Investigating the mechanics of retrograde neurotransmission in the cerebellum

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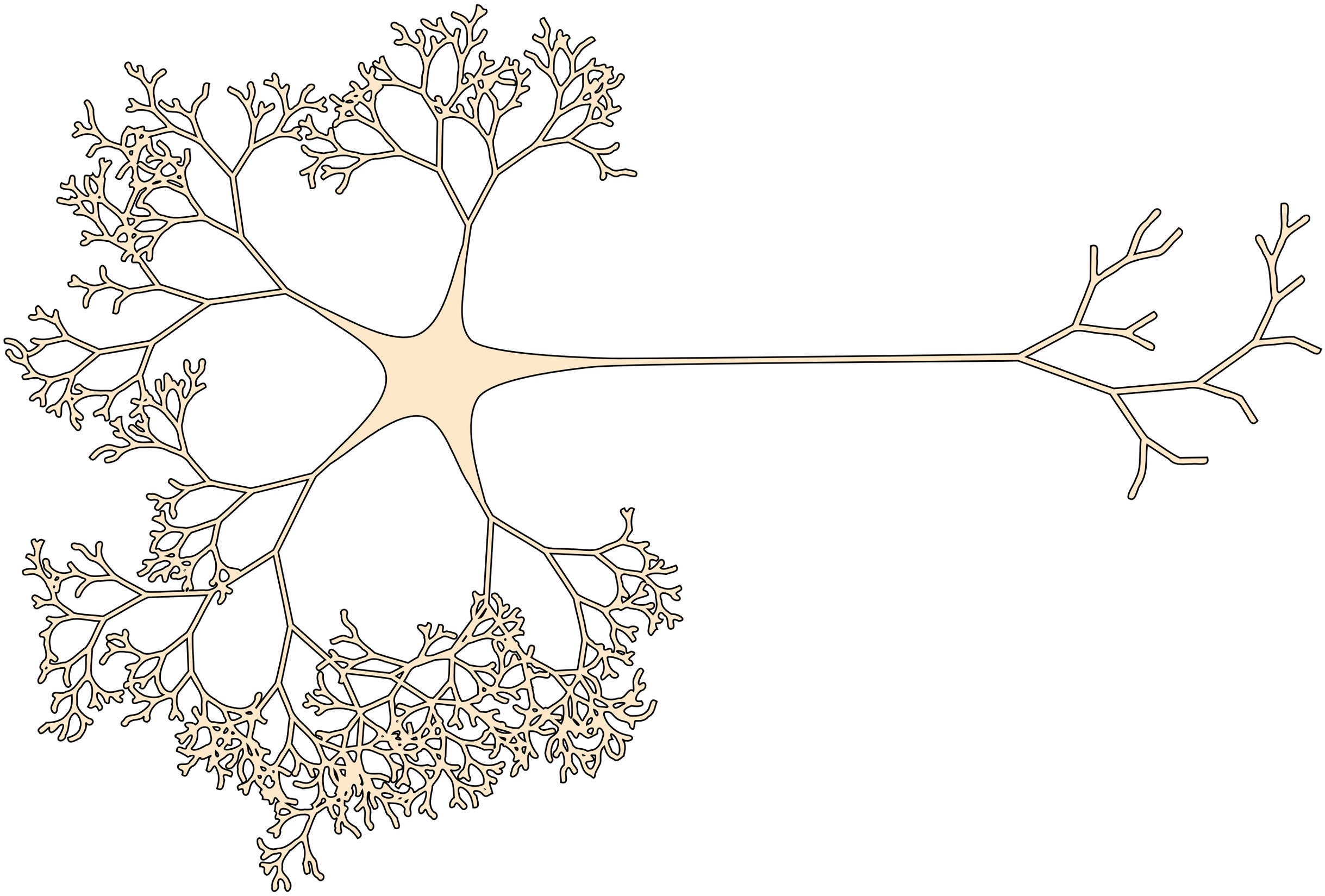
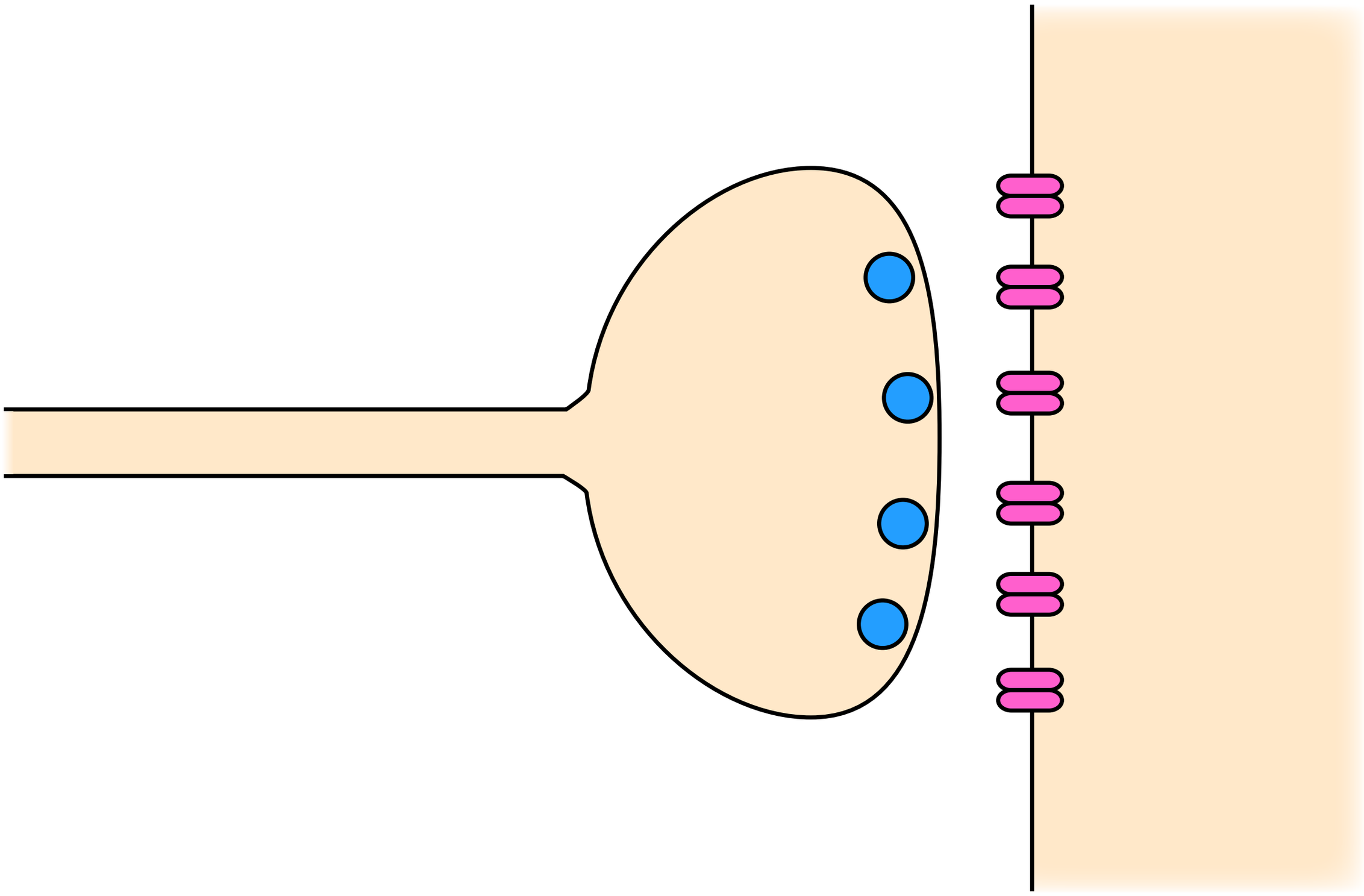
We tend to think of neural communication as a rather directional process. There are good anatomical and physiological reasons for thinking this: both neurons themselves and the majority of the connections between them -- chemical synapses -- are highly asymmetrical, specialised for transferring information one way rather than the other.

Image: Johnny Jet



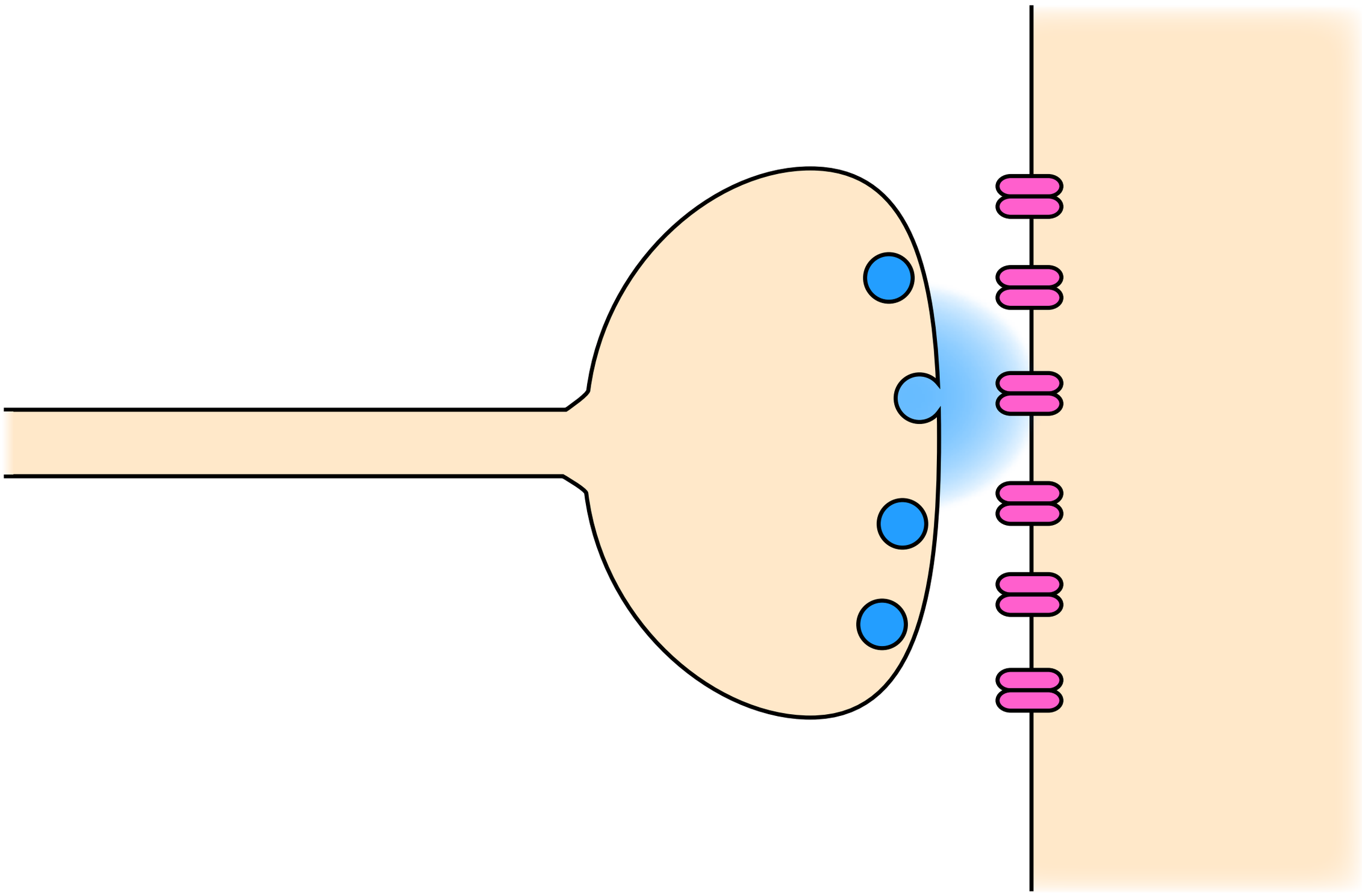
In neurons, for example, many synaptic inputs -- often a huge number -- are integrated to produce a single result -- in the form of a time course of firing -- which is then sent off down a specialised output line.

