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Targeting Trends

Reporting the latest news in Molecular Surgery

Motivation's modulation of attention through the mesolimbic-corticopetal cholinergic circuitry

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Increasing attentional "effort" as a result of challenging circumstances, and as a function to maintain or recover attentional performance, is pervasive in our daily lives.[1] Attentional effort is more than a function of task difficulty; it is also a function of the subject's motivation to perform. Consider, for instance, your attention focused on driving when you are alone on a well-travelled road with no traffic around, in contrast to when a police vehicle is behind you. Indeed, in many real-world scenarios, attention and motivation are interwoven.

Motivational-incentive processing involves mesolimbic circuitry, particularly the dopaminergic midbrain and the nucleus accumbens (NAc).[2,3] Top-down control of attention relies on frontoparietal cortical regions.[4,5] However, the precise circuitry underlying motivation's modulation of attention had remained largely undefined.

Here, we show that interactions between the NAc and the basal forebrain corticopetal cholinergic projection system are essential components of the circuitry involved in the motivated recruitment of attention. We do this by using the operant sustained attention task (SAT). This task requires animals to press a lever to indicate the presence (signal trials) or absence (nonsignal trials) of a cue light. Correct responses to signal and nonsignal trials ("hits" and "correct rejections," respectively) result in a water reward. Incorrect responses ("misses" and "false alarms," respectively), are not rewarded. Animals undergo daily 40-minute sessions, which are divided into five 8-minute blocks for analyses. Once trained to reach criterion (70% correctly identified signal and non-signal trials) animals are also exposed to the more challenging distractor version of the task (dSAT). Overall attentional performance was determined using SAT/dSAT scores, that are derived from performance in both signal and non-signal trials. This measure ranges from -1.0 to +1.0, with -1.0 indicating that all responses were misses and false alarms, 0 indicating an inability to discriminate between signal and nonsignal events, and +1.0 indicating that all responses were hits and correct rejections. For a more comprehensive description of the methods and results, please see reference 6.

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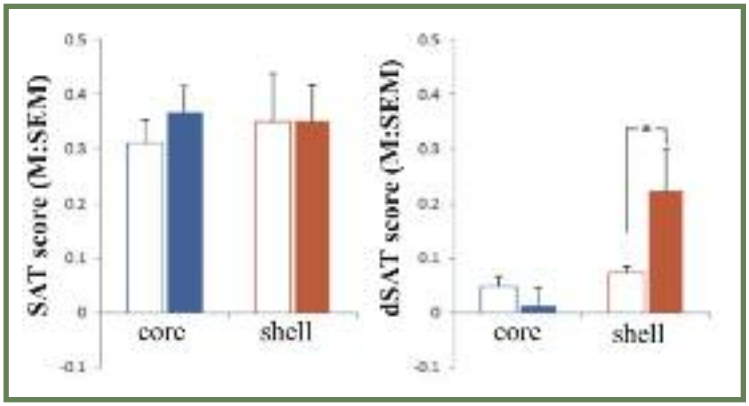


Figure 1. Attentional performance in animals bilaterally-infused with saline (open bars) or 0.33 nmol NMDA (shaded bars) in the core and shell subregions of the NAc. Attentional performance is shown for both the standard SAT and the more challenging dSAT versions. During SAT performance (left panel), stimulation of NMDA receptors in the core nor shell had an effect on attentional performance. Core stimulation had no effect during dSAT either. However, stimulation of NMDA receptors in the shell NAc enhanced performance during distractor presentation (*p<.05; LSD).

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Poster of the Year awarded by ATS at SFN 2011

For the first time in twelve years of giving out the Poster of the Year Award, ATS has selected two posters to be co-winners at the 2011 Society for Neuroscience meeting. The posters both used a conjugate of Neurokinin B and saporin that was prepared as a custom service by Advanced Targeting Systems. The posters are entitled:

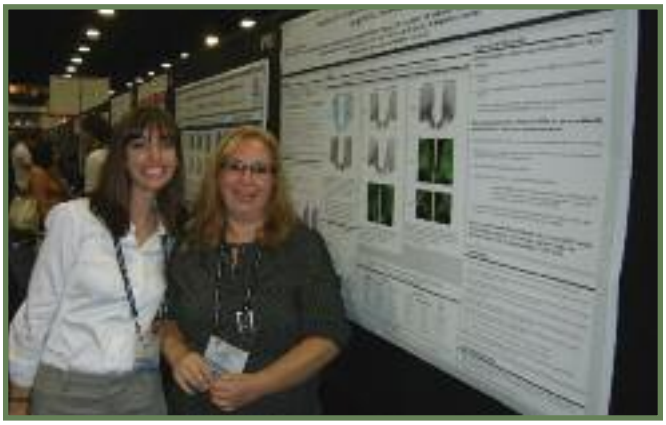
Ablation of NK3 receptor-expressing KNDy neurons in the rat arcuate nucleus using [MePhe7]Neurokinin B-Saporin

and

Arcuate NK3 receptor-expressing KNDy neurons are essential for estrogen modulation of LH secretion and body weight in the female rat

These posters combined all of the things one looks for in a winning poster: great idea, great histology, great demonstration of specificity and great, and meaningful, *in vivo* data. The fact that these were KNDy neurons was well, yes, sweet! There was obviously too much data for one poster, and the two posters made a great story together.

The award and our congratulations go to the presenters at the poster: Melinda Smith and Sally Krajewski from University of Arizona College of Medicine. We also congratulate Dr. Naomi Rance for putting together the splendid team we met at SFN and the resulting work.



Co-Winners of Poster of the Year Award: Melinda Smith and Sally Krajewski

Amer Assoc Cancer Research
Mar 30 - Apr 4, 2012
Chicago, IL
Booth #745

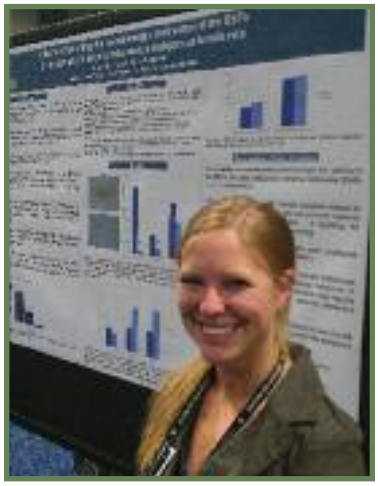
Upcoming Events

Experimental Biology
Apr 21 - 25, 2012
San Diego, CA
Booth #315

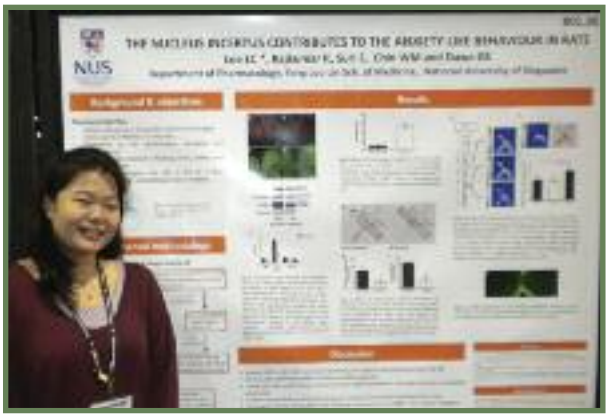
Honorable Mentions for ATS Poster of the Year at SFN 2011

The poster *Diminished norepinephrine release in the BSTv decreases anxiety but does not promote maternal behavior in nulliparous female rats*, presented by M.A. Holschbach at the 20ll Society for Neuroscience meeting, was a candidate for the Poster of the Year Award from Advanced Targeting Systems. Ms. Holschbach's poster has won Honorable Mention in a very competitive year, and we congratulate her and her colleagues at Michigan State University.

We very much appreciated this work using Anti-DBH-SAP (Cat. #IT-03), which included excellent histology and interesting behavioral effects in addressing the issues being studied. It was also an excellent opportunity to learn what 'nulliparous' means.



Honorable Mention for Poster of the Year Award: M.A. Holschbach



Honorable Mention for Poster of the Year Award: C. Lee

The poster *The nucleus incertus contributes to the anxiety-like behaviour in rats*, presented by C. Lee at the 20ll Society for Neuroscience meeting, was a candidate for the Poster of the Year Award and Ms. Lee's poster has also won Honorable Mention.

We very much appreciated this work using CRF-SAP (Cat. #IT-13). Congratulations to Ms. Lee and her collaborators at National University of Singapore.

Join us next year at the annual Society for Neuroscience meeting in New Orleans, Louisiana, October 13-17, 2012, Booth 616.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Phox2b-expressing neurons of the parafacial region regulate breathing rate, inspiration, and expiration in conscious rats.

Abbott SB, Stornetta RL, Coates MB, Guyenet PG.
J Neurosci 31(45):16410-16422, 2011.

Neurons in the retrotrapezoid nucleus (RTN) are involved in the CO_2-dependent control of breathing in conscious and anesthetized rats. In this work the authors specifically examined Phox2b-expressing glutaminergic neurons in the RTN. Rats received 44 ng of anti-DBH-SAP (Cat. #IT-03) into the lateral horn of the second thoracic segment in order to eliminate C1 neurons that project to the spinal cord. The data demonstrate regulation of lung ventilation by RTN-Phox2b neurons, and also that these neurons are not rhythmogenic in adults.

MAP kinases couple hindbrain-derived catecholamine signals to hypothalamic adrenocortical control mechanisms during glycemia-related challenges.

Khan AM KKL, Sanchez-Watts, Ponzio TA, Kuzmiski JB, Bains JS, Watts AG.
J Neurosci 31(50):18479-18491, 2011.

This work uses *in vivo* and *ex vivo* techniques to clarify how hypothalamic afferent pathways use intracellular mechanisms to modulate glycemia-related adrenocortical responses. Rats received 42-ng injections of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus of the hypothalamus. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The results establish a relationship between neurons from nutrient-sensing regions and intracellular mechanisms in hypothalamic corticotropin-releasing hormone neuroendocrine neurons.

Cholinergic Control in Developing Prefrontal-Hippocampal Networks.

Janiesch PC, Kruger HS, Poschel B, Hanganu-Opatz IL.
J Neurosci 31(49):17955-17970, 2011.

In this work the authors examined the role of acetylcholine in the maturation of cognitive processing due to oscillatory rhythms entraining neuronal networks. Rats received 50 ng of 192-IgG-SAP (Cat. #IT-01) into each lateral ventricle, or 25 ng directly into the medial septum. Among other results, cholinergic input was shown to reach the prefrontal cortex toward the end of the first postnatal week, initially targeting GABAergic neurons. Reduction of this activity by lesioning cholinergic neurons may cause global diminishment of neocortical activity.

Unidirectional Cross-Activation of GRPR by MOR1D Uncouples Itch and Analgesia Induced by Opioids.

Liu XY, Liu ZC, Sun YG, Ross M, Kim S, Tsai FF, Li QF, Jeffry J, Kim JY, Loh HH, Chen ZF.
Cell 147(2):447-458, 2011.

Recent work has begun to define the different pathways used by itch and pain. This study was designed to investigate whether opioids cause the itch sensation by gastrin-releasing peptide receptor activation. Mice received intrathecal injections of bombesin-SAP (Cat. #IT-40) in order to investigate the coexpression of various signaling molecules in the spinal cord. Blank-SAP (Cat. #IT-21) was used as a control. The data suggest that opioid-induced itch is independent of opioid analgesia, and is controlled through a mu-opioid receptor isoform.

Minireview: The value of looking backward: the essential role of the hindbrain in counterregulatory responses to glucose deficit.

Ritter S, Li AJ, Wang Q, Dinh TT.
Endocrinology 152(11):4019-4032, 2011.

This review examines work addressing how particular glucose-sensing cells function in glucoregulation under specific physiological or pathological conditions. There are specific populations of norepinephrine (NE) and epinephrine (E) neurons in the hindbrain that mediate these responses. The use of anti-DBH-SAP (Cat. #IT-03) to eliminate selective NE/E subgroups without disrupting basic functions is discussed.

Recognition of novel objects and their location in rats with selective cholinergic lesion of the medial septum.

Cai L, Gibbs RB, Johnson DA.
Neurosci Lett Epub2011.

This work examined object recognition and object location recognition as specific components of memory. Rats received 0.22 µg of 192-IgG-SAP (Cat. #IT-01) infused into the medial septum followed by testing in novel object recognition (NOR) and object location recognition (OLR) models. Substantial

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

decreases in choline acetyltransferase activity in the hippocampus and frontal cortex produced no difference in NOR but caused a significant impairment in OLR – highlighting the role that septo-hippocampal cholinergic projections play in OLR.

Depletion of Endogenous Noradrenaline Does Not Prevent Spinal Cord Plasticity Following Peripheral Nerve Injury.

Hayashida KI, Peters CM, Gutierrez S, Eisenach JC.

J Pain Epub2011.

The authors examined what involvement noradrenergic fibers in the spinal cord have in neuronal and glial plasticity associated with neuropathic pain states. Rats received 5-µg intrathecal injections of anti-DBH-SAP (Cat. #IT-03). Lesioned animals did not display altered mechanical withdrawal thresholds, but L5-L6 spinal nerve ligation in these animals caused enhanced mechanical hypersensitivity and analgesia induced by intrathecal clonidine. The data suggest that endogenous noradrenaline may play an inhibitory role on glial activation.

Impaired Visual Search in Rats Reveals Cholinergic Contributions to Feature Binding in Visuospatial Attention.

Botly LC, De Rosa E.

Cereb Cortex Epub2011.

Previous work established the role of acetylcholine from the nucleus basalis magnocellularis in attentional processing and visuospatial attention. In order to investigate the necessity of cortical cholinergic input for support of feature binding in visuospatial attention the authors administered bilateral intraparenchymal injections of 192-IgG-SAP (Cat. #IT-01, 4 injections, 40 ng per injection). Lesioned animals took longer to locate targets during type-specific search trials, demonstrating that cholinergic input influences feature binding during visuospatial attention tasks.

Control of the central chemoreflex by A5 noradrenergic neurons in rats.

Taxini CL, Takakura AC, Gargaglioni LH, Moreira TS.

Neuroscience Epub2011.

The A5 group of noradrenergic neurons in the ventrolateral pons is involved in the control of sympathetic and respiratory networks. Using anti-DBH-SAP (Cat. #IT-03) the authors eliminated TH+ neurons in order to clarify which aspects of respiration are modulated by A5 neurons. Rats received bilateral 4.2-ng injections of the toxin into the A5 region. The results suggest that A5 noradrenergic neurons are involved in control of mean arterial pressure, splanchnic sympathetic nerve activity, and phrenic nerve activity.

