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Volume 9, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Role of medial septal GABAergic neurons in learning and extinction: Effects of the novel GABA immunotoxin GAT1-SAP

Contributed by Kevin C.H. Pang, Xilu Jiao and Richard J. Servatius
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The medial septum and diagonal band of Broca (MSDB) provide a major afferent pathway to the hippocampus.[1] Cholinergic and GABAergic neurons are the main components of this pathway, but glutamatergic and peptidergic neurons also contribute.[1-5] Damage of MSDB neurons or the septohippocampal pathway impairs learning and memory, and disrupts hippocampal theta rhythm.[6-7] Although the role of cholinergic septal neurons in learning and memory and hippocampal function is well studied, little is known about the contribution of noncholinergic neuronal populations. In previous studies, intraseptal kainic acid was found to preferentially damage noncholinergic MSDB neurons, to drastically reduce hippocampal theta rhythm, and to impair spatial reversal learning.[8-11] The current study was performed to assess the cytotoxic and behavioral effects of a novel GABAergic toxin.

GAT1-SAP is a rabbit antibody to the GABA transporter GAT1 that is conjugated to the ribosome-inactivating protein, saporin. The behavioral effect of GAT1-SAP administration into the MSDB was assessed in two versions of the water maze and in an active avoidance task. In one version of the water maze, the escape platform remained in the same location every day (reference memory). In the second version, the escape platform was moved to a new location every day (working memory). For this study, rats

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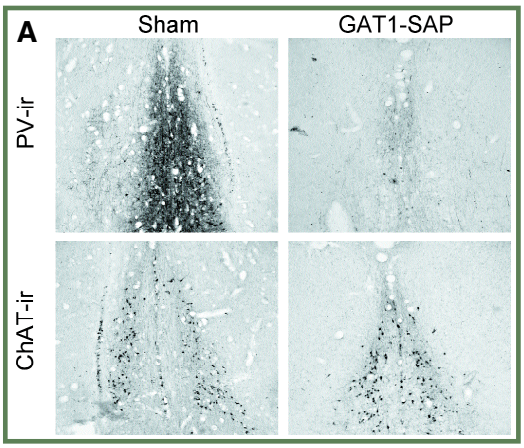

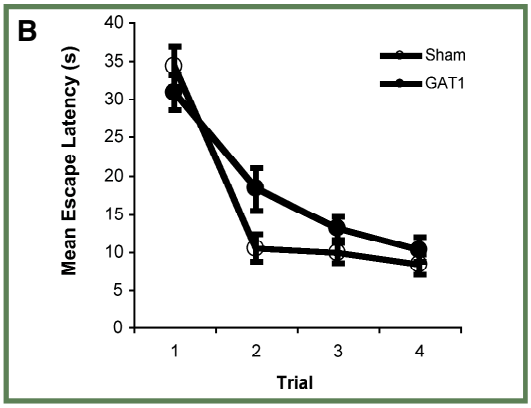

Figure 1.
A. Photomicrographs of the medial septum following a sham (left) or GAT1-SAP (right) lesion. Intraseptal GAT1-SAP reduced the number of GABAergic septohippocampal neurons, as demonstrated by parvalbumin-immunoreactivity (top). In contrast, GAT1-SAP did not remarkably affect the number of cholinergic cells, as visualized by choline acetyltransferase-immunoreactivity (bottom).
B. Within session learning was impaired in the working memory version of the water maze following GAT1-SAP treatment.

Inside this issue:

Newsletter Highlights

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Denise Higgins, Editor

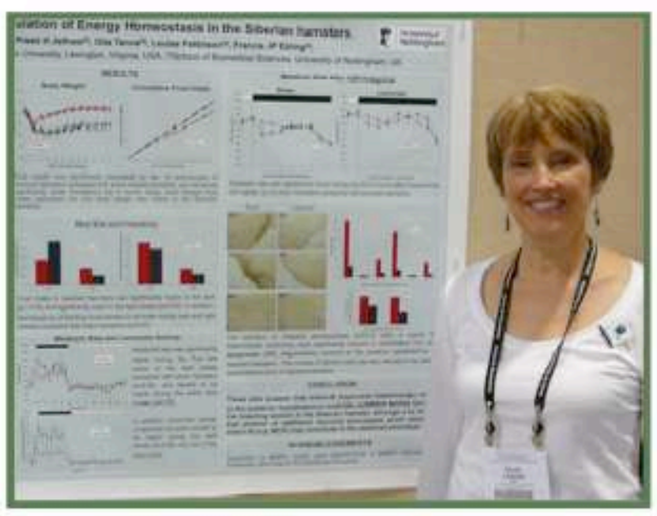
ADVANCED TARGETING SYSTEMS

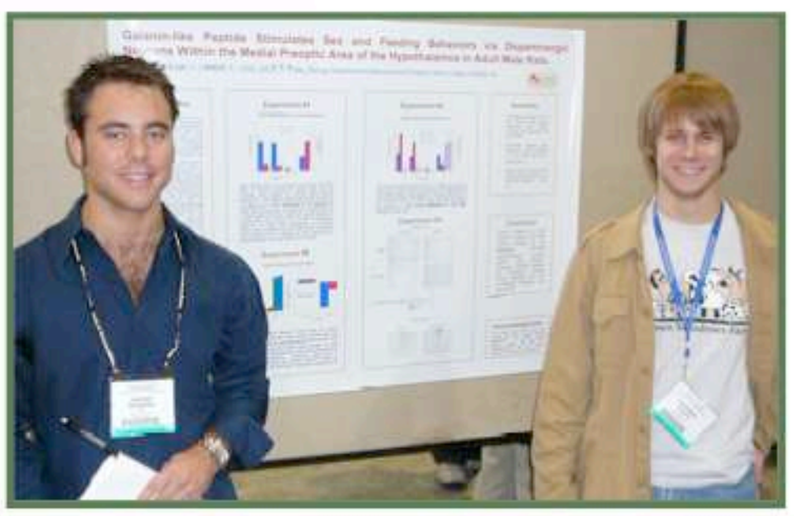
Annual Society for Neuroscience Poster Award

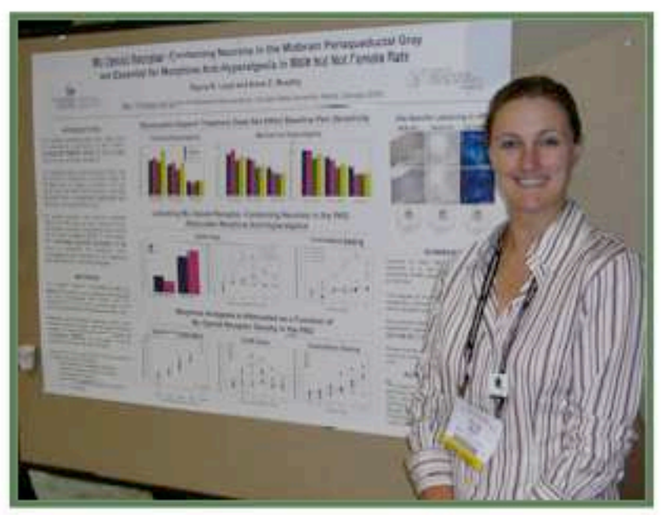
Dr. Kevin Pang and Dr. Douglas Lappi
2007 Poster Award Winner

On the cover of this issue we have the pleasure of presenting to you an article by the winner of this year's Poster of the Year Award at the Society for Neuroscience meeting. This year's winner is Dr. Kevin Pang for his presentation of "Understanding the role of non-cholinergic medial septal neurons in learning and memory: Implications for disease- and aging-related impairments." He studied the relationship between cholinergic and GABAergic neurons in the basal forebrain and their roles in behavior. This poster presented for the first time the much-requested specific GABAergic neurotoxin, GAT1-SAP (Cat. #IT-32) and showed its specificity and what lessons can be learned through its use.

This year there were a number of interesting and innovative uses of saporin toxins that were contenders for the Poster of the Year Award. Helen I'Anson presented







Poster Contributors--Honorable Mention
Left: Helen I'Anson. Center: Vincent Ganapini and Alexander Taylor. Right: Dayna Loyd.

"Histaminergic regulation of energy homeostasis in the Siberian hamster" in collaboration with her co-authors PH Jethwa, GA Tanna, LM Pattinson, and FJP Ebling. This poster discussed the use of orexin-SAP (Cat. #IT-20) in the hypothalami of Siberian hamsters and the resulting increased metabolism and reduced weight with no change in feeding.

Vincent Ganapini and Alexander Taylor collaborated with K Kuper, A Taylor, and GS Fraley on "Galanin-like peptide stimulates feeding and sexual behavior via dopaminergic fibers within the medial preoptic area of adult male rats." They used Anti-DAT-SAP (Cat. #IT-25) in the medial preoptic area to eliminate dopaminergic fibers that resulted in a loss of "all aspects of male sexual behavior" and loss of stimulatory effects of galanin-like peptide.

Dayna Loyd, in collaboration with AZ Murphy presented their work with dermorphin-SAP (Cat. #IT-12) in the ventrolateral periaqueductal gray to lesion MOR-expressing neurons, and found male rats to be less sensitive to morphine while little change was seen in female rats. Their poster was titled "Lesioning mu opioid receptor-containing neurons in the ventrolateral periaqueductal gray attenuates morphine analgesia in male but not female rats."

We congratulate these authors on their fascinating work.

Upcoming Events

Experimental Biology
April 5-9, 2008
San Diego, CA
Booth #1135

Amer Assoc for Cancer Research
April 12-16, 2008
San Diego, CA
Booth #1139

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Conjugation of an anti transferrin receptor IgG3-avidin fusion protein with biotinylated saporin results in significant enhancement of its cytotoxicity against malignant hematopoietic cells

Daniels TR, Ng PP, Delgado T, Lynch MR, Schiller G, Helguera G, Penichet ML
Mol Cancer Ther 6(11):2995-3008, 2007.

The human transferrin receptor (hTfR) is overexpressed in malignant cells. Using Advanced Targeting System's custom biotinylation service, the authors combined an anti-hTfR antibody-avidin fusion protein with biotinylated saporin (Cat. #PR-01, saporin alone), and examined the effect of the combined complex on cancer cells *in vitro*. Although the antibody-avidin fusion protein has an intrinsic cytotoxic effect, the fusion protein-saporin complex was able to overcome the resistance that some cells showed to the fusion protein alone.

Selective immunolesion of cholinergic neurons leads to long-term changes in 5-HT2A receptor levels in hippocampus and frontal cortex

Severino M, Pedersen AF, Trajkovska V, Christensen E, Lohals R, Veng LM, Knudsen GM, Aznar S
Neurosci Lett 428(1):47-51, 2007.

Changes in several neurotransmitter systems, including serotonin and 5HT2A receptors, are associated with early Alzheimer's disease (AD). The authors gave rats intracerebroventricular injections of either 2.5 or 5 μg of 192-IgG-SAP (Cat. #IT-01) then examined both of these systems. 5HT2A receptor levels were markedly decreased in the frontal cortex and markedly increased in the hippocampus of animals lesioned with 5 μg of 192-IgG-SAP. The change in 5HT2A receptor number suggests that the AD effect stems from interaction with the cholinergic system.

The role of the nucleus basalis of Meynert and reticular thalamic nucleus in pathogenesis of genetically determined absence epilepsy in rats: A lesion study

Berdiev RK, Chepurnov SA, Veening JG, Chepurnova NE, van Luijtelaar G
Brain Res 1185(266-274, 2007.

Absence seizures due to epilepsy usually occur during passive behavior. This work investigated the role of the cholinergic nucleus basalis of Meynert (NB) and the reticular thalamic nucleus (RT) in these seizures. Rats received either 75 ng of 192-IgG-SAP (Cat. #IT-01) or the control, mouse IgG-SAP (Cat. #IT-18), into the NB and the RT. Loss of cholinergic neurons in the NB resulted in an increased number of spike-and-wave discharges (SWD), a marker for absence seizures.

Food-elicited increases in cortical acetylcholine release require orexin transmission

Frederick-Duus D, Guyton MF, Fadel J
Neuroscience 149(3):499-507, 2007.

In these experiments the authors examine the hypothesis that orexin fibers from the hypothalamus are necessary for basal forebrain cholinergic system (BFCS) activation in a food restriction model. Rats received 200 ng of orexin-SAP (Cat. #IT-20) into the lateral hypothalamus/perifornical area. Lesioned animals that were also deprived of food displayed increased feeding latency when presented with food. This and other data indicate orexin in the BFCS is involved in attention to stimuli associated with homeostatic challenges.

Lesions of the basal forebrain impair reversal learning but not shifting of attentional set in rats

Tait DS, Brown VJ
Behav Brain Res 187(1):100-108, 2008.

The authors compared specific lesions of the basal forebrain using 192-IgG-SAP (Cat. #IT-01) with non-specific lesions generated by ibotenic acid. Rats were given 0.12 μg per 0.5 μl bilateral injections of 192-IgG-SAP. The treated animals were then tested in food reward tasks involving two-choice discriminations and reversal of stimulus-reward. Animals with specific lesions did not show impairment with any of the tasks suggesting that non-cholinergic neurons are involved in reversal learning. This work also demonstrates the similarities between monkey and rodent basal forebrain function.

Amyloid beta protein modulates glutamate-mediated neurotransmission in the rat basal forebrain: involvement of presynaptic neuronal nicotinic acetylcholine and metabotropic glutamate receptors

Chin JH, Ma L, MacTavish D, Jhamandas JH
J Neurosci 27(35):9262-9269, 2007.

