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Targeting Trends

Reporting the latest news in Molecular Surgery

A specific immunotoxin elucidates a causal role of striatal cholinergic system in behavioral flexibility

by Sho Aoki and Jeffery R. Wickens

Neurobiology Research Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna, Kunigami, Okinawa 904-0495, Japan

Behavioral flexibility is broadly defined as the ability to change behavioral strategy, according to a change of governing rules. Accumulated evidence suggests the involvement of particular brain areas such as prefrontal cortex and striatum in this function, in which specific brain regions play their own roles. An extension of understanding on neural substrates mediating behavioral flexibility needs a next step beyond the specificity of brain regions: the specific role of different neuronal subtypes. A method utilizing specific neurotoxins enabled us to target and elucidate the role of neurochemically-specific neurons in this ability. In our recent study,[1] we demonstrated a causal role of rat cholinergic interneurons in the striatum in behavioral flexibility, using a new specific immunotoxin targeting neurons containing choline acetyltransferase (ChAT). Comparing non-selective neuronal labeling and specific immunostaining of ChAT neurons indicated that local injections of the immunotoxin successfully and selectively damaged cholinergic neurons (Fig. 1). This result is consistent with a previous study that used Anti-ChAT-SAP to study the medial prefrontal cortex (Cat. #IT-42).[2]

Using the selective lesion, we compared intact rats injected with saline and rats without cholinergic interneurons of either dorsomedial or ventral striatum in a set-shifting task.[3] This task required animals to shift their attention from one stimulus dimension to

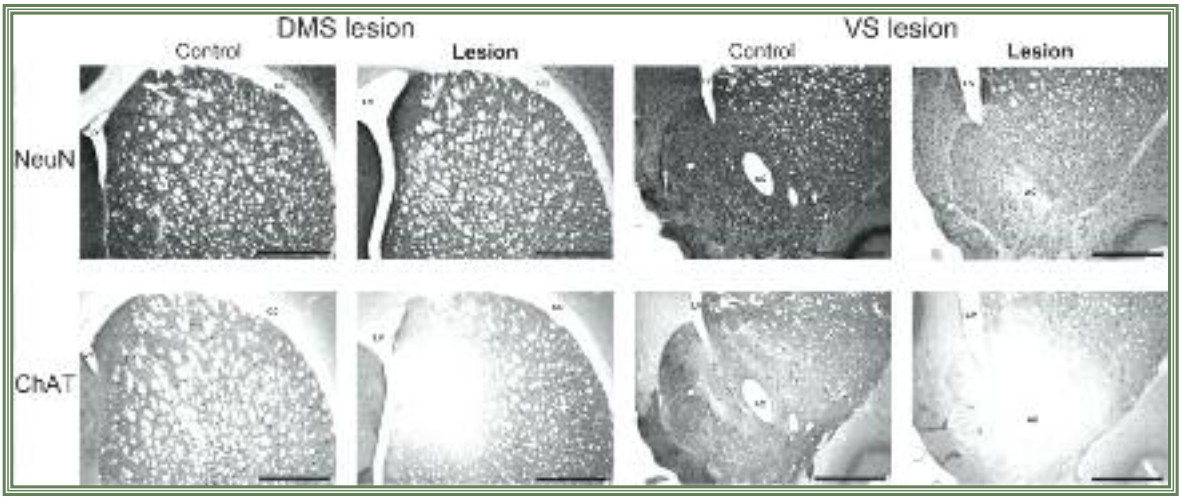

Fig. 1: Representative coronal sections of the rat striatum show intact nuclei (NeuN) staining but clear ablation of the cholinergic interneurons with ChAT staining in lesioned cases (DMS or VS). Abbreviations; DMS: dorsomedial striatum, VS: ventral striatum, LV: lateral ventricle, CC: corpus callosum, AC, anterior commissure. Scale bar: 1 mm. Reprinted from Aoki *et al.* (2015).