Image adapted from one by Nicolas Rougier

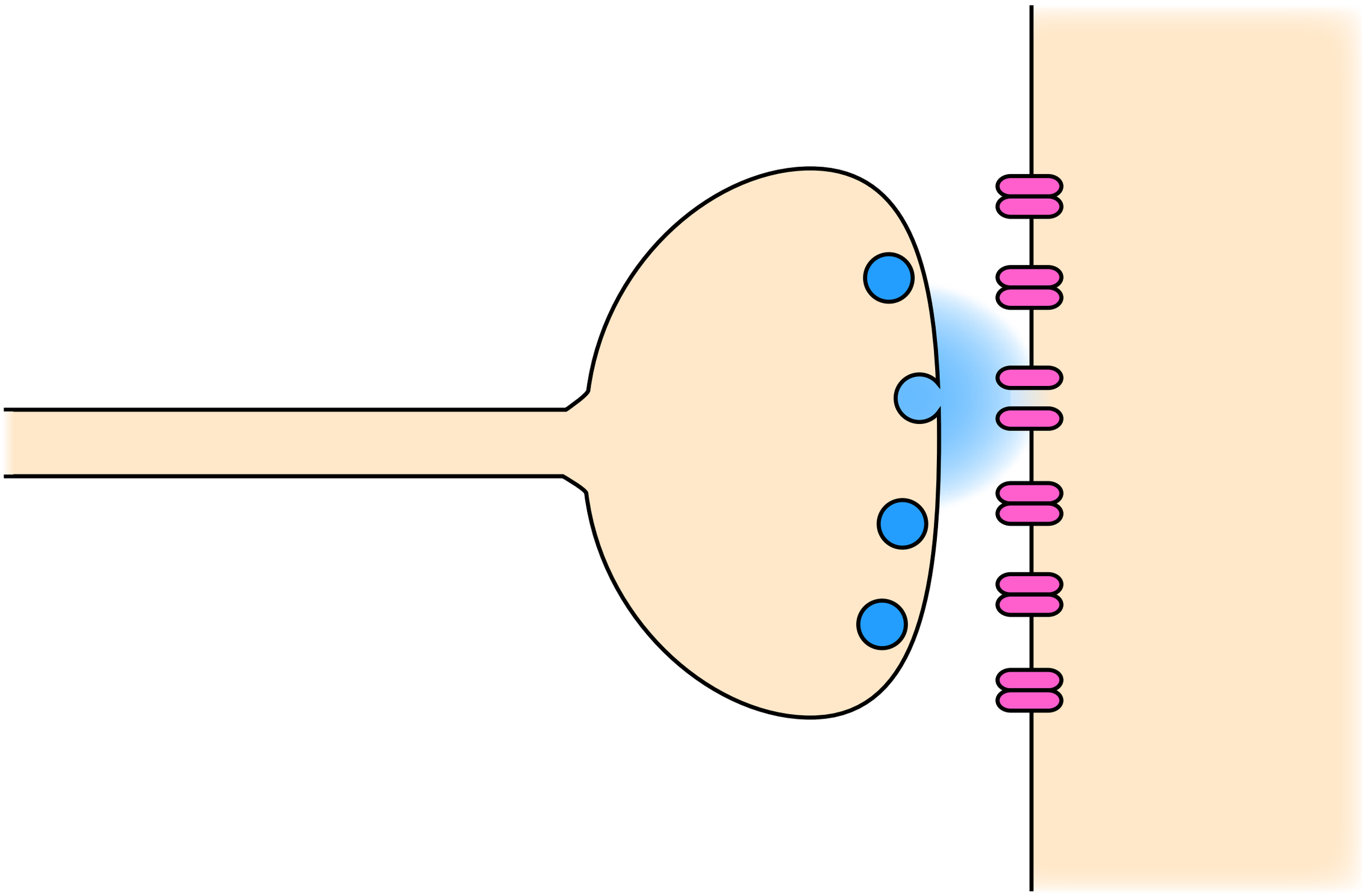


Synapses are specialised structures at the close apposition of two neurons that have a bunch of transmission machinery on one side and receiving machinery on the other side.

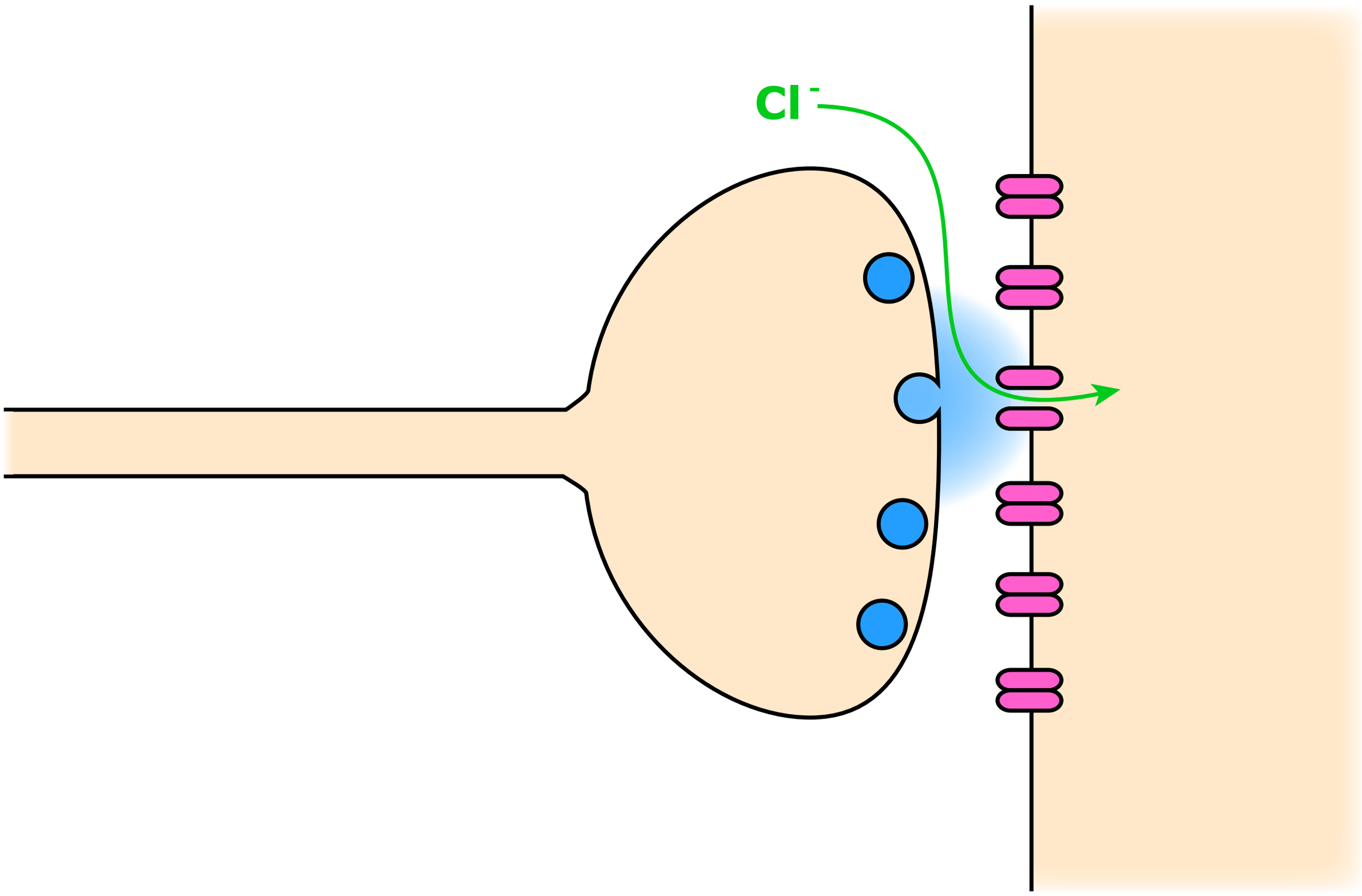
The sending side contains vesicles of neurotransmitter, while the other is packed with receptors sensitive to that transmitter. When an action potential comes along on the sending side, it leads (via a series of events that we don't need to go into here) to an increased probability that one of the vesicles will...



...fuse with the membrane and release its contents into the synaptic cleft. The neurotransmitter then diffuses across the cleft and may bind to postsynaptic receptors, leading to various forms of signal transduction in the post-synaptic cell. Very often this will take the form of...

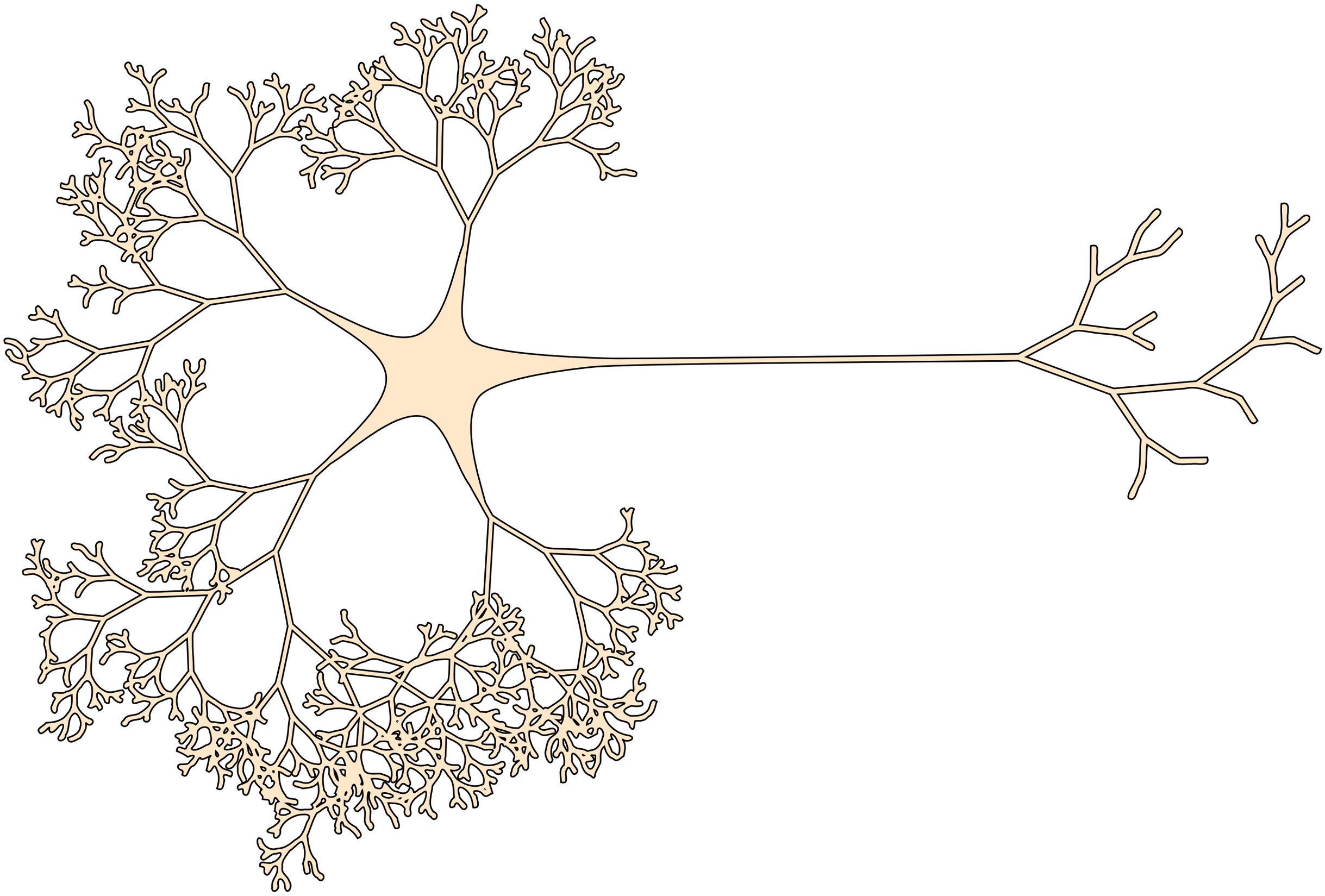


...ion channels opening, allowing some specific ions to cross the membrane into or out of the downstream cell, thereby modifying its behaviour.



