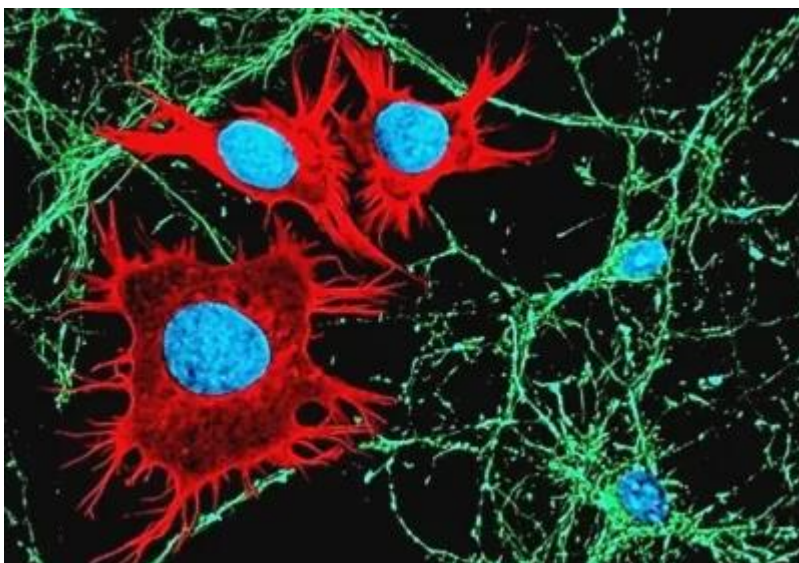


# D

Brain neurons form excitatory glutamatergic synapses with metastatic cells of non-neural cancers, mediated by AMPA glutamate receptors, with metastatic cancer cells on the postsynaptic side of the synapses | 1

Synapses are fundamental information-processing units of neuronal circuits. They form the basis for all brain functions by controlling the excitation-to-inhibition balance. Various cellular and molecular mechanisms underlie synapse formation. High-grade gliomas form synapses that hijack electrical signals from healthy nerve cells to drive their growth. In this study, authors from Germany investigated synapses between neurons and metastatic cells of non-neural cancers in the brain. They investigated at which stage of the brain metastasis cascade synapses between neurons and individual metastatic cancer cells are formed, and whether these synaptic contacts support metastasis and cancer progression.

Glutamate (Glu) AMPA receptors are heterotetrameric complexes composed of subunits GluR 1-4. These receptors mediate fast excitatory synaptic transmission in the central nervous system (CNS). The expression of glutamate AMPA receptors permeable for Ca<sup>2+</sup> in the glioma cells is a key feature of neuron-glioma synapses. The neuron-tumor synapses were not detected in brain tumors of lower malignancy (oligodendrogliomas or meningiomas). In contrast, very aggressive, incurable, primary, or secondary brain tumors receive neuronal synaptic input that drives a disease progression. It has been reported that the formation of excitatory synapses between presynaptic neurons and postsynaptic cancer cells in certain cancer types of neural origin stimulates tumor growth and invasion (Venkataramani, V. *et al. Nature* 2019; 573, 532-538. <https://www.nature.com/articles/s41586-019-1564-x>)





Brain neurons form excitatory glutamatergic synapses with metastatic cells of non-neural cancers, mediated by AMPA glutamate receptors, with metastatic cancer cells on the postsynaptic side of the synapses | 2

### ***About the study***

The authors assessed whether and at what stage of the brain metastatic cascade synapses between neurons and individual metastatic cancer cells were formed.

Human brain metastatic cells of non-neural cancers were injected into the left ventricle of mice older than 8 weeks. To investigate synaptic connections between neurons and brain metastatic cells, the researchers performed patch-clamp recordings of single breast cancer or melanoma cells during early growth in the perivascular niche. Female mice were used for the breast cancer model of brain metastases, and male mice for the melanoma model. To conduct intravital correlative microscopy, a chronic cranial window with a titanium ring was created in the mice at least three weeks before the injection of human metastatic cells.

