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Volume 4, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Deep Lumbar Neurons Control Ejaculation

Contributed by William Truitt, PhD, Department of Cell Biology, Neurobiology, and Anatomy, Neuroscience Graduate Program, University of Cincinnati, College of Medicine, Post Office Box 670521, Cincinnati, OH 45267-0521, USA.

Previously we demonstrated the existence of a spinothalamic pathway in the male rat where neural activation is specifically induced by ejaculation.[1] This pathway includes the parvocellular subparafascicular thalamic nucleus (SPFp) and projection neurons located in the lumbar spinal cord. These lumbar spinothalamic (LSt) neurons are located in lumbar segments 3 and 4 and can be identified by neural peptide content including galanin. To test the behavioral significance of these lumbar spinothalamic (LSt) neurons, effects of lesions of the LSt population on sexual behavior were investigated.[2]

Methodology: LSt neurons are sparsely distributed lateral to the central canal in lamina X and in the medial portion of lamina VII of L3 and L4, and are difficult to lesion by traditional methods. We thus identified a membrane target located on the LSt neurons. It was demonstrated that 93% of LSt neurons express neurokinin-1 receptor (NK-1r) and conversely 85% of NK-1r containing cells in the area surrounding the central canal at L3-4 express galanin (Fig. 1). We therefore used the targeted

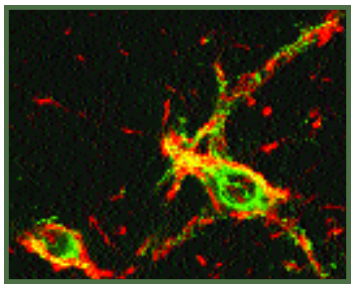

Fig. 1
Confocal image of LSt neurons demonstrating co-expression (yellow) of galanin (green) and NK-1r (red).

toxin SSP-SAP (a stable form of Substance P conjugated to Saporin) for specific ablation of LSt cells. A series of 6-8 bilateral 1-μl SSP-SAP injections (4 ng) was infused into the L3-4 spinal cord at the location of the LSt cells in male rats. Control animals were injected with non-conjugated equimolar concentrations of SAP (Saporin). Sexual behavior was first tested 10 days after surgery, and during 5 subsequent twice-weekly tests. Following the final behavior test, animals were perfused and spinal cord tissue was immuno-processed for galanin, NK-1r, or neuronal marker N (NeuN). Labeled cells were counted in a standard area surrounding the central canal of L3-4 sections representative of the location of LSt cells.

(continued on page 6)

2002 Society for Neuroscience ATS Award Winner

Congratulations to Dr. William Truitt for winning this year's award for best poster. Pictured here with Dr. Lique Coolen and Dr. Doug Lappi, he shows off his prizes: an ATS cap, mug, and polo shirt. The competition was stiff this year—57 abstracts utilized ATS products in a variety of interesting and innovative reports. For more info about the winning poster and a brief summary of some other top contenders, see the article on page 2.

SP-SAP as a Chronic Pain Therapeutic: An Update

Advanced Targeting Systems continues to search for funding to develop Substance P-Saporin (SP-SAP) as a chronic pain therapeutic. In May of last year we decided to seek venture capital funding and began traveling to make presentations to potential investors.

The response from most everyone we spoke to was positive and they thought the potential of SP-SAP was tremendous. However, the economy and prior portfolio commitments kept them from investing.

So, where does that leave SP-SAP now? Over the next couple of months we will be completing dose-ranging and preliminary safety studies in a second animal. The FDA will require two GLP toxicology studies—one in rat and one in dog—prior to granting approval to begin clinical trials in humans. Costs to take SP-SAP to that point are in the $2.5 million range.

Up to this point, all of the SP-SAP development costs have been covered by grants from the National Institutes of Health (NIH) and ATS research and development funds. We recently applied for a drug development grant from the NIH, National Institute of Mental Health, that may provide substantial support, if approved. We will hear about the probability of this funding in May or June of this year.

In the meantime, we continue to talk to potential investors and corporate partners to search for a quicker way to bring SP-SAP to the many people suffering from chronic pain. It's an important effort that receives a lot of our time and attention.

President Doug Lappi takes the front seat accompanied by the ATS staff. L to R: Leo Fernandez, Kristina Majer, Matthew Kohls, Denise Higgins, Brian Russell, and Cynthia Wilson.

Scientists Present Innovative Results Using ATS Products

This year's Society for Neuroscience meeting was a great success in the wealth of posters presented using ATS products. Our cover article and award-winning poster were presented by Dr. William Truitt of the University of Cincinnati.

The subject of his abstract is expanded in this issue's cover article.

LESIONS OF SPINOTHALAMIC NEURONS IN LUMBAR SPINAL CORD DISRUPT EJACULATORY REFLEXES IN MALE RATS. *(SSP-SAP)*
W.A. Truitt*; K.E. McKenna; L.M. Coolen (Cell Biol Neurobiol & Anat, Univ Cincinnati)

Matthew Kohls and Doug Lappi in the ATS booth at the Society for Neuroscience meeting in Orlando, Florida.

The competition for this year's ATS Poster of the Year award made choosing a single winner a difficult decision. Here are some of the top contenders:

CHANGES IN ROSTRALVENTROMEDIAL MEDULLA (RVM) NEURONS AFTER THE SELECTIVE LOSS OF MU-OPIOID RECEPTOR EXPRESSING CELLS *(Dermorphin-SAP)*
I.D. Meng*; I. Harasawa; J. Lai; F. Porreca; H.L. Fields (Neurology, UCSF)

SELECTIVE LESION OF NEUROPEPTIDE Y (NPY)-RECEPTOR NEURONS IN HYPOTHALAMUS INHIBITS FOOD INTAKE AND REDUCES BODY WEIGHT IN RATS *(Avidinylated-SAP)*
S.T. Sheriff*; C. Xiao; W.T. Chance; J.W. Kasckow; A. Balasubramaniam (Dept Surg, Univ Cincinnati Med Ctr)

LACK OF CAPSAICIN (CAP)-EVOKED SENSITIZATION FOLLOWING SP-SAP TREATMENT IS NOT ATTRIBUTED TO DECREASED CAP-EVOKED EXCITATION *(SP-SAP)*
S.G. Khasabov*; S.D. Rogers; J.R. Ghilardi; C.M. Peters; P.W. Mantyh; D.A. Simone (Preventive Sciences, Univ Minnesota)

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Basal forebrain cholinergic neurons are necessary for estrogen to enhance acquisition of a delayed matching-to-position T-maze task.

Gibbs RB
Horm Behav 42(3):245-257, 2002

The author investigated the role of cholinergic neurons of the basal forebrain in cognitive function using a long-term hormone replacement model in rats. Septal infusions of either 1.0 μg or 0.22 μg 192-Saporin (Cat. #IT-01) prevented the therapeutic effects of hormone replacement on cognitive function.

Long-term plastic changes in galanin innervation in the rat basal forebrain.

Hartonian I, Mufson EJ, de Lacalle S
Neuroscience 115(3):787-795, 2002

One hallmark of Alzheimer's disease is the hyperinnervation of surviving cholinergic basal forebrain neurons with galanin-IR fibers. This may exacerbate the cholinergic deficit. The authors injected 192-Saporin (140 nl of 0.075 mg/ml, Cat. #IT-01) into the diagonal band of Broca of rats. An increase in galanin immunoreactivity was observed as early as 1 hour post-injection, and persisted as long as 6 months.

Effects of cholinergic lesions produced by infusions of 192 IgG-saporin on glucocorticoid receptor mRNA expression in hippocampus and medial prefrontal cortex of the rat.

Helm KA, Han JS, Gallagher M
Neuroscience 115(3):765-774, 2002

The authors investigated the loss of cholinergic support from the basal forebrain, a hallmark of aging, on glucocorticoid receptor mRNA expression in various target sites. 192-Saporin (Cat. #IT-01) was injected into either the nucleus basalis magnocellularis/substantia innominata (0.2 μl of 0.25 mg/ml) or the medial septum/vertical limb of the diagonal band (0.3 μl of 0.25 mg/ml). Treated rats sustained a significant decrease in glucocorticoid receptor mRNA levels in the hippocampus and medial prefrontal cortex.

Changes in activity and expression of phosphofructokinase in different rat brain regions after basal forebrain cholinergic lesion.

Zeitschel U, Schliebs R, Rossner S, Bigl V, Eschrich K, Bigl M
J Neurochem 83(2):371-380, 2002

The authors used intraventricular injections of 4 μg of 192-Saporin (Cat. #IT-01) in rats to investigate whether impaired cholinergic transmission may cause metabolic changes. Although the results demonstrate an initial increase in a cortical glucose metabolic marker, this increase was transient. The authors conclude that cholinergic systems do not control cortical glucose metabolic mechanisms affected by Alzheimer's disease.

Early neonatal 192 IgG saporin induces learning impairments and disrupts cortical morphogenesis in rats.

Ricceri L, Hohmann C, Berger-Sweeney J
Brain Res 954(2):160-172, 2002

Previous data have shown that cholinergic lesions on postnatal day (pnd) 7 in rats produce learning impairments on pnd 15. Using 0.2 μg injections of 192-Saporin (Cat. #IT-01) into the lateral ventricles, the authors investigated the effect of lesioning animals at pnd 1 and 3. The treated animals demonstrated sex-specific deficits in some cognitive behaviors, as well as changes in neurochemistry and cortical organization.

Selective lesions of basal forebrain cholinergic neurons produce anterograde and retrograde deficits in a social transmission of food preference task in rats.

Vale-Martinez A, Baxter MG, Eichenbaum H
Eur J Neurosci 16(6):983-998, 2002

Injections of 0.2 μg 192-Saporin (Cat. #IT-01) were made into either the medial septum/vertical limb of the diagonal band (MS/VDB), or the nucleus basalis magnocellularis/substantia innominata (NBM/SI) of rats. MS/VDB lesions had no effect on anterograde memory, while NBM/SI lesions strongly impaired immediate and 24-hour retention. In contrast, MS/DVB lesions produced significant memory deficits in a long-delay retrograde memory test.

Substance P-saporin lesion of neurons with NK1 receptors in one chemoreceptor site in rats decreases ventilation and chemosensitivity.

Nattie EE, Li A
J Physiol 544(Pt 2):603-616, 2002

The authors injected 0.1 pmol SP-SAP (Cat. #IT-07) into the retrotrapezoid nucleus/parapyramidal region of rats. The lesioned animals demonstrated hypoventilation while at rest, decreased response to high CO_2 levels, and a tendency to sleep less.

Efferent projections from the striatal patch compartment: anterograde degeneration after selective ablation of neurons expressing mu-opioid receptor in rats.

Tokuno H, Chiken S, Kametani K, Moriizumi T
Neurosci Lett 332(1):5-8, 2002

Taking advantage of the fact that neurons in patch compartments of the striatum express μ-opioid receptors, the authors injected 8.5 ng of dermorphin-SAP (Cat. #IT-12) into the striatum of rats. This lesion produced a degeneration of patch neurons as well as anterograde degeneration of efferent fibers from patch compartments, allowing further elucidation of the functional organization of the striatum.

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

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Targeting Topics: Recent Scientific References

(continued from page 3)

Selective immunolesioning of the basal forebrain cholinergic neurons in rats: effect on attention using the 5-choice serial reaction time task.
Risbrough V, Bontempi B, Menzaghi F
Psychopharmacology 164:71-81, 2002

The authors used 0.067 μg injections of 192-Saporin (Cat. #IT-01) into the nucleus basalis magnocellularis to investigate attentional performance in rats. The treated animals exhibited a very specific subset of attentional deficits, many centered around increased difficulty completing tasks in the presence of distractions.

Spinal neurons that possess the substance P receptor are required for the development of central sensitization.
Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA
J Neurosci 22(20):9086-9098, 2002

Using 5 x 10^{-5} M intrathecal injections of SP-SAP (Cat. #IT-07) the authors examined the role of SPR-expressing neurons in modulation of pain and hyperalgesia. Treated animals exhibited highly attenuated sensitization to stimuli after capsaicin treatment as compared to controls, but normal responses in the absence of capsaicin.

***In vivo* labeling of rabbit cholinergic basal forebrain neurons with fluorochromated antibodies.**
Hartig W, Varga C, Kacza J, Grosche J, Seeger J, Luiten PG, Brauer K, Harkany T
NeuroReport 13(11):1395-1398, 2002

To investigate *in vivo* labeling of p75 low-affinity neurotrophin receptor the authors conjugated Cy3 to ME20.4 (Cat # ABN07) and performed either unilateral or bilateral icv injections in rabbits. The antibody labeled only cholinergic neurons demonstrating its potential as a p75 marker.

Reactivity to object and spatial novelty is normal in older Ts65Dn mice that model Down syndrome and Alzheimer's disease.
Hyde LA, Crnic LS
Brain Res 945:26-30, 2002

The authors hypothesized that a mouse model for Down syndrome may show some of the same cognitive deficits exhibited by rats lesioned with 192-Saporin (Cat. #IT-01), which eliminates cholinergic cells in the basal forebrain. The results suggest that in this Down syndrome model, cell loss has a much greater cognitive effect if it happens early in development as opposed to in adulthood.

Motoneuron-derived neurotrophin-3 is a survival factor for PAX2-expressing spinal interneurons.
Bechade C, Mallecourt C, Sedel F, Vyas S, Triller A
J Neurosci 22(20):8779-8784, 2002

In the rat, half of motoneurons die between embryonic day 15 and postnatal day 1. Programmed cell death of interneurons is not as well characterized. The authors cultured explants of brachial neural tubes from rat embryos in the presence of 200 ng/ml of 192-Saporin (Cat. #IT-01). Although 192-Saporin had no direct effect on interneurons in culture, elimination of p75-neurotrophin receptor-expressing neurons caused the interneurons to die.

