hartman, Denise Higgins.

Back Row, Lto R: Stephen Abreu, David Dunbar, Douglas Lappi, Brian Russell, Leonardo Ancheta, Majid Siadat-Pajouh
Front Row Lto R: Nicholas Jolson, Courtney Gosch, Thea Marlinga
Not pictured: Kristen Hartman, Denise Higgins, Matthew Kohls

Rosalind Ridley

Doors of San Marco basilica (watercolor)

Accomplished Scientist. . . Talented Artist - Rosalind Ridley

by Douglas Lappi

Rosalind Ridley is an internationally recognized expert on prion diseases and learning and memory in primates; the latter is a reason that we have interacted with her often. We discovered that we have in common a fascination with artwork and architecture of Renaissance Italy. Dr. Ridley has been drawing some of the beautiful places of that country, and we are extremely pleased to be able to present them to you over the next few quarters in *Targeting Trends*.

Dr. Ridley's subject is one of the five famous doors of the façade of the Basilica of San Marco, highlight of the square that Napoleon called the finest drawing room in Europe ("le plus bon salon d'Europe"). In 828, the remains of the apostle Mark were transported to Venice and a Basilica was built to house them. The building was seriously damaged by fire in 976 and rebuilt between 1050 and 1090. The bronze doors were constructed between the 11th and 14th Centuries, and during the 12th and 13th Centuries, the bas-reliefs surrounding the doors were made. The beautiful structures above the doors are typical Venetian, and make a pattern that is widely recognizable.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Relationship between CSF hypocretin levels and hypocretin neuronal loss.

Gerashchenko D, Murillo-Rodriguez E, Lin L, Xu M, Hallett L, Nishino S, Mignot E, Shiromani PJ
Exp Neurol 184(2):1010-1016, 2003

Narcolepsy has recently been shown to be a neurodegenerative disease. Data from several different sources indicate that narcoleptics have very low levels of hypocretin (HCRT)-containing neurons. The authors sought to verify a direct linkage between HCRT-containing neurons and HCRT levels in the CSF. Rats were lesioned with 45-90 ng of orexin-SAP (Cat. #IT-20) bilaterally into the lateral hypothalamus. Loss of HCRT neurons correlated with decreased levels of HCRT in the CSF.

Cholinergic modulation of visual attention and working memory: Dissociable effects of basal forebrain 192-IgG-saporin lesions and intraprefrontal infusions of scopolamine.

Chudasama Y, Dalley JW, Nathwani F, Bouger P, Robbins TW
Learn Mem 11(1):78-86, 2004

It is hypothesized that cortical cholinergic dysfunction underlies the cognitive impairments associated with dementia and normal aging. The authors examined the role of these neurons in both attentional and mnemonic functions, using either bilateral infusions of 125 ng of 192-Saporin (Cat. #IT-01) into the bregma of rats or infusions of scopolamine. The results suggest that attentional and working memory capacities can be tested separately during the same session. It is also indicated that the CBF system is a modulator of both attentional and mnemonic processing.

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

Selective cholinergic denervation of the cingulate cortex impairs the acquisition and performance of a conditional visual discrimination in rats.

Winters BD, Robbins TW, Everitt BJ
Eur J Neurosci 19(2):490-496, 2004

Performance in conditional discrimination tasks is thought to be controlled at least in part by the cingulate cortex and its basal forebrain afferents. Using bilateral 0.5 µl injections of 0.02 µg/ml 192-Saporin (Cat. #IT-01) into the cingulate cortex of rats, the authors investigated the role of cholinergic projections from the vertical limb nucleus of the diagonal band to the cingulate cortex in specific types of learning. The results reinforce the idea that cholinergic projections to the cortex are involved in processing sensory information as well as task-related stimuli.

Medullary serotonergic neurons and adjacent neurons that express neurokinin-1 receptors are both involved in chemoreception in vivo.

Nattie EE, Li A, Richerson GB, Lappi DA
J Physiol Apr 1;556(Pt 1):235-253, 2004

The retrotrapezoid nucleus contains neurokinin-1 receptor (NK-1r)-expressing neurons that are involved in chemoreception. NK-1r-expressing neurons are also present in areas that contain medullary serotonergic neurons. These serotonergic neurons have been shown to be chemosensitive *in vitro*. With two 100-nl injections of 1 µM SP-SAP (Cat. #IT-07), anti-SERT-SAP (Cat. #IT-23), or both, the authors examined whether both cell populations are involved in chemoreception *in vivo* in rats. The results support that separate populations of serotonergic and NK-1r-expressing neurons are each involved in chemoreception *in vivo*.

Testosterone manipulation protects motoneurons from dendritic atrophy after contralateral motoneuron depletion.

Fargo KN, Sengelaub DR
J Comp Neurol 469(1):96-106, 2004

The authors wished to investigate the therapeutic effects of testosterone on motoneuron dendrites in nerve injury models. 1 µl of a 0.1% solution of CTB-SAP (Cat. #IT-14) solution was unilaterally injected into the ispilateral bulbocavernosus and levator ani muscles of rats, and the contralateral motoneuron morphology was examined. In castrated rats receiving testosterone, dendrites in the spinal nucleus of the bulbocavernosus grew after CTB-SAP treatment. This is a demonstration of the neuroprotective/neurotherapeutic role of testosterone in the nervous system.

Increased calcium influx and ribosomal content correlate with resistance to endoplasmic reticulum stress-induced cell death in mutant leukemia cell lines.

Zhang Y, Berger SA
J Biol Chem 279(8):6507-6516, 2004

Ca^{2+} plays a vital role in many cell processes. To investigate events associated with Ca^{2+} and endoplasmic reticulum (ER) stress-induced cell death, the authors developed a mutant cell line with resistance to several ER stress-inducing agents. One of the assays used to define the characteristics of this cell line was treatment of the cells with

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

3 μg/ml of Saporin (Cat. #PR-01) and subsequent analysis of protein expression. The suppression of ribosome function partially reversed the resistance to ER stress-induced cell death.

Extrinsic regulation of injury/growth-related gene expression in the inferior olive of the adult rat.

Buffo A, Carulli D, Rossi F, Strata P
Eur J Neurosci 18(8):2146-2158, 2003

Inferior olive (IO) cells of the CNS have the ability to regenerate axons after injury, even when the injury is close to the terminal field. After administration of 2.2 μg of 192-Saporin (Cat. #IT-01) and a control immunotoxin (mouse IgG-SAP, Cat #IT-18) to each ventricle in rats, two subsets of IO cells were discovered. Each subset responded differently to injury indicating that multiple mechanisms are responsible for their intrinsic regenerative potential.

Transfer effects and conditional learning in rats with selective lesions of medial septal/diagonal band cholinergic neurons.

Janisiewicz AM, Baxter MG
Behav Neurosci 117(6):1342-1352, 2003

Conditional learning appears to require cholinergic input to the hippocampus and cingulate cortex. Using a total of 0.5 μl of 0.12 μg/μl 192-Saporin (Cat. #IT-01) injected into the medial septal area of rats, the authors investigated the role of cholinergic input in conditional learning. The results suggest that cholinergic neurons of the medial septum/vertical limb of the diagonal band play a role in the transfer of behavioral experience rather than in conditional learning itself.

Environment-spatial conditional learning in rats with selective lesions of medial septal cholinergic neurons.

Janisiewicz AM, Jackson O, Firoz EF, Baxter MG
Hippocampus 14:265-273, 2004

192-Saporin (Cat. #IT-01) has produced varied results when used to determine the role of cholinergic neurons of the medial septum/vertical limb of the diagonal band (MS/VDB) in spatial working memory. The authors used a total of 0.5 μl of 0.12 μg/μl 192-Saporin injected into the MS/VDB to examine "environment-spatial" conditional learning. The findings suggest that cholinergic neurons of the MS/VDB are involved in some aspects of conditional associative learning.

Septohippocampal acetylcholine: involved in but not necessary for learning and memory?

Parent MB, Baxter MG
Learn Mem 11:9-20, 2004

In this review the authors describe some of the methods and rationale behind the investigation of hippocampal acetylcholine and its role in the support of learning and memory processes. Results produced by the use of 192-Saporin (Cat. #IT-01) are discussed, as well as the differences that have been found between the effects of 192-Saporin and those of less specific lesioning agents.

Extensive lesions of cholinergic basal forebrain neurons do not impair spatial working memory.

Vuckovich JA, Semel ME, Baxter MG
Learn Mem 11:87-94, 2004

The authors wished to examine whether cerebellar Purkinje cells damaged during a cholinergic basal forebrain lesion might be the cause of impaired working memory. Four injections of 0.2-0.3 μl (0.12-0.15 μg/ml, 192-Saporin, Cat. #IT-01) into the medial septum/vertical limb of the diagonal band, two injections into the horizontal limb of the diagonal band of Broca, and four injections into the nucleus basalis magnocellularis/ substantia innominata of rats were used to produce a very specific lesion. The results indicate that the cholinergic basal forebrain does not play a substantial role in spatial working memory.

Effects of complete immunotoxin lesions of the cholinergic basal forebrain on fear conditioning and spatial learning.

