Activating memory

By Lev Osherovich, Senior Writer

Despite more than a decade of research suggesting histone acetyltransferases play a role in learning and memory, the targets have proven difficult to selectively activate in the brain. Now, a French and Indian team has preclinical proof of concept for activating histone acetyltransferases with a small molecule formulated to cross the blood brain barrier.[1] The compound enhanced brain activity in wild-type mice, but its selectivity and utility in neurodegenerative disease remain to be seen.

Histone acetyltransferases (HATs) are a family of epigenetic regulators that, in the brain, broadly modulate gene expression in response to neural stimulation. HATs work by adding acetyl groups to histones, which are nuclear proteins that package and organize chromosomal DNA. Acetylation generally causes tightly wound and transcriptionally silent chromatin to loosen up and become active.

The new report describes the molecular and behavioral effects of activating two HATs linked to learning and memory—CREB binding protein (CREBBP; CBP) and a related protein called E1A binding protein p300 (EP300; p300).

In the late 1990s, academic researchers identified CBP as a critical regulator of learning and memory in sea slugs, flies and mice.[2,3] Despite genetic evidence that activating CBP and other HATs can improve brain function, identifying small molecule activators of enzymes is a tall order and for HATs is compounded by the blood brain barrier's exclusion of most chemical classes from the CNS.

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> ***—Anne-Laurence Boutillier, University of Strasbourg***

Researchers at the **Jawaharlal Nehru Centre for Advanced Scientific Research** and the **University of Strasbourg** have made progress on both the chemistry and delivery fronts and have designed a HAT-activating compound that appears to work in mice.

"Our results, obtained *in vivo*, show that new HAT activator molecules provide therapeutic options for brain diseases," said Anne-Laurence Boutillier, the team's coleader and the director of research at **Centre National de la Recherche Scientifique** (CNRS) at the University of Strasbourg.

The team began by making brain-permeating derivatives of an EP300-activating compound previously identified in the laboratory of the team's coleader Tapas Kundu.

Kundu is a professor in the Transcription and Disease Laboratory at the Jawaharlal Nehru Centre.[4]

The researchers first synthesized a molecule, TTK21, that increased EP300 and CBP activity *in vitro* compared with vehicle control. To increase TTK21's ability to enter cells, the team conjugated the molecule to carbon nanospheres.

Cultured neuroblastoma cells treated with TTK21-covered nanoparticles had higher levels of histone acetylation than controls receiving unconjugated nanoparticles.

In mice, intraperitoneal injection of TTK21 particles led to their accumulation in the brain, liver and spleen. The nanoparticles were eliminated from the liver and spleen in a matter of days but substantial amounts remained in the brain for up to two weeks. Effects on spleen and liver function were not reported.

Animals receiving the molecule had higher levels of histone acetylation in the cortex and hippocampus than nanoparticle carrier–treated controls, resulting in greater expression of genes associated with neuronal growth. The functional consequence of TTK21 treatment was increased neurogenesis and better performance in assays of memory duration compared with controls receiving drug-free nanoparticles.

Results were reported in *The Journal of Neuroscience*. TTK21 is the subject of a pending patent in India and is available for licensing.

HAT trick

The findings suggest TTK21 could be a starting point for the development of a brain-permeating chemical class that activates HATs. However, substantial questions remain about the compound's selectivity and applicability in disease.

"Molecules that favor and promote the *in vivo* maturation and differentiation of newly generated neurons in the adult present an obvious advantage in several neurodegenerative diseases," said Boutillier, citing Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) as potential indications in which to try TTK21.

Her team is focusing on AD. Prior work by other researchers has shown that overexpression of CBP enhances cognitive performance in a mouse model for AD,[5] so activating the protein with a small molecule could yield similar results.

"We are currently testing this compound in two transgenic AD mouse models, one presenting with microtubule-associated protein-τ (MAPT; TAU; FTDP-17) aggregates and the other one with amyloid plaques," said Boutillier.

"The novel hook is not that activating histone acetyltransferases is good for memory, but rather that they came up with chemistry" that leads to HAT activation, said **Atlas Venture** partner Bruce Booth.

In June, Atlas launched **Rodin Therapeutics Inc.** to pursue epigenetic targets for neurodegenerative disease, with Booth taking on the CEO role. **Johnson & Johnson**'s venture arm, **Johnson & Johnson Development Corp.**, invested an undisclosed sum in Rodin's tranched series A round.

Booth said that TTK21 likely needs further medicinal chemistry optimization of its potency and selectivity. He noted that HATs regulate the acetylation of a multitude of proteins besides histones, so broad-spectrum activation of the class might have undesirable effects.

Although TTK21 could activate CBP and EP300 in an *in vitro* assay in the absence of other enzymes, it is not clear whether these relatively low-affinity compounds can act on other HATs inside cells.

Activating both CBP and EP300 may mean "acetylating hundreds of proteins, so you might have broad effects on tons of genes and tons of proteins," said Booth. "The big question is whether there are other nonhistone targets that are affected. The challenge is to get to the specific compounds with a high selectivity."

One way to test TTK21's selectivity would be to assess the compound's effects in cells or mice lacking Cbp or Ep300.

An alternative to activating HATs is to inhibit histone deacetylases (HDACs), a family of epigenetic regulators that removes acetyl groups from histones. Indeed, researchers at **The University of Texas Southwestern Medical Center** reported in April that inhibiting HDAC2 improves learning and memory in similar assays to those used by Boutillier and Kundu's team.[6]

Booth said Rodin is focusing on CNS diseases including AD but did not disclose the company's specific targets.

"We're not talking about which specific epigenetic targets are in our scope and which ones aren't, but the general area of histone and epigenetic modification, transferase activity, demethylation and acetylation is very much at the heart of what we're doing," said Booth.

Rodin is partnered with **Proteros biostructures GmbH** and with Johnson & Johnson's Janssen Research & Development LLC unit and Boston Innovation Center to develop compounds and assays.

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