

Mitochondrial plasticity: An emergent concept in neuronal plasticity and memory

Typhaine Comyn, Thomas Preat^{*}, Alice Pavlowsky^{*}, Pierre-Yves Plaçais^{*}

Energy & Memory, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, 10 rue Vauquelin, 75005 Paris, France

ARTICLE INFO

Keywords:

Mitochondria dynamics
Energy
Memory
Glia
TCA cycle

ABSTRACT

Mitochondria are classically viewed as ‘on demand’ energy suppliers to neurons in support of their activity. In order to adapt to a wide range of demands, mitochondria need to be highly dynamic and capable of adjusting their metabolic activity, shape, and localization. Although these plastic properties give them a central support role in basal neuronal physiology, recent lines of evidence point toward a role for mitochondria in the regulation of high-order cognitive functions such as memory formation. In this review, we discuss the interplay between mitochondrial function and neural plasticity in sustaining memory formation at the molecular and cellular levels. First, we explore the global significance of mitochondria in memory formation. Then, we will detail the memory-relevant cellular and molecular mechanisms of mitochondrial plasticity. Finally, we focus on those mitochondrial functions, including but not limited to ATP production, that give mitochondria their pivotal role in memory formation. Altogether, this review highlights the central role of mitochondrial structural and functional plasticity in supporting and regulating neuronal plasticity and memory.

1. Introduction

A long-standing view in the field of neuroenergetics is that mitochondria support neuronal activity through acting as ‘on demand’ energy providers to neurons. Neurons are indeed among the most energy-demanding cells in the body, requiring high amounts of ATP to maintain their functions (Rumpf et al., 2023). Even at rest, neurons consume significant amounts of energy to maintain their membrane potential and support membrane turnover, i.e. the continuous synthesis, recycling and degrading of membrane components crucial for maintaining structural integrity and functionality of the neuron's membrane (Harris et al., 2012; Purdon and Rapoport, 2007; Rumpf et al., 2023). This energy is essential for sustaining ionic gradients across the neuronal membrane, which are in turn vital to the cell's excitability and overall function. Upon neuronal activity, the energy demands of electrical signaling processes increase significantly through the generation of action potentials and postsynaptic potentials (Harris et al., 2012). The synaptic vesicle cycle, which includes the loading of neurotransmitters as well as the exocytosis and endocytosis of synaptic vesicles (SVs), is an energy-intensive process (Pulido and Ryan, 2021; Rangaraju et al., 2014). Neurotransmitter biosynthesis also relies on ATP production and precursors provided by mitochondrial metabolism (Mergenthaler et al.,

2013). Additionally, cytoskeleton dynamics and synapse remodeling, including the endocytosis and exocytosis of neurotransmitter receptors, contribute to these high energy demands. Translation (especially the local translation that involves protein synthesis at the synapse) also adds to this energy burden, as it is essential to the synaptic plasticity and long-term potentiation (LTP) of the excited synapse (Rangaraju et al., 2019).

These specific energy requirements highlight the critical role played by mitochondria in neurons. Mitochondria have a pivotal role in cellular energy production, in which they produce ATP through oxidative phosphorylation. Mitochondria also serve many other functions that are equally vital to neuronal health and function, including calcium buffering to maintain ion homeostasis during neuronal firing, the regulation of lipid and carbohydrate metabolism, the biosynthesis of several precursors and the production of reactive oxygen species (ROS) for signaling, and apoptosis (Mattson et al., 2008). To adapt to their wide array of demands, mitochondria need to be highly dynamic and capable of adjusting their metabolic activity, shape, and localization. Because they exhibit such dynamic properties, mitochondria can exist in and switch between different states depending on the “history” of each cell. As a result, we can view these properties as comprising the functional plasticity of mitochondria, which positions them as pivotal players in neuronal physiology. Regarding this plasticity, recent evidence obtained

^{*} Corresponding authors.

E-mail addresses: thomas.preat@espci.fr (T. Preat), alice.pavlowsky@espci.fr (A. Pavlowsky), pierre-yves.placais@espci.fr (P.-Y. Plaçais).

<https://doi.org/10.1016/j.nbd.2024.106740>

Received 4 October 2024; Received in revised form 14 November 2024; Accepted 14 November 2024

Available online 17 November 2024

0969-9961/© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

in fruit flies suggests that mitochondria play a regulatory role in higher order cognitive functions including long-term memory formation, which surpasses the view of them as powerhouses of neurons (Plačais et al., 2017; Rabah et al., 2023a; Comyn et al., 2024; Pavlowsky et al., 2024). In this review, we highlight the interplay between mitochondrial function and neural plasticity in sustaining memory formation, at the cellular and molecular scales. First, we focus on the global significance of neuronal and glial mitochondria in plasticity and memory formation. In the second part, we examine the cellular and molecular mechanisms of mitochondrial plasticity, from the regulation of their metabolism to their shape and motility, that are relevant to the formation of memory. Finally, we ask what are the outputs of mitochondrial activity, from ATP production to metabolites and ROS production, that are relevant for memory formation. Altogether, the current knowledge reviewed here presents a picture of mitochondria as cellular gatekeepers of memory consolidation, endowed with an unexpected role as active regulators of memory formation.

2. Mitochondrial activity underlies the control of long-term memory formation

The idea that mitochondria support neuronal transmission is well-established, given the high energy cost of synaptic vesicle cycling and Na^+/K^+ ATPase function in maintaining the ionic gradient across the neuronal membrane (Harris et al., 2012). But beyond providing support for neurotransmission in general, what role do mitochondria play in neuronal plasticity and the resulting memory? We categorized the publications discussed in this review into a bibliographical table (Table 1) based on their focus regarding mitochondrial function and neuronal activity, spanning from *in vitro* neurotransmission and plasticity to *in vivo* studies assessing memory formation.

Synaptic plasticity is fundamental to learning and memory, as it allows the brain to adapt and store information by modifying the efficiency of synaptic transmission. These plasticity processes can present various temporal dynamics that last from a few minutes for transient short-term potentiation (STP) of the synapse, to hours or weeks for long-term potentiation (LTP) or long-term depression (LTD), which respectively enhance or reduce synaptic strength (Hagena and Manahan-Vaughan, 2024). These processes have been widely studied *in vitro* in a great variety of models, particularly in hippocampus slices and hippocampus neuronal cultures of rodents, as the hippocampus is the major mammalian associative memory center (Hagena and Manahan-Vaughan, 2024). These preparations allow the controlled induction of neuronal stimulation, as well as pre- and postsynaptic observation of response mechanisms. As a result, they are widely used for investigating the role of mitochondria in neuronal plasticity (Todorova and Blokland, 2017).

Long-term synaptic plasticity relies on increased neurotransmitter release, enhanced excitability, gene expression, *de novo* protein synthesis, and the formation of new synapses (Bailey et al., 2015), which all critically depend on mitochondria function (Glock et al., 2021; Li et al., 2004; Raefsky and Mattson, 2017; Rangaraju et al., 2014, 2019; Smith et al., 2016). Pharmacologically inhibiting mitochondrial activity (using inhibitors of mitochondrial calcium uptake and release) thus impairs LTP (Tang and Zucker, 1997), and doing so during intense stimulation leads to depressed synaptic transmission (Billups and Forsythe, 2002; Medler and Gleason, 2002). On the other hand, boosting mitochondrial respiration *via* creatine increases synaptic density (Li et al., 2004). However, the precise contributions of pre- and postsynaptic mitochondria remains unanswered by these experiments.

At the presynapse, glycolysis is critical in sustaining steady-state ATP demand (Ashrafi et al., 2017; Ikemoto et al., 2003; Rangaraju et al., 2014; Wang et al., 2024; Zala et al., 2013), whereas the additional ATP production required to sustain electrical activity or long-term synaptic plasticity is supplied by both glycolysis and mitochondrial oxidative phosphorylation (Devine and Kittler, 2018; Rangaraju et al., 2014).

Interestingly, presynaptic terminals can switch from glycolytic to oxidative metabolism upon high-intensity stimulation (Ashrafi et al., 2020). Voltage-evoked calcium entry into the cell triggers calcium uptake by mitochondria through the mitochondrial calcium uniporter regulator MICU3, which stimulates ATP production (Ashrafi et al., 2020).

Postsynaptically, LTP leads in cultured hippocampal neurons to the phasic occurrence of “mitoflashes”, transient events involving heightened mitochondrial activity, depolarization, spikes in peaks of mitochondrial calcium concentration, and ROS production (Fu et al., 2017). These fast mitochondrial calcium transients show similar kinetics to cytosolic calcium transients, and have been reported in pyramidal neurons upon LTP (Stoler et al., 2022). Interestingly, the coincident detection of a single excitatory postsynaptic potential and of back-propagating action potential induced in these dendritic mitochondria a localized calcium transient that enhanced ATP production, which could play a role in another type of neuronal plasticity called spike-timing dependent plasticity (STDP) (Stoler et al., 2022). These studies suggest that postsynaptic mitochondria have the unique ability, *via* fluctuations in mitochondrial calcium levels, to decode both firing frequency and simultaneous neuronal firing patterns. In turn, it is suggested that these mitoflashes could act as putative signals for stabilizing long-term synaptic plasticity. Indeed, it was shown that dendritic mitochondria play a critical role in long-term regulation of synaptic strength, notably by fueling local translation for plasticity (Rangaraju et al., 2019). LTP also induces postsynaptic elevation in the expression of genes encoded by mitochondrial DNA, which was seen to last for up to two weeks (Williams et al., 1998). Altogether, these studies point toward the fact that mitochondrial function is intricately linked to synaptic activity, since mitochondria both respond to and actively regulate synaptic plasticity.

Despite the valuable insights gained from studying LTP *in cellulo*, these results do not fully capture the complexity of the physiological process of memory formation. *In vivo* approaches have been instrumental in firmly establishing the role of mitochondria in memory, bridging the gap between cellular models and cognitive processes. In rats, the chronic administration of sodium azide, a potent inhibitor of mitochondrial complex IV that blocks mitochondrial respiration, induced learning and memory deficits in spatial and non-spatial memory tasks (Bennett et al., 1992; Bennett and Rose, 1992). These defects are coupled with synaptic plasticity impairment in the hippocampus (Bennett et al., 1992). However, while these *in vivo* studies confirmed the critical role of mitochondria in memory, they did not determine in which phase of the memory process or in which cell type they are required. Indeed, doing so would require experimental strategies that can both monitor and manipulate mitochondrial activity in a more acute way. This has only become achievable recently thanks to the increasing sophistication of manipulations available in genetically tractable species, and the development of new tools for *in vivo* metabolic imaging at a cellular resolution.

Memory formation encompasses several distinct but connected processes, *i.e.* learning (memory encoding), storage, consolidation, and recall. In particular, the long-term consolidation of a memory trace into long-lasting memory mobilizes important cognitive and energy resources. For the optimized allocation of such resources, long-term memory (LTM) formation is inhibited by default in order to avoid wasteful expenses (Abel et al., 1998; Scheunemann et al., 2018). Lifting this inhibition, a process known as LTM gating, can notably be achieved through spaced repetition of learning sessions. This phenomenon, called the spacing effect, is highly conserved across species (Glas et al., 2021; Nishijima and Maruyama, 2017; Shaughnessy, 1977; Sutton et al., 2002; Tully et al., 1994). Recent reports using classical Pavlovian olfactory conditioning in *Drosophila melanogaster* have offered crucial insights into the regulatory role that mitochondria play in LTM gating. In this aversive protocol that involves the pairing of an odorant with mild electric shock delivery, a single-cycle learning trial gave rise to short-lived

Table 1

Classification of the reviewed studies according to the mitochondrial function under investigation.

