non-enriched samples and proteomes from glia. This group also took the work a step further, analyzing the excitatory hippocampal neuron proteome from mice exposed to an enriched environment and identifying over 200 differentially expressed proteins.

Combining AAV expression in striatum with a neuron-specific promoter, Krogager *et al.*[4] label 1,780 proteins in medium spiny neurons. The enriched proteins included those expected of γ-amino-butyric acid-producing (GABAergic) medium spiny neurons, as well as proteins associated with glutamatergic and dopaminergic neurotransmission. They also demonstrated feasibility for more specific cell-type expression using Cre-driver lines and an AAV carrying a double-floxed inverted open reading frame for *Methanosarcina mazei* pyrrolysine–tRNA ligase (*MmPylS*) preceded by the red fluorescent protein marker mCherry (AAV FLEX approach).

Although these results are encouraging, there is still room for improvement considering that both studies used samples pooled from multiple mice. Moreover, the total number of proteins identified in both studies is relatively low compared with likely upper limits of ~12,000 proteins in a specific cellular proteome[9,10]; indeed, greater depth may be required to identify and quantify cell-type-specific biomarkers. Although neither study established the stoichiometry of labeling, the modified tRNA should be expressed at a level that ensures optimal incorporation of the non-canonical amino acid into the target proteome, which is sufficient for a reasonable protein yield without interfering with protein function. Variations in amino acid intake and/or absorption could also affect the rate of incorporation into the proteome and ultimately alter the proteomics results. The AAV method could be particularly useful for experiments requiring the use of specific mouse models, and would avoid extensive animal breeding compared with the Cre-dependent transgenic mouse method. It could also be used in other animal species. On the other hand, the AAV approach could potentially be associated with higher variability in levels of tRNA expression among biological replicates, as it depends on precise injections. The requirement for administering a non-canonical amino acid for up to 21 days and provision of a low-methionine diet[3] might be a limitation for some applications.

Collectively, however, these methods demonstrate promising applications for neuronal cell-type-specific proteomic analyses *in vivo*. The continued rapid development of mass-spectrometry-based technologies should enable researchers to reach greater depths of proteomic coverage using this type of approach. Either method should also be extendable using the increasing numbers of mouse and other rodent and primate models that are available for cell-type-specific analyses. Integrated with other approaches to interrogate the transcriptome and translatome, cell-type-specific proteomics will add a further layer of molecular understanding to studies of the mammalian brain.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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