This work focused on the effect of amyoid beta on glutamate-mediated neurotransmission in the diagonal band of Broca. Using neurons identified by staining with Cy3-labeled 192-IgG (Cat. #FL-01, 5 μl of 1:1 diluted antibody injected into the left and right ventricle) the authors monitored the response to amyloid beta by measuring excitatory

(continued on page 4)

Targeting Topics:

(continued from page 3)

postsynaptic currents via whole-cell patch-clamp recordings. The results suggest that glutamate neurotransmission might be vulnerable to Alzheimer's disease, and may also be a therapeutic target.

Effects of saporin-induced lesions of three arousal populations on daily levels of sleep and wake

Blanco-Centurion C, Gerashchenko D, Shiromani PJ
J Neurosci 27(51):14041-14048, 2007.

Orexin neurons in the basal forebrain, tuberomammillary nucleus (TMN), and locus ceruleus (LC) are thought to regulate arousal. Rats were injected with 2 or 3 of the following targeted conjugates: anti-DBH-SAP (Cat. #IT-03), 0.25 μl bilateral injections of 1 μg/μl into the LC; orexin-SAP (Cat. #IT-20), 0.25 μl injection of 0.25 μg/μl into the TMN; 192-IgG-SAP (Cat. #IT-01), 3 μl injection of 2 μg/μl into the lateral ventricle. Small differences were observed in sleep architecture, but the data does not support the traditional hypothesis that these 3 areas of the brain are essential links in the control of wake levels.

Elimination of rat spinal substance P receptor bearing neurons dissociates cardiovascular and nocifensive responses to nicotinic agonists

Khan IM, Wart CV, Singletary EA, Stanislaus S, Deerinck T, Yaksh TL, Printz MP
Neuropharmacology [Epub Oct 17](2007.

Nocifensive behavior and cardiovascular responses due to nicotinic agonists may be sustained by substance P positive primary afferents. Rats received 10 μl intrathecal injections of 10 μM SP-SAP (Cat. #IT-07), unconjugated saporin (Cat. #PR-01) was used as a control. Lesioned animals displayed reduced nocifensive response to nicotinic agonists, but cardiovascular responses were not changed. Tachycardia and pressor responses were enhanced upon administration of cytisine and epibatidine.

Respiratory plasticity in response to changes in oxygen supply and demand

Bavis RW, Powell FL, Bradford A, Hsia CCW, Peltonen JE, Soliz J, Zeis B, Fergusson ED, Fu Z, Gassmann M, Kim CB, Maurer J, McGuire M, Miller BM, O'Halloran KD, Paul RJ, Reid SG, Rusko HK, Tikkanen HO, Wilkinson KA
Integ and Comp Biol 47(4): 532-551, 2007

This paper covers data presented at the First Annual Congress of Respiratory Biology. One of the subjects discussed is the use of SP-SAP (Cat. #IT-07) to elucidate the role of central chemoreceptors in the nucleus tractus solitarius during ventilatory acclimitization to hypoxia.

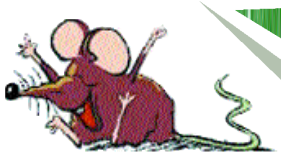
Please visit www.ATSbio.com to see a complete list of references.

Targeting Teaser Winners

The solution to the puzzle was:

Across:
1. SAP
3. CRICK
5. LANOLIN
7. BASEBOARD
9. ELASTIC
10. ROUGE
11. RED

Down:
1. SOLUBLE
2. PANES
3. CALIBRATE
4. ION
6. INDUCED
8. ALTER
9. EMU



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

WINNERS: Mason Hartman- Nordstrom, Men's Shoe Dept, Clackamas, OR * YanYan Hong- City of Hope Natl Med Center, Surgical Research Dept, Duarte CA * Peter Yuen-NIH, NIDDK, Bethesda MD * Angela Repoli- Panacea Pharmaceuticals Inc, Gaithersburg MD * Michael Garrick- SUNY, Dept of Biochemistry, Buffalo NY * Thea Marlinga- Libertyville, IL * Vivian Lee- Medical College of Wisconsin, Dept of Pediatrics, Milwaukee WI * Bruce Pappas- Carleton University, Ottawa, ON CANADA * Seto Chice- SUNY HSC at Brooklyn, Brooklyn NY * Robert Speth- University of Mississippi, School of Pharmacy, University MS

Targeting Talk

by Dr. Douglas Lappi

Secondary Conjugates

Q: I'm using your secondary conjugate Mab-ZAP and it's not killing my cells.

A: Are you following the protocol on the data sheet? It's described in detail in the article by Kohls *et al.*[1]

Q: No, I'm doing flow cytometry. I have 70,000 cells per well. I mix Mab-ZAP with my primary antibody and add it. When I count the cells, there is no decrease.

A: That's a lot of cells per well. We use between 500 and 2500 over a 72-hour period and then develop with MTS.

Q: My cells grow very slowly. I didn't see anything after 72 hours.

A: If your cells are slow-growing, you may want to wait a little longer to develop the assay, because the whole metabolism process is slowed. This is a weakness of the MTS system--you have to have a certain number of cells in the end in the control cells to get a decent reading on your plate reader.

In this case, you might want to try a more sensitive assay such as protein synthesis inhibition. You can use protein or DNA synthesis inhibition with radiolabeled leucine or thymidine.

1. Kohls MD, Lappi DA (2000) BioTechniques 28(1):162-165.

Reducing Agents

Q: I have a question about what solutions might be incompatible with theconjugated saporins. We have done an experiment where we injected a mixture of saporin conjugate (same batch we've used in previous studies here) and a cocktail of 5,7-dihydroxytryptamine and 6-hydroxydopamine (in 0.1% ascorbic acid) to try to deplete multiple neurotransmitters.

The way we did this was to prepare both solutions at double strength and to mix them immediately before loading the syringe and placing the injections. So the final solution has 0.05% ascorbate, 0.01 ug/ul saporin conjugate, and I think 6 ug/ul 5,7-DHT and 4 ug/ul 6-OHDA.

Anyway, we are doing the histology now and the cholinergic lesion didn't work. I'm wondering whether the ascorbic acid might have either damaged the conjugation of the saporin to the antibody, or have inactivated the saporin molecule itself somehow.

A: You have well-described what the problem is. A reducing agent will inactivate the toxin, and of, course, ascorbic acid is a potent reducing agent. We have now decided, because of your experience, to put a line in the data sheet to caution people. This is the first report of this happening in nearly 14 years of business, so it just had not been an issue. That was a lack of foresight on our part.

Role of medial septal GABAergic neurons in learning and extinction

(continued from page 1)

were tested in the reference memory version prior to the working memory version. Another group of rats was assessed in a lever press avoidance task. In this task, a tone (warning signal) was presented at the start of a trial. If a lever press occurred less than sixty seconds from the start of the trial, the warning signal was terminated and an intertrial interval signaled by a flashing light began with a duration of three minutes; this constituted an avoidance response. If a lever press did not occur prior to sixty s from the start of the trial, intermittent foot shock (0.5-second shock every three seconds) was delivered and continued until a lever press or five minutes occurred. Either lever press or the end of the five-minute duration resulted in termination of the warning signal and foot shock and the start of the intertrial interval. A session consisted of twenty trials, each session occurring three times a week. Acquisition of the lever press avoidance procedure was followed by extinction sessions in which the foot shock was not delivered. Following behavioral testing, immunocytochemistry was performed to assess the damage produced by GAT1-SAP.

Intraseptal GAT1-SAP reduced the number of GABAergic septohippocampal neurons containing the calcium-binding protein, parvalbumin (Figure 1A).[2] The number of cholinergic neurons, identified by the presence of choline acetyltransferase, was not markedly affected. Behaviorally, GAT1-SAP did not alter performance on the reference memory version of the water maze (data not shown), but did impair learning in the working memory version (Figure 1B). The pattern of results was similar to that following MSDB damage with kainic acid.[8] In the avoidance procedure, GAT1-SAP did not alter acquisition (Figure 2) but impaired extinction of the avoidance response (Figure 2). Overall, the results support the view that damage of GABAergic MSDB neurons enhances proactive interference and perseveration, possibly by interfering with hippocampal theta rhythm.[12]

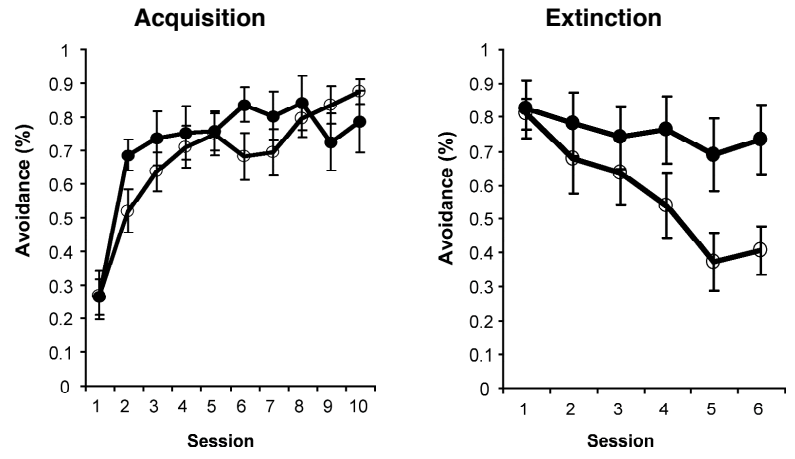


Figure 2. Extinction but not acquisition of an active avoidance response was impaired by intraseptal GAT1-SAP.

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2. Freund TF (1989) GABAergic septohippocampal neurons contain parvalbumin. Brain Res 478(2):375-381.
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9. Malthe-Sørenssen D, Odden E, Walaas I (1980) Selective destruction by kainic acid of neurons innervated by putative glutamergic afferents in septum and nucleus of the diagonal band. *Brain Res* 182(2):461-465.
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Targeting Tools: Featured Products

Stimulate Instead of Eliminate

SP-CTA (Cat. #IT-39) is an exciting new tool for use in the research of neurokinin (NK-1) receptor-expressing cells of the central nervous system. A conjugate of the substance P molecule and the catalytic subunit of Cholera toxin, this product can be used very effectively *in vivo* for increasing sensitization of these neuronal cells. Selectively targeting the NK-1r-expressing cells with the substance P moiety allows the researcher to stimulate only the cells of interest by amplifying their cAMP production with the CTA, without altering the neighboring cells. This effect lasts for a few days, and gives the researcher an opportunity to study behaviors such as those related to the perception of pain or the control of breathing. See Figure 1 and Cover article of *Targeting Trends Vol. 8, Iss. 4.*

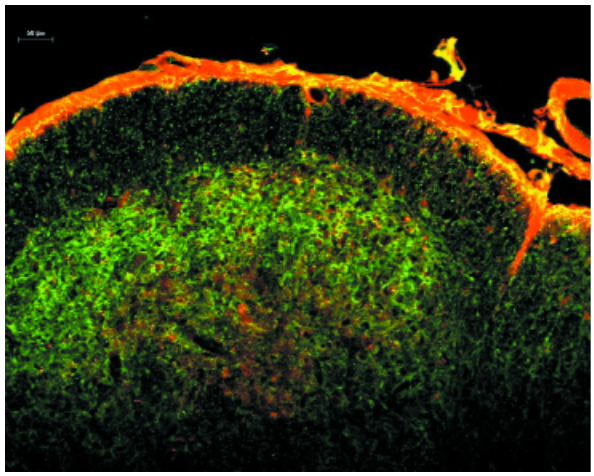

Figure 1. Immunohistochemical localization of SP-CTA uptake in the dorsal horn of the cervical spinal cord. SP-CTA (10 μg) was injected intracisternally into rats via a percutaneous puncture under isoflurane anesthesia. One hour later the animals were euthanized and sections (20 μ) of the brain stem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

Anti-AChR (mAb 35) Rat Monoclonal

Species Reactivity: Human, Rat, Mouse, Rabbit

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

Reference

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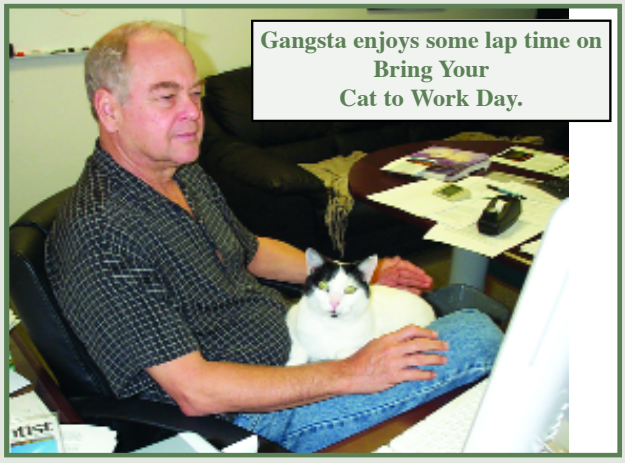

Targeting FGF Receptors

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

References

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Targeting Technology

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

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

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

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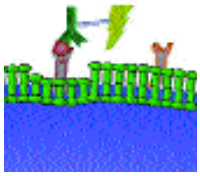
[§]or anything recognized on the cell surface and internalized.