Effect of 192 IgG-saporin on circadian activity rhythms, expression of P75 neurotrophin receptors, calbindin-D28K, and light-induced Fos in the suprachiasmatic nucleus in rats.
Beaule C, Amir S
Exp Neurol 176(2):377-389, 2002

The authors used bilateral icv injections of 200 ng of 192-Saporin (Cat. #IT-01) to investigate the contribution of $p75^{NTR}$-expressing neurons to the determination of a circadian rhythm. The data show that $p75^{NTR}$-expressing neurons are not essential for this process.

Rivastigmine antagonizes deficits in prepulse inhibition induced by selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis.
Ballmaier M, Casamenti F, Scali C, Mazzoncini R, Zoli M, Pepeu G, Spano PF
Neuroscience 114(1):91-98, 2002

The authors injected 300 nl of 400 ng/μl 192-Saporin (Cat. #IT-01) bilaterally into the nucleus basalis magnocellularis of rats, then treated the lesioned animals with rivastigmine, a cholinesterase inhibitor. Animals treated with rivistagmine exhibited raised levels of cortical acetylcholine, in contrast to undetectable acetylcholine levels in lesioned animals not treated with rivastigmine.

Mnemonic deficits in animals depend upon the degree of cholinergic deficit and task complexity.
Pizzo DP, Thal LJ, Winkler J
Exp Neurol 177:292-305, 2002

In this study, the authors compared icv and intraparenchymal injections of 192-Saporin (Cat. #IT-01, 3.3 μg and 450 ng, respectively). While a similar reduction in choline acetyltransferase activity was observed with each strategy, and performance in certain allocentric tasks was similar, an egocentric task showed a marked difference between the two groups.

Targeting Talk: *Custom Conjugations*

by Dr. Douglas A. Lappi

Part II: Targeted Toxins (Peptide-Saporin conjugates)

Q: How do I know my peptide will work as a targeted toxin?

A: There is a rich literature that demonstrates peptides can usher proteins that inhibit protein synthesis (such as saporin) into cells and result in cell death. Peptide ligands that bind to the cell surface (i.e., to their receptors) are internalized—in fact, often quite rapidly. As with all saporin cytotoxins, internalization is necessary; antagonists that do not internalize would not be expected to be proper agents for a saporin cytotoxin. All agonists that have a decent affinity and internalization rate should work as a targeted toxin.

Q: How much peptide do I need to provide for a custom conjugation?

A: Actually, we will consult with you on the structure-function properties of your peptide. We will need to synthesize an entirely new peptide for conjugation to saporin. We will, in collaboration with you and/or by examination of the literature, design the new peptide and have it synthesized (it is our plan to have peptide synthesis capabilities in 2003). We pass the price of the peptide, usually quite reasonable, directly to you without any increase.

Q: How much of the saporin conjugate will that give me?

A: We strive to give you 2-3 mg of peptide-saporin cytotoxin. These often are effective in the nanogram range.

Q: What is the ratio of saporin to antibody?

A: We synthesize the conjugate such that there is one mole of saporin per mole of peptide.

Q: What quality control is involved?

A: We monitor the reactions and purification by several means. The product is confirmed by gel electrophoresis. A data sheet will be provided when we ship the immunotoxin to inform you of final average molecular weight.

Q: What is the cost of a custom targeted toxin preparation? How long will it take to complete?

A: The standard cost of a peptide-saporin conjugation is US$3500.00, plus the price of the peptide. From the time we receive the peptide to the time we ship out the finished targeted toxin is 2-3 weeks.

Second Immunotoxins are most effective in determining the specificity of your targeting agent (peptide, antibody, ligand) and suitability for conjugation as a primary immunotoxin.

ATS recommends that you order a custom conjugation of your antibody to saporin when the *in vitro* results confirm the desired specificity.

Avidinylated-SAP (Cat. # IT-09)
a conjugate of avidin
and the ribosome-inactivating protein, saporin
Converts biotinylated materials into targeted toxins.

Hum-ZAP (Cat. # IT-22)
a conjugate of affinity purified goat anti-human IgG
and the ribosome-inactivating protein, saporin
Cells that internalize your human monoclonal antibody will be eliminated.

Mab-ZAP (Cat. # IT-04)
a conjugate of affinity purified goat anti-mouse IgG
and the ribosome-inactivating protein, saporin
Cells that internalize your mouse monoclonal antibody will be eliminated.

Rab-ZAP (Cat. # IT-05)
a conjugate of affinity purified goat anti-rabbit IgG
and the ribosome-inactivating protein, saporin
Cells that internalize your rabbit polyclonal antibody will be eliminated.

Upcoming Events

Experimental Biology
April 11-15, 2003
San Diego, California
Booth #545

Society for Neuroscience
November 8-12, 2003
New Orleans, Louisiana

Deep Lumbar Neurons Control Ejaculation

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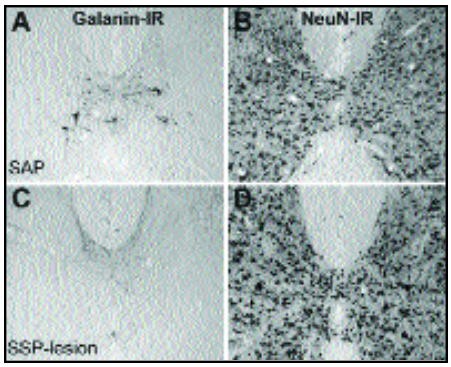

Fig. 2 Photomicrographs of galanin-IR (A, C) and NeuN-IR (B, D) in L4 of a representative SAP(Aand B) and SSP-les (C and D) animal. Galanin is visibly reduced, while NeuN labeling shows no reduction in SSP-les males.

SSP-SAP treatment resulted in two groups, rats with complete lesions of LSt neurons (SSP-les; defined by less than 1/3 of the number of LSt cells observed in untreated rats), and rats with incomplete or misplaced lesions (SSP-il). No lesions were present in SAP-treated males (SAP). SSP-les animals had fewer galanin-immunoreactive (IR; Fig. 2) and NK-1r-IR neurons than SSP-il or SAP animals. Despite the severe reduction in LSt neurons in SSP-les rats, there was no overall reduction in numbers of NeuN-IR cells (Fig. 2), indicating the selectivity of the lesions. Furthermore, the lesions were restricted to the area surrounding the central canal and did not affect the number of NK-1r expressing neurons in the dorsal horn.

LSt lesions had dramatic effects on sexual behavior. Lesions completely disrupted display of ejaculatory behavior in SSP-les males and seminal plugs were uniformly absent upon examination of the female partner throughout the testing session. In contrast, SSP-il and SAP males continued to ejaculate regularly following surgery. Furthermore, ablation of LSt neurons selectively blocked ejaculatory behavior without affecting other components of sexual behavior. SSP-les animals did not differ from the SSP-il or SAP animals in number of mounts or intromissions (Fig. 3).

It is well established that ejaculation is a reflex and ejaculatory reflexes remain intact when control by supraspinal sites is eliminated, suggesting the existence of a spinal ejaculation generator. However, the anatomical site of such an ejaculation generator is yet unknown. Our data suggest that LSt cells form a critical component of the ejaculation generator.

To further test this hypothesis, effects of LSt cell lesions on expression of ejaculatory reflexes were investigated. We utilized a model that reliably mimics ejaculatory responses in anesthetized, spinalized male rats, i.e. the urethrogenital reflex model.[3] In short, urethral stimulation induces characteristic

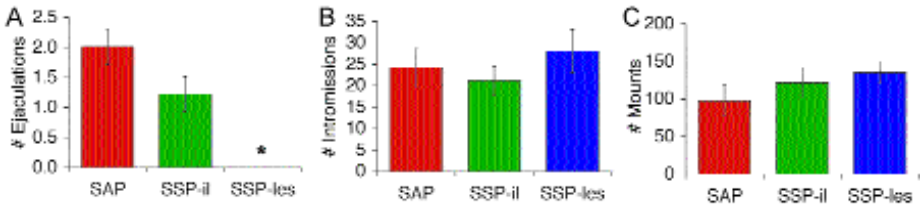

Fig. 3 LSt neuronal lesioning abolishes ejaculatory behavior but has no effect on number of mounts or intromissions. Presented here are group means ± SEM of numbers of ejaculations (A), intromissions (B), and mounts (C).

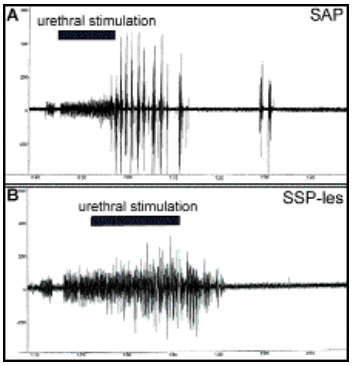

Fig. 4 EMG of BCM bursting in urethral genital reflex model. Characteristic bursting patterns evoked by urethral distention observed in SAPcontrol rats (A) are disrupted in LSt lesioned rats (B).

organized bursting patterns of peripheral nerves, smooth muscles and striated muscles, including the bulbocavernosus muscle (BCM). In the present study, urethrogenital reflexes were investigated in males with complete and incomplete LSt lesions, and SAP controls. LSt cell lesions were performed using SSP-SAP as described above and BCM bursting patterns were monitored. LSt lesions dramatically disrupted ejaculatory reflexes. In SSP-les males, urethral stimulation resulted in asynchronous, low amplitude, short bursts of the BCM (Fig. 4B). In contrast, urethral stimulation resulted in a characteristic organized and synchronized series of 8 to 12 BCM bursts in SSP-il and SAP control males (Fig. 4A). Together these data demonstrate that LSt cells play a pivotal role in control of ejaculatory reflexes and behavior.

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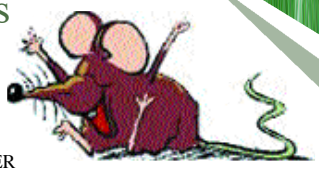
2. Truitt WA, Coolen LM (2002) *Science* 297(5586):1566-1569.
3. McKenna KE, Chung SK, McVary KT (1991) *Am J Physiol* 261(5 Pt 2) R1276-R1285.

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: CELLULAR DORSAL NEONATAL HYBRID LOWER

Answer: What the cloners said when they entered the barnyard — WELLHELLO, DOLLY!

WINNERS: Dr. Shannon Burgess, Univ Arizona Health Science Center * Kristen Phend, Univ North Carolina, Cell Biology & Anatomy * Asha Sahu, Univ Maryland, Pharmacology * Sukie Rayas, Univ Virginia, Psychobiology * Brian Bacskai, Ph.D., Massachusetts General Hospital, Neurology * Teresa Milner, Cornell Univ, Weill Medical College

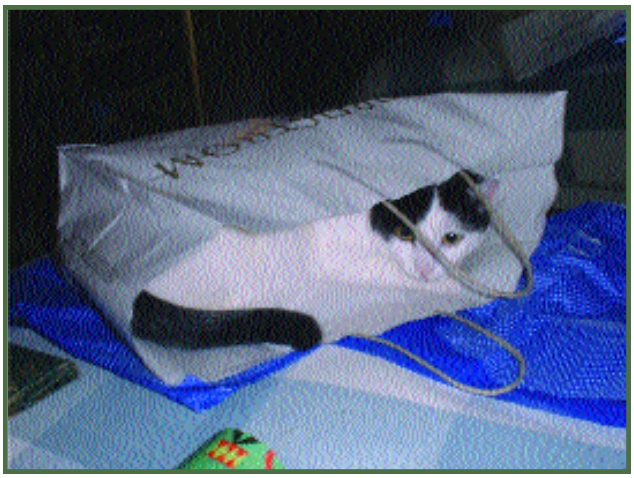
Targeting Tools: Featured Products

Dermorphin-SAP

Even though it only became available recently, Dermorphin-SAP (Cat. #IT-12) has already begun to help researchers unlock the mysteries of brain function. Dermorphin-SAP is a chemical conjugate between an analog of the frog skin peptide dermorphin and the ribosome-inactivating protein saporin. Dermorphin, a peptide of seven amino acids characterized by Ersparmer and his colleagues,[1] shows excellent affinity for the mu-opioid receptor, with a much lower affinity for the delta receptor and virtually no binding to the kappa receptor.[2] These properties make it an excellent targeting vehicle for cells that express the mu receptor.

Porreca and his group have used Dermorphin-SAP to characterize the descending pathway that transmits the signal for hyperalgesia and allodynia. In 2001, these workers published that injection of Dermorphin-SAP in the rostroventromedial medulla caused a specific loss of mu receptor neurons. In behavioral tests, rats treated with the material failed to exhibit the expected increase in sensitivity to non-noxious mechanical or noxious thermal stimuli applied to the paw after spinal nerve ligation,[3] the widely-accepted model of neuropathic pain. They went on to extend these studies in 2001 and determined that, while establishment of the neuropathic state is not dependent on these neurons, maintenance is.[4] Their work supports the idea that enhanced afferent discharge is an important component of the neuropathic state at both the initial stage and at subsequent stages after injury.

At the Society for Neuroscience meeting in Orlando last year, the group of Howard Fields, in collaboration with Porreca's group, presented data that the neurons eliminated by Dermorphin-SAP show properties of On and Off cells (see page 2). The former have been postulated to be important for nociceptive transmission, while the latter are inhibitory neurons.[5] Both are sensitive to morphine antagonists, and they express the mu receptor. Disruption of these neurons would be expected to cause changes in maintenance of neuropathic pain.



There's no mystery to unlock here.
Gangsta finds more fun with the bag than the gift!

Dermorphin-SAP has made a large contribution in the understanding of this extremely complex system. Perhaps Dermorphin-SAP can help you in dissecting the biological system with which you work.