Mitochondrial function	Model system	Cell type	Experimental assay/behavioral task
Pyruvate metabolism			
Yang et al., 2021	<i>In vitro</i> : mouse hippocampal slices; culture of primary mouse hippocampal neurons; forebrain synaptosomes	Hippocampus neurons	KCl, bicuculline or BDNF-induced depolarization
Plaçaïs et al., 2017	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons	Aversive olfactory conditioning
Comyn et al., 2024	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons	Aversive olfactory conditioning
Rabah et al., 2023a	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons	Aversive olfactory conditioning
Raun et al., 2023	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons	Courtship conditioning
Descalzi et al., 2019 (see also Translation)	<i>In vivo</i> : rat	Dorsal hippocampus neurons	Inhibitory avoidance
Respiratory chain and ATP production			
Li et al., 2004 (see also Morphology: fission/fusion dynamics)	<i>In vitro</i> : culture of primary rat hippocampal neurons; hippocampal slice	Hippocampus neurons; CA1 pyramidal neurons	KCl-induced depolarization; electric stimulation (tetanic stimulus)
Bennett et al., 1992	<i>In vivo</i> : rat	Nonspecific	Spatial and nonspatial learning: eight-arm radial maze (appetitive) and two-way shuttle box task (aversive)
Bennett and Rose, 1992	<i>In vivo</i> : rat	Nonspecific	Spatial learning: Morris water maze (aversive)
Underwood et al., 2023 (see also Morphology: fission/fusion dynamics)	<i>In vitro</i> : isolated rat hippocampus punches; hippocampal synaptosomes	CA1 pyramidal neurons	Context fear conditioning
Rangaraju et al., 2014 (see also Translation)	<i>In vitro</i> : culture of primary rat hippocampal neurons	Hippocampus neurons	Glutamate uncaging (structural LTP)
Verstreken et al., 2005 (see also Vesicle cycling)	<i>In vivo</i> : <i>Drosophila</i>	Neuromuscular junction	Electric stimulation
Olesen et al., 2020 (see also ROS production)	<i>In vivo</i> : mice <i>In vitro</i> : mice hippocampus synaptosomes	Hippocampus neurons	Spatial and nonspatial learning: Morris water maze test, Barnes maze test, novel object recognition test, novel object localization test
Calcium dynamics			
Tang and Zucker, 1997	<i>In vitro</i> : crayfish neuromuscular junction	Motor neuron	Electric stimulation (tetanic stimulus)
Medler and Gleason, 2002	<i>In vitro</i> : cultured chick retinal cells	Amacrine cells	KCl-induced depolarization; electric stimulation
Xiong et al., 2002	<i>In vitro</i> : mouse cerebellar slices	Cerebellar granule neurons	KCl-induced depolarization
Stoler et al., 2022	<i>In vitro</i> : mouse somatosensory neocortical slices	L5 pyramidal neurons	Electric stimulation (spike-timing-dependent plasticity (STDP))
Billups and Forsythe, 2002 (see also Neurotransmitter release)	<i>In vitro</i> : rat transverse brainstem slices	Auditory brainstem neuron (calyx of Held synapse)	Electric stimulation
Ashrafi et al., 2020	<i>In vitro</i> : culture of primary rat hippocampal neurons	Hippocampus neurons	Electric stimulation
Fu et al., 2017 (see also ROS production)	<i>In vitro</i> : Culture of primary rat hippocampal neurons	Hippocampus neurons	Glutamate uncaging (structural LTP)
Vishwanath et al., 2024	<i>In vivo</i> : <i>Drosophila</i> ; Mouse; <i>In vitro</i> : Culture of primary rat hippocampal neurons	Mushroom body neurons; Hippocampus neurons	Aversive olfactory memory; Electric stimulation; Glutamate uncaging
Kim et al., 2011 (see also ROS production)	<i>In vivo</i> : Mouse <i>In vitro</i> : Slices of mouse spinal cord	Dorsal horn neurons	Mechanical sensitivity of hindpaw upon mechanical stimuli, NMDA or capsaicin injection; Electric stimulation (spinal LTP)
Morphology: Fission/fusion dynamics			
Li et al., 2004 (see also Respiratory chain)	<i>In vitro</i> : culture of primary rat hippocampal neurons; hippocampal slice	Hippocampus neurons; CA1 pyramidal neurons	KCl-induced depolarization; electric stimulation (tetanic stimulus)
Divakaruni et al., 2018	<i>In vitro</i> : culture of primary rat hippocampal neurons; mouse hippocampal slices	Hippocampus neurons; CA1 pyramidal neurons	Glycine application (chemical LTP); glutamate uncaging (structural LTP); electric stimulation (high frequency stimulation LTP)
Liu et al., 2022	<i>In vivo</i> : mice	Auditory cortex neurons	Auditory fear conditioning
Oettinghaus et al., 2016	<i>In vivo</i> : mice	Hippocampus neurons	Spatial working memory task: spontaneous alternation tasks in an eight-arm radial maze
Virga et al., 2024	<i>In vivo</i> : mice <i>In vitro</i> : culture of primary mouse hippocampal neurons	CA1 pyramidal neurons; hippocampus neurons	Spontaneous activity; PTX-induced synaptic activity
Underwood et al., 2023 (see also Respiratory chain)	<i>In vivo</i> : rat <i>In vitro</i> : isolated rat hippocampus punches; hippocampal synaptosomes	CA1 pyramidal neurons	Context fear conditioning
(Hara et al., 2014)	<i>In vivo</i> : Rhesus monkey	Dorsolateral prefrontal cortex Brodmann's area neurons	Delayed response test of visuospatial working memory
Motility: transport and docking			
Kang et al., 2008	<i>In vitro</i> : culture of primary rat and mouse hippocampal neurons	Hippocampus neurons	Electric stimulation (tetanic stimulus)
Li et al., 2020	<i>In vitro</i> : culture of primary mouse hippocampal neurons	Hippocampus neurons	PTX-induced synaptic activity; electric stimulation (tetanic stimulus)

(continued on next page)

Table 1 (continued)

Mitochondrial function	Model system	Cell type	Experimental assay/behavioral task
Sun et al., 2013	<i>In vitro</i> : culture of primary mouse neurons and hippocampal slices	Hippocampus neurons	Electric stimulation
Pavlovsky et al., 2024	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons	Aversive olfactory memory
Sajic et al., 2013	<i>In vivo</i> : mouse	Saphenous nerves	Electric stimulation; capsaicin application
Smit-Rigter et al., 2016	<i>In vivo</i> : mouse	Visual cortex	Retinal lesion (structural cortical plasticity)
ROS production			
Esparza-Moltó et al., 2021	<i>In vivo</i> : mouse <i>In vitro</i> : culture of primary mouse hippocampal neurons; mouse hippocampal slices	Hippocampus neurons; CA1 pyramidal neurons	Novel object recognition task; electric stimulation (theta-burst stimulation LTP)
Olesen et al., 2020 (see also ATP production)	<i>In vivo</i> : mice <i>In vitro</i> : mice hippocampus synaptosomes	Hippocampus neurons	Spatial and nonspatial learning: Morris water maze test, Barnes maze test, novel object recognition test, novel object localization test
Kim et al., 2011 (see also Calcium dynamics)	<i>In vivo</i> : mouse <i>In vitro</i> : slices of mouse spinal cord	Dorsal horn neurons	Mechanical sensitivity of hindpaw upon mechanical stimuli, NMDA or capsaicin injection; electric stimulation (spinal LTP)
Morant-Ferrando et al., 2023	<i>In vivo</i> : mouse	Astrocytes	Novel object recognition task
Vicente-Gutierrez et al., 2019	<i>In vivo</i> : mouse	Astrocytes	Novel object recognition task
Fueling translation			
Rangaraju et al., 2019	<i>In vitro</i> : culture of primary rat hippocampal neurons	Hippocampus neurons	Glutamate uncaging (structural LTP)
Rangaraju et al., 2014 (see also ATP production)	<i>In vitro</i> : culture of primary rat hippocampal neurons	Hippocampus neurons	Glutamate uncaging (structural LTP)
Descalzi et al., 2019 (see also Pyruvate metabolism)	<i>In vivo</i> : rat	Dorsal hippocampus neurons	Inhibitory avoidance
Neurotransmitter release/vesicle cycling			
Billups and Forsythe, 2002 (see also Calcium dynamics)	<i>In vitro</i> : rat transverse brainstem slices	Auditory brainstem neuron (calyx of Held synapse)	Electric stimulation
Smith et al., 2016	<i>In vitro</i> : rat hippocampal slices	CA1 pyramidal neurons	Electric stimulation (theta-burst stimulation LTP)
Verstreken et al., 2005 (see also ATP production)	<i>In vivo</i> : <i>Drosophila</i>	Neuromuscular junction	Electric stimulation
Miscellaneous			
Ketone body metabolism			
Silva et al., 2022	<i>In vivo</i> : <i>Drosophila</i>	Mushroom body neurons, cortex glia	Aversive olfactory memory upon starvation
Mitochondrial gene expression			
Williams et al., 1998	<i>In vitro/in vivo</i> : rat hippocampal homogenates	Perforant path-dentate gyrus synapses; CA3 and CA1 region	Electric stimulation (high frequency stimulation LTP)

memories that started to decay after 3 h (Quinn et al., 1979; Tully and Quinn, 1985). Multiple training sessions make it possible to reinforce memory and increase its persistence, albeit with different dynamics, depending on the pattern of training over time (Tully et al., 1994). Multiple consecutive trials (massed training) induce a memory that decays within 1–2 days, whereas repeated trials spaced in time (spaced training) lead to protein synthesis-dependent LTM formation that lasts up to one week (Tully et al., 1994). These different forms of memory involve defined and well-described neuronal networks that have been mapped to specific subpopulations of neurons within a brain region known as the mushroom bodies (MBs), the *Drosophila* olfactory memory center (Bouzaiane et al., 2015).

In this context, the use of a genetically encoded pyruvate sensor coupled to pharmacological blockade of the mitochondrial respiratory chain allowed for *in vivo* measurement at the cellular scale (in a cell-specific manner) of mitochondrial influx of pyruvate. As an input substrate for the tricarboxylic acid (TCA) cycle and subsequent oxidative phosphorylation, this can serve as a proxy to measure mitochondrial metabolic activity. Combined with behavioral experiments, this approach has been instrumental in uncovering links between concepts linking energy metabolism and memory in *Drosophila*. After one cycle of training, upregulation of pyruvate metabolism in MB neuronal

mitochondria (Rabah et al., 2023a) occurs both at the level of the somas and the axons. Preventing this upregulation yields memory impairment (Rabah et al., 2023a). However, this increased metabolic activation is not sustained, as MB neurons return to their basal energy state 3 h after training (Comyn et al., 2024). Importantly, memory is still present at that time point, indicating that mitochondrial activation is not necessary for the behavioral expression of memory, while it is involved in the early establishment of the memory trace. On the other hand, spaced training upregulates mitochondrial pyruvate flux (Plaçaïs et al., 2017) in the same compartments (somas and axons of the MB neurons), but specific mechanisms maintain this activated state specifically in the axons beyond 8 h (Comyn et al., 2024). These analyses demonstrate that the spatiotemporal organization of mitochondrial metabolic activation is a determinant factor of memory persistence (Plaçaïs et al., 2017; Rabah et al., 2023a; Comyn et al., 2024; Pavlovsky et al., 2024) (see also Fig. 1 for a global schema). Here again, at the time of memory recall (24 h after training), the metabolic activation has finished, showing that upregulation of pyruvate metabolism does not constitute the memory trace itself. Remarkably, genetic interventions constitutively boosting MB pyruvate mitochondrial metabolism (see Part II for more details) allowed flies to form LTM with fewer learning trials (Comyn et al., 2024; Plaçaïs et al., 2017). Moreover, knock-down of Letm1, a mitochondrial ion

