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

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

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

Anti-DBH-SAP injections virtually eliminated noradrenergic fibers and varicosities from the mPFCd, whereas control injections of the untargeted toxin (IgG-saporin) or CSF left these inputs intact (Fig. 1, middle). The specificity of the noradrenergic denervation of the mPFCd was assessed by examining the extent to which damage from the IT injection (continued on page 6)
SP-SAP Treatment for Chronic Pain... in non-Humans

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

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

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

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

ATS Receives SBIR Phase I Grant

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

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

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

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

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Selective ablation of GABA neurons in the ventral tegmental area increases spontaneous locomotor activity.
Shank EJ, Seitz PK, Bubar MJ, Stutz SJ, Cunningham KA

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

Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice.
Kublaoui BM, Gemelli T, Tolson KP, Wang Y, Zinn AR
Mol Endocrinol [Epub May 1], 2008.

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

Environmental enrichment mitigates the effects of basal forebrain lesions on cognitive flexibility.
De Bartolo P, Leggio MG, Mandolesi L, Foti F, Gelfo F, Ferlazzo F, Petrosini L
Neuroscience [Epub Apr 7], 2008.

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

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

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

Selective ablation of dorsal horn NK1 expressing cells reveals a modulation of spinal alpha2-adrenergic inhibition of dorsal horn neurones.
Rahman W, Suzuki R, Hunt SP, Dickenson AH

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

(continued on page 4)
Targeting Topics: Recent Scientific References

(continued from page 3)

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

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

Craig LA, Hong NS, Kopp J, McDonald RJ

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

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

Wiley RG

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

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

Laalou FZ, de Vasconcelos AP, Oberling P, Jeltsch H, Cassel JC, Pain L

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

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

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

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

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

Harati H, Barbelivien A, Cosquer B, Majchrzak M, Cassel JC

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

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

Joseph EK, Chen X, Bogen O, Levine JD

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

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

Takakura AC, Moreira TS, Stornetta RL, West GH, Gwilt JM, Guenet PG

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

Additional Product References


Dhaka A et al. (2008) J Neurosci 28(3):566-575. (Cat. #AB-N04: Ab to NK-1 Receptor)

Hub CY et al. (2008) J Neurosci 28(6):1404-1409. (Cat. #FL-01: Cy3-labeled 192-IgG)

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

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

Momiyama T et al. (2007) J Physiol 580(1):103-117. (Cat. #FL-01: Cy3-192-IgG)

Xu J et al. (2007) Endocrinology 148(11):5385-5395. (Cat. #AB-02: Ab to CRH/CRF)


Shekhar A et al. (2006) J Neurosci 26(36):9205-9215. (Cat. #AB-N27a: Ang IIR (AF-1r))
Q: Do targeted toxin-treated cells die by apoptosis?

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

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


Saporin and apoptosis gives 25 hits in PubMed.

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


Saporin and necrosis gives 11 hits in PubMed.

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

Targeting Teaser

**Winners**

*The solution to the puzzle was:*

Jumbles: ENZYME, METABOLIC, ORGANELLE, MITOCHONDRIA, AFFERENT

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

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

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

**An important note about shipping and storage temperatures.**

ATS is testing all of the targeted toxins for activity and stability when stored at room temperature for one week. When products are proven to retain their performance after one week, we are able to reduce your shipping costs by eliminating dry ice or freezer packs from the container.

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

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

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

Selected References for Cover Article (continued from pages 1 and 6):

involved dopaminergic fibers and terminals in dual immunofluorescence preparations for tyrosine hydroxylase (TH) and DBH (Fig. 1). TH converts tyrosine to dihydroxyphenylalanine, a precursor of both dopamine (DA) and norepinephrine (NE). Immunolabeling of this enzyme represents both DA and NE fibers and terminals, whereas staining for DBH in cortex is specific to NE.

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

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

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

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

**Article References are on page 5.**

This article was edited due to space constraints. You can view the entire article at [www.ATSbio.com/08Q3cover.html](http://www.ATSbio.com/08Q3cover.html)
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Additional Product References

Singh B et al. (2006) J Neurosci 26(27):7189-7200. (Cat. #AB-N01 and Cat. #AB-N01α: Ab to Nerve Growth Factor (p75) Receptor)


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Schechter LE et al. (2005) J Pharmacol Exp Ther 314(3):1274-1289. (Cat. #IT-05: ME20.4-SAP)


(continued from page 4)


Wu M et al. (2003) J Pharmacol Exp Ther 307(2):535-543. (Cat. #FL-01: Cy3-labeled 192-IgG)


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

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**IBITHIN**

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

How the scientist stopped the disease from spreading.

**Answer:**

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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**.

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