Reassessment of the structural basis of the ascending arousal system.

Fuller P, Sherman D, Pedersen NP, Saper CB, Lu J.

J Comp Neurol 519(5):933-956, 2011.

Traditional thought has been that electroencephalogram activity is mainly generated by the thalamocortical system. In this work the authors investigated the effects of basal forebrain lesions on various measurments of wakefulness. Rats received four 50-ng injections of 192-IgG-SAP (Cat. #IT-01) into the basal forebrain. The effects of these lesions showed that the parabrachial nucleus/precoeruleus region projection relayed by the basal forebrain to the cerebral cortex plays a critical role in behavioral and electrocortical arousal.

Redefining the components of central CO2 chemosensitivity - towards a better understanding of mechanism.

Huckstepp RT, Dale N.

J Physiol 589(Pt 23):5561-5579, 2011.

This review discusses advances in the field of CO_2 chemosensitivity over the past few years. Discussion of the role that locus coeruleus (LC) neurons play in this process includes the use of anti-DBH-SAP (Cat. #IT-03) to reduce the hypercapnic ventilatory response. Data from these and other experiments support a role of the LC in modulation of the ventilatory response to hypercapnia.

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

Targeting Talk: Product Questions

Q: I have a question regarding your antibody to NGF (p75) receptor antibody (Cat. #AB-N01AP). Could you please tell me how you determined that it is a blocking antibody? Has this information been published?

A: Thank you for your interest in our products and your message via our website. I would be happy to help answer your question regarding AB-N01AP, affinity-purifed anti-NGFr (p75). We list on our website that one application for this antibody is for blocking the function of nerve growth factor receptor. This information was presented in an abstract at the Society for Neuroscience Meeting held in 1994.

Huber LJ, Lee K-F, Dreyfus CF, Chao MV (1994) Generation and characterization of a murine p75 receptor blocking antibody. *Soc Neurosci Mtg, Miami Beach FL*, Abstract #23-12.

Here is a link to the references page on our website that lists other publications describing applications for this antibody.
http://www.atsbio.com/reference/abn.html#abn01ap

Send a message on our website to get answers to your targeting questions.

Q: I plan to use your Secondary Antibody Conjugates, Rab-ZAP (Cat. #IT-05), and Fab-ZAP Rabbit (Cat. #IT-57) with my primary antibody and would like to observe eliminated cells using a fluorescence microscope. The idea is to co-culture cancer cells and fibroblast cells, and kill fibroblast cells only with a specific primary antibody. Then I want to observe the eliminated fibroblast cells and take pictures with a fluorescence microscope. Can you recommend a protocol?

A: In order to stain and visualize the cells that are being eliminated, it would be best to stain for Saporin using a fluorescently-tagged antibody such as the FITC-labeled Saporin antibody (Cat. #FL-02). By washing off the media after a day, and then staining for saporin, one would illuminate only the cells that have internalized the saporin (marking them for death). The cells that do not stain for saporin will live.

Q&A Products

Antibody to Nerve Growth Factor (p75) Receptor, Affinity-Purified (AB-N01AP)
FITC-labeled Anti-Saporin (FL-02)

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles:	COMMON
	CONCEPT
	NOCIFENSIVE
	HYPERSENSITIVITY
	DORSAL
Answer:	MONSTER MASH!

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

WINNERS: Yasuhiro Maeda, VA Medical Center, East Orange, NJ * Kim Van Vliet, Univ Florida Biochemistry, Gainesville, FL * Abel Shalom, Syngene International, Bangalore, Karnataka, INDIA * E Polin-Purch, Montefiore Hospital, Bronx, NY * Andres Rodriguez, Univ Puerto Rico * Bob Speth, Nova Southeastern Univ, Fort Lauderale, FL

Solve this quarter's Teaser online at: www.ATSbio.com/news/12q1_teaser.html

Motivation's modulation of attention through the mesolimbic-corticopetal cholinergic circuitry

(continued from page 1)

In this first set of studies, we examined whether stimulation of NAc benefits attentional performance during the SAT and dSAT. After animals were trained to criterion, they were implanted with bilateral guide cannulas in either the shell or the core region of the NAc. After surgery, animals were habituated to performing the task while being tethered. Each animal received four infusions – two during SAT and two during dSAT. For each condition, the animal received an infusion of vehicle (saline; 0.9%) or NMDA (Sigma-Aldrich), dissolved in 0.9% saline, so that each animal served as its own control. Animals received 0.5 µL of drug or vehicle into each site simultaneously, using a microinfusion pump (Model CMA/100; Carnegie Medicine) at the rate of 0.5 µL/minute. Infusions occurred during the first two minutes of block 2 to enable demonstration of similar SAT performance pre-infusion.

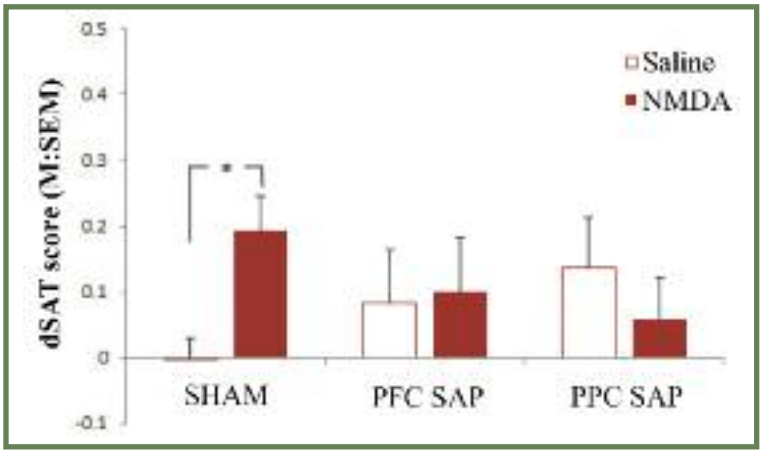


Figure 2. Infusions of NMDA into the shell of the NAc restored performance during distractor blocks in animals that received sham surgeries for cholinergic deafferentation of the PFC or PPC, but not following removal of PFC ("PFC SAP"), or PPC ("PPC SAP") cholinergic inputs. In deafferented animals, NMDA infusions failed to benefit dSAT performance (*p<0.05; LSD).

Effects of NAc shell and core infusions on SAT and dSAT performance (omnibus test). The analysis of the effects of group (shell vs. core), task type (SAT vs. dSAT), block of trials (t1–t5), signal duration (500, 50, 25 ms), and dose of NMDA (0, 0.067, 0.33, 1.01 nmol/hemisphere) indicated a significant interaction between all factors ($F(8,464) = 2.72$, $p = 0.01$). No differences were found between conditions pre-infusion (data shown in reference 6). To be concise, the most effective dosage found for infusions are reported in this summary. Post hoc analyses revealed that stimulation of NMDA receptors in the shell or core had no effect during SAT performance. When attentional demands were increased by the dSAT, stimulation of NMDA receptors (0.33 nmol) in the shell enhanced performance (see Figure 1). NMDA stimulation continued to have no effect when infused in the core during dSAT.

In the second set of studies, we wanted to see if the benefits afforded by stimulation of the NMDA receptors in the shell were related to its neural connectivity with the cortical cholinergic system. Animals were randomly assigned to receive sham surgeries, cholinergic deafferentation of the prefrontal cortex (PFC), or cholinergic deafferentation of the posterior parietal cortex (PPC). Sham surgeries were performed using the control immunotoxin, mouse IgG-SAP (Cat. #IT-18), whereas cholinergic deafferentation was produced using 192 IgG-SAP (Cat. #IT-01). All animals that received these surgeries (sham, PFC, or PPC) also received bilateral implantation of guide cannulas in the shell region of the NAc. They were tested using identical parameters to the previous study, receiving saline and NMDA infusions. As shown in Figure 2, the infusions in the sham animals replicated the results in Experiment 1 – namely, infusions of NMDA enhanced performance during dSAT (see left panel, Figure 2). The benefits of NMDA during dSAT were not observed in the PFC or PPC deafferented animals (middle and right panels, Figure 2).

These results demonstrate that motivation's modulation of attention is reliant on the interactions between the shell of the NAc and the basal forebrain corticopetal cholinergic projection system. Interestingly, this interaction is observed only when attentional systems are taxed during dSAT, as the benefits of NMDA infusions were not observed during SAT, suggesting this circuitry specifically underlies the motivated recruitment of the basal forebrain corticopetal cholinergic projection system. Further, stimulation of the NAc core offered no benefits to attentional performance, showing the selectivity of the neural circuitry. Our results define a mesolimbic-basal forebrain cortical system that mediates the motivated activation of attentional mechanisms. Strategies designed to treat attentional impairments or enhance attentional performance may benefit from adopting broader concepts that integrate motivational-attentional interactions and from exploiting the multiple targets known to influence mesolimbic-basal forebrain circuitry.

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2. Knutson B *et al.* (2001) Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci* 21:RC159.
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5. Lim J, Wu WC, Wang J, Detre JA, Dinges DF, Rao H (2010) Imaging brain fatigue from sustained mental workload: an ASL perfusion study of the time-on-task effect. *Neuroimage* 49:3426 –3435.
6. St. Peters M, Demeter E, Lustig C, Bruno JP, & Sarter M (2011) Enhanced control of attention by stimulating mesolimbic-corticopetal cholinergic circuitry. *J Neurosci* 31: 9760-71.

Targeting Tools: Featured Products

Antibody to Metabotropic Glutamate Receptor 2

There are eight known metabotropic glutamate receptors (mGluR) playing diverse roles in brain function and pathology. They are 7-transmembrane domain receptors involved in learning, memory, anxiety, synaptic plasticity, and pain perception. The ligand for these receptors is glutamate, which functions as an excitatory neurotransmitter. mGluR2 is involved in the inhibition of the cAMP cascade. Potentiation of mGluR2 has recently emerged as a new approach for the treatment of schizophrenia.

Anti-mGluR2 (Cat. #AB-N32) is a mouse monoclonal antibody against a GST fusion protein containing a 47-amino acid sequence from the C-terminal domain of mGluR2. It has been tested in western blot on rat cortical tissue extracts.

Currently in Production: anti-mGluR5

References

1. Neki A, Ohishi H, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N (1996) Pre- and postsynaptic localization of a metabotropic glutamate receptor, mGluR2, in the rat brain: an immunohistochemical study with a monoclonal antibody. *Neurosci Lett* 202(3):197.
2. Neki A, Ohishi H, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N (1996) Metabotropic glutamate receptors mGluR2 and mGluR5 are expressed in two non-overlapping populations of Golgi cells in the rat cerebellum. *Neuroscience* 75(3):815-826.

Anti-mGluR2 (Cat. #AB-N32)

Rat cortical tissue extracts were run on SDS-PAGE (5 µg in lane 1, 25 µg in lane 2 for each blot). AB-N32 was applied to blot A at a 1:500 dilution, and blot B at 1:2000. Both blots were incubated with an anti-mouse-HRP secondary antibody at 1:10,000. The protein was run under reducing conditions (40 mM DTT); both monomer and dimer forms of mGluR2 are recognized.

Data provided by Marek Schwendt

Tag-Targeted Toxins

Tag-targeted toxins are new conjugates in the Targeted Toxins family. Antibodies specific for tags on expressed proteins are conjugated to the ribosome-inactivating protein, saporin (from the seeds of the plant, *Saponaria officinalis*). The conjugate binds to your tag that is expressed on the cell surface and then internalizes by antibody-mediated endocytosis. Saporin breaks away from the targeting agent, and inactivates the ribosomes which causes protein synthesis inhibition and, ultimately, cell death. Cells that do not have the tag are not affected.

Perhaps Gangsta should wear his safety glasses when he eats cat grass!

Anti-6 His-ZAP (Cat. #IT-52)

Specifically eliminates cells expressing your **6 His-tagged recombinant proteins on the cell surface.**

Anti-GFP-ZAP (Cat. #IT-53)

Specifically eliminates your cells demonstrating **extracellular expression of green fluorescent protein (GFP).**

Anti-V5-SAP (Cat. #IT-58)

Specifically eliminates cells expressing your **extracellular V5.**

Anti-FLAG (M5)-ZAP (Cat. #IT-58)

Specifically eliminates cells expressing your **FLAG-tagged recombinant proteins on the cell surface.**

Control for Tag-Targeted Toxins

Mouse IgG-SAP (Cat. #IT-18) is the same molecular weight, consists of similar, comparable materials and is synthesized with the same protocols as the tag-targeted toxins. The difference between targeted toxins and controls is the cell-specific targeting agents are replaced with "blanks," antibodies or peptides that have no specificity, and no ability to target cells. Mouse IgG-SAP is the perfect control molecule to be used with these Tag-Targeted Toxins.

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Choose an ANTIBODY§ specific to your cell type.

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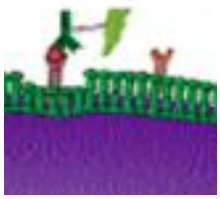
ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

§ or anything recognized on the cell surface and internalized.

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

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



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

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

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

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

CRYTURCII

ONSNALING

FRECEPROMAN

ROBENFAIR

DIVOTEMAT

Why the surgeon took his job so seriously.

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

ANSWER: HE HAD NO ... _____ _____ !

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 March 1, 2012.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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Apr-May-Jun 2012
Volume 13, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Use of a novel saporin conjugate (NK3-SAP) to study the function of neurokinin 3 receptor (NK3r)-expressing kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the rat arcuate nucleus

Contributed by Naomi E. Rance, Melinda. A. Mittelman-Smith and Sally J. Krajewski-Hall
Department of Pathology, University of Arizona College of Medicine

This year, our two posters shared the ATS Poster of the Year Award at the Society for Neuroscience Meeting. These posters documented the use of NK3-SAP, a custom conjugate of saporin with a neurokinin 3 receptor (NK3r) agonist, as a tool to ablate NK3r-expressing KNDy neurons in the rat brain. In the first poster, we demonstrated the effectiveness of NK3-SAP for selective ablation of KNDy neurons in the rat arcuate nucleus. In the second poster, this technique was used to examine the role of KNDy neurons in the estrogen regulation of LH secretion and body weight. These data have recently been accepted for publication in *Endocrinology*.[1]

Although estrogen withdrawal has profound effects on gonadotropin secretion and body weight, the neural circuitry underlying these effects is not well understood. Recent studies have focused on a group of arcuate neurons expressing estrogen receptor alpha and the KNDy peptides in the estrogen modulation of reproduction.[2-3] Because these neurons express NK3r,[4] we hypothesized that they could be ablated using saporin conjugated to a selective NK3r agonist. NK3-SAP was obtained as a custom conjugate from Advanced Targeting Systems. Pilot studies validated the selectivity of NK3-SAP for ablation of NK3r neurons in the rat brain.

