nature structural & molecular biology

SERIES | REVIEW ARTICLE

https://doi.org/10.1038/s41594-019-0263-5

Local translation in neurons: visualization and function

Christine E. Holt[1]*, Kelsey C. Martin[2]* and Erin M. Schuman[3]*

Neurons are among the most compartmentalized and interactive of all cell types. Like all cells, neurons use proteins as the main sensors and effectors. The modification of the proteome in axons and dendrites is used to guide the formation of synaptic connections and to store information. In this Review, we discuss the data indicating that an important source of protein for dendrites, axons and their associated elements is provided by the local synthesis of proteins. We review the data indicating the presence of the machinery required for protein synthesis, the direct visualization and demonstration of protein synthesis, and the established functional roles for local translation for many different neuronal functions. Finally, we consider the open questions and future directions in this field.

The function of a neuron is to receive, process and potentially store information from other neurons, and then transmit an integrated signal to other cells in its network. A typical excitatory neuron receives information from 1–10,000 other neurons and transmits information to 50–100,000 neurons; information transfer occurs at specialized junctions called synapses. This complex morphology requires unique solutions to maintain and modify the proteins that are required for the nervous system's correct wiring during development and for its function and plasticity during adulthood.

Neurons and numbers

Compared with other cell types in the body, neurons have a unique morphology. Each neuron has a cell body from which a single axon and multiple dendrites emanate. Whereas the cell body contains the nucleus, the axons and dendrites of a neuron can contain as much as 99% of the cytoplasm. Axons can be extremely long, extending hundreds of centimeters in length in vertebrates. Dendrites are highly branched and also quite long; for example, the average dendritic length of hippocampal pyramidal neurons is 13.5 mm (ref. [1]) (Fig. 1).

The contacts between neurons occur at specialized junctions called synapses. This unique cellular junction usually forms between a specialized ending (axon bouton or axon terminal) of a neuron transmitting the information (the presynaptic neuron) and special compartments within dendrites (often protrusions called 'spines') of a receiving neuron (the postsynaptic neuron). During development, neurons send out long axons that navigate over hundreds or even thousands of cell-body lengths to reach their postsynaptic targets. This navigational feat is accomplished by 'growth cones', the specialized tips of growing axons. These growth cones detect and react rapidly to local guidance cues and consequently steer their movement along the route; when they reach their destination, axons branch and form synapses. A single axon with a length of ~200 cm forms approximately ~50,000 synapses (CA3 region of the hippocampus[2]). Axonal boutons contain synaptic vesicles filled with neurotransmitter as well as scaffolding and regulatory molecules and organelles that organize, promote and regulate the release of neurotransmitter. A typical pyramidal neuron hosts approximately 54,000 synapses in its dendritic tree (~4.0 synapses per μm^2 in the CA1 region of the hippocampus[3]). The synaptic compartments within dendrites, such as the dendritic spines that form excitatory synapses, contain a special complement of proteins that include neurotransmitter receptors as well as scaffolding, regulatory and specialized adhesion molecules. The union of proteomic studies of biochemical preparations that enrich for synapses (for example, 'synaptosomes') has identified more than 2,500 proteins present at synapses[4] (Fig. 1). This finding does not mean that all 2,500 proteins are present at a single synapse—the proteome of a single synapse has not been determined. Within an individual pre- or postsynaptic compartment, however, the copy numbers of some proteins have been determined. For example, most excitatory pre-synaptic proteins (for example, scaffolding molecules, synaptic-vesicle proteins and membrane-associated proteins) have been reported to exhibit between 100 and 10,000 copies per bouton[5,6]. Much less quantitative information is available regarding the postsynaptic compartment. Although the relative abundance of ~30 postsynaptic proteins has been determined[7], approximate copy-number estimates per synapse have been obtained for a relatively smaller number of proteins including CamKIIa (5,000 copies), the glutamate receptors (30–80 copies) and PSD-95 (300 copies); most other measured postsynaptic proteins exhibit copy numbers between 10 and 1,000 (ref. [8]) (Fig. 1). In addition, like all proteins, neuronal proteins have a limited half-life. Neuronal proteins measured in vitro exhibit half-lives of ~5.5 days (refs. [9,10]), and those measured in vivo exhibit half-lives of ~10 days (ref. [11]). Interestingly, as a group, proteins in synapses and axonal growth cones exhibit a half-life shorter than the average population of cellular proteins[9–12].

The number, size and strength of synapses in the brain change with experience through a process known as synaptic plasticity. Experience-dependent changes in connectivity provide a means of forming and storing memories. Long-lasting forms of synaptic plasticity, like long-term memory, require new RNA and protein synthesis[13,14]. Stimulation of individual synapses or synapse clusters has demonstrated that stimulated synapses can undergo long-lasting changes in synaptic strength, whereas neighboring unstimulated synapses remain unchanged[15,16]. Because long-term plasticity can occur at some but not all synapses made by a given neuron, mechanisms must also exist to compartmentalize the changes in gene expression to those synapses undergoing plasticity.

Together, the morphological complexity of neurons, the volumetric dominance of the neuronal processes and their capacity for plasticity indicate that there are special challenges in setting up, maintaining and modifying the proteome in axonal and dendritic

[1]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK. [2]Department of Biological Chemistry, and Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [3]Max Planck Institute for Brain Research, Frankfurt am Main, Germany. *e-mail: ceh33@cam.ac.uk; KCMartin@mednet.ucla.edu; erin.schuman@brain.mpg.de

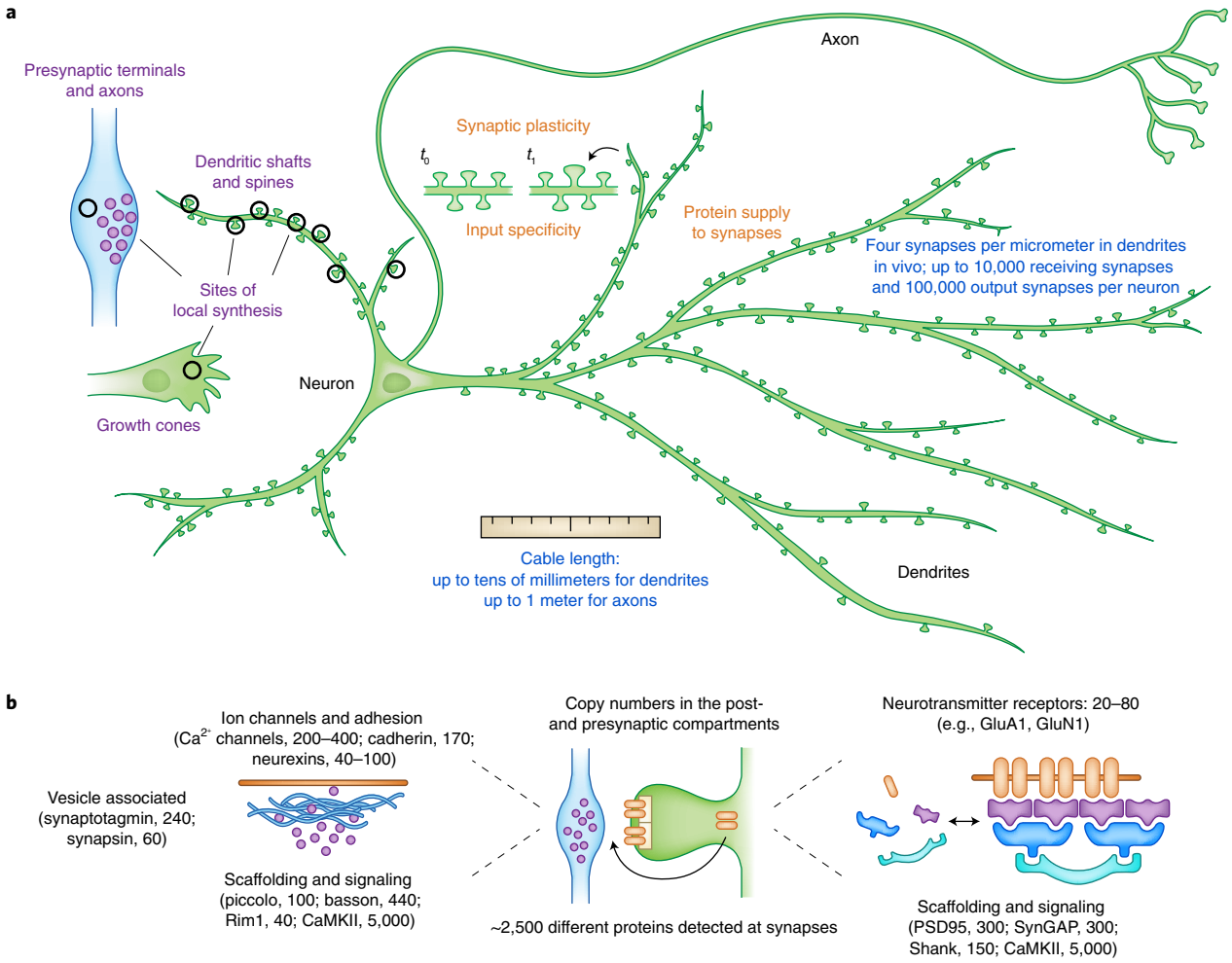

Fig. 1 | Neurons and numbers. a, Anatomy of a neuron. Neuron-specific cell parts; the sites of local protein translation and neuronal functions dependent on local translation are indicated. *t*, time. **b**, Localization, functions and per-synapse copy numbers of various synaptic proteins. Protein copy numbers are from Wilhelm et al.[6] and Sheng and Hoogenraad[8].

synaptic compartments. The fastest protein transport times measured in axons (1 µm per second) indicate that 11.6 days would be required to traffic a protein from the cell body to a distal axon terminal (1 m away)[17]. The processing of local information and the ensuing response (for example, turning in response to a navigational cue during development or synaptic plasticity in adults) usually requires responses on the time scale of minutes rather than hours. As discussed below, the localization of messenger RNA and the on-site synthesis of proteins is a conserved mechanism that appears to have evolved to fulfill local demand for new proteins at short time scales and to provide subcellular functions for development, synaptogenesis, experience-dependent plasticity and survival in dendrites and axons.

