Supplementary Information

Genetic code expansion in the mouse brain

Russell J. Ernst[1,2], Toke P. Krogager [1,2], Elizabeth S. Maywood[1], Roberto Zanchi[1], Vaclav Beranek[1], Thomas S. Elliott[1], Nicholas P. Barry[1], Michael H. Hastings[1], Jason W. Chin[1,3]

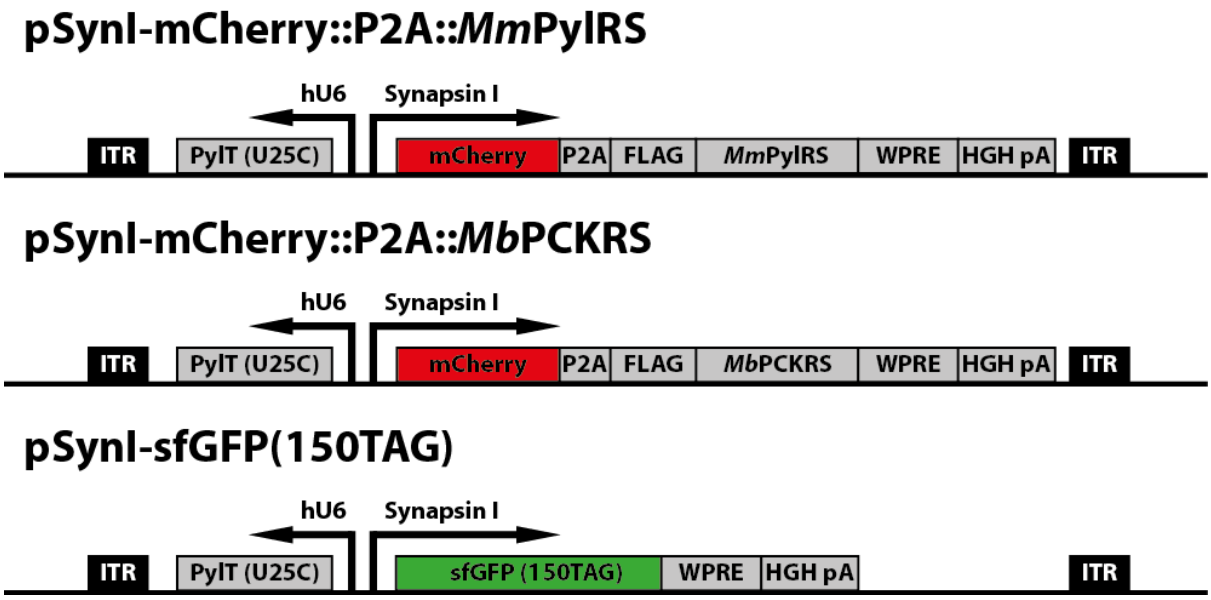
[1]Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, England, UK

[2]contributed equally to this work

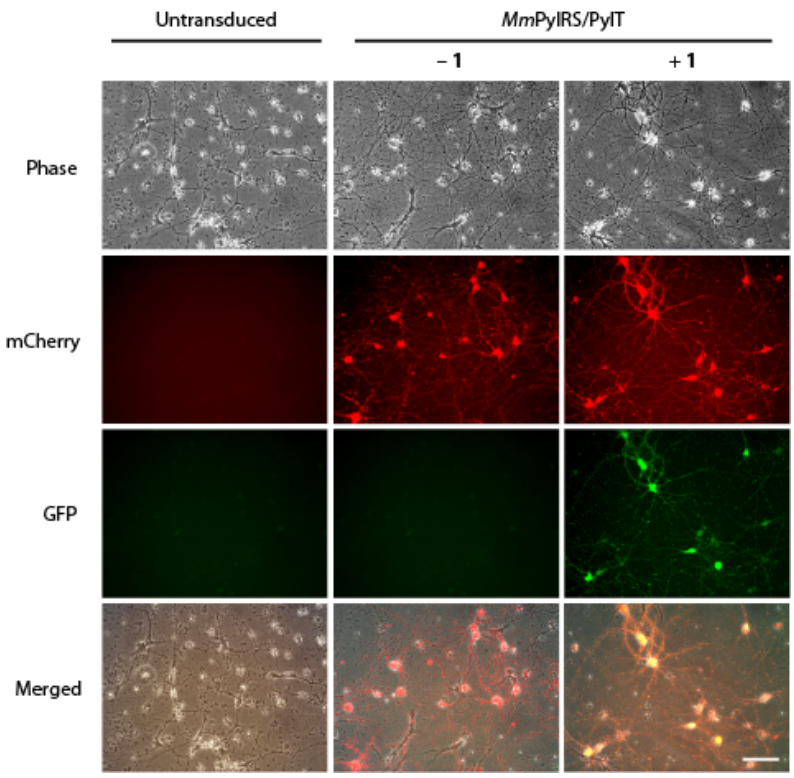
[3]Correspondence: chin@mrc-lmb.cam.ac.uk