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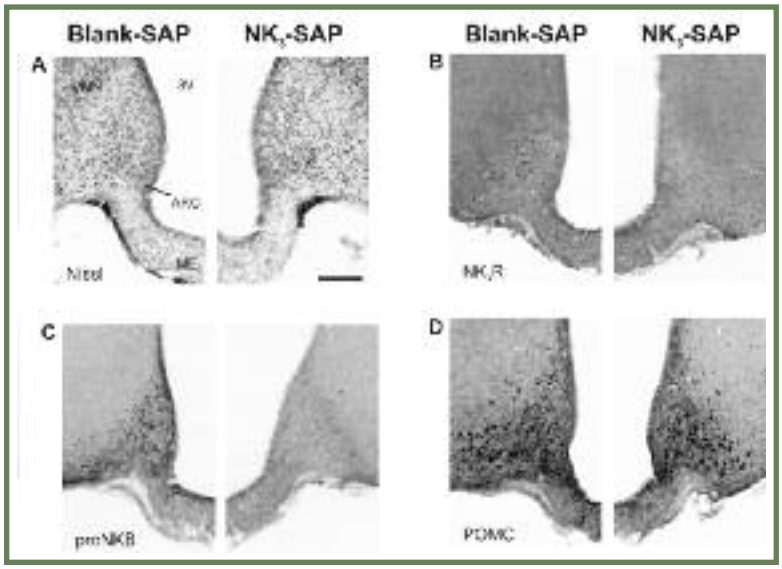


Figure 1. *Representative photomicrographs of sections from rats with injections of Blank-SAP (left) or NK3-SAP (right) in the arcuate nucleus, stained with Cresyl-violet (A) or immunohistochemistry (B-D).* **A:** Cresyl violet-stained sections show preservation of the Nissl-architecture in NK3-SAP rats. **B-C**: NK3-SAP rats exhibited near total depletion of NK3r and proNKB immunoreactive neurons and fibers in the arcuate nucleus and median eminence. **D:** There was no difference in the number of proopiomelanocortin (POMC) neurons between Blank-SAP and NK3-SAP rats, demonstrating specificity of NK3-SAP.

Abbreviations: 3V, third ventricle; ARC, arcuate nucleus; ME, median eminence; NK3r, neurokinin B receptor; proNKB, proneurokinin B; VMN, ventromedial nucleus. Scale bar in A = 100 μm and applies to all.

Inside this issue:

Newsletter Highlights

- ATS Celebrates 18th Birthday; New Shipping Boxes *(page 2)*
- OX7-SAP, Chick-ZAP, Gibberellic Acid Antibody *(page 5)*
- Teaser Winners *(page 5)*
- NK3-SAP, Chick-ZAP, Anti-RFP *(page 7)*

Denise Higgins, Editor

ATS Celebrates 18 Years Serving the Research Community

Advanced Targeting Systems became a company on April 22, 1994, based on the saporin targeting technology Dr. Douglas Lappi refined in his academic laboratory. In collaboration with Dr. Ronald Wiley, this technology was developed still further to become a powerful research technique known as 'Molecular Surgery.'

Denise Higgins was working at Invitrogen when the three founders (Lappi, Wiley and Higgins) decided to form ATS. From the beginning it has been the ATS mission to bring innovative targeting tools to scientists around the world to further their research.

Today, ATS targeted toxins are used in the finest laboratories (academic and industrial) around the world. The premise is simple: use a targeting agent that is recognized on the cell surface and is internalized to deliver a powerful toxin inside a cell. Scientists use this technique to get rid of unwanted cells in culture, to set up behavioral or disease models in animals, or to screen antibodies for internalization and specificity.

ATS hopes the next eighteen years will provide our customers with many exciting, new targeting tools!

From Left to Right: Darlene Martineau, Douglas Lappi, Brian Russell, Miranda Ramirez, Matthew Kohls, Denise Higgins, Brandon Preddy, Leonardo Ancheta, Khrysten Taylor, David Young

Upcoming Events

Amer Assoc Immunologists
May 4 - May 8, 2012
Boston, MA
Booth #405

Amer Pain Society
May 17-19, 2012
Honolulu, HI
Booth #229

Check Out Our New Product Boxes!

All of the ATS employees participated in a contest to design our new product shipping boxes. Brian Russell was our winner!

Brian Russell came to Advanced Targeting Systems eleven years ago as a graduate of University of California, San Diego. He started out as a research technician at ATS with a dual role as flow cytometry technician for Cytometry Research (an in-house service organization that is a subsidiary of ATS).

Over the years, Brian became adept in targeted toxin production, flow cytometry analysis and sorting, product development and management, and customer service. He works in the booth at the Society for Neuroscience meeting, answers technical questions from customers, and even pitches in occasionally to take orders or prepare shipments.

It is a tradition at ATS, that on your tenth anniversary you are given three options for a special gift. Brian decided he wanted to take a trip to South Africa. He took some spectacular photos and even made a 2012 calendar depicting some of the awesome animals and scenery from his trip. Here he is in the picture to the right getting up close and personal with a major cat!

Mr. Russell is a senior Product Manager and Head of Custom Saporin Conjugations.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Regulation of Ejaculation via Intraspinal Connections.

Staudt MD, Truitt WA, McKenna KE, de Oliveira CV, Lehman MN, Coolen LM.
J Sex Med Epub 2011.

The authors examined the hypothesis that specific lumbar spinothalamic (LSt) cells control ejaculation through intraspinal connections. Rats received six bilateral injections of SSP-SAP (Cat. #IT-11) into the spinal cord, 48 ng in total. Saporin (Cat. #PR-01) was used as a control. It was found that while erectile function and emission were not affected, the usual rhythmic contractions of the bulbocaveronosus muscle during ejaculation were prevented.

Carrageenan induced phosphorylation of Akt is dependent on neurokinin-1 expressing neurons in the superficial dorsal horn.

Choi JI, Koehrn FJ, Sorkin LS.
Mol Pain 8(1):4, 2012.

In this work the authors administered 100 ng SSP-SAP (Cat. #IT-11) into the intrathecal space of rats (saporin, Cat. #PR-01 was used as a control). Lesioned animals displayed decreased carrageenan-induced mechanical allodynia, and carrageenan-induced phosphorylation of Akt was blocked throughout the spinal cord gray matter. Anti-NK-1 (Cat. #AB-N33AP) was used for immunohistochemistry.

Subplate neurons promote spindle bursts and thalamocortical patterning in the neonatal rat somatosensory cortex.

Tolner EA, Sheikh A, Yukin AY, Kaila K, Kanold PO.
J Neurosci 32(2):692-702, 2012.

Immature cortices in both human and rat have spontaneous activity associated with the maturation of cortical synapses and neuronal circuits. In order to investigate what cells are controlling these events the authors administered 400 ng of 192-IgG-SAP (Cat. #IT-01) to the S1 cortex hindlimb/forelimb area of rats. mu p75-SAP (Cat. #IT-16) and mouse-IgG-SAP (Cat. #IT-18) were used as controls. This lesion eliminates subplate neurons which results in a significant loss of evoked spindle burst activity.

Cholinergic modulation of a specific memory function of prefrontal cortex.

Croxson PL, Kyriazis DA, Baxter MG.
Nat Neurosci 14(12):1510-1512, 2011.

The authors investigated loss of acetylcholine in the large and highly differentiated PFC's of rhesus monkeys. The monkeys received 80-92 20-ng injections of ME20.4-SAP (Cat. #IT-15) per hemisphere. Lesioned animals were severely impaired on tasks involving spatial working memory.

Neuromodulation targets intrinsic cardiac neurons to attenuate neuronally mediated atrial arrhythmias.

Gibbons DD, Southerland EM, Hoover DB, Beaumont E, Armour JA, Ardell JL.
Am J Physiol Regul Integr Comp Physiol 302(3):R357-64, 2012.

Cardiac arrhythmias can be generated by excessive activation of specific inputs to the intrinsic cardiac nervous system. The authors sought to determine whether subpopulations of neurons were responsible for this activation, and therefore potential therapeutic targets. A series of studies were done following electrical stimuli to the mediastinal nerves. Choline acetyltransferase levels were assessed using anti-ChAT (Cat. #AB-N34) in immunohistochemistry. The data suggest activation of certain neurons by mediastinal nerve stimulation results in atrial arrhythmias leading to atrial fibrillation.

Application of anti-CD103 immunotoxin for saving islet allograft in context of transplantation.

Zhang L, Hadley GA.
Chin Med J (Engl) 123(24):3644-3651, 2010.

This work investigates whether depletion of CD103-positive cells protects transplanted islets from host-immune cell attack. Diabetes was induced in mice, followed by an islet transplant. Anti-CD103-SAP (Cat. #IT-50) was administered via i.p. injection (1.0 mg/kg or 2.0 mg/kg). Rat IgG-SAP (Cat. #IT-17) was used as a control. Diabetic mice treated with anti-CD103-SAP after islet transplantation had an indefinite survival time as compared to untreated mice that survived fewer than 20 days.

Spinal bombesin-recognized neurones mediate more nonhistaminergic than histaminergic sensation of itch in mice.

Han N, Zu JY, Chai J.
Clin Exp Dermatol Epub 2012.

The authors administered 400 ng of Bombesin-SAP (Cat. #IT-40) to the lumbar spinal subarachnoid space of rats and evaluated the distribution of Fos-positive cells in the dorsal horn after stimulation. Saporin (Cat. #PR-01) was used as a control. The results demonstrate that the neurons eliminated by Bombesin-SAP are critical to both acute and chronic itch pathways,

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

although they have more effect on nonhistaminergic sensation.

IB4-saporin attenuates acute and eliminates chronic muscle pain in the rat.

Alvarez P, Gear RW, Green PG, Levine JD.
Exp Neurol 233(2):859-865, 2012.

In order to clarify the roles of isolectin B4-positive and IB4-negative nociceptors in inflammatory and ergonomic muscle pain, the authors administered 3.2 µg of IB4-SAP (Cat. #IT-10) into the intrathecal space of rats. Although the baseline mechanical nociceptive threshold was not affected in the lesioned animals, mechanical hyperalgesia had a shorter duration. In the ergonomic models peak hyperalgesia was attenuated, and prolongation of PGE2-induced mechanical hyperalgesia was completely prevented.

Two patterns of thrombopoietin signaling suggest no coupling between platelet production and thrombopoietin reactivity in thrombocytopenia-absent radii syndrome.

Fiedler J, Strauss G, Wannack M, Schwiebert S, Seidel K, Henning K, Klopocki E, Schmugge M, Gaedicke G, Schulze H.
Haematologica 97(1):73-81, 2012.

Lower than normal blood platelet counts result from a congenital disorder called thrombocytopenia (thrombocytopenia absent radii syndrome, or TAR). Recent work indicates a complex pattern of inheritance, and possibly that TAR is at least a digenic disorder. The authors performed an extended study investigating signal transduction via immunoblotting, gel electrophoretic shift assays, and flow cytometry. One of the antibodies used was anti-p70 S6K (Cat. #AB-241). The authors conclude that there are defects in both platelet production and function in TAR.

Age-related Accumulation of Non-heme Ferric and Ferrous Iron in Mouse Ovarian Stroma Visualized by Sensitive Non-heme Iron Histochemistry.

Asano Y.
J Histochem Cytochem 60(3):229-242, 2012.

The mammalian ovary engages in continuous growth and cellular differentiation as long as the animal is capable of reproduction. During these processes iron ions are released from heme structures; these ions are capable of generating free radicals. The purpose of this study was to investigate non-heme iron distribution in ovarian tissue, and how this distribution changes during aging. Lipid peroxidation was monitored by immunohistochemistry using anti-conjugated malondialdehyde (Cat. #AB-T090). The data indicate that increasing oxidative stress, non-heme iron accumulation in ovarian stromal tissue, and aging are related.

Selective cholinergic depletion in medial septum leads to impaired long term potentiation and glutamatergic synaptic currents in the hippocampus.

Kanju PM, Parameshwaran K, Sims-Robinson C, Uthayathas S, Josephson EM, Rajakumar N, Dhanasekaran M, Suppiramaniam V.
PLoS One 7(2):e31073, 2012.

Long term potentiation (LTP) is dependent on excitatory neurotransmission in the hippocampus, which plays a major role in learning and memory. The authors examine whether cholinergic lesions in the medial septum result in LTP alteration or affect synaptic glutamate receptor subtypes. After bilateral administration of 192-IgG-SAP (Cat. #IT-01, 50 ng per injection) into the medial septum of rats, hippocampal slices were made and the LTP of the slices was measured. The data show modulation of medial septal LTP and hippocampal glutaminergic currents by cholinergic afferents.

Strongly amphiphilic photosensitizers are not substrates of the cancer stem cell marker ABCG2 and provides specific and efficient light-triggered drug delivery of an EGFR-targeted cytotoxic drug.

Selbo PK, Weyergang A, Eng MS, Bostad M, Maelandsmo GM, Hogset A, Berg K.
J Control Release Epub 2012.

Many anti-cancer drugs are substrates of the ATP-binding cassette transporter ABCG2. Unfortunately ABCG2 is also thought to play an important role in multi-drug resistance and the protection of cancer stem cells against chemotherapeutics and photodynamic therapy. This paper examined whether photosensitizers used in photochemical internalization (PCI) are substrates for ABCG2. Streptavidin-ZAP (Cat. #IT-27) was combined with biotinylated EGF and applied to cells in culture; saporin (Cat. #PR-01) was used as a control. The data show that PCI with the EGF-saporin toxin did not utilize ABCG2 to enter cells.

Targeting Talk: Product Questions

Q&A Products

Anti-Conjugated Gibberellic Acid (AB-T130)
OX7-SAP (IT-02)
Chick-ZAP (IT-62)

Q: I was wondering whether it could be possible to receive more information about the Gibberellic acid antibody (AB-T130)? Is it possible to use this antibody to recognize free gibberellic acid by a direct ELISA system?

A: The most common use for this product is immunohistochemistry. The tissue is perfused with a gluteraldehyde component, and that gluteraldehyde provides the epitope complement needed for the antibody to recognize gibberellic acid. On its own, gibberellic acid is too small a molecule to provide a complete effective epitope.

