NEURAL REGENERATION RESEARCH
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Specific dendritic spine modifications and dendritic transport: From *in vitro* to *in vivo*

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Dendritic spines are small protrusions along dendrites that contain most of the excitatory synapses in principal neurons, playing a crucial role in neuronal function by creating a compartmentalized environment for signal transduction. The plasticity of spine morphologies provides a tunable handle to regulate calcium signal dynamics, allowing rapid regulation of protein expression necessary to establish and maintain synapses (Cornejo et al., 2022). If excitatory inputs were to be located primarily on dendritic shafts, dendrites would frequently short-circuit, preventing voltage signals from propagating (Cornejo et al., 2022). It is thus not surprising that the structural plasticity of dendritic spines is closely linked to synaptic plasticity and memory formation (Berry and Nedivi, 2017). While comprehensive *in vitro* studies have been conducted, *in vivo* studies that directly tackle the mechanism of dendritic transport and translation in regulating spine plasticity spatiotemporally are limited.

To the best of our knowledge, there are only two studies prior to ours that demonstrated tracking of RNA binding protein or mRNA transport *in vivo* in vertebrate systems. *Bestman and Cline* electroporated *Xenopus laevis* tadpoles with fluorescence-tagged cytoplasmic polyadenylation element binding protein 1 (CPEB1) and tracked the transport of CPEB1 in optic tectum by two-photon microscopy (Bestman and Cline, 2009). However, neurons in *Xenopus laevis* tadpole optical tectum are not spiny. In another study, Nwokafor et al. (2019) adopted knock-in mice containing MS2 binding stem loop sequences inserted at the 3′ untranslated region of *β-actin* to track *in vivo β-actin* transcripts tagged with GFP in the Layer 2/3 neurons of visual cortex. This study aimed at demonstrating the methodology, and an in-depth investigation of dendritic spine plasticity regulation by *β-actin* transport and translation was not in its scope.

Our recent studies thus aimed to address the *in vivo* mechanism of synaptic activity-dependent transport and translation at dendritic spines (Zhao et al., 2020; Fok et al., 2024). After demonstrating the functional specificity of kinesin 1 isoform, KIF5B, using *in vitro* primary hippocampal neurons, we generated a conditional knockout mouse model of *kif5b* to study the effects of impaired dendritic transport on the dendritic spine plasticity *in vivo* by two-photon imaging (Zhao et al., 2020). By using *in utero* electroporation, both Cre recombinases and Cre-dependent fluorescent proteins were simultaneously expressed in a *kif5b*-floxed transgenic mouse. This approach enables us to study the effects of postsynaptic knockout of KIF5B on dendritic spine plasticity (Fok et al., 2024). These experiments show that ablated dendritic KIF5B-mediated transport heightened basal dendritic spine turnover and impaired activity-dependent dendritic spine plasticity that would otherwise be necessary for mice to acquire freezing behaviors in auditory-cued fear conditioning and fear extinction (Fok et al., 2024). In addition to fear associative learning and extinction, mice with impaired KIF5B-mediated dendritic transport were also found to have other memory deficits, such as working memory, spatial memory, and social memory (Zhao et al., 2020). To visualize the impaired KIF5B-mediated dendritic transport, fluorescently tagged KIF5B cargoes, including FMRP, PSD95, and gephyrin, were exogenously expressed in Layer 2/3 pyramidal neurons in the frontal association cortex (Fok et al., 2024). The dynamics of FMRP and PSD95 dendritic transport were specifically implicated in KIF5B conditional knockout neurons and were correlated with spine instability and abnormal learning-dependent spine plasticity. To our knowledge, these studies are the first to provide *in vivo* evidence to support the important role of dendritic transport in regulating dendritic spine plasticity necessary for memory formation. While our study confirmed the specific need for KIF5B-dependent transport in dendrites to regulate plasticity-related proteins and RNA binding proteins, such as PSD95 and FMRP, and dendritic spine plasticity, it is noteworthy that the behavior deficits observed in *Camk2a-Cre;Kif5b*$^{fl/fl}$ conditional knockout mice could result from the combinatory deletion of *Kif5b* in both axons and dendrites involved in a memory circuit. Similar to CPEB1, FMRP is a well-studied RNA binding protein that assembles ribonucleoprotein granules. Therefore, these findings highlight the crucial role of localizing mRNA transcripts in dendrites and enabling their translation to facilitate activity-dependent spine plasticity, which is associated with the formation of memories.