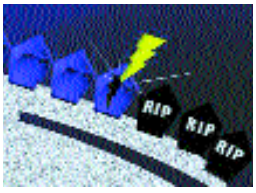
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

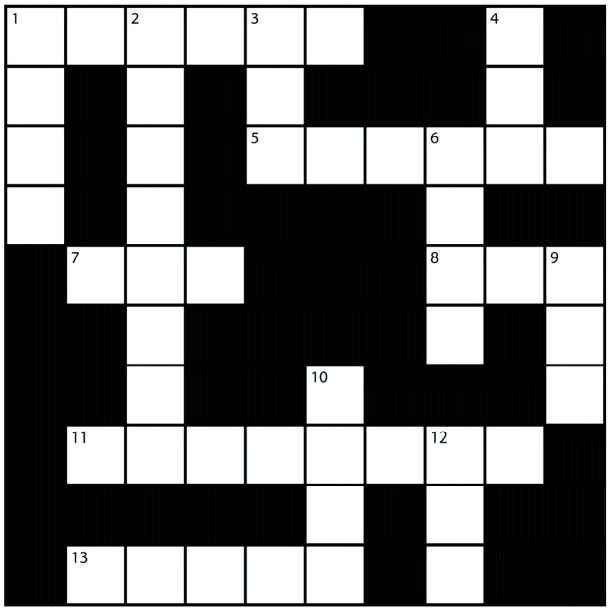
The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser



Across
1. to aim for
5. an in crease in size
7. org. that monitors air and water
8. lower appendage
11. chemical componen or a small partt
13. poisonous substance

Down
1. cylindrical container
2. structure on surface of cell
3. unit of energy
4. Gangsta
6. reservoir for liquid
9. colloid combined with dispersion medium
10. to daze
12. slippery mineral substance

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Limit one entry per laboratory.

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2. Fax in this entire page with the correct solution by February 29, 2008
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See last quarter's winners, page 5.

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Targeting Trends

Reporting the latest news in Molecular Surgery

Selective lesion of basal forebrain cholinergic neurons in mice with the mu p75-saporin immunotoxin: Neuroanatomy and behavior

Contributed by Pierre-Henri Moreau, Brigitte Cosquer, Hélène Jeltsch, Jean-Christophe Cassel, Chantal Mathis
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The basal forebrain cholinergic neurons (BFCNs) are dramatically affected in several neurodegenerative diseases such as Alzheimer's disease or Rett syndrome. The characterization of the behavioral consequences of selective BFCN lesions is necessary to study the implication of these neurons in cognitive functions. Until recently, this model was not available in mice, despite the growing interest in this species, due to the creation of a wide variety of genetically modified mouse lines modeling neurodegenerative diseases. The first version of a new cholinergic immunotoxin, mu p75-SAP, induced specific lesions of the BFCNs associated with dramatic memory performance deficits, but it also showed side effects and poor survival rates (Berger-

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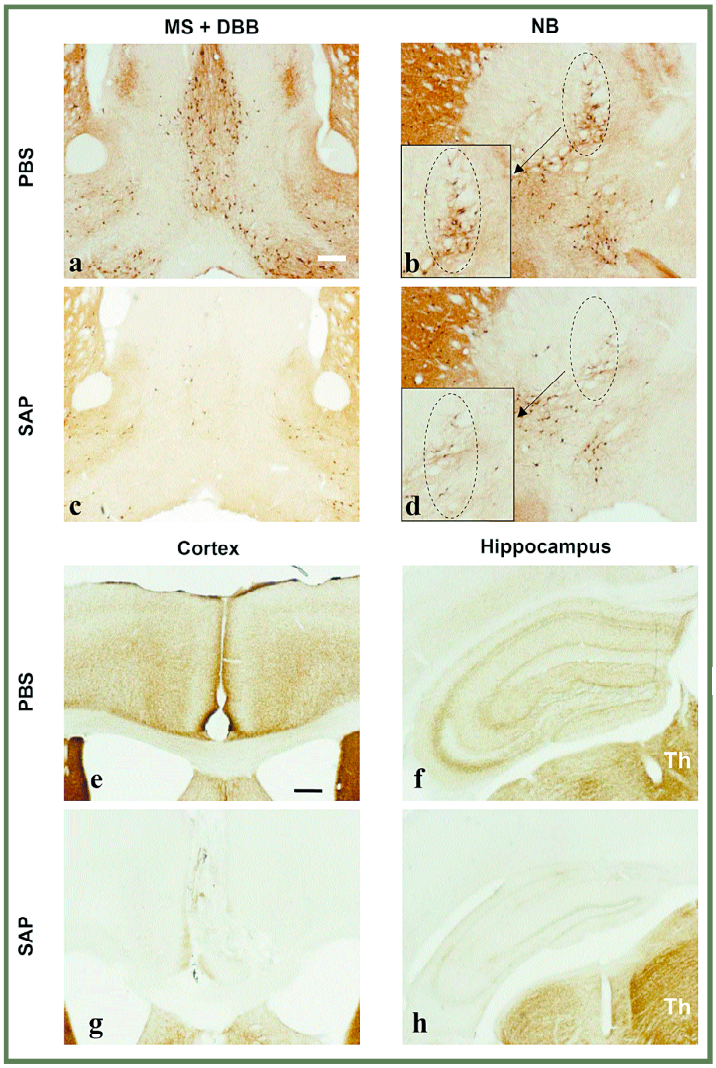


Figure 1. Micrographs of brain coronal sections of mice treated with PBS or mu p75-saporin (SAP). In lesioned mice, the number of ChAT-positive neurons is dramatically reduced in the medial septum (MS) and the diagonal band of Broca (DBB) (a,c) and in the nucleus basalis (NB) (b,d). AChE staining is massively depleted in the cortical mantle (e,g) and the hippocampus (f,h), but not in the thalamus (Th). Scale bar = 200 μm.

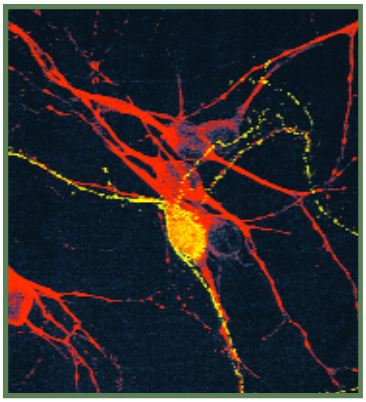
Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

ATS Licenses SP-SAP to Advanced Pain Therapeutics



Substance P receptor-positive neuron eliminated with SP-SAP

Advanced Targeting Systems is pleased to announce that it has licensed its Substance P receptor-targeted chronic pain drug (SP-SAP). Advanced Pain Therapeutics, LLC (APT) obtained exclusive, worldwide rights to develop, manufacture, use, and sell SP-SAP for the treatment of severe chronic pain. Cato Research, a global contract research and development organization, will provide CRO services to the new company.

SP-SAP is a single-dose, non-opioid, substance P receptor-targeted treatment designed to specifically bind to and eliminate a subset of neurons that send the chronic pain signal to the brain. Preclinical studies in animal models have shown that SP-SAP eliminates chronic pain without disrupting other sensory modalities or motor function and is well tolerated.

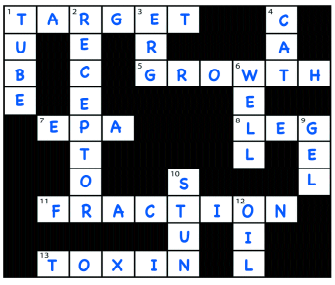
"When we partner early, as we have here, we can make a major difference in the overall development of promising drug candidates such as SP-SAP," said Lynda Sutton, COO of Cato Research and CEO of Advanced Pain Therapeutics. "The flexible, broad-based relationships among APT, ATS, and Cato Research position us well to execute our innovative drug development model."

Denise Higgins, Vice President of ATS, affirms that this arrangement allows for a unique synergy between the companies. "We are enthusiastic about working with APT and Cato Research to help address the under-served chronic pain population. Our preclinical work with SP-SAP makes us very optimistic about the impact this innovative treatment can have for those who are suffering."

Chronic disease states and tissue damage can lead to chronic pain. In particular, terminally ill patients often experience severe, chronic pain due to advancement of disease or unwanted side effects of treatment. Although in many cases, standard treatments, such as opioids, can control pain, there is a significant subset of patients who cannot find relief through standard care. Use of opioids can also be associated with unwanted, severe side-effects. In these cases, sedation or cordotomy may be a patient's only option for pain relief. Hence, severe chronic pain represents a serious, poorly met medical need. SP-SAP offers a novel approach to target a specific set of neurons involved in chronic pain and as such, has the potential to revolutionize the way severe chronic pain is treated.

Targeting Teaser Winners

The solution to the puzzle was:



Across:

1. TARGET
5. GROWTH
7. EPA
8. LEG
11. FRACTION
13. TOXIN

Down:

1. TUBE
2. RECEPTOR
3. ERG
4. CAT
6. WELL
9. GEL
10. STUN
12. OIL

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

WINNERS: Valery Nelson, Panacea Pharmaceuticals, Gaithersburg MD * Barry Marguiles, Towson University, Towson MD * Seto Chice- SUNY HSC at Brooklyn, Brooklyn NY

Upcoming Events

Experimental Biology
April 5-9, 2008
San Diego, CA
Booth #1135

Amer Assoc for Cancer Research
April 12-16, 2008
San Diego, CA
Booth #1139

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neuroanatomical and behavioral effects of a novel version of the cholinergic immunotoxin mu p75-saporin in mice

Moreau PH, Cosquer B, Jeltsch H, Cassel JC, Mathis C
Hippocampus [Epub Feb 27], 2008.

192-IgG-SAP (Cat. #IT-01) has been used for over a decade to examine the cholinergic system in the basal forebrain of rats. Establishing the same reagent for mice has been problematic. Here the authors describe the use of a mouse-specific lesioning agent, mu p75-SAP (Cat. #IT-16). After deciding on a dosage of 0.4 μg administered in the form of bilateral intracerebroventricular injections, mice were lesioned and tested. Lesioned animals displayed increased locomotor activity, and spatial learning and memory deficits, with minimal side effects. (see cover article)

Selective impairment of the cerebellar C1 module involved in rat hind limb control reduces step-dependent modulation of cutaneous reflexes

Pijpers A, Winkelman BH, Bronsing R, Ruigrok TJ
J Neurosci 28(9):2179-2189, 2008.

The cerebellar cortex is arranged in a series of modules. Elucidation of module-specific function has been difficult because of the closely arranged structure of these modules. Here the authors lesioned the C1/C3 hindlimb module of the rat with CTB-SAP (Cat. #IT-14). Rats received 75-125 ng injections of CTB-SAP into the C1 zone of the copula pyramidis or the paramedian lobule of the right cerebellar hemisphere. C1-injected animals displayed marked diminishment of cutaneously induced reflexes with no significant impact on walking or stepping pattern.

Septal grafts restore cognitive abilities and amyloid precursor protein metabolism

Aztiria E, Cataudella T, Spampinato S, Leanza G
Neurobiol Aging [Epub Feb 5], 2008.

Although cholinergic loss and the presence of ß-amyloid plaques are well documented in Alzheimer's disease, it is unknown whether restoration of regulatory cholinergic inputs affects *in vivo* β-amyloid precursor protein (APP). 5 μg of 192-IgG-SAP (Cat. #IT-01) was split between the lateral ventricles of rats. 6 months post-surgery the animals were implanted with cholinergic-rich septal tissue grafts. Grafted animals exhibited normal or near-normal levels of APP. APP levels, as well as improved spatial navigation performance, correlated strongly with graft-induced cholinergic changes.

The pedunculopontine tegmental nucleus and the nucleus basalis magnocellularis: do both have a role in sustained attention?

Rostron CL, Farquhar MJ, Latimer MP, Winn P
BMC Neurosci 9:16, 2008.

This study provided further investigation into the role of the pedunculopontine tegmental nucleus (PPTg) in control of sustained attention. Rats were given 0.13 μg injections of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis. The immunotoxin-treated animals were compared to animals receiving ibotenate injections into the PPTg. Results suggest that ibotenate lesions cause impaired selection of conditioned response as shown by an increase in unconditioned behaviors. 192-IgG-SAP treated animals exhibited difficulty obtaining successful lever presses linked to attention.

Spinal mu-opioid receptor-expressing dorsal horn neurons: role in nociception and morphine antinociception

Kline RHt, Wiley RG
J Neurosci 28(4):904-913, 2008.

The authors used Dermorphin-SAP (Cat. #IT-12) to investigate the function of spinal cord mu-opioid receptor (MOR)-expressing dorsal horn neurons in nociception and morphine analgesia. Rats were treated with 500 ng intrathecal injections of Dermorphin-SAP; 500 ng of Blank-SAP (Cat. #IT-21), and up to 1 μg of Saporin (Cat. #PR-01) were used as controls. The data indicate that MOR-expressing dorsal horn neurons are necessary for morphine action and play a role in nocifensive responses to persistent pain in the formalin test.

Basal forebrain and saporin cholinergic lesions: the devil dwells in delivery details

Kalinchuk AV, Porkka-Heiskanen T, McCarley RW
Sleep 29(11):1385-1387; discussion 1387-1389, 2006.

The authors of this commentary discuss results presented by Blanco-Centurion *et al*. (*J Neurosci* 26: 8092-8100, 2006). The topic is the role of adenosine in the basal forebrain in the control of sleep homeostasis. Discussion covers the potential differences found when 192-IgG-SAP (Cat. #IT-01) is administered locally as compared to an intracerebroventricular injection.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Cholinergic Deafferentation of Prefrontal Cortex Increases Sensitivity to Cross-Modal Distractors during a Sustained Attention Task

Newman LA, McGaughy J
J Neurosci 28(10):2642-2650, 2008.

