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. 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). Top-down control of attention relies on frontoparietal cortical regions. 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.
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.

---

**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 2011 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.

The poster *The nucleus incertus contributes to the anxiety-like behaviour in rats*, presented by C. Lee at the 2011 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.
Cholinergic Control in Developing Prefrontal-Hippocampal Networks.
Janiesch PC, Kruger HS, Poschel B, Hanganu-Opatz IL.

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, Jeffrey J, Kim JY, Loh HH, Chen ZF.

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 isof orm.

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.

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.

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...
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.


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.

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.

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 measurements 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.

This review discusses advances in the field of CO2 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.
**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-purified 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.


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

**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 an Anti-SAP 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-SAP (FL-02)**

**Targeting Teaser Winners**

The solution to the puzzle was:

Jumbles: COMMON
CONCEPT
NOCIFENSIVE
HYPERSENSITIVITY
DORSAL

Answer: MONSTER MASH!

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-Puch, Montefiore Hospital, Bronx, NY * Andres Rodriguez, Univ Puerto Rico * Bob Speth, Nova Southeastern Univ, Fort Lauderdale, FL

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

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](image_url) 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.

**References**

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**


**Tag-Targeted Toxins**

Tag-targeted toxins are new conjugates in the Targeted Toxins family. Antibodies specific for tags on expressed proteins are bound 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.

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

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

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.

Please correct the address information above and provide the following:

Your Name:
Phone:
Fax:
Email:

See last quarter’s winners, page 5.