As a framework for this Review, we consider which of the following criteria must be met to establish that protein synthesis occurs in a particular subcellular compartment to supply proteins and to allow for proteome remodeling: (i) the localization of the protein template (mRNA), the machinery (ribosomes) and regulatory elements required for protein synthesis; (ii) the detection of newly synthesized proteins; and (iii) the observation that blocking local synthesis decreases protein levels or affects plasticity.

We conclude the Review with a discussion of some of the open questions and issues that are particularly interesting and timely.

The localization of protein-synthesis machinery

mRNAs. The initial identification of mRNAs in distal dendrites was a fortuitous finding from in situ hybridization studies of several transcripts, including *Map2* (ref. [18]) and *Camk2a*[19], and *Actb* in axons[20]. Although the limited number of mRNAs detected in this manner initially suggested that only a select number of transcripts localize to synapses, the development of more sensitive fluorescence in situ hybridization (FISH) approaches and modern genomic technologies subsequently allowed for more comprehensive and unbiased detection of dendritically, axonally and synaptically localized transcripts (Fig. 2). Microarray analysis and quantitative PCR of mRNAs present in isolated neuronal processes from *Aplysia* sensory neurons[21], rat DRG neurons[22], *Xenopus* or mouse retinal ganglion neurons[23,24], or hippocampal neurons[25–27] have revealed the presence of up to hundreds of distally localized transcripts. In-depth RNA sequencing combined with FISH and Nanostring analysis of the stratum radiatum in the rat hippocampus increased the resolution of detection and identified ~2,500 mRNAs that localize to the dendrites and axons of hippocampal pyramidal neurons[28] (Fig. 2a). More recently, purification of excitatory synaptosomes (from vGlut1-GFP mice) has identified more than 450 transcripts that are enriched in excitatory presynaptic nerve terminals, including many components of the active zone[29] (Fig. 2e).

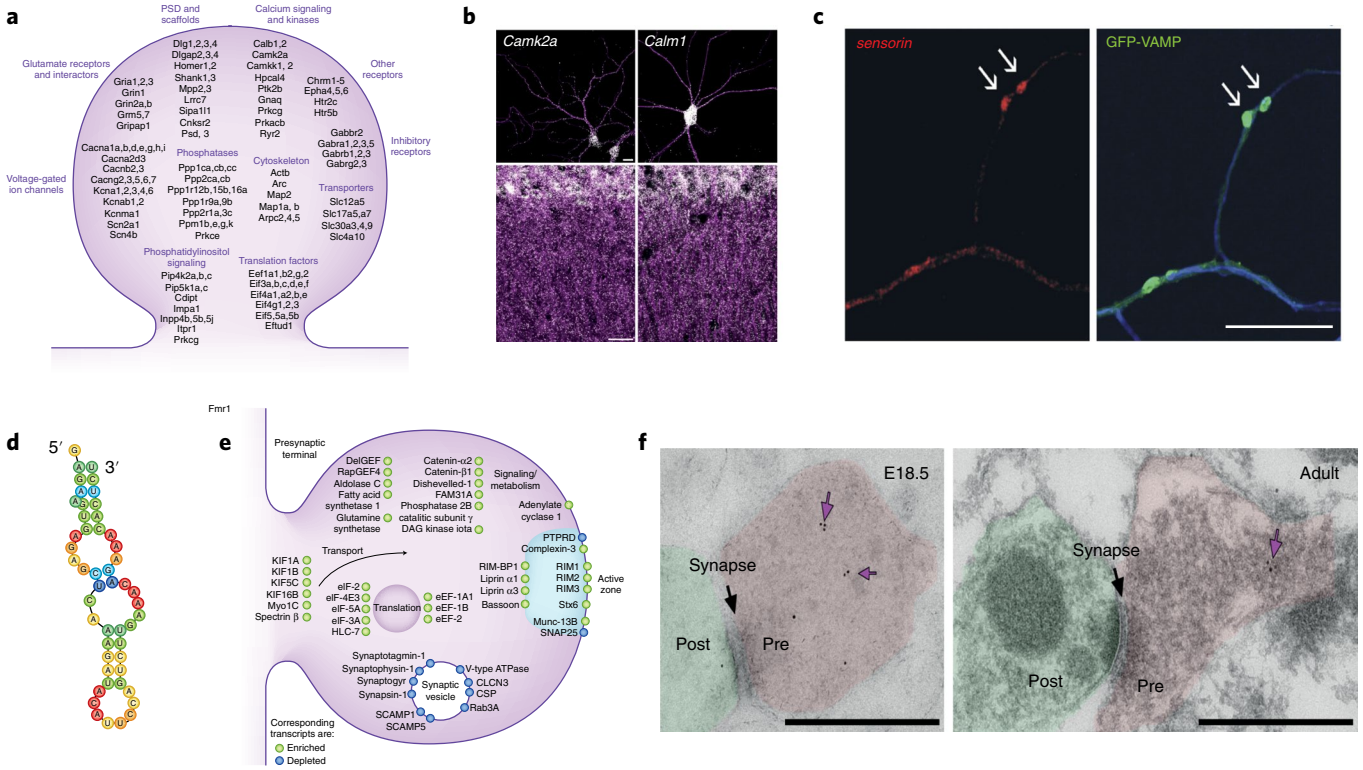

Fig. 2 | The localization of the protein template (mRNA), the machinery (ribosomes) and regulatory elements required for protein synthesis. a, Scheme of a postsynaptic compartment, highlighting some of the transcript families identified by RNA-seq and encoding synaptically relevant proteins that are present in the synaptic neuropil in area CA1 of the hippocampus. PSD, postsynaptic density. Image reproduced with permission from ref. [28], Elsevier. **b**, FISH signal in cultured rat hippocampal neurons (top) or adult rat hippocampal slices (bottom), on the basis of probes designed to detect the indicated mRNAs. Dendritic processes were immunostained with anti-MAP2 (purple). Scale bars, 15 and 25 μm for top and bottom panels, respectively. Image reproduced with permission from ref. [33], Elsevier. **c**, FISH for *sensorin* mRNA (red; left) in *Aplysia* sensory-motoneuron cultures, showing the localization of the mRNA at presynaptic sites, labeled by VAMP-GFP (green; right). An Alexa dye (blue) represents the membrane. Scale bar, 50 μm. Image reproduced with permission from ref. [110], Elsevier. **d**, The stem-loop structure present in the *sensorin* mRNA 5′ UTR that is responsible for localization. Image reproduced with permission from ref. [134], National Academy of Sciences. **e**, Schematic representation of a vGLUT1+ presynaptic terminal with the localization of a subset of proteins encoded by mRNA detected with next-generation RNA sequencing of fluorescently sorted synaptosomes. Many presynaptic active-zone-related mRNAs are enriched in vGluT1+ presynaptic terminals relative to unsorted (generic) synaptosomes, whereas synaptic-vesicle-related mRNAs are either significantly depleted (magenta) or not enriched by sorting. Image reproduced with permission from ref. [29], AAAS. f, Hemagglutinin (HA) immuno-gold electron microscopy to label ribosomes containing the Rpl22-HA tagged protein. HA-tagged ribosomes localize to retinal ganglion cell axon terminals in the superior colliculus in embryonic day (E) 18.5 (left) or adult (right) mice. Scale bars, 500 nm. Image reproduced with permission from ref. [42], Elsevier.

mRNAs are trafficked to distal compartments in a microtubule-dependent manner[20], and the 3′ and 5′ untranslated (UTR) regions of mRNAs play key roles in transport and localization selectivity[30–34]. However, despite the identification of multiple localization-required motifs[35], no single motif has emerged as a canonical sequence for targeting mRNAs specifically to axons, parts of axons or dendrites. Small changes in UTR sequences can change the secondary structure of mRNAs and consequently alter the binding of RNA-binding proteins[36]. Indeed, there is great diversity in the 3′ UTRs of neuronal mRNAs: more than half the mRNA population can use alternative 3′ UTRs that give rise to differences in localization, half-life and presumably translational regulation, owing to the presence or absence of microRNA (miRNA) seed sequences[33]. Interestingly, plasticity changes 3′-UTR choice[34], and 3′ UTRs of mRNAs have also been suggested to be locally remodeled after plasticity[33,37].