The authors injected 5 ng of 192-IgG-SAP (Cat. #IT-01) into the prefrontal cortex of rats to investigate the effect of cholinergic loss on distractors to attentional demand. Where all animals experienced impaired performance in the presence of visual distractions, lesioned animals were more sensitive to auditory distractions. While these results indicate compromised top-down processing, lesioned animals showed improved performance in bottom-up processing, possibly caused by a shift in circuit dynamics after the lesion.

Effects of ibotenate and 192-IgG-saporin lesions of the nucleus basalis magnocellularis/substantia innominata on spontaneous sleep and wake states and on recovery sleep after sleep deprivation in rats

Kaur S, Junek A, Black MA, Semba K
J Neurosci 28(2):491-504, 2008.

The caudal basal forebrain of rats was lesioned with 0.26-μg bilateral injections of 192-IgG-SAP (Cat. #IT-01) in order to examine the role of this area of the brain in several facets of sleep behavior. The results suggest that cholinergic neurons and non-cholinergic neurons in the basal forebrain play different, but important roles in non-rapid eye movement sleep and EEG delta power after sleep loss. Non-cholinergic basal forebrain neurons inhibit delta waves, whereas cholinergic neurons promote wakefulness.

Elimination of rat spinal substance P receptor bearing neurons dissociates cardiovascular and nocifensive responses to nicotinic agonists

Khan IM, Wart CV, Singletary EA, Stanislaus S, Deerinck T, Yaksh TL, Printz MP
Neuropharmacology 54(2):269-279, 2008.

The intrathecal (IT) administration of nicotinic agonists produces both nocifencive behavior and cardiovascular responses. In this work the authors treated rats with 10 μl of 10-μM SP-SAP (Cat. #IT-07) IT injections; 10 μl of 10-μM Saporin (Cat. #PR-01) was used as a control. Lesioned animals displayed a reduction in response to all nicotinic agonists, but cardiovascular responses to IT nicotine were left intact. The results indicate subunit-specific interactions between the NK-1 receptor and nicotinic receptor systems.

Reactive oxygen species generation by the ethylene-bis-dithiocarbamate (EBDC) fungicide mancozeb and its contribution to neuronal toxicity in mesencephalic cells

Domico LM, Cooper KR, Bernard LP, Zeevalk GD
Neurotoxicology 28(6):1079-1091, 2007.

This work explores the mechanisms of neuronal damage associated with the ethylene-bis-dithiocarbamate fungicide mancozeb (MZ). In order to obtain a purified rat mesencephalic culture, the authors treated neuronal cultures with Mac-1-SAP (Cat. #IT-33) at a final concentration of 2 μg/ml. The microglia-free cultures did not display attenuated reactive oxygen species (ROS) production when treated with MZ. The data suggest that microglia are not required for ROS production in the presence of MZ.

Brainstem catecholaminergic neurons modulate both respiratory and cardiovascular function

Li A, Emond L, Nattie E
Adv Exp Med Biol 605:371-376, 2008.

The authors examined the role of brainstem catecholamine (CA) neurons in various aspects of breathing and chemoreception. Rats received 5-μg injections of anti-DBH-SAP (Cat. #IT-03) into the 4th ventricle; mouse IgG-SAP (Cat. #IT-18) was used as a control. This method of lesioning left the CA neurons in the peripheral nervous system intact. Lesioned animals displayed a constant decrease in breathing frequency, reduced response to CO_2, and increased variability of breathing during REM sleep. Inhibitory cardiovascular effects were also seen.

Targeting Talk: Cytotoxicity Assays

by Dr. Douglas Lappi

One of the tests you can use to test your targeting agent for internalization is the *in vitro* Cytotoxicity Assay. Protocols to assist in preparing for, executing and interpreting results are now posted on our website. From the Home page (www.ATSbio.com) click on Protocols.

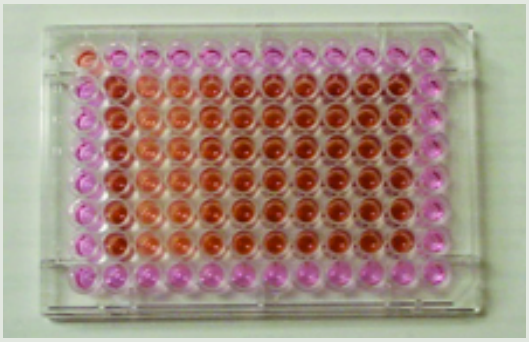
There are several protocols available.

1. ***Preparing for a Cytotoxicity Assay using Secondary Conjugates***. *This protocol will be helpful when using our secondary antibody-saporin conjugates with your primary antibody. These include Anti-M-ZAP (Cat. #IT-30), Goat-ZAP (Cat. #IT-36), Hum-ZAP (Cat. #IT-22), Mab-ZAP (Cat. #IT-04), Rab-ZAP (Cat. #IT-05), and Rat-ZAP (Cat. #IT-26).*

2. ***Preparing for a Cytotoxicity Assay using Streptavidin-ZAP***. *This protocol will be helpful when using our streptavidin-saporin conjugate with your biotinylated targeting agent (peptide, ligand, cytokine, growth factor, antibody, etc.).*

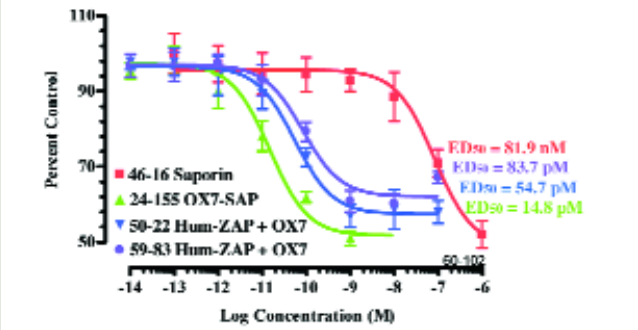
3. ***Concentration Calculation: Convert molarity to mg/ml and mg/ml to molarity.*** *This protocol will help in determining the correct amount of material to use in your assay. There is also a link to an Online Calculator.*

4. ***Cytotoxicity Assay for Targeted Toxins* in vitro.** *This protocol includes photos of what your plates should look like during the assay process. It takes five days to complete this assay. Start on a Monday and develop on Friday. There are many factors that go into a successful cytotoxicity assay. This protocol should help you design and execute appropriately.*



Day Five. Lighter-colored wells signify dead cells. Your targeted toxin is working.

5. ***Preparing Cytotoxicity Data***. *This protocol will give an example of how to process the data from a Cytotoxicity Assay. ATS uses SOFTMax Pro software connected to a plate reader to determine the A490 value. Then we import this data into Prism software (GraphPad) to conduct further data analysis. Here is a figure generated with Prism.*



This graph gives important information about how the potency of your targeted toxin. The ED50 is the Median Effective Dose (produces desired effect in 50% percent of population). The lower this number is, the more potent the targeted toxin.

We hope these protocols will be helpful to you in your research. If there are additional protocols or tutorials we can provide, please do not hesitate to ask.

Selective lesion of BFCN with mu p75-SAP

(continued from page 1)

Sweeney *et al.*, 2001; Hunter *et al.*, 2004). Therefore, as soon as the improved version of mu p75-saporin was released by ATS, we characterized the neuroanatomical and behavioral effects of this new cholinergic neurotoxin.

Mu p75-saporin was administered bilaterally in the cerebral ventricles of male C57BL/6J at the dose of 0.4 μg/mouse. At this dose, all treated mice survived and none of them showed abnormal loss of weight or epileptic-like episodes as obtained with higher doses or reported with the previous version of the toxin. The loss of choline acetyltransferase-immunoreactive neurons was more pronounced in the medial septum (-82%, see figure 1) and the diagonal band of Broca than in the nucleus basalis (-55%). The cholinergic specificity of the lesion was suggested by preserved parvalbumin immunostaining. The hippocampus and several regions of the cortical mantle exhibited a marked drop in the levels of acetylcholinesterase-positive staining. This suggests that septo-hippocampal and basal-cortical projections of the BFCNs underwent massive degeneration. As opposed to the cholinergic toxin 192 IgG-SAP used in rats, mouse Purkinje cells remained undamaged by mu p75-saporin as suggested by preserved anti-calbindin immunostaining in the cerebellum (Traissard *et al.*, 2007). The lesion of the BFCNs affected both locomotor activity as well as learning and memory performances. Nocturnal, and to a lesser extent, diurnal locomotor activity was increased in lesioned mice. The rate of acquisition of a water-maze reference memory task was significantly slower in mice treated with mu p75-saporin (see figure 2). Acquisition performance was also affected in the Barnes maze as suggested by an increase in the total number of holes and in the latency to find the target hole. Retention performance of lesioned mice was lower than those of control mice in both spatial memory tasks, although the effect was significant only in the Barnes-maze probe trial. Motivation, visual capacities and sensorimotor coordination appeared unaffected in the water-maze visual discrimination task and the beam-walking test, respectively.

The lesion of BFCNs with mu p75-saporin induces behavioral deficits similar to those reported after ICV 192 IgG-SAP in rats, but without Purkinje cell damage. In addition, this new version of the mouse immunotoxin has fewer side effects and appears more efficient than its predecessor. This safer and more powerful tool may be particularly adapted to improve transgenic models of AD in which amyloid and/or tangle pathologies are expressed, but do not result in extensive loss of cholinergic neurons.

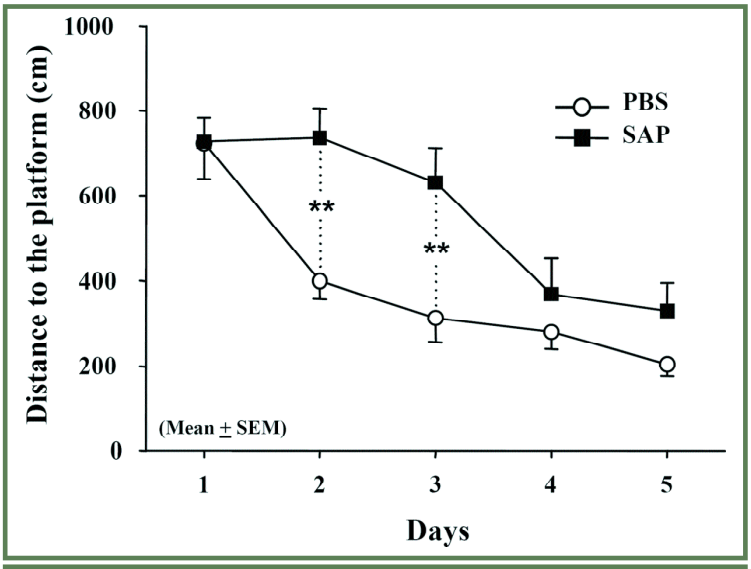


Figure 2. Mu p75-saporin (SAP) treated mice show a slower learning compared to PBS controls in the water-maze spatial reference memory task. * $p < 0.01$, significantly different from PBS.

References

1. Berger-Sweeney J, Stearns NA, Murg SL, Floerke-Nashner LR, Lappi DA, Baxter MG. 2001. Selective immunolesions of cholinergic neurons in mice: Effects on neuroanatomy, neurochemistry, and behavior. *J Neurosci* 20:8164-8173.
2. Hunter CL, Quintero EM, Gilstrap L, Bhat NR, Granholm AC. 2004. Minocycline protects basal forebrain cholinergic neurons from mu p75-saporin immunotoxic lesioning. *Eur J Neurosci* 12:3305-3316.
3. Traissard N, Herbeaux K, Cosquer B, Jeltsch H, Ferry B, Galani R, Pernon A, Majchrzak M, Cassel JC. 2007. Combined damage to entorhinal cortex and cholinergic basal forebrain neurons, two early neurodegenerative features accompanying Alzheimer's disease: effects on locomotor activity and memory functions in rats. *Neuropsychopharmacol* 4:851-871.

Targeting Tools: Featured Products

mu p75-SAP

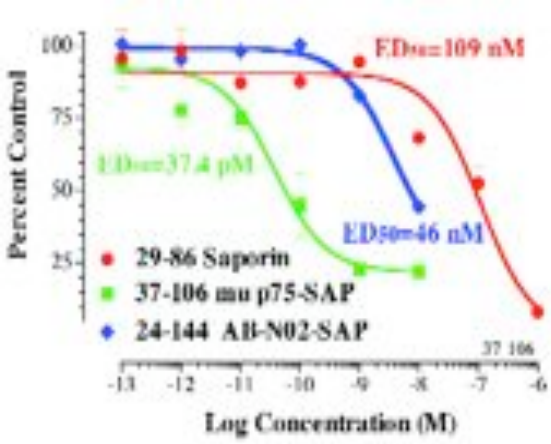
In 2004, ATS re-designed the anti-murine p75-SAP targeted toxin (mu p75-SAP, Cat. #IT-16) and produced a conjugate that is much more potent in our *in vitro* cell cytotoxicity assays. Previously, we used a rat monoclonal antibody. This antibody had been outperformed by our rabbit polyclonal (Cat. #AB-N01), in several assays, especially flow cytometry analysis of murine p75-expressing cells. This is an important indicator of being able to bind to the cell surface, which is fundamental for a targeted toxin.

To create this toxin, we affinity-purified the rabbit polyclonal (Cat. #AB-N01AP) with the immunogen bound to a solid support, and conjugated the affinity-purified antibody to saporin. As can be seen in the cytotoxicity assay on the right, the new mu p75-SAP is orders of magnitude more potent than the previous conjugate. The new and more active version of mu p75-SAP has an ED50 in the picomolar range compared to an ED50 in the nanomolar range for the previous product. We believe that the greater potency will translate to smaller amounts used for elimination of p75-positive neurons in the mouse brain, and that this will result in a greater index of efficacy and lesser non-specific cytotoxicity. (see cover article for *in vivo* results).