The differences observed between *in vitro* and *in vivo* studies of synapses indicate potential variations in the demands and strategies involved in dendritic transport and local translation, suggesting the intriguing possibility of a more complex transport mechanism *in vivo*. Cultured neurons have a higher percentage of excitatory synapses located on the dendritic shaft and demonstrate a different developmental pattern compared to age-matched *in vivo* neurons (Boyer et al., 1998). The speed of dendritic transport also differs. Kinesin-1 is known to be the common motor for *β-actin* mRNA, FMRP, and CPEB1. We did not observe apparent FMRP translocation *in vivo* within 1 hour (Fok et al., 2024), albeit *in vitro* consensus of translocation at around 1–1.3 m per second (Zhao et al., 2020). Similarly, *β-actin* mRNA and CPEB1 were also observed to exhibit slower motility *in vivo* compared to their *in vitro* counterpart (Bestman and Cline, 2009; Nwokafor et al., 2019). Furthermore, it is also noteworthy that *in vivo* studies so far have tracked dendritic transport in cortical neurons, while *in vitro* studies were predominantly conducted in hippocampal neurons. Although this may explain partially their discrepancies, it also suggests an interesting hypothesis whereas dendritic transport mechanisms may vary among neuronal types. Finally, the transportation of cargoes *in vivo* could be limited to specific time windows corresponding to animal experience and brain activity, which could easily elude detection without prior knowledge of such regulatory mechanisms in different animal experiences. Awake intravital imaging of dendritic transport, similar to how our recent study was conducted, would be preferable compared to the imaging of anesthetized animals to address this question.

Synaptic activities upon artificial stimulations have been shown to induce dendritic transport and local translation at dendritic spines, which is necessary for structural plasticity. Several *in vitro* studies have elegantly adopted glutamate uncaging in live cells to demonstrate the recruitment of mRNAs to the stimulated spines that support the translation of new proteins and spine remodeling (Rangaraju et al., 2019). Sequestration of *β-actin* mRNA into condensates of membrane-less assemblies has also been shown to block *β-actin* translation at dendritic spines and induce spine shrinkage, as demonstrated by the optoMCP-FUS system in dissociated hippocampal neurons. This technique utilizes light to induce sequestration of MSB-tagged *β-actin* into small assemblies to disrupt translation (Lee et al., 2024). Interestingly, stimulation of spines does not always lead to an increase in local translation, but also leads to the reduction of translation through miRNA-mediated degradation (Sambandan et al., 2017). The enrichment of translation organelles near stimulated spines also supports this view. Studies on ribosomes, endoplasmic reticulum, and mitochondria have revealed that these organelles become less mobile and cluster near dendritic spines upon synaptic stimulation, creating an ideal environment to promote local translation. Depletion of such machineries is shown to impair spine modifications and protein synthesis (Rangaraju et al., 2019).

While *in vitro* studies have made significant advancements, it remains unclear how these processes translate to *in vivo* conditions. Owing to technological advancement, similar approaches previously carried out *in vitro* could be attempted *in vivo*, including the use of *in vivo* glutamate uncaging. Nevertheless, the controlled stimulation of dendritic spines by *in vivo* glutamate uncaging is ultimately a mimicry of a subset of synaptic cues at spines. It still poses a challenge to translate these artificial spine stimulations into normal physiological conditions.