Supplementary Results

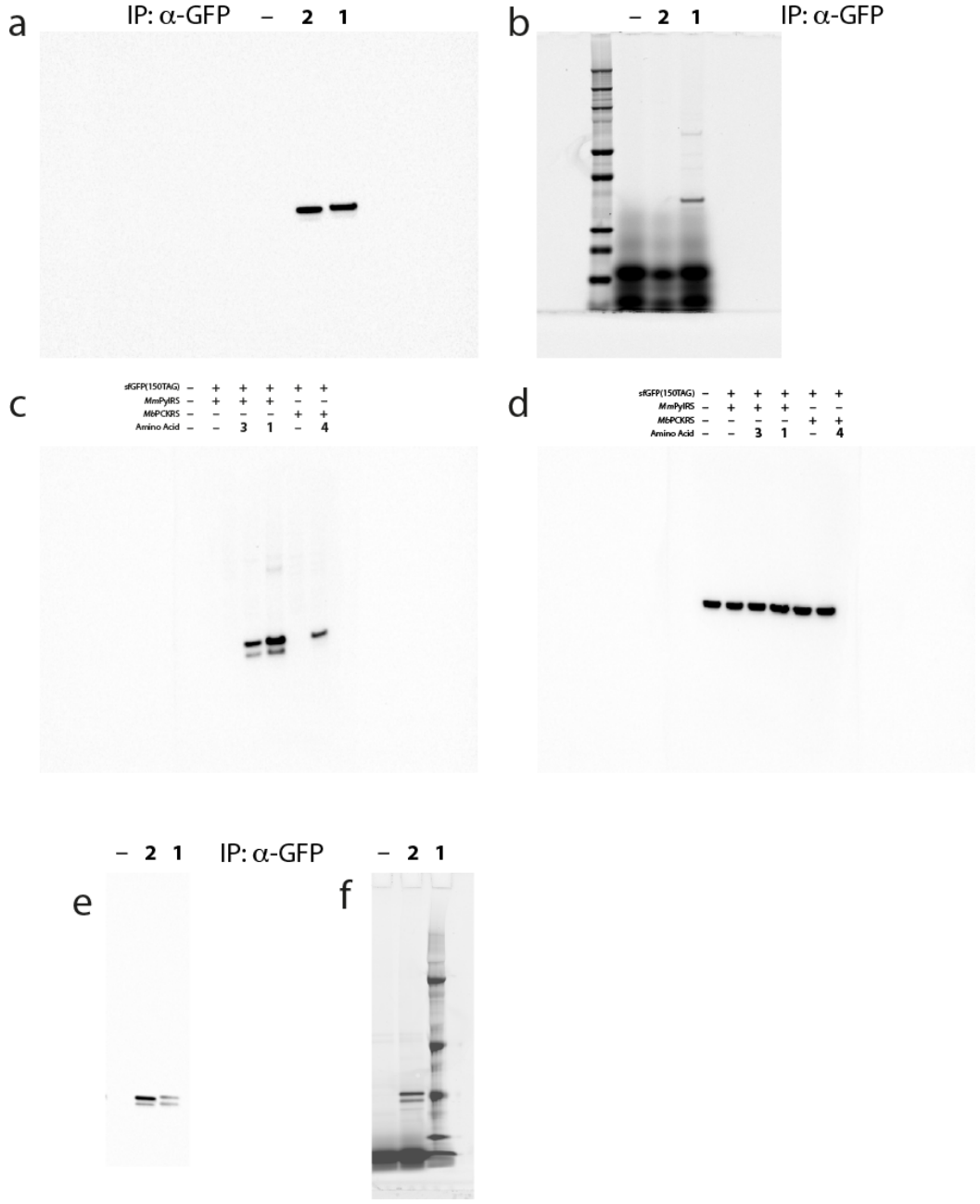
Supplementary Figures



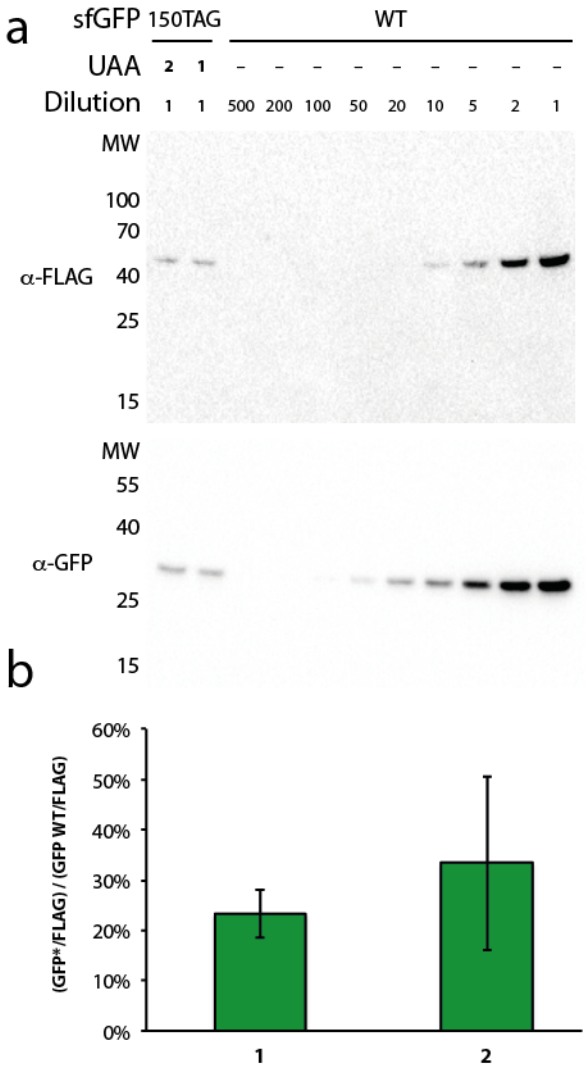
Supplementary Figure 1. AAV Vectors. Vectors for AAV mediated unnatural amino acid mutagenesis. ITR is Inverted Terminal Repeat, WPRE is Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element, PylT/PylRS are the genes encoding the pyrrolysyl tRNA/tRNA synthetase pair from *M. mazei*, P2A is the self-cleaving