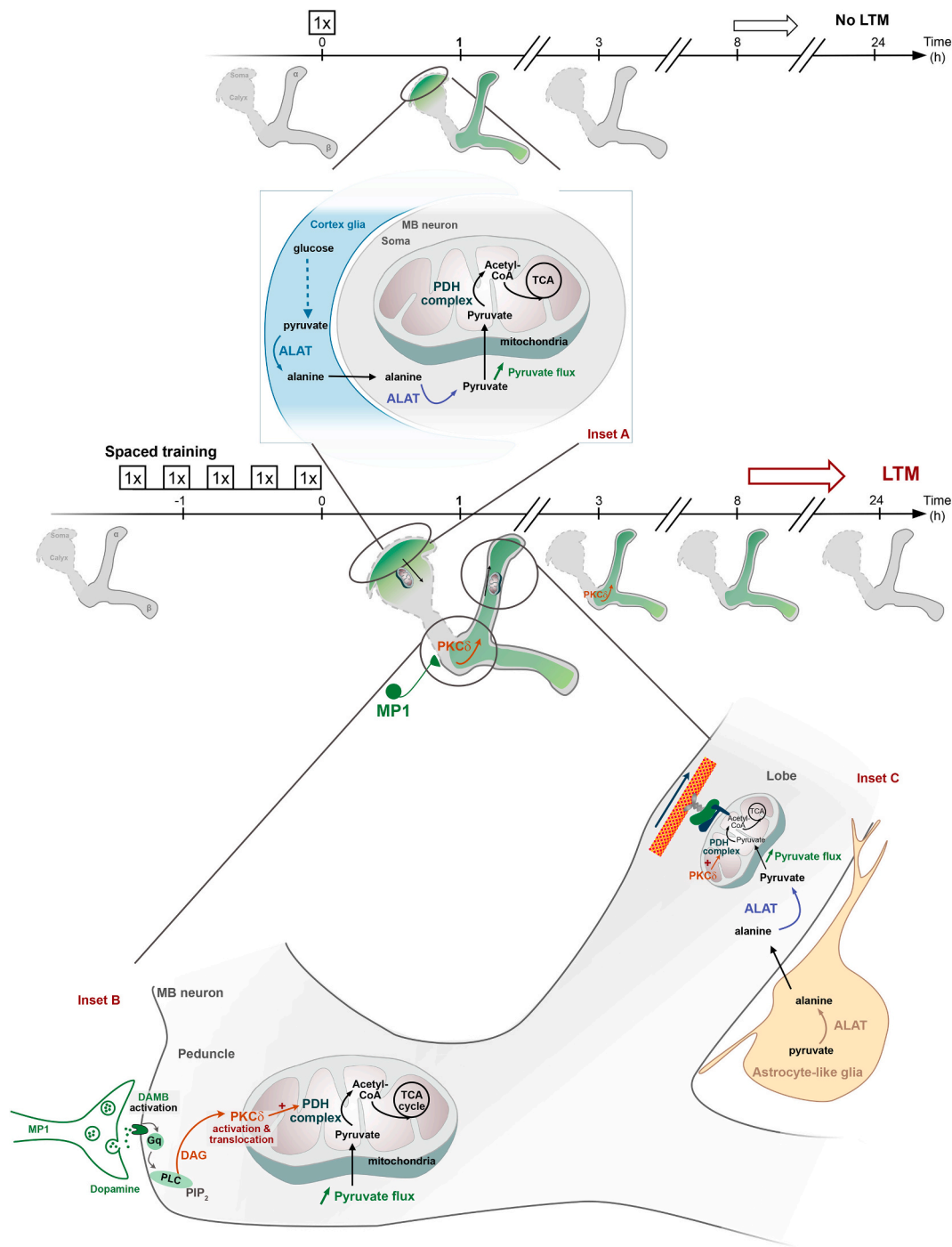


Fig. 1. Mitochondrial plasticity and memory: mechanisms gating LTM formation in *Drosophila*. After one-cycle training, pyruvate flux to mitochondria in the MB neuronal somas and the vertical and medial lobes is increased (in green). This is supported by alanine transfer from cortex glia surrounding the MB neuronal soma (inset A). This increase in mitochondrial metabolic activity is not maintained, but is progressively turned off. Upon spaced training, in addition to the mechanism occurring after one-cycle training, the enhanced oscillatory activity of MP1 dopaminergic neurons activates DAMB signaling in the efferent α/β MB neurons (inset B). The DAMB receptor preferentially couples with Gq and the produced DAG activates PKC δ , resulting in its translocation to mitochondria. There, PKC δ can activate the PDHC, by releasing the inhibition exerted by PDK. The enhanced PDHC activity promotes an increase in mitochondrial pyruvate flux, and this enhanced mitochondrial metabolism gates memory consolidation, thereby enabling LTM formation. In these first hours following spaced training, additional alanine is provided locally to the lobes by the astrocyte-like glia, whereas Miro-Milton mediated mitochondria transport supports the increased mitochondrial metabolic activity in the lobes (inset C). After 3 h, the lasting effect of activated PKC δ on mitochondrial metabolism maintains a high pyruvate flux in the vertical and medial lobes (in green), while it returns to its baseline levels in the somas. PKC δ activation ends after 8 h, whereas mitochondrial metabolic activity is still upregulated (in green), ultimately resulting in the LTM gating. After 24 h, once LTM has been encoded and can be tested by behavioral assays, mitochondrial metabolism in the MB neurons returns to its basal state, indicating that pyruvate metabolism upregulation does not represent the memory trace in itself. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

channel that mediates calcium efflux, prolonged the increased pyruvate consumption in MB neurons beyond 3 h after single-cycle training (Vishwanath et al., 2024). At the behavioral level, these flies were able to form LTM after a single cycle of conditioning, demonstrating that the occurrence of long-lasting mitochondrial activation is the driving factor for long-term memory formation. These studies in fruit flies thus revealed the instructive role played by mitochondrial energy fluxes in lifting the default inhibition of LTM. Interestingly, some evidence in the literature suggests that such a mechanism could be conserved in mammals. Upregulated mitochondrial respiration was measured in rat hippocampus following context fear conditioning. This upregulation was only observed for presynaptic mitochondria of the Schaffer collaterals-CA1 synapses, i.e. mitochondrial respiration was not increased in the cell body CA1 region (Underwood et al., 2023). Although the dynamics and function of this effect remain to be clarified, it seems that, like in flies, the specific localization of mitochondrial metabolic activation is an important factor in LTM formation. Perhaps the most compelling evidence of a conserved role for mitochondria in LTM control comes from a recent study reporting that, similar to what was observed in flies, knock-down of *Letm1* in mouse hippocampus facilitated the formation of LTM in an aversive associative olfactory memory task (Vishwanath et al., 2024).

In parallel to the role of neuronal mitochondria as a cognitive regulator, recent studies have revealed alterations of mitochondrial metabolic activity in glial cells linked to memory formation. It is well established, both in mammals and invertebrates, that glycolytic output from glial cells can be diverted to neurons to support enhanced mitochondrial activity for memory formation (Newman et al., 2011; Suzuki et al., 2011; Rabah et al., 2023a). Under physiological conditions, the major molecular mediator of the neuron-glia metabolic coupling underlying memory is lactate in mammals (Newman et al., 2011; Suzuki et al., 2011), and alanine in flies (Rabah et al., 2023a). But how do glial cells metabolically adapt to this coupling and maintain metabolite production during memory formation? Two recent studies in mice demonstrated that astrocytic mitochondria are required for memory formation, as evaluated by a novel object recognition task (Morant-Ferrando et al., 2023; Vicente-Gutierrez et al., 2019). In mice, conditional overexpression of mitochondrially addressed catalase in astrocytes allowed a decrease in production of mitochondrial ROS, which consequently impaired memory formation (Vicente-Gutierrez et al., 2019). It was then found that astrocytes oxidize fatty acids in their mitochondria to favor an ‘electron-leaky’ configuration state of the oxidative phosphorylation chain. This inefficient conformation prevents the assembly of complex I into supercomplexes, which ultimately favors glycolysis and ROS generation rather than oxidative phosphorylation (Lopez-Fabuel et al., 2016; Morant-Ferrando et al., 2023). Memory in mice is also impaired when knocking out a key enzyme of mitochondrial fatty acid oxidation in adult astrocytes (Morant-Ferrando et al., 2023). However, the precise mechanism leading to the observed memory deficit remains unknown. Do astrocytes oxidize fatty acid to favor glycolysis in order to efficiently provide lactate for memory formation? Or do ROS play an indispensable signaling role in memory?

Overall, research in recent years has made it clear that mitochondrial metabolic adaptation, both in neurons and glial cells, is integral to memory formation. In neurons, the spatiotemporal dynamics of mitochondrial activation is causally linked to the onset of long-term memory formation, establishing a cognitive function of mitochondria in neurons. In the following section, we review the cellular and molecular mechanisms that govern learning-induced mitochondrial plasticity in neurons.

3. Memory-relevant cellular and molecular mechanisms of mitochondrial plasticity

Upon associative memory formation, metabolic activation of neuronal mitochondria can be observed in *Drosophila* MBs, the duration and location of which depends on the type of conditioning and memory

persistence (Fig. 1) (Comyn et al., 2024; Plaçais et al., 2017; Rabah et al., 2023a). In addition, genetic inhibition of either pyruvate mitochondrial import or of the pyruvate dehydrogenase complex (PDHC; the enzymatic complex that converts pyruvate into acetyl-CoA to fuel the TCA cycle) in the adult MB neurons leads to memory defect after single-cycle training as well as after spaced training. Upregulation of mitochondrial activity via pyruvate consumption can occur as a passive mechanism, which is made thermodynamically favorable by the increased consumption of mitochondrial output products such as ATP. If such a mechanism could be sufficient to support the short-lasting upregulation of pyruvate metabolism observed after one-cycle training (Fig. 1, inset A), we could ask whether additional mechanisms are involved in the axonal-specific prolonged activation of mitochondrial metabolism triggered by spaced conditioning. In support of this, increasing PDHC activity by down-regulating its inhibitor, the pyruvate dehydrogenase kinase (PDK) (Lavington et al., 2014), does not improve memory performance after single-cycle or spaced training but facilitates the formation of LTM; in that case, it can be formed after a single cycle of training instead of the five cycles usually required (Comyn et al., 2024; Plaçais et al., 2017). Thus, an inability to respond to the acute increase in energy demand upon LTM formation is detrimental to memory formation, but increasing mitochondrial metabolic capacity is sufficient to allow LTM formation. The mechanisms that support this coupling between LTM formation and mitochondrial plasticity will now be discussed.

The upregulation of pyruvate metabolism in MB neurons required for LTM formation is triggered by post-learning dopamine signaling from a specific pair of MB-afferent neurons, alternately referred to as MP1 neurons (Plaçais et al., 2017) or PPL1-γ1pedc neurons (Aso et al., 2014). These neurons display sustained calcium rhythmic activity in the first hours after spaced training, and activate MB neurons via the dopaminergic metabotropic receptor DAMB (also named Dop1R2) (Plaçais et al., 2012). Intracellularly, DAMB is coupled to Gq signaling (Cassar et al., 2015; Himmelreich et al., 2017), whose signaling utilizes the second messengers calcium and DAG (Mizuno and Itoh, 2009). Upon DAMB activation, PKCδ, a ‘novel PKC’ enzyme known to be exclusively activated by DAG (Duquesnes et al., 2011), translocates to mitochondria, where it releases the PDHC inhibition and thereby increases the pyruvate flux to mitochondria (Comyn et al., 2024). Importantly, the LTM defect caused by PKCδ knock-down in the MB at the adult stage was rescued by the co-inhibition of PDK, demonstrating that the role of PKCδ in LTM formation is entirely mediated through its effects on mitochondrial metabolism. PKCδ is therefore a key molecular actor in the coupling between dopaminergic signaling and mitochondrial metabolism specifically upon LTM formation (Fig. 1, insets B-C).

As an alternative or parallel pathway to PKCδ, mitochondrial calcium signaling is well-known for increasing the activity of mitochondrial metabolic enzymes, particularly the activity of the PDHC (Denton et al., 1972; Glancy and Balaban, 2012). In cultured rodent hippocampal neurons, the coupling between neuronal activity and synaptic ATP production relies on mitochondrial calcium. Therefore, the inhibition of mitochondrial calcium uptake prevents ATP production upon neuronal activation (Ashrafi et al., 2020), whereas the inhibition of mitochondrial calcium extrusion via *Letm1*, a $\text{Ca}^{2+}/\text{H}^{+}$ antiporter mediating mitochondrial calcium efflux (Jiang et al., 2009), results in the over-activation of metabolism of synaptic mitochondria, as evaluated by increased mitochondrial consumption of pyruvate and increased ATP production (Vishwanath et al., 2024). Consistent with these observations, knock-down of *Letm1* in *Drosophila* MB neurons prolonged the upregulated pyruvate metabolism for more than three hours after a single cycle of conditioning, a time when the effect is extinct in control flies (Vishwanath et al., 2024). At the behavioral level, this *Letm1* knock-down effect leads to the facilitation of LTM formation in *Drosophila*, in which only a single session of training is required to consolidate memory into LTM. Strikingly, this is replicated in adult mice upon *Letm1* knock-down in the hippocampus for the formation of olfactory aversive LTM, in which a single session of training is enough to

induce a 15-day persistent memory that is already extinguished at that time in control mice (Vishwanath et al., 2024). Whether in *Drosophila* this mitochondrial calcium coupling of mitochondrial metabolism to neuronal activity upon LTM formation is downstream of DAMB-mediated dopaminergic signaling will require further investigation. Collectively, these results point toward an evolutionarily conserved role for mitochondrial calcium in upregulating mitochondrial metabolism within integrative memory centers, thereby controlling the formation of long-term memories.

In the mammalian brain, induction of synaptic plasticity is associated with the transient and rapid activation of the expression of specific genes known as the immediate early genes (Lanahan and Worley, 1998; Minatohara et al., 2016). Among these genes, Rheb, a member of the Ras family of small GTP-binding proteins, is of particular interest in the context of metabolic coupling to neuronal activity. Thus, while its expression is rapidly and transiently induced in the hippocampus upon NMDA-dependent synaptic plasticity *in vitro* (Yamagata et al., 1994), it was recently described as an activator of PDP upon neuronal stimulation, thereby increasing the PDHC activity independently from its well-known interaction with mTORC1 (Yang et al., 2021). However, because Rheb is also upstream of the TOR pathway involved in cell development and growth, the memory defect associated with its overexpression in the *Drosophila* MB described by Brown et al. cannot be linked specifically to a role in mitochondrial metabolic coupling to neuronal activity *in vivo* (Brown et al., 2012). Further exploration with appropriate tools will be necessary to distinguish between the two roles of Rheb. Nevertheless, the coupling of mitochondrial metabolism to neuronal activity *via* the expression of the immediate early genes could allow a second temporality complementary to the rapid activation of enzymatic activity upon neurotransmitter receptor activation, as in the case of the dopamine/PKC δ pathway (Comyn et al., 2024).