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Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

New Product: Mono-Biotin Saporin

The conjugation specialists at Advanced Targeting Systems are proud to announce a new addition to the catalog:

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Researchers now have more freedom in selecting conjugation strategies. Users have taken advantage of the ability to use biotinylated targeting agents with Streptavidin-ZAP (Cat. #IT-27) a chemical conjugate between saporin and streptavidin. Streptavidin-ZAP converts biotinylated materials into targeted toxins. Streptavidin is a tetrameric protein (molecular weight 53 kDa in its recombinant form), with each subunit able to bind a single biotin molecule. The bond between streptavidin and biotin is rapid and essentially non-reversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. It is the strongest known noncovalent biological interaction ($Ka = 10^{15}\ M^{-1}$) between protein and ligand. The streptavidin used to make Streptavidin-ZAP contains no carbohydrate group and has a neutral isoelectric point, which therefore reduces the nonspecific binding as compared to avidin. A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with streptavidin-labeled probes or other detection reagents for use in biological assays.

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- ***LD_{50} in mice is 4 mg/kg***
- ***Saporin does not have a method of cell entry on its own***

Reference

Minami SS, Sun B, Popat K, Kauppinen T, Pleiss M, Zhou Y, Ward ME, Floreancig P, Mucke L, Desai T, Gan L. (2012) Selective targeting of microglia by quantum dots. *J Neuroinflammation* 9:22.

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Upcoming Events

Targeting Teaser Solution

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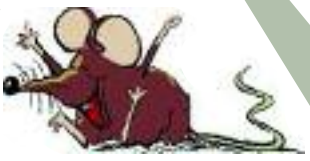

LAST QUARTER'S WINNERS: Seto Chice, SUNY HSC at Brooklyn * Corinne Kiessling, Binghamton University, NY

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Alterations in the rostral ventromedial medulla after the selective ablation of mu-opioid receptor expressing neurons.

Harasawa I, Johansen JP, Fields HL, Porreca F, Meng ID.

Pain Epub2015.

The rostral ventromedial medulla (RVM) has both excitatory and inhibitory control over nociceptive neurons in the medullary dorsal horn and spinal cord. Previous work has demonstrated that elimination of mu-opioid receptor-expressing neurons in the RVM reduces stress and injury-induced behavioral hypersensitivity, but the effect of losing these cells on the descending inhibitory system has not been examined. The authors administered 1.2 pmol of Dermorphin-SAP (Cat. #IT-12) to each side of the RVM of rats. Saporin (Cat. #PR-01) was used as a control. Characterization of RVM neurons in lesioned animals showed a reduction in on- and off-cells, but no change in the number of neutral cells. These data indicate that mu-opioid receptor-expressing cells in the RVM are not needed for analgesia produced by activation of RVM neurons.

CD103+ Dendritic Cells Elicit CD8+ T Cell Responses to Accelerate Kidney Injury in Adriamycin Nephropathy.

Cao Q, Lu J, Li Q, Wang C, Wang XM, Lee VW, Wang C, Nguyen H, Zheng G, Zhao Y, Alexander SI, Wang Y, Harris DC.

J Am Soc Nephrol Epub2015.

Although it is known that dendritic cells (DCs) are involved in chronic kidney disease, it is not well understood how they either resolve or aggravate the condition. CD103+ dendritic cells in particular, are known to maintain tolerance through interaction with regulatory T cells, as well as protect against infection through interactions with CD8+ T cells. In this work the authors depleted CD103+ DCs by administering 1 mg/kg of anti-CD103-SAP (Cat. #IT-50) to the intraperitoneal space of mice subject to adriamycin nephropathy. Rat IgG-SAP (Cat. #IT-17) was used as a control. Elimination of the CD103+ DCs attenuated the kidney injury, indicating that in murine chronic kidney disease CD103+ DCs are pathogenic rather than therapeutic.

Anti-EFNA4 Calicheamicin Conjugates Effectively Target Triple-Negative Breast and Ovarian Tumor-Initiating Cells to Result in Sustained Tumor Regressions.

Damelin M, Bankovich A, Park A, Aguilar J, Anderson W, Santaguida M, Aujay M, Fong S, Khandke K, Pulito V, Ernstoff E, Escarpe P, Bernstein J, Pysz M, Zhong W, Upeslacis E, Lucas J, Lucas J, Nichols T, Loving K, Foord O, Hampl J, Stull R, Barletta F, Falahatpisheh H, Sapra P, Gerber HP, Dylla SJ.

Clin Cancer Res 21(18):4165-4173, 2015.