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3. Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan TP Jr, Ossipov MH, Lappi DA, Lai J (2001) *J Neurosci* 21(14):5281-5288.
4. Burgess SE, Gardell LR, Ossipov MH, Malan TP, Jr., Vanderah TW, Lai J, Porreca F (2002) *J Neurosci* 22(12):5129-5136.
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Fluorescent Conjugates

FL-01 Cy3-labeled 192-IgG

Specificity: rat $p75^{NTR}$ cells

Applications: immunofluorescence; immunocytochemistry

Reference: Wu *et al*. (2000) *J Neurosci* 20(10):3900-3908.

FL-02 FITC-labeled anti-SAP

Specificity: native and recombinant saporin

Applications: FACS analysis

Reference: Gerashchenko *et al*. (2001) *J Neurosci* 21(18):7273-83.

FL-03 Alexa488-labeled 192-IgG

Specificity: rat $p75^{NTR}$ cells

Applications: spectroscopy; anisotropy; microscopy; radioligand competition binding

Reference: Harikumar *et al*. (2002) *J Biol Chem* 277(21):18552-18560.

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.

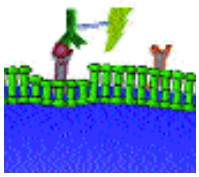
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

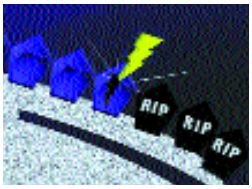
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



Cells which 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 used in science.

TERSEWN

FORPLIED

CROMELLUA

ITAPSYTICS

NICEJOINT

WHAT THE STUDENT TURNED IN TO WHEN HE TRIED TO FINISH THE CHEMISTRY EXPERIMENT

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

Answer:

A ____ of "____ ____"!

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by February 28, 2003.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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Apr-May-Jun 2003
Volume 4, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Biotinylated targeting: A viable option?

For the IB4-SAP illustration (Figure 1), our thanks to Christopher N. Honda, Ph.D., Associate Professor, Department of Neuroscience, University ofMinnesota, 6-145 Jackson Hall, 321 Church Street, Minneapolis, MN 55455

This issue of our quarterly newsletter addresses the use of biotinylated materials in targeting. ATS is now offering a biotinylation service (see p. 7) that gives scientists options for expanding their experimental capabilities. In this first example, Dr. Honda presents data using the targeted toxin IB4-SAP and biotinylated IB4. His laboratory used IB4-SAP to investigate the role of IB4-binding neurons in nociception.

Figure 1 shows ipsilateral dorsal root ganglion (DRG) neurons 3 days after a sciatic nerve injection of IB4-SAP (A, B, E, F), unconjugated IB4 (C), or unconjugated SAP (D). Anti-BSI (*Bandaireae simplicifolia I*) was used to label neurons that internalize IB4 after a sciatic nerve injection (A and C), and biotinylated IB4 was used to label all IB4-binding neurons regardless of internalization (E). The images were adjusted for contrast and brightness, and double-labeled images were pseudocolored and digitally merged (Adobe Photoshop).

(A, B) IB4-SAP injected into sciatic nerve was detected in DRG neurons 3 days later using anti-BSI (A) or anti-SAP (B). The pattern of staining of the two antisera in neurons was similar. The staining was diffusely distributed throughout the cytoplasm or aggregated in the center of the cell. Labeled neurons had irregular eccentric nuclei and irregular cell perimeters.

(C) Unconjugated IB4 injected into sciatic nerve was detected in DRG neurons 3 days later using anti-BSI. Anti-BSI staining was seen near the cell perimeter and in well-defined puncta surrounding the nuclei of neurons.

(D) Unconjugated SAP injected into sciatic nerve was not detected in DRG.

(E) Double labeling with anti-SAP (represented in red) and biotinylated IB4 (represented in green) after IB4-SAP injection.

The majority of anti-SAP-stained neurons were also labeled by biotinylated IB4, as indicated by the presence of red and green puncta in the same neurons. IB4-binding neurons that did not internalize IB4-SAP are shown in green. The arrow indicates an example of disfigured neurons that were positive only for anti-SAP.

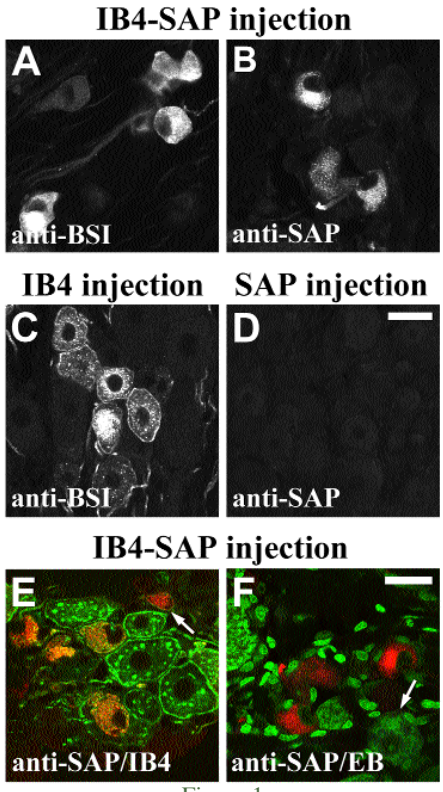


Figure 1

(F) Double labeling with anti-SAP (represented in red) and ethidium bromide (EB); represented in green). The neurons containing IB4-SAP were not stained by EB, as indicated by the lack of overlap of red and green pseudo-colored neurons. The arrow indicates an example of the normal appearance of EB staining in sensory neurons. Scale BARS=20 mm.[1]

This figure demonstrates the internalization of IB4-SAP and its selectivity for IB4-binding neurons.

(continued on page 6)

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Funding Awarded for SBIR Phase II Grant

On February 1, 2003 ATS was awarded an $800,000 grant from the National Institutes of Health, National Institute of Mental Health. This three-year grant will support the creation of monoclonal antibodies to the extracellular domains of G protein-coupled receptors. These receptors play an important role in all aspects of human health, reflecting their widespread presence in the human physiology, especially the nervous systems.

The five somatostatin receptors have been selected as the first candidates for antibody development because of their importance in neuronal systems, biology, and nervous system pathologies. ATS plans to create recombinant antibodies to these receptors. The best of these antibodies will be modified to demonstrate that biologically active molecules can be inserted into cells that express the somatostatin receptors (SSTRs).

There are no commercially available monoclonals to extracellular domains of SSTRs. The availability of new monoclonal antibodies would be an outstanding contribution to scientific discovery, e.g. the pain research field.

The innovations in this project are several. The development of reagents for insertion of biologically active cells will be important for developing new treatments for neurodegenerative diseases and pain pathologies, among others. Despite widespread use of recombinant antibodies in cancer biology with their ability to be modified through the construction of fusion proteins, they are still extremely rare and make this a novel approach.

Kristina Majer and Matthew Kohls do a test run on the BioMek.

In preparation for this project, ATS acquired a Biomek 2000 robotic workstation (Beckman Coulter) which will be used for high throughput liquid handling such as the large number of ELISAs required for the development of antibodies.

With sophisticated equipment and talented, experienced personnel, ATS expects to release the first five monoclonal antibodies early in 2004. After comparing the monoclonals to recombinant antibodies for specificity, immunotoxins will be developed using the best performers.

Has it been a YEAR already?!

Malia and Sam Kohls had their first birthday on February 14, 2003. Proud papa, Matt may be getting more sleep, but during waking hours, life's getting busier.

Targeting Tickler

Two hydrogens are walking along a street. The first one says, "Hey! I think I lost an electron!" The second one replies, "Are you sure?" The first one says, "Yeah, I'm POSITIVE."

* * * * *

The Difference Between Dogs and Cats

A dog thinks: Hey, these people I live with feed me, love me, provide me with a nice warm, dry house, pet me, and take good care of me ... They must be gods!

A cat thinks: Hey, these people I live with feed me, love me, provide me with a nice warm, dry house, pet me, and take good care of me ... I must be a god!

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Effects of lateral hypothalamic lesion with the neurotoxin hypocretin-2-saporin on sleep in Long-Evans rats

Gerashchenko K, Blanco-Centurion C, Greco MA, Shiromani PJ
Neuroscience 116:223-235, 2003

Recent data has linked narcolepsy to the loss of neurons containing the neuropeptide hypocretin, also known as orexin. The authors wished to investigate whether the variance in severity of narcolepsy could be explained by the extent of loss of these neurons. After injection of 90 or 490 ng of orexin-SAP (Cat. #IT-20) into the lateral hypothalamus, the measurement of several parameters demonstrated the severity of narcolepsy may be linked to the degree of loss of neurons expressing the orexin receptor.

Ablation of NK1 receptors in rat nucleus tractus solitarii blocks baroreflexes

Riley J, Lin LH, Chianca DA, Talman WT
Hypertension 40(6):823-826, 2002

Stimulation of arterial baroreflexes releases the neuropeptide substance P (SP) from vagal afferent nerves within the nucleus tractus solitarii. To ascertain whether the neurons taking up this SP are critical to baroreflex transmission, the authors injected 18 ng SP-SAP (Cat. #IT-07) into the nucleus tractus solitarii of rats. In animals that received bilateral injections, baroreflex gain was significantly reduced, indicating that neurons expressing SP receptors play a critical role in mediation of this process.

Effects of lesions of basal forebrain cholinergic neurons in newborn rats on susceptibility to seizures

Silveira DC, Cha BH, Holmes GL
Dev Brain Res 139:277-283, 2002

It has previously been shown that adult rats treated with the cholinergic lesioning agent 192-Saporin (Cat. #IT-01) display increased susceptibility to generalized seizures. Here, the authors studied the effects of 200 ng intracerebroventricular injections of 192-Saporin in neonatal rats. Although treated rats did not demonstrate differences in seizure duration or EEG ictal duration, a significantly shorter latency to seizure onset was observed. No significant differences were observed in spatial learning between treated and control rats.

Immunolesion of norepinephrine and epinephrine afferents to medial hypothalamus alters basal and 2-deoxy-D-glucose-induced neuropeptide Y and agouti gene-related protein messenger ribonucleic acid expression in the arcuate nucleus

Fraley GS, Ritter S
Endocrinology 144(1):75-83, 2003

Neuropeptide Y (NPY) and agouti gene-related protein (AGRP) are important peptides in the control of food intake. Prior studies have shown that mRNAs for both these peptides are increased in the arcuate nucleus of the hypothalamus (ARC) by glucoprivation. Using bilateral 42 ng intracranial injections of anti-DBH-SAP (Cat. #IT-03) in rats, the authors investigated the role of hindbrain catecholamine afferents in this increased ARC NPY and AGRP gene expression. The results indicate that these afferents contribute to basal NPY and AGRP gene expression as well as mediate the responsiveness of NPY and AGRP neurons to glucose deprivation.

Specific contributions of the basal forebrain corticopetal cholinergic system to electroencephalographic activity and sleep/waking behaviour

Berntson GG, Shafi R, Sarter M
Eur J Neurosci 16(12):2453-2461, 2002

There is a large amount of data suggesting the basal forebrain cholinergic system plays an important part in arousal and REM sleep. In this study the authors used 192-Saporin (Cat. #IT-01, 0.05 μg injected into the basal forebrain of each hemisphere) to lesion the corticopetal projection and examined cortical EEG activity across sleep/wake states. Lesioned animals displayed significantly reduced high frequency EEG activity across all stages of sleeping and wakefulness, indicating that the basal forebrain cholinergic system may exert a general activational effect on the cortical mantle.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Neurotransmitter release and its presynaptic modulation in the rat hippocampus after selective damage to cholinergic or/and serotonergic afferents

Birthelmer A, Ehret A, Amtage F, Förster S, Lehmann O, Jeltsch H, Cassel JC, Jackisch R
Brain Res Bull 59(5):371-381, 2003

Previous studies have investigated some of the modulatory mechanisms present in the denervated hippocampus. These studies have used nonselective denervation models, therefore it is difficult to assign results to the lesion of any specific system. This study examined the interaction of lesions caused by 192-Saporin (Cat. #IT-01, 0.4 μg injected into the medial septum/diagonal band of broca) and 5,7-DHT. The authors were able to establish controlled and selective damage to more than one transmitter system, allowing examination of the interaction between multiple-lesioned systems.

A group of glutaminergic interneurons expressing high levels of both neurokinin-1 receptors and somatostatin identifies the region of the pre-Bötzinger complex

Stornetta RL, Rosin DL, Wang H, Sevigny CP, Weston MC, Guyenet PG
J Comp Neurol 455(4):499-512, 2003

Study of the pre-Bötzinger complex (pre-BötC) has been hindered by the lack of a specific marker. Using SSP-SAP (Cat. #IT-11, three 0.313-ng unilateral injections in the rostral part of the ventral respiratory group) coupled with *in situ* hybridization and the labeling of selected markers, the authors examined whether somatostatin (SST) might be a marker for this region. The data suggest that a subgroup of cells containing high levels of SST and neurokinin-1 receptor immunoreactivity may identify the pre-BötC.

Effects of septal cholinergic lesion on rat exploratory behavior in an open-field

Lamprea MR, Cardenas FP, Silveira R, Walsh TJ, Morato S
Braz J Med Biol Res 36(2):233-238, 2003

Exploratory behavior triggered by novelty involves the medial septum. The authors lesioned the medial septum in rats with 237.5-ng injections of 192-Saporin (Cat. #IT-01) and examined the behavior of these animals in a model for novelty. The results suggest not only do septohippocampal cholinergic mechanisms contribute to the motivation to explore new environments, they also are related to the acquisition and storage of spatial information.