Ribosomes. Ribosomes were first identified in the dendrites of primate spinal cord motoneurons[38] and were then observed in the dendrites of hippocampal neurons[39]. Ribosomes were detected in axons as early as 1970 (ref. [40]) in embryonic rabbit spinal cord and in vitro[41], but have only recently been detected in adult CNS axons[29,42]. Polysomes (clusters of mRNA and two or more ribosomes) have been detected in dendritic spines and throughout the length of the dendrite[43]. Recently, ribosomes were observed in axon terminals through electron microscopy detection of a tagged knock-in ribosomal protein in adult mouse retinal ganglion cell axons[42] (Fig. 2f). One of the reasons why ribosomes eluded discovery in adult CNS axons for so long is that they are likely to be sparsely scattered and are only rarely seen grouped into polysomes, for example, in the nodes of Ranvier[44]. Even in growing axons and in growth cones, where their presence is less controversial, polysomes are not numerous; instead, ribosomes appear to be commonly scattered within the cytoplasm as monosomes (single ribosomes engaged with an mRNA), which are sometimes lined up along the plasma membrane[45]. In yeast, translation of short proteins or proteins subject to nonsense-mediated decay can occur on monosomes[46]. In small cytoplasmic compartments, such as dendritic spines, axonal growth cones or boutons, where the supply of only a few new molecules of a protein can make a large difference functionally (see protein copy numbers above), monosomal translation might possibly be sufficient. Indeed, translation on monosomes could allow for

synthesis of a greater number of different protein species by using relatively limited ribosomal resources.

Regulatory elements: co- and post-translational processing machinery. Ribosomes and co-translational chaperones are sufficient to synthesize, fold and traffic cytoplasmic proteins, but additional machinery is required for integral membrane proteins and secreted proteins. Membrane and secreted proteins are processed through multiple membrane-bound organelles (including the endoplasmic reticulum (ER), the ER–Golgi intermediate compartment (ERGIC), the Golgi apparatus and the *trans*-Golgi network, where they are folded, assembled and glycosylated before their delivery to the plasma membrane. Although the ER and several of its intrinsic proteins have been detected in dendrites and axons, for example[47–50], a conventional Golgi apparatus is largely lacking[51], and only sparse small Golgi outposts have been detected. Interestingly, many neuronal membrane proteins appear to exhibit glycosylation patterns consistent with Golgi bypass[52]. Given the abundance of membrane proteins in the dendritic and axonal transcriptomes, this finding suggests that membrane proteins could indeed be synthesized locally and might have a glycosylation signature that would indicate their site of synthesis. In line with this hypothesis, a dendritic ER–ERGIC–Golgi satellite–retromer microsecretory system has been described recently[53].

Detection of local protein synthesis

Evidence of local protein synthesis in distal neuronal processes began with the demonstration of the metabolic incorporation of amino acids in axons in the 1960s[54,55]. Similar observations have since been made in a variety of biochemical fractions enriched in synaptic components (that is, 'synaptosomes' or 'synaptoneurosomes')[56–58]. These early experiments were often met with skepticism that indicated a potential contribution of contaminating proteins synthesized in the cell body. Given these concerns, four basic approaches have been taken to establish a local source of protein synthesis: (i) physical isolation of the compartment of interest from the cell body, (ii) use of photoconvertible or bleachable fluorescent reporters, (iii) high-resolution purification of the fraction or subcellular compartment and (iv) metabolic labeling with very short labeling periods, to eliminate the contributions of proteins made in the soma.

The physical isolation of the compartment is perhaps the best evidence of local translation and is further supported by a demonstration that the observed protein-synthesis signal can be blocked by a protein-synthesis inhibitor. Local protein-synthesis-dependent plasticity was first observed in some brain slices (such as from the hippocampus), in which, for example, the synaptic neuropil could be physically isolated from the neuronal cell bodies[59,60]. In *Aplysia* neurons, physically isolated neurites have been treated with [^{35}S] methionine, and serotonin-induced protein synthesis has been detected[61]. Physical isolation is easily accomplished in axons that can be surgically separated from their somas in vitro or in vivo[62–64]. For example, in isolated retinal ganglion growth cones, [^{3}H]leucine incorporation has been observed to be stimulated by different guidance molecules[63]. More recently, 1,000–2,000 mRNAs undergoing active translation in isolated embryonic and adult axons in vivo have been identified through an axon-specific translating ribosome affinity purification (TRAP) approach[42,65]. In addition, a highly sensitive proteomic approach combining stable isotope labeling by amino acids in cell culture with single-pot solid phase-enhanced sample preparation (SP3) has revealed that hundreds of nascent proteins are rapidly synthesized in response to guidance molecules[66,67].

Fluorescent reporters that can be targeted to subcellular compartments and switched on or off or converted to different colors to define epochs of protein synthesis have been extremely useful. For example, expression of a green fluorescent protein (GFP) reporter in which the mRNA was targeted to the dendrites has been followed by the cutting of a dendrite and stimulation of protein synthesis by a neurotrophin[68] (Fig. 3a). Through use of translational reporters based on photoconvertible fluorophores, stimulus-induced protein synthesis has also been visualized in the growth cones of retinal axons and at synapses of *Aplysia* neurites isolated from their cell bodies[69,70]. At cultured *Aplysia* sensory-motoneuron synapses, the guidance cue netrin has been found to stimulate protein synthesis in sensory neuron neurites isolated from their cell bodies[71] (Fig. 3). In addition, fluorescence recovery after photobleaching (FRAP) experiments have demonstrated the synthesis of β-actin in branch tips in vivo[72] (Fig. 3b). Continuous FRAP/image capture with a fast-folding fluorescent reporter has enabled single-molecule imaging of newly synthesized proteins in dendrites of hippocampal neurons and retinal growth cones[73–75]; moreover, single-molecule imaging of nascent peptides performed in conjunction with single-molecule FISH to detect local mRNAs has revealed 'bursting' translation in distal dendrites of hippocampal neurons[76].

An alternative approach makes use of new metabolic labeling methods that enable brief labeling and/or the ability to mark or tag nascent proteins. For example, non-canonical amino acids (for example, methionine analogs such as azidohomoalanine) can be taken up by the cell, charged by the cell's own tRNA synthetase and incorporated into protein[77]. Through click-chemistry and fluorescent labeling, newly synthesized proteins have been detected in situ (fluorescence noncanonical amino acid tagging; FUNCAT)[78]. Many demonstrations of local translation have made use of the antibiotic puromycin, which covalently associates with the nascent chain during protein synthesis[79]. After a very brief (~5–10 min) treatment with low concentrations of puromycin, protein synthesis can be visualized in situ by recognition with antibodies[71,80,81]. In brain tissue subjected to expansion microscopy to increase the spatial resolution, a positive protein-synthesis signal is detectable in ~60% of dendritic spines and ~40% of excitatory and inhibitory nerve terminals after just 5 minutes of metabolic labeling with puromycin[29]. The combination of puromycin labeling with the proximity ligation assay (puro-PLA) has allowed the direct visualization of newly synthesized proteins of interest[82]. To date, several locally newly synthesized proteins have been detected at synapses and in growing axons through puro-PLA, such as CamKIIa[82,83], Bassoon[29,82], SNAP25 (ref. [84]), RapGEF[29], Tau protein[85], LB2 (ref. [50]), VDAC2 (ref. [50]), Limk1 (ref. [86]) and several others[66] (Table 1).