* * *

Q: I'd like to know which of your products are the pan-neural targeting toxins? I need an agent to kill all nerves in tissue preps.

A: OX-7-SAP (IT-02) should be perfect for this application. We recommend you examine your neurons with OX-7 antibody to see if they are positive. The only complication would be if you want to look at T-lymphocytes that also express Thy 1.

Q: We are interested in having a custom conjugation of saporin and our antibody. Do you remove unconjugated antibody from the final material you send us?

A: We do remove the unconjugated antibody and saporin from the final product that we send to you. And perhaps to answer a question you may not be asking, but may be curious about, the unconjugated material is not particularly usable, after it has been removed from the final product as it has been slightly modified in preparation for the conjugation.

* * *

Q: We will be using your chick-ZAP secondary conjugate (IT-62) and noticed that in your protocol you mention not to use a reducing agent in your media. Our normal growth media contains beta mercaptoethanol at 100 µM. Will this be a problem?

A: Officially, we would recommend allowing the cells to acclimate to media that contains NO BMe, and then proceed with your experiments. However, some of our in-house experiments use cells that are cultured in media containing 50 µM BMe, and we have not seen that concentration affect the toxin's effectiveness, but we have not tried a concentration as high as 100 µM.

Send a message on our website to get answers to your targeting questions.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: CIRCUITRY
NONSIGNAL
PERFORMANCE
FOREBRAIN
MOTIVATED

Answer: He had no FUNNY BONE!

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

WINNERS: Sherie Ma, Florey Neuroscience Institutes, Parkville Australia * Glenn H. Kageyama, Cal Poly Pomona University, Pomona, CA * Roger Guillemin, La Jolla, CA * Seto Chice, SUNY-HSC, Brooklyn, NY * Nakon Aroonsakool, University California San Diego, San Diego, CA * Khalid Touzani, CUNY Brooklyn, NY

Solve this quarter's Teaser online at: www.ATSbio.com/news/12q2_teaser.html

Use of NK3-SAP to study the function of NK3r-expressing KNDy neurons in the rat arcuate nucleus

(continued from page 1)

Twenty-four female rats were ovariectomized and received bilateral arcuate microinjections of either NK3-SAP or Blank-SAP (control). Three weeks later, animals were implanted with silastic capsules containing 17β-estradiol (E_2) and 11 days after that, they were sacrificed. Body weights and blood samples were taken at the beginning of the experiment, three weeks after ovariectomy, and at 11 days after E_2 -treatment. Immunohistochemical studies revealed ablation of KNDy neurons by NK3-SAP. There was near-complete loss of NK3r neurons in the arcuate nucleus with a 94-98% reduction in the number of kisspeptin and neurokinin B neurons, compared to Blank-SAP rats (Figure 1). In contrast, proopiomelanocortin, neuropeptide Y and GnRH immunoreactive elements were preserved.

We found that ablation of KNDy neurons resulted in marked changes in LH secretion and the E_2 modulation of body weight. In control animals, ovariectomy markedly increased serum LH and body weight and these effects were reversed by replacement with E_2. In contrast, NK3-SAP-injected rats did not exhibit a significant rise in serum LH in response to ovariectomy, and serum LH was lower in these animals regardless of estrogen status (Figure 2A). Surprisingly, the effects of ovariectomy and E_2 on body weight were blocked in rats with KNDy neuron ablation (Figure 2B). These data show an essential role of arcuate KNDy neurons for tonic LH secretion, the rise in LH in response to ovariectomy, and the E_2 modulation of body weight.

References

1. Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, Lai J, Ciofi P, McMullen NT, Rance NE. (2012) Arcuate kisspeptin/neurokinin B/dynorphin (KNDy) neurons mediate the estrogen suppression of gonadotropin secretion and body weight. *Endocrinology* April 16 Epub ahead of print.
2. Rance NE. (2009) Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. *Peptides* 30:111-122.
3. Lehman MN, Coolen LM, Goodman RL. (2010) Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arucate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology* 151:3479-3489.
4. Burke MC, Letts PA, Krajewski SJ, Rance NE. (2006) Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: morphologic evidence of interrelated function within the arcuate nucleus. *J Comp Neurol* 498:712 -726.

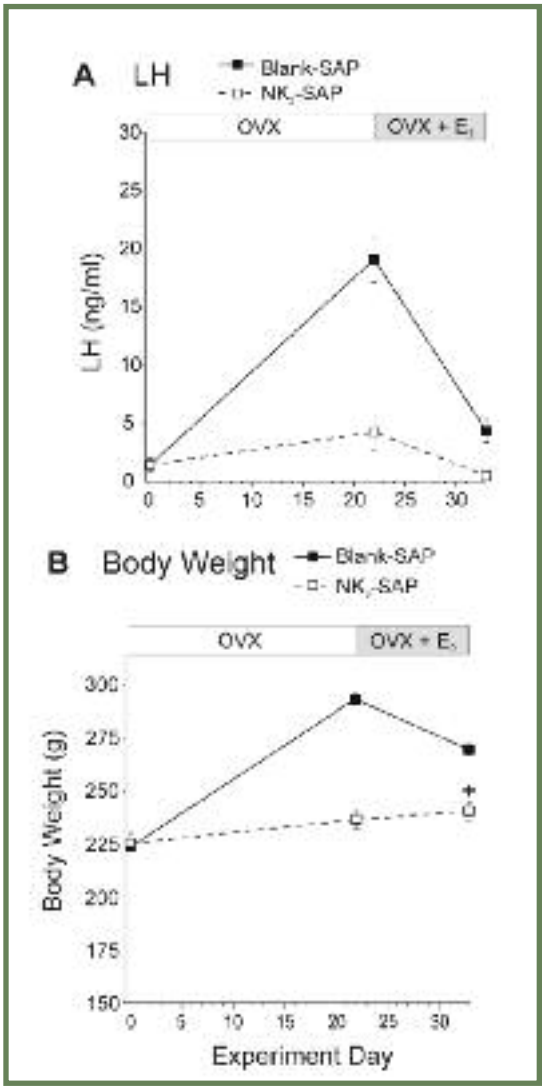


Figure 2. *Effects of KNDy neuron ablation on Serum LH (A) and body weight (B).* Rats were OVX and injected with NK3-SAP or Blank-SAP in the arcuate nucleus on day 0, given E_2 capsules 20-23 days later (shown as day 22), and sacrificed after 11 days of E_2.

A: Serum LH was markedly increased by OVX and reduced by E_2 treatment in Blank-SAP animals. In contrast, serum LH after OVX was not significantly different from intact values in NK3-SAP rats. Serum LH was significantly lower in KNDy-ablated rats than Blank-SAP controls regardless of estrogen treatment.

B: Following ovariectomy, Blank-SAP animals were 25% heavier relative to their initial body weight and lost weight following E_2 treatment. Rats with arcuate NK3-SAP injections gained small amounts of weight throughout the study, regardless of E_2 status.

Modified with permission from reference 1. Copyright 2012, *The Endocrine Society*

Targeting Tools: Featured Products

Antibody to Red Fluorescent Protein (RFP)

Red fluorescent protein (RFP) is derived from *Discosoma* sea anemone. This antibody recognizes native and denatured forms of RFP. The antigen for this antibody is present in all of these fluorescent molecules: RFP, tag-RFP, turbo-RFP, DeRed, mCherry, and mOrange. The epitope for the antibody is part of the RFP N-terminal sequence.

RFP is similar to GFP and can be used as a reporter of expression and transcription marker. It is effective for ELISA, immunoblotting (dot blot and western blot), immunoprecipitation, and immunostaining.

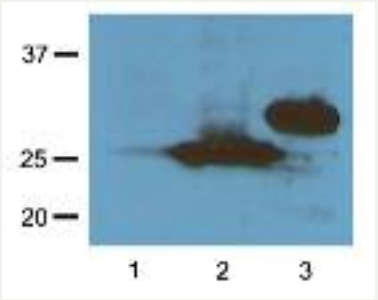
Anti-RFP (Cat. #AB-332)

1:1000 (1 µg/mL) Ab dilution probed against HEK293 cells transfected with RFP-tagged protein vector.

Lane 1: untransfected control

Lane 2: transfected with Turbo-RFP

Lane 3: transfected with DeRed



AB-332 Anti-RFP
100 micrograms

NK3-SAP

Neurokinin B (NKB), a neuropeptide that is a selective agonist of the NK3 receptor, allows for the selective elimination of a subpopulation of neurons expressing kisspeptin, neurokinin B, and dynorphin (KNDy neurons) through their co-expression of the NK3 receptor. These neurons have been shown to reside within the arcuate nucleus of many mammalian species, and the correlating peptides are critical for reproductive function.

NK3-SAP (molecular weight 31.5 kDa) is a chemical conjugate of neurokinin B and the ribosome-inactivating protein, saporin, and has been used to examine the role of KNDy neurons in the estrogen regulation of LH secretion and body weight in female rats (see cover article, pages 1 and 6).

IT-63 NK3-SAP
25 µg, 100 µg or 250 µg available individually or in a kit with Saporin (Cat. #PR-01) and Blank-SAP (Cat. #IT-21)

Chick-ZAP

Chick-ZAP is the most recent in our line of Secondary Conjugates. It is a chemical conjugate of affinity-purified rabbit anti-chicken IgY and the ribosome-inactivating protein, saporin. The conjugate will target and eliminate cells that recognize your primary antibody. This reagent can be utilized for screening chicken IgY antibodies for internalization and/or their suitability to make potent immunotoxins.

IT-62 Chick-ZAP
25 µg, 100 µg or 250 µg available individually or in a kit with Goat IgG-SAP (Cat. #IT-19)

**It wasn't me!
I didn't do it!
I've been in this cupboard all day!**

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

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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.

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

§ or anything recognized on the cell surface and internalized.

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

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

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

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

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

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

CATEURA

LAMITOONDU

BANALITO

GEWITH

JANGOCUTE

What the scientist thought was the hottest issue in science.

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

ANSWER: ☐☐☐☐☐☐ ☐☐☐☐☐☐☐ !

WIN $100.00

Limit one entry per laboratory.

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

See last quarter's winners, page 5.

Please correct the address information above and provide the following:

Your Name:
Phone:
Fax:
Email:

Jul-Aug-Sep 2012
Volume 13, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

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- LD_{50} of Saporin immunotoxins in mice; Antagonists or Targeted Toxins? *(page 5)*
- Teaser Winners *(page 5)*
- Mac-1-SAP, anti-GST, anti-SERT-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

A pivotal role of lumbar spinothalamic cells in regulation of ejaculation via intraspinal connections

Contributed by Lique M. Coolen, University of Michigan

Introduction from Dr. Lappi: *An article by Lique Coolen's group[5] was the 1000th article in PubMed in response to the search word 'saporin.' Dr. Coolen has graciously agreed to describe the latest in her fascinating work.*

Male sexual behavior consists of many different aspects, with ejaculation being the most rewarding. Ejaculation is a complex reflex controlled by a central pattern generator in the lumbosacral spinal cord, named the spinal ejaculation generator.[1] This generator receives sensory inputs during mating via the dorsal penile nerve and triggers emission and expulsion of seminal fluids via projections to autonomic and motor neurons in the lumbosacral spinal cord. For a long time it was hypothesized that the integration of sensory inputs and autonomic/motor outflow is mediated by a key group of interneurons located in the lumbar spinal cord. Indeed, 10 years ago, our laboratory identified this key population of interneurons as a group of spinothalamic cells located in lumbar levels L3-4 surrounding the central canal in laminae VII and X.[2] Based on their location and axonal projections, this cell group is referred to as lumbar spinothalamic (LSt) cells. These neurons express several neuropeptides, including galanin, and exclusively express neurokinin-1 (NK-1) receptors (Figure 1A), thus allowing us to test questions concerning the functional role of LSt cells using saporin conjugated to SSP (Cat. #IT-11), the substance P analog [Sar^9, $Met(O_2)^{11}$], from Advanced Targeting Systems. In our previous paper we demonstrated that cell-specific lesions of the LSt cells, completely eliminated ejaculatory behavior, demonstrating the pivotal role of this neural population for ejaculation as the key component of the spinal ejaculation generator.[2] Moreover, we showed that LST cells have projections to the autonomic preganglionic and motor centers controlling ejaculation and are activated by sensory inputs from the dorsal penile nerve;[3,4] stimulation of this nerve triggers ejaculation in mammals (Figure 2).[5] However, several questions remained to be addressed. The spinal ejaculation generator, and thus the ejaculatory reflex, is influenced by inhibitory and excitatory projections from supraspinal sites.[1] Therefore, in the current study we determined that LSt cells were essential for control of ejaculatory reflexes in the absence of these supraspinal influences, using several different

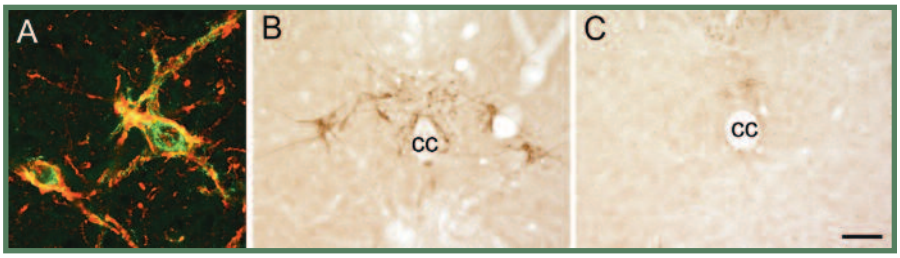


Figure 1:
A) LSt cells express NK-1 receptors (immunoreactivity for galanin in green and NK-1 in red.[6] Representative images showing presence of galanin-immunoreactive LSt cells surrounding central canal (cc) in control males (B), and absence in LSt-lesioned males (C). Scale bar indicates 20 µm (A) and 100 µm (B,C).

(continued on page 6)

SBC Advances Brain Research

The 24th Annual Spring Brain Conference will be held at Poco Diablo Resort, Sedona, Arizona, on March 20-23, 2013. SBC is a broad-brush meeting in which neuroscientists from all disciplines come to meet and exchange ideas in a beautiful environment. The meeting is small, usually with 50-75 scientists. The format is designed to allow for extensive discussion with people outside of your immediate field. This is very attractive to academic scientists that have come for years because, in a short period of time, they can become aware of what's happening in the Neurosciences by actually chatting and directly asking questions with experts in and outside of sessions in a relaxed setting. It is the objective of this conference to bring together scientists with varied backgrounds, interests and approaches to the study of brain function to promote the development of new strategies necessary to better understand the complexities of neural systems.