Frick KM, Jeansok JK, Baxter MG
Hippocampus 14:244-254, 2004

The authors examined the hypothesis that basal forebrain cholinergic neurons are critical for acquisition and consolidation of fear conditioning by lesioning the medial septum/vertical limb of the diagonal band, the horizontal limb of the diagonal band of Broca, and the nucleus basalis magnocellularis of rats with 192-Saporin (Cat. #IT-01). The lesions did not impair contextual fear conditioning, implying that impairments induced by scopolamine may not be mediated by cholinergic input to the hippocampus and neocortex.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: Streptavidin versus Avidin

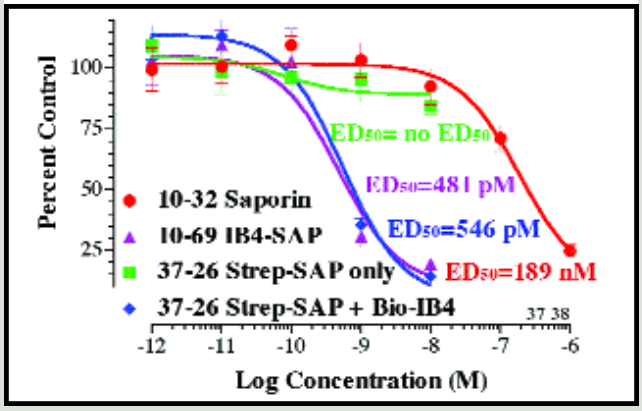
Q I recently tried to order avidinylated-SAP and was told that this product has been replaced with a new product, streptavidin-SAP. Why did you replace avidinylated-SAP?

A We initially had good results with avidinylated-SAP. It combined well with biotinylated antibody to produce extremely potent cytotoxic materials, and had low toxicity itself. However, the weaknesses of avidin are well-documented. Probably the most severe is its high isoelectric point that has been suggested to cause non-specific binding. As we produced more batches of avidinylated-SAP and completed comparative studies, we in fact, found this to be the case.

Q I use avidinylated-SAP to demonstrate that my antibody internalizes. It worked quite well for me.

A A couple of months ago we received two reports from customers that they were seeing that, even in batches that had performed well in quality control testing, there was a non-specific cytotoxicity with some cells and/or cell lines. Since a major use of this material is to demonstrate internalization of the biotinylated targeting agent, this was an unacceptable situation.

KNRK cells are plated at 2500 cells/well and incubated overnight. Streptavidin-SAP is premixed with Biotinylated-IB4 in equimolar concentrations or added to a plate alone. Saporin, IB4-SAP, and the Streptavidin-SAP + Biotinylated-IB4 mixture are added in 10-μl volumes and plates incubated 72 hrs. PMS/MTS is added and the plates are incubated 15-30 min, then read at 490 nm.



We changed to streptavidin to overcome these specificity issues. As shown in the figure above, streptavidin-SAP has an excellent capacity to transform a biotinylated reagent into a potent cytotoxic targeting vehicle, while streptavidin-SAP alone has no detectable cytotoxicity.

Streptavidin-SAP Pricing

IT-27-25	25 micrograms	$165 ($190)
IT-27-100	100 micrograms	$625 ($725)
IT-27-250	250 micrograms	$1450 ($1775)

Kits (pricing in parentheses) includes equal amounts of saporin

Upcoming Events

FENS
July 10-14, 2004
Lisbon, Portugal
Booth 42

Society for Neuroscience
October 23-27, 2004
San Diego, CA
www.atsbio.com/golf

Join ATS at the Torrey Pines Golf Course

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: PLANETS OPTICS CALCULUS WESTMINSTER VELOCITY

Answer: SIR ISAAC NEWTON

WINNERS: Armando Poeppl, Univ Health Network * Dr. Hilda Yu, Univ of CAIrvine * Ching-Hui Yang, Univ Texas Health Ctr * Douglas J. Taatjes, Univ Vermont * Filomena Dimayuga, Univ Kentucky * Greg Hickey, Massachusetts General Hospital * Tanja Babic, Univ Western Ontario * Seto Chice, SUNY HSC Brooklyn * Michael Lebowitz, Panacea Pharmaceuticals Inc * Robert Speth, Univ Mississippi * Thomas Breithaupt, Des Moines Univ * Dr. Carmen Diaconu, Institute of Virology

Isaac Newton was born December 25, 1642 in Lincolnshire, England. His accomplishments in mathematics, optics, and physics laid the foundations for modern science.

As a mathematician, Newton invented integral calculus, and jointly with Leibnitz, differential calculus.

Newton made a huge impact on theoretical astronomy. He defined the laws of motion and universal gravitation which he used to predict precisely the motions of stars, and the planets around the sun. The first law dealt with forces and changes in velocity. Newton also constructed the first reflecting telescope.

Newton died in London on March 20, 1727 and was buried in Westminster Abbey, the first scientist to be accorded this honor.

192 IgG-Saporin-Induced Lesions Identify an Inhibitory Role in Cocaine Reward

(continued from page 1)

p75 and ChAT gene expression in the MS-DB and VP with little effect on the caudate putamen (CP) or NAcc of the saporin-treated rats (Figure 2). These data indicate that the net impact of cholinergic neurons in these three brain regions is inhibitory to the processes underlying cocaine self-administration. p75 has been assumed to be a selective marker for cholinergic neurons in the basal forebrain (1) and not associated with other cholinergic interneurons in the CP, NAcc and VP. These findings were based on data obtained with immunohistochemical techniques for p75 that had limited sensitivity for regions where cholinergic neurons are less dense. The real-time RT-PCR procedure used here was more sensitive, permitting identification of p75 in the CP, NAcc and VP. However, whether this low affinity nerve growth factor receptor is associated exclusively on cholinergic neurons in these brain regions is yet to be demonstrated.

The significant decreases in p75 and ChAT in the MS-DB and VP in the saporin-lesioned animals suggest that cholinergic neurons in these regions may produce a negative feedback on the actions of cocaine through dopaminergic neurons. The MS-DB neurons receive excitatory glutamatergic input from the entorhinal cortex, inhibitory GABAergic input from the hippocampus and both monoaminergic and peptidergic input from the brainstem. There are also cholinergic interneurons within the MS-DB that synapse onto cholinergic projection neurons in this structure. The output of the MS-DB consists almost exclusively of cholinergic and GABAergic projection neurons, with the cholinergic afferents comprising approximately two-thirds of the neuronal population. These projection neurons innervate the hippocampus, olfactory bulb and the entire cortical mantle. A septo-hippocampal-septal loop appears to serve as a feedback inhibition from the hippocampus to the MS-DB that contains GABAergic projections from the hippocampus that synapse onto cholinergic projection neurons within the MS-DB. Increased turnover rates of ACh were seen in the hippocampus of cocaine self-administering rats suggesting that the balance in this loop shifts toward the activation of cholinergic neurons that innervate this structure. The interaction of MS-DB neurons with the monoaminergic efferents from the brainstem are complex, with noradrenergic neurons producing excitation of cholinergic MS-DB neurons and dopaminergic inputs producing inhibition of these cells. The substantia nigra and VTA send dopaminergic projections that synapse onto cholinergic neurons in the MS-DB and the MS-DB contains relatively dense populations of D3 receptors. Electrolytic lesions of the septum and MS-DB regions increase the sensitivity of rats to cocaine in conditioned place preference, also suggesting an inhibitory modulation of cocaine reinforcement by this region that is consistent with the present data.

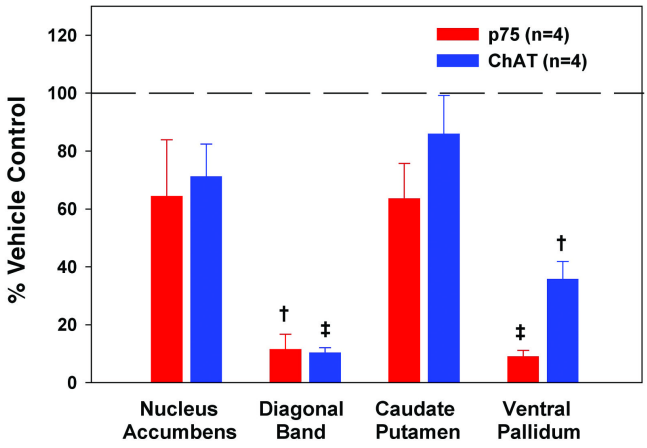


Figure 2. p75 and choline acetyltransferase gene expression. p75 and choline acetyltransferase gene expression in four brain regions of four pairs of rats intravenously self-administrating cocaine that received either 192-IgG-saporin or sham vehicle treatment into the posterior nucleus accumbens-ventral pallidum. The diagonal band of Broca and the ventral pallidum showed significant decreases in both. Significance of difference between means are: † = p<0.01; ‡ = p<0.001.

These data indicate that cholinergic innervations of the VP and DB are inhibitory to the brain processes that underlie cocaine self-administration. In addition, the nucleus basalis magnocellularis which contains cell bodies for a major cholinergic system innervating the forebrain may also be involved since AMPA-induced lesions shifted the cocaine dose-intake relationship to the left suggesting an enhancement of reinforcing efficacy which is consistent with the role of these neurons in the MS-DB and VP. Discrete intracranial injections of 192 IgG-saporin can be used to investigate the involvement of cholinergic innervations of other forebrain sites where the low affinity nerve growth factor receptor is localized exclusively on cholinergic neurons as is the case for the MS-DB. Identification of the specific cholinergic receptors that are responsible for this shift to the left in the dose-intake function could provide potential targets for the treatment of cocaine abuse. In addition, since drugs of abuse are thought to have addictive properties because they modulate brain systems dedicated to reinforcement and since cholinergic receptors have been shown to participate in the processes underlying brain stimulation reinforcement (2,3), it is likely that some of the cholinergic neurons represent neuronal systems mediating reinforcement in general and thus may have broader applications to understanding the biological basis of behavior.