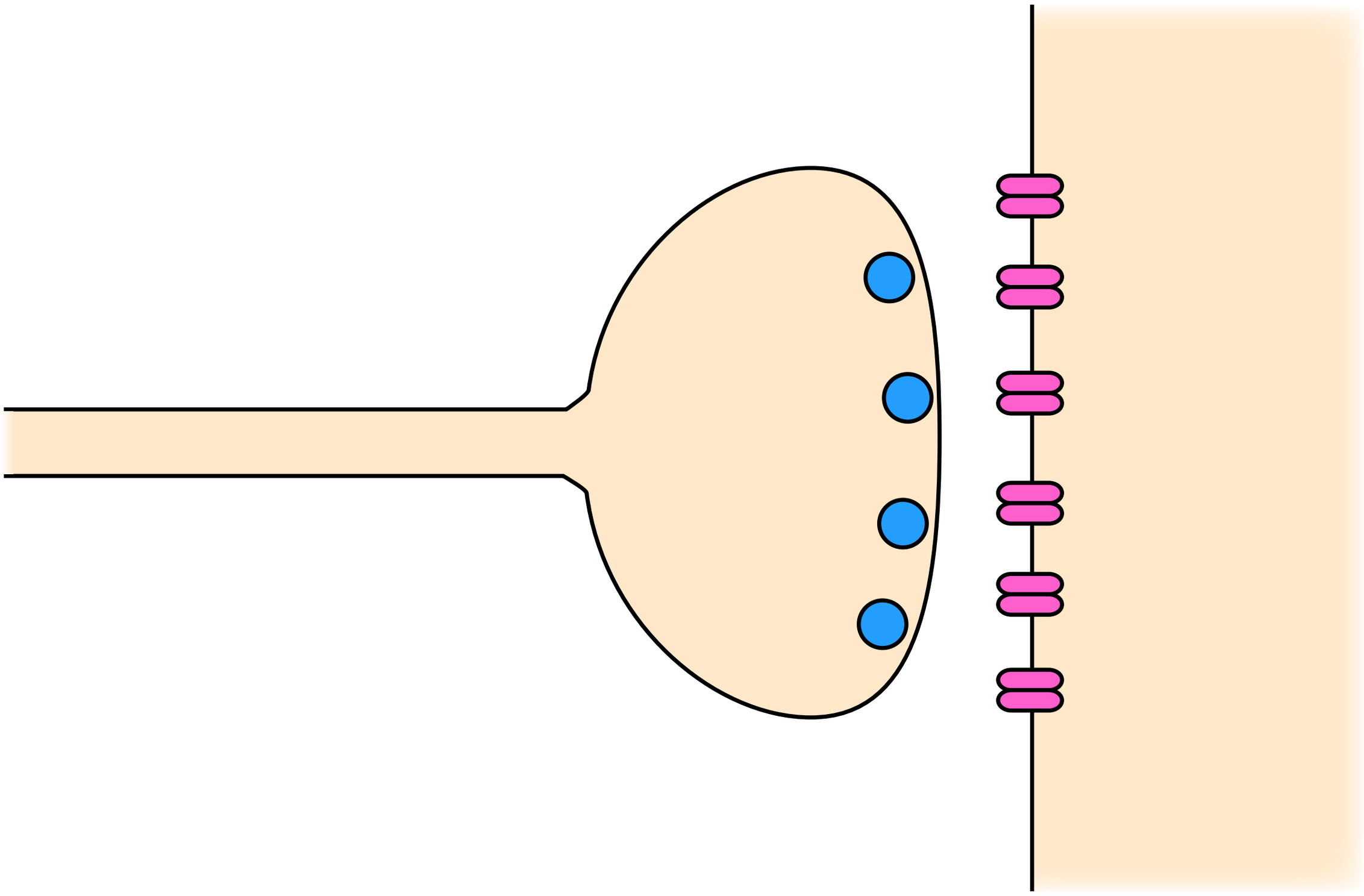
For example, at the sort of synapses I'm mostly interested in here, the neurotransmitter GABA may activate GABA-A receptors, which are ion channels permeable to chloride ions. These modify the excitability of the receiving neuron, leading in most cases to a reduction in the probability of firing. This is an inhibitory effect.

In other synapses the effect may be different, but the overall stucture is usually similar.



The behaviour of a neuron is to a large extent driven by its synapses -- just as the behaviour of the network is driven by its neurons. For the neurons to modulate their activity in response to a given set of inputs -- ie, for them to learn or adapt -- they must be able to change the significance placed on different inputs -- that is, the weight accorded each synapse.

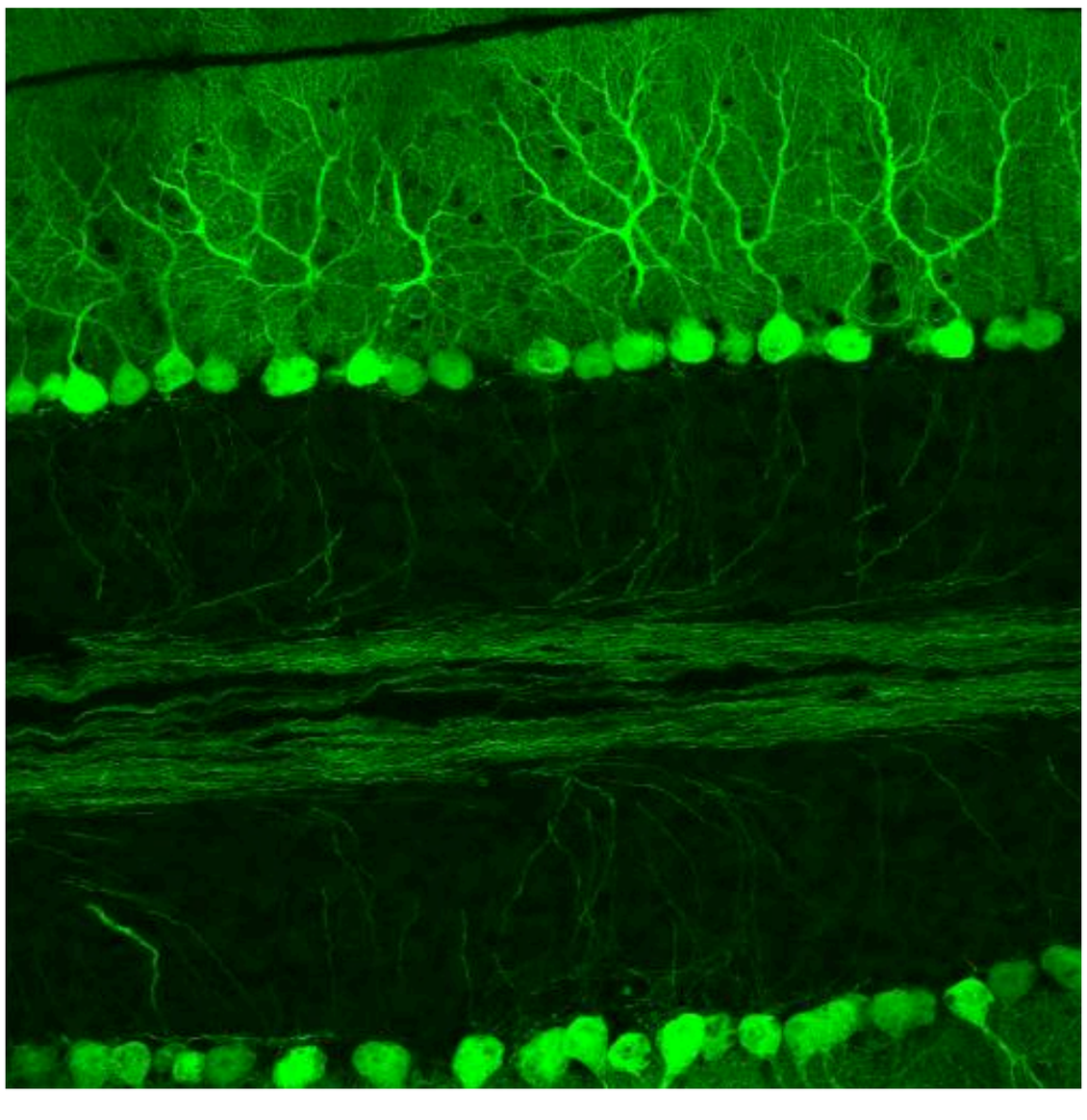
Synaptic strength is thus a major locus of plasticity in the central nervous system.