Photo by Brandon Preddy

The format consists of an opening session with a keynote address on Wednesday evening. On Thursday and Friday mornings there will be morning sessions, usually with three 20-30 minute talks. The afternoon is free to enjoy the remarkable Sedona, with its great tourism possibilities of golf, hiking, and horseback or jeep tours. In the evenings, there will be either a distinguished scientist giving a talk or a session relevant to key issues in the field. Saturday morning will conclude the meeting with sessions and discussions of key clinical matters. An example of a previous meetings agenda is available at www.springbrain.org.

The Spring Brain Conference is not a high tension meeting in which it is necessary to run from room to room in a convention center. It is a casual meeting in a wonderful environment that will enlarge your view of the Neurosciences. And who knows, you may even find that vortex you needed.

Upcoming Events

FENS
July 14-18, 2012
Barcelona, Spain
Booth #61

SFN • October 13-17, 2012
New Orleans, LA

ASCB • December 15-19, 2012
San Francisco, CA

ATS at FENS Meeting in Barcelona

Advanced Targeting Systems has a booth at the upcoming meeting in Barcelona. Please stop by Booth #61 and discuss your targeting projects with Dr. Douglas Lappi. You can pick up your own ATS cotton shopping bag, M&Ms, and coupon for discounts on your next ATS order. Orders placed in Europe are shipped via overnight delivery from our warehouse in the Netherlands. We look forward to seeing you soon!

Taz was hoping it was "take your cat to work" day.
Taz currently resides in Oregon with Kristen Hartman, ATS Data & Website Administrator

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Cholinergic denervation attenuates phencyclidine-induced c-fos responses in rat cortical neurons.

Savage S, Mattsson A, Olson L.
Neuroscience 216:38-45, 2012.

Phenylcyclidine (PCP) has been used to model aspects of schizophrenia in animals. 81 ng of 192-IgG-SAP (Cat. #IT-01) was injected into the nucleus basalis magnocellularis of rats to assess the effects of low dose PCP in a cholinergically-deprived system. Saporin (Cat. #PR-01) was used as control. Results demonstrate basalocortical cholinergic neurons are necessary for PCP to have full effect.

C1 neurons excite locus coeruleus and A5 noradrenergic neurons along with sympathetic outflow in rats.

Abbott SB, Kanbar R, Bochorishvili G, Coates MB, Stornetta RL, Guyenet PG.
J Physiol 590(12):2897-2915, 2012.

C1 neurons are known to activate sympathetic tone and stimulate the hypothalamic-pituitary-adrenal axis. C1 activation is also reported to inhibit locus coeruleus (LC) neurons. Rats received 0.6 ng of SSP-SAP (Cat. #IT-11) injected under the caudal edge of the facial motor nucleus to destroy the retrotrapezoid nucleus, increasing the proportion of C1 ChR2-expressing neurons. Stimulation of C1 neurons resulted in activation of noradrenergic neurons that are involved in hypoxia and hypotension.

Prior pathology in the basal forebrain cholinergic system predisposes to inflammation-induced working memory deficits: reconciling inflammatory and cholinergic hypotheses of delirium.

Field RH, Gossen A, Cunningham C.
J Neurosci 32(18):6288-6294, 2012.

The authors lesioned the basal forebrain of mice with 0.08 µg or 0.4 µg icv injections of mu p75-SAP (Cat. #IT-16) to establish an early dementia-associated cholinergic loss model. The mice were then challenged with systemic inflammation using low-dose lipopolysaccharide (LPS). The mu p75-SAP lesion left hippocampal-dependent reference and working memory relatively intact. LPS-induced inflammation created acute working memory deficits; an acetylcholinesterase inhibitor protected against this deficit.

TrkA gene ablation in basal forebrain results in dysfunction of the cholinergic circuitry.

Sanchez-Ortiz E, Yui D, Song D, Li Y, Rubenstein JL, Reichardt LF, Parada LF.
J Neurosci 32(12):4065-4079, 2012.

The authors created a conditional trkA knockout mouse line. Anti-trkA (Cat. #AB-N03) was used for immunohistochemistry (1:1000) and western blots (1:4000). The data demonstrate the importance of trkA in the establishment of basal forebrain cholinergic circuitry, and choline acetyltransferase expression.

c-Maf is required for the development of dorsal horn laminae III/IV neurons and mechanoreceptive DRG axon projections.

Hu J, Huang T, Li T, Guo Z, Cheng L.
J Neurosci 32(16):5362-5373, 2012.

The molecular mechanisms responsible for development of laminae III/IV neurons are not yet well understood. In this work the authors investigated the role of c-Maf, a basic leucine-zipper transcription factor from the AP-1 superfamily. Anti-trkA (Cat. #AB-N03: 1:100) was used for immunohistochemistry.

Involvement of brain-derived neurotrophic factor and sonic hedgehog in the spinal cord plasticity after neurotoxic partial removal of lumbar motoneurons.

Gulino R, Gulisano M.
Neurosci Res 73(3):238-247, 2012.

In this work the authors created a motoneuron depletion with bilateral 6.0-µg injections of CTB-SAP (Cat. #IT-14) into the medial and lateral gastrocnemius muscles of rats. The results indicate BDNF and sonic hedgehog may collaborate in modulating synaptic plasticity after loss of motoneurons.

Adenosine inhibits glutamatergic input to basal forebrain cholinergic neurons.

Hawryluk JM, Ferrari LL, Keating SA, Arrigoni E.
J Neurophysiol 107(10):2769-2781, 2012.

Using patch-clamp recordings from mouse brain slices the authors demonstrate that adenosine not only directly inhibits cholinergic neurons in the basal forebrain, it also reduces excitatory inputs to these neurons as well. Cy3-anti-mu p75 (Cat. #FL-05, 40-60 ng) was injected into the lateral cerebroventricle.

Brainstem facilitations and descending serotonergic controls contribute to visceral nociception but not pregabalin analgesia in rats.

Sikandar S, Bannister K, Dickenson AH.
Neurosci Lett 519(1):31-36, 2012.

Neurons in the rostral ventromedial medulla (RVM) are classified as ON, OFF, or NEUTRAL based on firing patterns in response to noxious somatic stimulation. ON cells express µ-opioid receptors, and are therefore a target for dermorphin-SAP (Cat. #IT-12). The authors injected the RVM of rats with 3 pmol of dermorphin-SAP; Saporin (Cat. #PR-01) was used as control.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Results show the μ-opioid receptor population is not needed for the function of analgesics through the serotonergic system.

Effects of noradrenergic alpha-2 receptor antagonism or noradrenergic lesions in the ventral bed nucleus of the stria terminalis and medial preoptic area on maternal care in female rats.

Smith CD, Holschbach MA, Olsewicz J, Lonstein JS.

Psychopharmacology (Berl) Epub 2012.

The authors investigated the function of norepinephrine in mothering. Lesioned animals received 55-ng infusions of anti-DBH-SAP (Cat. #IT-03) into the ventral bed nucleus of the stria terminalis. Mouse-IgG-SAP (Cat. #IT-18) was used as a control. The results demonstrate that downregulated noradrenergic activity is necessary for postpartum maternal behavior, but is not enough to elicit maternal behavior in nulliparous females.

Vestibular stimulation enhances hippocampal long-term potentiation via activation of cholinergic septohippocampal cells.

Tai SK, Leung LS.

Behav Brain Res 232(1):174-182, 2012.

It is known that vestibular stimulation induces acetylcholine release in the hippocampus. The authors hypothesized that this stimulation enhances long-term potentiation (LTP) in CA1 and depends on the activation of septohippocampal cholinergic neurons. Rats received 105-ng bilateral infusions of 192-IgG-SAP (Cat. #IT-01) into the medial septum. The data suggest that LTP enhancement during vestibular stimulation is mediated by cholinergic septohippocampal cells.

Sudden Death and Myocardial Lesions after Damage to Catecholamine Neurons of the Nucleus Tractus Solitarii in Rat.

Talman WT, Dragon DN, Jones SY, Moore SA, Lin LH.

Cell Mol Neurobiol Epub 2012.

Previous work has shown that elimination of neurons expressing the neurokinin-1 receptor (NK1r) from the nucleus tractus solitarii (NTS) causes various circulatory system dysfunctions, often leading to sudden death. The authors injected the brainstem of rats with 42 ng anti-DBH-SAP (Cat. #IT-03). to eliminate catecholaminergic neurons in the NTS that express tyrosine hydroxylase. This elimination had similar cardiac and cardiovascular effects to the elimination of NK1r-expressing neurons.

Consequences of the ablation of nonpeptidergic afferents in an animal model of trigeminal neuropathic pain.

Taylor AM, Osikowicz M, Ribeiro-da-Silva A.

Pain 153(6):1311-1319, 2012.

The authors used IB4-SAP (Cat. #IT-10; 3.2 μg injected into the mental nerve) to eliminate C-fibers in the lower lip of rats to see if this was enough to induce the sprouting of autonomic fibers. Saporin alone (Cat. #PR-01) was used as control. Only parasympathetic fibers sprouted in these animals, but after nerve ligation surgery both sympathetic and parasympathetic fibers sprouted.

Analgesia Targeting IB4-Positive Neurons in Cancer-Induced Mechanical Hypersensitivity.

Ye Y, Dang D, Viet CT, Dolan JC, Schmidt BL.

J Pain 13(6):524-531, 2012.

DOR (δ opioid receptor) agonists produce minimal side effects and do not lead to tolerance, making them potential alternatives to the widely used μ opioid receptor agonists. Utilizing the fact that DOR's are expressed by IB4-positive neurons, the authors injected the subarachnoid space between the L4 and L5 vertebrae of rats with 2.4 μg of IB4-SAP (Cat. #IT-10). 3 μg of saporin (Cat. #PR-01) was used as control. The results indicate that pharmacological agents targeting IB4-positive neurons may have use in cancer pain treatment.

Immunogold Detection of L-glutamate and D-serine in Small Synaptic-Like Microvesicles in Adult Hippocampal Astrocytes.

Bergersen LH, Morland C, Ormel L, Rinholm JE, Larsson M, Wold JFH, Roe AT, Stranna A, Santello M, Bouvier D, Ottersen OP, Volterra A, Gundersen V.

Cereb Cortex 22(7):1690-1697, 2012.

Verifying the presence of D-serine in astrocyte vesicles would help resolve whether astrocytes produce rapid gliotransmitter exocytosis for the purpose of neuromodulation. The authors looked at D-serine levels with anti-L-glutamate (Cat. #AB-T08). The results suggest that domains of astrocytes can acquire local Ca2+ increases that trigger glutamate and D-serine release.

Disrupted serotonergic system in patients with pulmonary hypertension may serve as novel biomarkers new therapeutic targets and to assess severity, progression and response to treatment.

Kirillova V, Prosviryakov E.

Cardiovasc Res 93 Suppl 1:P209, 2012.

The authors examined the role serotonin and serotonin transporters play in pulmonary hypertension. Anti-SERT (Cat. #AB-N09, 1:500) was used in immunohistochemistry to detect the serotonin transporter in the myocardium. The data demonstrate that serotonin levels in the blood and serotonin transporter levels in the myocardium are both increased in patients with pulmonary hypertension.

Targeting Talk: Product Questions

Antagonists vs. Targeted Toxins

Q: Recently, I attended a talk where the speaker said that a targeted toxin was able to work when an antagonist did not. Can you explain how your technology is different?

A: Certainly. It is a very interesting question and one that helps explain the Targeting Technology quite well. An antagonist is used to block a receptor on a cell to keep it from binding a target molecule and activating the cell. For example, a substance P antagonist binds to the substance P (NK-1) receptor. The hypothesis was that if the antagonist binds to the receptor, substance P cannot bind and the cell will not be activated. The reality is that there are other receptors besides the substance P receptor on that cell. If any of these other receptors bind to their target molecules, then the cell will still be activated.

It sometimes is not enough to block one particular receptor. The ATS targeting technology (Molecular Surgery) can use any of these cell surface receptors to target and completely eliminate the cell. That way, there are no receptors left to bind; no cell left to be activated. Importantly, Molecular Surgery cleanly removes one particular cell type and does not damage bystander cells. Once the debris from the targeted cell is cleared away, there is nothing remaining to interfere or affect the normal action/interaction of other cells.

Q&A Products

Saporin (PR-01)
Targeted Toxins

Lethal Dose of Saporin in Mice

Q: What is the LD_{50} of saporin in mice? Do you have references for this?

A: Thank you for your question. It is very helpful to have this information to calculate the appropriate dose for systemic administration.

According to the work of Thorpe *et al.* (*J Natl Cancer Inst*. 75(1):151-159, 1985), saporin alone has an acute LD_{50}, when delivered intravenously, of 6.8 mg/kg in mice. Histologic examination of kidneys from mice receiving near-lethal doses of saporin revealed necrosis of the convoluted tubules. Other major organs had only minor changes.

Once saporin is attached to an immunoglobulin, the LD_{50} drops dramatically to 1.0 mg/kg in systemic administration. Near-lethal doses of the conjugates, by contrast to saporin alone, inflicted major damage to the liver and spleen of the mice while the kidneys (and other organs) appeared normal under histologic examination.

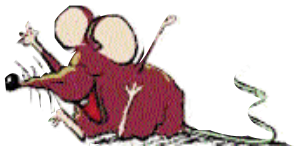
Send a message on our website to get answers to your targeting questions.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: ARCUATE
MODULATION
ABLATION
WEIGHT
CONJUGATE

Answer: GLOBAL WARMING!