peptide sequence derived from porcine teschovirus-1, HGH pA is the Human Growth Hormone poly-adenylation signal, hU6 is the human U6 RNA Pol III promoter, and Synapsin I is the human Synapsin I promoter.



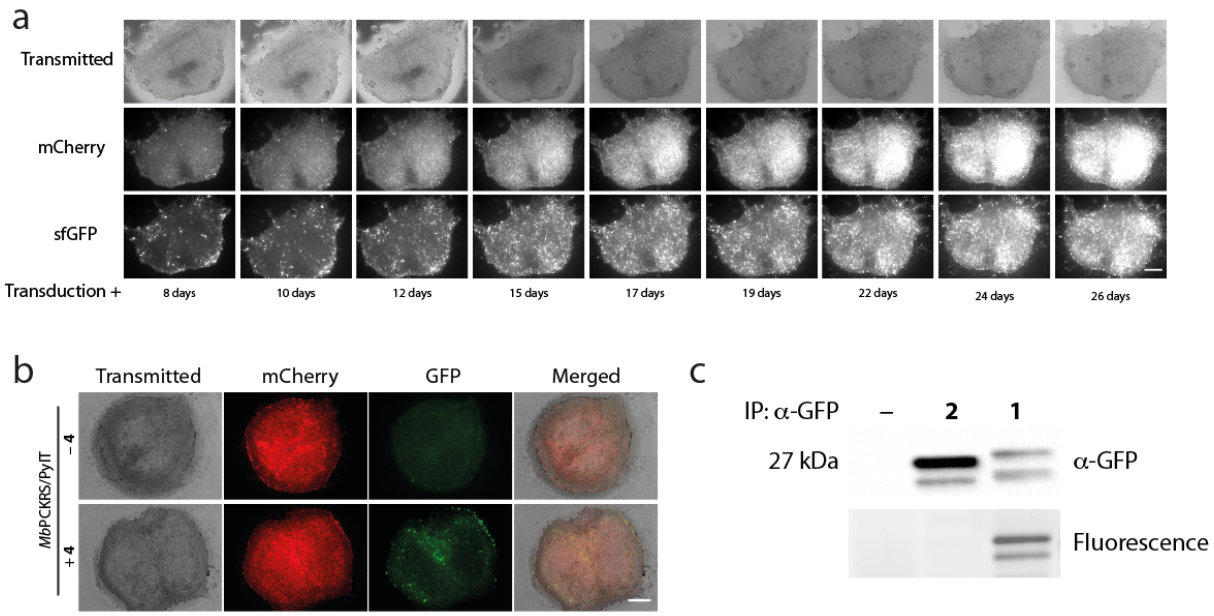
Supplementary Figure 2. AAV-mediated incorporation of unnatural amino acids into proteins. Unnatural amino acid incorporation in dissociated rat cortical neurons. Scale bar is 200 μm.