Selective joint denervation promotes knee osteoarthritis in the aging rat

Salo PT, Hogervorst T, Seerattan RA, Rucker D, Bray RC
J Orthop Res 20(6):1256-1264, 2002

Noting that mice lose joint afferents with aging, and that this loss precedes osteoarthritis development, the authors investigated the effects of denervating the knee joints of young rats. Injection of 10 μl OX7-SAP (Cat. #IT-02) into the knee joint space produced severe degenerative cartilage changes as well as a significant reduction in the number of joint afferents. These changes indicate that joint denervation predisposes a joint to osteoarthritic changes more severe than those found with aging alone.

Neurobiology of substance P and the NK1 receptor

Mantyh PW
J Clin Psychiatry 63(Suppl 11):6-10, 2002

The NK-1 receptor system is somewhat unusual in that it is expressed on only 5-7% of neurons in the central nervous system. Dr. Patrick Mantyh reviews how tools such as SP-SAP (Cat. #IT-07) have been used to begin defining the roles of substance P and the NK-1 receptor in affective behavior.

Visit our website at www.ATSbio.com for a complete list of references for ATS targeted toxins, antibodies and other reagents.

Arranged by Product:

192-Saporin (295)
Anti-DBH-SAP (55)
SP-SAP (24)
OX7-SAP (16)
ME20.4-SAP (15)
Dermorphin-SAP (14)
SSP-SAP (14)
Orexin-SAP (9)
IB4-SAP (9)

Targeting Talk: Retrograde Transport

by Dr. Ronald G. Wiley

Q. I'm interested in using SAP to eliminate cells through retrograde transport, like OX7-SAP and IB4-SAP have been used. Can you explain how retrograde transport works and if it is possible for this to work with dermorphin-SAP? What determines whether a targeted toxin will be able to be used in retrograde transport?

A. Current evidence indicates that effective suicide transport agents undergo endocytosis at nerve terminals followed by retrograde axonal transport of the endocytic vesicles containing the toxin. Experiments using vincristine have shown that the retrograde axonal transport of suicide transport toxins utilizes the fast transport system (microtubules). However, it is not known what determines whether or not a specific toxin-ligand undergoes axonal transport after internalization.

Empirically, it has been observed that immunotoxins (OX7-SAP, 192-Saporin, anti-DBH-SAP) and lectin-toxins (ricin, volkensin, IB4-SAP) all undergo retrograde axonal transport and are therefore effective suicide transport agents. This is not true, however, for neuropeptide-toxin conjugates, such as dermorphin-SAP. For example, in an unpublished study, we injected large doses (1-2 μg) of dermorphin-SAP into the lumbar intrathecal space of rats. After 2-3 days, rats were sacrificed and lumbar dorsal root ganglia examined for evidence of toxin effect (striking chromatolysis). None was found after examining numerous ganglia and >15,000 primary afferent neurons. Apparently, dermorphin-SAP is not retrogradely transported even if it is taken into the primary afferent terminals that express the mu opioid receptor (MOR).

Q. If a targeted toxin cannot be used in retrograde transport, will it only kill cell bodies in the injection site or will it also kill terminals?

A. Current evidence suggests that applying dermorphin-SAP to the population of MOR-expressing neurons in the dorsal horn of the spinal cord results in destruction only of the neurons in lamina II and not the primary afferent terminals that also express MOR. This may be a general principle but it has not been tested in any other situation for dermorphin-SAP, nor have SP-SAP and SSP-SAP been evaluated for terminal uptake and suicide transport. Any saporin taken into a nerve terminal should not be toxic unless retrogradely transported to the cell body since there are no ribosomes (site of saporin action) or protein synthesis in the nerve terminal.

Suggested Reading:

1. Oeltmann TN, Wiley RG (1986) Wheat germ agglutinin-ricin A-chain conjugate is neuronotoxic after vagal injection. Brain Res 377:221-228.
2. Wiley RG, Stirpe F, Thorpe P, Oeltmann TN (1989) Neuronotoxic effects of monoclonal anti-Thy 1 antibody (OX7) coupled to the ribosome inactivating protein, saporin, as studied by suicide transport experiments in the rat. Brain Res 505:44-54.
3. Contestabile A, Fasolo A, Virgili M, Migani P, Villani L, Stirpe F (1990) Anatomical and neurochemical evidence for suicide transport of a toxic lectin, volkensin, injected in the rat dorsal hippocampus. Brain Res 537(1-2):279-286.
4. Pangalos MN, Francis PT, Pearson RC, Middlemiss DN, Bowen DM (1991) Destruction of a sub-population of cortical neurones by suicide transport of volkensin, a lectin from Adenia volkensii. J Neurosci Methods 40(1):17-29.
5. Wiley RG (1992) Neural lesioning with ribosome-inactivating proteins: suicide transport and immunolesioning. Trends in Neurosci 15:285-290.
6. Roberts RC, Harrison MB, Francis SMN, Wiley RG (1993) Differential effects of suicide transport lesions of the striatonigral or striatopallidal pathways on subsets of striatal neurons. Exp Neurol 124:242-252.
7. Contestabile A, Stirpe F (1993) Ribosome-inactivating proteins from plants as agents for suicide transport and immunolesioning in the nervous system. Eur J Neurosci 5:1292-1301.
8. Wiley RG, Lappi DA(1994) Suicide Transport and Immunolesioning. R.G. Landes, Houston.
9. Roberts RC, Strain-Saloum C, Wiley RG (1995) Effects of suicide transport lesions of the striatopallidal or striatonigral pathways on striatal ultrastructure. Brain Res 710:227-237.
10. Wiley RG, Kline IVRH (2000) Neuronal lesioning with axonally transported toxins. J Neurosci Methods 103:73-82.

Experimental Biology
April 11-15, 2003
San Diego, California
Booth #545

Society for Neuroscience
November 8-12, 2003
New Orleans, Louisiana

Biotinylated materials (continued)

(continued from page 1)

Brian Russell is the flow cytometry, fluorescence labeling, and biotinylation specialist at ATS

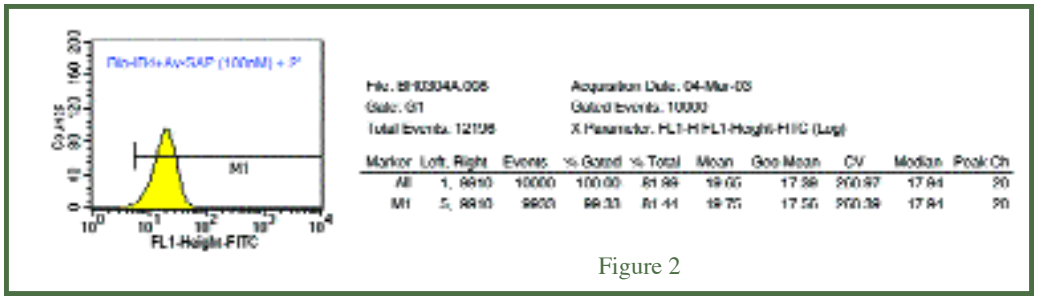


Figure 2

In Figure 2, IB4, a tetramer isolectin from *Bandeiraea simplicifolia*, was bound to biotin through an amide bond with a primary amine on the IB4 molecule. After incubation with the biotin, IB4 was run over a desalting column, and sample containing fractions determined by absorbance at 280 nm. A BCA was performed to determine protein concentration, and then binding evaluated through FACS analysis.

Paraformaldehyde-fixed KNRK cells, normal rat kidney cells positive for α-D-galactose, were used for a FACS analysis with the biotinylated IB4 plus avidinylated-SAP (Cat. #IT-09). Cells were treated with biotinylated-IB4 and Avidinylated-SAP, both at a 100 nM concentration, and incubated for 1 hr. Cells were subsequently incubated with FITC-labeled anti-SAP (Cat. #FL-02) at a 1:50 dilution for 30 min. A 99% shift is seen as compared to the non-treated control.

In Figure 3, the biotinylated IB4 was combined with avidinylated-SAP (Cat #IT-09) and compared to the directly conjugated IB4-SAP (Cat # IT-10) in a cytotoxicity assay. KNRK cells were plated at 2500 per well in a 96-well plate and incubated overnight. IB4-SAP, biotinylated-IB4, avidinylated-SAP, and saporin were added in 10-μl volumes, and the plates incubated 72 hours. The plates were developed with PMS/MTS for 1-2 hours, then read at 492 nm in a plate reader. Data analysis was done by PRISM (GraphPad, San Diego).

Results show cytotoxicity levels for the directly conjugated IB4-SAP are 4 times higher than for the biotinylated IB4/avidinylated-SAP. Cells targeted by biotinylated materials will be eliminated using avidinylated-SAPand this is a powerful and economical research tool for scientists in trying to determine the most specific targeting agent for their applications. Potency of avidinylated-SAPmay vary according to the specificity and affinity of the biotinylated material to its receptor. ATS recommends the direct conjugation of your material to saporin when the *in vitro* results confirm the desired specificity.

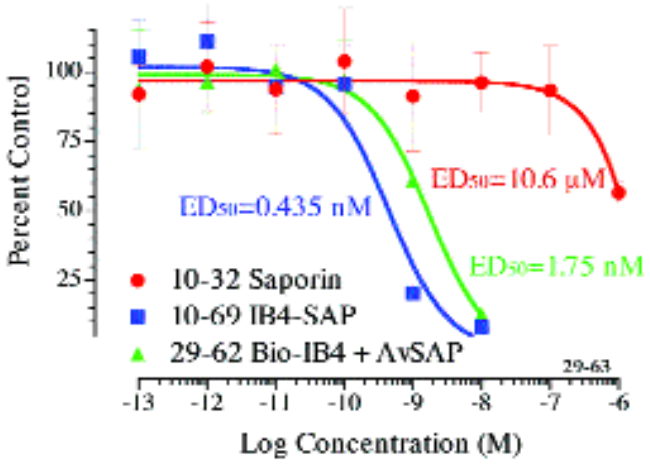
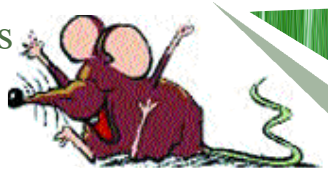


Figure 3

1. Vulchanova L, Olson TH, Stone LS, Riedl MS, Elde R, Honda CN. (2001) Cytotoxic targeting of isolectin IB4-binding sensory neurons. *Neuroscience* 108(1):143-155.

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: WESTERN PROFILED MOLECULAR SPASTICITY INJECTION

Answer: What the student became when he tried to finish the chemistry experiment — A WEAPON OF "MASS DISRUPTION"!

WINNERS: Bryan Hudson, Washington State University, Dept Vet & Comp Anatomy * Geda C Unabia, University of Texas Medical Branch, Dept Anatomy & Neuroscience * Bob Speth, Washington State University, VCAPP * Kristen Phend, University of North Carolina, Dept Cell Biology & Anatomy

Targeting Tools: Featured Products

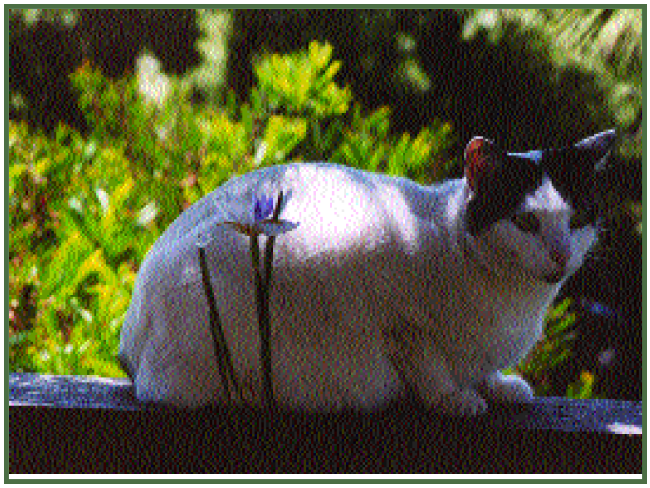
Avidinylated-SAP

a chemical conjugate of avidin and the ribosome-inactivating protein, saporin

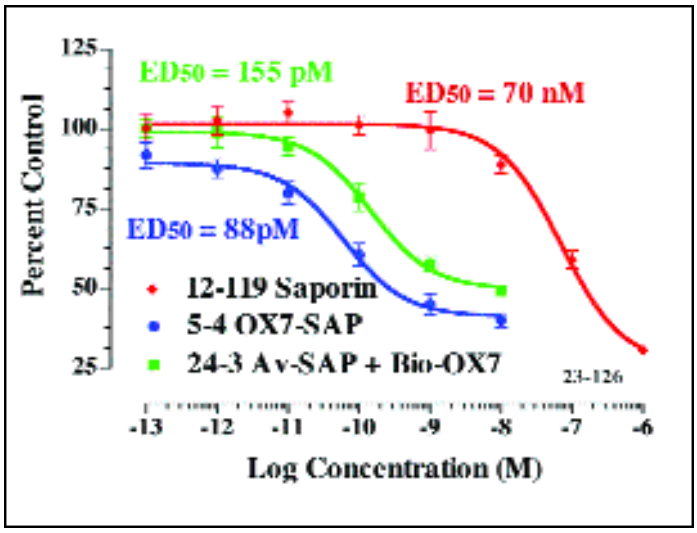
Avidin is a glycoprotein found in egg white and in tissues of birds, reptiles, and amphibians. This protein is composed of four subunits, each of which can bind one molecule of biotin. Biotin, a 244-dalton vitamin found in tissue and blood, binds with high affinity to avidin. In fact, the avidin-biotin interaction is the strongest known noncovalent biological interaction (Ka = 10^{15} M^{-1}) between protein and ligand. The bond formation between avidin and biotin is rapid and essentially non-reversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. Extensive chemical modification has little effect on the activity of avidin, and biotin's small size allows it to be conjugated to many proteins without significantly altering the biological activity of the protein. The avidin-biotin interaction has found extensive use as a research tool. A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with avidin-labeled probes or other detection reagents for use in biological assays.