In addition to the temporal dimension of this increase in energetic demand, neurons must face compartment-specific needs given the specific morphology of highly polarized cells, which introduces a spatial constraint. Thus, upon memory formation, places in high demand of mitochondrial energy production and Ca²⁺ buffering (the synapses) can be distant from the place of mitochondria biogenesis (the soma). To adjust to compartment-specific needs, mitochondria can modulate their shape through fission and fusion so as to influence their energy production, Ca²⁺ buffering ability and motility, thereby impacting their intracellular distribution and presence at sites of intense synaptic activity (Lovas and Wang, 2013; Pekkurnaz and Wang, 2022; Saxton and Hollenbeck, 2012; Yu and Pekkurnaz, 2018). In the soma and dendrites, mitochondria form an elongated and interconnected network, whereas axonal and presynaptic mitochondria are discrete, granular, small organelles (Lee et al., 2018). The occurrence of these antagonistic fusion and fission events are finely balanced to maintain mitochondrial homeostasis: whereas fusion produces long, filamentous shapes, fission promotes short, bean-shaped mitochondria that are more suited for transport along axons (Rossi and Pekkurnaz, 2019; Yu and Pekkurnaz, 2018). *In vitro* studies suggest that the balance between fission and fusion is regulated by neuronal activity (Li et al., 2004). More specifically, upon synaptic plasticity such as NMDA-dependent LTP, mitochondrial fission is increased through a coupling mediated by CaMKII phosphorylation of Drp1, the main actor of mitochondrial fission (Divakaruni et al., 2018). *In vivo* neuronal activity favors fission over fusion in specific dendritic compartments (Virga et al., 2024) *via* AMPK, the master energy sensor of the cell that is activated when intracellular ATP is depleted or ATP consumption is high. Furthermore, extensive electronic microscopy analysis of mitochondria morphology in the mouse auditory cortex after auditory fear conditioning revealed that mitochondria size was significantly decreased while their number increased (Liu et al., 2022). This study suggests that mitochondrial fission occurs at these cortical synapses upon associative fear conditioning to sustain memory formation. Precisely assessing the role of mitochondrial fission as a way to adjust mitochondria physiology to the

neuronal needs of memory formation requires loss-of-function experiments. However, the importance of this process in the basal physiology of neurons makes such loss-of-function experiments complicated to perform without generating global neuronal dysfunction. Chemical inhibition of Drp1 *via* intracerebroventricular injection in mice impaired contextual fear memory as well as the training-associated enhancement of mitochondrial oxygen consumption in isolated hippocampal tissues (Underwood et al., 2023). However, due to the injection site and a lack of cell-specific inhibitors, this manipulation cannot be interpreted as targeting a particular brain region or cell type. The inducible genetic ablation of Drp1 in memory-relevant structures is another approach that could be used to investigate whether mitochondrial fission is coupled to neuronal activity to support memory formation. Conditional knock-out was thus performed in adult hippocampal neurons, causing a short-term spatial working memory defect without neurodegeneration (Oettinghaus et al., 2016). However, at the cellular level, the conditional ablation of Drp1 resulted in depletion of synaptic mitochondria, due to alterations in mitochondrial transport caused by an increase in mitochondrial size. These *in vivo* and *in vitro* studies suggest that the imbalance between fusion and fission could be regulated upon memory formation as a way to adjust mitochondrial metabolism. Although this hypothesis has not been properly demonstrated yet in a memory formation context, a possible experimental strategy could be to induce ablation of mitochondrial fission factor (MFF), which was shown to result in mitochondrial elongation and alter mitochondrial calcium handling in cortical neurons (Lewis et al., 2018).

Mitochondrial dynamics also includes their long-range transport along microtubules, and indirectly affecting their transport *in vivo* by inhibiting fission impairs cognitive functions (Oettinghaus et al., 2016). In addition, numerous *in vitro* studies show that maintaining energy homeostasis throughout the neuron requires the efficient delivery of mitochondria from the soma to distal areas such as the axonal terminals, and that mitochondrial dynamics and, in particular, mitochondria motility are critical upon synaptic plasticity (Chang and Reynolds, 2006; Li et al., 2004; Sheng, 2017; Verstreken et al., 2005; Yu and Pekkurnaz, 2018). Anterograde transport (from the soma to synaptic terminals) distributes these organelles down the axons, while retrograde transport (toward the soma) is essential for removing defective or stressed mitochondria from the periphery when necessary. Both anterograde and retrograde transport rely on microtubule motor proteins (dynein and kinesin, respectively) and the adaptor proteins Miro and Milton, which anchor mitochondria to the microtubule cytoskeleton (Yu and Pekkurnaz, 2018). While opposite conclusions have been drawn regarding the importance of mitochondrial transport along axons in sustaining plastic change *in vivo* in mammalian neurons (Sajic et al., 2013; Smit-Rigter et al., 2016), there is compelling evidence that this transport supports local mitochondrial metabolic upregulation in the context of memory formation in *Drosophila* (Pavlovsky et al., 2024). Thus, when mitochondria transport is specifically reduced by down-regulation of either Milton or Miro in the MB neurons, only LTM is impaired, and not any other type of less-persistent memory. After spaced conditioning yielding LTM, the number of small mitochondria in the cell bodies of MB neurons was decreased, due to their axonal transport *via* the Miro/Milton machinery. There, mitochondrial transport contributed to the increase in mitochondrial pyruvate consumption measured in the axons (but not in the somas). As this mitochondrial motility is activated in the hours following spaced conditioning (Pavlovsky et al., 2024), it could contribute to the prolonged activation of mitochondrial metabolism that extends for more than 8 h after conditioning, by importing increasingly more mitochondria into the MB axons. The nature of the signal that triggers *in vivo* this active transport of mitochondria toward the axonal compartment is still unknown. Based on *in vitro* studies, it has been proposed that neurons employ a peculiar mechanism to activate mitochondrial biogenesis and deliver mitochondria to distal axons by sensing energy requirements, a pathway mediated by AMPK (Sheng, 2017; Tao et al., 2014; Watters et al., 2020). On the other hand, it is not known

whether this increase in mitochondrial motility is downstream of MP1 dopaminergic signaling, as is PKC δ activation. Intriguingly, increased mitochondrial PKC δ activity is detected both at the site of MP1 projection (the peduncle) and in the axonal compartment (Fig. 1, insets B-C). Two possible mechanisms could be at play, the first of which involves the passive diffusion of activated PKC δ to the axons, where it subsequently translocates to the mitochondria. Alternatively, PKC δ could be activated and translocated to the mitochondria at the level of the peduncle, after which mitochondria containing the activated PKC δ are transported to the axons.

Once they have reached the axonal compartment *via* microtubule-dependent transport, mitochondria must halt and anchor to places of high energy and/or calcium buffering demand. *In vitro*, direct impairment of mitochondrial anchoring to the local synaptic actin cytoskeleton *via* deletion of syntaphilin alters neuronal plasticity (Kang et al., 2008; Sun et al., 2013). But how can intense synaptic activity be sensed by mitochondria, and translated into a 'stop and detach' signal from the microtubule cytoskeleton and an anchoring signal at the appropriate synapses? Several mechanisms targeting either Miro or Milton have been identified *in vitro* as interesting candidates to mediate the coupling between intense synaptic plasticity activity and the arrest of mitochondria movement along microtubules. As synaptic activity induces a transient elevation in the presynaptic Ca²⁺ level, it induces conformational changes in Miro *via* Ca²⁺ binding to its EF-hands, resulting in the detachment of the Miro-Milton complex from the kinesin-microtubule system (MacAskill et al., 2010; Wang and Schwarz, 2009). However, testing whether synaptic calcium elevation mediates the coupling of mitochondrial arrest with neuronal activity to support the energetic demand at the relevant synapses during memory formation *in vivo* would require the development of new tools that specifically target Miro's sensitivity to calcium. As an alternative to metabolic coupling by presynaptic calcium elevation, AMPK has been shown *in vitro* to be locally involved in two mechanisms that could facilitate mitochondrial recruitment at the synapse to rescue local energy deficits due to intense synaptic activity. The first mechanism is based on the upregulation of glucose import at the synapse *via* glucose transporters upon AMPK activation and the subsequent elevation of cytoplasmic glucose (Ashrafi et al., 2017). This mechanism could mediate the local increase of glucose that has been shown to increase O-glycosylation of Milton, limiting its ability to bind kinesin and thus reducing mitochondrial motility (Glatter et al., 2006; Pekurnaz et al., 2014). The second mechanism is mediated by the AMPK downstream kinase PAK, which facilitates the switch of mitochondria from axonal microtubules to the presynaptic actin cytoskeleton *via* phosphorylation of the myosin-syntaphilin anchoring complex (Li et al., 2020). Both mechanisms should allow energy-sensitive regulation of mitochondrial recruitment to ensure that metabolically active synapses are adequately supplied with ATP during intense synaptic activity. However, because of the pleiotropic functions regulated by AMPK, it is difficult to assess whether these specific mechanisms are at play *in vivo* upon LTM formation. A first step would be to investigate, using an AMPK activity sensor (Konagaya et al., 2017), the local activation of AMPK in neurons *in vivo* during LTM formation.

Altogether, several actors are involved in neurons to couple neuronal activity to mitochondrial plasticity. This concept extends beyond structural dynamics to encompass functional adaptability, including mechanisms of metabolic adjustments. Some of these actors, such as PKC δ or mitochondrial Ca²⁺, act as triggers to boost mitochondrial metabolism upon specific neuronal activation. In contrast, others such as Miro and Milton, AMPK, and Rheb are more likely required to maintain and stabilize an enhanced neuronal metabolic state, which ultimately allows long-term memory consolidation. Although we do not yet fully understand the precise temporal and spatial coordination of these actors in response to neuronal needs during memory formation, this does raise the question of the ultimate purpose of mitochondrial plasticity in memory formation.

4. The outcome of mitochondrial activation

As described in the previous sections, recent studies have highlighted a detailed (yet incomplete) spatiotemporal and molecular characterization of mitochondrial activation induced by various forms of learning. The final objective of this activation is much less understood, although it is essential to bridging the gap with what is known about the neuronal plasticity mechanisms underlying memory. The large diversity of mitochondria output offers several interesting tracks to explore, which are not mutually exclusive.

In line with the prevailing view of mitochondria as the 'powerhouse' of the cell, ATP is the primary output of mitochondria biochemical activity. The TCA cycle, which in neurons is thought to be essentially fueled by pyruvate-derived acetyl-CoA, generates reduced equivalents (NADH and FADH₂) that can be used by the respiratory chain to produce ATP through oxidative phosphorylation (Fig. 2). Several *in vitro* studies show that increased local ATP production is necessary for intensive synaptic transmission and synaptic plasticity (Rangaraju et al., 2014, 2019; Rumpf et al., 2023). In the presynaptic compartment, basal ATP levels are maintained by glycolysis, but an acute increase in ATP demand is provided by both glycolysis and mitochondrial oxidative phosphorylation (Devine and Kittler, 2018; Rangaraju et al., 2014), notably for the mobilization, refilling and recycling of synaptic vesicles to sustain long-term synaptic plasticity (MacAskill et al., 2010; Rangaraju et al., 2014; Smith et al., 2016; Sun et al., 2013; Verstreken et al., 2005). Support for this hypothesis also comes from a recent study reporting increased mitochondrial oxygen consumption from hippocampus synaptic layers in mice upon LTM formation following fear conditioning (Underwood et al., 2023).

Why would LTM formation be costly in terms of ATP consumption? The main hypothesis is based on the significant energy cost of *de novo* protein synthesis, which LTM formation depends on at later stages (Davis and Squire, 1984; Helmstetter et al., 2008; Jarome and Helmstetter, 2014). In mammalian cells, approximately 20 % of cellular ATP is dedicated solely to mRNA translation, excluding the energy needed for the biosynthesis of translational machinery like ribosome biogenesis (Buttgereit and Brand, 1995), and it is well-characterized that translation rates elevate in response to learning and stimuli that induce long-term plasticity (Costa-Mattioli et al., 2009; Descalzi et al., 2019; Richter and Klann, 2009; Sutton and Schuman, 2006; Wang et al., 2009). Specifically, one study has demonstrated in adult rats that a critical role of astrocyte-derived lactate supply to neurons for LTM is to support *de novo* mRNA translation in hippocampal excitatory and inhibitory neurons (Descalzi et al., 2019), although mitochondrial ATP production in itself was not directly assessed (as discussed below). In *Drosophila* MB neurons, increased uptake and conversion of pyruvate into acetyl-CoA by mitochondria after aversive learning has been well-described (Comyn et al., 2024; Pavlovsky et al., 2024; Pla  ais et al., 2017; see Part II), but the dynamics of ATP production and consumption in this paradigm have not been investigated yet.

Interestingly, the role of mitochondria in ATP production has been questioned in the axons of certain types of neurons, notably cortical pyramidal neurons (Hirabayashi et al., 2024). Instead, it is proposed that axonal mitochondria may be more involved in electron transport chain-independent anabolic functions, such as amino acid biosynthesis, in order to support local protein synthesis and generate glutamate, the primary excitatory neurotransmitter used by these neurons. Indeed, aside from ATP production, the TCA cycle can also generate metabolites involved in a large array of cellular processes relevant to memory formation, in particular amino acid or neurotransmitter synthesis, as well as epigenetic modifications (Fig. 2). Up to nine amino acids can be synthesized from two precursors corresponding to TCA cycle intermediates, alpha-ketoglutarate and oxaloacetate. Local translation has been shown to be necessary for synaptic plasticity (Rajgor et al., 2021; Rangaraju et al., 2014, 2019; Wang et al., 2009). As previously discussed, *de novo* protein synthesis during LTM formation in rats requires