References

1. Woolf NJ,Gould E, Butcher LL (1989) *Neuroscience* 30:143-152.
2. Panagis G, Kastellakis A, Spyraki C, Nomikos G (2000) Psychopharmacology 149: 338-396.
3. Yeomans JS, Takeuchi J, Baptista M, Flynn DD, Lepik K, Nobrega J, Fulton J, Ralph MR (2000) *J Neurosci* 20: 8861-8867.

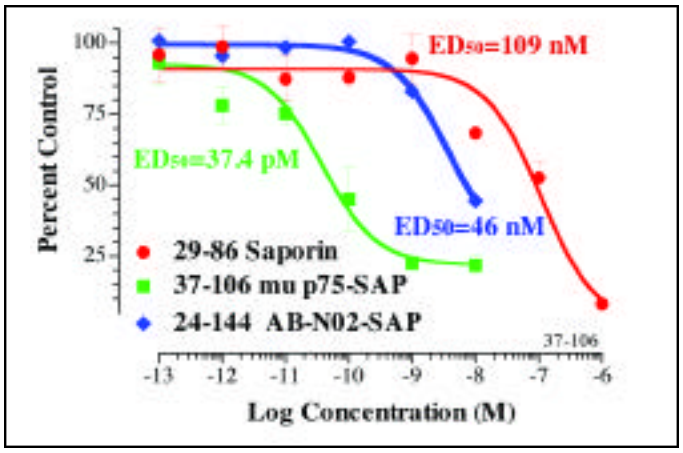
Targeting Tools: Featured Products

mu p75-SAP

Improved

We have re-designed the anti-murine p75-Saporin targeted toxin (mu p75-SAP, Cat. #IT-16) and have produced a conjugate that is much more potent in our *in vitro* cell cytotoxicity assays. Previously, we used a rat monoclonal antibody. This antibody had been outperformed by our rabbit polyclonal AB-N01, in several assays, especially FACS analysis of murine p75-expressing cells. This is an important indicator of being able to bind to the cell surface, which is fundamental for a targeted toxin. We still consider the rat monoclonal (AB-N02) to be an important antibody since it provides an alternative to the rabbit polyclonal.

To create this toxin, we have affinity-purified the rabbit polyclonal with the immunogen bound to a solid support, and conjugated the affinity-purified antibody to saporin. As can be seen in the cytotoxicity assay on the right, the new mu p75-SAP is orders of magnitude more potent than the previous conjugate. We believe that the greater potency will translate to smaller amounts used for elimination of p75-positive neurons in the mouse brain, and that this will result in a greater index of efficacy and lesser non-specific cytotoxicity.



NG3 cells are plated at 1000 cells/well and incubated overnight. Saporin, mu p75-SAP(conjugate of the affinity-purified rabbit polyclonal to mouse NGFr and saporin), and AB-N02-SAP are added in 10 μl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 1-2 hours, then read at 490 nm.

mu p75-SAP Pricing

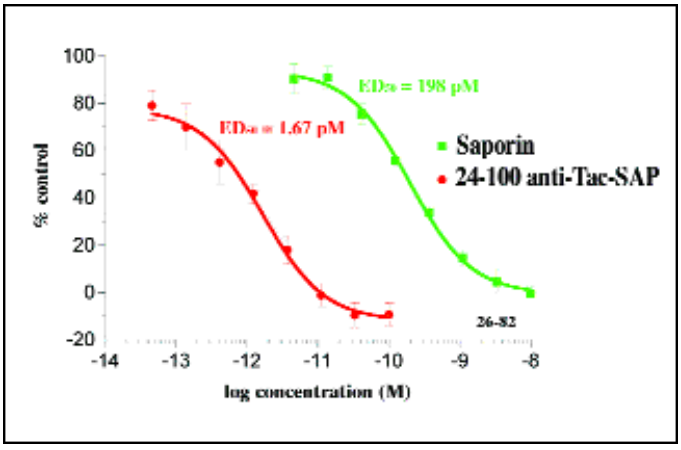
IT-16-50	50 micrograms	$350 ($600)
IT-16-100	100 micrograms	$700 ($1100)
IT-16-250	250 micrograms	$1400 ($2600)

Kits (pricing in parentheses) include equal amounts of saporin and antibody

Anti-Tac (CD25)-SAP

NEW!

Advanced Targeting Systems has introduced important new toxins for investigation into cells that express the interleukin-2 (IL-2) receptor and especially the workings of the immune system. Anti-CD25-saporin (Anti-Tac-SAP) conjugates are directed to the human IL-2 receptor or to the mouse IL-2 receptor. This marker for activated T lymphocytes allows removal of this important group of cells and determination of their function in numerous important biological responses. The graph below shows the potent cytotoxic activity of the anti-human CD25-saporin toxin (anti-Tac-SAP, Cat. #IT-24).



Cytotoxicity of anti-Tac-SAP and of non-conjugated saporin (Saporin) to SR cells in culture. Cells were plated at 5000 cells/well and 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).

Spring Cleaning?

Gangsta's solution to the Spring Cleaning bug - find a nice warm spot for a nap!

Anti-Tac-SAP (human) Pricing

IT-24-25	25 micrograms	$200 ($300)
IT-24-100	100 micrograms	$700 ($900)
IT-24-250	250 micrograms	$1400 ($1800)

Kits (pricing in parenthesis) include equal amounts of saporin and antibody

Anti-Tac-SAP (mouse) Pricing

IT-29-25	25 micrograms	$200 ($300)
IT-29-100	100 micrograms	$700 ($900)
IT-29-250	250 micrograms	$1400 ($1800)

Kits (pricing in parenthesis) include equal amounts of saporin and antibody

Visit the ATS website for a complete list of products.

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.

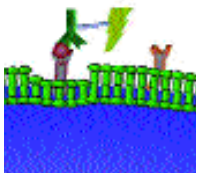
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

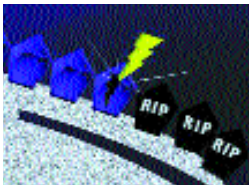
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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 form the name of this noted scientist.

ROCATTOED

SHOPIL

SHIRTEMCY

VOICARATIED

EMULEIKA

This scientist was so hot, she won TWO Nobel Prizes!

Arrange the circled letters to form the name of this noted scientist, as suggested by the above clue.

Answer:

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by May 31, 2004.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

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Volume 5, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

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Newsletter Highlights

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- Acetylated LDL-SAP; FITC-labeled second antibodies *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

The Discovery of Saporin

Contributed by Dr. Fiorenzo Stirpe, Dipartimento di Patologia sperimentale, Università di Bologna, I-40125 Bologna, Italy

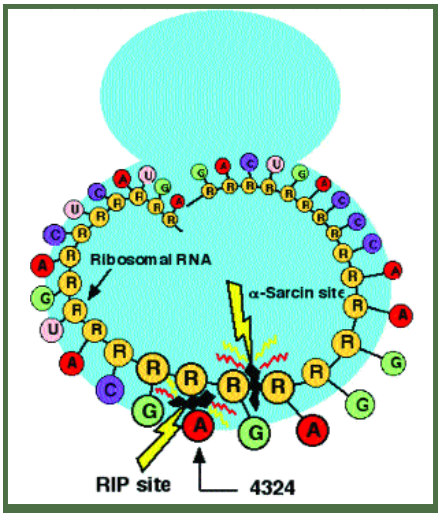
Saporin from *Saponaria officinalis* (soapwort plant of the Caryophyllaceae family) was discovered as part of the research on plant toxins we undertook around 1970, when we became interested in the study of the mechanism of action of ricin.

Ricin, from *Ricinus communis* (castor bean plant of the Euphorbiaceae family) and abrin from *Abrus precatorius* (of the Leguminosae family), are two potent toxins known since the end of the 19th century, when Paul Ehrlich prepared the first antibodies with them.

The study of these toxins was almost completely neglected until in 1970 it was found that they were more toxic to malignant than to normal cells (1), and that they inhibited protein synthesis both by cells and cell-free systems. While investigating the mechanism of this inhibition, in collaboration with L. Montanaro and S. Sperti of our Department, we had the rather simple idea that if two plants taxonomically very far from each other contained very similar toxins, it was possible that other

Saponaria officinalis

(continued on page 6)



The site of action
Saporin inactivates the ribosome and stops protein synthesis.