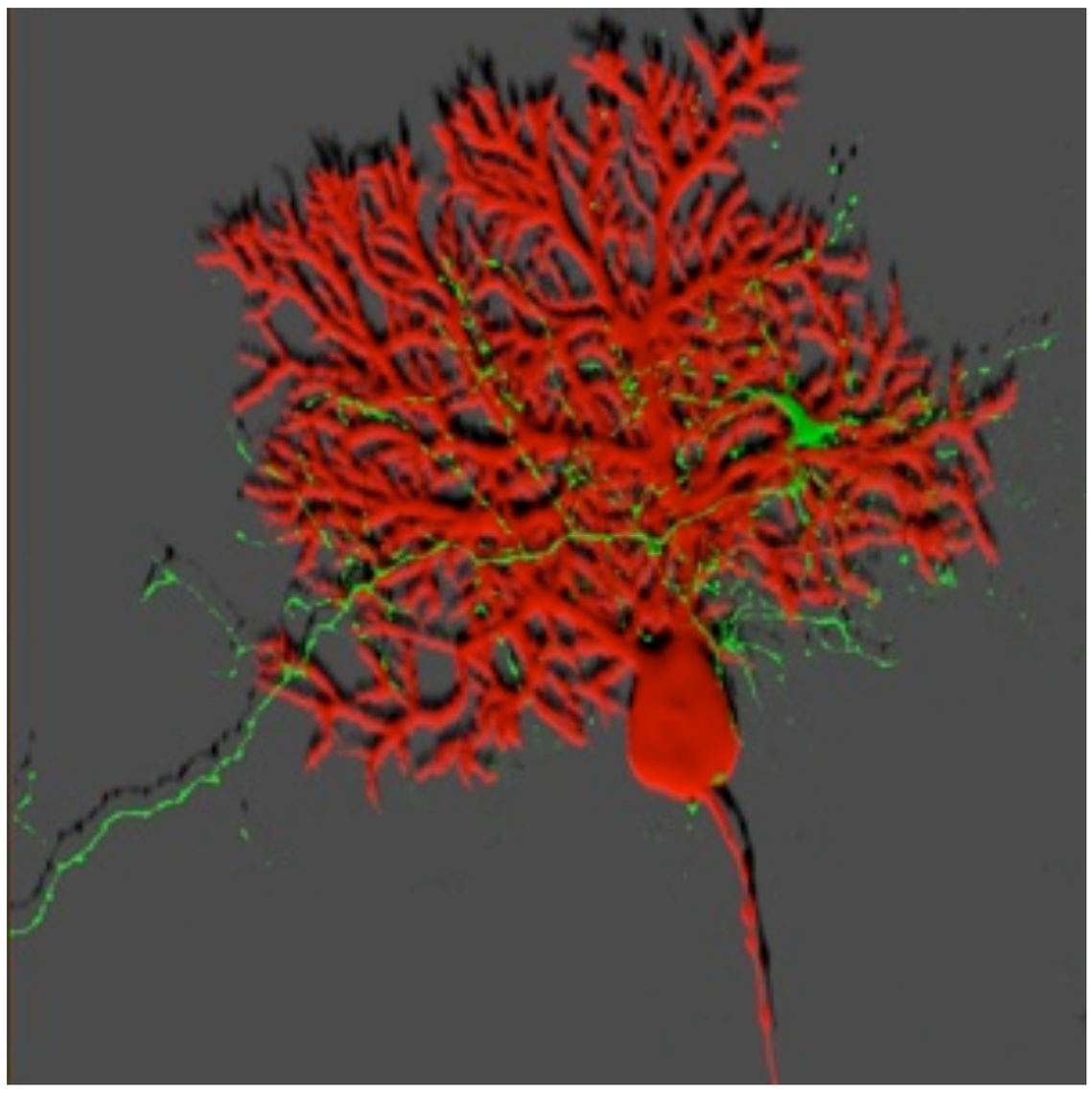


But how is that strength to be adjusted, given the cartoon view of synaptic function we've so far presented? There are two main ways. On the presynaptic side, the probability of release can be altered; while on the post-synaptic side, the sensitivity of reception can -- which usually means changing the population of receptors in various ways.

Naturally, both kinds of change can take place simultaneously -- and most of the time probably do.

The interneuron-Purkinje synapse





So let's introduce the particular synapses we're immediately concerned with, which are those between the molecular layer interneurons and the Purkinje cells in the cerebellum.

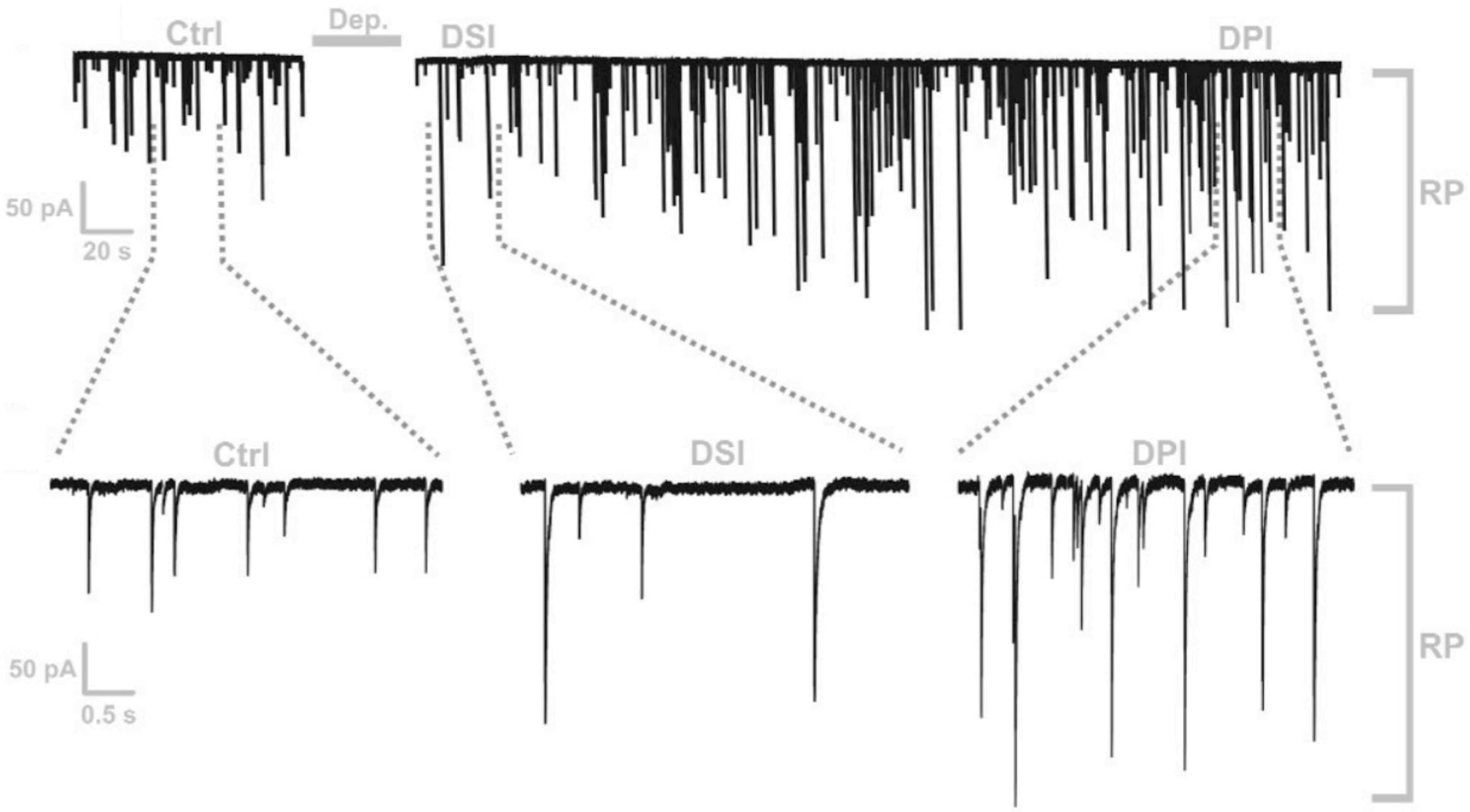
Purkinje cells are the big output neurons from the cerebellar cortex, delivering inhibitory signals to the deep cerebellar nuclei that fundamentally shape coordinated motor behaviour.

The interneurons are internal, also inhibitory, acting to cross-reference a vast range of sensory and other signals to tune and synchronise motor responses.

These synapses are incredibly important to the physical behaviour of all higher animals, and they exhibit some very interesting properties. But it's important to note that there is no very good reason to suppose that their complexities are unique: it's likely that many other synapses have similarly complicated processes of modulation.

Images: NIH, Michael Häusser

...exhibits several forms of plasticity driven by post-synaptic depolarisation



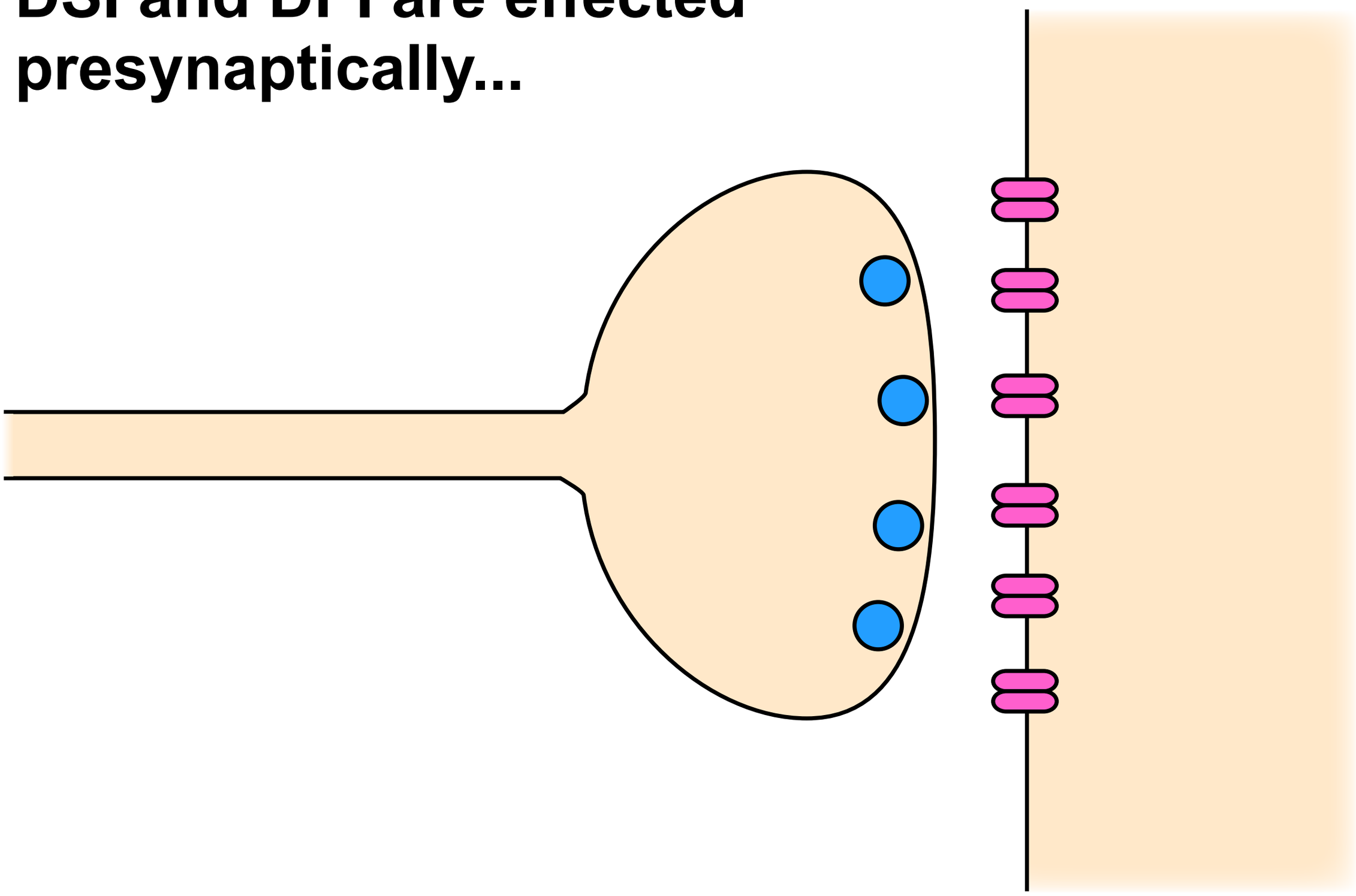
At least three forms of plasticity have been demonstrated experimentally in these synapses in response to repeated post-synaptic depolarisation -- which is to say, the Purkinje cells firing several times in quick succession. These forms overlap and interact in interesting ways. All three are illustrated in this figure.

Rebound potentiation (RP) is a long term increase in the amplitude of currents detected in the Purkinje cells in response to neurotransmitter release.
Depolarisation-induced suppression of inhibition (DSI) is a short-term decrease in the frequency of such currents.
Depolarisation-induced potentiation of inhibition (DPI) is a longer-term increase in the frequency of these currents.
The combined effect of these changes is, obviously, complex!