The mu p75-SAP kit includes, in addition to the immunotoxin, equal aliquots of saporin (Cat. #PR-01), the affinity-purified rabbit polyclonal antibody (AB-N01AP), and the control immunotoxin, Rabbit-IgG-SAP (Cat. #IT-35).

Also available are fluorescent conjugates of AB-N01AP: Cy3-labeled Anti-murine NGFr (Cat. #FL-05), and Cy5-labeled Anti-murine NGFr (Cat. #FL-06).

Ahh. The joys of Spring!



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

Flow Cytometry Tools

Advanced Targeting Systems is excited to offer two new tools for flow cytometry.

WBLyse™ (Cat. #FL-08) is a gentle erythrocyte lysing reagent matched with a leukocyte preservative. This kit can be used to enumerate lymphocyte subsets, detect a large variety of antigens on lymphocytes, and to identify other leukocyte subsets, including CD34 stem cells, and granulocytes. WBLyse™ works on many types of specimens and is active against all erythrocytes and is available in the 100 or 500 test size.

The CFSE (carboxyfluorescein-succinimidyl ester) Compensation Kit (Cat. #FL-13) provides a reproducible and convenient source of CFSE particles for evaluating and correcting for the spectral overlap between CFSE and other dyes. Fluorescence compensation is the process wherein a portion of the primary dye signal is removed from all non-primary dye channels. Compensation is generally required whenever more than a single dye (color) is used in flow cytometric analysis. This 1-color CFSE Compensation Kit is sufficient for 25 tests and contains negative/unstained microspheres and CFSE microspheres. Cells, once labeled with CFSE, can be stimulated *in vitro* and cell proliferation measured by changes in the staining intensity of the cells.

Targeting Technology

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

Choose an ANTIBODY§ specific to your cell type.

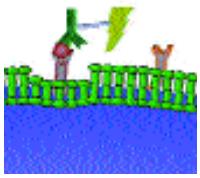
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

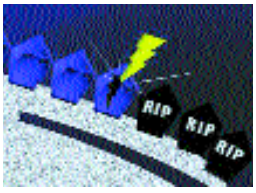
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

ZENEMY
◯□□◯□□

BOATMICEL
□□◯□□□◯□□

GREENLOLA
□◯□◯□□□□◯

IRANCHMOODIT
□◯□□□◯□□◯◯□□

FENTFEAR
□◯□□□□◯□

Why the scientist made her media green.

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

Answer:
She wanted her lab to be ... ◯◯◯◯◯ ◯◯◯◯◯◯◯◯

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, 2008
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 2.

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Jul-Aug-Sep 2008
Volume 9, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- SP-SAP veterinary applications *(page 2)*
- ATS receives SBIR grant *(page 2)*
- Teaser Winners *(page 5)*
- New Sample-Size Antibodies *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Noradrenergic Innervation of the Dorsal Medial Prefrontal Cortex Modulates Hypothalamo-Pituitary-Adrenal Responses to Acute Emotional Stress

Contributed by Jason J. Radley, Ph.D.
Laboratory of Neuronal Structure and Function; The Salk Institute of Biological Studies and Foundation for Medical Research; La Jolla, CA 92037, USA

The medial prefrontal cortex (mPFC) has been proposed to play a role in the inhibition of hypothalamo-pituitary-adrenal (HPA) responses to emotional stress via influences on neuroendocrine effector mechanisms housed in the paraventricular hypothalamic nucleus (PVH).[1,2] The locus coeruleus (LC) is the principal noradrenergic cell group in the brain, and plays established roles in promoting behavioral adaptations to a variety of alerting stimuli, including stressful ones.[3,4] While the PVH receives a substantial catecholaminergic innervation, the bulk of this arises not from the LC, but from medullary cell groups,[5] which are implicated in mediating HPA responses to physiologic, but not emotional, stressors.[6] This raises the possibility that LC's influence on stress-induced HPA activation might be mediated indirectly, through its projections to limbic and forebrain regions implicated in HPA control. The mPFC is involved in the processing of convergent cognitive and emotionally relevant information, and the LC noradrenergic projections to this region have been proposed to play a critical role in the modulation of working memory and attention.[7] These operations are likely to be involved in the mPFC's capacity to evaluate the contextual relevance and emotional valence of potentially threatening stimuli in order to effect adaptive responses. Nonetheless, the involvement of the LC-to-mPFC pathway in HPA regulation has not been tested, and is problematic, since dorsal mPFC (mPFCd) lesions have been shown to enhance,[1,2] while LC lesions attenuate,[8] HPA activation in response to acute emotional stressors.

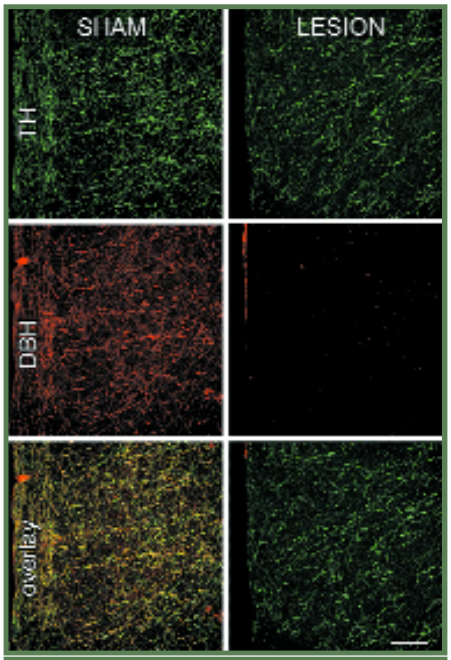

Figure 1. Specificity of anti-DBH-SAP-mediated denervation of mPFCd.

We assessed the effects of selectively ablating noradrenergic inputs into the mPFC, employing the axonally-transported catecholamine immunotoxin (IT), saporin-conjugated anti-dopamine-beta-hydroxylase (anti-DBH-SAP, Cat. #IT-03), on acute restraint stress-induced activation of HPA output.[9] Rats received dorsal mPFC injections of IT or sham injections of IgG-saporin (mouse IgG-SAP, Cat. #IT-18) or saline. Fourteen days later, rats were subjected to 30 min of restraint stress and perfused 2 h later.

Anti-DBH-SAP injections virtually eliminated noradrenergic fibers and varicosities from the mPFCd, whereas control injections of the untargeted toxin (IgG-saporin) or CSF left these inputs intact (Fig. 1, middle). The specificity of the noradrenergic denervation of the mPFCd was assessed by examining the extent to which damage from the IT injection

(continued on page 6)

SP-SAP Treatment for Chronic Pain. . . in non-Humans

ATS recently licensed SP-SAP for development as a chronic pain therapeutic in humans. Much progress has been made in the past three months to prepare the pre-IND package for a meeting with the FDA. This meeting has been requested and the FDA has assigned a meeting date of October 2nd. The purpose of this meeting will be to present data and determine the next steps for moving SP-SAP into human clinical trials.

Marlow Russell and Codi Sansone
Companions to Brian and Jessica

Part of the data that will be presented at the pre-IND meeting will be from the veterinary clinical trial now going on with SP-SAP in companion dogs with bone cancer. We are very encouraged by the mid-term results of this trial and are hopeful that we can find a veterinary pharmaceutical company that will market this chronic pain drug for use in animals.

As part of the veterinary development, we hope to begin a trial in cats in the near future. Felines have a unique need because they are intolerant to treatment with standard non-steroidal, non-inflammatory medications, due to the way their livers function.

Check the October issue for more updates on SP-SAP.

ATS Receives SBIR Phase I Grant

ATS begins work on an exciting new line of targeting products with a newly funded grant from the National Institute of Mental Health. The grant is entitled “Inhibition of Neurotransmission in Specific Neuronal Populations” and proposes to develop a new tool set for the understanding of cell function and systems biology.

In extensive work over the last decade, it has become clear that biologically active molecules can be inserted into specific cell types through targeting to molecules on the cell surface. The new project will direct this technology to specific neuronal cell types with the purpose of temporarily inhibiting their capacity of releasing neurotransmitters.

Inhibition of neurotransmitter release would be a short-term phenomenon, because slowly, over time, normal function would resume. This would be about a one-month process to move from inhibition back to normal function.

The demonstration of efficacy would usher in a new technology with applications as research reagents. Targeted agents could be used to shut down neuronal sub-types, allowing observation of the effect and greater understanding of the function of the cell in systems biology, while the return to homeostasis and function would act as a control for the experiment.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Noradrenergic innervation of the dorsal medial prefrontal cortex modulates hypothalamo-pituitary-adrenal responses to acute emotional stress.

Radley JJ, Williams B, Sawchenko PE
J Neurosci 28:5806-5816, 2008.

Rats were injected with 90-120 nl of 0.475-μg/μl anti-DBH-SAP (Cat. #IT-03) into the cortical field containing noradrenergic neurons that project to the dorsal medial prefrontal cortex. The results indicate that the locus ceruleus functions as an upstream component in medial prefrontal cortex modulation of hypothalamo-pituitary-adrenal activation due to emotional stress.

Vascular smooth cell proliferation in perfusion culture of porcine carotid arteries.

Liao D, Lin PH, Yao Q, Chen C
Biochem Biophys Res Commun [Epub Jun 2], 2008.

The authors used FGF-SAP (Cat. #IT-38) to help characterize a model of vascular smooth muscle cell proliferation with porcine carotid arteries. Arteries isolated from pigs were cultured under several different conditions, one of which included FGF-SAP at a concentration of 0.4 nM. In all cases the arteries maintained viability for up to 96 hours.

Neuromodulatory role of acetylcholine in visually-induced cortical activation: Behavioral and neuroanatomical correlates.

Dotigny F, Ben Amor AY, Burke M, Vaucher E
Neuroscience [Epub Apr 25], 2008.

After rats were treated with 192-IgG-SAP (Cat. #IT-01, 2 μl of 2.4 μg/μl into the lateral ventricle) visual acuity and performance in a visual water maze task were analyzed. Lesioned animals displayed no loss in acuity, but were less able to learn a new orientation discrimination task.

Selective ablation of GABA neurons in the ventral tegmental area increases spontaneous locomotor activity.

Shank EJ, Seitz PK, Bubar MJ, Stutz SJ, Cunningham KA
Behav Neurosci 121:1224-1233, 2007.

To further examine the importance of the ventral tegmental area (VTA) γ-aminobutyric acid (GABA) neurons in behavioral function, the authors lesioned the VTA of rats. Animals received 1 or 2 pmol/200 nl bilateral injections of dermorphin-SAP (Cat. #IT-12); blank-SAP (Cat. #IT-21) was used as a control. Rats treated with dermorphin-SAP displayed significantly elevated motility as compared to control animals.

Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice.

Kublaoui BM, Gemelli T, Tolson KP, Wang Y, Zinn AR
Mol Endocrinol [Epub May 1], 2008.

Central administration of neuropeptides in the paraventricular nucleus (PVN) is known to inhibit feeding. Hypothalamic expression of several neuropeptides, including corticotrophin releasing hormone (CRH) was measured. To do so, anti-CRH (Cat. #AB-02, 1:800) was used in immunohistochemistry.

Environmental enrichment mitigates the effects of basal forebrain lesions on cognitive flexibility.

De Bartolo P, Leggio MG, Mandolesi L, Foti F, Gelfo F, Ferlazzo F, Petrosini L
Neuroscience [Epub Apr 7], 2008.

This work examines whether environmental enrichment can reduce the effect of cholinergic lesions on learning and memory tasks. Rats received 0.4-μg bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the cholinergic projection to the neocortex. Deficits caused by the lesion were attenuated in rats experiencing an enriched environment.

Targeting CUB domain-containing protein 1 with a monoclonal antibody inhibits metastasis in a prostate cancer model.

Siva AC, Wild MA, Kirkland RE, Nolan MJ, Lin B, Maruyama T, Yantiri-Wernimont F, Frederickson S, Bowdish KS, Xin H
Cancer Res 68:3759-3766, 2008.

After demonstrating *in vitro* activity of the monoclonal antibody 25A11 with Mab-ZAP (Cat. #IT-04) and Hum-ZAP (Cat. #IT-22) the authors had ATS do a direct conjugation of 25A11 and saporin. Goat-IgG-SAP (Cat. #IT-19) was used as a control for *in vivo* experiments, and saporin (Cat. #PR-01) was the control *in vitro*. In treated mice, the direct conjugate significantly inhibited tumor growth as well as metastasis *in vivo*.

Selective ablation of dorsal horn NK_1 expressing cells reveals a modulation of spinal alpha2-adrenergic inhibition of dorsal horn neurones.

Rahman W, Suzuki R, Hunt SP, Dickenson AH
Neuropharmacology 54:1208-1214, 2008.

In this work the spinal origin of the major descending noradrenergic inhibitory pathway is examined with the help of SP-SAP (Cat. #IT-07). Rats

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

received a 10-μl infusion of 1 mM SP-SAP (Saporin, Cat. #PR-01, was used as a control) into the sub-arachnoid space terminating in the L4-5 region. Results suggest that NK1r-expressing cells are involved with activity in noradrenergic pathways and descending facilitation.

Emergence of spatial impairment in rats following specific cholinergic depletion of the medial septum combined with chronic stress.