SAP FACTS

Saporin
(from the seeds of the plant *Saponaria officinalis*)
29.5 kDa

SO6 isoform

Single-chain ribosome-inactivating protein (RIP)

Extremely stable

Non-glycosylated

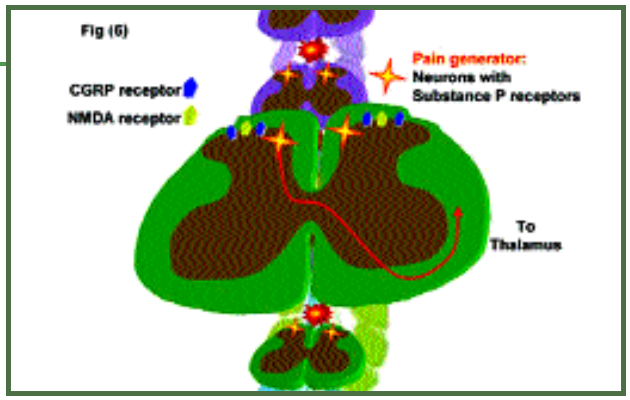
Most active RIP

Safely handled in the laboratory

Drug Development Update

Chronic pain conditions are often caused by ongoing disease states or tissue damage that create sensitization of both the primary afferents (nerves that convey impulses from the outer part of the body) and spinal cord neurons. This sensitization results in a persistent increased sensitivity to both noxious (hyperalgesia) and non-noxious (allodynia) stimuli. Many persistent pain states have proven difficult to treat using currently available pharmacological or surgical approaches that may cause significant unwanted side effects, and often result in significant reduction in quality of life.

Advanced Targeting Systems is continuing to develop Substance P-Saporin (SP-SAP) as a chronic pain therapeutic. The first of two toxicology studies has begun and we expect to have results from that study in early 2005. All of the preclinical data and scientific publications support the idea that SP-SAP eliminates the chronic pain signal. It accomplishes this by removing a small subset of neurons in the spinal cord.



Location and Function of Substance P receptor (SPR)-positive neurons in the spinal cord. SP-SAPspecifically targets and eliminates cells that express SPR. Intrathecal administration in the spinal cord kills these spinothalamic neurons that make up less than 5% of the neurons in the spinal cord. These SPR-positive neurons also express CGRP(calcitonin gene-related peptide) and NMDA(N-methyl-D-aspartate or glutamate) receptors. These SPR-positive neurons are labeled "Pain Generators" and it has been shown that their elimination greatly reduces the perception of chronic pain in animal models.

One patient population that should be targeted is terminal cancer patients who are unresponsive to opioid treatment. These patients have run out of options. The surgical and chemical interventions that are often made by physicians destroy more neurons in a less-specific manner and can even cause a central pain state. We believe SP-SAP can greatly reduce the perception of chronic pain in patients with minimal or no side effects. We believe this because we have seen the effects in two animal models in preclinical studies. Once we have toxicology results the FDA accepts, clinical trials in humans can begin.

Doorway of Campo Sant'Angelo

Accomplished Scientist. . . Talented Artist - Rosalind Ridley

As promised, we are pleased to present another drawing from Dr. Rosalind Ridley, internationally recognized expert on prion diseases and learning and memory in primates.

The doorway is the 15th century entrance to an older Augustinian mendicant friary in the corner of the Campo Sant'Angelo next to the Ponte dei Frati. The Campo Sant'Angelo is situated in the western part of the San Marco district. Being in the inner curve of the meander of the Grand Canal, this area was the most marshy and unstable in Venice. Many campanile in this area collapsed under subsidence and earthquake. The *campanili* of San Michele Arcangelo, which stood a few feet from this doorway, fell in 1347. Its replacement fell on its day of completion and a third attempt was finished in 1456, damaged by a thunderbolt in 1487, and finally demolished in 1837.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Modulation of photic resetting in rats by lesions of projections to the suprachiasmatic nuclei expressing p75 neurotrophin receptor.

Erhardt C, Galani R, Jeltsch H, Cassel JC, Klosen P, Menet JS, Pevet P, Challet E
Eur J Neurosci 19(7):1773-1788, 2004

The circadian clock in mammals is located within suprachiasmatic nuclei of the hypothalamus (SCN). The authors investigated how cholinergic afferents from the basal forebrain may be involved in control of the circadian clock. 3 μg of 192-Saporin (Cat. #IT-01) was injected intracerebroventricularly, or 1 μg was injected in SCN of rats, and various aspects of the circadian system were investigated. The data suggest that the forebrain cholinergic system is involved in the phase resetting properties of light.

Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-Saporin-induced lesions of the medial prefrontal cortex.

Dalley JW, Theobald DE, Bouger P, Chudasama Y, Cardinal RN, Robbins TW
Cereb Cortex Apr 14, 2004

Prior work has demonstrated that lesions of the cortical cholinergic system of the basal forebrain impair performance in attentional tasks. The authors examined the effects of selective depletion of acetylcholine from the prefrontal cortex (PFC) on these same attentional tasks. 50 or 100 ng of 192-Saporin (Cat. #IT-01) was infused into the PFC of rats. Treated animals displayed deficits in specific aspects of the attentional tasks, indicating a modulatory role in PFC function by basal forebrain cholinergic neurons.

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

Involvement of cholinergic neuronal systems in intravenous cocaine self-administration.

Smith JE, Co C, Yin X, Sizemore GM, Liguori A, Johnson WE 3rd, Martin TJ
Neurosci Biobehav Rev 27(8):841-850, 2004

Recent studies have demonstrated that cholinergic neurons take part in the process of cocaine self-administration. In this study the authors used 0.25 μg-injections of 192-Saporin (Cat. #IT-01) into the posterior nucleus accumbens (Nacc)-ventral pallidum (VP) region of rats to identify specific subsets of cholinergic neurons that are involved. The results indicate that cholinergic innervations of the VP, the medial septum/diagonal band of Broca, and the Nacc are inhibitory to the underlying processes of cocaine self-administration.

Altered neurotrophin receptor function in the developing prefrontal cortex leads to adult-onset dopaminergic hyperresponsivity and impaired prepulse inhibition of acoustic startle.

Rajakumar N, Leung LS, Ma J, Rajakumar B, Rushlow W
Biol Psychiatry 55(8):797-803, 2004

Neurodevelopmental abnormalities are suspected to play a role in the pathogenesis of schizophrenia. The authors injected 0.75 μl of 192-Saporin (Cat. #IT-01) bilaterally into the prefrontal cortex of postnatal day 1 rats. The rats were then evaluated in tests designed to measure behavioral abnormalities relevant to schizophrenia. The behavior of the treated animals indicated that damage to p75-receptor-expressing neurons in the prefrontal cortex may be involved in the manifestation of schizophrenia.

Spinal neurons involved in the generation of at-level pain following spinal injury in the rat.

Yezierski RP, Yu CG, Mantyh PW, Vierck CJ, Lappi DA
Neurosci Lett 361(1-3):232-236, 2004

The elimination of substance P receptor-expressing neurons in lamina I of the spinal cord using SSP-SAP (Cat.#IT-11) has been shown to reduce behavior associated with chronic pain. The authors investigated the effects of 150 or 300 ng SSP-SAP treatment during or after intraspinal administration of quisqualic acid in rats. Both treatments resulted in a reduction of pain-associated behavior. These results demonstrate that pain following spinal cord injury involves a population of spinal neurons expressing the substance P receptor.

Effects of hypocretin2-saporin and antidopamine-beta-hydroxylase-saporin neurotoxic lesions of the dorsolateral pons on sleep and muscle tone.

Blanco-Centurion C, Gerashchenko D, Salin-Pascual RJ, Shiromani PJ
Eur J Neurosci 19(10):2741-2752, 2004

Narcolepsy is linked to the loss of orexin (or hypocretin)-containing neurons in the brain. These neurons are located in the perifornical region of the posterior hypothalamus and innervate the locus coeruleus (LC). To investigate the role of the LC in sleep the authors injected 0.3 μl of 192-Saporin (Cat. IT-01) or anti-DBH-SAP (Cat. #IT-03) at 1 μg/μl. They

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

also used 0.3 μl of orexin-SAP (Cat. #IT-20) at either 90 ng/μl or 60 ng/μl in a separate group of animals. The results indicate that orexin innervation to the pons plays a role in arousal from sleep.

Glucoprivation increases expression of neuropeptide Y mRNA in hindbrain neurons that innervate the hypothalamus.

Li AJ, Ritter S
Eur J Neurosci 19(8):2147-2154, 2004

It is suspected that hypothalamic neuropeptide Y (NPY) innervation of the hypothalamus contributes to glucoregulatory feeding. Along with mRNA studies, the authors injected 42 ng of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus. Elimination of the hindbrain catecholamine/NPY neurons abolished increases in NPY expression due to glucoprivic conditions. This response suggests that NPY hindbrain neurons play a role in glucoprivic feeding and other glucoregulatory responses.

Impaired and spared cholinergic functions in the hippocampus after lesions of the medial septum/vertical limb of the diagonal band with 192 IgG-saporin.

Chang Q, Gold PE
Hippocampus 14(2):170-179, 2004

192-Saporin has been widely used to eliminate cholinergic neurons in the basal forebrain. These lesions can produce near-total loss of choline acetyltranferase (ChAT)-positive neurons in the medial septum/vertical limb of the diagonal band of Broca (MS/VDB). There is evidence that this ChAT deficit produces an upregulation of cholinergic mechanisms providing input to the hippocampus. Using either 0.2 or 0.3 μl of 0.5 μg/μl 192-Saporin (Cat. #IT-01) injected into the MS/VDB the authors examined the residual release of acetylcholine in the hippocampus after lesioning.

Cognitive effects of neurotoxic lesions of the nucleus basalis magnocellularis in rats: differential roles for corticopetal versus amygdalopetal projections.

Beninger RJ, Dringenberg HC, Boegman RJ, Jhamandas K
Neurotox Res 3(1):7-21, 2001

The cholinergic hypothesis states that projections of cholinergic neurons from the nucleus basalis magnocellularis to cortical and amygdalar targets are important in memory. This review discusses the work done on the cholinergic hypothesis using non-specific lesioning agents such as ibotenate and quisqualate, and the specific targeted conjugate 192-Saporin (Cat. #IT-01). The authors conclude that cholinergic targets in both the cortex and amygdala are important for the control of memory.