Triple-negative breast cancer (TNBC) is characterized by tumors lacking HER2, estrogen receptor, and progesterone receptor. TNBC has proved to be very difficult to treat, in large part because of the absence of consensus targets on the surface of the tumor cells. In this work the authors empirically established a set of surface markers associated with TNBC tumor initiating cells, as produced by patient-derived xenografts. Ephrin-A4 was selected as a therapeutic target, and a cell line transfected with the ephrin-A4 gene was challenged with two versions of biotinylated anti-ephrin-A4 coupled to Streptavidin-ZAP (Cat. #IT-27). Both the mouse monoclonal and the humanized antibodies reach an EC_{50} of 10 ng/ml, indicating that ephrin-A4 has promise as a therapeutic target for TNBC.

KNDy neurons modulate the magnitude of the steroid-induced luteinizing hormone surges in ovariectomized rats.

Helena CV, Toporikova N, Kalil B, Stathopoulos AM, Pogrebna VV, Carolino RO, Anselmo-Franci JA, Bertram R.

Endocrinology Epub2015.

Maturation and reproductive function in mammals is controlled by the kisspeptin neuropeptide. Kisspeptin modulates numerous systems within this framework including the mediation of positive and negative feedback effects of estradiol on luteinizing hormone (LH). In the rat, two kisspeptin neuronal populations exist; one in the anteroventral periventricular nucleus (AVPV), and the KNDy (kisspeptin/neurokinin B/dynorphin) neurons in the arcuate nucleus. In this work the authors examine the role of KNDy neurons in estradiol positive feedback effects by administering 10-ng bilateral injections of NK3-SAP (Cat. #IT-63) into the arcuate nucleus of rats. The results indicate that KNDy neurons use dynorphin to inhibit AVPV neurons, establishing a regulatory mechanism for the amplitude of steroid-induced LH surges.

Membrane associated cancer-oocyte neoantigen SAS1B/ovastacin is a candidate immunotherapeutic target for uterine tumors.

Pires ES, D'Souza RS, Needham MA, Herr AK, Jazaeri AA, Li H, Stoler MH, Anderson-Knapp KL, Thomas T, Mandal A, Gougeon A, Flickinger CJ, Bruns DE, Pollok BA, Herr JC.

Oncotarget Epub2015.

Ovastatin is a zinc matrix metallo-proteinase thought to play roles in sperm-egg interaction and the prevention of polyspermy in eutherians. This protein is not found in normal adult tissues, but is expressed by uterine carcinosarcomas. The authors investigated the possibility of targeting ovastatin as a tumor surface neoantigen for therapeutic purposes. SNU539 cells, a uterine malignant mixed Müllerian tumor-derived cell line, were challenged with 1 µM, 0.1 µM, and 0.01 µM rabbit polyclonal anti-ovastatin coupled to 5.42 nM Fab-ZAP rabbit (Cat. #IT-57). Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The results indicate that for this form of uterine cancer, ovastatin is a viable therapeutic target.

Neuropsin (OPN5)-mediated photoentrainment of local circadian oscillators in mammalian retina and cornea.

Buhr ED, Yue WW, Ren X, Jiang Z, Liao HR, Mei X, Vemaraju S, Nguyen MT, Reed RR, Lang RA, Yau KW, Van Gelder RN.

Proc Natl Acad Sci U S A Epub2015.

Circadian clocks are found in most mammalian tissues. These clocks are

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Targeting Topics: Recent Scientific References

(continued from page 3)

synchronized by the suprachiasmatic nuclei (SCN) in the brain. The local clock found in the retina does not require rods, cones, intrinsically photosensitive retinal ganglion cells, or the SCN. In order to determine what photopigments are responsible for local retinal photoentrainment, the authors used a candidate gene approach. For immunohistochemical studies on flat mount retinas they used a melanopsin antibody (Cat. #AB-N38) at a 1:1000 dilution. The data indicate that OPN5, also known as neuropsin, has a light-sensing function and is involved in retinal photoentrainment.

Retrograde transport is not required for cytosolic translocation of the B-subunit of Shiga toxin.

Garcia-Castillo MD, Tran T, Bobard A, Renard HF, Rathjen SJ, Dransart E, Stechmann B, Lamaze C, Lord M, Cintrat JC, Enninga J, Tartour E, Johannes L.

J Cell Sci 128(13):2373-2387, 2015.

Bacterial and plant toxins rely on various trafficking pathways to reach intracellular targets. Shiga and Shiga-like toxins have been found to be moved via vesicular transport through several subcellular structures on the way to the cytosol. Shiga toxin (STx) is the cause of hemolytic uremic syndrome, for which there is no effective treatment. In order to better understand the mechanisms of STx membrane translocation the authors used a custom conjugate of the receptor-binding B-subunit of STx (STxB) and saporin (Custom conjugation provided by Advanced Targeting Systems). *In vitro* assays demonstrated that STxB-SAP did not use retrograde transport to the Golgi complex in order to reach the cytosol. This information has relevance to antigen cross-presentation of antigen-presenting cells.