How specific is localized translation? There is evidence for tight control over mRNA translation specificity in neuronal compartments, especially in signal-regulated translation. In growing axons, there appears to be a high degree of specificity to translation, and specific extrinsic cues trigger the synthesis of distinct proteins[66]. Hints of this specificity were first observed with a candidate approach in which different guidance cues that exerted translation-dependent responses in turning growth cones were found to induce the synthesis of different proteins. Netrin-1, an attractant, triggers the local synthesis of proteins that build up the cytoskeleton (for example, β-actin;[70]), whereas the repellents Sema3A and Slit-2 induce synthesis of cytoskeletal-disassembly factors[87,88]. Unbiased proteomic analyses have extended this differential translation model and revealed that a single cue typically triggers the rapid and specific regulation of ~100 proteins, and different cues induce distinct proteomic signatures[66]. Another likely important factor in the specificity of cue-induced translation is receptor activation. Different guidance cues or different neurotransmitters bind and activate specific receptors, some of which are directly coupled to ribosomes[89]. In *Aplysia* neurons, local translation of the neuropeptide sensorin is induced by stimuli that produce translation-dependent long-term facilitation but not by stimuli that induce translation-dependent long-term depression of sensory-motor synapses[56]. In rodent neurons, a large-scale proteomic analysis of two opposite forms of homeostatic scaling

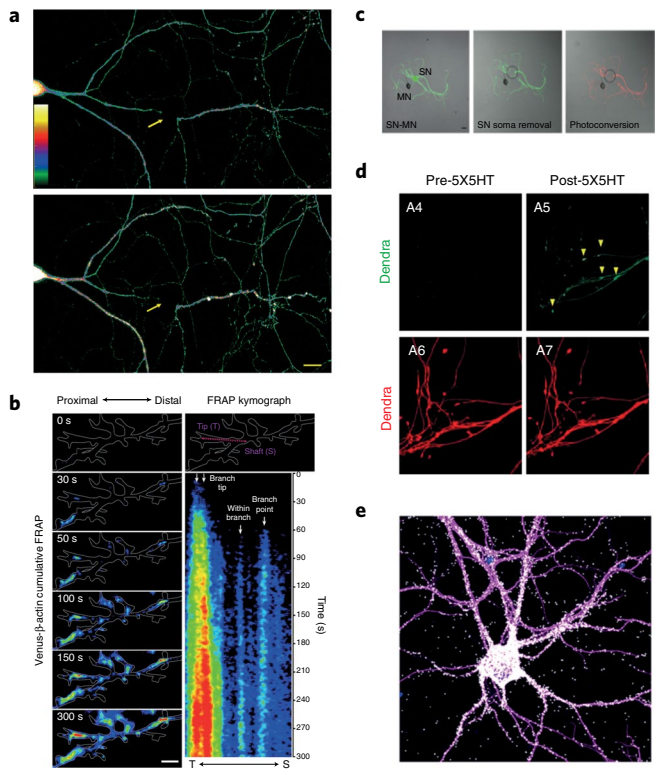

Fig. 3 | Visualization of local protein synthesis. a, Fluorescence reporter combined with physical isolation. A cultured hippocampal neuron was transfected with a GFP reporter containing the 3′ UTR from *Camk2a* for dendritic targeting of the mRNA. Images of a transfected neuron before (top) and 120 min after (bottom) BDNF treatment; arrow points to the region of transfection. The fluorescence signal in the transected dendrite increases after BDNF treatment, indicating a stimulation of local protein synthesis. Scale bar, 15 µm. Image reproduced with permission from ref. [135], Elsevier. **b**, Fluorescence reporter combined with photobleaching (FRAP). Venus-β-actin was electroporated into *Xenopus* retinal ganglion cells, and fluorescence was monitored in axonal branches. Shown are Venus-β-actin hotspots forming at different sub-compartments of an axonal branch. The kymograph displays the FRAP after 300 s, along the dotted magenta arrow. At least four distinct translational hotspots can be identified in this single branch. Image reproduced with permission from ref. [72], Elsevier. **c**,**d**, Fluorescence reporter combined with physical isolation. A reporter consisting of *sensorin* mRNA fused to the photoconvertible fluorescent protein dendra2 was expressed in cultured *Aplysia* sensory neurons (SN). Image reproduced with permission from ref. [69], AAAS. **c**, The SN soma was removed (dotted circle), and 12–18 h later the dendra2 signal was photoconverted from green to red (right). MN, motoneuron. **d**, Higher-magnification panels show the green (top) and the red channels (volume control, bottom). Increased green signal (yellow arrowheads) represents newly translated reporter after spaced application of 5-hydroxytryptamine. **e**, A newly synthesized protein of interest demonstrating the utility of Puro-PLA, a metabolic labeling strategy that couples puromycin as a metabolic tag (recognized by an anti-puromycin antibody) together with an antibody against the protein of interest, followed by the proximity-ligation assay[82]. Shown is a cultured hippocampal neuron labeled for 15 min with puromycin; newly synthesized *Camk2a* is detected. Scale bar, 25 µm.

has revealed both commonly and differentially regulated newly synthesized proteins[90,91]. Another level of specificity may be the individual synaptic compartment in which protein synthesis is regulated by plasticity. For example, a recent study has demonstrated that three different forms of plasticity (for example, induced by brain-derived neurotrophic factor (BDNF), metabotropic glutamate receptor (mGluR) activation or endocannabinoids) result in different signatures of protein synthesis in post-synaptic, excitatory or inhibitory presynaptic compartments[29]. Several mechanisms might underlie this specificity, such as miRNA regulation[92], mRNA modification[93], modulation of the phosphorylation of eukaryotic initiation factors[94] and/or RNA-binding protein phosphorylation[95,96]. This important topic was recently reviewed in more detail[97,98].

Established roles of local translation in protein supply and plasticity

The detection of mRNAs and the translational machinery in axonal, dendritic and synaptic compartments, and the visualization of local protein synthesis at synapses indicate that translation can occur at synapses, thereby prompting questions about the physiological function of local translation in neural-circuit development, survival and plasticity. An experimental challenge in demonstrating functions of local translation is the need to differentiate local translation from somatic translation with protein transport to synapses. For example, synaptic potentiation elicited by the neurotrophin BDNF requires local translation, as demonstrated by recordings from hippocampal slices in which the synaptic neuropil was physically isolated from the cell-body layer[59] (Fig. 3a and Table 2). A similar approach has demonstrated a requirement for local translation in mGluR-dependent long-term depression of synaptic transmission[60] and late-phase long-term potentiation (LTP)[99] (Table 2). In electron microscopy analyses, the induction of long-term potentiation is associated with an increased number of polyribosomes detected in dendritic spines[43]. Restricted extracellular application of protein-synthesis inhibitors to different synaptic layers has also been used to demonstrate that local protein synthesis is required for late-phase long-term potentiation[100] (Table 2). Another form of plasticity, local homeostatic scaling, is mediated via regulation of local protein synthesis. Acute blockade of synaptic activity at the synapse results in the stimulation of local translation[101,102]. At the calyx of Held synapse, located approximately 3 millimeters from the cell body in mice, a block of protein synthesis leads to an upregulation of spontaneous release events[103] (Table 2). In addition, long-term depression of inhibitory transmission induced by endocannabinoids requires protein synthesis in the inhibitory presynaptic nerve terminal[104] (Table 2). In the above experiments, protein-synthesis inhibitors were injected, bath applied or perfused in a restricted manner. Clearly, a new generation of protein-synthesis inhibitors that can be expressed in individual cells or enabled with light (for example, refs. [105,106]) would enable better spatial and temporal control of protein-synthesis inhibition and hence make roles for local translation easier to ascertain Table 2.

As noted above, *Camk2a* is one of the most prominent dendritically localized mRNAs. Within 5 minutes after LTP induction, newly synthesized *Camk2a* can be detected in the dendrites[107]. To probe the function of local translation of dendritically localized *Camk2a* mRNA in vivo, Miller et al.[108] have generated mice in which the *Camk2a* 3′ UTR that targets the mRNA to dendrites is deleted, such that the protein is expressed, but the mRNA is not dendritically localized. The phenotype of these mice includes impairments in hippocampal-dependent forms of memory, diminished late-phase LTP and a striking decrease in the amount of CamKIIa protein present in post-synaptic densities. Using a similar approach, Kuklin et al. have observed that loss of the *CaMKII* 3′ UTR in *Drosophila* results in diminished spontaneous neurotransmission and capacity for plasticity[109]. Together, these findings indicate that local translation of one dendritically localized mRNA is required for functional and structural synaptic plasticity in vivo.

Cultured *Aplysia* sensory-motor synapses provide a simple preparation for investigating the function of local translation in synapse formation and plasticity. Decreasing the levels of a localized

Table 1 | Newly synthesized proteins of interest detected with Puro-PLA

Protein detected	Species/model system	Compartment	Method	References
CamK2a	Rat hippocampal neuron culture	Dendrite	Endogenous	82,83
Bassoon	Rat hippocampal neuron culture	Along dendrites VGluT1+ presynaptic terminals	Endogenous	29,82
RapGEF	Rat hippocampal neuron culture	VGluT1+ presynaptic terminals	Endogenous	29
Tau	Mouse hippocampal neuron culture	Dendrite	Endogenous	85
LaminB2	*Xenopus* retinal ganglion cell culture	Axon	Endogenous	50
VDAC2	*Xenopus* retinal ganglion cell culture	Axon	Endogenous	50
LimK1	Rat cortical neuron culture	Dendrite	Endogenous	86
DSCR1.4	Mouse hippocampal neuron culture	Axon	5′- and 3′-UTR-containing expression reporter	136
DAP5	Mouse hippocampal neuron culture	Axon	Endogenous	136
CDC42 isoform	Mouse embryonic-stem-cell-derived neuron culture	neurites	CDS-E7-3′ UTR expression reporter	137
Neurogranin	Mouse cortical neuron culture	Dendrite	Endogenous	138
Calmodulin	Mouse cortical neuron culture	Dendrite	Endogenous	138
SNAP25	Rat hippocampal neuron culture	Axon nascent presynapses	Endogenous	84

mRNA—without alterations in protein levels—has been found to block synapse formation[110], thus indicating that synaptic translation of that single localized mRNA is required for synapse formation. Local perfusion of translational inhibitors to distal sensory neurites has been found to inhibit serotonin-induced plasticity at translationally inhibited synapses but not at other stimulated synapses formed by that neuron, and sensory neurons severed from their cell bodies are capable of undergoing serotonin-induced, translation-dependent long-term facilitation lasting 24 hours (ref. [61]). Together, these findings demonstrate a role of the translation of localized mRNAs in both synapse formation and plasticity in this simple culture preparation.