Effects of isolectin B4-conjugated saporin, a targeting cytotoxin, on bladder overactivity induced by bladder irritation.

Nishiguchi J, Sasaki K, Seki S, Chancellor MB, Erickson KA, de Groat WC, Kumon H, Yoshimura N
Eur J Neurosci in press, 2004

It has been demonstrated that IB4-binding non-peptidergic C-fiber neuronal populations are present in afferent pathways to the bladder. The authors used intrathecal administration of 8 μl of 2.5 μM IB4-SAP (Cat. #IT-10) to investigate what roles these neurons play in bladder function. Treated animals displayed a reduction of IB4 afferent nerve terminal staining, as well as a suppression of bladder overactivity due to bladder irritation, without a change in normal bladder function.

Immunotoxins and neuropeptide-toxin conjugates experimental applications.

Lappi DA, Wiley RG
Mini Rev Med Chem 4(5):585-595, 2004

The use of targeted toxins in research is rich and varied; here the authors describe some of the exciting results that researchers have made in the neurosciences.

[Interaction between sensory and cognitive processes in visual recognition: the role of the associative areas of the cerebral cortex] *Russian*

Dudkin KN, Chueva IV, Makarov FN
Ross Fiziol Zh Im I M Sechenova 89(10):1226-1239, 2003

The authors used ME20.4-SAP (Cat. #IT-15).

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: *Eliminating CBF Neurons*

by Dr. Ronald G. Wiley

Q. What dosage of 192-Saporin should be used in the lateral ventricle to eliminate cholinergic neurons in the basal forebrain, including substantia innominata (SI)? I read that Calza et al (1) used 2 or 3 micrograms/4.5 ul and found this was highly effective.

A. It has been our experience that two- or three-micrograms into the lateral ventricle is necessary to obtain a maximum cholinergic basal forebrain (CBF) lesion. However, these doses typically kill some cerebellar Purkinje cells. Another issue is that some cholinergic neurons in the NBM region are never killed by 192-Saporin.

Q. Should we expect to be able to kill all or almost all ChAT SI neurons?

A. Mesulam's lab has some data (2,3) to suggest that these neurons innervate the amygdala and adjacent cortex. Generally lesions of the septum and diagonal band are complete, but when you get more caudal, i.e. SI region, there will be some cholinergic neurons left. When you do ChAT or AChE stains, the amygdala and adjacent cortex are not denervated whereas the hippocampus, olfactory system and all the rest of the cortex are devoid of cholinergic terminals.

Q. Is there another toxin that will eliminate the remaining ChAT SI neurons?

A. There may be other targeted conjugates that could clean out the residual cells in the SI region if we knew what markers they co-express. For example, our SSP-saporin conjugate is very good at removing cells that express the NK-1 receptor such as striatal cholinergic interneurons.

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Upcoming Events

FENS
July 10-14, 2004
Lisbon, Portugal
Booth 42

Society for Neuroscience
October 23-27, 2004
San Diego, CA
www.atsbio.com/golf

Join ATS at the Torrey Pines Golf Course

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: DOCTORATE
POLISH
CHEMISTRY
RADIOACTIVE
LEUKEMIA

Answer: MARIE CURIE

WINNERS: Angela Finney, Panacea Pharmaceuticals Inc * Brian Bacskai, Massachusetts General Hospital * Fiona Hemming, Univ Joseph Fourier * Thomas Breithaupt, Des Moines Univ * Dr. Douglas J. Taatjes, Univ Vermont * Seto Chice, SUNYHSC Brooklyn * Filomena Dimayuga, Univ Kentucky * Ching-Hui Yang, Univ Texas Health Ctr * Kristen Phend, Univ North Carolina * Julie Bielawski, Univ Health Network

Marie Curie was born Maria Sklodowska, in Warsaw on November 7, 1867. She received a general education in **Polish** schools and then went to Paris to continue her studies at the Sorbonne where she gained her **Doctorate** of Science in 1903, the first awarded to a woman in Europe. Following the tragic death of her husband, Pierre Curie, in 1906, she took his place as Professor of General Physics in the Faculty of Sciences, the first time a woman had held this position.

Madame Curie developed methods for the separation of radium from **radioactive** residues in sufficient quantities to allow for its characterization and the careful study of its properties, therapeutic properties in particular. Together with her husband, she was awarded half of the Nobel Prize for Physics in 1903, for their study into the spontaneous radiation discovered by Becquerel, who was awarded the other half of the Prize. In 1911 she received a second Nobel Prize, this time in **Chemistry**, in recognition of her work in radioactivity.

Unfortunately, the repeated contact with radioactive elements took their toll on Madame Curie. She was afflicted with a double cataract and then died from **leukemia** on July 4, 1934.

The Discovery of Saporin

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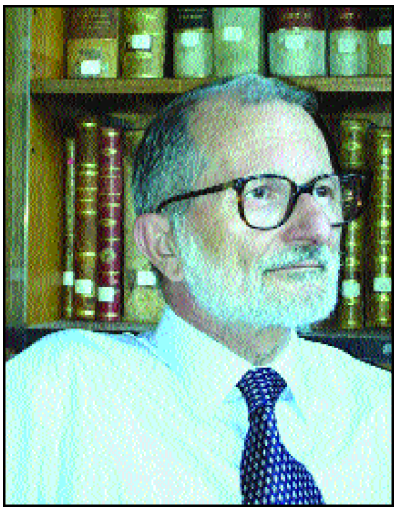

Professor Fiorenzo Stirpe
The discoverer of Saporin

toxins of the same kind existed in the plant kingdom. This began our search for other toxins and prepared extracts from all toxic plants we could get, mostly from tropical countries. Instead of testing these extracts for toxicity to animals or cells, for the sake of simplicity and rapidity, we assayed their capacity of inhibiting protein synthesis in a cell-free system. Indeed, in the course of some years we succeeded in identifying new toxins, namely modeccin, viscumin and volkensin. However, we soon found that some extracts inhibited cell-free protein synthesis, but were only some thousand-fold less toxic than ricin to cells or animals. At first we did not know how to interpret these "disturbing" results, until it was found that PAP, the pokeweed antiviral protein, inhibited protein synthesis with the same mechanism as ricin (2). It was then easy to ascertain that our active extracts contained proteins similar to PAP, which later on we called "ribosome-inactivating proteins" (RIPs). We decided to search for other RIPs, and since PAP had antiviral activity, we examined extracts from plants known to contain antiviral properties. Among these was *Dianthus caryophyllus* (carnation), from which we purified two RIPs, dianthin 30 and dianthin 32 (3).

Another approach was to explore plants in the same families as those plants with antiviral properties. Among these there was the Caryophyllaceae family, to which the carnation belongs. One of the plants investigated was *Saponaria officinalis* (soapwort), which was growing in the garden outside our department in Bologna, exactly under the window of my office. Much to our astonishment, the seeds of *Saponaria* turned out to contain several very active RIPs we called saporin (4). Saporin-S6 was used in the construction of immunotoxins (5) and subsequently by Drs. Douglas Lappi and Ronald Wiley for the preparation of a range of conjugates that are excellent experimental tools for the study of the nervous system.

So, after having struggled to search for seeds from all over the world, more or less by chance we isolated one of the most widely used RIPs from something that was literally under our feet.

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3. Stirpe F, Williams DG, Onyon LJ, Legg RF, Stevens WA (1981) Dianthins, ribosome-damaging proteins with antiviral properties from Dianthus caryophyllus L. (carnation). *Biochem J* 195(2):399-405.
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Golf Torrey Pines this October!

Torrey Pines Golf Course
San Diego, California

The 2004 SfN meeting will once again be in San Diego, home of one of the most beautiful golf courses . . . Torrey Pines. The name itself stirs the imagination of golf enthusiasts all over the world. Situated atop cliffs towering above the Pacific Ocean in San Diego, California, golfers marvel at the views of the coastline and deep ravines. Nature lovers can spot wildlife and the famous Torrey Pines trees that are indigenous to the area.

Each year, the PGA Buick Invitational is held at Torrey Pines Golf Course and in 2008, the US Open Championship will be held on the newly remodeled South Course. Saturday, October 23, 2004 is YOUR opportunity to play the South Course. Format will be either a shotgun beginning at 7am (if sufficient golfers enroll) or set tee times beginning at 7:30am. Green fees, including golf cart will be about $150 per person.

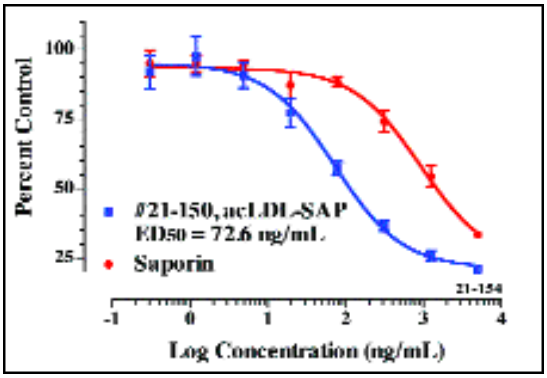
Limited space available! Contact ATS as your San Diego area hosts to reserve your tee time at www.ATSbio.com/golf.