Craig LA, Hong NS, Kopp J, McDonald RJ
Eur J Neurosci 27:2262-2271, 2008.

Rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum and vertical limb of the diagonal band of Broca totaling 0.075 μg. Animals were not impaired in a water maze task, but lesioning combined with stress caused significant reduction in performance.

Substance P receptor-expressing dorsal horn neurons: Lessons from the targeted cytotoxin, substance P-saporin.

Wiley RG
Pain 136:7-10, 2008.

This review covers some of the more recent work utilizing SP-SAP (Cat. #IT-07) and SSP-SAP (Cat. #IT-11) in the dorsal horn. The potential of these conjugates as pain therapeutics is explored.

Involvement of the basal cholinergic forebrain in the mediation of general (propofol) anesthesia.

Laalou FZ, de Vasconcelos AP, Oberling P, Jeltsch H, Cassel JC, Pain L
Anesthesiology 108:888-896, 2008.

192-IgG-SAP (Cat. #IT-01) was injected three ways: icv injection of 2 μg, 0.4 μg into the nucleus basalis magnocellularis, and 0.8 μg into the medial septum/ vertical diagonal band of Broca. The results suggest that loss of cholinergic neurons in the cortex and hippocampus leads to potentiation of the anesthetic effects of Propofol.

Unilateral Ablation of preBötzinger Complex Disrupts Breathing During Sleep but not Wakefulness.

McKay LC, Feldman JL
Am J Respir Crit Care Med [Epub Apr 17], 2008.

Here rats received a unilateral injection of SP-SAP (Cat. #IT-07, 6.7 ng) into the left preBötC. SP plus unconjugated saporin (Cat. #PR-01) was used as control. Unilaterally-treated rats did not develop disrupted breathing patterns during wakefulness.

Selective cholinergic lesions in the rat nucleus basalis magnocellularis with limited damage in the medial septum specifically alter attention performance in the five-choice serial reaction time task.

Harati H, Barbelivien A, Cosquer B, Majchrzak M, Cassel JC
Neuroscience 153:72-83, 2008.

Here the authors examined the effect of lesions in the nucleus basalis magnocellularis (NBM) when septal damage was kept to a minimum. The NBM received bilateral 0.2-μg injections of 192-IgG-SAP, and the animals were then tested in a 5-choice serial reaction time task. The disruption of sustained visual attention remained, but other variables were close to normal.

Oxaliplatin Acts on IB4-Positive Nociceptors to Induce an Oxidative Stress-Dependent Acute Painful Peripheral Neuropathy.

Joseph EK, Chen X, Bogen O, Levine JD
J Pain 9:463-472, 2008.

The authors administered 3.2-μg intrathecal injections of IB4-SAP (Cat. #IT-10), using saporin (Cat. #PR-01) as a control. Lesioning IB4-binding neurons in the dorsal horn completely prevented oxaliplatin-induced hyperalgesia.

Selective lesion of retrotrapezoid Phox2b-expressing neurons raises the apnoeic threshold in rats.

Takakura AC, Moreira TS, Stornetta RL, West GH, Gwilt JM, Guyenet PG
J Physiol 586.12: 2975-2991, 2008

Injections of SSP-SAP (Cat. #IT-11) into the retrotrapezoid nucleus eliminated $Phox2b^{+}TH^{-}$ neurons but spared other neuron classes. Several different amounts of the conjugate were used (0.15, 0.3, or 0.6 ng in 1 or 2 injections). Elimination of ≥70% of $Phox2b^{+}TH^{-}$ neurons markedly attenuated the central chemoreflex.

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Lau T *et al.* (2008) FASEB J 22(6):1702-1714. *(Cat. #AB-N09: Ab to Serotonin Transporter)*

Lorier AR *et al.* (2007) J Neurosci 27(5):993-1005. *(Cat. #AB-N04: Antibody to NK-1r)*

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Momiyama T *et al.* (2006) J Neurophysiol 96(2):686-694. *(Cat. #FL-01: Cy3-192-IgG)*

Shekhar A *et al.* (2006) J Neurosci 26(36):9205-9215. *(Cat. #AB-N27AP: Ang IIr (AT-1r)*

(continued on page 7)

Targeting Talk: Saporin death: Apoptosis or Necrosis

by Dr. Douglas Lappi

Q: Do targeted toxin-treated cells die by apoptosis?

A: There are, allegedly, two ways for cells to die: by apoptosis or necrosis. According to Fiorenzo Stirpe (the discoverer of saporin), saporin-intoxicated cells die both ways, some by one, others by the other.

There is a good literature that states that cells die by apoptosis, for instance:

Bergamaschi G, Perfetti V, *et al*. (1996). Saporin, a ribosome-inactivating protein used to prepare immunotoxins, induces cell death via apoptosis. *Brit J Haemat* 93:789-794.

Saporin and apoptosis gives 25 hits in PubMed.

However, Seeger *et al*., did not find evidence of apoptosis in an electron microscopy study with cells dying from 192-IgG-SAP and concluded they die from necrosis:

Seeger G, Hartig W, *et al*. (1997). Electron microscopic evidence for microglial phagocytotic activity and cholinergic cell death after administration of the immunotoxin 192IgG-saporin in rat *J Neurosci Res* 48:465-476.

Saporin and necrosis gives 11 hits in PubMed.

So, saporin-treated cells seem to die by both apoptosis and necrosis. The customer is always right.

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Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: ENZYME
METABOLIC
ORGANELLE
MITOCHONDRIA
AFFERENT

Answer: She wanted her lab to be. . .EARTH FRIENDLY

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

WINNERS: Wiktor Janczewski, UCLA Medical School, Neurobiology, Los Angeles CA * Jack Feldman, UCLA Medical School, Neurobiology, Los Angeles CA * Indira Jutooru, Texas A&M Univ, Toxicology, College Station TX * Seto Chice-SUNY HSC at Brooklyn, Brooklyn NY

An important note about shipping and storage temperatures.

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Enclosed with each shipment are instructions for storage and handling. Storage information is also attached on the outside of each package. Please read these instructions carefully. All targeted toxins should be stored frozen (-20°C or -80°C).

When you receive a targeted toxin, realiquot to the amounts you expect to use for experimental doses and then freeze. DO NOT DILUTE until just before administering. Repeated freezing and thawing can reduce the activity of the material.

If you have any questions, do not hesitate to contact us.

Noradrenergic Innervation of the mPFCd Modulates HPA Responses to Acute Emotional Stress

(continued from page 1)

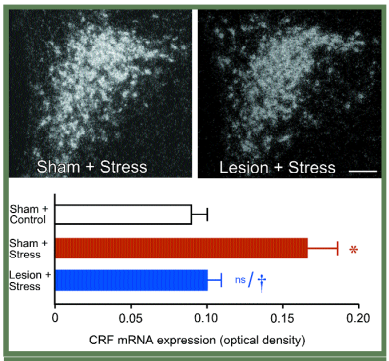

Figure 2. Effects of noradrenergic denervation of mPFCd on restraint-induced upregulation of CRF mRNA in PVH.

involved dopaminergic fibers and terminals in dual immunofluorescence preparations for tyrosine hydroxylase (TH) and DBH (Fig. 1). TH converts tyrosine to dihydroxyphenylalanine, a precursor of both dopamine (DA) and norepinephrine (NE). Immunolabeling of this enzyme represents both DA and NE fibers and terminals, whereas staining for DBH in cortex is specific to NE. Thus, the overlay of TH and DBH stained fibers and varicosities represents the subpopulation of inputs into mPFCd that are noradrenergic, whereas fibers singly-labeled for TH are dopaminergic (Fig. 1, top). Following anti-DBH-SAP injections into PL, the density of dopaminergic fibers and varicosities was comparable to controls in density and distribution, while there was a near complete elimination of DBH staining in mPFCd, as well as of elements doubly-labeled for both enzymes (Fig. 1, bottom). Ancillary analyses revealed that IT injection in mPFCd resulted in a 23% decrease in the number of LC neurons and a corresponding decrease in stress-induced LC activational responses, compared to sham-lesioned controls.[9] Anti-DBH-SAP is an effective tool for achieving focal noradrenergic denervation by ablating the neurons from LC that project to targeted terminal fields in mPFCd.

We initially surveyed the effects of lesions in mPFCd on stress-induced expression of Fos protein, a generic marker of neuronal activation, in the PVH. Acute stress resulted in a marked increase in Fos expression in the sham-lesioned animals, focused in the CRF-rich hypophysiotropic zone of PVH. This effect was reduced by 28% in anti-DBH-SAP lesioned animals. Ancillary analyses from sham- and IT-lesioned groups failed to reveal any effect of lesion status on the number of Fos immunoreactive neurons in the PVH of unstressed rats. We examined relative levels of CRF mRNA in PVH using densitometry (Fig. 2, top). Consistent with the Fos expression data, restraint stress resulted in a two-fold increase in CRF mRNA expression in the hypophysiotropic zone of PVH in sham-lesioned animals compared to unstressed controls (Fig. 2, bottom). In contrast, IT lesions diminished this effect to levels that did not differ significantly from those of unstressed controls.

HPA secretory responses before and after the 30-min restraint stress were examined in separate groups of sham- and IT-lesioned animals (Fig. 3). Blood samples were obtained from indwelling jugular catheters that were implanted 2 days prior to stress exposure. Stress exposure significantly increased plasma levels of ACTH in both sham- and IT-lesioned animals. While these data suggest a difference between peak plasma levels of ACTH in IT- as compared to sham-lesioned animals, they did not differ significantly. Nonetheless, there was a significant reduction in total integrated plasma ACTH levels in lesioned compared to sham groups, assessed by calculating areas under the curve. Stress exposure also significantly increased plasma levels of corticosterone in sham- and IT-lesioned animals. While there were no significant differences at any individual time point between sham and lesioned groups, there was an overall main effect for treatment, and a decrease in integrated corticosterone levels in lesioned as compared to unlesioned groups. Sham-lesioned animals also showed a prolonged increase in stress-induced plasma corticosterone levels, whereas lesioned animals show a more rapid recovery.

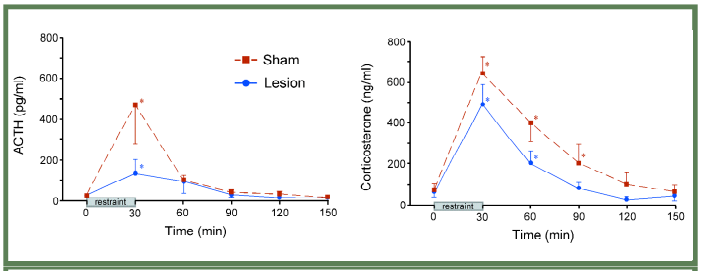

Figure 3. Noradrenergic denervation of mPFCd attenuates pituitary-adrenal secretory responses to acute restraint.

The present findings localize previously documented HPA-facilitatory influences of LC, at least in part, to its projections to mPFCd and help to clarify the extended circuitry underlying mPFC modulation of HPA responses to acute emotional stress. In addition to participating in the regulation of stress responses, the mPFC is also a target of them. Repeated exposure to emotional stress gives rise to dendritic atrophy and synapse loss in this region,[10,11] findings that have clinical parallels in reports of mPFC shrinkage and functional impairment in posttraumatic stress disorder (PTSD).[12,13] NE has been linked to the mediation of maladaptive, as well as adaptive, consequences of stress exposure, being implicated in various psychiatric conditions, including PTSD.[14] Drugs that modulate noradrenergic transmission have demonstrated efficacy in treating such mood disorders via actions that may be exerted, at least in part, on the mPFC.[15] Further progress in unraveling the broader circuitry governing HPA responses to emotional stress, and the places of the LC and mPFC within it, should foster more informed management of stress-related psychiatric conditions.

Article References are on page 5.

This article was edited due to space constraints.
You can view the entire article at
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AB-39 Anti-GAPDH Mouse Monoclonal, 100 µl

Anti-GAPDH is a mouse monoclonal antibody, affinity-purified from mouse ascites fluid to recognize native and denatured forms of GAPDH in human, mouse, rat, rabbit, bacteria, etc.

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(continued from page 4)

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§ or anything recognized on the cell surface and internalized.

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

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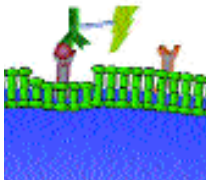
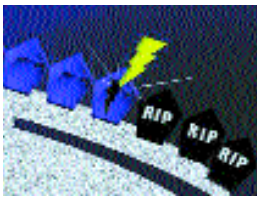


Cells that do not have the receptor will not be affected.

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

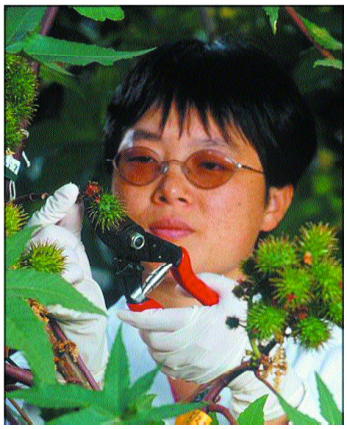
YERTAR

FRAPROLTEN

SITPRUD

TEASESHANI

IBITHIN



How the scientist stopped the disease from spreading.

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

Answer:
She had to . . .

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
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Oct-Nov-Dec 2008
Volume 9, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Selective lesions of amygdala intercalated neurons using the Dermorphin-SAP immunotoxin reveal their role in extinction of conditioned fear.