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

WINNERS: Norma Huff, Univ California San Diego, La Jolla, CA * Roger Guillemin, La Jolla, CA * Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA * Seto Chice, SUNY-HSC Brooklyn, Brooklyn, NY

Solve this quarter's Teaser online at: www.ATSbio.com/news/12q3_teaser.html

A pivotal role of lumbar spinothalamic cells in regulation of ejaculation via intraspinal connections

(continued from page 1)

sensory stimulation and pharmacological paradigms in anesthetized male rats with transections of the spinal cord.[5]

Methods: Male rats (anesthetized with a ketamine/xylazine mixture) received a laminectomy to expose the L3-L4 spinal segment and bilateral injections (12 x 1 µL) of the selective neurotoxin saporin conjugated to SSP [SSP-SAP (Cat. #IT-11); 4 ng/µL] into the spinal cord targeting the entire LSt population (Figure 1B,C). We previously demonstrated that this technique results in specific lesions of the LSt cells without loss of other surrounding neurons in the spinal cord, without loss of NK-1 receptor neurons in the dorsal horn, and without changes in thermal pain perception.[2] Control males received saporin conjugated to a nonsense peptide (Blank-SAP, Cat. #IT-21; 3.68 ng/mL) or had misplaced injections of SSP-SAP. Two weeks following lesion surgeries, males were tested for ejaculatory reflexes induced by stimulation of the dorsal penile nerve, stimulation of the urethra, or by treatment with the D3 dopamine receptor agonist 7-hydroxy-2-N,N-dipropylaminotetralin (7-OH-DPAT; 1 mg/kg s.c.). All males were anesthetized with urethane and the spinal cord was completely transected between thoracic levels T6-8. Ejaculatory reflexes were measured by EMG recordings of the striated perineal muscle, the bulbocavernosus muscle (BCM), as rhythmic bursting of this muscle is characteristic of the ejaculatory reflex in mammals.

Specific lesions of LSt cells completely ablated the BCM bursting characteristic of ejaculation upon stimulation of the dorsal penile nerve, urethra, or dopamine D3 receptors in anesthetized, male rats with spinal transections, while BCM bursting was reliably triggered in all control animals (Figure 3). These data extend our previous findings that LSt cells are essential for ejaculatory behavior in mating animals, thus providing further evidence for the pivotal role of LSt cells in control of ejaculation. Moreover, these data demonstrate the LSt cells control ejaculation via projections within the spinal cord, presumably to autonomic and motor neurons, and independent of supraspinal influences.

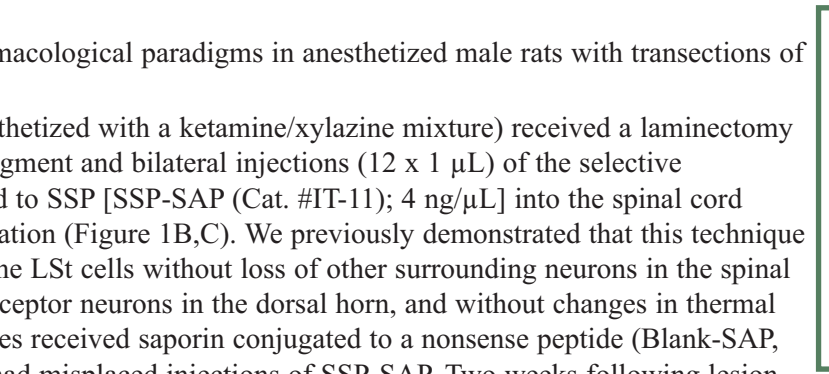


Figure 2: Schematic overview of the spinal ejaculation generator. Lumbar spinothalamic (LSt) cells receive sensory inputs via the dorsal penile nerve (DPN) and send axonal projections to preganglionic sympathetic neurons in the central autonomic nucleus (CAN) and intermediolateral cell column (IML), to preganglionic parasympathetic neurons in the sacral parasympathetic nucleus (SPN), and to pudendal motor neurons in the sacral nucleus of the bulbocavernosus (SNB). Spinal lumbar levels are indicated on the right. Modified from Kozyrev *et al*.[5]

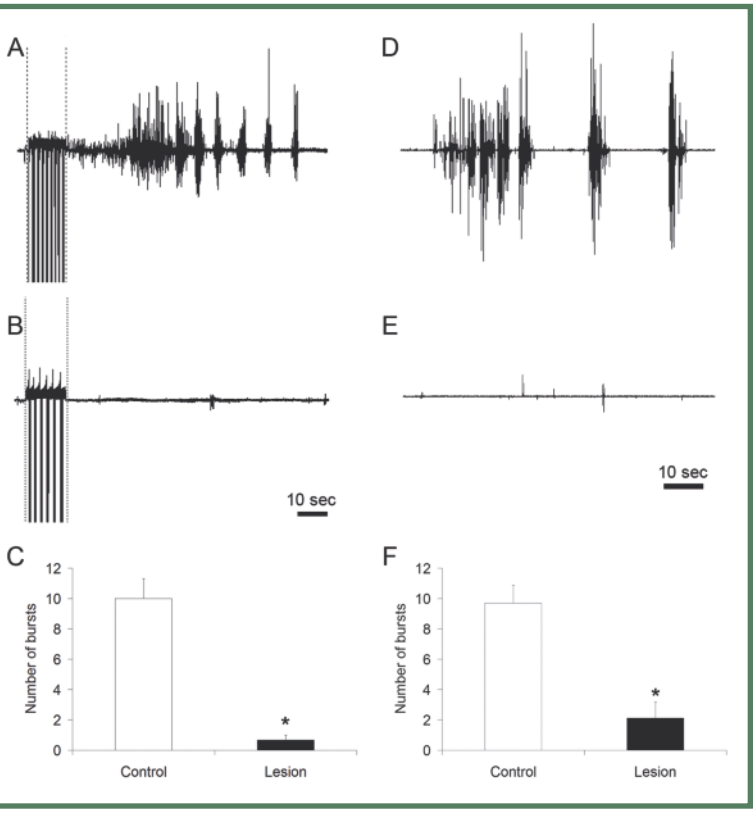


Figure 3: Representative examples of bulbocavernosus muscle bursting in control (A, D) and LSt-lesioned males (B, E) following stimulation of the dorsal penile nerve (indicated by dashed lines in A, B) or systemic injections of 7-OH-DPAT (D, E). Quantitative analysis of bulbocavernosus muscle EMG is shown as numbers of bursts (C, F).
* indicates significant difference from control animals.

References

1. Coolen, L.M. (2005) Neural control of ejaculation. *J Comp Neurol* 493:39-45.
2. Kozyrev, N., Lehman, M.N. & Coolen, L.M. (2012) Activation of Gastrin-releasing Peptide Receptors in the Lumbosacral Spinal Cord is Required for Ejaculation in Male Rats. *J Sex Med*. Mar 16. [Epub ahead of print]
3. Staudt, M.D., de Oliveira, C.V., Lehman, M.N., McKenna, K.E. & Coolen, L.M. (2010) Activation of MAP kinase in lumbar spinothalamic cells is required for ejaculation. *J Sex Med* 7:2445-2457.
4. Staudt, M.D., de Oliveira, C.V., Lehman, M.N., McKenna, K.E. & Coolen, L.M. (2011) Activation of NMDA receptors in lumbar spinothalamic cells is required for ejaculation. *J Sex Med* 8:1015-1026.
5. Staudt, M. D., Truitt, W. A., McKenna, K. E., de Oliveira, C. V.R., Lehman, M. N. and Coolen, L. M. (2011), A Pivotal Role of Lumbar Spinothalamic Cells in the Regulation of Ejaculation via Intraspinal Connections. *J Sex Med*. doi: 10.1111/j.1743-6109.2011.02574
6. Truitt, W.A. & Coolen, L.M. (2002) Identification of a potential ejaculation generator in the spinal cord. *Science* 297:1566-1569.

Targeting Tools: Featured Products

Anti-SERT-SAP

Anti-SERT-SAP (Cat. #IT-23) utilizes a monoclonal antibody to the fourth extracellular domain of the serotonin re-uptake transporter (SERT). SERT is the major determinant of serotonin inactivation following release at synapses, is the site of action for many tricyclic antidepressants and the SSRIs (serotonin-selective reuptake inhibitors), and is also targeted by a number of psychostimulants including cocaine, methylphenidate, and MDMA 'ecstasy.' SERT is produced from a single gene and is expressed in both the CNS and GI system. Decreased serotonergic neurotransmission has been proposed to play a key role in the etiology of depression. Recent findings suggest that SERT might be linked to both neurotic and sexual behavior as well as to obsessive-compulsive disorder (OCD).

ATS has developed a new version of Anti-SERT-SAP and is **looking for experienced researchers to test its activity in an *in vivo* system**. If you are interested in testing this new targeted toxin, please contact us with a brief description of your experimental design and how much material you will need to complete it. You will be provided with Anti-SERT-SAP and a control conjugate (Mouse IgG-SAP; Cat. #IT-18) at no charge. In return ATS asks to be able to share an image or visual result from your experiment that will inform other researchers of how the conjugate works.

Anti-SERT-SAP #IT-23
25 µg, 100 µg or 250 µg available individually
or in a kit with Saporin (Cat. #PR-01) and Mouse IgG-SAP (Cat. #IT-18)

Mac-1-SAP

The Mac-1-SAP targeted toxins recognize Mac-1-positive (CD11b) cells in mouse/human (Cat. #IT-06) or in rat (Cat. #IT-33). The toxin is excellent for removing contaminating macrophages from primary cultures. Elimination of macrophages *in vivo* is useful to determine their role(s) in autoimmune diseases and in degenerative diseases such as Alzheimer's. Both toxins are extremely potent, with EC_{50}'s three to five orders of magnitude less than non-targeted saporin.

The Mac-1-SAP mouse/human-specific antibody (Cat. #AB-N05) is from the well-characterized M1/70 clone, and has been created by immunization from mouse macrophages, but reacts well with the human epitope also. It does not recognize the rat form. The antibody is also available biotin-labeled (Cat. #BT-N05) and Alexa488-labeled (Cat. #FL-N05).

The Mac-1-SAP rat-specific antibody (Cat. #AB-N06) was developed in mice immunized with rat neutrophils. It does not cross-react with the human or mouse epitope. The antibody is also available biotin-labeled (Cat. #BT-N06) and Alexa488-labeled (Cat. #FL-N06).

Mac-1-SAP mouse/human #IT-06
5 µg, 25 µg, 100 µg or 250 µg available individually or 25µg and 100µg in a kit with Saporin (Cat. #PR-01), antibody (Cat. #AB-N05), and Rat IgG-SAP (Cat. #IT-17)

Mac-1-SAP rat #IT-33
25 µg, 100 µg or 250 µg available individually or in a kit with Saporin (Cat. #PR-01), antibody (Cat. #AB-N06), and Mouse IgG-SAP (Cat. #IT-18)

Gangsta practices his 'upward facing cat' pose.

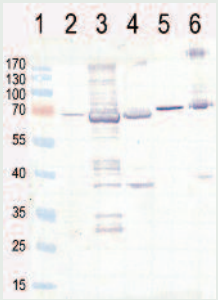
Anti-GST

The Glutathione S-Transferase (GST) family of enzymes contains cytosolic, mitochondrial, and microsomal proteins that are capable of multiple reactions with a multitude of substrates, both endogenous and xenobiotic. GST catalyzes the conjugation of reduced glutathione which is useful in the detoxification of endogenous compounds such as peroxidized lipids, as well as the metabolism of xenobiotics. GST is used to create the GST gene fusion system in which the GST moiety is used to detect and purify proteins of interest. This GST-fusion protein can then be purified from cell extracts via its high affinity for glutathione. Fusion proteins offer an important biological assay for direct protein-to-protein interactions.

Advanced Targeting Systems announces the release of a new rabbit polyclonal antibody to GST (unlabeled, Cat. #AB-45AP). It is also available labeled with HRP (Cat. # AB-45HRP). Both are useful for the detection of GST fusion proteins.

GST rabbit polyclonal #AB-45AP
HRP-labeled GST rabbit polyclonal #AB-45HRP
Get a free sample now!
Enter Code GST-July when placing your order.

Cultures of *E. coli* transformed with a vector expressing a GST-Cre recombinase fusion protein were grown under varying conditions. Soluble protein was extracted, and expression compared between soluble and insoluble fractions on SDS-PAGE which was then transferred to a PVDF membrane. Membrane was incubated with anti-GST-HRP overnight at a 1:500 dilution.



Lane 1: Page Ruler 5 µl
Lane 2: 200 ng GST-Cre
Lane 3: Supernate 20 µg
Lane 4: Supernate 20 µg
Lane 5: Pellet 5 µl
Lane 6: Pellet 5 µl

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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.

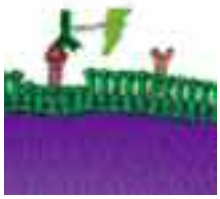
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

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

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



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

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

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

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

ALEXUS

DISFUL

CLUMES

REGENTORA

BURMAL

How the budding scientist viewed the idea of vacation time at Science Camp.

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

ANSWER: HE THOUGHT IT WAS . . . ◯◯◯◯◯◯ ◯◯◯ !

WIN $100.00

Limit one entry per laboratory. Credits expire after one year.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by September 15, 2012.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

Please correct the address information above and provide the following:

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Oct-Nov-Dec 2012
Volume 13, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Partial basal forebrain cholinergic depletion leaves working memory susceptible to the effects of systemic inflammation

Contributed by Dr. Colm Cunningham, Trinity College, Institute of Neuroscience & School of Biochemistry and Immunology, Dublin, Ireland

It is well established that peripheral inflammation can signal the intact CNS to bring about adaptive changes in behavior during the sickness response. However, during aging and dementia, the brain is particularly susceptible to the deleterious effects of such insults. Delirium is an acute and severe disturbance in cognitive function with particular deficits in attention, memory, orientation and perception. The pathophysiology of delirium remains poorly understood but it is clear that dementia and prior cognitive impairment are major risk factors for delirium, and systemic inflammation is a frequent trigger. Hypocholinergia and increased neuroinflammation have been proposed as major players in delirium pathophysiology but no clinical or animal studies have investigated the interplay between systemic inflammation and cholinergic function.

In a paper published in *The Journal of Neuroscience,*[1] we hypothesized that making limited lesions of the basal forebrain cholinergic neurons (BFCN) would leave these animals vulnerable to the CNS effects of systemic inflammation. The BFCN, which comprises the medial septum, the diagonal band and the nucleus basalis, is the source of most acetylcholine in the forebrain and this area degenerates markedly during Alzheimer's disease. To specifically lesion this region we used the ribosomal toxin saporin, covalently linked to an antibody directed against the p75 neurotrophin receptor that is highly expressed on BFCN. We showed that intracerebroventricular (icv) injection of this toxin (mu p75-SAP, Cat. #IT-16) at low doses could induce approximately 20% destruction of the cholinergic cells of the medial septum without obvious effect on working memory function (see Fig 1). However, if these animals were allowed to recover for 40 days post-surgery, and then challenged systemically with bacterial endotoxin to mimic mild-moderate systemic bacterial infection (100 µg/kg LPS intraperitoneal), only those animals with prior lesions showed acute working memory deficits.