Effective Tool

Using avidinylated SAP and specific targeting agents that have been biotinylated, specific cytotoxins can be created JUST BY MIXING!



Spring is in the air and Gangsta ponders the mysteries of life.



PC12 cells are plated at 5000 cells/well and incubated overnight. Avidinylated-SAP is premixed with biotinylated-OX7 in equimolar concentrations. Saporin, OX7-SAP, and the avidinylated-SAP + biotinylated-OX7 mixture are then 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.

Biotinylation Service

$250 for 2-5 mgs (includes 25 μg Avidinylated-SAP)

Step 1: Send ATS your targeting agent (antibody, lectin, etc.).

Step 2: ATS biotinylates your targeting agent and returns it to you with avidinylated-SAP.

Step 3: You mix together your biotinylated targeting agent and avidinylated-SAP to specifically eliminate cells that recognize and internalize your targeting agent.

avidinylated-SAP (Cat. # IT-09)
a conjugate of avidin
and the ribosome-inactivating protein, saporin
Converts biotinylated materials into targeted toxins.

Available Individually and as a Kit (with unconjugated saporin)
25 μg. $100 ($150)
100 μg. $350 ($450)
250 μg. $778 ($975)

ATS recommends that you order a custom conjugation of your targeting agent to saporin when the *in vitro* results confirm the desired specificity.

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.

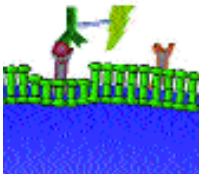
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

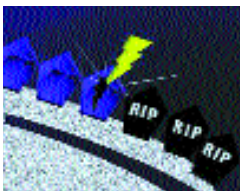
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



Cells which 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 used in science.

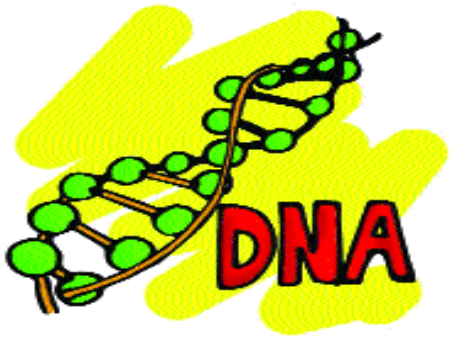
GROOLYUNE

TENMUSTRIN

GNASTINI

THIGEW

METEGA



WHY THE SCIENTISTS MISSED THE LABORATORY PRESENTATION

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

Answer:

THEY DIDN'T... ○○○ ○○○

"○○○○○○○○"!

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, 2003.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6

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Jul-Aug-Sep 2003
Volume 4, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

A New Immunotoxin for Targeting Dopaminergic Neurons

Targeting results with anti-DAT-SAP shown in Figure 1 are reprinted by permission of Kluwer Academic/Plenum Publishers. The original was published in Cell Mol Neurobiol *23:839-850, 2003.*

Dopaminergic neurons are widely studied because of their role in one of the devastating diseases of old age, Parkinson's. Lesioning studies using 6-hydroxydopamine or MPTP have been useful in research on Parkinson's Disease. However, these reagents have limitations such as MPTP affecting catecholaminergic neurons and 6-OHDA stability and specificity issues.

Targeting dopaminergic neurons with an antibody provides maximal specificity and overcomes the limitations of previous methods. Here we announce the availability of an immunotoxin against cells that express the dopamine transporter (DAT). DAT, because of its importance in dopamine use, is an indentifier of dopaminergic neurons. The DAT antibody used as the targeting agent in the immunotoxin recognizes a unique extracellular domain sequence and does not recognize other transporters.[1] This new immunotoxin is called anti-DAT-SAP and is described in Wiley *et al*.[2]

Figure 1 shows the removal of dopaminergic neurons in a dose-dependent manner after direct injection into the striatum. Cells can also be eliminated by intracerebroventricular injection; the immunotoxin will seek out the dopamine transporter, and the saporin moiety will be transported to the cell body to inhibit protein synthesis.

References

1. Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI (1997) Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J Comp Neurol* 388(2):211-227.
2. Wiley RG, Harrison MB, Levey A Lappi DA (2003) Destruction of midbrain dopaminergic neurons by using an immunotoxin to the dopamine transporter. *Cell Mol Neurobiol* 23:839-850.

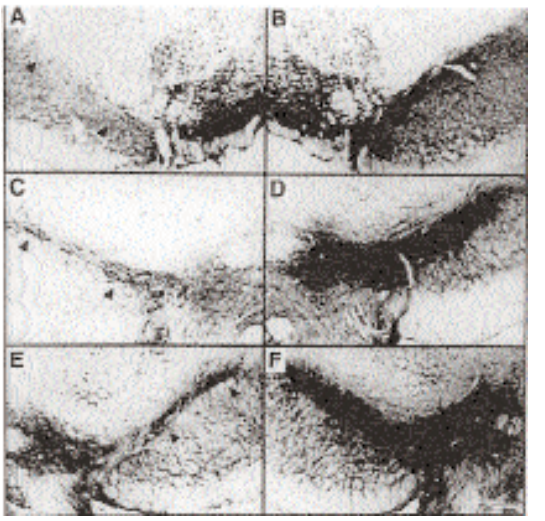

Figure 1. Representative sections stained for tyrosine hydroxylase from rats with intrastriatal injections of anti-DAT-SAP two weeks prior to sacrifice. Panels A, C, and E are ipsilateral to the striatal injections. Panels B, D, and F are contralateral, from the same sections. The anti-DAT-SAP doses were 2.8 μg in A, 0.56 μg in C, and 0.28 μg in E. Note loss of dopaminergic neurons from the ipsilateral substantia nigra, pars compacta (arrowheads) with the greatest extent of cell loss in A and the least in E. The magnification bar in F indicates 100 μm and applies to all panels.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Dr. Rania Siam Joins ATS Team of Scientists

ATS is happy to welcome Dr. Rania Siam to our team of scientists. Rania joined ATS in April to lend her expertise to a recently awarded SBIR Phase II grant from the National Institutes of Health, National Institute of Mental Health.

Dr. Siam examines the latest data on the development of somatostatin receptor antibodies.

Rania is originally from Egypt and moved to Montreal, Canada in 1994 to pursue graduate studies. She obtained her Ph.D. degree from the Department of Microbiology and Immunology, McGill University under the supervision of Dr. Gregory Marczynski. Her Ph.D. thesis focused on cell cycle regulation by a signal transduction cascade. She then moved in 2001 to The Salk Institute for Biology Studies in San Diego, California to pursue post-doctoral studies in the laboratory of Dr. Susan Forsburg. Her post-doctoral study involved the regulation of eukaryotic cell cycle by a checkpoint and replication protein. Rania is happy to have recently joined ATS as a research scientist to develop monoclonal and recombinant antibodies against the extracellular domains of the five somatostatin receptors.

On a more personal note, Rania's husband, Mohamed Khedr, is director of engineering technology of an Illinois-based company. They have two lovely children. Omar was born in Egypt; he is 9 years old. He attends Doyle school and loves to skateboard and play soccer. Salma is almost 2 years old and was born in San Diego. She loves to read—right-side up or upside down, just as long as she has a book in her hands, she's happy.

Omar, age 9, loves skateboarding, soccer

Salma, nearly 2, can't wait for her first library card

Substance P-Saporin (SP-SAP) Drug Development Update

Substance P-Saporin (SP-SAP) is under development as a chronic pain therapeutic with tremendous potential to permanently eliminate suffering in many painful diseases and conditions. ATS is awaiting news from the National Institutes of Health on further funding to fulfill FDA requirements to bring SP-SAP to clinical trial in humans.

ATS has a patent on SP-SAP and has completed extensive preclinical studies in animal models. All the results to date demonstrate SP-SAP as an effective, safe treatment for the elimination of chronic pain. With such positive data, intense patient demand, and few options for treatment, it is surprising that this project would not move forward more quickly.

The biggest reason for not rushing forward to FDA-mandated toxicology studies and on to clinical trials is not due to the usual suspects. It's not negative preclinical data. It's not lack of positive preclinical data. It's not proof of efficacy. It's not indications of toxicity. It's money.

The United States economy has hurt many businesses and the biotechnology industry is among the hardest hit. Investors are few and most are saving their cash to try to keep their present portfolio companies alive. The $5 million it will take to bring SP-SAP to clinical trial is not a huge sum, and ATS continues to search for foundations, investors, or partners to help speed the process.

ATS remains dedicated to bringing SP-SAP to market to meet the unmet needs of a suffering population. If you have an interest in sponsoring this development, please contact Denise Higgins at ats@atsbio.com.

Targeting Topics: Recent Scientific References

Intrathecal substance P-saporin attenuates operant escape from nociceptive thermal stimuli.

Vierck CJ, Kline RH, Wiley RG
Neuroscience 119(1):223-232, 2003.

Administration of SP-SAP (Cat. #IT-07) eliminates sensitization of nocifensive reflexes. The authors investigate whether SP-SAP elimination of neurokinin-1 receptor-expressing neurons in the lumbar spinal cord affects nociceptive sensitivity in general, or preferentially affects nociception dependent on spinal and brainstem, or cerebral processing. Rats treated with spinal intrathecal injections of 175 ng of SP-SAP showed attenuated thermal hyperalgesia, and no secondary hyperalgesia, while innate reflexes were unaffected by SP-SAP treatment.

Ablation of striatal interneurons influences activities of entopeduncular neurons.

Chiken S, Tokuno H
Neuroreport 14(5):675-678, 2003.

To investigate the role of the basal ganglia in informational processing of voluntary movement, the authors used SP-SAP (Cat. #IT-07) to lesion SP receptor-expressing neurons in the striatum. A 0.5 μl injection of 40 ng/μl SP-SAP into the dorsolateral portion of the striatum decreased the spontaneous discharge of entopeduncular neurons. These data indicate that SP receptor-positive striatal interneurons indirectly regulate activity of basal ganglia output neurons.

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

Food- and light-entrained circadian rhythms in rats with hypocretin-2-saporin ablations of the lateral hypothalamus.

Mistlberger RE, Antle MC, Kilduff TS, Jones M
Brain Res 2003, avail online at http://dx.doi.org/10.1016/S0006-8993(03)02755-0

Food-anticipatory behaviors in mammals can follow circadian rhythms entrained by daily feeding schedules. Lateral hypothalamic (LH) neurons express hypocretin (also known as orexin) receptors, therefore rats were treated with four 500-ng injections of orexin-SAP (Cat. #IT-20) to eliminate these neurons. Lesioned animals displayed altered dietary behavior, but maintained anticipatory activity before the daily meal.

Neural stem cells and cholinergic neurons: Regulation by immunolesion and treatment with mitogens, retinoic acid, and nerve growth factor.

Calza L, Giuliani A, Fernandez M, Pirondi S, D'Intino G, Aloe L, Giardino L
Proc Natl Acad Sci U S A 100(12):7325-7330, 2003.

The authors explore the influence of exogenous administration of hormones, cytokines, and neurotrophins on stem cells following a lesion. Rats were treated with 2 or 3 μg of 192-Saporin (Cat. #IT-01) into the cerebral ventricles, which induced a lesion of the cholinergic system in the basal forebrain. The surgery was followed by infusion of EGF, bFGF, and NGF into the lesioned area, as well as addition of retinoic acid to the food pellets. This pharmacological control of endogenous neural stem cells increased the number of proliferating cells in both lesioned and non-lesioned animals, as well as improved performance in a water maze test.

Long-term effects of decreased noradrenergic central nervous system innervation on pain behavior and opioid antinociception.

Jasmin L, Boudah A, Ohara PT
J Comp Neurol 460(1):38-55, 2003.

Noradrenaline (NA) is an essential element of the endogenous pain inhibitory system. The authors injected 5 μg of anti-DBH-SAP (Cat. #IT-03) into either the cerebral ventricles or lumbosacral cistern of rats to investigate whether a permanent reduction of noradrenergic innervation of the spinal cord leads to a chronic decreased nociceptive threshold. Although treated animals were less responsive to the antinociceptive effects of morphine, the results suggest that NA makes only a modest contribution to the nociceptive threshold.

Role of the medial septum diagonal band of Broca cholinergic neurons in oestrogen-induced spine synapse formation on hippocampal CA1 pyramidal cells of female rats.

Lam TT, Leranth C
Eur J Neurosci 17(10):1997-2005, 2003.

Estrogen effects on the hippocampus are known to be mediated by subcortical structures. The authors examined the role that the medial septum diagonal band of Broca (MSDB) plays in this mediation. An injection of 0.5 μg of 192-Saporin (Cat. #IT-01) into the right lateral ventricle of rats was used to specifically

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Targeting Topics: Recent Scientific References

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investigate the role of cholinergic MSDB neuron projections to the hippocampus, since many of these neurons express estrogen receptors. The data suggest that septo-hippocampal cholinergic neurons are involved in mediating estrogen effects on the hippocampus.

Immunotoxin lesion of hypothalamically projecting norepinephrine and epinephrine neurons differentially affects circadian and stressor-stimulated corticosterone secretion.

Ritter S, Watts AG, Dinh TT, Sanchez-Watts G, Pedrow C
Endocrinology 144(4):1357-1367, 2003.

Hindbrain norepinephrine (NE) and epinephrine (E) neurons are important in the distribution of internal sensory signals. Injecting 42 ng of anti-DBH-SAP (Cat. #IT-12) into the paraventricular nucleus of rat hypothalamus, the authors were able to specifically destroy NE and E neurons. This study revealed the contribution of NE/E afferents to hypothalamo-pituitary-adrenal activation during stress and confirmed that NE and E neurons are required for specific stress responses.