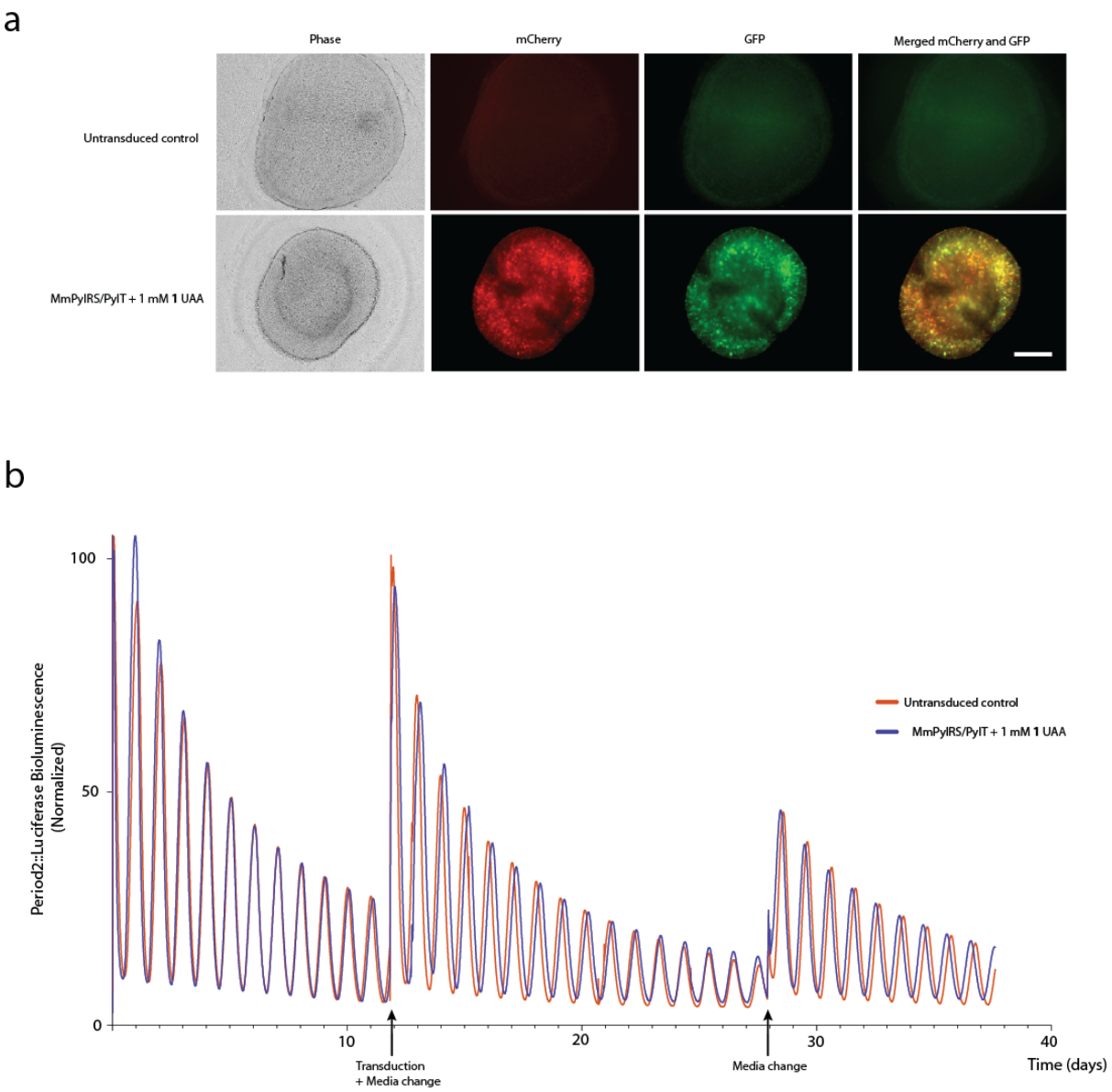
Supplementary Figure 3. Full gel and membrane images for all figures. A. Full membrane for anti-GFP WB from Figure 1C. **B.** Full fluorescence scan of gel from Figure 1C. **C.** Full membrane for anti-GFP WB from Figure 2B. **D.** Full membrane for anti-actin WB from Figure 2B. **E.** Full lanes for anti-GFP WB from Supplementary Figure 5C. **F.** Full lanes for fluorescence scan of gel from Supplementary Figure 5C.