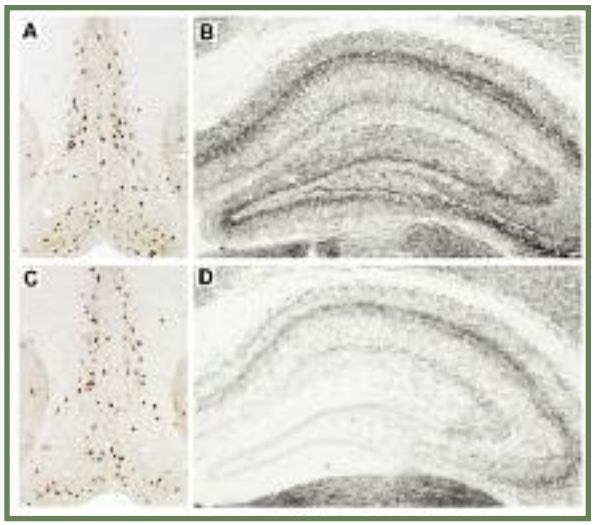

Fig 1. Limited selective lesions of the basal forebrain cholinergic system induced by mu p75-SAP.
Representative photomicrographs of ChAT-positive neurons in the medial septum/diagonal band in PBS (A) and 0.08 µg mu p75-SAP (C).
Histochemical AChE enzyme activity using the thiocholine method in the hippocampus of PBS (B) and 0.08 µg mu p75-SAP (D).

(continued on page 6)

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

24th Annual Spring Brain Conference - Register NOW to Attend

This is your invitation to the 2013 Spring Brain Conference (SBC). The conference will be held at the Poco Diablo Resort in Sedona, AZ, March 20-23, 2013. The SBC is a small, informal meeting. The goal is to stimulate neuroscientists to share ideas across broad areas of neuroscience. The primary objective of each session is to introduce the audience to what is interesting and important in a field while emphasizing key research areas and new findings. The program will include 11 non-competing plenary sessions with lectures by leading scientists. You are encouraged to submit a proposal for a session.

There will also be a poster session for scientists to display their posters from the Society for Neuroscience meeting. If you plan to come to Sedona for SBC, you can drop off your poster tubes at the ATS booth at SfN in New Orleans (Booth #824). ATS will transport the posters to the SBC meeting so they can be displayed and presented by you.

PROGRAM AT A GLANCE

Wednesday, March 20	
6pm – 8:30pm	RECEPTION (food and beverages provided) Poster Session 7p – 8p: Introduction of Conference Speakers
Thursday, March 21 and Friday, March 22	
7am – 8am	BREAKFAST BUFFET
8am – 12:15pm	Four Plenary Sessions
12:15pm – 1:15pm	LUNCH BUFFET
1:15pm – 7pm	FREE TIME Golf, Hike, Tennis, Spa, Jeep Tour, etc.
7pm – 8pm	DINNER BUFFET
7:30pm – 8:30pm	KEYNOTE ADDRESS
Saturday, March 23	
7am – 8am	BREAKFAST BUFFET
8am – 11:15am	Three Plenary Sessions
11:15am – 12:15pm	BUSINESS MEETING
12:15pm – 1:15pm	LUNCH BUFFET

Plenary session proposals should be sent to Dr. Douglas Lappi (proposals@springbrain.org) by October 26, 2012. Sessions (1 - 1.5 hours each) will include 5-minute discussions led by each of the diverse speakers. One-page proposals should contain a session title, name/affiliation/email of the organizer and speakers, title for each talk and a half-page abstract summarizing the session's goal and issues that each speaker will address. Proposals will be evaluated promptly and the program will be finalized for distribution in November.

For additional information, please visit the SBC website (www.SpringBrain.org).

Upcoming Events

SfN • October 13-17, 2012
New Orleans, LA Booth #824

ASCB • December 15-19, 2012
San Francisco, CA Booth #1138

Spring Brain Conference
March 20-23, 2013 • Sedona, AZ

AACR • April 4-10, 2013
Washington, DC

Suggest It . . .

Do you have an idea for a new target? Contact us with your suggestion. If your target is chosen for development of a targeted toxin, we will provide the conjugate to you at no charge.

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Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Time to pay attention: attentional performance time-stamped prefrontal cholinergic activation, diurnality, and performance.

Paolone G, Lee TM, Sarter M.
J Neurosci 32(35):12115-12128, 2012.

The authors performed bilateral 160-ng infusions of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis and substantia innominata of the basal forebrain of rats that had reached stable performance on a sustained attention task. Both lesioning and altering the task training time impaired task performance.

A nociceptive signaling role for neuromedin B.

Mishra SK, Holzman S, Hoon MA.
J Neurosci 32(25):8686-8695, 2012.

Specific subsets of dorsal horn interneurons were eliminated by administering either 10 µg of the custom conjugate Neuromedin B-SAP, 0.13 µg of SSP-SAP (Cat. #IT-11), or 1.3 µg of Bombesin-SAP (Cat. #IT-40). Blank-SAP (Cat. #IT-21) was used as a control. The data indicate that Neuromedin B may be involved in the perception of thermal sensation, but not mechanical or pruritic sensation.

PET imaging of cholinergic deficits in rats using [(18)F]fluoroethoxybenzovesamicol ([(18)F]FEOBV).

Parent M, Bedard MA, Aliaga A, Soucy JP, Landry St-Pierre E, Cyr M, Kostikov A, Schirrmacher E, Massarweh G, Rosa-Neto P.
Neuroimage 62(1):555-561, 2012.

In order to better understand and evaluate neurodegenerative diseases, imaging agents are necessary to visualize the affected systems. ([(18)F]FEOBV) is one such agent that shows promise for labeling the vesicular acetylcholine transporter with positron emission tomography. The authors injected 0.2 µg of 192-IgG-SAP (Cat. #IT-01) into the left hemisphere of rats to model cholinergic terminal loss as seen in aged animals. Loss of these terminals was found to reduce ([(18)F]FEOBV) binding in the ventral frontal cortex on the lesioned side, and also in the homologous region of the contralateral hemisphere, allowing detection of both physiological and pathological reduction of cholinergic terminals.

Infusion of GAT1-saporin into the medial septum/vertical limb of the diagonal band disrupts self-movement cue processing and spares mnemonic function.

Koppen JR, Winter SS, Stuebing SL, Cheatwood JL, Wallace DG.
Brain Struct Funct Epub2012.

Rats received a total of 350 ng of GAT-1-SAP (Cat. #IT-32) infused into the medial septum-diagonal band of Broca. Although lesioned animals performed normally in tasks involving spatial cues, food hoarding was affected indicating that self-movement cue processing was interfered with by the loss of these GABAergic neurons.

Cholinergic denervation exacerbates amyloid pathology and induces hippocampal atrophy in Tg2576 mice.

Gil-Bea FJ, Gerenu G, Aisa B, Kirazov LP, Schliebs R, Ramirez MJ.
Neurobiol Dis 48(3):439-446, 2012.

This work sought to examine the interaction between cholinergic denervation, amyloid precursor protein (APP) processing, and hippocampal integrity. Tg2576 transgenic mice received 2 µg of mu p75-SAP (Cat. #IT-16) injected into the third ventricle. Lesioned animals displayed various aspects of Alzheimer's disease such as hippocampal synaptic pathology and neurodegeneration.

Medial septal cholinergic neurons are necessary for context-place memory but not episodic-like memory.

Easton A, Fitchett AE, Eacott MJ, Baxter MG.
Hippocampus 21(9):1021-1027, 2011.

The authors administered a total of 150 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum and vertical limb of the diagonal band of Broca in rats. The results suggest that hippocampal spatial representations might not be essential for episodic memory function.

Septohippocampal GABAergic neurons mediate the altered behaviors induced by n-methyl-D-aspartate receptor antagonists.

Ma J, Tai SK, Leung LS.
Hippocampus Epub2012.

In this work the authors administered bilateral injections totaling 140 ng of orexin-SAP (Cat. #IT-20; discontinued) into the medial septum of rats.

Nucleus of the Solitary Tract catecholaminergic neurons modulate the cardiovascular response to psychological stress in rats.

Daubert DL, McCowan M, Erdos B, Scheuer D.
J Physiol Epub2012.

Rats received 22-ng bilateral injections of anti-DBH-SAP (Cat. #IT-03) into the NTS.

(continued on page 4)

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

Targeting Topics: Recent Scientific References

(continued from page 3)

CXB-909 Attenuates Cognitive Deficits in the Mu-P-75 Saporin Mouse Model of Alzheimer's Disease.

Lowrance S, Matchynski J, Rossignol J, Dekorver N, Sandstrom M, Dunbar G.
Neuroscience & Medicine 3(1):65-68, 2012.

In this work the authors lesioned cholinergic cells in the basal forebrain of mice with bilateral 0.8-µg intracerebroventricular injections of mu p75-SAP (Cat. #IT-16).

Cholinergic depletion in nucleus accumbens impairs mesocortical dopamine activation and cognitive function in rats.

Laplante F, Zhang ZW, Huppe-Gourgues F, Dufresne MM, Vaucher E, Sullivan RM.
Neuropharmacology 63(6):1075-1084, 2012.

Rats received bilateral injections totaling 500 ng of anti-ChAT-SAP (Cat. #IT-42) into the nucleus accumbens; Rabbit IgG-SAP (Cat. #IT-35) was used as a control.

Leptin-sensitive neurons in the arcuate nuclei contribute to endogenous feeding rhythms.

Li AJ, Wiater MF, Oostrom MT, Smith BR, Wang Q, Dinh TT, Roberts BL, Jansen HT, Ritter S.
Am J Physiol Regul Integr Comp Physiol 302(11):R1313-26, 2012.

Rats received bilateral injections of Leptin-SAP (Cat. #IT-47, 56.5 ng per dose) into each arcuate nucleus. Blank-SAP (Cat. #IT-21) was used as a control.

A2 noradrenergic lesions prevent renal sympathoinhibition induced by hypernatremia in rats.

Pedrino GR, Freiria-Oliveira AH, Almeida Colombari DS, Rosa DA, Cravo SL.
PLoS One 7(5):e37587, 2012.

Rats received bilateral 6.3-ng injections of anti-DBH-SAP (Cat. #IT-03) into the nucleus of the solitary tract. An equimolar amount (1.3 ng) of Saporin (Cat. #PR-01) was used as a control.

Chronic treadmill exercise in rats delicately alters the Purkinje cell structure to improve motor performance and toxin-resistance in the cerebellum.

Huang TY, Lin LS, Cho KC, Chen SJ, Kuo YM, Yu L, Wu FS, Chuang JI, Chen HI, Jen CJ.
J Appl Physiol Epub2012.

Rats were given a 2-µg injection of OX7-SAP (Cat. #IT-02) into the lateral ventricle.

Intact Catecholamine Inputs to the Forebrain Are Required for Appropriate Regulation of CRH and Vasopressin Gene Expression by Corticosterone in the Rat Paraventricular Nucleus.

Kaminski KL, Watts AG.
J Neuroendocrinol Epub2012.

The authors administered bilateral 42 ng injections of anti-DBH-SAP (Cat. #IT-03) into the PVH of both normal and adrenalectomized rats. Mouse IgG-SAP (Cat. #IT-18) was used as a control.

Local serotonin mediates cyclic strain-induced phenotype transformation, matrix degradation, and glycosaminoglycan synthesis in cultured sheep mitral valves.

Lacerda CM, Kisiday J, Johnson B, Orton EC.
Am J Physiol Heart Circ Physiol 302(10):H1983-90, 2012.

Anti-SERT (Cat. #AB-N09) was used in immunoblotting experiments.

Lamina I NK1 expressing projection neurones are functional in early postnatal rats and contribute to the setting up of adult mechanical sensory thresholds.

Man SH, Geranton SM, Hunt SP.
Mol Pain 8(1):35, 2012.

Rats at postnatal day 3 were treated with 2 µl of 5 µM SP-SAP (not available; see Cat. #IT-11 SSP-SAP) injected into the intrathecal space. Blank-SAP (Cat. #IT-21) was used as a control.

Intrinsic voltage dynamics govern the diversity of spontaneous firing profiles in basal forebrain noncholinergic neurons.

Ovsepian SV, Dolly JO, Zaborszky L.
J Neurophysiol 108(2):406-418, 2012.

The authors labeled cholinergic neurons by injecting 0.8-1.6 µg of Cy3-192-IgG (Cat. #FL-01) into the lateral ventricles of rats.

Neonatal cholinergic lesion alters the acoustic structure of infant rat vocalization but not the early cognitive development.

Kruger HS, Hanganu-Opatz IL.
Dev Psychobiol Epub2012.

The authors administered 0.1 µg of 192-IgG-SAP (Cat. #IT-01) to each lateral ventricle of rats on the day of birth.

Control of sleep and wakefulness.

Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW.
Physiol Rev 92(3):1087-1187, 2012.

Several targeted conjugates are mentioned, such as 192-IgG-SAP (Cat. #IT-01), anti-DBH-SAP (Cat. #IT-03), and orexin-SAP (discontinued).

Summaries have been abbreviated due to number of publications. Please visit

www.ATSbio.com/news/12q4_news.html

to see the complete summaries.

Targeting Talk: SfN 2012 Product Abstracts

The Society for Neuroscience meeting is always a wonderful opportunity to see what scientists are doing with ATS products. Here are some abstracts using our products. Don't forget to stop by and see us at booth #824.

