

at high frequency. Air bubbles added to the liquid below a critical depth sank to the bottom of the container. The authors inflated sunken bubbles to produce a stable air layer with the liquid levitating on top. The maximum volume of levitating liquid studied was 0.5 litres, and the maximum width was 20 centimetres. Remarkably, Apffel *et al.* observed that small objects (up to 7 grams in mass and 2.5 cm in length or diameter) floated upside down on the lower side of the air–liquid interface (Fig. 1).

To explain their observations, the authors suggest that the effective gravity exerted on the fluid – the apparent gravitational force that acts on a vertically accelerating system – as well as that exerted on submerged and floating bodies, oscillates with time when the system is vibrated vertically. The immersed volume of the body floating on the lower interface of the fluid also oscillates with time. Apffel and co-workers propose that this causes a time-averaged force to be applied to the body. This force has an ‘antigravity’ effect that, for vertical vibrations of frequencies of 80 Hz or above, enables the body to float on the lower interface of the fluid. As is the case for the Stephenson–Kapitza pendulum<sup>6</sup> and the Chelomei pendulum<sup>2</sup>, the stable states of Apffel and colleagues’ vibrating system correspond to potential-energy maxima, rather than minima.

The authors suggest a relatively simple mathematical description of the inverse-floating phenomenon. This description involves some simplifying assumptions, for example by supposing that the relationship between the pressure in the air layer and the height of the layer is linear. The simplifications somewhat limit the accuracy with which the authors’ theory describes the behaviour of the experimental system, leading to minor discrepancies with the observations.

It is also worth noting that the speed of sound in gas-saturated fluids is surprisingly low for a wide range of volumetric gas concentrations, and this has also been observed to produce antigravity effects<sup>3</sup>. For example, for air concentrations of 30–70%, the speed of sound is only 20 metres per second (ref. 3); this compares with about 340 m s<sup>-1</sup> in air, and about 1,450 m s<sup>-1</sup> in water. When the speed of sound is this low, one or even several longitudinal (compression) standing waves can fit into a vibrating volume 1 m in height at frequencies of the order of 50 Hz. Heavy, rigid particles and gas bubbles are attracted to the points of minimum and maximum amplitude of these standing waves, leading to gravity-counteracting effects.

Apffel and colleagues’ work suggests that many remarkable phenomena arising in vibrating mechanical systems are yet to be revealed and explained, particularly at interfaces between gases and fluids, implying great

potential for future research. More broadly, the analysis of the effects of high-frequency excitations on systems from other fields of science, such as chemistry, physics and biology, is another promising research topic<sup>8</sup>. In these systems, the excitation can be any periodic change in a property of the environment or medium in which a process is taking place. It will be exciting to discover what counter-intuitive phenomena can be induced by high-frequency excitations in non-mechanical systems – is there a chemical or biological counterpart of inverse gravity?

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### Cell biology

# Connections that couple brain activity to blood flow

**Chiara Zurzolo**

Structures similar to closed-ended tunnelling nanotubes have now been seen connecting pericyte cells in the mouse retina. The structures enable pericytes to coordinate their responses to neural activity, thereby modulating blood flow. **See p.91**

The discovery of membrane protrusions that form open-ended channels between cells has revolutionized our understanding of cell-to-cell communication. These tunnelling nanotubes (TNTs) enable the exchange of compounds, including organelles, pathogens and genetic material<sup>1–3</sup>, and have been reported – mainly on the basis of *in vitro* and *ex vivo* cultures – to participate in a range of cell behaviours, from differentiation to neurodegeneration and immune responses<sup>4</sup>. However, the lack of markers for TNTs has made their identification *in vivo* particularly challenging<sup>3</sup>. Consequently, there has been debate over whether they actually exist, how to define bona fide TNTs *in vivo*, and how many types there might be<sup>3,4</sup>. On page 91, Alarcon-Martinez *et al.*<sup>5</sup> now provide perhaps the first *in vivo* evidence for the existence of a type of TNT-like protrusion that is closed at one end, in the retina of living mice.

The authors set out to investigate a process called neurovascular coupling<sup>6</sup>, in which signalling between neurons and non-neuronal cells leads to altered blood flow in response to changes in neural activity – thereby ensuring that more blood flows towards more-active brain regions. One cell type that might be involved is the pericyte, which surrounds capillaries throughout the body. Pericytes

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1. Bleich, H. H. *J. Jet Propuls.* **26**, 958–964 (1956).
2. Chelomei, V. N. *Sov. Phys. Dokl.* **28**, 387–390 (1983).
3. Blekhman, I. I., Blekhman, L. I., Sorokin, V. S., Vasilkov, V. B. & Yakimova, K. S. *J. Mech. Eng. Sci.* **226**, 2028–2043 (2012).
4. Wolf, G. H. *Phys. Rev. Lett.* **24**, 444–446 (1970).
5. Apffel, B., Novkoski, F., Eddi, A. & Fort, E. *Nature* **585**, 48–52 (2020).
6. Kapitza, P. L. *Sov. Phys. JETP* **21**, 588–597 (1951).
7. Blekhman, I. I. *Vibrational Mechanics: Nonlinear Dynamic Effects, General Approach, Applications* (World Scientific, 2000).
8. Blekhman, I. I. & Sorokin, V. S. *Nonlinear Dynam.* **83**, 2125–2141 (2016).

regulate microcirculatory blood flow by contracting and relaxing, but whether and how this process is synchronized with neurovascular coupling has been unknown.