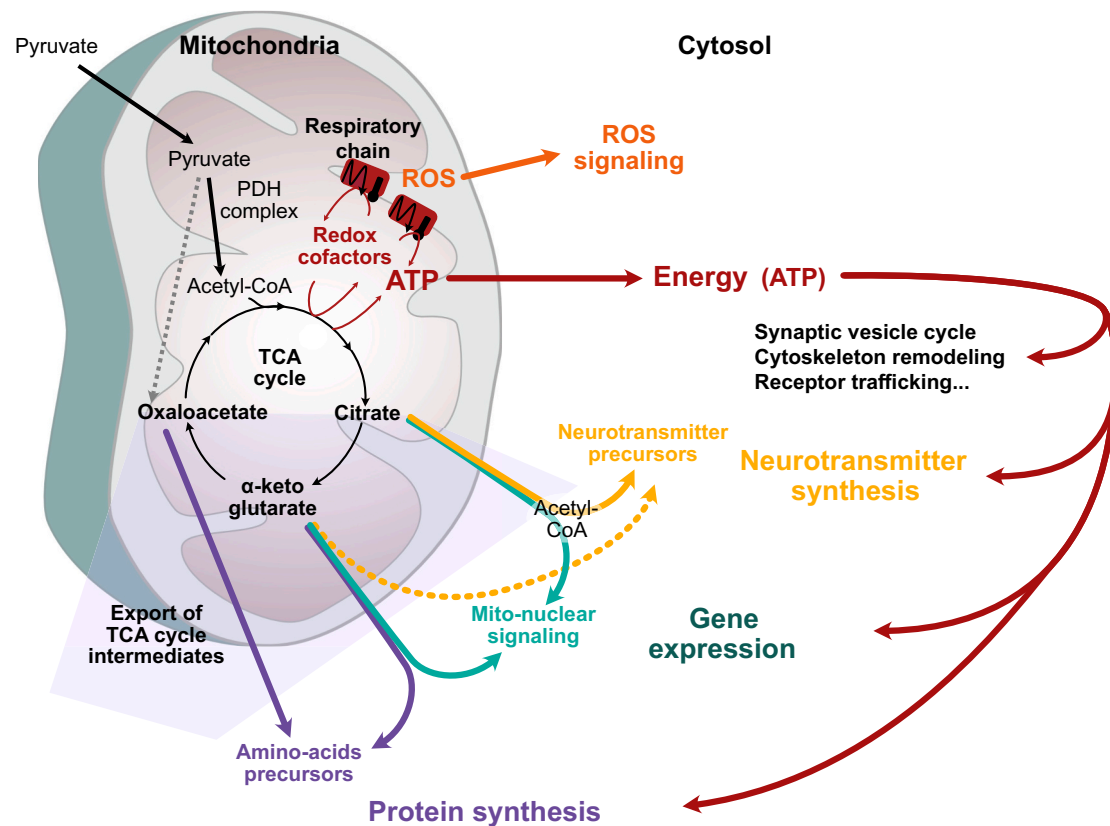


Fig. 2. Pyruvate-derived mitochondrial metabolic outputs involved in neuronal plasticity mechanisms underlying memory. Spaced conditioning triggers a prolonged increase in mitochondrial pyruvate consumption in the axonal compartment of MB neurons. Shown are the various outputs of pyruvate mitochondrial metabolism (ATP, TCA intermediates, ROS, etc.) involved in key processes of LTM formation that could be specifically supported by upregulated pyruvate mitochondrial metabolism.

lactate transfer to neurons from astrocytes (Descalzi et al., 2019). In this study, the role of lactate as a mitochondrial metabolic substrate for the TCA cycle was established by the fact that intrahippocampal injection of pyruvate or ketone bodies could rescue a deficient astrocytic lactate production, as with L-lactate injection. This does not exclude that, in addition to ATP production, TCA cycle intermediates may be diverted to new amino acid synthesis. Such a mechanism would allow the local production of amino acids by mitochondria on site, which seems more efficient than relying on amino acid production from mitochondria located remotely in the soma. This is also consistent with observations in *Drosophila* and mice of a differential activation of mitochondria metabolism across cellular compartments (Comyn et al., 2024; Underwood et al., 2023).

In addition to amino acid precursors, the biosynthesis of several neurotransmitters also relies on metabolites produced by the mitochondrial TCA cycle. Enhanced neurotransmitter release is another prominent feature of LTM formation (Miranda, 2007). In mammals, glutamate is the main excitatory neurotransmitter. Glutamate and GABA are thought to be primarily recycled from glutamine derived from the astrocytic reuptake and metabolism of neurotransmitters, i.e. the glutamate/GABA-glutamine shuttle (Bak et al., 2006). In principle at least, glutamate, and subsequently GABA, can be produced from the TCA intermediate α -ketoglutarate, so that one can speculate that increased neurotransmission demand, mitochondrial activation of the PDH complex and the TCA cycle could sustain neurotransmitter production for memory formation. It should be noted, however, that such a scheme would require anaplerotic pathways to be activated to compensate for carbon derivation out of the TCA cycle due to glutamate synthesis. This hypothesis could be even more relevant in invertebrates, where acetylcholine is the main excitatory neurotransmitter. In

particular, memory-relevant neurotransmission by *Drosophila* MB neurons is cholinergic (Barnstedt et al., 2016). Although acetylcholine synthesis occurs in the cytosol, it requires an acetyl group derived from the TCA cycle. As acetyl-CoA cannot be directly exported through the mitochondrial membrane, it is converted to citrate in the first step of the TCA cycle, which is then transported out and back-converted in the cytosol by ATP citrate lyase (ACLY) (Kopel et al., 2021). It still remains to be investigated whether acetylcholine levels during LTM formation in flies are dependent on the upregulation of mitochondrial pyruvate uptake.

Several metabolites produced by mitochondria can also be involved in epigenetic modification, another memory-relevant process (Creighton et al., 2020). Three main classes of epigenetic modifications, i.e. histone acetylation, histone demethylation and DNA demethylation, use TCA-related metabolites, which have led to an emerging concept of “mito-nuclear signaling” in the regulation of gene expression (Matilainen et al., 2017). Acetyl-CoA used for histone acetylation can be generated directly in the cytoplasm from acetate via acetyl-CoA synthetase 2 (ACSS2), or from citrate exported from the mitochondria, as explained earlier. Several studies have clearly established that epigenetic modifications such as histone acetylation are required for long-term memory formation both in vertebrates and invertebrates (Alexander et al., 2022; Day and Sweatt, 2010; Levenson et al., 2004; Mews et al., 2017; Raun et al., 2023). Currently, only the ACSS2 pathway has been proven necessary for LTM formation in mammals (Alexander et al., 2022; Mews et al., 2017), whereas the ACLY pathway remains to be investigated.

The demethylation of either histone or DNA is performed with demethylase enzymes of which several classes exist, including a family of enzymes called 2-oxoglutarate dependent dioxygenase enzymes that are activated by Fe(II) and α -ketoglutarate (also known as oxoglutarate).

This enzyme family encompasses the main lysine histone demethylases known as JMJD (for Jumonji C domain demethylases) and the DNA demethylation enzyme TET (for ten-eleven translocation) (Matilainen et al., 2017). Several studies have shown that DNA demethylation is involved in memory formation (Alaghband et al., 2016; Day and Sweatt, 2010). In particular, among the three tet mammalian genes, Tet3 is transcriptionally upregulated by synaptic activity, and Tet3 conditional KO mice display spatial memory defects (Li et al., 2014; Yu et al., 2015), whereas Tet1 is involved in the regulation of well-known neuronal activity-dependent genes such as c-fos, Arc, and BDNF (Alaghband et al., 2016). As for acetylation, it is still an open question whether mitochondrial activation of the TCA cycle sustains memory-relevant DNA demethylation.

As a general comment about TCA cycle derivatives, it should be noted that diverting carbon atoms away from the TCA cycle, whether for biosynthesis or epigenetic regulation, requires a compensatory input of new TCA intermediates through the anaplerotic action of the pyruvate carboxylase mitochondrial enzyme. Among the various metabolites that can be converted into acetyl-CoA to fuel the TCA cycle, only pyruvate can also fuel anaplerosis. This could provide a rationale for the critical requirement of pyruvate mitochondrial uptake for LTM, as revealed in flies (Plaçaïs et al., 2017; Rabah et al., 2023a; Raun et al., 2023). In particular, aversive LTM formation is disabled in flies upon starvation (Plaçaïs and Preat, 2013). Remarkably, it was shown that in such a prolonged fasting context, less-persistent aversive memory formation can still occur, but relying on ketone body consumption by mitochondria in MB neurons, rather than pyruvate (Silva et al., 2022).

In addition to ATP generation, the mitochondrial respiratory chain can lead to the production of ROS. The electron transport chain generates ROS primarily at complexes I and III. Complex I (and eventually complex II) produces ROS directed toward the mitochondrial matrix, which can be dissipated by matrix antioxidant defense, while complex III generates ROS that can diffuse both into the matrix and the intermembrane space, allowing easier diffusion into the cytosol (Chen et al., 2003). Beyond their intrinsic toxicity, ROS are increasingly recognized as neuronal signaling molecules (Biswas et al., 2022; Kishida and Klann, 2007; Oswald et al., 2018), notably involved in the processes of synaptic plasticity (Oswald et al., 2018; Vicente-Gutiérrez et al., 2021) and memory formation (Rabah et al., 2023b). In mice, the production of ROS by astrocytic mitochondria has been shown to be critical for some forms of memory, although their precise role is not fully understood (Morant-Ferrando et al., 2023; Vicente-Gutiérrez et al., 2019; Vicente-Gutiérrez et al., 2021). Notably, ROS can also be produced by cytosolic enzymes such as NADP-oxidase, and one recent report showed in *Drosophila* that glia-derived cytosolic ROS production is required in MB neurons for LTM formation (Rabah et al., 2023b), likely for signaling purposes.

Regarding neuronal mitochondria, several pieces of evidence point toward a role for ROS produced by mitochondria in memory-related processes. For example, mitoflashes, which stabilize the short-term enlargement of hippocampal neuron spines giving rise to persistent synaptic LTP, induce localized elevations of ROS levels at the dendrites (Fu et al., 2017). Pharmacological ROS scavengers suppress mitoflash activity, which only allows the formation of short-term LTP and prevents its long-term expression, indicating a role of ROS in the stabilization of postsynaptic changes (Fu et al., 2017). Furthermore, in the context of spinal LTP involved in pain persistence, increases in mitochondrial calcium concentration were found to be accompanied by ROS production in neurons likely via activation of mitochondria metabolism (Kim et al., 2011). *In vivo*, the crucial role of mitochondrial ROS in memory formation was recently revealed in mice. Specifically, ablation of the mitochondrial ATPase inhibitory factor 1 (IF1) in neurons, which reduced ROS production, significantly impaired long-term memory assessed by the novel object recognition task. On the other hand, IF1 overexpression enhanced synaptic transmission and memory. These effects were partially lost upon antioxidant MitoQ administration, suggesting a role of mitochondrial ROS increase in response to IF1

overexpression (Esparza-Moltó et al., 2021). However, this does not exclude additional contributions from other mitochondrial outputs. In particular, the impact of IF1 modulation on ATP production in the context of memory formation has not been assessed.

Although these reports are consistent with a role for mitochondrial ROS in plasticity and memory, direct evidence of increased ROS production by neuronal mitochondria, resulting from the activation of their metabolic flux, has not yet been reported. Whatever the source of ROS, we must determine whether ROS-mediated redox signaling actually underlies memory formation, and what classes of proteins (*i.e.* redox-sensitive transcription factors, metabolic enzymes, protein complexes involved in synaptic plasticity) it may target.

5. Conclusion and perspectives

In light of the literature discussed in this review, mitochondria appear as an integral component of the cognitive process leading to memory formation, in particular long-term memory storage. Together, the regulation of mitochondrial metabolic activity, the spatial reorganization of the mitochondrial network in neurons, the recruitment of specific signaling pathways acting on mitochondria following learning, and possibly the re-allocation of mitochondrial output fluxes sketch an emerging concept of learning-induced mitochondrial plasticity that is pivotal in controlling the strength and persistence of memory.

Understanding the mechanisms through which mitochondria intervene in memory formation also opens new perspectives regarding age-related memory deficits. Indeed, mitochondrial dysfunction and defects affect various aspects of synaptic plasticity, and are linked to cognitive defects in numerous human conditions, especially in neurodegenerative diseases such as Alzheimer's or Parkinson's disease (Camandola and Mattson, 2017; Mattson et al., 2008). A large number of studies have shown impaired metabolism in aged brains due to alterations in mitochondrial structure (Dorszewska, 2013; Toescu et al., 2000) such as microvacuolization, broken cristae, and accumulation of paracrystalline inclusions, as well as a characteristic donut shape associated with a working memory defect in aged monkeys (Hara et al., 2014). Functionally, it was shown that the neuronal mitochondrial defects that accompany synaptic and memory impairment with age are i) reduced ATP production (Olesen et al., 2020); ii) increased ROS generation (Knapp and Klann, 2002; Olesen et al., 2020; Serrano and Klann, 2004); iii) mitochondrial calcium homeostasis impairment (Jung et al., 2020; Toescu et al., 2000; Xiong et al., 2002); and iv) mitochondrial DNA mutations (Indo et al., 2007; Sedelnikova et al., 2004). However, a key challenge in the field is to prove whether mitochondria deficits are a cause of aging and its accompanying memory deficits, or if they are markers of the aging process.

Funding

T.C. was funded by doctoral fellowships from the French Ministry of Research for three years, and from the Fondation pour la Recherche Médicale (FRM) for one year (grant number FDT202304016704).