RP is managed post-synaptically, but...

Image: Duguid & Smart 2004

DSI and DPI are effected presynaptically...

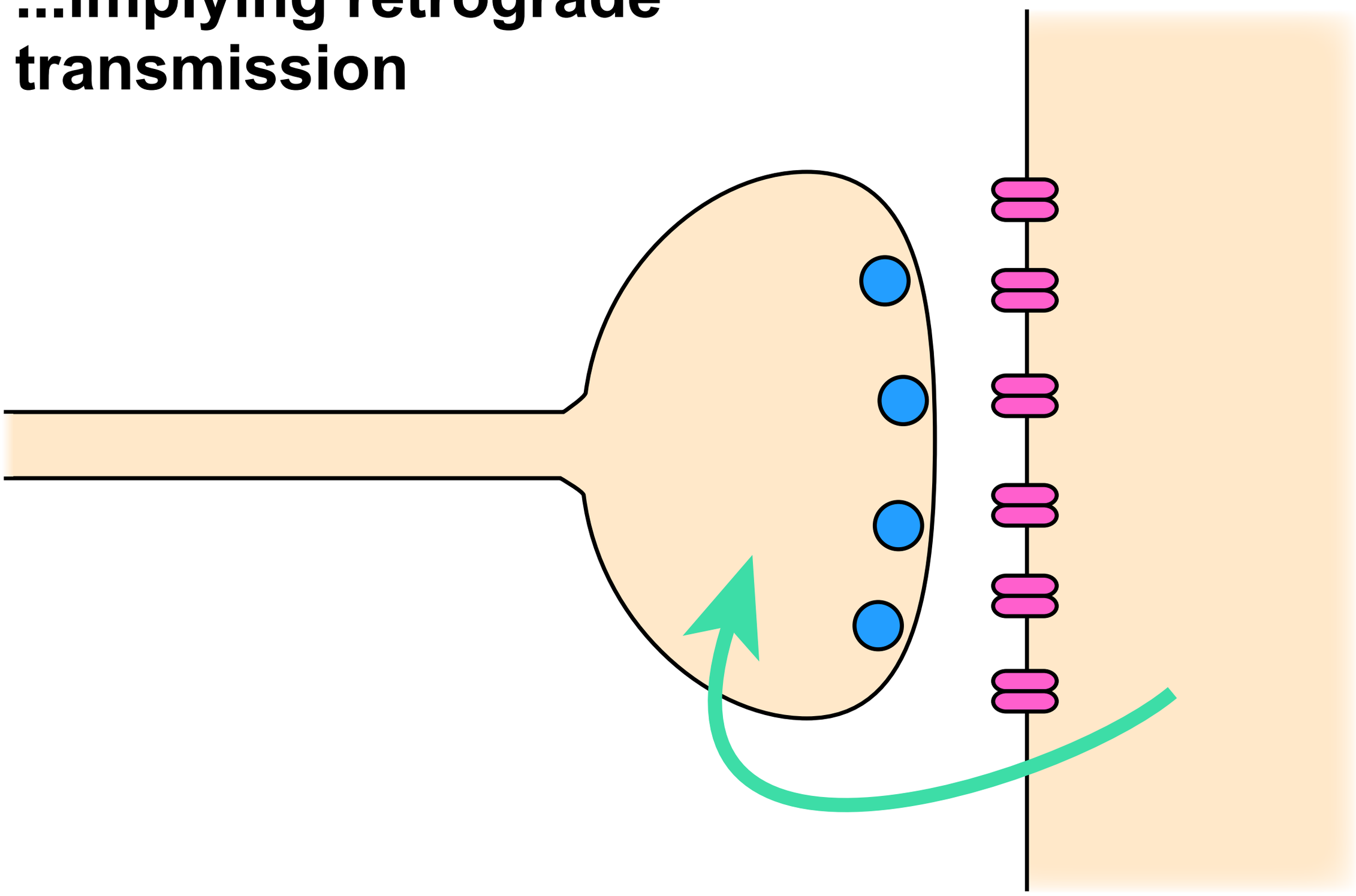


DSI and DPI are effected presynaptically. That is, the changes in synaptic strength are a result of changes in the probability of vesicular release from the presynaptic terminal.

But, remember that the causative events -- the action potential firings -- take place in the downstream neuron. It is not dependent on any presynaptic involvement. The effect occurs irrespective of whether the transmitting neuron has contributed to the post-synaptic depolarisation

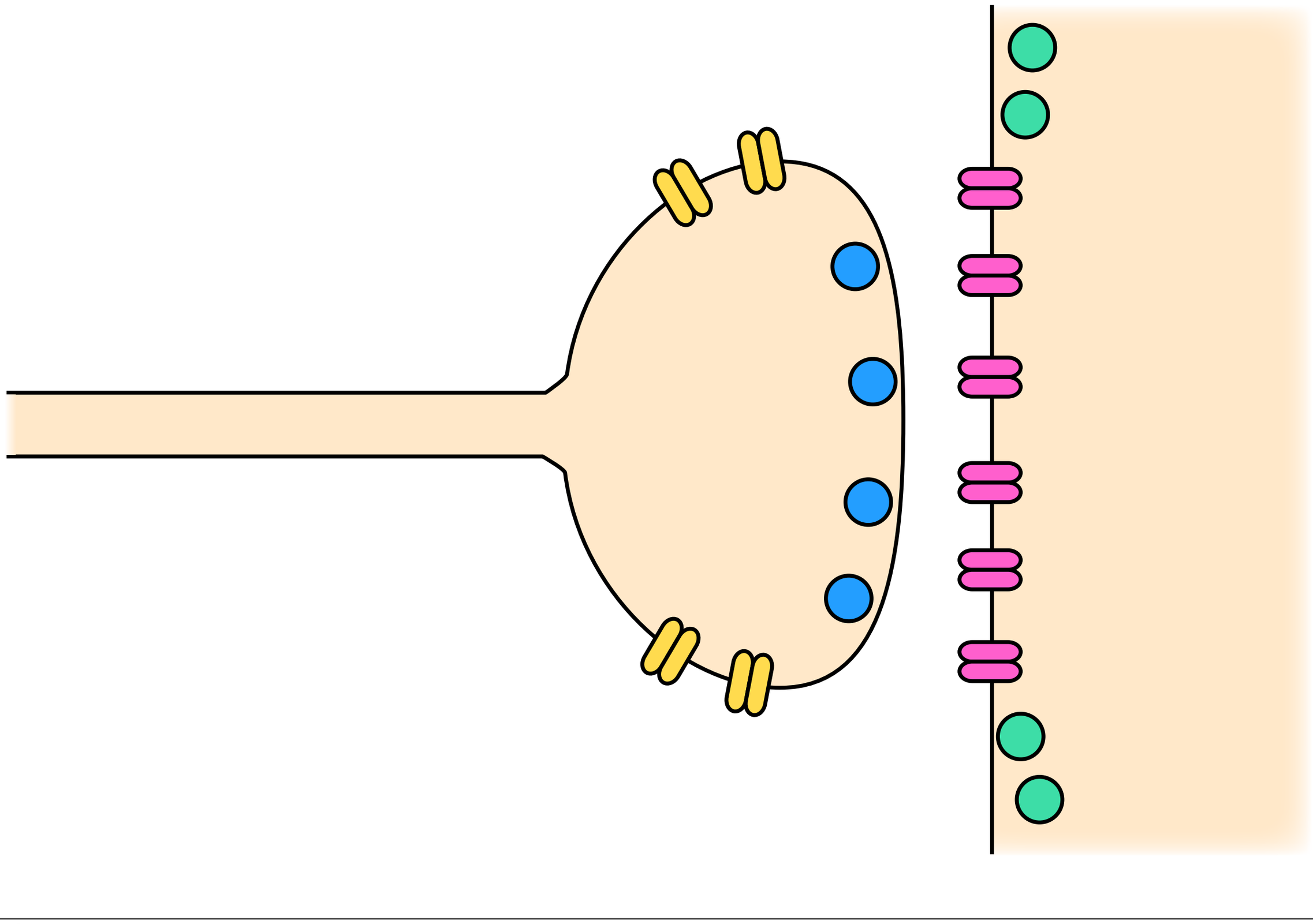
THUS:

...implying retrograde transmission



...some kind of signal must be being sent “backwards” from the post-synaptic cell to the presynaptic one. And in fact, there’s ample evidence for both DSI and DPI that this signal is structurally very similar to the familiar forward or anterograde signal.

So, really, our cartoon neuron picture ought probably to look more like...

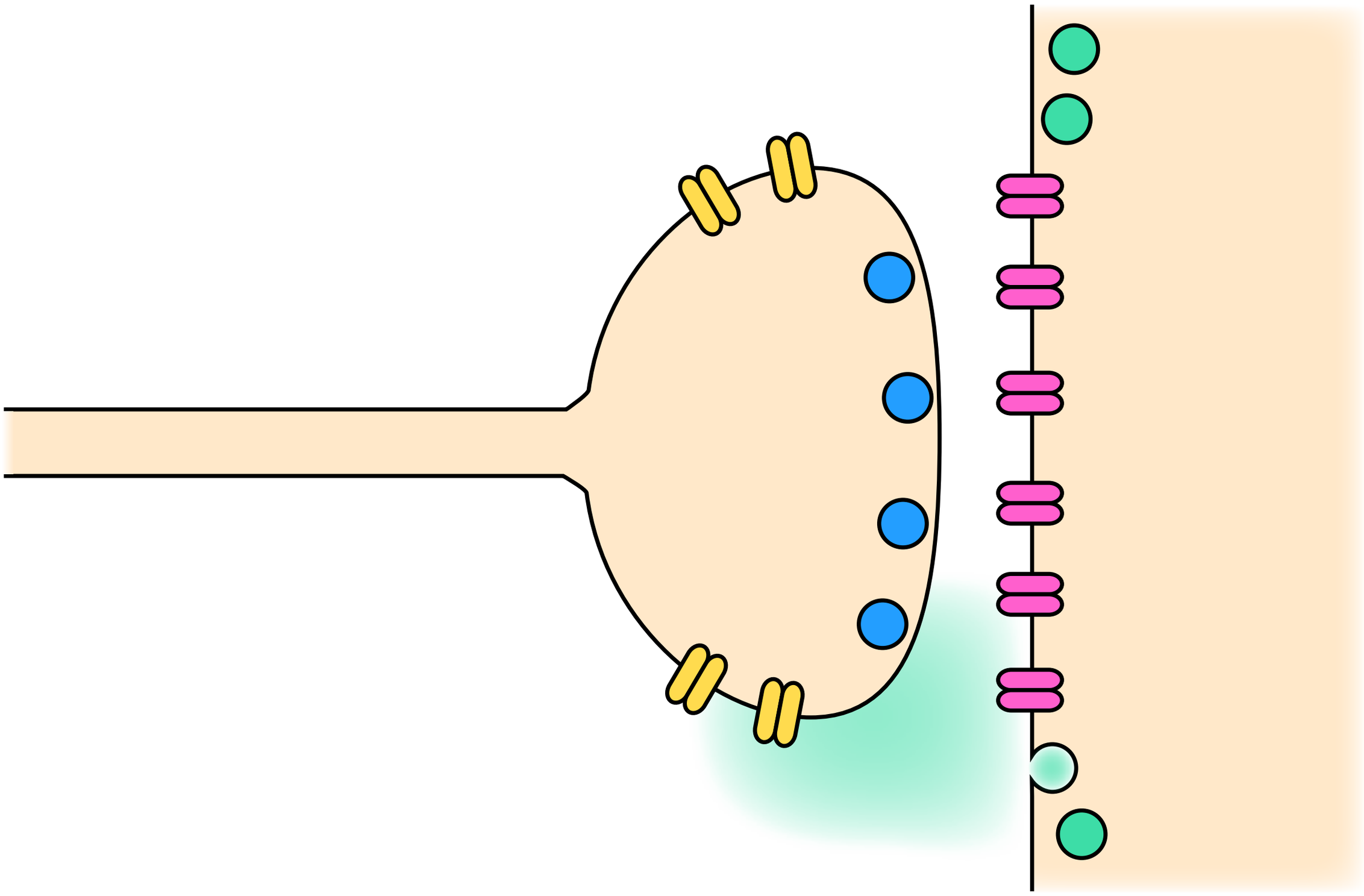


...this.