Contributed by Ekaterina Likhtik
Columbia University, New York Psychiatric Institute, 1051 Riverside Drive, New York, NY 10032

Introduction by Douglas Lappi, Ph.D., President/CSO:

This quarter's Cover Article is from Ekaterina Likhtik who reprises her recently published work in Nature *454:642-645. In the News and Views, Sah and Westbrook comment that "Neuronal receptors in these circuits—such as those targeted with saporin in Likhtik and colleagues' study—are likely to become targets for the development of specific treatments for many anxiety disorders."*

The amygdala is a key subcortical structure in the neural circuit that processes acquisition as well as extinction of conditioned fear. Although a large body of literature details how amygdala activity results in fear conditioning, fear extinction circuits are less well understood. In particular, it is difficult to study the role that one of its potentially important cell groups, the intercalated (ITC) cells, may play in this behavior. These cells likely constitute an important interface between the input and output nuclei of the amygdala, gating information flow out of the amygdala during fear conditioning and extinction.[1,2]

To date the obstacle to studying the ITC cells is that they occur in small, anatomically distributed clusters[3] and are therefore difficult to selectively lesion using conventional methods. In order to circumvent this issue, we took advantage of the high levels of μ-opioid receptor expression observed in ITC cells in the light microscope. Indeed, a more detailed analysis using electron microscopy, revealed that the ITC cells express μ-opioid receptors post-synaptically at 3-6 times the rate of surrounding amygdala nuclei (Fig

(continued on page 6)

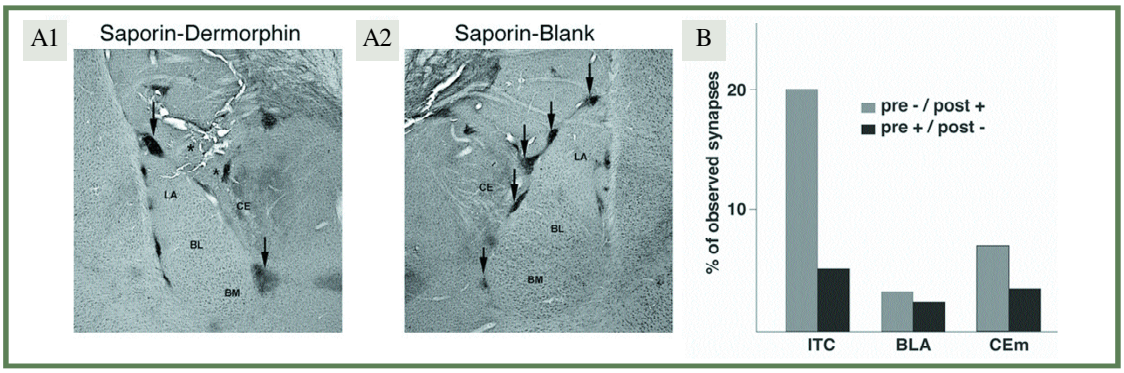


Figure 1. Dermorphin-SAP infusions lead to a spatially circumscribed loss of μ-opioid receptor immunoreactivity. **(A)** Coronal sections from rats that received either Dermorphin-SAP (A1) or Blank-SAP **(A2)** injections in the vicinity of ITC cells. Arrows indicate the remaining ITC cell clusters in the two animals. Asterisks point to injection tracks. Note that only cell clusters adjacent to tracks are affected by the infusion. **(B)** Electron microscopy: proportion of synapses where μ-opioid receptor immunoreactivity was found in post-synaptic (grey) or pre-synaptic (black) elements of the basolateral (BLA), medial central (CEm) or ITC nuclei.

Inside this issue:

Newsletter Highlights

- Melanopsin-SAP *(page 2)*
- European office open *(page 2)*
- Teaser Winners *(page 5)*
- New Antibodies: HSA and saporin *(page 7)*

Denise Higgins, Editor

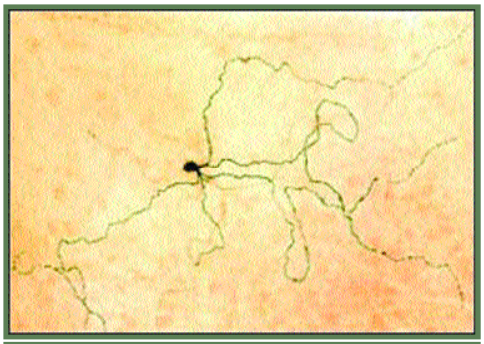
ADVANCED TARGETING SYSTEMS

Control Circadian Rhythm?

Advanced Targeting Systems announces the release of Melanopsin-SAP, a reagent that specifically eliminates intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain melanopsin. This material is described in Göz *et al.*[1] and consists of an antibody to an extracellular domain conjugated to saporin. The ipRGCs, with their amazing long processes, have become major players in the perception of light and dark, and their role as circadian rhythm determinants is a hot topic. Use this new tool to determine who's right!

1. Göz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms. ***PLoS One 3(9), e3153, 2008***

2. http://www.innovations-report.com/html/reports/life_sciences/report-39491.html



Intrinsically photosensitive retinal ganglion cells – ipRGCs – were discovered in 2002.[2]

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Bas Hulshof and pal, Indy.

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Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Tomoregulin internalization confers selective cytotoxicity of immunotoxins on prostate cancer cells.

Zhao XY, Liu HL, Liu B, Willuda J, Siemeister G, Mahmoudi M, Dinter H
Transl Oncol 1:102-109, 2008.

Tomoregulin is a type 1 transmembrane protein with a short cytoplasmic tail, and is found in the brain and prostate. After confirming cell surface localization by flow cytometry, and determining expression levels by whole-cell binding assays, the authors evaluated the use of tomoregulin as a target for immunotoxin therapy. Cells transfected with tomoregulin were treated with anti-tomoregulin + Mab-ZAP (IC_{50} = 160 pM; Cat. #IT-04) *in vitro*. The results demonstrate the potential for tomoregulin in prostate cancer treatment.

Reduced cholinergic status in hippocampus produces spatial memory deficits when combined with kainic acid induced seizures.

Craig LA, Hong NS, Kopp J, McDonald RJ
Hippocampus [Epub Jul 23], 2008.

The loss of cholinergic neurons in the medial septum and seizures are both associated with Alzheimer's disease. The authors investigated links between these factors using 192-IgG-SAP (Cat. #IT-01) and kainic acid. Rats received 0.15 μg of 192-IgG-SAP delivered to the medial septum and vertical limb of the diagonal band of Broca in four injections. Animals receiving both 192-IgG-SAP and kainic acid performed significantly worse in water maze tests than control animals, indicating that loss of cholinergic neurons and seizures interact in Alzheimer's disease.

GDNF hyperalgesia is mediated by PLCgamma, MAPK/ERK, PI3K, CDK5 and Src family kinase signaling and dependent on the IB4-binding protein versican.

Bogen O, Joseph EK, Chen X, Levine JD
Eur J Neurosci 28:12-19, 2008.

C-fiber nociceptors have been divided into NGF and GDNF classes. Here the authors examined the function of an isolectin B4-binding subpopulation of these nocicepetors. Rats received 40 ng of IB4-SAP (Cat. #IT-10) into the intrathecal space between the fifth and sixth lumbar vertebrae. The results demonstrate that GDNF sensitizes $IB4^+$ C-fiber nociceptors and causes mechanical hyperalgesia.

Amygdala intercalated neurons are required for expression of fear extinction.

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Pare D
Nature 454:642-645, 2008.

Scientists have been using fear learning in animals to study human anxiety disorders. In order to investigate the contribution of amygdala plasticity to fear learning, rats received 0.25-μl bilateral infusions of 3-μM dermorphin-SAP (Cat. #IT-12) into the amygdala. Blank-SAP (Cat. #IT-21) was used as a control. Lesioned rats displayed extinction expression deficits, indicating that the eliminated intercalated amygdala neurons play a large role in the extinction process. (see cover story)

IB4 afferent sprouting contributes to bladder dysfunction in spinal rats.

Zinck ND, Downie JW
Exp Neurol 213:293-302, 2008.

Spinal cord injury can cause inefficient bladder function, but the direct cause is not well understood. Most work has focused on afferent neurons that contain CGRP and respond to NGF. Here the authors investigate the role of isolectin B4 (IB4)-expressing neurons that are supported by GDNF. Rats received intrathecal injections of either 2.4 μg IB4-SAP (Cat. #IT-10) or 3 μg control saporin (Cat. #PR-01). The data suggest that IB4-afferent sprouting is involved in bladder dysfunction following spinal cord transection.

Renal sympathoinhibition induced by hypernatremia: Involvement of A1 noradrenergic neurons.

Pedrino GR, Rosa DA, Korim WS, Cravo SL
Auton Neurosci [Epub Aug 8], 2008.

A1 noradrenergic neurons in the caudal ventrolateral medulla (CVLM) are thought to contribute to body fluid homeostasis and cardiovascular regulation. In order to examine the role these neurons play on inhibition of renal sympathetic nerve activity (RSNA) induced by hypertonic saline infusion, rats received 6.3 ng of anti-DBH-SAP (Cat. #IT-03) into the CVLM. Saporin (Cat. #PR-01) was used as a control.

(continued on page 4)

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

Targeting Topics: Recent Scientific References

(continued from page 3)

Animals treated with anti-DBH-SAP displayed lengthened duration of the pressor response and sustained RSNA.

Lesions of medullary catecholaminergic neurons increase salt intake in rats.

Colombari DS, Pedrino GR, Freiria-Oliveira AH, Korim WS, Maurino IC, Cravo SL
Brain Res Bull 76:572-578, 2008.

Catecholaminergic neurons in the caudal ventrolateral medulla (CVLM) are thought to contribute to cardiovascular regulation and body fluid homeostasis. Bilateral 6.3-ng injections of anti-DBH-SAP (Cat. #IT-03) were administered to the CVLM of rats. Saporin (Cat. #PR-01) was used as a control. After lesioning and challenge with either furosemide/captopril or water deprivation, intake of 0.3 M NaCl and water were observed. The data indicate medullary catecholaminergic neurons play an inhibitory role in sodium appetite.

Effects of hypocretin (orexin) neuronal loss on sleep and extracellular adenosine levels in the rat basal forebrain.

Murillo-Rodriguez E, Liu M, Blanco-Centurion C, Shiromani PJ
Eur J Neurosci [Epub Sep 9], 2008.

Adenosine levels in the basal forebrain are thought to regulate the waxing and waning of sleep drive. Rats received bilateral 100-ng injections of orexin-SAP (Cat. #IT-20) into the lateral hypothalamus – resulting in a 94% loss of orexin-containing neurons. Lesioned animals displayed several changes in sleep characteristics, but no increase of adenosine levels after sleep deprivation. The results indicate that sleep changes due to orexin-SAP lesioning occur independently of adenosine levels.

Hyperphagia and obesity produced by arcuate injection of NPY-saporin do not require upregulation of lateral hypothalamic orexigenic peptide genes.

Li AJ, Dinh TT, Ritter S
Peptides [Epub Jun 5], 2008.

It has already been shown that lesioning NPY receptor-expressing cells in the arcuate nucleus (Arc) and basomedial hypothalamus produces obesity in rats. The authors examined the contribution of orexigenic peptides, orexins, and melanocortin-concentrating hormone to the lesion effects. Rats received bilateral 24 ng injections of NPY-SAP (Cat. #IT-28) into the dorsal border of the Arc. Blank-SAP (Cat. #IT-21) was used as a control. The data suggest that obesity produced by NPY-SAP lesion is different than dietary obesity or obesity associated with leptin or leptin receptor deficiency.

The neonatal injury-induced spinal learning deficit in adult rats: central mechanisms.

Young EE, Baumbauer KM, Hillyer JE, Patterson AM, Hoy KC, Jr., Mintz EM, Joynes RL
Behav Neurosci 122:589-600, 2008.

This report examined whether neonatal injuries had any contralateral effects in adult life, and evaluated the role of the NK1 receptor of adult animals that had been subjected to neonatal trauma. Rats were injected with 5 μl of SP-SAP (Cat. #IT-07, 30 ng/μl, 100 ng/μl, or 300 ng/μl) into the intrathecal space. Blank-SAP (Cat. #IT-21) was used as a control. The results indicate both that injury effects are isolated in the injured limb, and NK1 receptor-expressing cells are involved in processing this pain.

Environmental Enrichment Provides a Cognitive Reserve to be Spent in the Case of Brain Lesion.

Mandolesi L, De Bartolo P, Foti F, Gelfo F, Federico F, Leggio MG, Petrosini L
J Alzheimers Dis 15(1):11-28, 2008.

The cognitive reserve model suggests individuals can develop resources that reduce the risk of later cognitive impairment. This theory was tested by raising rats in standard vs. enriched environments then lesioning the animals with 192-IgG-SAP (Cat. #IT-01). A total of 0.8 μg of 192-IgG-SAP was administered in bilateral injections, followed by various behavioral tests. It was found that animals raised in an enriched environment had reduced cognitive impairment following forebrain lesions.

Noradrenergic inputs to the paraventricular hypothalamus contribute to hypothalamic-pituitary-adrenal axis and central Fos activation in rats after acute systemic endotoxin exposure.

Bienkowski MS, Rinaman L
Neuroscience [Epub Aug 13], 2008.

Noradrenergic (NA) neurons in the central nervous system are activated during the immune response to systemic lipopolysaccharide (LPS). The authors tested whether these neurons with axonal inputs to the paraventricular nucleus (PVN) were necessary for LPS-directed Fos expression and increase of plasma corticosterone. Rats received 44-ng bilateral injections of anti-DBH-SAP (Cat. #IT-03) into the medial PVN then were challenged with i.p. LPS. Lesioned animals had attenuated Fos activation and smaller than normal increases in plasma corticosterone.