CRediT authorship contribution statement

Typhaine Comyn: Writing – original draft. **Thomas Preat:** Writing – review & editing. **Alice Pavlowsky:** Writing – original draft, Writing – review & editing. **Pierre-Yves Plaçaïs:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- Abel, T., Martin, K.C., Bartsch, D., Kandel, E.R., 1998. Memory suppressor genes: inhibitory constraints on the storage of long-term memory. *Science* 279, 338–341. <https://doi.org/10.1126/science.279.5349.338>.
- Alaghband, Y., Bredy, T.W., Wood, M.A., 2016. The role of active DNA demethylation and Tet enzyme function in memory formation and cocaine action. *Neurosci. Lett.* 625, 40–46. <https://doi.org/10.1016/j.neulet.2016.01.023>.
- Alexander, D.C., Corman, T., Mendoza, M., Glass, A., Belity, T., Wu, R., Campbell, R.R., Han, J., Keiser, A.A., Winkler, J., Wood, M.A., Kim, T., Garcia, B.A., Cohen, H., Mews, P., Egervari, G., Berger, S.L., 2022. Targeting acetyl-CoA metabolism attenuates the formation of fear memories through reduced activity-dependent histone acetylation. *Proc. Natl. Acad. Sci.* 119, e2114758119. <https://doi.org/10.1073/pnas.2114758119>.
- Ashrafi, G., Wu, Z., Farrell, R.J., Ryan, T.A., 2017. GLUT4 mobilization supports energetic demands of active synapses. *Neuron* 93, 606–615 e3. <https://doi.org/10.1016/j.neuron.2016.12.020>.
- Ashrafi, G., de Juan-Sanz, J., Farrell, R.J., Ryan, T.A., 2020. Molecular tuning of the axonal mitochondrial Ca²⁺ uniporter ensures metabolic flexibility of neurotransmission. *Neuron* 105, 678–687 e5. <https://doi.org/10.1016/j.neuron.2019.11.020>.
- Aso, Y., Hattori, D., Yu, Y., Johnston, R.M., Iyer, N.A., Ngo, T.T.B., Dionne, H., Abbott, L. F., Axel, R., Tanimoto, H., Rubin, G.M., 2014. The neuronal architecture of the mushroom body provides a logic for associative learning. *eLife* 3, e04577. <https://doi.org/10.7554/eLife.04577>.
- Bailey, C.H., Kandel, E.R., Harris, K.M., 2015. Structural components of synaptic plasticity and memory consolidation. *Cold Spring Harb. Perspect. Biol.* 7, a021758. <https://doi.org/10.1101/cshperspect.a021758>.
- Bak, L.K., Schousboe, A., Waagepetersen, H.S., 2006. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* 98, 641–653. <https://doi.org/10.1111/j.1471-4159.2006.03913.x>.
- Barnstedt, O., Oswald, D., Felsenberg, J., Brain, R., Moszynski, J.-P., Talbot, C.B., Perrat, P.N., Waddell, S., 2016. Memory-relevant mushroom body output synapses are cholinergic. *Neuron* 89, 1237–1247. <https://doi.org/10.1016/j.neuron.2016.02.015>.
- Bennett, M.C., Rose, G.M., 1992. Chronic sodium azide treatment impairs learning of the Morris water maze task. *Behav. Neural Biol.* 58, 72–75. [https://doi.org/10.1016/0163-1047\(92\)90967-9](https://doi.org/10.1016/0163-1047(92)90967-9).
- Bennett, M.C., Diamond, D.M., Stryker, S.L., Parks, J.K., Parker, W.D., 1992. Cytochrome oxidase inhibition: a novel animal model of Alzheimer's disease. *J. Geriatr. Psychiatry Neurol.* 5, 93–101. <https://doi.org/10.1177/002383099200500206>.
- Billups, B., Forsythe, I.D., 2002. Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses. *J. Neurosci.* 22, 5840–5847. <https://doi.org/10.1523/JNEUROSCI.22-14-05840.2002>.
- Biswas, K., Alexander, K., Francis, M.M., 2022. Reactive oxygen species: angels and demons in the life of a neuron. *NeuroSci* 3, 130–145. <https://doi.org/10.3390/neurosci3010011>.
- Bouzaiane, E., Trannoy, S., Scheunemann, L., Plaçaïs, P.Y., Preat, T., 2015. Two independent mushroom body output circuits retrieve the six discrete components of *Drosophila* aversive memory. *Cell Rep.* 11, 1280–1292. <https://doi.org/10.1016/j.celrep.2015.04.044>.
- Brown, H.L.D., Kaun, K.R., Edgar, B.A., 2012. The small GTPase Rheb affects central brain neuronal morphology and memory formation in *Drosophila*. *PLoS One* 7, e44888. <https://doi.org/10.1371/journal.pone.0044888>.
- Buttgereit, F., Brand, M.D., 1995. A hierarchy of ATP-consuming processes in mammalian cells. *Biochem. J.* 312, 163–167.
- Camandola, S., Mattson, M.P., 2017. Brain metabolism in health, aging, and neurodegeneration. *EMBO J.* 36, 1474–1492. <https://doi.org/10.15252/emboj.201695810>.
- Cassar, M., Issa, A.R., Riemensperger, T., Petitgas, C., Rival, T., Coulou, H., Iché-Torres, M., Han, K.A., Birman, S., 2015. A dopamine receptor contributes to paraquat-induced neurotoxicity in *Drosophila*. *Hum. Mol. Genet.* 24, 197–212. <https://doi.org/10.1093/hmg/ddu430>.
- Chang, D.T.W., Reynolds, L.J., 2006. Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. *Neuroscience* 141, 727–736. <https://doi.org/10.1016/j.neuroscience.2006.01.034>.
- Chen, Q., Vazquez, E.J., Moghaddas, S., Hoppel, C.L., Lesnefsky, E.J., 2003. Production of reactive oxygen species by mitochondria: CENTRAL ROLE OF COMPLEX III*. *J. Biol. Chem.* 278, 36027–36031. <https://doi.org/10.1074/jbc.M304854200>.
- Comyn, T., Preat, T., Pavlowsky, A., Plaçaïs, P.-Y., 2024. PKCδ is an activator of neuronal mitochondrial metabolism that mediates the spacing effect on memory consolidation. *eLife* 13. <https://doi.org/10.7554/eLife.92085.1>.
- Costa-Mattioli, M., Sossin, W.S., Klann, E., Sonenberg, N., 2009. Translational control of long-lasting synaptic plasticity and memory. *Neuron* 61, 10–26. <https://doi.org/10.1016/j.neuron.2008.10.055>.
- Creighton, S.D., Stefanelli, G., Reda, A., Zovkic, I.B., 2020. Epigenetic mechanisms of learning and memory: implications for aging. *Int. J. Mol. Sci.* 21, 6918. <https://doi.org/10.3390/ijms21186918>.
- Davis, H.P., Squire, L.R., 1984. Protein synthesis and memory: a review. *Psychol. Bull.* 96, 518–559.
- Day, J.J., Sweatt, J.D., 2010. DNA methylation and memory formation. *Nat. Neurosci.* 13, 1319–1323. <https://doi.org/10.1038/nn.2666>.
- Denton, R.M., Randle, P.J., Martin, B.R., 1972. Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. *Biochem. J.* 128, 161–163. <https://doi.org/10.1042/bj1280161>.
- Descalzi, G., Gao, V., Steinman, M.Q., Suzuki, A., Alberini, C.M., 2019. Lactate from astrocytes fuels learning-induced mRNA translation in excitatory and inhibitory neurons. *Commun. Biol.* 2, 247. <https://doi.org/10.1038/s42003-019-0495-2>.
- Devine, M.J., Kittler, J.T., 2018. Mitochondria at the neuronal presynapse in health and disease. *Nat. Rev. Neurosci.* 19, 63–80. <https://doi.org/10.1038/nrn.2017.170>.
- Divakaruni, S.S., Van Dyke, A.M., Chandra, R., LeGates, T.A., Contreras, M., Dharmasri, P.A., Higgs, H.N., Lobo, M.K., Thompson, S.M., Blanpied, T.A., 2018. Long-term potentiation requires a rapid burst of dendritic mitochondrial fission during induction. *Neuron* 100, 860–875 e7. <https://doi.org/10.1016/j.neuron.2018.09.025>.
- Dorszewska, J., 2013. Cell biology of normal brain aging: synaptic plasticity-cell death. *Aging Clin. Exp. Res.* 25, 25–34. <https://doi.org/10.1007/s40520-013-0004-2>.
- Duquesnes, N., Lezoualc'h, F., Crozatier, B., 2011. PKC-delta and PKC-epsilon: foes of the same family or strangers? *J. Mol. Cell. Cardiol.* 51, 665–673. <https://doi.org/10.1016/j.yjmcc.2011.07.013>.
- Esparza-Moltó, P.B., Romero-Carramiñana, I., de Arenas, C.N., Pereira, M.P., Blanco, N., Pardo, B., Bates, G.R., Sánchez-Castillo, C., Artuch, R., Murphy, M.P., Esteban, J.A., Cuezva, J.M., 2021. Generation of mitochondrial reactive oxygen species is controlled by ATPase inhibitory factor 1 and regulates cognition. *PLoS Biol.* 19, e3001252. <https://doi.org/10.1371/journal.pbio.3001252>.
- Fu, Z.-X., Tan, X., Fang, H., Lau, P.-M., Wang, X., Cheng, H., Bi, G.-Q., 2017. Dendritic mitoflash as a putative signal for stabilizing long-term synaptic plasticity. *Nat. Commun.* 8, 31. <https://doi.org/10.1038/s41467-017-00043-3>.
- Glancy, B., Balaban, R.S., 2012. Role of mitochondrial Ca²⁺ in the regulation of cellular energetics. *Biochemistry* 51, 2959–2973. <https://doi.org/10.1021/bi2018909>.
- Glas, A., Hübener, M., Bonhoeffer, T., Goltstein, P.M., 2021. Spaced training enhances memory and prefrontal ensemble stability in mice. *Curr. Biol.* 31, 4052–4061 e6. <https://doi.org/10.1016/j.cub.2021.06.085>.
- Glater, E.E., Megeath, L.J., Stowers, R.S., Schwarz, T.L., 2006. Axonal transport of mitochondria requires Milton to recruit kinesin heavy chain and is light chain independent. *J. Cell Biol.* 173, 545–557. <https://doi.org/10.1083/jcb.200601067>.
- Glock, C., Biever, A., Tushev, G., Nassim-Assir, B., Kao, A., Bartnik, I., Tom Dieck, S., Schuman, E.M., 2021. The translatome of neuronal cell bodies, dendrites, and axons. *Proc. Natl. Acad. Sci. USA* 118, e2113929118. <https://doi.org/10.1073/pnas.2113929118>.
- Hagena, H., Manahan-Vaughan, D., 2024. Interplay of hippocampal long-term potentiation and long-term depression in enabling memory representations. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 379, 20230229. <https://doi.org/10.1098/rstb.2023.0229>.
- Hara, Y., Yuk, F., Puri, R., Janssen, W.G.M., Rapp, P.R., Morrison, J.H., 2014. Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. *Proc. Natl. Acad. Sci. USA* 111, 486–491. <https://doi.org/10.1073/pnas.1311310110>.
- Harris, J.J., Jolivet, R., Attwell, D., 2012. Synaptic energy use and supply. *Neuron* 75, 762–777. <https://doi.org/10.1016/j.neuron.2012.08.019>.
- Helmstetter, F.J., Parsons, R.G., Gafford, G.M., 2008. Macromolecular synthesis, distributed synaptic plasticity, and fear conditioning. *Neurobiol. Learn. Mem.* 89, 324–337. <https://doi.org/10.1016/j.nlm.2007.09.002>.
- Himmelreich, S., Masuho, I., Berry, J.A., MacMullen, C., Skamangas, N.K., Martemyanov, K.A., Davis, R.L., 2017. Dopamine receptor DAMB signals via Gq to mediate forgetting in *Drosophila*. *Cell Rep.* 21, 2074–2081. <https://doi.org/10.1016/j.celrep.2017.10.108>.
- Hirabayashi, Y., Lewis, T.L., Du, Y., Virga, D.M., Decker, A.M., Coceano, G., Alvelid, J., Paul, M.A., Hamilton, S., Kneis, P., Takahashi, Y., Gaublomme, J.T., Testa, I., Polleux, F., 2024. Most Axonal Mitochondria in Cortical Pyramidal Neurons Lack Mitochondrial DNA and Consume ATP. *bioRxiv*. <https://doi.org/10.1101/2024.02.12.579972>, 2024.02.12.579972.
- Ikemoto, A., Bole, D.G., Ueda, T., 2003. Glycolysis and glutamate accumulation into synaptic vesicles. Role of glyceraldehyde phosphate dehydrogenase and 3-phosphoglycerate kinase. *J. Biol. Chem.* 278, 5929–5940. <https://doi.org/10.1074/jbc.M211617200>.
- Indo, H.P., Davidson, M., Yen, H.-C., Suenaga, S., Tomita, K., Nishii, T., Higuchi, M., Koga, Y., Ozawa, T., Majima, H.J., 2007. Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. *Mitochondrion* 7, 106–118. <https://doi.org/10.1016/j.mito.2006.11.026>.
- Jarome, T.J., Helmstetter, F.J., 2014. Protein degradation and protein synthesis in long-term memory formation. *Front. Mol. Neurosci.* 7, 61. <https://doi.org/10.3389/fnmol.2014.00061>.
- Jiang, D., Zhao, L., Clapham, D.E., 2009. Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca²⁺/H⁺ antiporter. *Science (New York, N.Y.)* 326, 144–147. <https://doi.org/10.1126/SCIENCE.1175145>.
- Jung, H., Kim, S.Y., Canbakis Cecen, F.S., Cho, Y., Kwon, S.-K., 2020. Dysfunction of mitochondrial Ca²⁺ regulatory machineries in brain aging and neurodegenerative diseases. *Front. Cell Dev. Biol.* 8.
- Kang, J.-S., Tian, J.-H., Pan, P.-Y., Zaid, P., Li, C., Deng, C., Sheng, Z.-H., 2008. Docking of axonal mitochondria by syntrophin controls their mobility and affects short-term facilitation. *Cell* 132, 137–148. <https://doi.org/10.1016/j.cell.2007.11.024>.
- Kim, H.Y., Lee, K.Y., Lu, Y., Wang, J., Cui, L., Kim, S.J., Chung, J.M., Chung, K., 2011. Mitochondrial Ca²⁺ uptake is essential for synaptic plasticity in pain. *J. Neurosci.* 31, 12982–12991. <https://doi.org/10.1523/JNEUROSCI.3093-11.2011>.