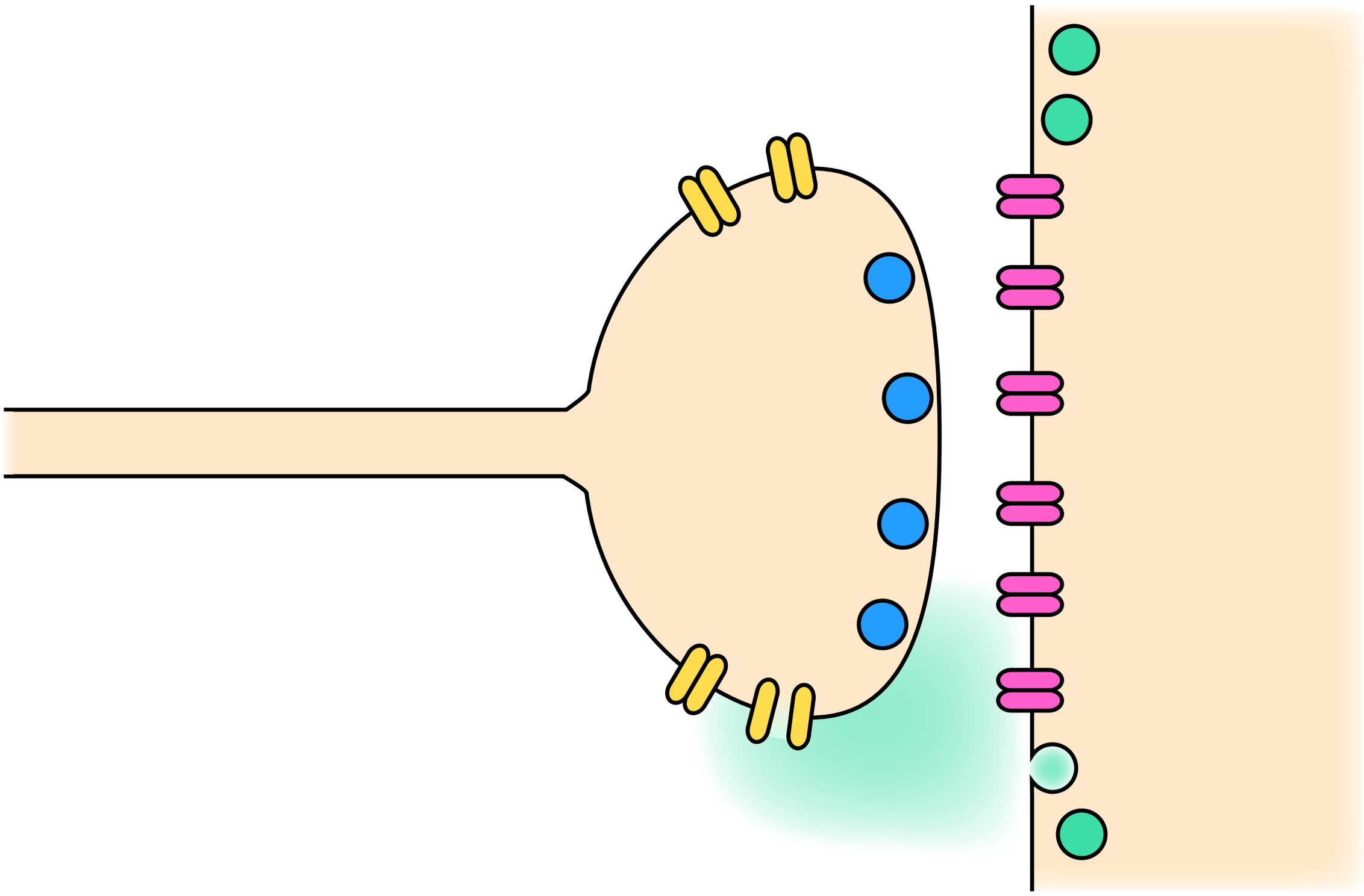
In addition to the presynaptic transmission machinery and post-synaptic receptors, there is also post-synaptic transmission machinery, and there are presynaptic receptors. The communication is not quite as one-way as we might have chosen, for convenience, to imagine.

Now, don't get me wrong: the retrograde signal is much weaker than the anterograde. The system is still massively asymmetric and specialised for forward communication. But the reverse communication is also there.

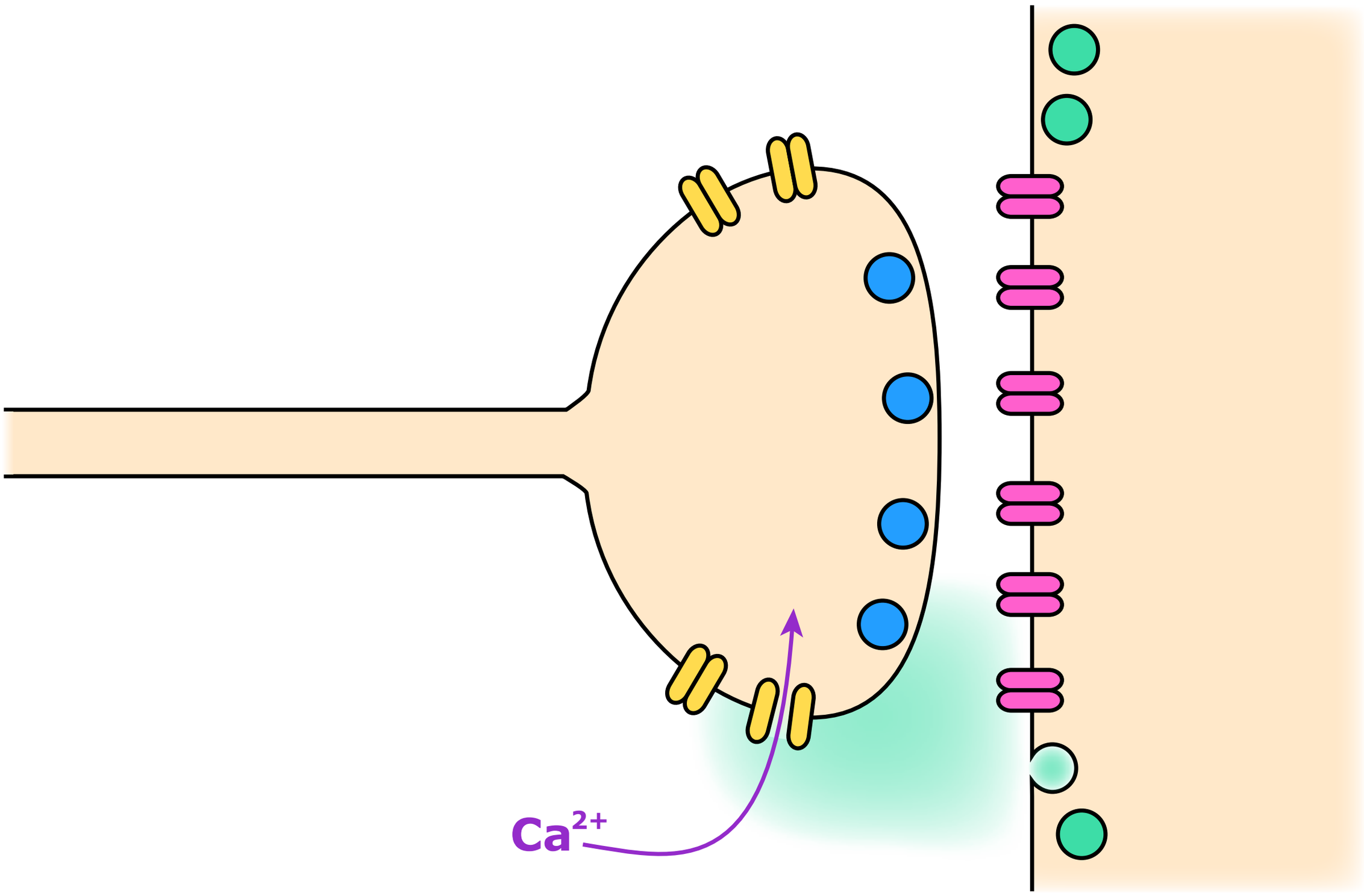
The process for both DSI and DPI is similar, though mediated by different pathways. I'll describe DPI because it's what I'm concerned with, and because it's somewhat less well characterised than DSI.



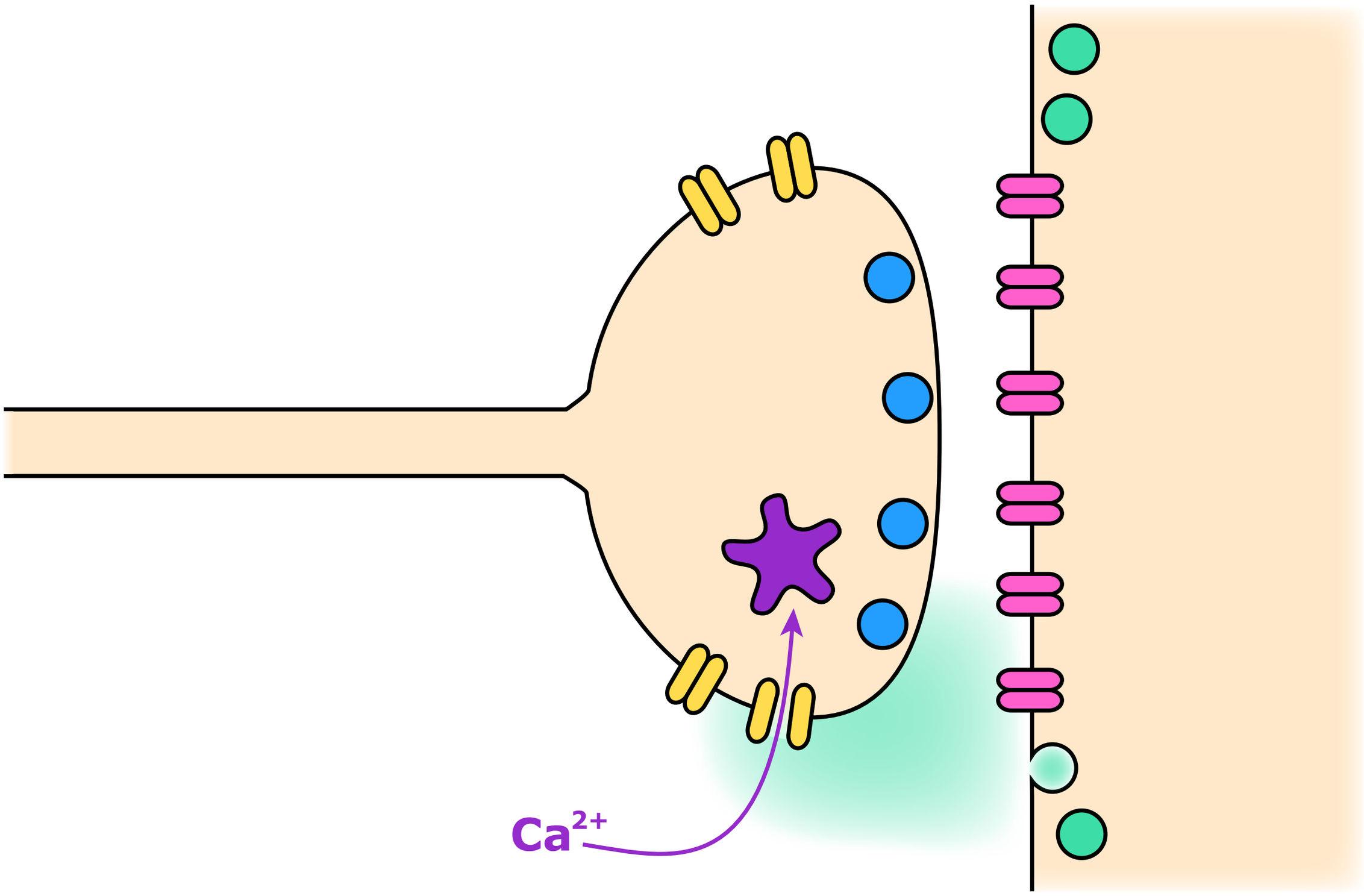
The Purkinje cell depolarisation leads to a rise in intracellular calcium concentration, which in turn can prompt fusion of post-synaptic neurotransmitter vesicles containing -- for DPI -- glutamate or something very similar to it. This is released into the extracellular space and diffuses back to receptors on or very near the presynaptic bouton.