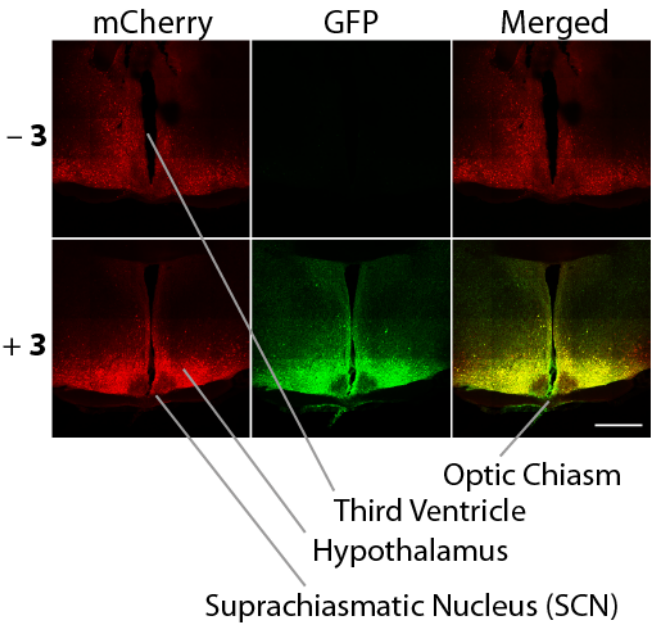
Supplementary Figure 4. Efficiency of unnatural amino acid incorporation in dissociated rat cortical neurons. **A.** Primary dissociated neurons from rat were transduced with a 1:1 mixture of the two AAV vectors encoding *Mm*PylRS/*PylT* and sfGFP(150TAG) in conditioned media. Protein extracts were prepared from the cultures 8 d after transduction and 6 d after addition of 1 mM amino acid **1** or **2**. The extracts were analyzed by SDS-PAGE and immunoblot against GFP and the N-terminal FLAG epitope tag of *Mm*PylRS. Expression in RCNs of sfGFP(150TAG) relative to the level of *Mm*PylRS, (GFP*/FLAG), was normalized to the expression in HEK293 of WT sfGFP relative to the level of *Mm*PylRS, (GFP WT/FLAG). The WT dilution series was prepared from an arbitrary concentration of protein extracts of HEK293 cells transiently transfected with a 1:1 mixture of plasmids encoding WT sfGFP and *Mm*PylRS under the AAV promoter, hSynI. **B.** The efficiencies of incorporation of amino acids **1** and **2** were determined to be 23.4% ± 4.8% and 33.3% ± 17.3%, respectively (n = 3, mean ± s.e.).



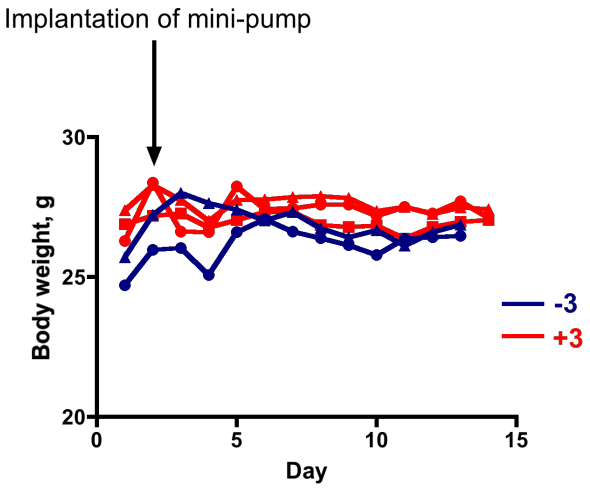
Supplementary Figure 5. AAV-mediated genetic code expansion in SCN slices. A. SCN slices were transduced with a 1:1 mixture of the two AAV vectors encoding *Mm*PylRS/*PylT* and sfGFP(150TAG). Times indicated are post transduction; at day 6, 1 mM amino acid **1** was added in fresh media, and the media containing **1** was replenished every seven days. Expression of mCherry and GFP continually increase over the duration of the experiment. Scale bar is 200 μm. **B.** Unnatural amino acid **4** dependent expression of GFP in SCN slices demonstrates genetic code expansion in brain tissue. Scale bar is 200 μm. **C.** Western blots confirm unnatural amino acid dependent production of sfGFP from sfGFP (150TAG). sfGFP incorporating **1**, but not **2**, is selectively labeled with **5** in an inverse electron demand Diels Alder reaction.