- Kishida, K.T., Klann, E., 2007. Sources and targets of reactive oxygen species in synaptic plasticity and memory. *Antioxid. Redox Signal.* 9, 233–244. <https://doi.org/10.1089/ars.2007.9.ft-8>.
- Knapp, L.T., Klann, E., 2002. Role of reactive oxygen species in hippocampal long-term potentiation: contributory or inhibitory? *J. Neurosci. Res.* 70, 1–7. <https://doi.org/10.1002/jnr.10371>.
- Konagaya, Y., Terai, K., Hirao, Y., Takakura, K., Imajo, M., Kamioka, Y., Sasaoka, N., Kakizuka, A., Sumiyama, K., Asano, T., Matsuda, M., 2017. A highly sensitive FRET biosensor for AMPK exhibits heterogeneous AMPK responses among cells and organs. *Cell Rep.* 21, 2628–2638. <https://doi.org/10.1016/j.celrep.2017.10.113>.
- Kopel, J.J., Bhutia, Y.D., Sivaprakasam, S., Ganapathy, V., 2021. Consequences of NaCT/SLC13A5/mINDY deficiency: good versus evil, separated only by the blood–brain barrier. *Biochem. J.* 478, 463–486. <https://doi.org/10.1042/BCJ20200877>.
- Lanahan, A., Worley, P., 1998. Immediate-early genes and synaptic function. *Neurobiol. Learn. Mem.* 70, 37–43. <https://doi.org/10.1006/nlme.1998.3836>.
- Lavington, E., Cogni, R., Kuczyński, C., Koury, S., Behrman, E.L., O'Brien, K.R., Schmidt, P.S., Eanes, W.F., 2014. A small system–high-resolution study of metabolic adaptation in the central metabolic pathway to temperate climates in *Drosophila melanogaster*. *Mol. Biol. Evol.* 31, 2032–2041. <https://doi.org/10.1093/molbev/msu146>.
- Lee, A., Hirabayashi, Y., Kwon, S.-K., Tommy, L., Lewis, J., Polleux, F., 2018. Emerging roles of mitochondria in synaptic transmission and neurodegeneration. *Curr. Opin. Physiol.* 3, 82. <https://doi.org/10.1016/j.cophys.2018.03.009>.
- Levenson, J.M., O'Riordan, K.J., Brown, K.D., Trinh, M.A., Molfese, D.L., Sweatt, J.D., 2004. Regulation of histone acetylation during memory formation in the hippocampus*. *J. Biol. Chem.* 279, 40545–40559. <https://doi.org/10.1074/jbc.M402229200>.
- Lewis, T.L., Kwon, S.-K., Lee, A., Shaw, R., Polleux, F., 2018. MFF-dependent mitochondrial fission regulates presynaptic release and axon branching by limiting axonal mitochondria size. *Nat. Commun.* 9, 5008. <https://doi.org/10.1038/s41467-018-07416-2>.
- Li, Z., Okamoto, K.-I., Hayashi, Y., Sheng, M., 2004. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 119, 873–887. <https://doi.org/10.1016/j.cell.2004.11.003>.
- Li, X., Wei, W., Zhao, Q.-Y., Widagdo, J., Baker-Andresen, D., Flavell, C.R., D'Alessio, A., Zhang, Y., Bredy, T.W., 2014. Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. *Proc. Natl. Acad. Sci. USA* 111, 7120–7125. <https://doi.org/10.1073/pnas.1318906111>.
- Li, S., Xiong, G.-J., Huang, N., Sheng, Z.-H., 2020. The cross-talk of energy sensing and mitochondrial anchoring sustains synaptic efficacy by maintaining presynaptic metabolism. *Nat. Metab.* 2, 1077–1095. <https://doi.org/10.1038/s42255-020-00289-0>.
- Liu, J., Qi, J., Chen, X., Li, Z., Hong, B., Ma, H., Li, G., Shen, L., Liu, D., Kong, Y., Zhai, H., Xie, Q., Han, H., Yang, Y., 2022. Fear memory-associated synaptic and mitochondrial changes revealed by deep learning-based processing of electron microscopy data. *Cell Rep.* 40, 111151. <https://doi.org/10.1016/j.celrep.2022.111151>.
- Lopez-Fabuel, I., Le Douce, J., Logan, A., James, A.M., Bonvento, G., Murphy, M.P., Almeida, A., Bolaños, J.P., 2016. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc. Natl. Acad. Sci. USA* 113, 13063–13068. <https://doi.org/10.1073/pnas.1613701113>.
- Lovas, J.R., Wang, X., 2013. The meaning of mitochondrial movement to a neuron's life. *Biochim. Biophys. Acta, Mol. Cell Res.* <https://doi.org/10.1016/j.bbamcr.2012.04.007>.
- MacAskill, A.F., Atkin, T.A., Kittler, J.T., 2010. Mitochondrial trafficking and the provision of energy and calcium buffering at excitatory synapses. *Eur. J. Neurosci.* 32, 231–240.
- Matilainen, O., Quirós, P.M., Auwerx, J., 2017. Mitochondria and epigenetics – crosstalk in homeostasis and stress. *Trends Cell Biol.* 27, 453–463. <https://doi.org/10.1016/j.tcb.2017.02.004>.
- Mattson, M.P., Gleichmann, M., Cheng, A., 2008. Mitochondria in neuroplasticity and neurological disorders. *Neuron* 60, 748–766. <https://doi.org/10.1016/j.neuron.2008.10.010>.
- Medler, K., Gleason, E.L., 2002. Mitochondrial Ca(2+) buffering regulates synaptic transmission between retinal amacrine cells. *J. Neurophysiol.* 87, 1426–1439. <https://doi.org/10.1152/jn.00627.2001>.
- Mergenthaler, P., Lindauer, U., Dienel, G.A., Meisel, A., 2013. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci.* 36, 587–597. <https://doi.org/10.1016/j.tins.2013.07.001>.
- Mews, P., Donahue, G., Drake, A.M., Luczak, V., Abel, T., Berger, S.L., 2017. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. *Nature* 546, 381–386. <https://doi.org/10.1038/nature22405>.
- Minatohara, K., Akiyoshi, M., Okuno, H., 2016. Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace. *Front. Mol. Neurosci.* 8, 78. <https://doi.org/10.3389/fnmol.2015.00078>.
- Miranda, M.I., 2007. Changes in neurotransmitter extracellular levels during memory formation. In: Bermúdez-Rattoni, F. (Ed.), *Neural Plasticity and Memory: From Genes to Brain Imaging*. Frontiers in Neuroscience. CRC Press/Taylor & Francis, Boca Raton (FL).
- Mizuno, N., Itoh, H., 2009. Functions and regulatory mechanisms of Gq-signaling pathways. *Neurosignals* 17, 42–54. <https://doi.org/10.1159/000186689>.
- Morant-Ferrando, B., Jimenez-Blasco, D., Alonso-Batan, P., Agulla, J., Lapresa, R., García-Rodríguez, D., Yunta-Sánchez, S., Lopez-Fabuel, I., Fernández, E., Carmeliet, P., Almeida, A., García-Macia, M., Bolaños, J.P., 2023. Fatty acid oxidation organizes mitochondrial supercomplexes to sustain astrocytic ROS and cognition. *Nat. Metab.* 5, 1290–1302. <https://doi.org/10.1038/s42255-023-00835-6>.
- Newman, L.A., Korol, D.L., Gold, P.E., 2011. Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS One* 6, e28427. <https://doi.org/10.1371/journal.pone.0028427>.
- Nishijima, S., Maruyama, I.N., 2017. Appetitive olfactory learning and long-term associative memory in *Caenorhabditis elegans*. *Front. Behav. Neurosci.* 11, 80. <https://doi.org/10.3389/fnbeh.2017.00080>.
- Oettinghaus, B., Schulz, J.M., Restelli, L.M., Licci, M., Savoia, C., Schmidt, A., Schmitt, K., Grimm, A., Morè, L., Hench, J., Tolnay, M., Eckert, A., D'Adamo, P., Franken, P., Ishihara, N., Mihara, K., Bischofberger, J., Scorrano, L., Frank, S., 2016. Synaptic dysfunction, memory deficits and hippocampal atrophy due to ablation of mitochondrial fission in adult forebrain neurons. *Cell Death Differ.* 23, 18–28. <https://doi.org/10.1038/cdd.2015.39>.
- Olesen, M.A., Torres, A.K., Jara, C., Murphy, M.P., Tapia-Rojas, C., 2020. Premature synaptic mitochondrial dysfunction in the hippocampus during aging contributes to memory loss. *Redox Biol.* 34, 101558. <https://doi.org/10.1016/j.redox.2020.101558>.
- Oswald, M.C.W., Garnham, N., Sweeney, S.T., Landgraf, M., 2018. Regulation of neuronal development and function by ROS. *FEBS Lett.* 592, 679–691. <https://doi.org/10.1002/1873-3468.12972>.
- Pavlovsky, A., Comyn, T., Minatchy, J., Geny, D., Bun, P., Danglot, L., Preat, T., Plaçais, P.-Y., 2024. Spaced training activates Miro/Milton-dependent mitochondrial dynamics in neuronal axons to sustain long-term memory. *Curr. Biol.* <https://doi.org/10.1016/j.cub.2024.03.050>. S0969982224003907.
- Pekkmuraz, G., Wang, X., 2022. Mitochondrial heterogeneity and homeostasis through the lens of a neuron. *Nat. Metab.* 4, 802–812. <https://doi.org/10.1038/s42255-022-00594-w>.
- Pekkmuraz, G., Trinidad, J.C., Wang, X., Kong, D., Schwarz, T.L., 2014. Glucose regulates mitochondrial motility via Milton modification by O-GlcNAc transferase. *Cell* 158, 54–68. <https://doi.org/10.1016/j.cell.2014.06.007>.
- Plaçais, P.-Y., Preat, T., 2013. To favor survival under food shortage, the brain disables costly memory. *Science* 339, 440–442. <https://doi.org/10.1126/science.1226018>.
- Plaçais, P.Y., Trannoy, S., Isabel, G., Aso, Y., Siwanowicz, I., Belliart-Guérin, G., Vernier, P., Birman, S., Tanimoto, H., Preat, T., 2012. Slow oscillations in two pairs of dopaminergic neurons gate long-term memory formation in *Drosophila*. *Nat. Neurosci.* 15, 592–599. <https://doi.org/10.1038/nn.3055>.
- Plaçais, P.Y., De Tredern, É., Scheunemann, L., Trannoy, S., Goguel, V., Han, K.A., Isabel, G., Preat, T., 2017. Upregulated energy metabolism in the *Drosophila* mushroom body is the trigger for long-term memory. *Nat. Commun.* 8, 1–14. <https://doi.org/10.1038/ncomms15510>.
- Pulido, C., Ryan, T.A., 2021. Synaptic vesicle pools are a major hidden resting metabolic burden of nerve terminals. *Sci. Adv.* 7, eabi9027. <https://doi.org/10.1126/sciadv.abi9027>.
- Purdon, A.D., Rapoport, S.I., 2007. 4.6 energy consumption by phospholipid metabolism in mammalian brain. In: Lajtha, A., Gibson, G.E., Dienel, G.A. (Eds.), *Handbook of Neurochemistry and Molecular Neurobiology: Brain Energetics. Integration of Molecular and Cellular Processes*. Springer US, Boston, MA, pp. 401–427. https://doi.org/10.1007/978-0-387-30411-3_15.
- Quinn, W.G., Sziber, P.P., Booker, R., 1979. The *Drosophila* memory mutant amnesiac. *Nature* 277, 212–214. <https://doi.org/10.1038/277212a0>.
- Rabah, Y., Francés, R., Minatchy, J., Guédon, L., Desnous, C., Plaçais, P.-Y., Preat, T., 2023a. Glycolysis-derived alanine from glia fuels neuronal mitochondria for memory in *Drosophila*. *Nat. Metab.* 1–18. <https://doi.org/10.1038/s42255-023-00910-y>.
- Rabah, Y., Sagar, N., Pasquer, L., Plaçais, P.-Y., Preat, T., 2023b. Long-Term Memory Formation Depends on an Astrocyte-to-Neuron H2O2 Signaling. <https://doi.org/10.1101/2023.07.11.548505>.
- Raefsky, S.M., Mattson, M.P., 2017. Adaptive responses of neuronal mitochondria to bioenergetic challenges: roles in neuroplasticity and disease resistance. *Free Radic. Biol. Med.* 102, 203–216. <https://doi.org/10.1016/j.freeradbiomed.2016.11.045>.
- Rajgor, D., Welle, T.M., Smith, K.R., 2021. The coordination of local translation, membranous organelle trafficking, and synaptic plasticity in neurons. *Front. Cell Dev. Biol.* 9, 711446. <https://doi.org/10.3389/fcell.2021.711446>.
- Rangaraju, V., Calloway, N., Ryan, T.A., 2014. Activity-driven local ATP synthesis is required for synaptic function. *Cell* 156, 825–835. <https://doi.org/10.1016/j.cell.2013.12.042>.
- Rangaraju, V., Lauterbach, M., Schuman, E.M., 2019. Spatially stable mitochondrial compartments fuel local translation during plasticity. *Cell* 176, 73–84 e15. <https://doi.org/10.1016/j.cell.2018.12.013>.
- Raun, N., Jones, S.G., Kerr, O., Keung, C., Ibrahim, V., Williams, M., Top, D., Kramer, J. M., 2023. *Trihorax* regulates long-term memory in *Drosophila* through epigenetic maintenance of mushroom body metabolic identity and translation capacity (preprint). *Mol. Biol.* <https://doi.org/10.1101/2023.08.08.549887>.
- Richter, J.D., Klann, E., 2009. Making synaptic plasticity and memory last: mechanisms of translational regulation. *Genes Dev.* 23, 1–11. <https://doi.org/10.1101/gad.1735809>.
- Rossi, M.J., Pekkmuraz, G., 2019. Powerhouse of the mind: mitochondrial plasticity at the synapse. *Curr. Opin. Neurobiol.* 57, 149–155. <https://doi.org/10.1016/j.conb.2019.02.001>.
- Rumpf, S., Sanal, N., Marzano, M., 2023. Energy metabolic pathways in neuronal development and function. *Oxf. Open Neurosci.* 2, kvad004. <https://doi.org/10.1093/oons/kvad004>.
- Sajic, M., Mastrolia, V., Lee, C.Y., Trigo, D., Sadeghian, M., Mosley, A.J., Gregson, N.A., Duchon, M.R., Smith, K.J., 2013. Impulse conduction increases mitochondrial transport in adult mammalian peripheral nerves in vivo. *PLoS Biol.* 11, e1001754. <https://doi.org/10.1371/journal.pbio.1001754>.