Local translation has an important role during axonal development. When retinal ganglion cell axons grow toward their targets, local translation is required for their appropriate response (for example, turning) to secreted guidance molecules[63]. Indeed, guidance-molecule-driven translation of β-actin is asymmetrically required on the near side of the growth cones (the side closest to the attractive guidance cue) to help growth cones turn in that direction[70], as is the interaction with a specific RNA-binding protein that interacts with elements in the 3′ UTR of the *Actb* mRNA in its transport to the near side[111]. In developing retinal axons in vivo, acute inhibition of protein synthesis or electroporation of morpholinos blocking β-actin translation in isolated axons (that is, with no somal contribution) impairs axonal branching in the optic tectum[72]. In adult sensory axons, Perry et al.[112] have taken advantage of the finding that the mRNA encoding the nuclear transport factor importin beta 1 has two distinct 3′ UTRs: a long 3′ UTR that localizes the mRNA to axons of sensory neurons and a short 3′ UTR that localizes the mRNA to cell bodies of sensory neurons. The authors targeted the long 3′ UTR to generate mice lacking importin beta 1 mRNA in axons, without altering the expression or function of importin beta 1 in the sensory cell body. The phenotype of these mice included delayed axonal loss in response to axonal injury, a result indicating a function of local, axonal translation of importin beta 1 in initiating the response to injury. Indeed, multiple examples exist in which local axonal translation is required for the axon to mount an injury response[113,114]. Another important role for local translation in axons is the promotion of axon maintenance and survival. The axonal translation of LaminB2 and Bclw, for example, has been shown to be required for axon viability[64,115,116].

Open questions and future directions

We highlight the following areas as particularly interesting, owing to recent progress, new ideas or persistent questions that remain unanswered.

Ribosome repair and remodeling. Little is known about the origin and life of ribosomes in distant neuronal compartments. Ribosomal proteins (RPs) synthesized in the cell body are presumed to be assembled into ribosomes in the nucleolus and then transported to the cytoplasm and ultimately to distal neuronal processes; however, recent findings indicate the possibility of on-site ribosome remodeling. Transcriptomic studies have repeatedly shown the abundant presence of RP-encoding mRNAs in axons and dendrites[21,23,25,28], thus raising the possibility that RP mRNAs might be locally translated. A translatomic analysis has confirmed this possibility by showing that RPs are the most enriched functional category of mRNAs translated in mouse retinal axons in vivo[42]. A proteomic study has further shown that RPs are the most enriched functional group of basally translated proteins in cultured retinal axons and that extrinsic cues, such as Netrin, trigger the rapid regulation of newly synthesized RPs[66]. In line with this finding, a recent subcellular transcriptomic/proteomic analysis of mouse callosal axons has identified the ribosome as one of the six most enriched categories[117]. Several RPs are known to have extra-ribosomal functions, but the sheer number of RP mRNAs detected (>50) in dendrites and axons suggests that at least some may have a ribosomal function. The gold-standard experimental approach to directly answering this question is proteomic analysis on biochemically isolated ribosomes. However, this approach presents a major challenge in neuronal compartments because only very small amounts of purified axonal and dendritic material from ribosomes can be isolated. Recent analysis of this type has been performed on isolated axons by growing hundreds of embryonic *Xenopus* eyes on filters in which the cell bodies reside on one side and the axons extend to the other side[65]. Stable isotope labeling by amino acids in cell culture labeling combined with axonal ribosome purification and mass spectrometry analysis has suggested that locally synthesized RPs may join pre-existing ribosomes in axons in response to cue (Netrin-1) stimulation[118].

If new RPs are added to ribosomes on site in dendrites and axons, what could their role be, and how would it fit with classical views on

Table 2 | Established roles for local translation in protein supply and plasticity

Function	Species/model system	Compartment	Method	References
BDNF-induced synaptic potentiation	Rat hippocampal slices	Synaptic neuropil of area CA1	Physical isolation	59
Serotonin-induced synaptic facilitation	Cultured *Aplysia* sensory and motoneurons	Sensory to motoneuron synapses	Physical isolation	61
mGluR1/5-induced long-term depression	Rat hippocampal slices	Synaptic neuropil of area CA1	Physical isolation	60
Guidance decisions	*Xenopus* retinal ganglion cells in culture	Axonal growth cones	Physical isolation and protein-synthesis inhibitors and translation inhibition of β-actin	63,70
Maintenance of synaptic CamKIIa	Mouse synapses	Postsynaptic density of forebrain synapses	Deletion of CamKIIa 3′ UTR	108
Late-phase long-term potentiation	Rat hippocampal slices	Synaptic neuropil of area CA1	Restricted perfusion of a protein-synthesis inhibitor	100
Late-phase long-term potentiation	Rat hippocampal slices	Synaptic neuropil of area CA1	Physical isolation	99
Local homeostatic scaling	Rat hippocampal neuron cultures	Dendrites	Local perfusion of a protein-synthesis inhibitor	101
Synapse formation	cultured *Aplysia* sensory and motoneurons	Sensory to motoneuron synapses	mRNA knockdown	110
Injury response	Mouse sensory neuron axons	Axons	Deletion of long 3′ UTR of importin beta 1	112
Maintenance of presynaptic β-catenin	Cultured hippocampal neurons in microfluidic chambers	Axon terminals	Fluidically isolated application of a protein-synthesis inhibitor	27
Endocannabinoid-induced plasticity of inhibitory transmission	Rat hippocampal slices	Inhibitory axon terminals	Injection of a protein-synthesis inhibitor	104
Presynaptic protein clustering and neurotransmistter release	Cultured hippocampal neurons in microfluidic chambers	Axon terminals	Fluidically isolated application of a protein-synthesis inhibitor	84
Spontaneous neurotrasmitter release	Mouse calyx of Held synapse	Axon terminals	Bath application of protein-synthesis inhibitor	103
Axon branching	*Xenopus* retinal neurons in vivo	Axon terminals	Translation inhibition of β-actin in somaless axons	72
Axon maintenance	*Xenopus* retinal neurons in vivo	Axons	Translation inhibition of LB2 in somaless axons	64
Axon maintenance	Rat dorsal root ganglion sensory neurons in vitro	Axons	Compartmentalized axon cultures and shRNA to Sfpq, LaminB2 or Bclw	115
Axon maintenance	Rat sympathetic superior cervical ganglion neurons in vitro	Axons	Compartmentalized axon cultures and protein-synthesis inhibitors	116

ribosome biogenesis and function? In cultured neurons, ribosomal proteins exhibit remarkably variable half-lives ranging from 3 to 9 days (ref. [10]), findings inconsistent with the entire ribosome being synthesized and degraded together. Little is known about neuronal ribosome biogenesis and the trafficking of ribosomes to dendrites and axons. A mechanism may conceivably exist for maintaining or prolonging ribosomal function in distant compartments. One possibility is that basally translated RPs may be needed for the constant on-site homeostasis of ribosomes. A second possibility—based on compelling evidence showing that mutations in individual RPs give rise to distinct, sometimes tissue-specific phenotypes rather than a general non-thriving phenotype expected from a global loss of protein synthesis—has given rise to the 'specialized ribosome' hypothesis[119]. In line with this idea is the observation that distinct guidance cues induce a specific signature of RP translational changes in axons[66], thus raising the possibility that localized RP synthesis might 'tune' ribosomes in a highly localized way in axons and dendrites, perhaps determining which mRNAs are translated.

RNA methylation and partitioning of transcripts at synapses. The discovery and development of tools to study reversible chemical modifications of RNAs[120,121] have raised interest in the possibility that epitranscriptomic modifications might have a distinct role in regulating local translation at synapses. The most abundant mRNA modification in mammalian cells involves the addition of a methyl group to the N^6 position of adenine to form N^6-methyladenine (m^6A). Methylation of RNA has been shown to affect many aspects of RNA metabolism, including stability, transport and translation[122], and m^6A expression is higher in the brain than in any other organ in the body[121]. Using m^6A immunoprecipitation to sequence the m^6A epitranscriptome from purified mouse forebrain synaptosomes, Merkurjev et al.[123] have identified 4,469 m^6A peaks in 2,921

genes that were selectively enriched in synaptosomes compared with whole forebrain extracts. More than 40% of these mRNAs were not themselves enriched at synapses, thus suggesting either that the methyl modification was deposited at synapses or that methylation directed the synaptic localization of the transcript. These authors have also shown that several of the enzymes involved in m^{6}A modification (m^{6}A 'writers', 'erasers' and 'readers') are present at synapses, and that short-hairpin-RNA-mediated decreases in m^{6}A readers result in immature spine morphology and in synaptic transmission deficits. Together, these findings indicate that the chemical modification of mRNAs partitions specific mRNAs at synapses in a manner that can alter multiple steps of mRNA metabolism (for example, stability and translation), thereby controlling the synaptic proteome.