Targeting Tools: Featured Products

MICROGLIA IN YOUR NEURONAL CULTURES BEHAVING BADLY?

ELIMINATE THEM WITH AcLDL-SAP

It's routine for primary neuronal cultures to have "contamination" with naturally occurring microglia. But what happens when you don't want these little creatures or the cytokines and cytotoxins that they release? Or you want to eliminate any effect that microglia have on neurons or astrocytes? ATS has the cheap, effective and very simple solution for this: acetylated LDL-SAP (Cat # IT-08). Just add it to the medium and microglia are eliminated. This material has been well described by Giulian and colleagues (1,2). AcLDL binds to the scavenger receptor that is well-expressed on microglia (3) and this results in internalization (4). When saporin is attached, it is carried inside, too and causes death of the microglial population. The graph below shows *in vitro* data from our laboratories that demonstrates the ability of acLDL-SAP to eliminate cells that express the scavenger receptor.



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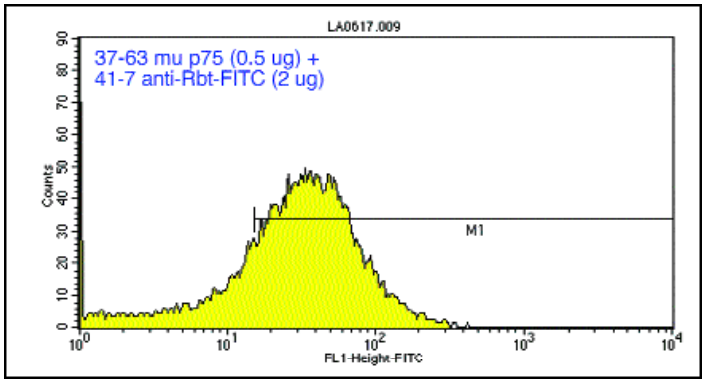
Gangsta thinks he's the preferred table decoration!



FITC-labeled goat anti-rabbit IgG

Advanced Targeting Systems announces its new fluorescent second antibody, FITC-labeled goat anti-rabbit IgG (Cat. #FL-04). This fluorescent conjugate is made with all the care that you expect from ATS. The figure above shows its use in flow cytometry on cells that express p75 and your results from immunohistochemistry should be just as clean. Look for our whole series of fluorescent second antibodies at great prices and splendid quality.

Visit the ATS website for a complete list of products.

Targeting Technology

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

Choose an NTIBODY[§] 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.

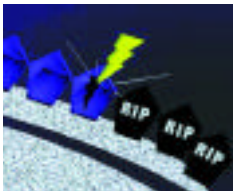
[§]or growth factor, peptide, ligand, or cytokine

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

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

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

The conjugate is internalized and APORIN 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 form the name of this noted scientist.

SHOYOPLYGI

AILARAM

SCLEL

INGASTIN

INTATER

This neuroscientist really knew how to network!

Arrange the circled letters to form the name of this noted scientist, as suggested by the above clue.

Answer:

Limit one entry per laboratory.

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by August 31, 2004.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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Oct-Nov-Dec 2004
Volume 5, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

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- CCK-SAP,NPY-SAP, Anti-M-ZAP, and new antibodies *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Effects of IB4-SAP on Bladder Overactivity

Contributed by Dr. Naoki Yoshimura, Dept Urology/Pharmacology, Univ Pittsburgh School of Medicine, Pittsburgh PA 15213. Dr. Yoshimura summarizes his work with IB4-SAP. A complete report was published in Eur J Neurosci *20(2):474-482, 2004.*

It has been demonstrated that hyperexcitability of C-fibers in bladder afferent pathways can contribute to bladder overactivity and/or bladder pain under pathological conditions such as spinal cord injury or chronic cystitis. We have also previously demonstrated that peptidergic and IB4-binding non-peptidergic C-fiber populations are present in the afferent pathways to the bladder and that the relative proportion of these two populations of neurons is different from that in the somatic afferents pathways. Therefore, in this present study, we investigated the effects of intrathecal application of the IB4-saporin conjugate at the level of L6-S1 spinal cord, where bladder afferent pathways terminate, on the normal bladder function and bladder overactivity induced by bladder irritation. The goal of this study was to elucidate the functional role of IB4-binding afferent pathways in bladder function.

We have found that intrathecal treatment with IB4-saporin at the level of L6-S1 spinal cord reduced IB4 afferent nerve terminal staining in the lamina II of the dorsal horn of L6 spinal cord as well as the number of IB4-binding neurons in L6 DRG (Figs. 1 and 2). IB4-saporin

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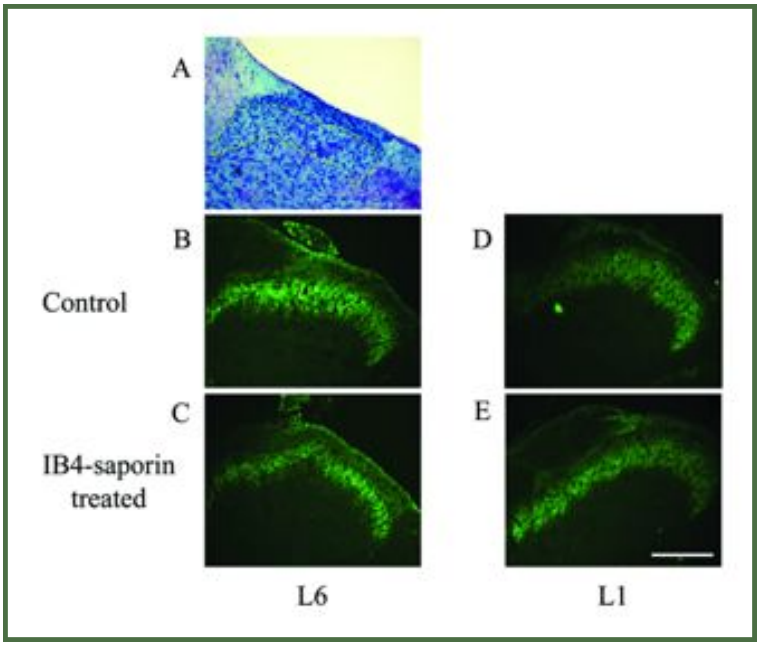

Fig. 1 Histochemical staining of the L1 and L6 spinal cord in normal rat (A). IB4-conjugated FITC staining of the L6 spinal cord from control (B) and IB4-saporin-treated rats (C) 3 weeks after the treatment. IB4-conjugated FITC staining of the L1 spinal cord from control (D) and IB4-saporin-treated rats (E) 3 weeks after the treatment. The lamina II area identified by Nissl's staining is indicated by dashed lines in A. Note that the staining density of IB4-binding afferent nerve terminals in the lamina II of the L6 spinal cord was depleted after the IB4-saporin treatment. Scale bar, 200 μm.

Take Me Out to the Ballgame. . .

On September 8th, ATS employees enjoyed an afternoon at the Padres/Cardinals game in San Diego. It was a hot day at Petco Park, but that made the peanuts and Crackerjacks taste all the better!

It was fun to celebrate the end of summer and close the office for a few hours — especially since the Padres won!

Don't worry. We're all back at work now, refreshed and ready to meet your targeting needs!

From left to right above: (back row) Brian Russell, Courtney Gosch, Doug Lappi, Denise Higgins. (front row) Thea Marlinga, Kristen Hartman, Majid Pajouh, Leonardo Ancheta. Photographer is new intern, Amalia Dingman.

Amalia is pictured in the blue hat in the photo at the left. Thanks to Doug's wife, Darlene, for shooting this photograph.

Palazzo Barbaro

Accomplished Scientist. . . Talented Artist - Rosalind Ridley

As promised, we are pleased to present another drawing from Dr. Rosalind Ridley, internationally recognized expert on prion diseases and learning and memory in primates.

The Palazzo Barbaro that this portico comes from was constructed in Venice in 1425. The building is typical of the several done by great Venetian architect Giovanni Bon. This beautiful portico and its palazzo is gloriously located on the Grand Canal.

The Gothic building was home to some famous people during its history, including the American Curtis family that hosted expatriates that were known as the Palazzo Barbaro Circle. Included were such luminaries as James Whistler and Henry James. James wrote "Letters from the Palazzo Barbaro" and "The Aspern Papers" while staying there.

Note that you can tie your gondola up in front!

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Facilitation of cholinergic transmission by combined treatment of ondansetron with flumazenil after cortical cholinergic deafferentation.

Gil-Bea FJ, Dominguez J, Garcia-Alloza M, Marcos B, Lasheras B, Ramirez MJ
Neuropharmacology 47(2):225-232, 2004.

Previous studies from this group demonstrated that 5-HT(3) receptor antagonists potentiated by GABA(A) antagonists increased acetylcholine (ACh) release in the rat cerebral cortex. This series of experiments investigated the effects of these antagonists on rats with 0.067 μg-bilateral infusions of 192-Saporin (Cat. #IT-01) into the nucleus basalis magnocellularis. Even after lesioning with 192-Saporin, rats treated with the 5-HT(3) and GABA(A) receptor antagonists displayed increased ACh release, indicating that these antagonists may have use as treatments for cognitive disorders.

Age-dependent effect of cholinergic lesion on dendritic morphology in rat frontal cortex.

Works SJ, Wilson RE, Wellman CL
Neurobiol Aging 25(7):963-974, 2004.