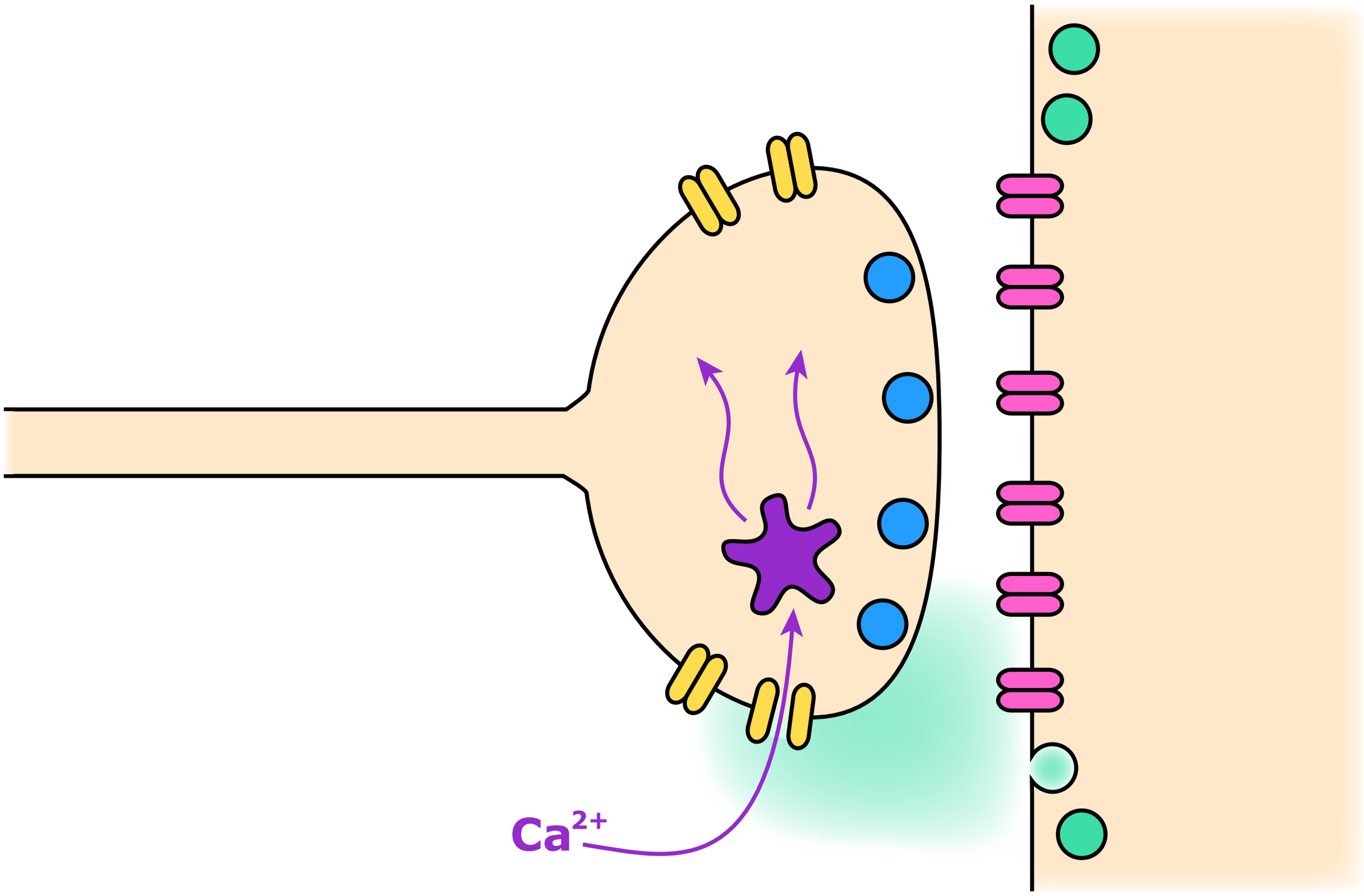
In the case of DPI, these are believed to be NMDA receptors, a subset of glutamate receptors with some interesting properties. They are ionotropic -- ie, are also ion channels -- and open to admit ions to the presynaptic bouton.



NMDA receptors are non-specific cation channels, but they are disproportionately permeable to calcium ions, and it is the influx of calcium that leads to DPI. However, the small calcium signal is not enough on its own to create the sustained concentration increase that leads to the long-term increase in release probability that manifests as DPI.

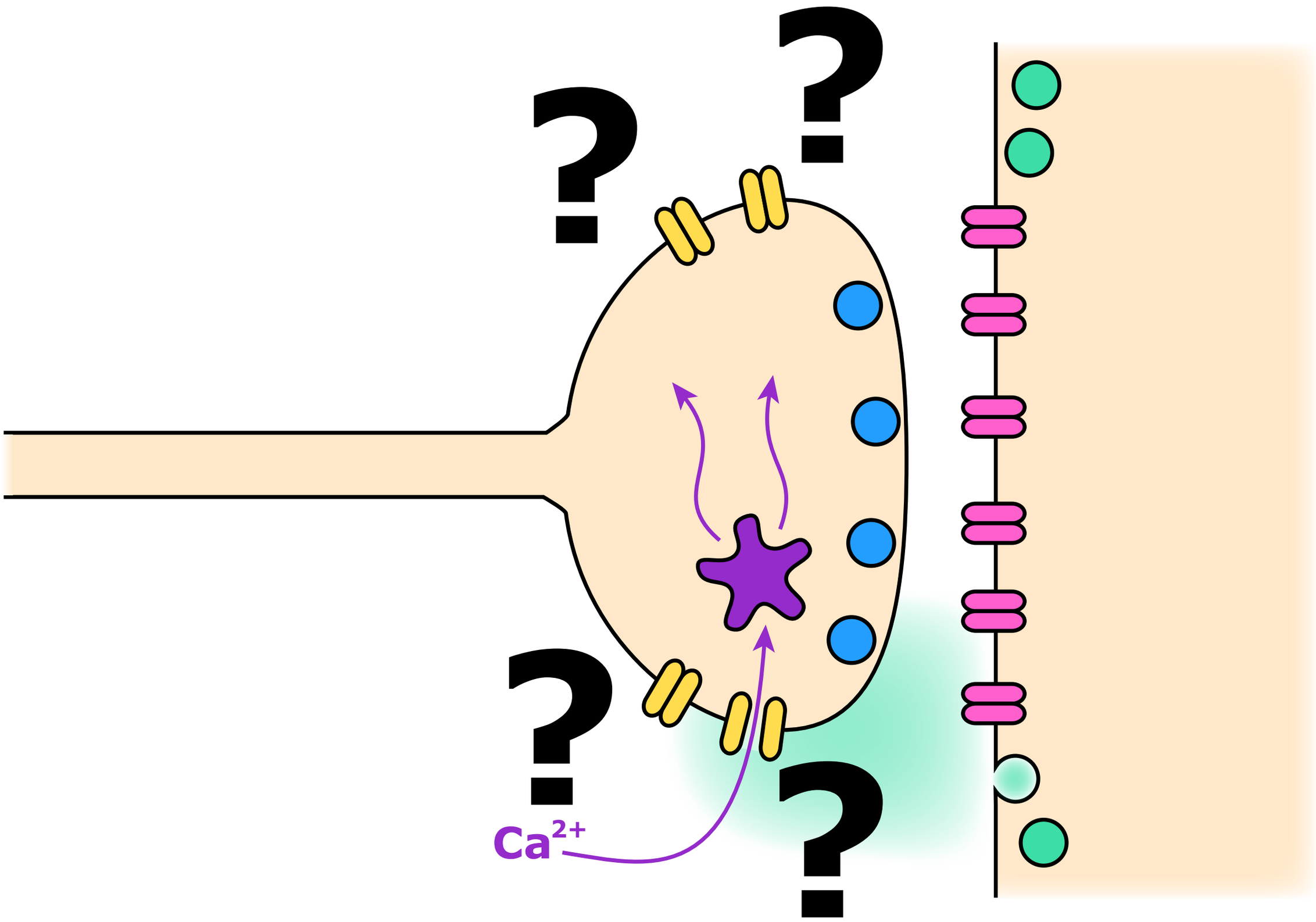


The boutons have their own internal calcium stores that help to maintain calcium concentration.