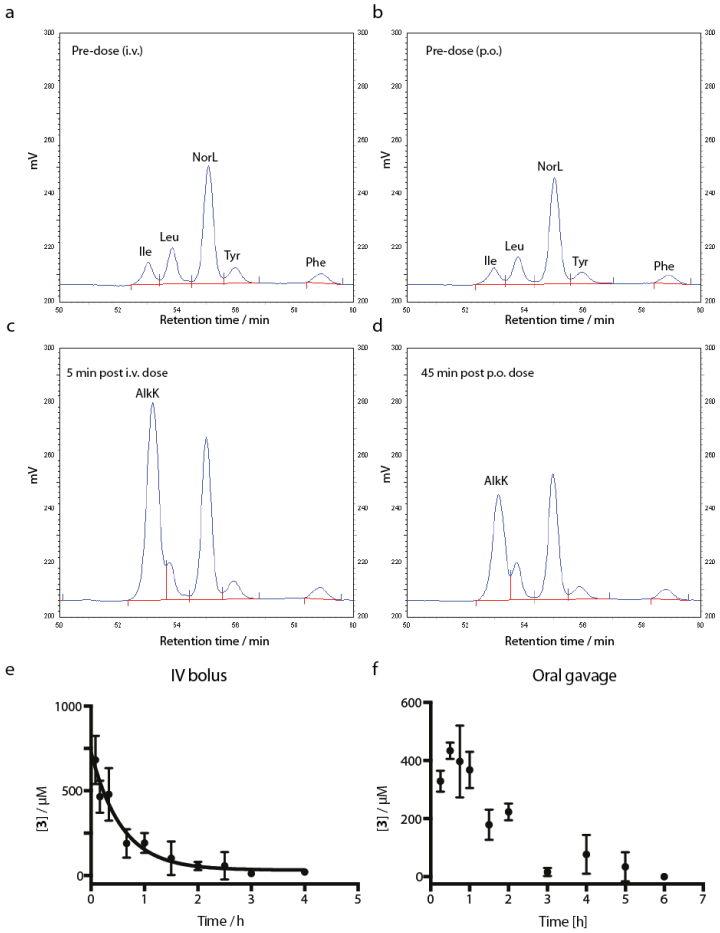
Supplementary Figure 6. AAV-mediated genetic code expansion does not disrupt function of the SCN. A. SCN slices were prepared from transgenic mice expressing a Period2:Luciferase fusion protein. AAV-mediated genetic code expansion results in sfGFP expression. Scale bar is 200 µm. **B.** AAV-mediated genetic code expansion does not disrupt the oscillations of the neural circuitry of the SCN, as seen by bioluminescence assay.



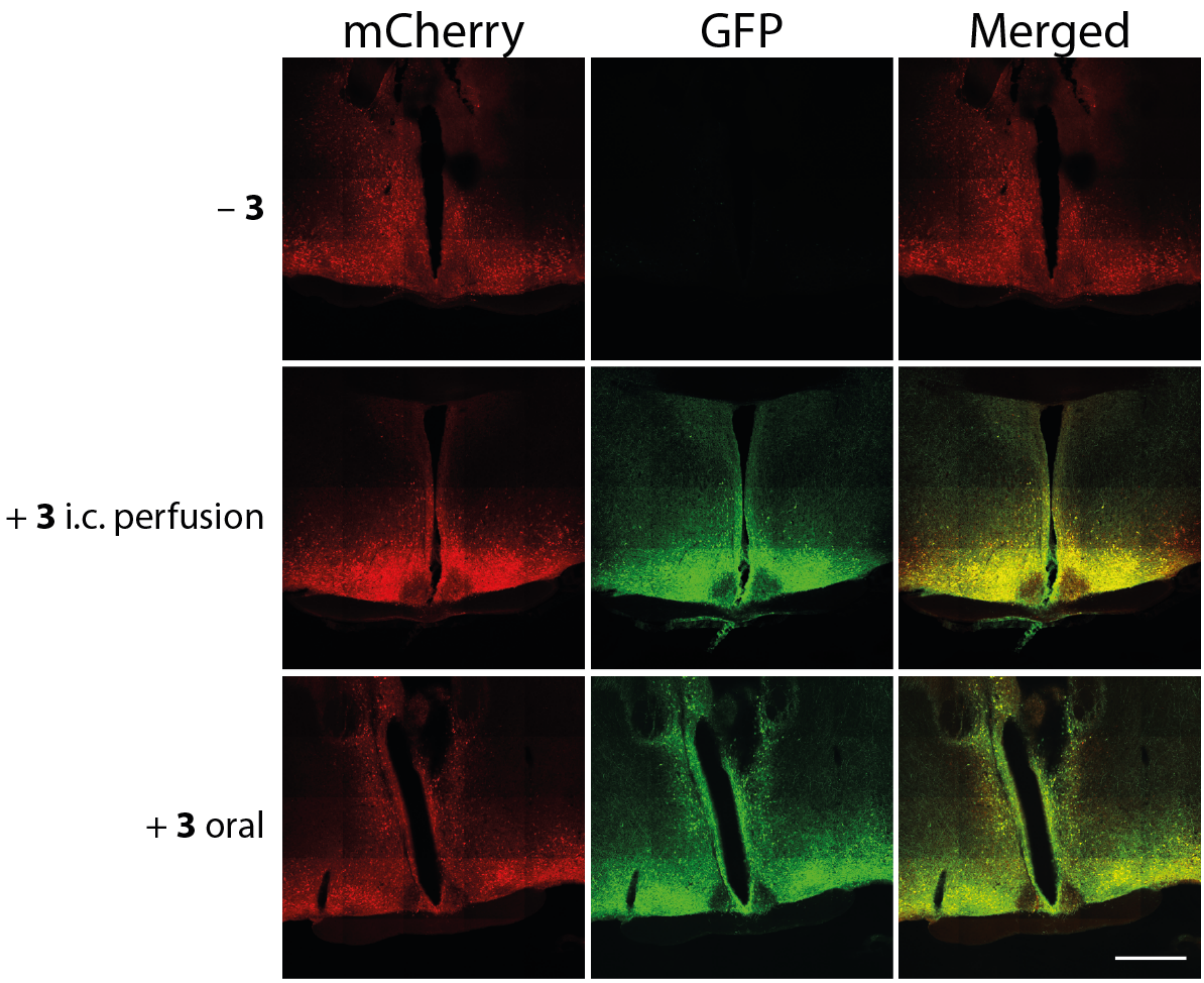
Supplementary Figure 7. Incorporation of unnatural amino acids into proteins in live mice with anatomical detail indicated. Unnatural amino acid dependent expression of GFP in coronal sections of the hypothalamus and SCN demonstrates genetic code expansion in the brains of live mice. Scale bar is 500 μm.