Targeting Talk: Saporin Clearance

by Dr. Douglas Lappi

Q: I am planning an experiment to investigate the effects of ablation of spinal NK-1r-expressing cells (using intrathecal injection of SSP-SAP). In the first part of the experiment I want to destroy the NK-1r-expressing cells before surgical modification. I am unsure how long after injection of SSP-SAP I should carry out the surgery. I was thinking of carrying out surgery at the two-week time point as in a 2007 Neuroscience paper by Wiley et al.[1] Their immunocytochemistry showed a large reduction in staining at this time point. Any advice you could give me would be much appreciated.

A: Two weeks is probably fine. Generally cells begin to lose function at four days, but people wait longer because there is a clean-up by microglia/macrophage that removes the markers that people use for detection/demonstration of efficacy. Mantyh *et al.* were conservative with a 30-day wait for saporin clearance.[2]

References

1. Wiley RG, Kline RHt, Vierck CJ, Jr. (2007) Anti-nociceptive effects of selectively destroying substance P receptor-expressing dorsal horn neurons using [Sar(9),Met(O(2))(11)]-substance P-saporin: Behavioral and anatomical analyses. *Neuroscience* 146:1333-1345.
2. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.

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Booth #1543

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: ARTERY
PREFRONTAL
DISRUPT
ANESTHESIA
INHIBIT

Answer: She had to . . .NIP IT IN THE BUD

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

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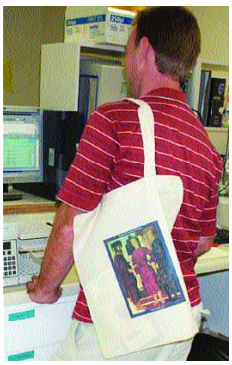
See you in Washington DC . . . . SfN - Booth 523!

Once again, ATS is reviewing abstracts and will be visiting each poster using our saporin conjugates. After discussion with our scientists and careful consideration, we will award the Poster of the Year. This award honors the scientist's innovation and presentation of results.

You can download a list of abstracts from our website (www.ATSbio.com). Click on References, then 2008 SfN Abstracts. Or you can stop by the ATS booth on Sunday, November 16 (9:30-5:00) and pick up an Itinerary. If you currently use targeted toxins or are considering using them in the future, you will find it very helpful to talk to other scientists about their experiences.



When you visit our booth, you can also pick up your 2009 ATS calendar and our earth-friendly canvas bag. And there might just be a special Gangsta surprise, too. We look forward to seeing you soon.

Selective lesions of ITC cells reveal their role in extinction of conditioned fear.

(continued from page 1)

1b). Given this imbalance in receptor expression, we were confident that local injections of the μ-opioid receptor-targeted Dermorphin-SAP would be the optimal way of testing the impact of these cells on behavior, without compromising the integrity of the surrounding tissue.

To test whether the ITC cells play a role in extinction expression, rats were habituated and fear conditioned in one context, and then extinguished in a second context. One day after extinction, animals underwent surgery where they received bilateral infusions stereotaxically aimed at the ITC cells of either Dermorphin-SAP (3 pmol/μl, infusion rate .01 μl/min, total of 0.25 μl per hemisphere) or an equivalent amount of control--a non-targeted peptide conjugated to saporin, Blank-SAP (Fig 1a). This protocol did not result in any adverse effect on post-operative weight gain, posture or exploratory behavior.

After recovery, rats were tested for how well they remembered extinction training. During the extinction recall session, sham-lesioned animals displayed low freezing to presentations of the conditioned stimulus, consistent with having retained the extinction training. On the other hand, animals with ITC cell lesions had a dramatic increase in the amount of freezing to the previously extinguished conditioned stimulus, indicating that their recall of the previous session was substantially diminished (Fig 2a). Importantly, rats that received identical Dermorphin-SAP infusions in nuclei surrounding the ITC cells, recalled extinction training as well as controls, suggesting an ITC-specific effect.

Stereological cell counts revealed that properly placed tracks delivering Dermorphin-SAP decreased the number of ITC cells in lesioned animals by 34% as compared to sham-lesioned controls (Fig 2b). In contrast, cells in the adjacent central nucleus of the amygdala were unaffected by the lesion. In addition, an inverse correlation between the number of surviving ITC cells and freezing during extinction recall (r=-0.67) was observed, whereas there was no such correlation between the number of cells in the central nucleus and freezing during extinction recall (r=-0.13).

In this study, Dermorphin-SAP has served as an important tool to seek out a potentially useful target for clinical intervention. Dermorphin-SAP lesions have allowed us to safely and selectively eliminate a proportion of amygdala ITC cells, revealing the importance of these neurons in the expression of extinction. Given that extinction failure is a robust model for a number of anxiety disorders,[4] we can now explore ways of pharmacologically manipulating μ-opioid and other receptors expressed on the ITC cells to control amygdala output and facilitate extinction.

References:

1. Paré D, Quirk GJ, Ledoux JE. (2004) *J Neurophysiol* 92:1-9.
2. Quirk GJ, Likhtik E, Pelletier JG, Paré D. (2003) *J Neurosci* 23:8800-8807.
3. Millhouse OE (1986) *J Comp Neurol* 247:246-271.
4. Ressler KJ, Mayberg HS (2007) *Nat Neurosci* 10:1116-1124.

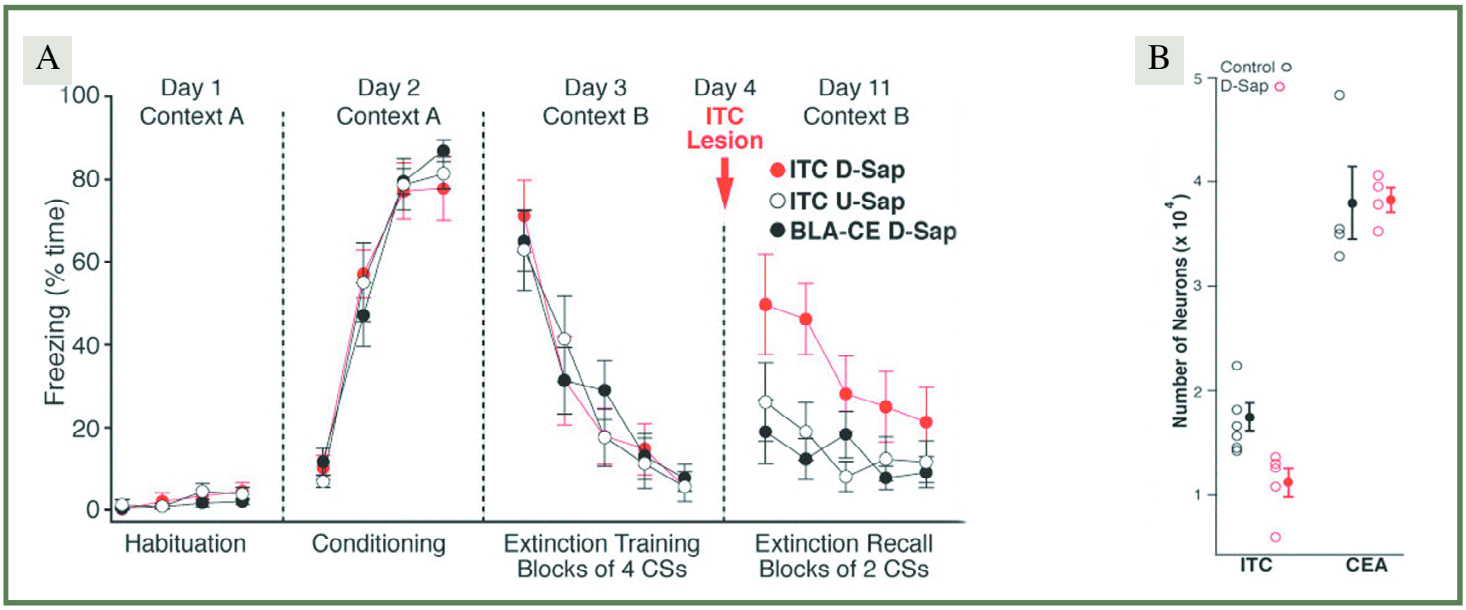
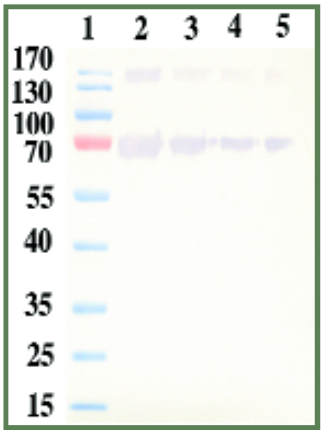


Figure 2. Infusions of Dermorphin-SAP decrease the number of ITC cells and lead to a deficit in extinction recall.
(A) Percent time freezing over experimental sessions in animals receiving Dermorphin-SAP injections in the ITC cells (red circles), in the BLA-CEA (black circles), or Blank-SAP injections in the ITC cells (white circles).
(B) Unbiased stereological estimates of cell numbers (mean ±sem, filled black circles) in the medial ITC cell clusters and central nucleus (CEA) in experimental (red circles) versus control (white circles) animals.

Targeting Tools: Featured Products

Anti-Human Serum Albumin NEW!



Human Serum Albumin (HSA) is a major constituent of human blood plasma comprising 55% of plasma proteins. Human serum albumin is produced in the liver and plays an important role in maintaining fluid balance in the blood.

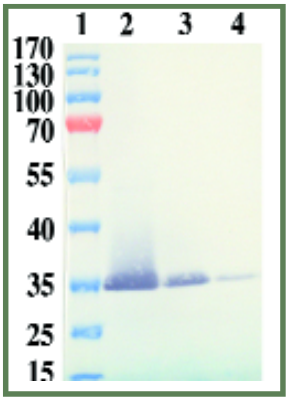
Affinity-purified Antibody to HSA, rabbit polyclonal,(Cat. #AB-40AP) is affinity-purified with HSA on CnBr-activated Sepharose 4 Fast Flow followed by affinity adsorption with human IgG. Applications for this antibody include ELISA and immunoblotting.

Lane 1: Molecular weight standards (Fermentas PageRuler)
Lane 2: 200 ng of HSA probed with Rabbit anti-HSA affinity-purified at 1:10,000
Lane 3: 100 ng of HSA probed with Rabbit anti-HSA affinity-purified at 1:10,000
Lane 4: 50 ng of HSA probed with Rabbit anti-HSA affinity-purified at 1:10,000
Lane 5: 25 ng of HSA probed with Rabbit anti-HSA affinity-purified at 1:10,000

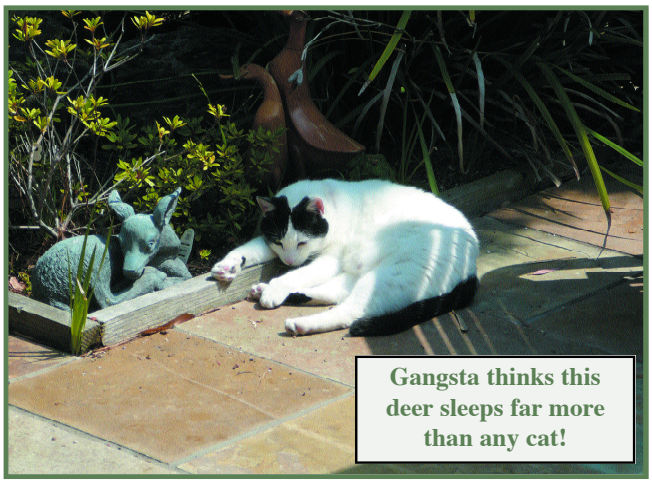
New Saporin Antibody NEW!

Saporin is a plant enzyme with N-glycosidase activity that depurinates a specific nucleotide in the ribosomal RNA 28S, thus irreversibly blocking protein synthesis. **Affinity-purified Anti-Saporin rabbit polyclonal (Cat. #AB-41AP)** is the most recent addition to our line of Saporin antibodies. These antibodies can be used as controls with our Saporin-conjugate immunotoxins, for immunoblotting, and in other applications.

AB-15	Anti-Saporin, goat polyclonal
AB-15AP	Anti-Saporin, affinity-purified goat polyclonal
AB-17AP	Anti-Saporin, affinity-purified chicken polyclonal
AB-41AP	**Anti-Saporin, affinity-purified rabbit polyclonal**



Lane 1: Molecular weight standards (Fermentas PageRuler)
Lane 2: 1 μg of Saporin probed with AB-41AP at 1:1000
Lane 3: 200 ng of Saporin probed with AB-41AP at 1:1000
Lane 4: 50 ng of Saporin probed with AB-41AP at 1:1000



Gangsta thinks this deer sleeps far more than any cat!

Targeting Technology

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

Choose an ANTIBODY§ specific to your cell type.

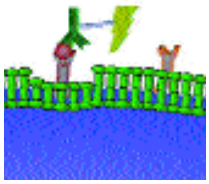
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

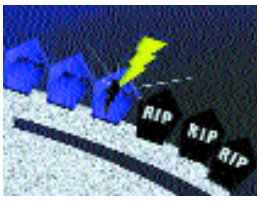
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to solve the puzzle.

OBAMAE

LUNGRARA

NITROPE

RUTUCEL

BRATUCOIN

What did the scientist use the microscope to see?

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

ANSWER: The . . .

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by November 30, 2008
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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