Aged rats display more dramatic and longer lasting effects due to brain injury than young animals. The authors examined the role cholinergic neurons may play in brain plasticity after injury in rats of varying ages. 0.15 μg of 192-Saporin (Cat. #IT-01) was injected into the nucleus basalis magnocellularis of young, middle-aged, and aged rats. Some types of injury were only seen in middle-aged and aged rats, and changes in dendritic morphology were least marked in the young animals.

Hindbrain catecholamine neurons mediate consummatory responses to glucoprivation.

Hudson B, Ritter S
Physiol Behav 82(2-3):241-250, 2004.

Norepinephrine (NE) and epinephrine (E) neurons appear to potently stimulate feeding behavior when administered to the hypothalamus. Previous work has indicated that these neurons play important roles in feeding responses due to glucoprivation. Bilateral 42 ng-injections of anti-DBH-SAP (Cat. #IT-03) were administered to rats to investigate the roles of NE and E neurons in the consummatory phase of the glucoprivic response. The results indicate that catecholaminergic neurons are involved in both appetitive and consummatory responses to glucoprivation.

Loss of cortical acetylcholine enhances amphetamine-induced locomotor activity.

Mattsson A, Pernold K, Ogren SO, Olson L
Neuroscience 127(3):579-591, 2004.

The authors have recently shown that cholinergic denervation of the basal forebrain in rats leads to an increased motor response to d-amphetamine, a hallmark of schizophrenia. In the present study 192-Saporin (Cat. #IT-01) was injected into the nucleus basalis magnocellularis or the medial septum/diagonal band of Broca, and OX7-SAP (Cat. #IT-02) was injected intracerebroventricularly. The dopaminergic hyper-reactivity was induced by lesions to the cortex cerebri, but not by damage to the cerebellum or hippocampus.

Recurrent paraplegia after remyelination of the spinal cord.

Jasmin L, Ohara PT
J Neurosci Res 77(2):277-284, 2004.

Previously, the authors demonstrated that a 3 μg-injection of CTB-SAP (Cat. #IT-14) into the lumbosacral intrathecal space caused a loss of motor function due to spinal demyelination. The motor function was recovered and stable for up to 9 months, after which the rats exhibited a slow deterioration of motor function, loss of spinal white matter, and the appearance of calcium deposits. The results indicate that the CTB-SAP-induced demyelination model is useful for investigating long term effects of axon and motoneuron loss.

Sound sequence discrimination learning is dependent on cholinergic inputs to the rat auditory cortex.

Kudoh M, Seki K, Shibuki K
Neurosci Res 50(1):113-123, 2004.

The auditory cortex (AC) is thought to play a role in the discrimination of sound sequences. The authors investigated the role of cholinergic inputs to the AC in processing these sequences by injecting 5 μg of 192-Saporin (Cat. #IT-01) into either the lateral ventricle or bilateral AC of rats. Treated animals displayed suppressed sound discrimination learning, but discrimination between two sound components was unaffected. The results suggest that cholinergic neurons in the AC are highly involved in sound sequence learning.

Habituation to stress and dexamethasone suppression in rats with selective basal forebrain cholinergic lesions.

Helm KA, Ziegler DR, Gallagher M
Hippocampus 14(5):628-635, 2004.

Basal forebrain cholinergic neurons may be involved in hippocampal and medial prefrontal cortex inhibition of glucocorticoid stress responses. The

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

authors investigated the effects of 0.05 to 0.075 μg injections of 192-Saporin (Cat. #IT-01) into the medial septum/vertical limb of the diagonal band of rats by measuring corticosterone levels during a restraint stress test. Lesioned rats displayed less stress suppression on the administration of dexamethasone than controls, indicating that cholinergic neurons are involved in these stress responses.

Cognitive and neurological deficits induced by early and prolonged basal forebrain cholinergic hypofunction in rats.

Ricceri L, Minghetti L, Moles A, Popoli P, Confaloni A, De Simone R, Piscopo P, Scattoni ML, di Luca M, Calamandrei G
Exp Neurol 189(1):162-172, 2004.

A distinctive feature of Alzheimer's disease is the loss of cholinergic neurons in the basal forebrain (BF). The authors investigated long-term effects of BF cholinergic lesions on several parameters. Administration of 0.21 μg of 192-Saporin (Cat. #IT-01) to the third ventricle of 7 day-old rats was followed by an evaluation of protein levels and cortical EEG patterns at 6 months of age. The findings indicate that permanent neonatal BF cholinergic damage may provide a model for abnormal adult cholinergic function.

Selective lesioning of the cholinergic septo-hippocampal pathway does not disrupt spatial short-term memory: a comparison with the effects of fimbria-fornix lesions.

Winters BD, Dunnett SB
Behav Neurosci 118(3):546-562, 2004.

The authors wished to investigate the role of the cholinergic system of the basal forebrain in delayed matching (DMTP)- and nonmatching (DNMTP)-to-position tasks after bilateral injections of 0.035 μg of 192-Saporin (Cat. #IT-01) into the dorsal and ventral hippocampus. The treated animals were compared to rats given fimbria-fornix (FF) lesions. Only the FF-lesioned animals showed impairment on DMTP and DNMTP tasks, demonstrating that the cholinergic septohippocampal system is not required for successful DMTP or DNMTP performance.

The effects of cerebellar damage on maze learning in animals.

Lalonde R, Strazielle C
Cerebellum 2(4):300-309, 2003.

Traditionally the cerebellum has been associated with motor control, but recent work has suggested that it plays a role in cognitive functions such as spatial learning as well. This study discusses the effects of cerebellar mutations in mice, and OX7-SAP (Cat. #IT-02) lesions in rats on water maze learning models. Results indicate that the cerebellum plays a role in working memory and the procedural aspect of maze learning, as well as being an important element of motor control.

NADPH oxidase contributes to angiotensin II signaling in the nucleus tractus solitarius.

Wang G, Anrather J, Huang J, Speth RC, Pickel VM, Iadecola C
J Neurosci 24(24):5516-5524, 2004.

Using immunoelectron microscopy the authors investigated whether NADPH oxidase is involved in angiotensin II signaling in central autonomic neurons. Angiotensin type 1 receptor (AT-1r)-expressing neurons were identified with an AT-1r antibody (Cat. #AB-N27AP) from which AT-1Ar (AB-N25AP) and AT-1Br (AB-N26AP) are affinity purified. Colocalization of AT-1r's and a NADPH oxidase subunit provides evidence that NADPH oxidase is involved in the effects of angiotensin II on autonomic neurons.

Minocycline protects basal forebrain cholinergic neurons from mu p75-saporin immunotoxic lesioning.

Hunter CL, Quintero EM, Gilstrap L, Bhat NR, Granholm AC
Eur J Neurosci 19(12):3305-3316, 2004.

In Alzheimer's disease basal cholinergic degeneration is accompanied by glial activation and the release of pro-inflammatory cytokines. To investigate whether neural events other than degeneration can cause effects of Alzheimer's disease, the authors treated mice with minocycline after lesioning the basal forebrain with 3.6 μg of mu p75-SAP (Cat. #IT-16). Administration of minocycline reduced the loss of cholinergic neurons, reduced glial response to the lesion, and lessened the cognitive impairment due to mu p75-SAP lesions.

Decreased neurogenesis after cholinergic forebrain lesion in the adult rat.

Cooper-Kuhn CM, Winkler J, Kuhn HG
J Neurosci Res 77(2):155-165, 2004.

Adult mammalian brains can produce new neurons, mainly in two areas: the interconnected system of the lateral ventricle and the olfactory bulb, and the dentate gyrus of the hippocampus. The authors used a 3.5 μg-injection of 192-Saporin (Cat. #IT-01) into the right ventricle of rats to determine whether cholinergic input is necessary for adult neurogenesis. The results suggest that acetylcholine, a product of cholinergic neurons, is necessary for the survival of newly-formed neurons.

Visit www.ATSbio.com for a complete list of references.

Targeting Talk: *Effective Toxins*

by Dr. Douglas Lappi

Q: Why do your directions for SSP-SAP state that it is to be used within hours after dissolution? To my knowledge, both proteins and peptides are stable in clean solution.

A: In fact, the two components of SSP-SAP (Stable Substance P and Saporin) are quite stable. However, we have found that many things happen in laboratories and some of them can impact stability. Probably the most severe is the loss of sterility. In that case, over time at room temperature or at 4°C, bacteria can grow on this rather excellent "medium." This would cause inactivation. Because many laboratories, due to molecular biology work, have high levels of resident bacteria, we prefer to emphasize playing it safe.

Even if saporin is a stable protein, it is a protein and can suffer denaturation. This occurs more rapidly at room temperature than at 4°C, and hardly at all in the frozen state (really, it is stable for years when stored at –80°C). The maintenance of precise activity is of extreme importance to our customers who use these materials *in vivo* (their assays are very sensitive), and so we choose to advise the most conservative course.

Q: I understand that theoretically only one molecule of Saporin taken up by a cell is enough to induce cell death. I have been looking for literature on this topic but have not come across anything.

A: Definitely theoretical. The only article that we know of that states anything close to that is: Yamaizumi *et al* (1978) One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell* 15(1): 245-250.

As you can see, this article speaks to the enzymatic chain of diphtheria toxin, which has a slightly different mechanism of action for shutting down protein synthesis, but otherwise is similar to saporin. In fact, we test all sorts of toxins against cells in controlled conditions, and we have only one candidate that is in this range; all the rest are orders of magnitude away. It takes more than thousands per cell. Another question would be: how many actually get in?