Intercellular RNA transfer via extracellular vesicles. The capacity of synaptic compartments to autonomously regulate their proteome via local translation decentralizes the regulation of gene expression from the nucleus to the synapse[124]. A series of intriguing and compelling studies over the past decade have revealed a role of extracellular vesicles in transporting mRNAs and miRNAs from one cell to another. Studies conducted in *Drosophila melanogaster*[125] and in mice[126] have revealed that the neuronal Arc protein assembles into viral-like capsids that contain the *Arc* mRNA and are transferred from one neuron to a target muscle (at the *Drosophila* neuromuscular junction) or to a target neuron (in cultured mouse cortical and hippocampal neurons). Moreover, the transferred *Arc* mRNA has been shown to be translated in recipient cells. The remarkable implication of these findings is that one cell can directly regulate the proteome of a neighboring cell via the local delivery of RNA. In the context of local translation at synapses, this idea is particularly provocative because it suggests that the regulation of gene expression during the development and plasticity of neural circuits is regulated not only in a compartmentalized fashion within neurons but also at the level of local spatial neighborhoods comprising multiple neurons.

Liquid–liquid phase separation and RNA localization. The regulated assembly of proteins and RNAs into membraneless organelles or compartments via liquid–liquid phase separation (LLPS[127]) has emerged as an important mechanism underlying RNA trafficking and metabolism in neurons. Many RNA-binding proteins associated with neurodegenerative disease, including FUS, TDP-43, tau, TIA1 and hnRNPA1, have been found to undergo LLPS in a manner that contributes to pathology[128]. One consequence of increased gel formation in hyperphosporylated FUS mutants is the impairment of new protein synthesis in axon terminals[129], and decreasing gelation of mutant FUS through overexpression of the nuclear transport protein Transportin rescues protein synthesis in these axons[130]. Further elucidation of the biochemistry of this phase-separation process promises to provide therapeutic insights into these diseases (for example, ref. [131]). By dynamically compartmentalizing RNAs and RNA-binding proteins, LLPS is also likely to play regulatory roles in synaptic RNA localization and translation.

Concluding remarks

The unique anatomy of neurons enables the vast information processing that they conduct. However, it also poses major challenges in how to manage protein supply and proteome remodeling at the temporal and spatial scales needed for axon guidance, branching and viability[132], the basic supply of an integral synaptic protein[108], the ongoing maintenance of synaptic function and the modification of individual proteins or proteomes required for synaptic plasticity. In each of these cases, the supporting data arise from studies both in vitro and in vivo, although in vivo visualizations of protein synthesis are relatively rare, owing to the extreme difficulty of the experiments (for example, refs. [72,133]). Therefore, it is clear that the ability to further assess the necessity of local translation will require new tools with enhanced spatial and temporal resolution.

Received: 19 April 2019; Accepted: 30 May 2019;
Published online: 3 July 2019

References

1. Ishizuka, N., Cowan, W. M. & Amaral, D. G. A quantitative analysis of the dendritic organization of pyramidal cells in the rat hippocampus. *J. Comp. Neurol.* **362**, 17–45 (1995).
2. Ishizuka, N., Weber, J. & Amaral, D. G. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J. Comp. Neurol.* **295**, 580–623 (1990).
3. Bourne, J. N. & Harris, K. M. Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. *Hippocampus* **21**, 354–373 (2011).
4. Pielot, R. et al. SynProt: a database for proteins of detergent-resistant synaptic protein preparations. *Front. Synaptic Neurosci.* **4**, 1 (2012).
5. Richter, K. N. et al. Comparative synaptosome imaging: a semi-quantitative method to obtain copy numbers for synaptic and neuronal proteins. *Sci. Rep.* **8**, 14838 (2018).
6. Wilhelm, B. G. et al. Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins. *Science* **344**, 1023–1028 (2014).
7. Cheng, D. et al. Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. *Mol. Cell. Proteom.* **5**, 1158–1170 (2006).
8. Sheng, M. & Hoogenraad, C. C. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu. Rev. Biochem.* **76**, 823–847 (2007).
9. Cohen, L. D. et al. Metabolic turnover of synaptic proteins: kinetics, interdependencies and implications for synaptic maintenance. *PLoS One* **8**, e63191 (2013).
10. Dörrbaum, A. R., Kochen, L., Langer, J. D. & Schuman, E. M. Local and global influences on protein turnover in neurons and glia. *eLife* **7**, e34202 (2018).
11. Fornasiero, E. F. et al. Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. *Nat. Commun.* **9**, 4230 (2018).
12. Deglincerti, A. et al. Coupled local translation and degradation regulate growth cone collapse. *Nat. Commun.* **6**, 6888 (2015).
13. Davis, H. P. & Squire, L. R. Protein synthesis and memory: a review. *Psychol. Bull.* **96**, 518–559 (1984).
14. Sutton, M. A. & Schuman, E. M. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**, 49–58 (2006).
15. Andersen, P., Sundberg, S. H., Sveen, O. & Wigström, H. Specific long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature* **266**, 736–737 (1977).
16. Govindarajan, A., Israely, I., Huang, S. Y. & Tonegawa, S. The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. *Neuron* **69**, 132–146 (2011).
17. Maday, S., Twelvetrees, A. E., Moughamian, A. J. & Holzbaur, E. L. Axonal transport: cargo-specific mechanisms of motility and regulation. *Neuron* **84**, 292–309 (2014).
18. Garner, C. C., Tucker, R. P. & Matus, A. Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. *Nature* **336**, 674–677 (1988).
19. Burgin, K. E. et al. In situ hybridization histochemistry of Ca2$^+$/calmodulin-dependent protein kinase in developing rat brain. *J. Neurosci.* **10**, 1788–1798 (1990).
20. Bassell, G. J. et al. Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. *J. Neurosci.* **18**, 251–265 (1998).
21. Moccia, R. et al. An unbiased cDNA library prepared from isolated *Aplysia* sensory neuron processes is enriched for cytoskeletal and translational mRNAs. *J. Neurosci.* **23**, 9409–9417 (2003).
22. Zheng, J. Q. et al. A functional role for intra-axonal protein synthesis during axonal regeneration from adult sensory neurons. *J. Neurosci.* **21**, 9291–9303 (2001).
23. Zivraj, K. H. et al. Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. *J. Neurosci.* **30**, 15464–15478 (2010).
24. Gumy, L. F. et al. Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization. *RNA* **17**, 85–98 (2011).
25. Poon, M. M., Choi, S. H., Jamieson, C. A., Geschwind, D. H. & Martin, K. C. Identification of process-localized mRNAs from cultured rodent hippocampal neurons. *J. Neurosci.* **26**, 13390–13399 (2006).
26. Zhong, J., Zhang, T. & Bloch, L. M. Dendritic mRNAs encode diversified functionalities in hippocampal pyramidal neurons. *BMC Neurosci.* **7**, 17 (2006).