Another approach would be to identify the stimulated spines retrospectively after the animal has gone through specific experience or learning task. Our study is an example of this approach, where the experience-associated spines related to fear conditioning and extinction are identified by the observed structural plasticity such as formation or elimination (Fok et al., 2024). By simultaneously tracking FMRP in the same dendrites, it is found that FMRP was transported to 2 µm proximity of the stimulated spines, suggesting the localization of mRNAs and translation machinery to support spine formation following stimuli in fear learning. Future studies can be carried out with the Targeted Recombination in Active Population (TRAP) transgenic mice to home in on the dendritic branches of activated neurons during memory formation (DeNardo et al., 2019). Furthermore, it is also possible to only label recently activated spines using a spine-directed fluorescent reporter expressed downstream of an immediate early gene promoter such as the e-GRASP technique (Lee et al., 2023). Combined with *in vivo* tracking of mRNAs (e.g., MS2-based mRNA reporting system), nascent protein synthesis (e.g., FRAP and FLIP microscopy), and plasticity-related protein transport/ turnover (e.g., PSD95-HaloTag mice) (Bulovaite et al., 2022), the relationship between spine plasticity, dendritic transport, and local translation can be analyzed retrospectively. It is also noteworthy that advances in correlated light and electron microscopy and high throughput electron microscopy technologies such as automated tape-collecting ultramicrotome combined with scanning electron microscopy allow a *post hoc* ultrastructural analysis of activity-related dendritic spines in a scale much higher than before to complement *in vivo* imaging of spine plasticity (Sohn et al., 2022). It is thus possible to validate local translation machinery increase in activity-related spines by electron microscopy analysis of polyribosomes, compared to non-related spines.

Moreover, there is an emerging perspective of an increasingly complex environment where dendritic local translation takes place. In the past two decades, the field has advanced a lot in the understanding of the decentralized capabilities of neurons to regulate translation machinery and synthesize proteins. Nonetheless, mRNA transcripts and ribosomes are believed to be originated from neuronal soma and are moved to distal dendrites through motor protein-dependent transport. Interestingly, emerging studies are introducing extracellular sources of mRNA and translation machinery such as ribosome and miRNA into the picture. Through the release and fusion of extracellular vesicles, inter-neuronal or glia-to-neuron transfer of mRNAs, miRNAs, and synaptic proteins were reported to impact dendritic signaling cascades, transport, local translation, and spine plasticity (see review such as Akbari-Gharalari et al., 2024). That being said, it remains to be answered about the prevalence and the extent neurons tap into these extracellular sources of translation materials. However, as the majority of studies heretofore about dendritic transport and local translation have come from cultured hippocampal neurons, it is expected to garner new insights when more *in vivo* studies are conducted in the future. For example, with the supply of extracellular sources of mRNAs and translation machinery, *in vivo* dendritic transport dynamics could simply rely less on long-distance transport from the soma. In our study, the effect of dendritic *Kif5b* knockout was not compensated by the endogenous presence of glial cells. However, we reported that the effect was not in the reduction of FMRP abundance in dendrites, but rather the fine localization of FMRP. As a proxy of mRNA presence in dendrites, our findings about KIF5B-dependent FMRP transport cannot rule out the role of glial cells as an external source of translation machinery. In addition, local translation regulation would also consider the states of the animal, which correlate with the activity of glia (Akbari-Gharalari et al., 2024). These are areas that can only be addressed through *in vivo* studies of dendritic transport and local translation.

Finally, dendritic spines are heterogeneous in their morphologies, molecular composition, and plasticity, which would pose different demands on dendritic transport and translation mechanisms (Berry and Nedivi, 2017). Spines have long been studied as the structural correlate for synapses, and their classification has been limited to the morphological perspective,

such as mushroom, thin, and stubby spines. Mushroom spines are believed to be the memory spines since they are the most stable and likely to contain the strongest synapses, while thin spines are believed to be the learning spines due to their plasticity (Berry and Nedivi, 2017). This forced morphological classification of dendritic spines is increasingly challenged as analyses reveal the continuum nature of spine morphology. Furthermore, *in vivo* visualization of synaptic markers such as PSD95 and gephyrin concurrently with spine structures has demonstrated another perspective to classifying spines, which is the molecular composition (Berry and Nedivi, 2017; Fok et al., 2024). Since spines contain most excitatory synapses, studies about the excitatory synaptome suggest that spines of the same morphological category still vary in their molecular composition and lifetime in different neuronal types and brain regions (Bulovaite et al., 2022). Why and how such heterogeneity of dendritic spines arises will then require further investigation in the future. Just like how synaptome complements our understanding of the connectome, the field would benefit from a more systemic and holistic description of spine heterogeneity across neuronal types and brain regions, which we term "spinome" in this perspective. Since spine plasticity (both formation and elimination) is a consistent indication of memory formation, it is intriguing to hypothesize memory-specific modifications to the "spinome." While most *in vivo* studies of dendritic spines randomly sample dendritic branches from one cortical area, future research that probes into these questions will require transgenic mouse line that labels endogenous synaptic markers to sort dendritic spine into subtypes, machine learning solutions to automatically score fluorescent puncta in a large scale, and super-resolution imaging by two-photon *in vivo* nanoscopy (e.g., STED) in multiple brain regions. With these technological advances, it will be possible to conduct high throughput imaging, for example, the dendritic spines in the somatosensory cortex and the thalamus in a sensory discrimination task with morphological and molecular composition details, to characterize the "spinome" in this behavior context.