Catecholaminergic neurons projecting to the paraventricular nucleus of the hypothalamus are essential for cardiorespiratory adjustments to hypoxia.

King TL, Ruyle BC, Kline DD, Heesch CM, Hasser EM.

Am J Physiol Regul Integr Comp Physiol Epub2015.

Catecholaminergic neurons in the brainstem are known to be involved in cardiorespiratory control and to modulate sensory function. Some of the projections from these neurons are to the paraventricular nucleus (PVN), and are involved in cardiorespiratory and neuroendocrine responses to hypoxia. While data have shown the PVN-projecting neurons are activated by hypoxia, their function in this context is not known. In this work the authors bilaterally injected 42 ng of Anti-DBH-SAP (Cat. #IT-03) into the PVN of rats. Mouse IgG-SAP (Cat. #IT-18) was used as control. Respiratory measurements of the lesioned animals indicates that PVN-projecting catecholaminergic neurons are involved in peripheral and central chemoreflex and arterial oxygen levels during exposure to hypoxic stimuli.

Catecholaminergic neurons in the comissural region of the nucleus of the solitary tract modulate hyperosmolality-induced responses.

Freiria-Oliveira AH, Blanch GT, Pedrino GR, Cravo SL, Murphy D, Menani JV, Colombari DS.

Am J Physiol Regul Integr Comp Physiol Epub2015.

Body fluid homeostasis and cardiovascular regulation are thought to be at least in part controlled by noradrenergic A2 neurons found in the nucleus of the solitary tract (NTS). In this work the authors investigated the involvement of A2 neurons of the commissural NTS in arterial pressure, as well as several body fluid homeostasis parameters. Rats received 12.6-ng injections of Anti-DBH-SAP (Cat. #IT-03) into the commissural NTS. Mouse IgG-SAP (Cat. #IT-18) was used as a control. Lesioned animals displayed increased c-Fos expression in the hypothalamic paraventricular nucleus when treated with hypertonic NaCl, and increased arterial pressure. The data indicate that commissural NTS A2 neurons are essential for inhibitory mechanisms that reduce water intake and pressor response to an acute increase in plasma osmolality.

Limited changes in spinal lamina I dorsal horn neurons following the cytotoxic ablation of non-peptidergic C-fibers.

Saeed AW, Pawlowski SA, Ribeiro-da-Silva A.

Mol Pain 11(1):54, 2015.

For the most part nociceptive information is moved from the periphery to the spinal cord through small diameter primary afferents. One subclass of these afferents is further divided into peptidergic and non-peptidergic populations. The authors examined the role of the non-peptidergic afferents in normal nociception and pain, especially the aspect that in rat neuropathic and inflammatory pain models there is novel expression of neurokinin-1 receptors in some neurons normally devoid of this protein. Rats received 4.8-µg injections of rIB4-SAP (Cat. #IT-10) into the left sciatic nerve, over three injection sites. While the number of non-peptidergic neurons was significantly reduced, de novo expression of the neurokinin-1 receptor was not increased in lamina I pyramidal projection neurons.

Selective elimination of isolectin B4-binding trigeminal neurons enhanced formalin-induced nocifensive behavior in the upper lip of rats and c-Fos expression in the trigeminal subnucleus caudalis.

Oyamaguchi A, Abe T, Sugiyo S, Niwa H, Takemura M.

Neurosci Res Epub2015.

In adult rats non-peptidergic neurons and peptidergic neurons innervate different areas and layers of the lamina. It is thought that these two neuronal populations play different roles in nociceptive processing, but the specific function of each group is not well understood. In order to investigate peptidergic and non-peptidergic neurons in orofacial pain processing the authors injected the cisterna magna of rats with 2.9 µg of rIB4-SAP (Cat. #IT-10). Blank-SAP (Cat. #IT-21) was used as a control. The lesioned

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(continued on page 5)

Targeting Talk: Product Q&A

Q: Hello, I have used your Anti-DBH-SAP (Cat. #IT-03) conjugate, and I'm having a hard time finding this citation: R.G. Wiley, D.A. Lappi. Suicide Transport and Immunolesioning. Molecular Biology Intelligence Unit, R.G. Landes Co, Austin, TX (1994). Do you know where I could find a copy?