27. Taylor, A. M. et al. Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J. Neurosci.* **29**, 4697–4707 (2009).
28. Cajigas, I. J. et al. The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron* **74**, 453–466 (2012).
29. Hafner, A. S., Donlin-Asp, P. G., Leitch, B., Herzog, E. & Schuman, E. M. Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. *Science* **364**, eaau3644 (2019).
30. Eom, T., Antar, L. N., Singer, R. H. & Bassell, G. J. Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J. Neurosci.* **23**, 10433–10444 (2003).
31. Andreassi, C. et al. An NGF-responsive element targets myo-inositol monophosphatase-1 mRNA to sympathetic neuron axons. *Nat. Neurosci.* **13**, 291–301 (2010).
32. Taliaferro, J. M. et al. Distal alternative last exons localize mRNAs to neural projections. *Mol. Cell* **61**, 821–833 (2016).
33. Tushev, G. et al. Alternative 3′ UTRs modify the localization, regulatory potential, stability, and plasticity of mRNAs in neuronal compartments. *Neuron* **98**, 495–511.e6 (2018).
34. Fontes, M. M. et al. Activity-dependent regulation of alternative cleavage and polyadenylation during hippocampal long-term potentiation. *Sci. Rep.* **7**, 17377 (2017).
35. Kislauskis, E. H., Zhu, X. & Singer, R. H. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J. Cell Biol.* **127**, 441–451 (1994).
36. Turner-Bridger, B. et al. Single-molecule analysis of endogenous β-actin mRNA trafficking reveals a mechanism for compartmentalized mRNA localization in axons. *Proc. Natl Acad. Sci. USA* **115**, E9697–E9706 (2018).
37. Andreassi, C. et al. 3′UTR cleavage of transcripts localized in axons of sympathetic neurons. Preprint at *bioRxiv* https://doi.org/10.1101/170100 (2019).
38. Bodian, D. A Suggestive relationship of nerve cell RNA with specific synaptic sites. *Proc. Natl Acad. Sci. USA* **53**, 418–425 (1965).
39. Steward, O. & Levy, W. B. Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *J. Neurosci.* **2**, 284–291 (1982).
40. Tennyson, V. M. The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. *J. Cell Biol.* **44**, 62–79 (1970).
41. Bunge, M. B. Fine structure of nerve fibers and growth cones of isolated sympathetic neurons in culture. *J. Cell Biol.* **56**, 713–735 (1973).
42. Shigeoka, T. et al. Dynamic axonal translation in developing and mature visual circuits. *Cell* **166**, 181–192 (2016).
43. Ostroff, L. E., Fiala, J. C., Allwardt, B. & Harris, K. M. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535–545 (2002).
44. Walker, B. A. et al. Reprogramming axonal behavior by axon-specific viral transduction. *Gene Ther.* **19**, 947–955 (2012).
45. Koppers, M. et al. Receptor-specific interactome as a hub for rapid cue-induced selective translation in axons. Preprint at *bioRxiv* https://doi.org/10.1101/673798 (2019).
46. Heyer, E. E. & Moore, M. J. Redefining the translational status of 80S monosomes. *Cell* **164**, 757–769 (2016).
47. Gardiol, A., Racca, C. & Triller, A. Dendritic and postsynaptic protein synthetic machinery. *J. Neurosci.* **19**, 168–179 (1999).
48. Horton, A. C. & Ehlers, M. D. Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. *J. Neurosci.* **23**, 6188–6199 (2003).
49. Merianda, T. T. et al. A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins. *Mol. Cell. Neurosci.* **40**, 128–142 (2009).
50. Cioni, J. M. et al. Late endosomes act as mRNA translation platforms and sustain mitochondria in axons. *Cell* **176**, 56–72.e15 (2019).
51. Hanus, C. & Ehlers, M. D. Secretory outposts for the local processing of membrane cargo in neuronal dendrites. *Traffic* **9**, 1437–1445 (2008).
52. Hanus, C. et al. Unconventional secretory processing diversifies neuronal ion channel properties. *eLife* **5**, e20609 (2016).
53. Mikhaylova, M., Bera, S., Kobler, O., Frischknecht, R. & Kreutz, M. R. A dendritic Golgi satellite between ERGIC and retromer. *Cell Rep.* **14**, 189–199 (2016).
54. Koenig, E. Synthetic mechanisms in the axon. I. Local axonal synthesis of acetylcholinesterase. *J. Neurochem.* **12**, 343–355 (1965).
55. Giuditta, A., Dettbarn, W. D. & Brzin, M. Protein synthesis in the isolated giant axon of the squid. *Proc. Natl Acad. Sci. USA* **59**, 1284–1287 (1968).
56. Rao, A. & Steward, O. Evidence that protein constituents of postsynaptic membrane specializations are locally synthesized: analysis of proteins synthesized within synaptosomes. *J. Neurosci.* **11**, 2881–2895 (1991).
57. Weiler, I. J. & Greenough, W. T. Potassium ion stimulation triggers protein translation in synaptoneurosomal polyribosomes. *Mol. Cell. Neurosci.* **2**, 305–314 (1991).
58. Torre, E. R. & Steward, O. Demonstration of local protein synthesis within dendrites using a new cell culture system that permits the isolation of living axons and dendrites from their cell bodies. *J. Neurosci.* **12**, 762–772 (1992).
59. Kang, H. & Schuman, E. M. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science* **273**, 1402–1406 (1996).
60. Huber, K. M., Kayser, M. S. & Bear, M. F. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* **288**, 1254–1257 (2000).
61. Martin, K. C. et al. Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell* **91**, 927–938 (1997).
62. Eng, H., Lund, K. & Campenot, R. B. Synthesis of beta-tubulin, actin, and other proteins in axons of sympathetic neurons in compartmented cultures. *J. Neurosci.* **19**, 1–9 (1999).
63. Campbell, D. S. & Holt, C. E. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron* **32**, 1013–1026 (2001).
64. Yoon, B. C. et al. Local translation of extranuclear lamin B promotes axon maintenance. *Cell* **148**, 752–764 (2012).
65. Shigeoka, T., Jung, J., Holt, C. E. & Jung, H. Axon-TRAP-RiboTag: affinity purification of translated mRNAs from neuronal axons in mouse in vivo. *Methods Mol. Biol.* **1649**, 85–94 (2018).
66. Cagnetta, R., Frese, C. K., Shigeoka, T., Krijgsveld, J. & Holt, C. E. Rapid cue-specific remodeling of the nascent axonal proteome. *Neuron* **99**, 29–46.e4 (2018).
67. Hughes, C. S., Sorensen, P. H. & Morin, G. B. A standardized and reproducible proteomics protocol for bottom-up quantitative analysis of protein samples using SP3 and mass spectrometry. *Methods Mol. Biol.* **1959**, 65–87 (2019).
68. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C. & Schuman, E. M. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489–502 (2001).
69. Wang, D. O. et al. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science* **324**, 1536–1540 (2009).
70. Leung, K. M. et al. Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat. Neurosci.* **9**, 1247–1256 (2006).
71. Kim, S. & Martin, K. C. Neuron-wide RNA transport combines with netrin-mediated local translation to spatially regulate the synaptic proteome. *eLife* **4**, e04158 (2015).
72. Wong, H. H. et al. RNA docking and local translation regulate site-specific axon remodeling in vivo. *Neuron* **95**, 852–868.e8 (2017).
73. Tatavarty, V. et al. Single-molecule imaging of translational output from individual RNA granules in neurons. *Mol. Biol. Cell* **23**, 918–929 (2012).
74. Ifrim, M. F., Williams, K. R. & Bassell, G. J. Single-molecule imaging of PSD-95 mRNA translation in dendrites and its dysregulation in a mouse model of fragile X syndrome. *J. Neurosci.* **35**, 7116–7130 (2015).
75. Ströhl, F. et al. Single molecule translation imaging visualizes the dynamics of local β-actin synthesis in retinal axons. *Sci. Rep.* **7**, 709 (2017).
76. Wu, B., Eliscovich, C., Yoon, Y. J. & Singer, R. H. Translation dynamics of single mRNAs in live cells and neurons. *Science* **352**, 1430–1435 (2016).
77. Dieterich, D. C., Link, A. J., Graumann, J., Tirrell, D. A. & Schuman, E. M. Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proc. Natl Acad. Sci. USA* **103**, 9482–9487 (2006).
78. Dieterich, D. C. et al. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nat. Neurosci.* **13**, 897–905 (2010).
79. Nathans, D. Puromycin inhibition of protein synthesis: incorporation of puromycin into peptide chains. *Proc. Natl Acad. Sci. USA* **51**, 585–592 (1964).
80. Smith, W. B., Starck, S. R., Roberts, R. W. & Schuman, E. M. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron* **45**, 765–779 (2005).
81. David, A. et al. Nuclear translation visualized by ribosome-bound nascent chain puromycylation. *J. Cell Biol.* **197**, 45–57 (2012).
82. tom Dieck, S. et al. Direct visualization of newly synthesized target proteins in situ. *Nat. Methods* **12**, 411–414 (2015).
83. Sambandan, S. et al. Activity-dependent spatially localized miRNA maturation in neuronal dendrites. *Science* **355**, 634–637 (2017).
84. Batista, A. F. R., Martínez, J. C. & Hengst, U. Intra-axonal SYnthesis of SNAP25 is required for the formation of presynaptic terminals. *Cell Rep.* **20**, 3085–3098 (2017).
85. Li, C. & Götz, J. Somatodendritic accumulation of Tau in Alzheimer's disease is promoted by Fyn-mediated local protein translation. *EMBO J.* **36**, 3120–3138 (2017).
86. Rajgor, D., Sanderson, T. M., Amici, M., Collingridge, G. L. & Hanley, J. G. NMDAR-dependent Argonaute 2 phosphorylation regulates miRNA activity and dendritic spine plasticity. *EMBO J.* **37**, e97943 (2018).