Empty, thin, long projections extending from and between pericytes have been seen before<sup>7,8</sup>. Alarcon-Martinez *et al.* show that, in the mouse retina, these interpericyte connections are not open bridges, but have one closed end: the protrusion extends from one cell (dubbed the proximal cell) to make contact with the other (the distal cell), and the tip of the protrusion is closed by a structure called a gap junction (Fig. 1). Small particles, such as ions, can pass through this junction, but larger objects, such as organelles, cannot. This type of closed-ended TNT has been shown to aid electrical coupling between cells *in vitro*<sup>9</sup>.

The authors found that, rather than being empty shells, these interpericyte TNTs (IP-TNTs) mediate communication between distant pericytes in separate capillary systems, through bidirectional transfer of calcium ions (Ca<sup>2+</sup>). Closed-ended TNTs are mainly formed from a network of the structural protein actin, but lack another structural protein, tubulin<sup>9</sup> – and the authors found actin, but not tubulin, in their IP-TNTs. In support of the supposition that IP-TNTs derive from

pericytes, the structures contained  $\alpha$ -SMA, a protein essential for pericyte contractility.

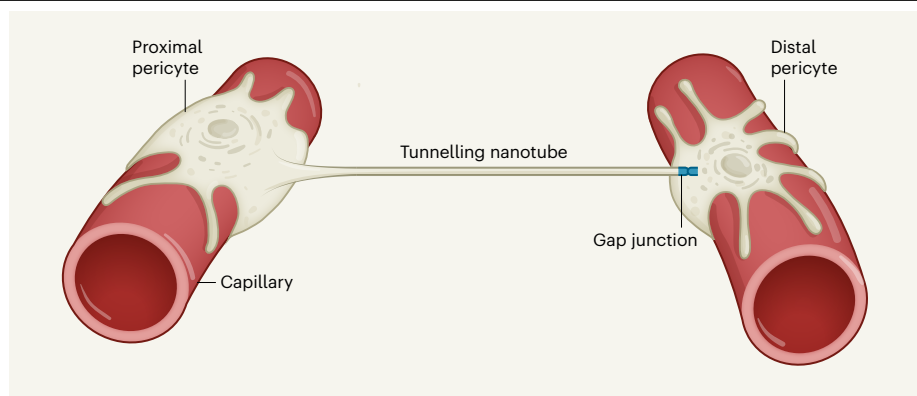
Do IP-TNTs have a role in coupling changes in microvasculature to neuronal activity? Alarcon-Martinez *et al.* addressed this question by stimulating the mouse retina with light (which triggers neural activity) and recording capillary dynamics and blood flow using a technique called two-photon *in vivo* imaging. They showed that a single flash of light triggers a coordinated response in the diameters of pairs of capillaries connected by IP-TNTs: one capillary dilates to increase blood flow and the other constricts to decrease flow. The response coincided with a transient increase in  $Ca^{2+}$  levels in the constricting pericyte, and a decrease in the cell that was causing capillary dilation. The response presumably allows rapid redistribution of a limited amount of blood between connected capillary systems, ultimately increasing blood flow around light-activated neurons.

Waves of synchronous  $Ca^{2+}$  increases can propagate down TNTs in response to electrical stimulation. The authors demonstrated that IP-TNT ablation reduced the frequency of  $Ca^{2+}$  waves, and prevented coordinated constriction and dilation in pericytes that had been connected. This key experiment confirms the role of IP-TNTs in the neurovascular response to light.

During a stroke, arteries close, impeding blood flow to the brain – a condition known as ischaemia. Subsequent constriction of pericytes is thought to restrict blood reflow after an artery has reopened, considerably reducing the chances of recovery<sup>10</sup>. Alarcon-Martinez *et al.* investigated the role of IP-TNTs in this process by blocking the central retinal arteries of mice or inducing local ischaemia with a laser. The authors found that, in either setting, ischaemia caused an unusually large influx of  $Ca^{2+}$  into IP-TNT-coupled pericytes, and led to the rupture of almost a quarter of IP-TNTs in the retina. This rupture substantially reduced the light-evoked capillary response and impaired capillary dilation. The researchers therefore hypothesize that the excessive  $Ca^{2+}$  influx throws normal  $Ca^{2+}$  regulation out of balance, and impairs neurovascular coupling by affecting the function and stability of IP-TNTs.