- Saxton, W.M., Hollenbeck, P.J., 2012. The axonal transport of mitochondria. *J. Cell Sci.* 125, 2095–2104. <https://doi.org/10.1242/jcs.053850>.
- Scheunemann, L., Plaçais, P.-Y., Dromard, Y., Schwärzel, M., Preat, T., 2018. Duncce phosphodiesterase acts as a checkpoint for *Drosophila* long-term memory in a pair of serotonergic neurons. *Neuron* 98, 350–365 e5. <https://doi.org/10.1016/j.neuron.2018.03.032>.
- Sedelnikova, O.A., Horikawa, I., Zimonjic, D.B., Popescu, N.C., Bonner, W.M., Barrett, J. C., 2004. Senescing human cells and ageing mice accumulate DNA lesions with unreparable double-strand breaks. *Nat. Cell Biol.* 6, 168–170. <https://doi.org/10.1038/ncb1095>.
- Serrano, F., Klann, E., 2004. Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res. Rev.* 3, 431–443. <https://doi.org/10.1016/j.arr.2004.05.002>.
- Shaughnessy, J.J., 1977. Long-term retention and the spacing effect in free-recall and frequency judgments. *Am. J. Psychol.* 90, 587–598. <https://doi.org/10.2307/1421733>.
- Sheng, Z.-H., 2017. The interplay of axonal energy homeostasis and mitochondrial trafficking and anchoring. *Trends Cell Biol.* 27, 403–416. <https://doi.org/10.1016/j.tcb.2017.01.005>.
- Silva, B., Mantha, O.L., Schor, J., Pascual, A., Plaçais, P.-Y., Pavlowsky, A., Preat, T., 2022. Glia fuel neurons with locally synthesized ketone bodies to sustain memory under starvation. *Nat. Metab.* 4, 213–224. <https://doi.org/10.1038/s42255-022-00528-6>.
- Smith, H.L., Bourne, J.N., Cao, G., Chirillo, M.A., Ostroff, L.E., Watson, D.J., Harris, K.M., 2016. Mitochondrial support of persistent presynaptic vesicle mobilization with age-dependent synaptic growth after LTP. *eLife* 5, e15275. <https://doi.org/10.7554/eLife.15275>.
- Smit-Rigter, L., Rajendran, R., Silva, C.A.P., Spierenburg, L., Groeneweg, F., Ruimschotel, E.M., van Versendaal, D., van der Togt, C., Eysel, U.T., Heimel, J.A., Lohmann, C., Levelt, C.N., 2016. Mitochondrial dynamics in visual cortex are limited in vivo and not affected by axonal structural plasticity. *Curr. Biol.* 26, 2609–2616. <https://doi.org/10.1016/j.cub.2016.07.033>.
- Stoler, O., Stavsky, A., Khrapunsky, Y., Melamed, I., Stutzmann, G., Gitler, D., Sekler, I., Fleidervish, I., 2022. Frequency- and spike-timing-dependent mitochondrial Ca²⁺ signaling regulates the metabolic rate and synaptic efficacy in cortical neurons. *eLife* 11, e74606. <https://doi.org/10.7554/eLife.74606>.
- Sun, T., Qiao, H., Pan, P.-Y., Chen, Y., Sheng, Z.-H., 2013. Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Rep.* 4, 413–419. <https://doi.org/10.1016/j.celrep.2013.06.040>.
- Sutton, M.A., Schuman, E.M., 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* 127, 49–58. <https://doi.org/10.1016/j.cell.2006.09.014>.
- Sutton, M.A., Ide, J., Masters, S.E., Carew, T.J., 2002. Interaction between amount and pattern of training in the induction of intermediate- and long-term memory for sensitization in aplysia. *Learn. Mem.* 9, 29–40. <https://doi.org/10.1101/lm.44802>.
- Suzuki, A., Stern, S.A., Bozdagi, O., Huntley, G.W., Walker, R.H., Magistretti, P.J., Alberini, C.M., 2011. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. <https://doi.org/10.1016/j.cell.2011.02.018>.
- Tang, Y., Zucker, R.S., 1997. Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. *Neuron* 18, 483–491. [https://doi.org/10.1016/s0896-6273\(00\)81248-9](https://doi.org/10.1016/s0896-6273(00)81248-9).
- Tao, K., Matsuki, N., Koyama, R., 2014. AMP-activated protein kinase mediates activity-dependent axon branching by recruiting mitochondria to axon. *Dev. Neurobiol.* 74, 557–573. <https://doi.org/10.1002/dneu.22149>.
- Todorova, V., Blokland, A., 2017. Mitochondria and synaptic plasticity in the mature and aging nervous system. *Curr. Neuropharmacol.* 15, 166–173. <https://doi.org/10.2174/1570159X1466616041411821>.
- Toescu, E.C., Myronova, N., Verkhratsky, A., 2000. Age-related structural and functional changes of brain mitochondria. *Cell Calcium* 28, 329–338. <https://doi.org/10.1054/ceca.2000.0167>.
- Tully, T., Quinn, W.G., 1985. Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J. Comp. Physiol. A* 157, 263–277. <https://doi.org/10.1007/BF01350033>.
- Tully, T., Preat, T., Boynton, S.C., Del Vecchio, M., 1994. Genetic dissection of consolidated memory in *Drosophila*. *Cell* 79, 35–47. [https://doi.org/10.1016/0092-8674\(94\)90398-0](https://doi.org/10.1016/0092-8674(94)90398-0).
- Underwood, E.L., Redell, J.B., Hood, K.N., Maynard, M.E., Hylin, M., Waxham, M.N., Zhao, J., Moore, A.N., Dash, P.K., 2023. Enhanced presynaptic mitochondrial energy production is required for memory formation. *Sci. Rep.* 13, 14431. <https://doi.org/10.1038/s41598-023-40877-0>.
- Verstreken, P., Ly, C.V., Venken, K.J.T., Koh, T.W., Zhou, Y., Bellen, H.J., 2005. Synaptic mitochondria are critical for mobilization of reserve pool vesicles at *Drosophila* neuromuscular junctions. *Neuron* 47, 365–378. <https://doi.org/10.1016/j.neuron.2005.06.018>.
- Vicente-Gutiérrez, C., Bonora, N., Bobo-Jimenez, V., Jimenez-Blasco, D., Lopez-Fabuel, I., Fernandez, E., Josephine, C., Bonvento, G., Enriquez, J.A., Almeida, A., Bolaños, J.P., 2019. Astrocytic mitochondrial ROS modulate brain metabolism and mouse behaviour. *Nat. Metab.* 1, 201–211. <https://doi.org/10.1038/s42255-018-0031-6>.
- Vicente-Gutiérrez, C., Jiménez-Blasco, D., Quintana-Cabrera, R., 2021. Intertwined ROS and metabolic signaling at the neuron-astrocyte interface. *Neurochem. Res.* 46, 23–33. <https://doi.org/10.1007/s11064-020-02965-9>.
- Virga, D.M., Hamilton, S., Osei, B., Morgan, A., Kneis, P., Zamponi, E., Park, N.J., Hewitt, V.L., Zhang, D., Gonzalez, K.C., Russell, F.M., Grahame Hardie, D., Prudent, J., Bloss, E., Losonczy, A., Polleux, F., Lewis, T.L., 2024. Activity-dependent compartmentalization of dendritic mitochondria morphology through local regulation of fusion-fission balance in neurons in vivo. *Nat. Commun.* 15, 2142. <https://doi.org/10.1038/s41467-024-46463-w>.
- Vishwanath, A.A., Comyn, T., Chintaluri, C., Ramon-Duaso, C., Fan, R., Sivakumar, R., Lopez-Manzaneda, M., Preat, T., Vogels, T.P., Rangaraju, V., Busquets-Garcia, A., Plaçais, P.-Y., Pavlowsky, A., de Juan-Sanz, J., 2024. Mitochondrial Ca²⁺ Efflux Controls Neuronal Metabolism and Long-Term Memory across Species. <https://doi.org/10.1101/2024.02.01.578153>.
- Wang, X., Schwarz, T.L., 2009. The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell* 136, 163–174. <https://doi.org/10.1016/j.cell.2008.11.046>.
- Wang, D.O., Kim, S.M., Zhao, Y., Hwang, H., Miura, S.K., Sossin, W.S., Martin, K.C., 2009. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science* 324, 1536–1540. <https://doi.org/10.1126/science.1173205>.
- Wang, H., Vant, J.W., Zhang, A., Sanchez, R.G., Wu, Y., Micou, M.L., Luczak, V., Whiddon, Z., Carlson, N.M., Yu, S.B., Jabbo, M., Yoon, S., Abushawish, A.A., Ghassemian, M., Masubuchi, T., Gan, Q., Watanabe, S., Griffiths, E.R., Hammarlund, M., Singharoy, A., Pekkurnaz, G., 2024. Organization of a functional glycolytic metabolon on mitochondria for metabolic efficiency. *Nat. Metab.* 6, 1712–1735. <https://doi.org/10.1038/s42255-024-01121-9>.
- Watters, O., Connolly, N.M.C., König, H.-G., Düsselmann, H., Prehn, J.H.M., 2020. AMPK preferentially depresses retrograde transport of axonal mitochondria during localized nutrient deprivation. *J. Neurosci.* 40, 4798–4812. <https://doi.org/10.1523/JNEUROSCI.2067-19.2020>.
- Williams, J.M., Thompson, V.L., Mason-Parker, S.E., Abraham, W.C., Tate, W.P., 1998. Synaptic activity-dependent modulation of mitochondrial gene expression in the rat hippocampus. *Brain Res. Mol. Brain Res.* 60, 50–56. [https://doi.org/10.1016/s0169-328x\(98\)00165-x](https://doi.org/10.1016/s0169-328x(98)00165-x).
- Xiong, J., Verkhratsky, A., Toescu, E.C., 2002. Changes in mitochondrial status associated with altered Ca²⁺ homeostasis in aged cerebellar granule neurons in brain slices. *J. Neurosci.* 22, 10761–10771. <https://doi.org/10.1523/JNEUROSCI.22-24-10761.2002>.
- Yamagata, K., Sanders, L.K., Kaufmann, W.E., Yee, W., Barnes, C.A., Nathans, D., Worley, P.F., 1994. Rheb, a growth factor- and synaptic activity-regulated gene, encodes a novel Ras-related protein. *J. Biol. Chem.* 269, 16333–16339.
- Yang, W., Pang, D., Chen, M., Du, C., Jia, L., Wang, L., He, Y., Jiang, W., Luo, L., Yu, Z., Mao, M., Yuan, Q., Tang, P., Xia, X., Cui, Y., Jing, B., Platero, A., Liu, Y., Wei, Y., Worley, P.F., Xiao, B., 2021. Rheb mediates neuronal-activity-induced mitochondrial energetics through mTORC1-independent PDH activation. *Dev. Cell* 56, 811–825 e6. <https://doi.org/10.1016/j.devcel.2021.02.022>.
- Yu, S.B., Pekkurnaz, G., 2018. Mechanisms orchestrating mitochondrial dynamics for energy homeostasis. *J. Mol. Biol.* 430, 3922–3941. <https://doi.org/10.1016/j.jmb.2018.07.027>.
- Yu, H., Su, Y., Shin, J., Zhong, C., Guo, J.U., Weng, Y.-L., Gao, F., Geschwind, D.H., Coppola, G., Ming, G., Song, H., 2015. Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. *Nat. Neurosci.* 18, 836–843. <https://doi.org/10.1038/nn.4008>.
- Zala, D., Hinckelmann, M.-V., Yu, H., Lyra da Cunha, M.M., Liot, G., Cordelières, F.P., Marco, S., Saudou, F., 2013. Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell* 152, 479–491. <https://doi.org/10.1016/j.cell.2012.12.029>.