87. Wu, K. Y. et al. Local translation of RhoA regulates growth cone collapse. *Nature* **436**, 1020–1024 (2005).
88. Piper, M. et al. Signaling mechanisms underlying Slit2-induced collapse of *Xenopus* retinal growth cones. *Neuron* **49**, 215–228 (2006).
89. Tcherkezian, J., Brittis, P. A., Thomas, F., Roux, P. P. & Flanagan, J. G. Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell* **141**, 632–644 (2010).
90. Schanzenbächer, C. T., Langer, J. D. & Schuman, E. M. Time- and polarity-dependent proteomic changes associated with homeostatic scaling at central synapses. *eLife* **7**, e33322 (2018).
91. Schanzenbächer, C. T., Sambandan, S., Langer, J. D. & Schuman, E. M. Nascent proteome remodeling following homeostatic scaling at hippocampal synapses. *Neuron* **92**, 358–371 (2016).
92. Bellon, A. et al. miR-182 regulates Slit2-mediated axon guidance by modulating the local translation of a specific mRNA. *Cell Rep.* **18**, 1171–1186 (2017).
93. Yu, C. C. et al. Epigenetic modulation on tau phosphorylation in Alzheimer's disease. *Neural Plast.* **2019**, 6856327 (2019).
94. Cagnetta, R. et al. Noncanonical modulation of the eIF2 pathway controls an increase in local translation during neural wiring. *Mol. Cell* **73**, 474–489. e5 (2019).
95. Lepelletier, L. et al. Sonic hedgehog guides axons via zipcode binding protein 1-mediated local translation. *J. Neurosci.* **37**, 1685–1695 (2017).
96. Sasaki, Y. et al. Phosphorylation of zipcode binding protein 1 is required for brain-derived neurotrophic factor signaling of local beta-actin synthesis and growth cone turning. *J. Neurosci.* **30**, 9349–9358 (2010).
97. Richter, J. D., Bassell, G. J. & Klann, E. Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nat. Rev. Neurosci.* **16**, 595–605 (2015).
98. Gkogkas, C., Sonenberg, N. & Costa-Mattioli, M. Translational control mechanisms in long-lasting synaptic plasticity and memory. *J. Biol. Chem.* **285**, 31913–31917 (2010).
99. Vickers, C. A., Dickson, K. S. & Wyllie, D. J. Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *J. Physiol. (Lond.)* **568**, 803–813 (2005).
100. Bradshaw, K. D., Emptage, N. J. & Bliss, T. V. A role for dendritic protein synthesis in hippocampal late LTP. *Eur. J. Neurosci.* **18**, 3150–3152 (2003).
101. Sutton, M. A. et al. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**, 785–799 (2006).
102. Sutton, M. A., Wall, N. R., Aakalu, G. N. & Schuman, E. M. Regulation of dendritic protein synthesis by miniature synaptic events. *Science* **304**, 1979–1983 (2004).
103. Scarnati, M. S., Kataria, R., Biswas, M. & Paradiso, K. G. Active presynaptic ribosomes in the mammalian brain, and altered transmitter release after protein synthesis inhibition. *eLife* **7**, e36697 (2018).
104. Younts, T. J. et al. Presynaptic protein synthesis is required for long-term plasticity of GABA release. *Neuron* **92**, 479–492 (2016).
105. Goard, M. et al. Light-mediated inhibition of protein synthesis. *Chem. Biol.* **12**, 685–693 (2005).
106. Elamri, I. et al. A new photocaged puromycin for an efficient labeling of newly translated proteins in living neurons. *Chembiochem* **19**, 2458–2464 (2018).
107. Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M. & Kennedy, M. B. Tetanic stimulation leads to increased accumulation of Ca^{2+}/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J. Neurosci.* **19**, 7823–7833 (1999).
108. Miller, S. et al. Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36**, 507–519 (2002).
109. Kuklin, E. A. et al. The long 3'UTR mRNA of *CaMKII* is essential for translation-dependent plasticity of spontaneous release in *Drosophila melanogaster*. *J. Neurosci.* **37**, 10554–10566 (2017).
110. Lyles, V., Zhao, Y. & Martin, K. C. Synapse formation and mRNA localization in cultured *Aplysia* neurons. *Neuron* **49**, 349–356 (2006).
111. Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J. & Zheng, J. Q. An essential role for beta-actin mRNA localization and translation in Ca^{2+}-dependent growth cone guidance. *Nat. Neurosci.* **9**, 1265–1273 (2006).
112. Perry, R. B. et al. Subcellular knockout of importin β1 perturbs axonal retrograde signaling. *Neuron* **75**, 294–305 (2012).
113. Koley, S., Rozenbaum, M., Fainzilber, M. & Terenzio, M. Translating regeneration: local protein synthesis in the neuronal injury response. *Neurosci. Res.* **139**, 26–36 (2019).
114. Willis, D. E. & Twiss, J. L. The evolving roles of axonally synthesized proteins in regeneration. *Curr. Opin. Neurobiol.* **16**, 111–118 (2006).
115. Cosker, K. E., Fenstermacher, S. J., Pazyra-Murphy, M. F., Elliott, H. L. & Segal, R. A. The RNA-binding protein SFPQ orchestrates an RNA regulon to promote axon viability. *Nat. Neurosci.* **19**, 690–696 (2016).
116. Hillefors, M., Gioio, A. E., Mameza, M. G. & Kaplan, B. B. Axon viability and mitochondrial function are dependent on local protein synthesis in sympathetic neurons. *Cell. Mol. Neurobiol.* **27**, 701–716 (2007).
117. Poulopoulos, A. et al. Subcellular transcriptomes and proteomes of developing axon projections in the cerebral cortex. *Nature* **565**, 356–360 (2019).
118. Shigeoka, T. et al. On-site ribosome remodeling by locally synthesized ribosomal proteins in axons. Preprint at *bioRxiv* https://doi.org/10.1101/500033 (2018).
119. Shi, Z. & Barna, M. Translating the genome in time and space: specialized ribosomes, RNA regulons, and RNA-binding proteins. *Annu. Rev. Cell Dev. Biol.* **31**, 31–54 (2015).
120. Dominissini, D. et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* **485**, 201–206 (2012).
121. Meyer, K. D. et al. Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. *Cell* **149**, 1635–1646 (2012).
122. Wang, Y. & Zhao, J. C. Update: mechanisms underlying N^6-methyladenosine modification of eukaryotic mRNA. *Trends Genet.* **32**, 763–773 (2016).
123. Merkurjev, D. et al. Synaptic N^6-methyladenosine (m^6A) epitranscriptome reveals functional partitioning of localized transcripts. *Nat. Neurosci.* **21**, 1004–1014 (2018).
124. Holt, C. E. & Schuman, E. M. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* **80**, 648–657 (2013).
125. Ashley, J. et al. Retrovirus-like Gag Protein Arc1 binds RNA and traffics across synaptic boutons. *Cell* **172**, 262–274.e11 (2018).
126. Pastuzyn, E. D. et al. The neuronal gene Arc encodes a repurposed retrotransposon gag protein that mediates intercellular RNA transfer. *Cell* **172**, 275–288.e18 (2018).
127. Hyman, A. A., Weber, C. A. & Jülicher, F. Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* **30**, 39–58 (2014).
128. Alberti, S. & Hyman, A. A. Are aberrant phase transitions a driver of cellular aging? *BioEssays* **38**, 959–968 (2016).
129. Murakami, T. et al. ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* **88**, 678–690 (2015).
130. Qamar, S. et al. FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation-pi Interactions. *Cell* **173**, 720–734.e15 (2018).
131. Seidler, P. M. et al. Structure-based inhibitors of tau aggregation. *Nat. Chem.* **10**, 170–176 (2018).
132. Cioni, J. M., Koppers, M. & Holt, C. E. Molecular control of local translation in axon development and maintenance. *Curr. Opin. Neurobiol.* **51**, 86–94 (2018).
133. Leung, L. C. et al. Coupling of NF-protocadherin signaling to axon guidance by cue-induced translation. *Nat. Neurosci.* **16**, 166–173 (2013).
134. Meer, E. J. et al. Identification of a cis-acting element that localizes mRNA to synapses. *Proc. Natl Acad. Sci. USA* **109**, 4639–4644 (2012).
135. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C. & Schuman, E. M. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489–502 (2001).
136. Seo, J. Y. et al. DAP5 increases axonal outgrowth of hippocampal neurons by enhancing the cap-independent translation of DSCR1.4 mRNA. *Cell Death Dis.* **10**, 49 (2019).
137. Ciolli Mattioli, C. et al. Alternative 3′ UTRs direct localization of functionally diverse protein isoforms in neuronal compartments. *Nucleic Acids Res.* **47**, 2560–2573 (2019).
138. Jones, K. J. et al. Rapid, experience-dependent translation of neurogranin enables memory encoding. *Proc. Natl Acad. Sci. USA* **115**, E5805–E5814 (2018).

Acknowledgements

We thank members of our laboratories, particularly S. tom Dieck for help with Fig. 1 and Table 1. C.E.H. is funded by the Wellcome Trust (203249/Z/16/Z) and an Advanced Investigator Award from the European Research council (322817). K.C.M. is funded by NIH R01MH077022 and NIH R21MH113102. E.M.S. is funded by the Max Planck Society, an Advanced Investigator Award from the European Research Council (743216), and DFG CRC 1080 and CRC 902.

Competing interests

The authors declare no competing interests.

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Correspondence should be addressed to C.E.H., K.C.M. or E.M.S.

Peer review information: Katarzyna Marcinkiewicz was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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