Alarcon-Martinez and colleagues' work provides impressive evidence of closed TNT-like structures *in vivo*, and delivers fresh perspectives for understanding the mechanism of neurovascular coupling. What makes the authors' work particularly interesting is the convincing evidence of a functional role for TNT-like connections in a living animal.

However, questions remain. For instance, do other cells – endothelial cells that line blood vessels, or neuron-supporting astrocytes – participate in the TNT-mediated neurovascular coupling reported? Although several lines of evidence led the authors to conclude



**Figure 1 | Interpericyte tunnelling nanotubes.** Tunnelling nanotubes (TNTs) are tubular connections between distant cells. Alarcon-Martinez *et al.*<sup>5</sup> observed, closed-ended TNT-like protrusions between cells called pericytes that are wrapped around different capillaries in the retina in mice. The TNTs protrude from one pericyte to contact the other, where they terminate in a structure called a gap junction. The gap junction permits the exchange of small molecules and calcium ions, but prevents the passage of large objects such as organelles.

that the distal cell of the pair is a pericyte, they did not confirm this by analysing the cell with pericyte-specific markers. The fact that the interconnected pericytes show coordinated oscillation suggests that the distal cell is indeed a pericyte, but there is still the possibility that the long protrusion from the proximal pericyte connects with an endothelial cell, which has been shown before<sup>7,8</sup>. However, if this is the case it is not clear how  $Ca^{2+}$  waves passing through the IP-TNT could cause constriction or dilation in the distal branch.

In addition, the neurovascular coupling analysed in this work was limited to a small area of the retina. It is possible that the coordinated

### “The authors’ work provides impressive evidence of closed tunnelling-nanotube-like structures *in vivo*.”

response of two capillary branches might arise from the need to balance blood over small areas, when blood stops flowing from an upstream region. The challenge will be to understand whether a similar phenomenon mediated by TNT-like connections between pericytes, possibly placed in series, can mediate neurovascular coupling in much larger regions of the brain.

It is possible that IP-TNTs have broader functions in the brain – in both health and disease. In the central nervous system, pericytes regulate blood-vessel formation and the highly controlled passage of molecules from the blood into the brain. Moreover, pericyte deficiency accompanies diseases such as multiple sclerosis, vision problems associated with diabetes, and neurodegenerative disorders<sup>11</sup>. In support of the idea that IP-TNTs might have

a role in these processes, human pericytes have been shown to connect to neuronal cells *in vitro* through TNTs, and to exchange both neurodegeneration-associated  $\alpha$ -synuclein protein and electrical signals<sup>12</sup>.

Finally, TNT-like protrusions that originate from pericytes and contact either another pericyte or an endothelial cell have been shown to connect distant vessels in fetal human cortex and in samples from brain tumours called glioblastomas<sup>13</sup>. In both settings, the protrusions are proposed to have an essential role in early phases of blood-vessel growth. However, the role in cell–cell communication for these TNT-like structures was not investigated<sup>13</sup>. Thanks to Alarcon-Martinez and colleagues’ advanced morphological and functional analysis, we now have a road map to investigate TNT-mediated communication in brain function and dysfunction.

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1. Rustom, A., Saffrich, R., Markovic, I., Walther, P. & Gerdes, H.-H. *Science* **303**, 1007–1010 (2004).
2. Goussset, K. *et al.* *Nature Cell Biol.* **11**, 328–336 (2009).
3. Sartori-Rupp, A. *et al.* *Nature Commun.* **10**, 342 (2019).
4. Ariazi, J. *et al.* *Front. Mol. Neurosci.* **10**, 333 (2017).
5. Alarcon-Martinez, L. *et al.* *Nature* **585**, 91–95 (2020).
6. Iadecola, C. *Neuron* **96**, 17–42 (2017).
7. Mendes-Jorge, L. *et al.* *Exp. Eye Res.* **98**, 79–87 (2012).
8. Franco, C. A. *et al.* *PLoS Biol.* **13**, e1002125 (2015).
9. Wang, X., Veruki, M. L., Bukoreshtliev, N. V., Hartveit, E. & Gerdes, H.-H. *Proc. Natl Acad. Sci. USA* **107**, 17194–17199 (2010).
10. O’Farrell, F. M. *et al.* *eLife* **6**, e29280 (2017).
11. Sweeney, M. D., Ayyadurai, S. & Zlokovic, B. V. *Nature Neurosci.* **19**, 771–783 (2016).
12. Dieriks, B. V. *et al.* *Sci. Rep.* **7**, 42984 (2017).
13. Errede, M. *et al.* *Fluids Barriers CNS* **15**, 28 (2018).

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