A role for the basal forebrain cholinergic system in estrogen-induced disinhibition of hippocampal pyramidal cells.

Rudick CN, Gibbs RB, Woolley CS
J Neurosci 23(11):4479-4490, 2003.

Estrogen plays a strong regulatory role in control of synaptic input to the hippocampus of female rats. Injection of 0.22 μg of 192-Saporin (Cat. #IT-01) directly into the medial septum eliminated NGFr-positive cholinergic neurons of the basal forebrain, producing evidence that estrogen-induced disinhibition is partially dependent on these neurons. GABAergic synapses were also found to be involved in this system.

Breathing: Rhythmicity, plasticity, chemosensitivity.

Feldman JL, Mitchell GS, Nattie EE
Annu Rev Neurosci 26:239-66, 2003.

Recent research has indicated that specific areas of the brain exert control over several aspects of breathing, such as rhythm generation, reaction to hypoxia, and regulation of carbon dioxide levels and pH. This review covers many of the latest advances, some of which utilize SP-SAP (Cat. #IT-07) and anti-SERT-SAP (Cat. #IT-23). The use of these targeted toxins allows altered breathing behavior through elimination of very specific cell populations.

192 IgG-saporin lesions to the nucleus basalis magnocellularis (nBM) disrupt acquisition of learning set formation.

Bailey AM, Rudisill ML, Hoof EJ, Loving ML
Brain Res 969:147-159, 2003.

Previous studies by Bailey and others have used quisqualic acid to lesion the nucleus basalis (nBM) in order to understand Alzheimer's disease. Injections of 75 ng of 192-Saporin (Cat. #IT-01) were made into each of four sites in the rat nBM. Behavioral tests showed initial learning set deficits followed by recovery, whereas with quisqualic acid lesions, the deficits were profound. The authors conclude noncholinergic neurons are involved in learning set formation.

Distinct roles of P2X receptors in modulating glutamate release at different primary sensory synapses in rat spinal cord.

Nakatsuka T, Tsuzuki K, Ling JX, Sonobe H, Gu JG
J Neurophysiol 89:3243-3252, 2003.

P2X receptors are important modulating neurons in the spinal cord. These authors used IB4-SAP (Cat. #IT-10) to target a neuronal subset, those neurons expressing $P2X_3$ receptors. 2 μg of IB4-SAP were injected directly into the sciatic nerve on one side. Histological examination showed efficient removal of IB4 and $P2X_3$-staining ipsilaterally in the dorsal horn outer laminae. Behavioral experiments showed intact modulation of glutamate release in the absence of $P2X_3$-positive neurons, indicating involvement by other P2X neurons.

Effects of septal grafts on acetylcholine release from rat hippocampus after 192 IgG-saporin lesion.

Hilgert M, Hartmann J, Loffelholz K, Jeltsch H, Cassel JC, Klein J
Neurochem Res 28:467-472, 2003.

A model for transplantation efficacy was created using injections of 400 ng each into the vertical limb of the rat diagonal band of Broca and the medial septum for the specific removal of cholinergic neurons. Thirteen months after lesioning, sham-operated animals had measured acetylcholine release at 20% of control. 192-Saporin (Cat. #IT-01)-lesioned animals were transplanted with fetal septal cells 15 days after lesioning. Thirteen months later, their septal level of acetylcholine release was near normal (71%) of controls. A serotonin uptake inhibitor briefly stimulated acetylcholine release similar to sham control animals.

Targeting Talk: Dosing, Volume, and Animal Care

by Dr. Ronald G. Wiley

Q: When performing intraparenchymal injections of immunotoxin, what is the proper volume to use? Is it better to induce two half-portions per hemisphere or is a higher concentration better? At what concentration do you expect necrosis or inflammation?

A: There is no one answer to the question of injection volume. Practically speaking, we have observed that large or extended structures such as the entire cholinergic basal forebrain (CBF) of rats are difficult to ablate with one single injection of 192-Saporin. The best results were obtained with 3-5 separate 0.5-1.0 µl injections. For even larger targets such as the CBF in primates, other strategies may be necessary. Oldfield and co-workers have reported success in delivering cytotoxic chemotherapy to large volumes of brain using long, slow infusions of solutions containing a low concentration of toxin (convective delivery). This procedure delivers toxin by bulk fluid flow rather than diffusion and avoids high local toxin concentrations around the infusion catheter or pipette. High local concentrations of toxin may compromise selectivity and produce non-specific cytotoxicity. This can occur when neurons and glia take up toxic amounts of saporin by bulk fluid phase endocytosis, rather than receptor-mediated endocytosis. With direct intraparenchymal injections, local necrosis can occur with surprisingly small doses of toxin. For example, 60 ng of SP-SAP or dermorphin-SAP into the rat striatum injected in 1 µl typically produces some necrosis in the center of the injection site. With immunotoxins such as 192-Saporin or anti-DBH-SAP, 200 ng in 0.5 µl may barely produce a trace of local damage.

Q: We are interested in the anti-Thy-1 nephritis model in rats. I want to know the titer of OX7-SAP and how much we have to expend for each rat to establish the model?

A: The "titer" of OX7-SAP is rather difficult to define. It is not known precisely how many molecules of this immunotoxin are necessary to kill a thymic-derived (Thy-1-expressing) lymphocyte *in vivo*. Also, since OX7-SAP kills Thy-1-expressing lymphocytes, it may prove difficult to induce Thy-1 nephritis with the immunotoxin. In humans, proteinuria was reported in clinical trials using immunotoxins for treatment of cancer, but we do not know of any comparable data in rats. Probably the only way to determine the appropriate dose would be a dose ranging study.

Selected references on convective delivery of toxin to brain:

1. Nguyen TT, Pannu YS, Sung C, Dedrick RL, Walbridge S, Brechbiel MW, Garmestani K, Beitzel M, Yordanov AT, Oldfield EH (2003) Convective distribution of macromolecules in the primate brain demonstrated using computerized tomography and magnetic resonance imaging. *J Neurosurg* 98(3):584-590.
2. Morrison PF, Chen MY, Chadwick RS, Lonser RR, Oldfield EH (1999) Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics. *Am J Physiol* 277(4 Pt 2):R1218-R1229.
3. Lonser RR, Corthesy ME, Morrison PF, Gogate N, Oldfield EH (1999) Convection-enhanced selective excitotoxic ablation of the neurons of the globus pallidus internus for treatment of Parkinsonism in nonhuman primates. *J Neurosurg* 91(2):294-302.
4. Wood JD, Lonser RR, Gogate N, Morrison PF, Oldfield EH (1999) Convective delivery of macromolecules into the naive and traumatized spinal cords of rats. *J Neurosurg* 90(1 Suppl):115-120.
5. Lonser RR, Gogate N, Morrison PF, Wood JD, Oldfield EH (1998) Direct convective delivery of macromolecules to the spinal cord. *J Neurosurg* 89(4):616-622.
6. Laske DW, Morrison PF, Lieberman DM, Corthesy ME, Reynolds JC, Stewart-Henney PA, Koong SS, Cummins A, Paik CH, Oldfield EH (1997) Chronic interstitial infusion of protein to primate brain: determination of drug distribution and clearance with single-photon emission computerized tomography imaging. *J Neurosurg* 87(4):586-594.
7. Lieberman DM, Laske DW, Morrison PF, Bankiewicz KS, Oldfield EH (1995) Convection-enhanced distribution of large molecules in gray matter during interstitial drug infusion. *J Neurosurg* 82(6):1021-1029.
8. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH (1994) Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci U S A* 91(6):2076-2080.
9. Morrison PF, Laske DW, Bobo H, Oldfield EH, Dedrick RL (1994) High-flow microinfusion: tissue penetration and pharmacodynamics. *Am J Physiol* 266(1 Pt 2):R292-305.

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Amer Assoc Cancer Research
July 12-14, 2003
Washington, DC

Society for Neuroscience
November 8-12, 2003
New Orleans, Louisiana

Targeting Talk (continued)

(continued from page 5)

Q: I have been doing research with 192-Saporin for 2-3 years now. I have read in the literature that animals with cholinergic lesions often get sick following surgery and require potatoes, apples, lettuce and saline injections. They may even stop eating or drinking all together. I have followed these practices in the past, but stopped when it didn't seem to make a difference. (I use a very small insignificant dose that is not prone to make animals ill). This is the first death I have had even remotely possibly related to the toxin. In short, the animal lost 64 grams over a period of 2 weeks, and expired 1-2 days thereafter. I weighed her at death and she was 139 grams (91 gram difference from her initial surgery weight). The rats in our colony are fed and given water ad libitum. However, we think that she dehydrated. She was given the same dose (1.1 microliters) as all of the other rats in the experiment. I do have other rats that were given injections from the same lot that do not appear to be sick or losing weight. I'm not sure what you can do with this information, but I would be grateful for whatever help you can offer.

A: In large series of intraventricular injections of 192-Saporin, I have never encountered quite the same sequence of events you describe. Death after intracranial toxin injection can reflect several possible misadventures including but not limited to:

1) Contamination of toxin solution with endotoxin resulting in death without awakening from anesthesia (usually due to bacterial contamination from prolonged exposure of toxin solution to room temperature),

2) Fatal intracranial hemorrhage which may result in delayed death depending on location and volume of bleeding,

3) Intracranial abscess (extremely unusual) from injection of contaminated solution,

or

4) Unrelated bacterial, viral or parasitic systemic disease.

Q: Another one of my 192-Saporin-treated rats is having an adverse reaction, including paralysis of the lower extremities. She has lost 40 grams in the past 3-4 days. Could this be Purkinje cell damage; does that happen after five weeks?

A: Purkinje cell damage after intraventricular injection of 192-Saporin typically is manifest not by hind limb paralysis but rather tremor (shaking) and ataxia (clumsiness, poor balance). At less than lethal doses, 192-Saporin does not produce paraparesis. Something else is going on. Rats develop hind limb paralysis from a variety of toxic or metabolic systemic insults in addition to specific nervous system disorders. The presence of rapid weight loss suggests the rat is systemically ill rather than an effect of a sub-lethal dose of 192-Saporin.

Please send your targeting questions to:

TargetingTalk@atsbio.com

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: NEUROLOGY INSTRUMENT STAINING WEIGHT GAMETE

Answer: Why the Scientists missed the laboratory presentation — THEY DIDN'T GET THE "MESSAGE"!

WINNERS: Lynn Young, Johnson & Johnson PRD * Bryan Hudson, Washington State Univ * Dr. Richard Robertson, UC Irvine College of Medicine * Bill Goodwin, Univ of Virginia * Key Kang, Raven biotechnologies * Dr. Ruth Stornetta, Univ of Virginia * Carmen C. Diaconu, Institute of Virology * Bob Speth, University of Mississippi * Joseph Menonna, E. Orange VA Medical Center * Dr. Douglas J. Taatjes, Univ of Vermont * Dr. Seto Chice, SUNY HSC at Brooklyn * Dr. Thomas J. Collins, UTMB * Kristen Phend, Univ of North Carolina * Sela James, Catholic Univ of America * Bruce Pappas, Carleton Univ * Heidi Day, Univ of Colorado

Targeting Tools: Featured Products

Targeting the Dopamine Transporter

Advanced Targeting Systems announces the availability of two monoclonal antibodies to the dopamine transporter (DAT). These antibodies have become a standard for work on DAT and have already played a prominent role in the characterization of the dopamine transporter. The two antibodies are rat monoclonals, so they can be excellent for multiple staining protocols.

AB-N18	Anti-DAT-NT	100 micrograms, $250

The first antibody (anti-DAT-NT) is made to the amino terminus of the human dopamine transporter, residues 1-66, an intracellular sequence that is expressed as a fusion protein with glutathione-S-transferase (GST). It gives a very dark band with immunoblotting of the transfected dopamine transporter.[1] This antibody has been used extensively and definitively for immunohistochemistry of DAT, and gave such an excellent signal that it could be used even in cases in which transporter expression was sparse.[2] It gives excellent staining of processes that express DAT,[1] and performs well in staining detection by both light microscopy and electron microscopy.

AB-N17	Anti-DAT-ECD	100 micrograms, $250

The second antibody, and one that has important properties, was raised to a GST fusion protein sequence from the second extracellular loop and is termed anti-DAT-ECD, emphasizing that it recognizes an extracellular domain. It also performs well in immunostaining,[1] though not as excellent as anti-DAT-NT. However, it is able to recognize DAT when injected *in vivo*.

Gangsta isn't usually on the fence about things; this must be about a decision over food, sleep or hassling his sister Ethel.

anti-DAT-SAP (Cat. # IT-25)
Available Individually and as a Kit (with unconjugated saporin, antibody, and control immunotoxin)

SPECIAL INTRODUCTORY PRICING

25 μg $100 ($250)
100 μg $350 ($650)
250 μg $775 ($1375)

Anti-DAT-ECD was used to create an immunotoxin that is highly specific for cells/neurons that express DAT. This immunotoxin is being released coincident with the first publication on its effects (see cover article). There are a number of advantages to using anti-DAT-SAP:

Absolute selectivity for mesencephalic dopamine neurons that express DAT (substantia nigra, pars compacta and ventral tegmental area). 6-OHDA is toxic to all types of catecholaminergic neurons and requires use of uptake inhibitors to minimize damage to noradrenergic and adrenergic neurons: this lesion exactly mimics the dopaminergic lesion of Parkinson's Disease. (There is also some neurochemical evidence that 6-OHDA and MPTP can damage serotonergic neurons.)