A: This book is available in many university libraries and can also be purchased here: http://www.amazon.com/Suicide-Transport-Immunolesioning-Molecular-Intelligence/dp/1570590958

Q: I'm trying to find out if enough Anti-DBH-SAP will be retrogradely transported and taken up by non targeted sympathetic neurons by bulk fluid-phase endocytosis. Does saporin become degraded after it kills the neuron or does it enter the extracellular matrix?

A: It is very unlikely that a targeted toxin such as Anti-DBH-SAP is freed from the targeted neuron in a meaningful condition. There has never been a reported identification of a targeted toxin, functionally or not, after it has eliminated its targeted neuron. Current evidence indicates that effective suicide transport agents undergo endocytosis at nerve terminals followed by retrograde axonal transport of the endocytic vesicles containing the toxin. Experiments using vincristine have shown that the retrograde axonal transport of suicide transport toxins utilizes the fast transport system (microtubules). However, it is not known what determines whether or not a specific toxin-ligand undergoes axonal transport after internalization.

Empirically, it has been observed that immunotoxins (OX7-SAP [Cat. #IT-02], 192-IgG-SAP [Cat. #IT-01], Anti-DBH-SAP) and lectin-toxins (ricin, volkensin, IB4-SAP) all undergo retrograde axonal transport and are therefore effective suicide transport agents. This is not true, however, for neuropeptide-toxin conjugates, such as dermorphin-SAP. For example, in an unpublished study, we injected large doses (1-2 µg) of Dermorphin-SAP (Cat. #IT-12) into the lumbar intrathecal space of rats. After 2-3 days, rats were sacrificed and lumbar dorsal root ganglia examined for evidence of toxin effect (striking chromatolysis). None was found after examining numerous ganglia and >15,000 primary afferent neurons. Apparently, dermorphin-SAP is not retrogradely transported even if it is taken into the primary afferent terminals that express the mu opioid receptor (MOR).

(continued from page 4)
animals displayed more frequent face-rubbing responses on the administration of formalin, indicating that IB4-binding neurons in the trigeminal nerve play an anti-nociceptive role in response to this type of pain.

Hippocampal acetylcholine depletion has no effect on anxiety, spatial novelty preference, or differential reward for low rates of responding (DRL) performance in rats.

McHugh SB, Francis A, McAuley JD, Stewart AL, Baxter MG, Bannerman DM.
Behav Neurosci 129(4):491-501, 2015.

It is unclear whether cholinergic lesions in the hippocampus affect both learning and behavior, or learning only. In this study the authors lesioned cholinergic neurons in the medial septum/vertical limb of the diagonal band of Broca of rats with bilateral 30-ng injections of 192-IgG-SAP (Cat. #IT-01). Although hippocampal cholinergic innervations were significantly reduced, with a concomitant reduction in choline acetyltransferase activity, the lesioned animals did not perform differently in several behavioral tests. The data do not provide evidence that the septo-hippocampal cholingeric projections play a role in anxiety, spatial novelty preference, or differential reward for low rates of responding tests.

Selective C1 Lesioning Slightly Decreases Angiotensin II Type I Receptor Expression in the Rat Rostral Ventrolateral Medulla (RVLM).

Bourassa EA, Stedenfeld KA, Sved AF, Speth RC.
Neurochem Res Epub2015.

Exogenous angiotensin II administered to the RVLM produces a significant pressor response that can be countered by angiotensin II type I receptor antagonists. In this work the authors examined the relative contribution of C1 and non-C1 neurons in the RVLM to this angiotensin II response. Rats received 10 or 15 ng of Anti-DBH-SAP (Cat. #IT-03) as unilateral injections into the RVLM. Mouse IgG-SAP (Cat. #IT-18) was used as control. The data indicate that the majority of angiotensin II type 1 receptors are expressed on non-C1 neurons or glia.

Pain from intra-articular NGF or joint injury in the rat requires contributions from peptidergic joint afferents.

Kras JV, Weisshaar CL, Pall PS, Winkelstein BA.
Neurosci Lett 604:193-198, 2015.