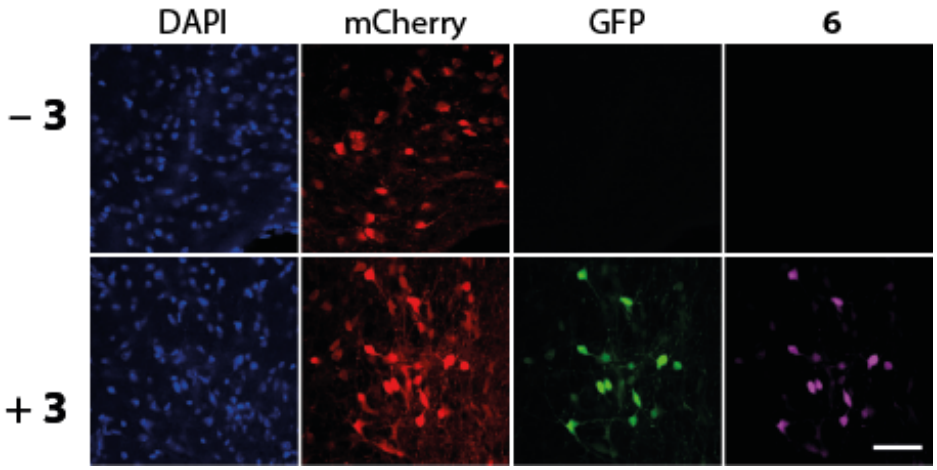
Supplementary Figure 8. Body weights of mice. Body weights of mice with or without continual perfusion of amino acid **3** into the region surrounding the hypothalamus and SCN were recorded daily throughout the two weeks perfusion period.



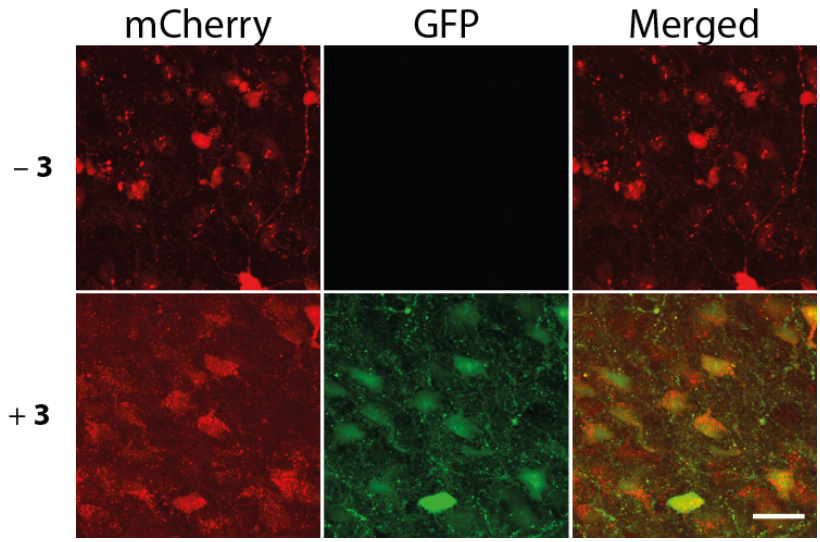
Supplementary Figure 9. Pharmacokinetic Analysis. Plasma amino acid analysis: in **A** and **B** the chromatograph of representative pre-dose plasma samples is shown. Following dosage with **3** additional area is observed (**C** and **D**). AlkK is amino acid **3**. Norleucine (NorL) was added as an internal standard to plasma samples. Estimation of pharmacokinetic parameters: time versus plasma concentration plots are shown for (**E**) intravenous (iv) bolus injection and (**F**) oral gavage. In **E** 100 mg/kg (380 µmol/kg) were administered by intra venous (iv) bolus injections in 3 cohorts of C57BL/6 mice. The observed $AUC_{0\text{-}4}$ was 503.9 ± 53.2 µM • h, from which we estimate iv clearance to be 0.755 L/h • kg^{-1}, the non-compartmental half-life to be 0.612 h, and the volume of distribution at steady state to be 0.667 L/kg. In **F** 250 mg/kg (950 µmol/kg) were administered by intra venous (iv) bolus injections in 3 cohorts of C57BL/6 mice. The observed $AUC_{0\text{-}6}$ was 811.8 ± 69.1 µM • h, from which we estimate po clearance to be 1.17 L/h • kg^{-1}, the non-compartmental half-life to be 1.09 h, and the oral bioavailability to be 64%. Error bars represent the standard deviation.