Much less traumatic, anti-DAT-SAP works best when injected intraventricularly, which does not involve disturbing the basal ganglia or mesencephalic structures, whereas 6-OHDA has to go directly into the target (substantia nigra or striatum).

The stability of anti-DAT-SAP is great, especially when compared to 6-OHDA which has to be made fresh with ascorbic acid, protected from light, and often kept under nitrogen.

These tools for the study of the dopamine transporter (one of the key proteins in neuronal systems) and dopaminergic neurons (one of the key neuronal subsets in brain systems biology and pathologies) will be important new additions to research and pharmaceutical toolboxes.

References:

1. Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI (1997) Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J Comp Neurol* 388:211-227.
2. Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* 18:2697-2708.

AB-N12	**Anti-GAD65 (B78)**
AB-N13	**Anti-GAD65 (B96)**
AB-N14	**Anti-TSHr (A7)**
AB-N15	**Anti-TSHr (A9)**
AB-N16	**Anti-TSHr (A10)**

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.

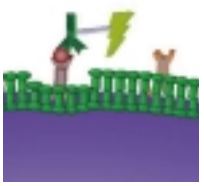
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

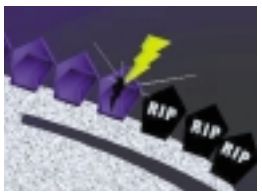
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



Cells which 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 used in science.

SMESTY

CUNDIE

GLOPNOR

ABGERM

CEPILOOM

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

Answer:

HE GAVE THEM...

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by August 30, 2003.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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Targeting Trends

Reporting the latest news in Molecular Surgery

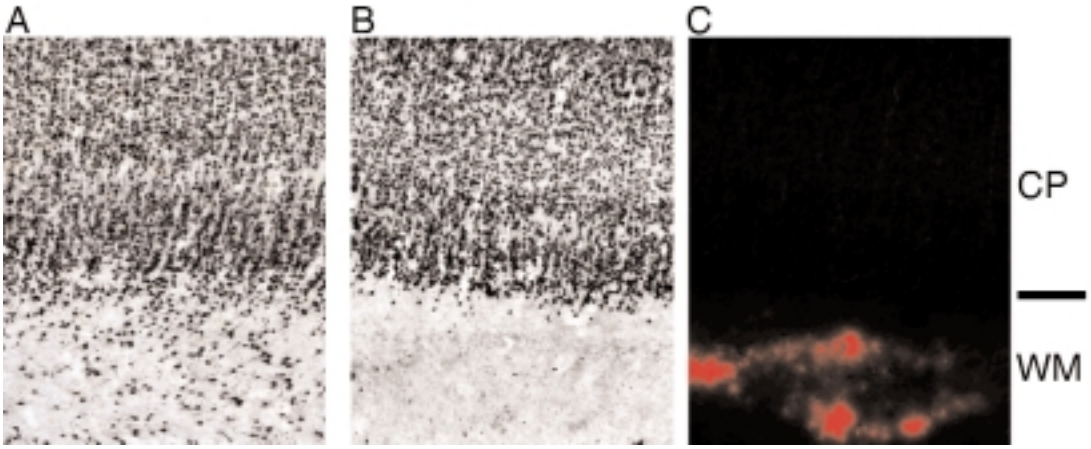
Subplate Neurons and Functional Maturation of Thalamocortical Synapses

Contributed by Dr. Patrick O. Kanold, Dept Neurobiology, Harvard Medical School, Boston, MA 02115. Dr. Kanold summarizes his work with ME20.4-SAP. A complete report was published in Science 301:521-525.

The processing of information in the visual system happens in multiple stages. Retinal neurons in the eye connect to neurons in the lateral geniculate nucleus (LGN), that in turn connect to neurons in the visual cortex. The axonal fibers grow to connect these regions during fetal development. For example, fibers from the LGN grow into visual cortex. Once these LGN axons reach their target area in visual cortex, they start to form synapses with neurons in the area. The axons also start to segregate into the ocular dominance columns (ODCs), stripes of cortex that are connected exclusively to the left eye or to the right eye. The prevailing hypothesis about the formation of ODCs is that they are shaped by activity-dependent competition between afferents from each eye and by selective growth and pruning of axonal projections as a means of refinement. The factors and circuits mediating the competition remain to be elucidated.

During this period of connecting and remodeling, a peculiar group of neurons is present in the developing brain: the subplate neurons. In contrast to other neuronal structures in the brain, this is a

(continued on page 6)



Immunohistochemistry using NeuN (neuron-specific marker) at P21 confirms selective loss of subplate neurons at injection site. Immunotoxin (ME20.4-SAP) was injected into subplate at P7 (1 mg/ml). A: Note the abundance of subplate neurons in the white matter (WM) of the control area of cortex. B: Loss of subplate neurons in white matter after immunotoxin injection. Note the abundance of neurons in the overlying cortical plate (CP) C: Fluorescent microspheres (coinjected with immunotoxin) in the WM indicating injection site (same section as B).

Inside this issue:

Newsletter Highlights

- "Surfboards for Spinal Cords" *(page 2)*
- $2.4M drug devel grant for SP-SAP *(page 2)*
- CCK-SAP binding studies *(page 5)*
- Rat-ZAP, the newest Second Immunotoxin *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Surfboards for Spinal Cords, September 27 in Ocean Beach, CA

Previously titled the Tony Mezzadri Surf Contest, "Surfboards for Spinal Cords" is a surf contest held at the Ocean Beach Pier, with all proceeds from the event to benefit spinal cord injury and disease research in the laboratory of Dr. Mark Tuszynski at University of California, San Diego. For the fifth year, ATS is pleased to be a supporting sponsor of this event. The cause is important and the event is a fun and entertaining day of music, food, and of course, surfing! Check out the action (including the disabled surfers' heat) captured by President Doug Lappi.

ATS Receives $2.4 M Award for SP-SAP Drug Development

ATS has received a Small Business Innovation Research (SBIR) grant from the National Institute of Mental Health (NIMH) under the program announcement for competing continuation awards for pharmacologic agents and drugs for mental disorders. This three-year, $2.4 million award is a continuation of Phase II funding for the development of a drug for the treatment of chronic pain. This new program launched by NIMH will allow ATS to complete toxicology studies and to prepare clinical-grade material for use in human trials. For small businesses like ATS, this latest expansion of the SBIR program provides important support at a time when alternative funding is expensive and difficult to find.

The revolutionary chronic pain drug under development is a targeted toxin called SP-SAP — a patented chemical conjugate composed of the neuropeptide Substance P, and the ribosome-inactivating protein saporin. According to Dr. Lappi, President and CSO, "SP-SAP targets delivery of a toxic compound to only those few and specialized nerve cells that transmit pain messages up the spinal cord to the brain. This precise method allows chronic pain to be permanently stopped without affecting normal pain transmission."

Dr. Lappi explains the urgency and importance of the development of SP-SAP. "Many people suffering from intractable chronic pain have exhausted all of their options. Their quality of life is diminished. We envision in the not too distant future offering a one-time injection that will end the pain. Chronic pain sufferers won't need to take a pill every day. Advanced Targeting Systems has excellent pre-clinical data that leads us to believe that SP-SAP will be safe and effective and compels us to develop SP-SAP for clinical use."

"We are fortunate to have a talented and knowledgeable team of collaborators and advisors," said Denise Higgins, VP of Business Development. "It's always good to have outside, expert input on projects of this magnitude, and in a small company of fewer than ten employees, it's critical."

The collaborative team is as diverse in their backgrounds as their locations. The idea for SP-SAP was first proposed by Ronald G. Wiley, M.D., Ph.D., scientific advisor to ATS and Chief of Neurology at the Veteran's Administration Medical Center, and Professor of Neurology and Pharmacology at Vanderbilt University, Nashville, TN. Initial pain model studies were performed in the laboratories of University of Minnesota pain expert, Dr. Patrick Mantyh, a subcontractor on ATS's SBIR Phase II grant. Results of the use of SP-SAP in chronic pain models was reported in a 1997 *Science* issue. Mantyh's laboratory published a second *Science* article in 1999 to present results demonstrating the long-term elimination of chronic pain with SP-SAP.

"We envision in the not too distant future offering a one-time injection that will end the pain. Chronic pain sufferers won't need to take a pill every day."

Dr. Tony Yaksh, Professor of Anesthesiology and Pharmacology at the University of California, San Diego, is a leading expert on the administration and pharmacology of drugs in the spinal cord and spinal fluid. His associate, Dr. Jeff Allen, completed preliminary toxicology studies with SP-SAP in one of the FDA-required animal models. UCSD will carry out the full toxicology studies with funding from the grant awarded to ATS.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Targeted toxins in pain.

Wiley RG, Lappi DA
Adv Drug Deliv Rev 55(8):1043-1054, 2003

The authors discuss the use of 'molecular neurosurgery' in the study of nociception. Applications using targeted toxins, which include immunotoxins, protein-toxin conjugates, or peptide-toxin conjugates, are illustrated. The authors describe the use of these molecules as research tools, as well as their potential for therapeutics. A helpful table is included that lists neuronal surface markers and class of cells targeted for each targeted toxin. Reagents discussed: CTB-SAP (Cat. #IT-14), IB4-SAP (Cat. #IT-10), OX7-SAP (Cat. #IT-02), 192-Saporin (Cat. #IT-01), ME20.4-SAP (Cat. #IT-15), Anti-DBH-SAP (Cat. #IT-03), Anti-DAT-SAP (Cat. #IT-25), SP-SAP (Cat. #IT-07), Dermorphin-SAP (Cat. #IT-12), Orexin-SAP (Cat. #IT-20), CRF-SAP (Cat. #IT-13), and acetylated LDL-SAP (Cat. #IT-08).

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

Mice lacking the neurokinin-1 (NK-1) receptor are insensitive to opiates in models of drug abuse. To assess what areas of the brain may be involved in this process, the authors used 1.0-μl injections of 1.0 μM SP-SAP (Cat. #IT-07) to eliminate NK-1 receptor-positive neurons in the nucleus accumbens, dorsomedial caudate putamen or amygdala of mice. Only mice with amygdala lesions displayed behavior comparable to NK-1 receptor knockout mice--increase in anxiety-like behavior, reduction in stimulant effect of morphine. These data suggest that the amygdala plays an important role in anxiety behaviors and the response to opiates.

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

Role of subplate neurons in functional maturation of visual cortical columns.

Kanold PO, Kara P, Reid RC, Shatz CJ
Science 301(5632):521-525, 2003

Subplate neurons play a role in the development of connections between the thalamus and cerebral cortex. The authors used 0.5-μl injections of 0.25-1.0 mg/ml of ME20.4-SAP (Cat. #IT-15) to eliminate p75 receptor-positive neurons in the subplate of cats to investigate whether these neurons are involved in the organization and maturation of the visual cortex. This study also uses mouse IgG-saporin (Cat. #IT-18) as a control. (see cover article "Subplate Neurons and Functional Maturation of Thalamocortical Synapses.")

Behavioral patterns under cholinergic control during development: lessons learned from the selective immunotoxin 192 IgG saporin.

Ricceri L
Neurosci Biobehav Rev 27(4):377-384, 2003

The author reviews the effects of 192-Saporin (Cat. #IT-01) neonatal lesions (0.42 μg in each hemisphere) on the cholinergic basal forebrain system in rats. Short-term effects are seen in pups in learning tasks, as well as ultrasound vocalizations. Longer term effects are seen in task-specific behaviors. Data suggest that the extent of these effects are linked to the attentional load of the task. The age of the animal when lesioned may also play a role in the extent of the deficits caused by 192-Saporin; studies show that early in the first week of life is a particularly vulnerable period.

Subtypes of substance P receptor immunoreactive interneurons in the rat basolateral amygdala.

Levita L, Mania I, Rainnie DG
Brain Res 981(1-2):41-51, 2003

SP-SAP (Cat. #IT-07) has been used to lesion substance P receptor (SPr)-expressing neurons in the basolateral amygdala (BLA), but the interneuron subgroups targeted by SP-SAP in the BLA have not yet been defined. The authors used dual-labeling immunofluorescence to examine SPr colocalization with calbindin-D28K, parvalbumin, calretinin, somatostatin, and neuropeptide Y (NPY). All neurons in the BLA that express NPY also express the SPr and therefore SP-SAP, which specifically eliminates SP receptor-positive neurons is a useful tool to study the role of NPY in the BLA.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

A double dissociation between serial reaction time and radial maze performance in rats subjected to 192 IgG-saporin lesions of the nucleus basalis and/or the septal region.

Lehmann O, Grottick AJ, Cassel JC, Higgins GA
Eur J Neurosci. 18(3):651-666, 2003

Using 0.4-μl injections containing 0.4 μg of 192-Saporin (Cat. #IT-01) into either the nucleus basalis magnocellularis, the medial septum/vertical limb of the diagonal band of Broca, or both, the authors examined the contributions of the p75 receptor-positive neurons on cognitive function in rats. Data indicate there is a functional dissociation between the two pathways in attention and memory.

Neonatal 192 IgG-saporin lesion of forebrain cholinergic neurons: focus on the life span?

Pappas BA, Sherren N
Neurosci Biobehav Rev 27(4):365-376, 2003

In this review, the authors discuss the use of 192-Saporin in the investigation of neurodevelopmental disorders, and propose that the effects of these lesions are amplified as the animal ages and experiences normal age-related synapse loss.

Hilar neuropeptide Y interneuron loss in the aged rat hippocampal formation.

Cadiacio CL, Milner TA, Gallagher M, Pierce JP
Exp Neurol 183(1):147-158, 2003

The authors investigate the loss of neuropeptide Y-immunoreactive (NPY-I) interneurons in the dentate gyrus of aged rats. Their results show a loss of a select group of interneurons in these animals. The behavioral as well as structural changes correlated with the results of previous studies on young rats treated with 192-Saporin (Cat. #IT-01). NPY-I neurons may therefore be affected by age-related losses of cholinergic neurons in the basal forebrain.