Both peptidergic and non-peptidergic neurons innervate the facet joint, which is the source of pain in a majority of neck trauma. In this work the authors examined these subpopulations of neurons to determine the contribution of each in facet joint pain. 100 ng of SSP-SAP (Cat. #IT-11) was injected into bilateral C6/C7 facet joints of rats. Alternatively, rats received 5 µg of rIB4-SAP (Cat. #IT-10) via the same method. Saporin (Cat. #PR-01) was used as control. SSP-SAP, but not rIB4-SAP was able to prevent NGF-induced mechanical and thermal hypersensitivity. SSP-SAP administration also prevented behavioral hypersensitivity and NGF upregulation in the dorsal root ganglion after facet joint distraction. The data indicate that interference with peptidergic signaling within the facet joint may be a treatment for pain originating in that location.

Anti-ChAT-SAP elucidates a causal role in behavioral flexibility

(continued from page 1)

another to change action strategies, based on a change of behavioral rules. We extended an established task[3] by setting three experimental conditions for a set-shift (Fig. 2), all of which required a change between two strategies involving attention to different stimuli. In all the conditions, animals initially learned to obtain a reward by choosing a Right lever (Fig. 2, Response strategy). Subsequently, after the set-shift, animals faced a change of behavioral rules in which

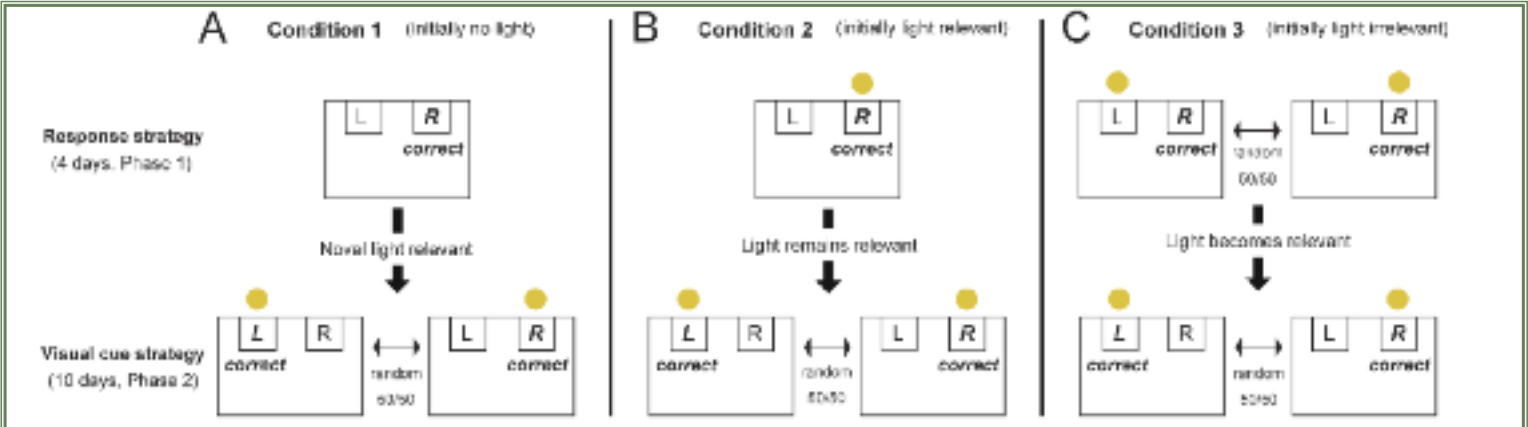

Fig. 2: A flow chart of three variations (A, B and C) of the set-shifting paradigm. A yellow circle shows a visual cue. Reprinted from Aoki *et al.* (2015).

animals had to learn to select a lever indicated by a light cue that randomly illuminated above either lever (Fig. 2, Visual cue strategy). Different manipulations of the light delivery in initial learning made it possible to test different attentional shifts in the next visual cue learning: attention to either 1) a previously absent but now novel light cue (Fig. 2A), 2) a previously relevant and remained relevant cue (Fig. 2B), and a previously irrelevant but now relevant cue (Fig. 2C).