### ***Results***

When circulating breast cancer and melanoma cells left the blood vessel and extravasated into the mouse brain, they were consistently found in a perivascular niche. This suggests that the perivascular niche has a survival-promoting function for metastatic non-neural cancer cells. Ca<sup>2+</sup> transients were detected *in vivo* during metastatic seeding of the perivascular niche and early proliferation. Ca<sup>2+</sup> activity in breast cancer micrometastases coincided with the increase in growth, which contrasted with brain metastases that were Ca<sup>2+</sup> silent.

20% of single breast cancer micrometastases in the perivascular niche made clear synapses with neurons. Similarly, electron microscopy showed synapses between neurons and melanoma brain micrometastases.

Intravital microscopy performed in mice with metastatic breast cancer or melanoma revealed that Ca<sup>2+</sup> transients in cancer cells were significantly reduced when the animals were anesthetized compared to the same brain regions in awake mice. These Ca<sup>2+</sup> transients recorded in cancer cells associated with neuronal activity in a crucial metastatic niche provided the first evidence of functional communication between neurons and metastatic cancer cells. The inability to induce action potentials in the brain metastatic cells suggests that these cells were the receivers of unidirectional synaptic input from neurons.



Brain neurons form excitatory glutamatergic synapses with metastatic cells of non-neural cancers, mediated by AMPA glutamate receptors, with metastatic cancer cells on the postsynaptic side of the synapses | 3

Postsynaptic currents generated in synapses between neurons and brain metastatic cancer cells during their early growth in the perivascular niche were mediated by AMPA receptors, according to patch-clamp recordings. The spontaneous excitatory postsynaptic currents (sEPSCs) recorded in tumor cells confirmed that synapses between presynaptic neurons and postsynaptic tumor cells were functional. These currents demonstrated a fast rise time and exponential decay, which are the hallmarks of AMPA receptor-mediated sEPSCs. The sEPSCs in tumor cells were completely blocked by cyanquixaline, a specific antagonist of AMPA receptors. This confirmed that AMPA receptors contribute to synapses between neurons and metastatic cancer cells.

To confirm the role of AMPA receptors in the growth of brain metastases *in vivo*, the scientists treated animals with perampanel, a selective and noncompetitive AMPA receptor antagonist and an FDA-approved medication for epilepsy treatment. The administration of perampanel resulted in a lower metastatic burden and fewer brain metastases *per mouse*.

## Conclusion

This study has shown, for the first time, that neurons can form physiologically relevant excitatory glutamatergic synapses with metastatic cells of non-neural cancers. This process started very early after the extravasation of cancer cells into the brain parenchyma, during their residence in the perivascular niche, a critical step for survival.

This research revealed that cancer cells always harbored the post-synapse and never exhibited pre-synaptic features. Most synapses between neurons and individual metastatic cancer cells were direct synapses between presynaptic neurons and postsynaptic cancer cells, without any neuronal structure co-located on the postsynaptic side. These findings indicate *de novo* synaptogenesis of metastatic cells from non-neural cancers, which is opposite to glioma cancer cells that showed a frequent hijacking of pre-existing brain synapses.

The excitatory postsynaptic currents generated in cancer cells were mediated by AMPA glutamate receptors, with metastatic cancer cells on the postsynaptic (receiving) side of the synapses. The discovery of Ca<sup>2+</sup> transients that depend on neuronal activity in metastatic breast cancer and melanoma cells suggests a common mechanism by which synaptic interactions between neurons and cancer cells can be translated into growth-promoting



Brain neurons form excitatory glutamatergic synapses with metastatic cells of non-neural cancers, mediated by AMPA glutamate receptors, with metastatic cancer cells on the postsynaptic side of the synapses | 4

signals.

The authors concluded that this discovery of synapses between neurons and metastatic cells from tumors originating outside the nervous system was unexpected. However, it opened a new chapter in cancer neuroscience. The selective AMPA receptor antagonist and the approved antiepileptic drug perampanel blocked the AMPAergic synapses between neurons and metastatic cells. This finding opened up the possibility of a new approach to metastasis prevention.

This study has been published on a preprint server and is currently being peer-reviewed.

### ***Journal Reference***

Venkataramani V, Karreman MA, Nguyen LC, et al. Direct excitatory synapses between neurons and tumor cells drive brain metastatic seeding of breast cancer and melanoma. 2024. BioRxiv preprint. <https://doi.org/10.1101/2024.01.08.574608>