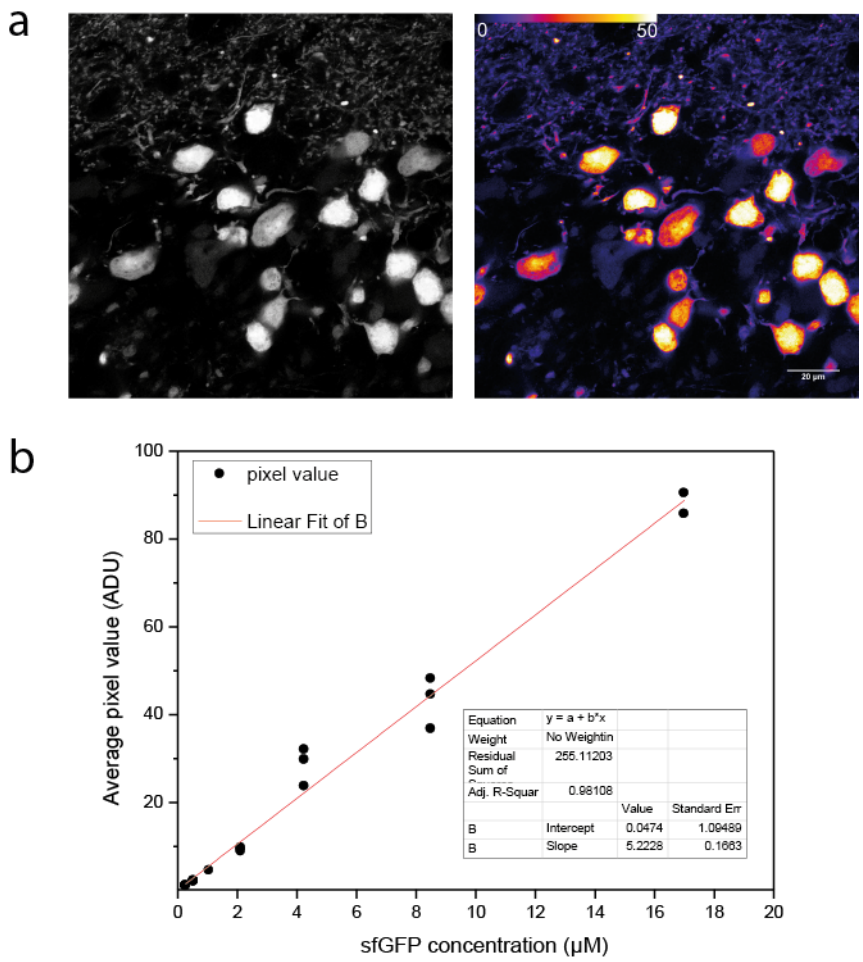
Supplementary Figure 10. Incorporation of unnatural amino acids into proteins in the brains of live mice. Unnatural amino acid dependent expression of GFP in coronal sections of the hypothalamus and SCN demonstrates genetic code expansion in the brains of live mice. Scale bar is 500 μm. Amino acid **3** was delivered either through continuous intra-cranial perfusion (0.25 μL/hr, 2 weeks) or through black currant-flavored water (30 mg/mL **3**).



Supplementary Figure 11. Incorporation of unnatural amino acids into live mice for bioorthogonal labeling. Labeling of cells within a coronal section from mice expressing sfGFP containing **3** with azide-sulfo-Cy5 fluorophore (**6**), via a Cu (I) catalyzed cycloaddition. Scale bar is 20 μm.



Supplementary Figure 12. Maximal intensity projections of GFP fluorescence in the hippocampus. Unnatural amino acid dependent expression of GFP in coronal sections of the hippocampus demonstrates genetic code expansion in the brains of live mice. Scale bar is 30 μm. AAV particles were injected into the lateral ventricle. Two weeks after transduction, amino acid **3** was delivered through continuous intra-cranial perfusion (0.25 μL/hr, 2 weeks). Images are maximal intensity projections of 30 optical sections taken at 1 μm intervals.



Supplementary Figure 13. Direct quantification of GFP in the hippocampus via confocal microscopy. A. Pixel intensity values of sfGFP expression in hippocampal sections map to GFP concentrations up to 50 µM. **B.** Regression fit of average pixel values to sfGFP standard calibration curve. The heat map goes from 0 to 50 µM and the scale bar is 20 µm.