The role of the septo-hippocampal cholinergic projection in T-maze rewarded alternation.

Kirby BP, Rawlins JN
Behav Brain Res 143(1):41-48, 2003

192-Saporin (Cat. #IT-01) has been used extensively to lesion cholinergic projections to the medial septum from the hippocampal region. It is not yet clear how post-lesion neural regeneration may affect the results. The authors used four 50-ng injections of 192-Saporin to investigate effects prior to any suspected neural regeneration. Significant microglia activation, loss of hippocampal acetylcholinesterase, and a clear inflammatory response were observed; but there was no impairment of spatial working memory.

Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning.

Conner JM, Culberson A, Packowski C, Chiba AA, Tuszynski MH
Neuron 38(5):819-829, 2003

Neuronal plasticity has been associated with normal learning. The authors wished to investigate the role of the cholinergic basal forebrain (CBF) system in learning motor skills. Rats received bilateral 95-ng injections of 192-Saporin (Cat. #IT-01) in either the medial septum, the nucleus basalis magnocellularis, or both. The results indicate that lesioned animals, with many aspects of attention still preserved, are unable to adapt attention to meet the demands of a particular task. The authors conclude that the CBF system may be implicated in learning forms that require plasticity of cortical representations.

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 examine whether afferent discharge produced by nerve injury and central changes in experimental neuropathic pain might interact at the spinal level. Rats were treated with 48 ng of dermorphin-SAP (Cat. #IT-12) and various markers for neuropathic pain were evaluated. The results link several consequences of the post-injury state, including support for increased afferent input as a driving force for neuropathic pain.

Targeting Tests: CCK-SAP in Binding Studies

Our thanks to Drs. Frank Porecca, Victory Hruby and Josephine Lai, Department of Pharmacology, The University of Arizona Health Sciences Center for sharing the results of their studies.

Cholecystokinin (CCK) is widely distributed in the central nervous system and the gastrointestinal tract. The 33-amino acid peptide contains a carboxyl terminal octapeptide sequence Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 which confers the biological activity of CCK, and where the tyrosine residue occurs in sulfated form. This octapeptide, CCK-8(SO_3), has high affinity for the two structurally-defined CCK receptors types, CCK_1 and CCK_2, based on radioligand binding analysis using [^{125}I]CCK8(SO_3) in transfected HEK293 cells that express either the human CCK_1 or human CCK_2 receptors (Table 1). On the other hand, the non-sulfated form of CCK-8 exhibits lower affinity for both CCK receptor types when compared with that of the sulfated form. The non-sulfated form is also moderately selective for the CCK_2 receptors (Table 1).

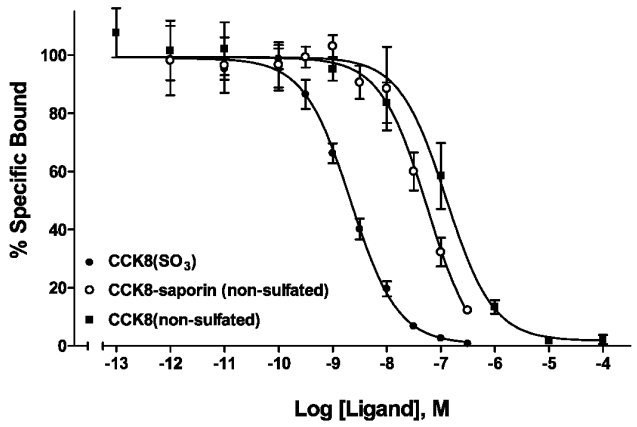


Figure 1. [^{125}I]-CCK-8(SO_3) / Ligand competition in transfected HEK 293 cells that express $hCCK_2$ receptors. Data represent mean ± S.E.M. of three independent experiments. Non-specific binding was defined by the amount of [^{125}I]-CCK-8(SO_3)(specific activity: 1200 μCi/mmol) bound in the presence of 1 μM CCK-8(SO_3).

Table 1

Ligand	Affinity (K_i, nM)	
	$hCCK_1$	$hCCK_2$
CCK8(SO_3) (sulfated)	0.8	1.5
CCK8 (non-sulfated)	800	125
CCK8-saporin (non-sulfated)	ND*	56
* ND; not determined. All data from at least 3 independent determinations.		

Table 1. Affinity of CCK-8 or CCK-8-saporin for the human CCK_1 and CCK_2 receptors based on competitive inhibition of [^{125}I]CCK-8 binding to HEK293 cells that stably express $hCCK_1$ or $hCCK_2$ receptors.

A covalent conjugate of non-sulfated CCK-8 to saporin (CCK-SAP) was synthesized and evaluated for the toxin conjugate's affinity for the human CCK receptors in transfected HEK293 cells (Figure 1 and Table 1). CCK-SAP exhibits similar affinity for the $hCCK_2$ receptors as non-sulfated CCK-8. The affinity of CCK-SAP for the $hCCK_1$ receptors was not determined because, based on the findings seen in the CCK_2 receptors, it is likely that the affinity of CCK-SAP for the CCK_1 receptors would be similar to that of non-sulfated CCK-8, and the cost of such assays would be prohibitive due to the high concentrations of CCK-SAP needed. Conjugation of saporin to CCK-8 does not significantly alter the affinity of CCK-8 for CCK receptors and should therefore be effective in targeted-lesion of CCK_2 expressing cells by CCK-mediated internalization of saporin.

Acknowledgments: The authors thank Dr. Alan Kopin for the generous gift of the cDNAs for $hCCK_1$ and $hCCK_2$, and the technical assistance of Dr. Shouwu Ma and Brian Lew.

Society for Neuroscience
November 8-12, 2003
New Orleans, Louisiana
Booth #640

Experimental Biology
April 17-21, 2004
Washington, DC

Subplate Neurons and Functional Maturation of Thalamocortical Synapses

(continued from page 1)

transient population of neurons. Subplate neurons are the first to mature in the developing cortex but then later die during further development. The axons of the subplate neurons reach into the developing cortex at a very early stage. Later, on the way to the cortex, thalamic axons pass through the subplate and wait there before they grow into the developing cortex. In part because of the brief life span of subplate neurons, very little is known about their function.

However, subplate neurons are in a key, intermediate position to control the flow of information into the developing cortex when first spontaneous (prenatal) and then visual (postnatal) activity are present in visual cortex. The fact that ODCs emerge even before the onset of patterned visual experience (1, 2), poses the question how subplate neurons influence the functional development of cortex. The subplate forms a transient circuit that is required for the development of axonal projections between thalamus and cerebral cortex. When subplate neurons are ablated after the arrival of thalamic inputs to layer 4 (P7-P10), ODCs do not form despite the robust presence of thalamic axons (3). We found that in addition to defects in the anatomical organization of visual cortex, late subplate ablation (using kainic acid or ME20.4-SAP) leads to profound functional deficits (4). Following the ablation, functional orientation maps are disorganized and visual responses are weak (4). Extracellular recordings *in vivo* showed that the orientation tuning of single neurons in visual cortex was weak and that these neurons were only weakly responsive to visual stimuli. *In vitro* slice recordings and *in vivo* current-source density measurements show that subplate ablation results in reduced efficacy of thalamocortical synaptic transmission consistent with the lower expression of GluR1 mRNA expression in layer 4 of the ablated area (4).

We conclude that subplate neurons and their connections are needed not only for the anatomical segregation of thalamic inputs into ODCs, but also for key steps in functional synaptic maturation and remodeling involved in creating the highly tuned responses of adult cortical neurons in all layers.

These observations are noteworthy because the thalamic axons from the LGN are all still present within cortical layer 4, however, they do not strengthen or refine themselves into adult patterns of connections (3, 4). In contrast, transmission from layer 4 to layer 2/3 is unaffected by subplate ablation (4) indicating that, apparently, cortex can continue to mature and develop even when not functionally activated by thalamic inputs.

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1. Crowley JC, Katz LC (2000) Early development of ocular dominance columns. *Science* 290(5495):1321-1324.
2. Crair MC, Horton JC, Antonini A, Stryker MP (2001) Emergence of ocular dominance columns in cat visual cortex by 2 weeks of age. *J Comp Neurol* 430(2):235-249.
3. Ghosh A, Shatz CJ (1992) Involvement of subplate neurons in the formation of ocular dominance columns. Science 255(5050):1441-1443.
4. Kanold PO, Kara P, Reid RC, Shatz CJ (2003) Role of subplate neurons in functional maturation of visual cortical columns. *Science* 301(5632):521-525, 2003.

Archives of *Targeting Trends* are available at www.ATSbio.com Just click on "NEWS"!

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: SYSTEM INDUCE PROLONG BREGMA PICOMOLE

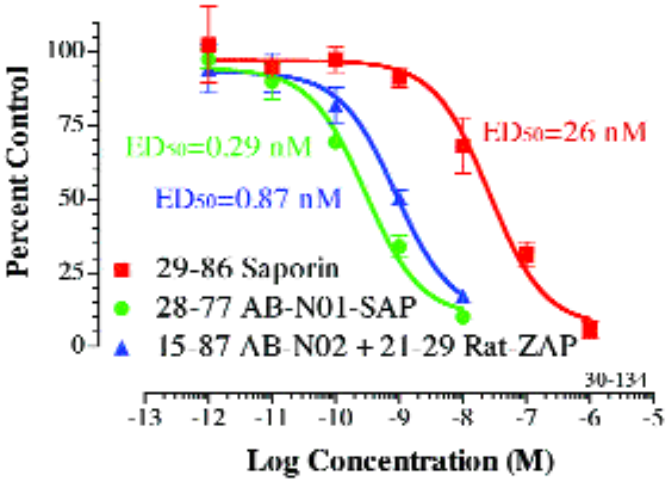
Answer: How Harry trained the new laboratory interns — He gave them SOME POINTERS

WINNERS: Armando Poeppl, Toronto Hospital * Ann Schreihofer, Medical College of Georgia (MCG Physiology) * Jim Fadel, Univ of South Carolina * Bryan Hudson, Washington State University * Joseph Menonna, VA Bio Med Center * Greg Hickey, Massachusetts General Hospital * Bob Speth, University of Mississippi, Dept Pharmacology * Alexsandra Chiez Demet, Univ of CAIrvine

Targeting Tools: Featured Products

Rat-ZAP

Advanced Targeting Systems is pleased to announce the arrival of its newest second immunotoxin, Rat-ZAP. This new screening agent is constructed from goat anti-rat IgG conjugated to saporin. Like the other second immunotoxins, Hum-ZAP, Mab-ZAP and Rab-ZAP, it converts your primary antibody, in this case a rat IgG, into a potent cytotoxic agent for cells that express the antigen of the rat IgG on their surface. The figure on the right shows the power of this second immunotoxin. So whether it's high-throughput screening, or just testing your antibody, this is a potent new tool to determine if your rat antibody internalizes.



NG3 cells were plated at 1000 cells/90 µl/well in a 96-well plate and incubated overnight. Saporin and ABN01-SAP dilutions were made in cell media, and 10 µl was added to each well. Anti-mu p75 was diluted in cell media containing, at a final concentration, 50 ng/10 µl of Rat-ZAP, and 10 µl was added to each well. The plates were incubated 72 hours and developed with MTS/PMS. Data was analyzed by Prism (GraphPad).

Rat-ZAP (Cat. # IT-26)
Available Individually and as a Kit
(with control immunotoxin)

PRODUCT PRICING

25 µg $165 ($215)
100 µg $625 ($825)
250 µg $1450 ($950)

Gangsta is a bit theatrical sometimes. This is his idea of a "screenplay"!

In the near future, ATS will be offering two new cytotoxins specific for interesting cell populations. Both are based on the extreme affinity of ligands for their receptors. The first is **CCK-SAP**, a conjugate between non-sulfated cholecystokinin and saporin. This targeted toxin shows excellent affinity for its receptor, and promises to be an important agent for determination of the systems biology function of CCK receptor-expressing cells, especially neurons. The second is **FGF-SAP**, a conjugate between basic fibroblast growth factor (FGF-2) and saporin. It has a lengthy resume on its efficacy for removal of FGF receptor-expressing cells.

Introducing. . .

Two new Mouse Monoclonal Antibodies

AB-T16	Anti-Histamine	50 microliters, $150
AB-T17	Anti-L-DOPA	50 microliters, $150

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.

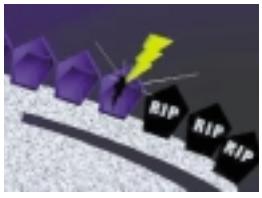
[§]or growth factor, peptide, ligand, or cytokine

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 used in science.

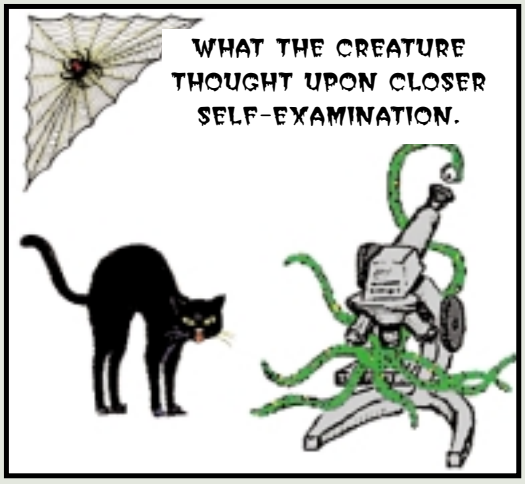
IMEANA

SOXEN

CAMETING

SLIFOS

PREMOLY



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

Answer:

WHAT A ...

Limit one entry per laboratory.

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by November 30, 2003.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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