In conclusion, dendritic spine plasticity is an important outcome of dendritic transport and local translation, as the constant fine-tuning of spine structures would require these processes to be well-regulated (**Figure 1**). By far, *in vivo* findings displayed different dendritic transport speeds and different sources of dendritic mRNAs and translation machinery. A global description of molecular heterogeneous spines would also provide a novel dimension, which we term "spinome," to understand memory formation and storage in addition to synaptome and connectome. How dendritic transport and local translation support the plasticity of molecularly heterogeneous spines in a memory context will be of great interest to the field in the future.

This work was supported by the National Natural Science Foundation of China (NSFC/RGC/JRF N_HKU735/21); Research Grant Council of Hong Kong, China (17102120, 17108821, 17103922, C1024-22GF, C7074-21G); Health and Medical Research Fund (HMRF 09200966) (to CSWL); and FRQS Postdoctoral Fellowship (to AHKF).

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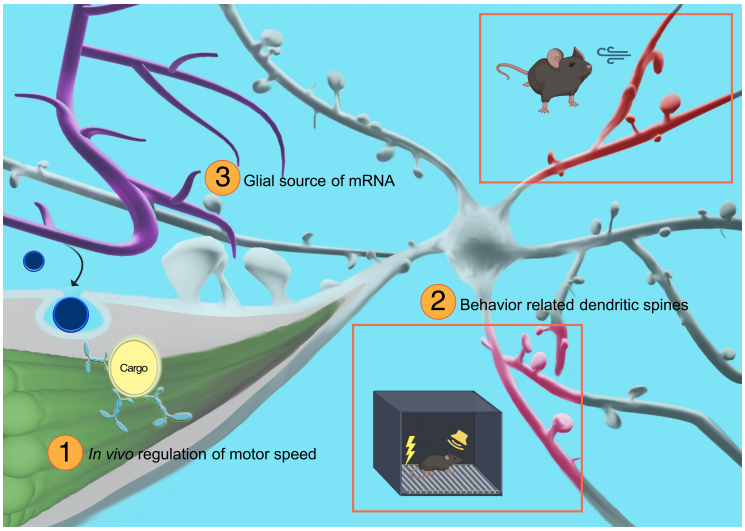

Figure 1 | **Summary schematic displaying the unique considerations for *in vivo* studies of dendritic transport and dendritic spine plasticity.**
(1) *In vivo* studies of dendritic transport thus far demonstrated significantly different transport dynamics of either mRNA or RNA-binding protein than *in vitro* findings. We speculate more complex mechanisms are involved *in vivo* to regulate motor protein (such as kinesin 1, light blue dimeric icon) speed on microtubules (green) such as synergistic movement of multiple motors for a cargo complex (yellow), coordination of multiple motor types attached to a cargo complex and stimulation dependent transport that is challenging to detect with anesthesia paradigms. (2) Studies of spine plasticity *in vivo* considering the behavior contexts. Mechanisms of dendritic transport and translation that support spine plasticity in one behavior context might be distinct from the other, given different synaptic cues, plasticity forms, signaling pathways involved. In the schematic, different behavior contexts (such as auditory-cued fear conditioning and whisker stimulation by air puffs) are designated to modulate different groups of spines under different mechanisms (colored in red and pink). Behavior related spine plasticity is the structural basis for memory formation. It is thus imperative to classify these spines a priori or a posteriori to investigate the underlying mechanisms of dendritic transport and translation with advanced genetic or analytic methodologies. (3) *In vivo* scenarios are further complicated by recent findings about inter-neuronal and glia-to-neuron transfer of mRNA and translational machinery (glia in purple, neuron in grey, extracellular vesicles in dark blue). The demand for long distance dendritic transport is speculated to be drastically different from *in vitro* scenarios as a result. Since glial secretory functions are closely tied to the behavior and internal states of the animals, mechanisms of dendritic transport, translation, and spine plasticity will likely depend on different behavior contexts or types of memories. These considerations add to the value of conducting *in vivo* studies of dendritic transport and translation. The models of neurons and glia are originally sketched by authors. Other graphics such as mice and icons of kinesin are adapted from BioRender.com.