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: PHYSIOLOGY
MALARIA
CELLS
STAINING
NITRATE

Answer: CAMILLO GOLGI

WINNERS: Douglas J Taatjes, Univ Vermont * Ching-Hui Yang, Univ Texas Health Ctr * Susan Grand, Univ North Carolina * Thad Lindsay, Univ Minnesota * Alfia Kaibullina, Natl Inst of Health * Linda Rogers, Tulane Regional Primate Res Ctr * Catherine Ulibarri, Washington State Univ * Audrey Vasauskas, Panacea Pharmaceuticals Inc * Sheela Vyas, INSERM U497 ENS * Lea Chaskiel, CNRS UMR 1244 Inst Magendie * Jean-Bernard Dietrich, INSERM U338 Ctr Neuroch * Marie-Christine Lombard, INSERM U378 Inst. Magendie * April Garcia, Univ Texas Health Cntr Dept of Pharm * Jane Quirk, NINDS/DMNB * Jerry Keith, NIDDK * Joseph Menonna, E. Orange VA Med Center * Robert Speth, Univ Mississippi

Camillo Golgi was born at Corteno July 7, 1843. He studied medicine at the University of Pavia. Golgi was appointed to the Chair for General Pathology in 1881. He became interested in the investigation of the causes of malaria and he must be credited for having determined the three forms of the parasite and the three types of fever. After prolonged studies he found a way of photographing the most characteristic phases in 1890.

The work of greatest importance that Golgi carried out was a revolutionary method of staining individual nerve and cell structures, which is referred to as the black reaction. This method uses a weak solution of silver nitrate and is particularly valuable in tracing the processes and most delicate ramifications of cells.

Golgi shared the Nobel Prize for Physiology or Medicine 1906 with Santiago Ramón y Cajal for their work on the structure of the nervous system.

Effects of IB4-SAP on Bladder Overactivity

(continued from page 1)

suppressed bladder overactivity induced by intravesical capsaicin or ATP without affecting normal micturition. These results indicate that elimination of IB4-binding afferents by IB4-SAP is effective for the treatment of bladder overactivity induced by bladder irritation. Thus, targeting IB4-binding, presumed non-peptidergic afferent pathways, which are sensitive to capsaicin and ATP, may be an effective treatment for overactivity and/or visceral pain responses in the bladder.

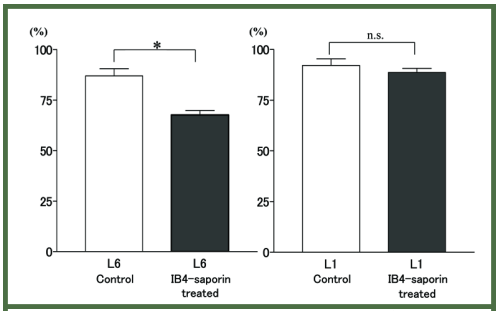

Fig. 2 The area positively stained with IB4 in the lamina II of the dorsal horn in the L1 and L6 spinal cord from control and IB4-saporin-treated rats (3 weeks after the treatment). Data are expressed as percentages of the positively stained area in total lamina II area (100%). *P< 0.01 (unpaired t-test)

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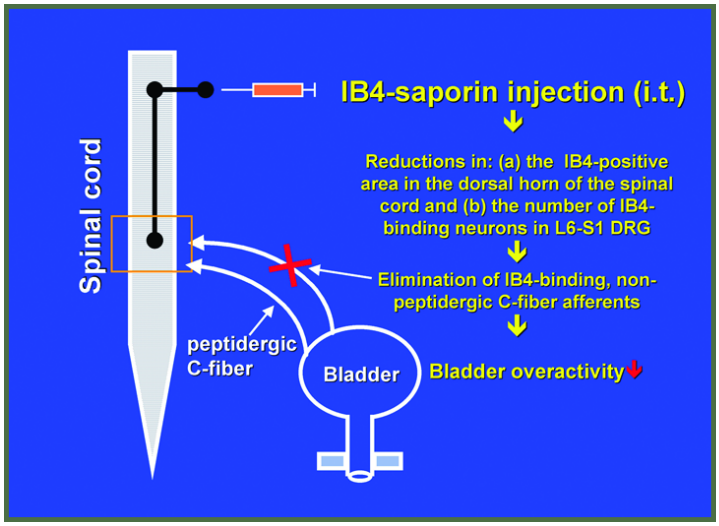


Upcoming Events

Society for Neuroscience
October 23-27, 2004
San Diego, CA
Booth 3117

The American Society for Cell Biology
December 4-8, 2004
Washington, DC
Booth 403

The Society for Neuroscience meeting comes to San Diego October 23-27

This year's SfN meeting is in our home town of San Diego. This gives more of our staff the opportunity to attend the meeting and interact with our customers.

The ATS Product Managers will be in the booth at the meeting to answer any questions you may have about our products. From left to right, Matthew Kohls, Brian Russell, and Leonardo Ancheta.

Dr. Doug Lappi, President and Chief Scientific Officer, will also be on hand to discuss custom saporin conjugations, potential collaborative efforts, and new products in the ATS pipeline.

Stop by Booth #3117 and pick up your free tube opener. You can also get a copy of our latest product catalog and 2005 calendar.

We look forward to seeing you!

Note: On Sunday, October 24, representatives from our distributor in Japan, Funakoshi Co., Ltd., will be with us in our booth to meet with any of our Japanese customers.

Targeting Tools: Featured Products

We are pleased to present two new targeted toxins, a new second immunotoxin, and six new antibodies.

With our expanded line of immunotoxins, controls, antibodies, and custom conjugates, ATS can meet all your targeting needs!

CCK-SAP *Cat. #IT-31*

This new targeted toxin is a chemical conjugate of sulfonated cholecystokinin (CCK) and the ribosome-inactivating protein, saporin. CCK is widely distributed in the central nervous system and the gastrointestinal tract. CCK-SAP is an effective tool to study eating disorders and pain transmission. CCK-SAP eliminates cells recognizing sulfonated CCK. All other cells are left untouched.

CCK-SAP

IT-31-25 . . . . . .25 micrograms
IT-31-100 . . . . . .100 micrograms
IT-31-250 . . . . . .250 micrograms

Kits are available and include equal amounts of saporin and control conjugate

NPY-SAP *Cat. #IT-28*

NPY-SAP is a chemical conjugate of neuropeptide Y (NPY) and the ribosome-inactivating protein, saporin. NPY is the most abundant neuropeptide in the brain and is involved in many processes from prenatal to mature animals. It promotes the proliferation of postnatal neuronal precursor cells and exhibits a diverse range of important physiologic activities, including effects on psychomotor activity, food intake, regulation of central endocrine secretion, and potent vasoactive effects on the cardiovascular system. Targeting cells expressing the NPY receptor makes this toxin an effective tool for studying the nervous and cardiovascular systems.

NPY-SAP

IT-28-25 . . . . . .25 micrograms
IT-28-100 . . . . . .100 micrograms
IT-28-250 . . . . . .250 micrograms

Kits are available and include equal amounts of saporin and control conjugate

New Antibodies

AB-15AP	Saporin, affinity purified goat polyclonal	.50 µg
AB-N29	GABA Transporter-1 (GAT-1), rabbit polyclonal	.100 µl
AB-N30	GABA Transporter-2 (GAT-2), rabbit polyclonal	.100 µl
AB-N31	GABA Transporter-3 (GAT-3), rabbit polyclonal	.100 µl
AB-N32	Metabotrophic Glutamate Receptor 2 (mGluR2), rabbit polyclonal	.100 µg
AB-N33AP	NK-1r, affinity purified rabbit polyclonal	.50 µg

Anti-M-ZAP *Cat. #IT-30*

Anti-M-ZAP is another product in the line of second immunotoxins. Second immunotoxins are conjugations of a secondary antibody to the ribosome-inactivating protein, saporin. The second immunotoxin uses the secondary antibody to "piggyback" onto YOUR primary antibody in order to evaluate the ability of the primary antibody to internalize. Anti-M-ZAP is a chemical conjugate of affinity-purified goat anti-mouse IgM and the ribosome-inactivating protein, saporin. Anti-M-ZAP uses your primary mouse monoclonal IgM antibody to target and eliminate cells. It's great for screening!

IT-30-25. . . . . 25 micrograms
IT-30-100. . . . . 100 micrograms
IT-30-250. . . . . 250 micrograms

Kits are available and include an equal amount of control conjugate

Visit the ATS website for a complete list of products.

"Hmm. I don't know what I think about that guy Hess."

(Psst. It's a clue from Gangsta!)

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.

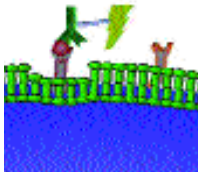
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

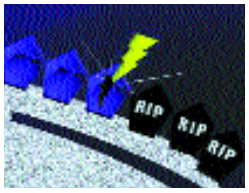
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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 form the name of this noted scientist.

CHEAPONNILED

NEWRIN

CHICSYP

HOTPALMOGHOLY

IRONPIRATES

Is it really a matter of Mind over manners?

Arrange the circled letters to form the name of this noted scientist, as suggested by the above clue.

Answer:

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by November 30, 2004.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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