Initial acquisition of response strategy was intact across conditions and treatments, indicating that the striatal cholinergic interneurons are unnecessary for initial learning. By contrast, after a change of behavioral rules occurred, both types of lesions made animals stick to an old strategy. They also showed less exploration for figuring a new rule out. Interestingly, ventral cholinergic ablation disrupted a strategic shift when it required attention to a novel light cue that was introduced as a new important stimulus (Fig. 2A). On the other hand, cholinergic loss in the dorsomedial striatum impaired a set shift when attention to a previously irrelevant cue was needed (Fig. 2C). There was no effect on a shift if the light remained relevant (Fig. 2B). These findings suggest that when facing a change of behavioral rules, striatal cholinergic interneurons play a specific role, namely inhibiting the use of an old strategy and facilitating exploration of a new rule. Furthermore, dorsomedial and ventral striatum cholinergic systems differentially contribute to this function in a highly context-dependent manner. Owing to the prominent targeting method by the Anti-ChAT-SAP, we found a causal role of a neurochemically-specific neuron in behavioral flexibility. This technique is undoubtedly powerful to deepen our knowledge of the causal relationship of particular neuronal types and behavior, and is encouraged for use in studies of different types of behavior.

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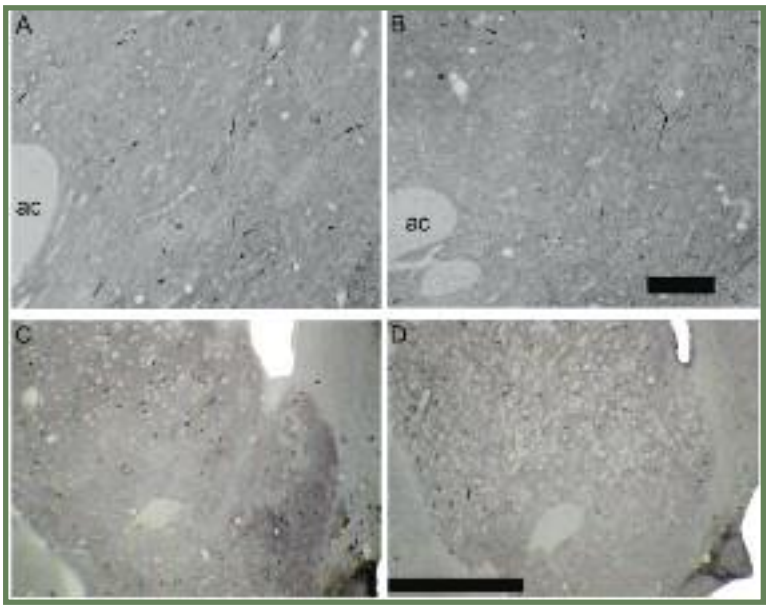
3. Floresco SB, Block AE, Tse MTL (2008) Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behavioural Brain Research 190:85-96.

Targeting Tools: Featured Products

Anti-ChAT-SAP

Choline acetyltransferase (ChAT) catalyzes the synthesis of the neurotransmitter acetylcholine (ACh) from choline and acetyl-CoA in cholinergic neurons. ChAT serves as a specific marker for cholinergic neurons in both peripheral and central nervous systems. Evidence shows that ChAT exists in two forms inside cholinergic nerve terminals, a soluble hydrophilic form and the membrane-associated amphiphilic form.[1-2] Membrane-bound ChAT has served as the feature condition that allows specific targeting with an affinity-purified antibody to ChAT conjugated to saporin to specifically target and eliminate those specific cells. Anti-ChAT-SAP is made with an antibody using a 22-amino acid peptide from porcine ChAT.

The targeted toxin has been shown in several papers to eliminate cholinergic neurons in the rat brain[3-6] (also see Cover Article) and is expected to cross-react with mouse, and many other species.



Representative sections of ChAT-immunostained tissues of N.Acc. from rats that (A and C) received an intra-accumbens micro-injection of Rabbit IgG-SAP (Cat. #IT-35; 250 ng; control), and (B and D) received an intra-accumbens micro-injection of Anti-ChAT-SAP (250 ng). Administration of Anti-ChAT-SAP reduced significantly the amount of cholinergic interneurons at the injection site while sparing adjacent areas. Scales A and B = 200 µm; C and D = 1 mm; ac: anterior commissure.
François LaPlante. Targeting Trends, 2013. 14(1): p. 1,6.

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SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles **taken from this issue's cover story**, one letter to each block, to solve the puzzle.

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LONKDWEEG

MATURIST

MOROSELADID

LARVENT

What chemistry can provide scientists.

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

ANSWER: A . . . ◯◯◯◯◯◯◯ OF POSSIBILITIES!

WIN!

SOLVE the puzzle online with the correct solution by December 31, 2015.

WIN a $100 product credit!

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