Saturday, October 13

2:00 - 3:00 pm U17 67.10
Y Ye: *IB4 (+) neurons contribute to force-induced cancer pain but not cancer proliferation* IB4-SAP

1:00 - 2:00 pm T14 93.05
A Jokiaho: *Catecholaminergic neurons in the ventrolateral medulla are differentially activated by the rate of fall in blood glucose during hypoglycemia, and are required for the rate-dependent hypoglycemic activation of sympathoadrenal responses.* Anti-DBH-SAP

2:00 - 3:00 pm UU7 93.18
A-J Li: *Lateral and fourth ventricular phloridzin injections stimulate feeding but do not produce hyperglycemia.* Anti-DBH-SAP

Sunday, October 14

10:00 - 11:00 am R11 164.19
R Kaushal: *Knockdown of noradrenergic locus coeruleus (LC) neurons alleviates chronic orofacial pain* Anti-DBH-SAP

11:00 am - 12:00 pm CCC37 203.28
C Mathis: *Combined loss of entorhinal and basal forebrain cholinergic hippocampal inputs deeply impairs spatial navigation memory in C57BL/6J and hAPPxapoE mice.* mu p75-SAP

4:00 - 5:00 pm SS5 281.28
LF Hayward: *Neurotoxic lesion of CRF-R1 neurons in the amygdala selectively attenuates the heart rate response to acute stress in the spontaneously hypertensive rat.* CRF-SAP

Monday, October 15

9:00 - 10:00 am H28 345.10
J Lee: The effects of basal forebrain cholinergic neuron on recognition tests. 192-IgG-SAP

11:00 am - 12:00 pm I9 345.20
S-Y Lee: *Effects of chronic stress on alterations of GR-PKA-NF-kappa B signaling and spatial learning in rats with cholinergic deafferentation.* 192-IgG-SAP

9:00 - 10:00 am Z2 360.06
A N Fricks-Gleason: *Evaluating the role of neuronal nitric oxide synthase-containing striatal interneurons in methamphetamine-induced dopamine neurotoxicity.* SSP-SAP

8:00 - 9:00 am DDD64 394.25
J R Koppen: *Infusion of GAT1-Saporin into the medial septum spares mnemonic function and impairs self-movement cue processing.* GAT1-SAP

2:00 - 3:00 pm Hall F-J 438.10
T. W. Nichols: *Emerging roles of pathogens in alzheimer's and moderate magnetic field therapy: dc emf 0.5 tesla* mu p75-SAP

2:00 - 3:00 pm FF7 471.22
S K Mishra: *Neuromedin B serves a role in nociceptive signaling.* Custom Conjugation

4:00 - 5:00 pm VV12 486.24
A V Kalinchuk: *Cholinergic basal forebrain neurons contribute to the biochemical and electrophysiological changes in the cortex during sleep deprivation.* 192-IgG-SAP

3:00 - 4:00 pm CCC54 496.03
K Zushida: *The GRP peptide and the GRPR-positive interneurons control fear acquisition and extinction.* Bombesin-SAP

Tuesday, October 16

9:00 - 9:15 am Rm 387 524.05
W T Talman: *Selective damage to glia in the nucleus tractus solitarii attenuates cardiovascular reflexes.* Anti-DBH-SAP, SSP-SAP

11:00 - 12:00 pm C25 536.08
C S Mallory: *Paying attention with a compromised cholinergic system: Attenuated activation of cholinergic neurotransmission in attentional task-performing CHT+/- mice.* mu p75-SAP

9:00 - 10:00 am TT3 585.02
S J Krajewski-Hall: *A role for kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the regulation of estrous cycles and the estrogen modulation of body temperature.* NK3-SAP, Blank-SAP

11:00 am - 12:00 pm DDD29 600.12
M L Anderson: *Acetylcholine and Learning: Are they related and does it matter for associating events across time?* 192-IgG-SAP

3:00 - 4:00 pm BBB38 696.15
M Grupe: *Positive allosteric modulation of nicotinic acetylcholine receptors augments the amplitudes of prefrontal nicotine-evoked glutamatergic transients.* 192-IgG-SAP

Wednesday, Oct. 17

8:00 - 9:00 am Rm 383 729.13
V Ljubojevic: *Cholinergic contributions to learned attentional suppression in the rat with touchscreens.* 192-IgG-SAP

11:00 am - 12:00 pm F13 751.12
O Ortiz-Barajas: *Limited effect of serotonergic denervation on beta-amyloid and cognitive impairment in APPswe/PS1dE9 mice.* mu p75-SAP

9:00 - 10:00 am K14 758.06
C Y Ostock: *Noradrenergic denervation by DBH saporin reduces behavioral responsivity to L-DOPA in the hemi-Parkinsonian rat.* Anti-DBH-SAP

10:00 - 11:00 am Hall F-J 781.07
L M Biggs: *Intercalated nucleus modulates chemosensory processing in medial amygdala.* Dermorphin-SAP

10:00 - 11:00 am OO17 785.07
F Carr: *Descending facilitation contributes to changes in dorsal horn gene expression in a rat model of inflammatory joint pain.* Dermorphin-SAP

10:00 - 11:00 am PP1 785.11
F Wang: *Depletion of spinal norepinephrine increases the duration of postoperative pain related behaviors following acute plantar incision and partial nerve injury in the rat.* Anti-DBH-SAP

10:00 - 11:00 am BBB24 799.23
M D Schwartz: *Effects of a dual hypocretin receptor antagonist on sleep and wakefulness in rats.* 192-IgG-SAP

1:00 - 2:00 pm C34 846.09
M Gulisano: *Neural plasticity in injured spinal cord.* CTB-SAP

1:00 - 2:00 pm D43 851.01
D Jeong: *Spatial memory facilitation by electrical stimulation of the medial septum in rats.* 192-IgG-SAP

2:00 - 3:00 pm DDD63 918.06
C A Rossi: *Evidence that focal hippocampal interneuron loss disrupts theta- and gamma-band activity.* SSP-SAP

Partial basal forebrain cholinergic depletion leaves working memory susceptible to the effects of systemic inflammation

(continued from page 1)

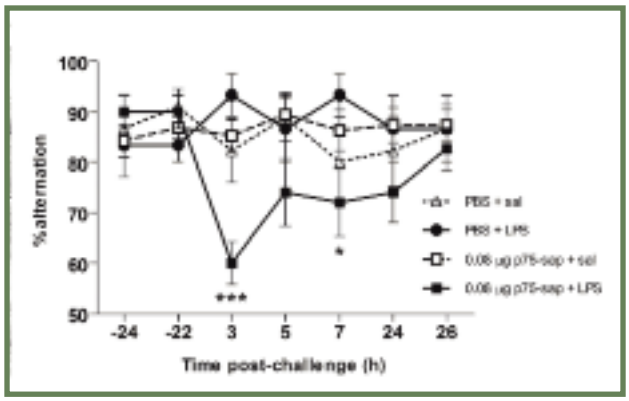


Fig 2. Working memory performance (T-maze alternation) of lesioned animals, LPS-treated (100 µg/kg LPS i.p.) animals and LPS-treated lesioned animals at 40 days post lesion, showing acute working memory deficits only in the latter group.

Many of our previous predictions about the interaction of prior pathology and systemic inflammation[2] were based on the original demonstration of microglial priming and subsequent exaggerated inflammatory responses to systemic inflammatory insult.[3] However, despite clear microglial activation at three days after mu p75-SAP lesions, microglial activation had resolved by 40 days post-lesion, and at this time microglia also did not show exaggerated inflammatory cytokine induction after systemic inflammation (Fig 2). Thus, the acute working memory deficits appear to occur in the absence of microglial priming. This implies that the 'vulnerability' in these animals represents a neuronal susceptibility to disruption of function. We showed, using the acetylcholine muscarinic receptor antagonist scopolamine, that the T-maze working memory task used in these studies was indeed dependent on cholinergic function and further showed that treatment with the acetylcholinesterase inhibitor donepezil, one hour after LPS, protected against the working memory deficit observed. Collectively these data show that the loss of 20% of cholinergic neurons of the basal forebrain cholinergic system does not robustly affect cognitive function under normal conditions, but leaves these animals vulnerable to significant cognitive disruption upon an acute systemic inflammatory insult.

We believe that these findings have significant implications for delirium, particularly in the setting of existing cognitive impairment/dementia and that this limited cholinergic lesion model will be an extremely useful tool in delineating molecular pathways to dysfunction arising from systemic inflammatory insults in older or cognitively-impaired individuals.

References

1. Robert H. Field, Anna Gossen, and Colm Cunningham (2012) Prior Pathology in the Basal Forebrain Cholinergic System Predisposes to Inflammation-Induced Working Memory Deficits: Reconciling Inflammatory and Cholinergic Hypotheses of Delirium. *J Neurosci* 32(18):6288–6294.
2. Murray C, Sanderson DJ, Barkus C, Deacon RM, Rawlins JN, Bannerman DM, Cunningham C (2012) Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. *Neurobiol Aging* 33:603– 616.e3.
3. Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH (2005) Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci* 25:9275–9284.

Send a message on our website to get answers to your targeting questions.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: SEXUAL
FLUIDS
MUSCLE
GENERATOR
LUMBAR

How the budding scientist viewed the idea of vacation time at Science Camp.

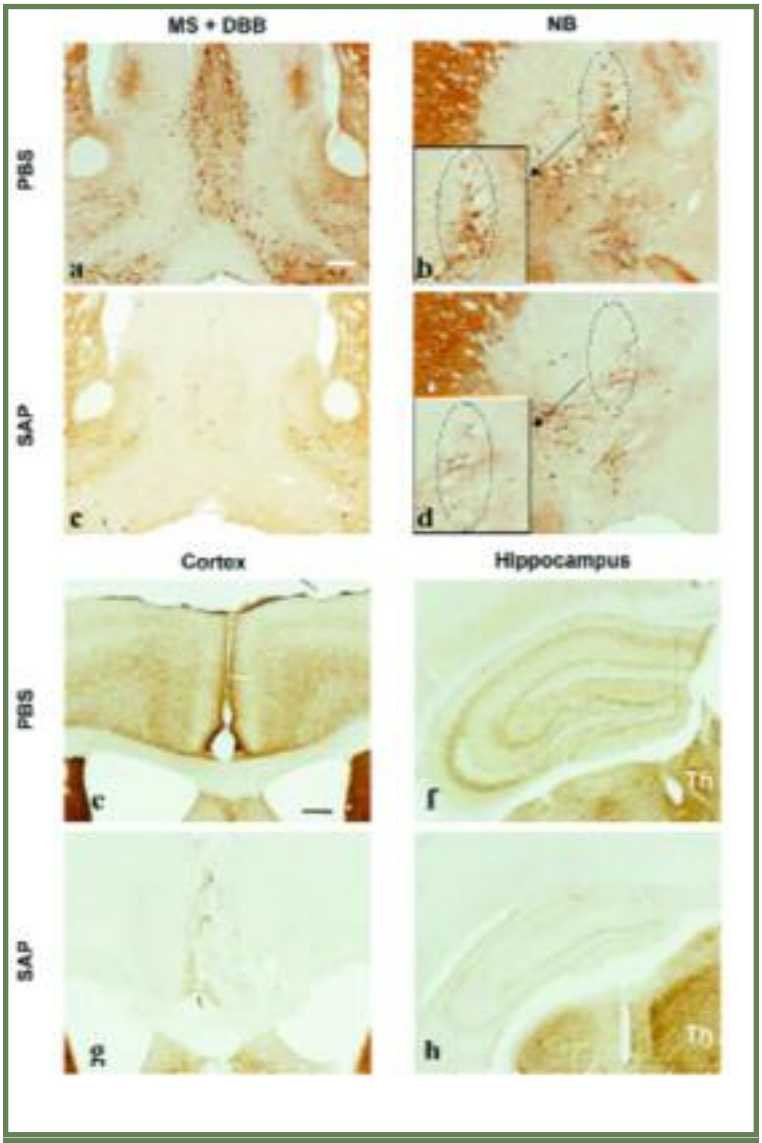
Answer: He thought it was... SUMMER FUN!

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

WINNERS: Graciela Sanchez-Watts, USC Neurobiology, Los Angeles, CA * Sathya Krishnasamy, Univ Louisville School of Medicine, Louisville, KY * Glenn Gakeyama, Cal Poly Pomona Bio Sciences, Pomona, CA * Kim Van Vliet, Univ Florida Biochem, Gainesville, FL * Darlene Martineau, Del Mar, CA * Aras Metrulis, Georgia State Neuroscience Inst, Atlanta, GA

Solve this quarter's Teaser online at: www.ATSbio.com/news/12q4_teaser.html

Targeting Tools: Featured Products



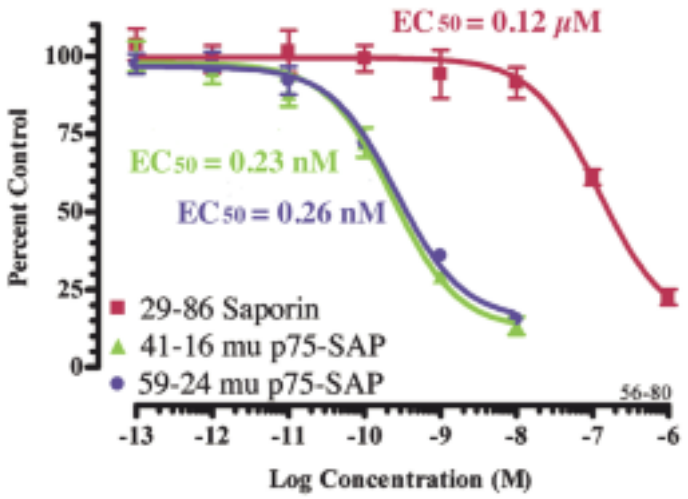
Micrographs of brain coronal sections of mice treated with PBS or mu p75-SAP. In lesioned mice, the number of ChAT-positive neurons is dramatically reduced in the medial septum (MS) and the diagonal band of Broca (DBB) (a,c) and in the nucleus basalis (NB) (b,d). AChE staining is massively depleted in the cortical mantle (e,g) and the hippocampus (f,h), but not in the thalamus (Th). Scale bar = 200 μm. (Fig 1 from Moreau *et al*. Targeting Trends, 9(2): pp. 1,6, 2008.)

mu p75-SAP

The cover article this quarter demonstrates usage of mu p75-SAP to study delirium. To create this toxin, we affinity-purified the rabbit polyclonal to the low affinity neurotrophin receptor, p75, (Cat. #AB-N01AP) with the immunogen bound to a solid support, and then conjugated the affinity-purified antibody to saporin (Cat. #PR-01). As can be seen in the cytotoxicity assay below, mu p75-SAP has an EC50 in the nanomolar range. This high potency translates to smaller amounts used for elimination of p75-positive neurons in the mouse brain which results in a greater index of efficacy and lesser non-specific cytotoxicity.

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).



NG6 cells are plated at 1000 cells/well and incubated overnight. Saporin and mu p75-SAP (conjugate of the affinity-purified rabbit polyclonal to mouse NGFr and saporin) 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.

Gangsta meows plaintively, "Have I been in 'time out' long enough? I'm getting hungry!"

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§ or anything recognized on the cell surface and internalized.

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

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

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

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

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

STORYBUL

ZUQI

TAMEDINE

GOVIETINC

SULTIN

What motivated the scientist to push forward in his research.

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

ANSWER: A . . . ○○○○○○○ ○○○○○○○○ !

WIN $100.00

Limit one entry per laboratory. Credits expire after one year.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by December 15, 2012.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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