Date of submission: September 27, 2024
Date of decision: November 12, 2024
Date of acceptance: December 4, 2024
Date of web publication: January 13, 2025

https://doi.org/10.4103/NRR.NRR-D-24-01159
How to cite this article: *Fok AHK, Lam CHM, Lai CSW (2026) Specific dendritic spine modifications and dendritic transport: From in vitro to in vivo. Neural Regen Res 21(2):665-666.*

Open peer reviewer: *Jun Noguchi, Kokuritsu Seishin Shinkei Center, Japan.*
Additional file: *Open peer review report 1.*

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P-Reviewer: Noguchi J; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y

特定的树突棘形态改变和树突运输：从体外到体内
文章特色分析

一、文题与摘要分析
- 文题：明确指出了文章的核心内容——树突棘的特异性修饰与树突运输机制，并强调从体外到体内的研究视角转变。
- 摘要：简要概述了树突棘在神经元兴奋性突触中的核心作用，指出其形态可塑性对钙信号、蛋白质表达及记忆形成的重要性。文章指出，尽管体外研究已较为深入，但体内研究仍较为有限，尤其是关于树突运输与局部翻译在时空上调控树突棘可塑性的机制。
二、文章重要性
1. 连接体外与体内研究：
   - 文章系统比较了体外与体内研究中树突运输速度、机制及调控方式的差异，强调体内环境的复杂性与行为背景的重要性。
2. 揭示记忆形成的结构基础：
   - 通过 KIF5B 条件性敲除模型，首次在体内证明树突运输对树突棘可塑性的必要性，并将其与多种记忆行为（恐惧记忆、空间记忆、社交记忆等）联系起来。
3. 提出“Spinome”概念：
   - 引入“Spinome”这一新概念，强调从分子组成与形态多样性角度全面理解树突棘的异质性，为理解记忆编码提供了新维度。
三、创新性特色
1. 体内研究的突破：
   - 使用双光子成像、在体电穿孔、条件性基因敲除等技术，首次在活体动物中追踪 RNA 结合蛋白（如 FMRP）和突触蛋白（如 PSD95）的树突运输，并揭示其与行为记忆的关联。
2. 多技术整合的研究策略：
   - 提出结合 TRAP 转基因小鼠、e-GRASP、MS2 mRNA 报告系统、电子显微镜等多种技术，实现对记忆相关树突棘的后验识别与机制解析。
3. 关注细胞外来源的翻译机制：
   - 提出神经元可能通过细胞外囊泡从胶质细胞获取 mRNA 与翻译机器，挑战了传统“所有翻译物质均来自胞体”的观点。
4. 强调行为背景对机制的影响：
   - 指出不同行为任务可能激活不同的树突运输与翻译机制，呼吁未来研究应在特定行为背景下分析树突棘的可塑性。
四、对学科的启示
1. 推动在体神经科学研究范式转变：
   - 强调从体外向体内研究的必要性，提倡在清醒动物中进行动态成像，以更真实地反映生理状态下树突运输与翻译的调控机制。
2. 促进多学科技术融合：
   - 文章展望了超分辨成像、机器学习、高通量电镜、分子标记技术等在解析“Spinome”中的应用，推动神经科学向更高精度、更高通量方向发展。
3. 拓展对记忆编码机制的理解：
   - 通过“Spinome”与“Synaptome”、“Connectome”的并列，提出应从结构、分子、功能多个层面综合理解记忆的形成与存储。
4. 启发对神经-胶质交互作用的研究：
   - 提出胶质细胞可能通过提供翻译物质参与树突棘可塑性的调控，为理解神经环路调控提供了新视角。

总结：该文章不仅在技术上展示了从体外到体内研究的跨越，在理论上也提出了“Spinome”等新概念，强调树突棘异质性、行为背景与细胞外来源物质对理解记忆机制的重要性。它为未来神经科学研究提供了方法论与概念框架上的双重启示，具有显著的前瞻性与引领性。