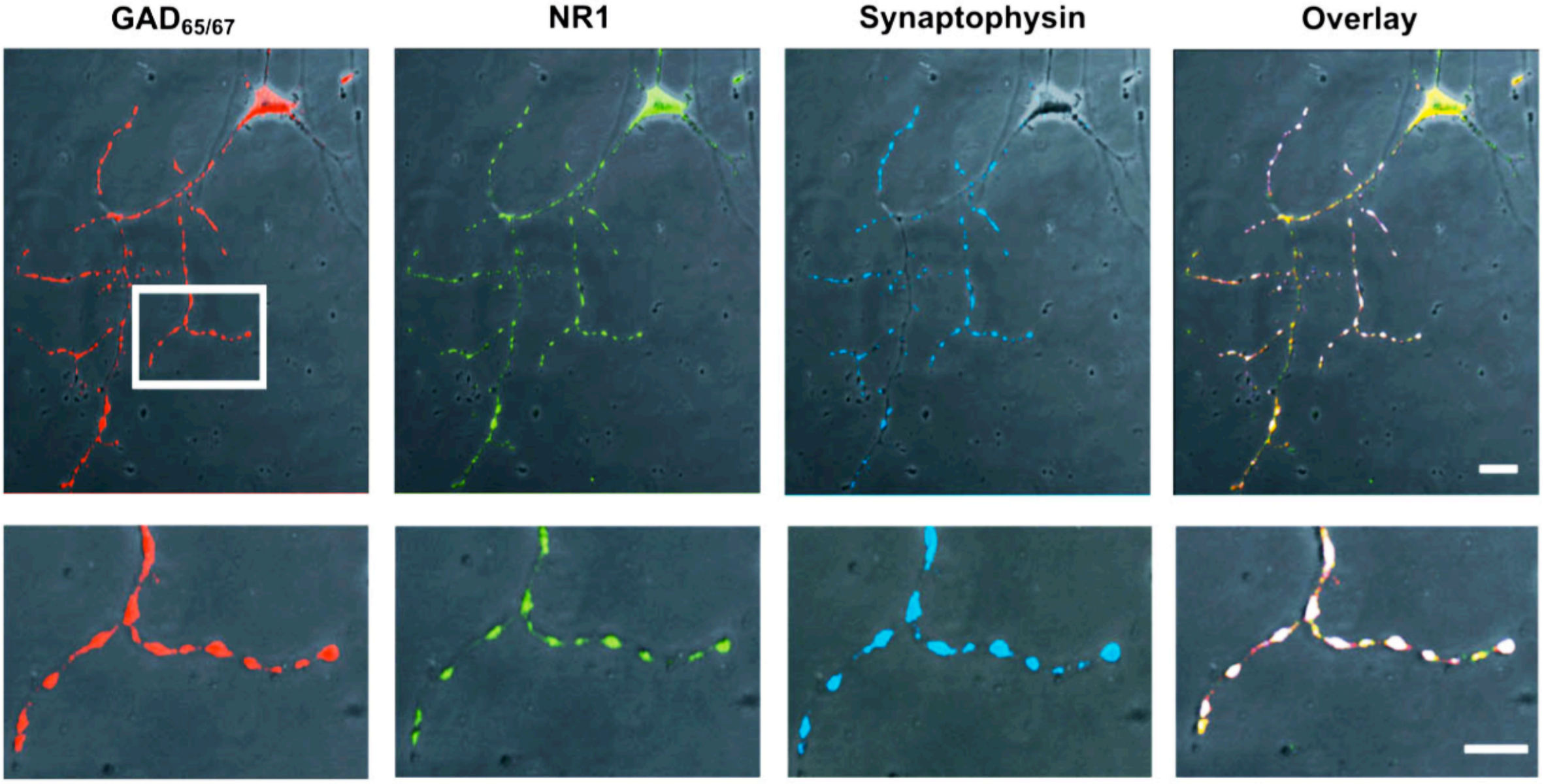


These stores participate in “calcium-induced calcium release”, detecting the incoming calcium and releasing more of their own -- effectively amplifying the signal to the point where it leads to DPI.

So this is our DPI story, and it seems pretty plausible and is in agreement with all the facts from a multitude of experiments. However, there’s a problem.

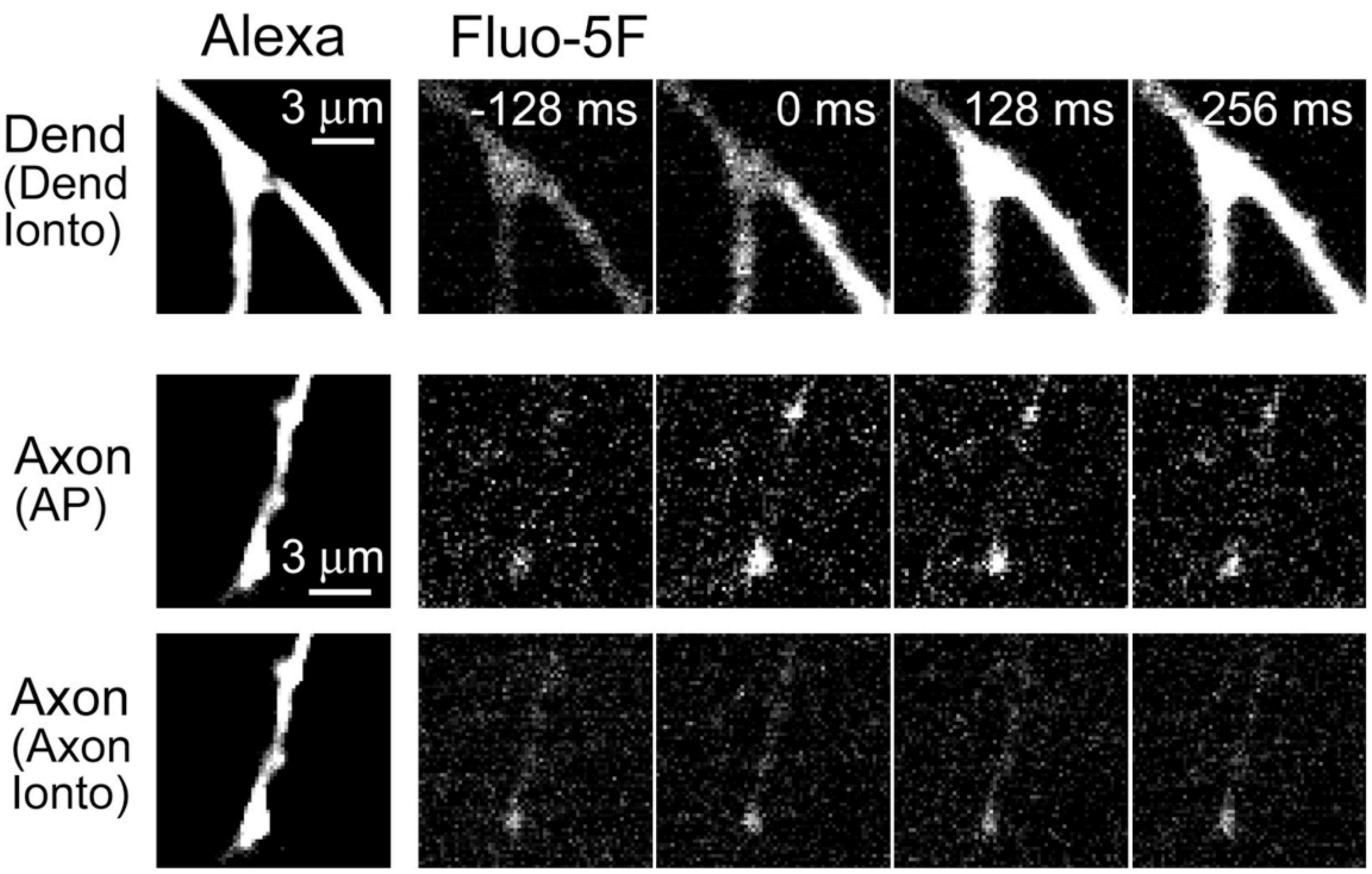


The process depends crucially on the presence of NMDA receptors in, or very close to, the presynaptic boutons. This is rather difficult to demonstrate experimentally, and there is conflicting evidence from different sources.



One approach has been to use antibody staining, but this can only detect the protein subunits that make up the NMDARs. The subunits are clearly present -- this image shows the colocalisation of the NR1 subunits with presynaptic markers in cultured cerebellar interneurons, and there are similar for the various NR2 subunits -- but the mere presence of the subunits does not prove that there are functional receptors.

Images: Duguid & Smart 2004

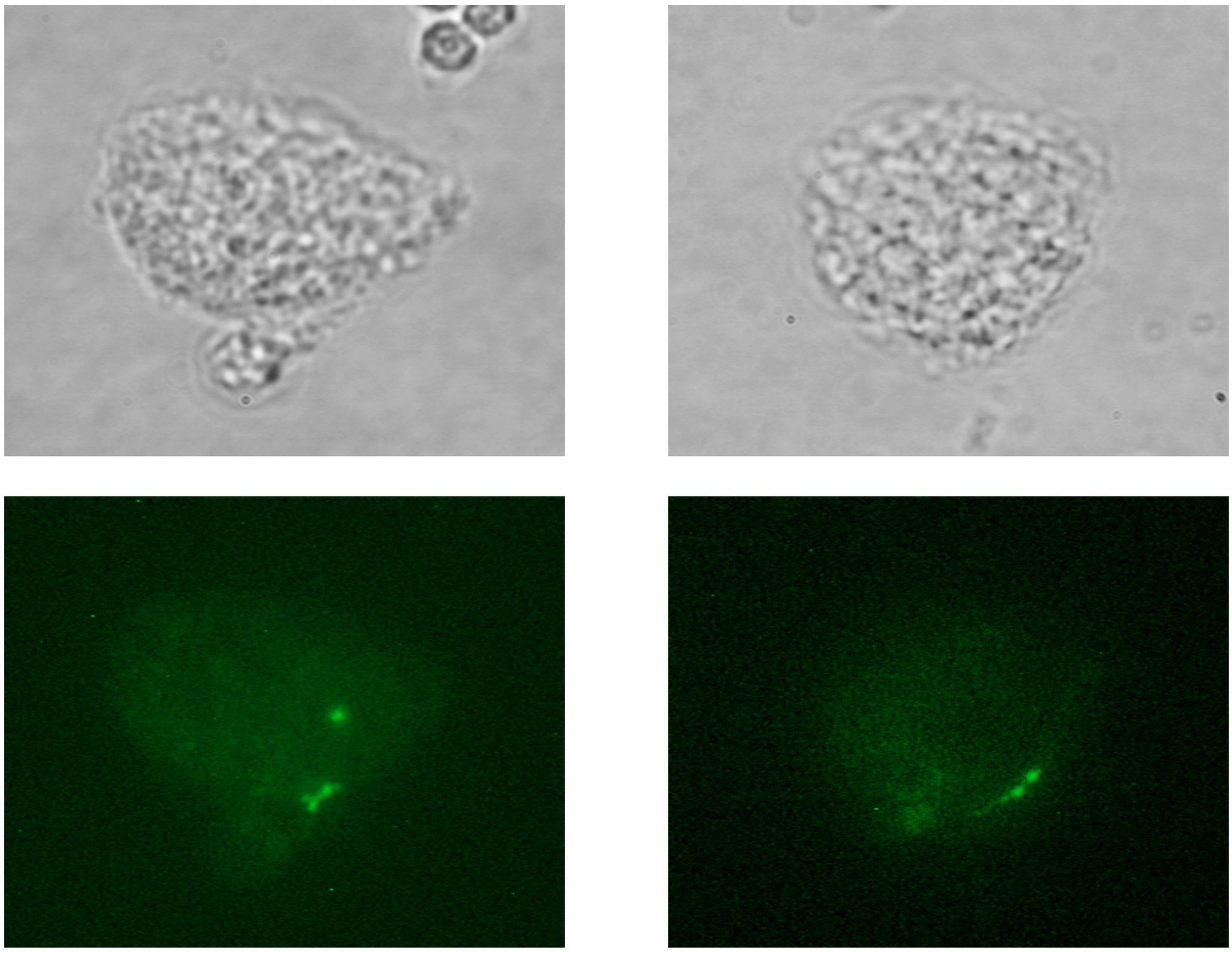


A different set of experiments published late last year cast doubt on the hypothesis. Christie & Jahr use calcium imaging to look for the calcium influx through NMDARs, and fail to detect it. They propose an alternative mechanism that involves somatic NMDARs and passively propagating depolarisation.

Now, let's be clear: Christie & Jahr are WRONG about this mechanism. The conclusions rest on shaky ground for some methodological reasons, particular that internal calcium stores were explicitly depleted before these tests were performed.

Moreover, the proposal that somatic calcium entry is required is provably false because...

Images: Christie & Jahr 2008

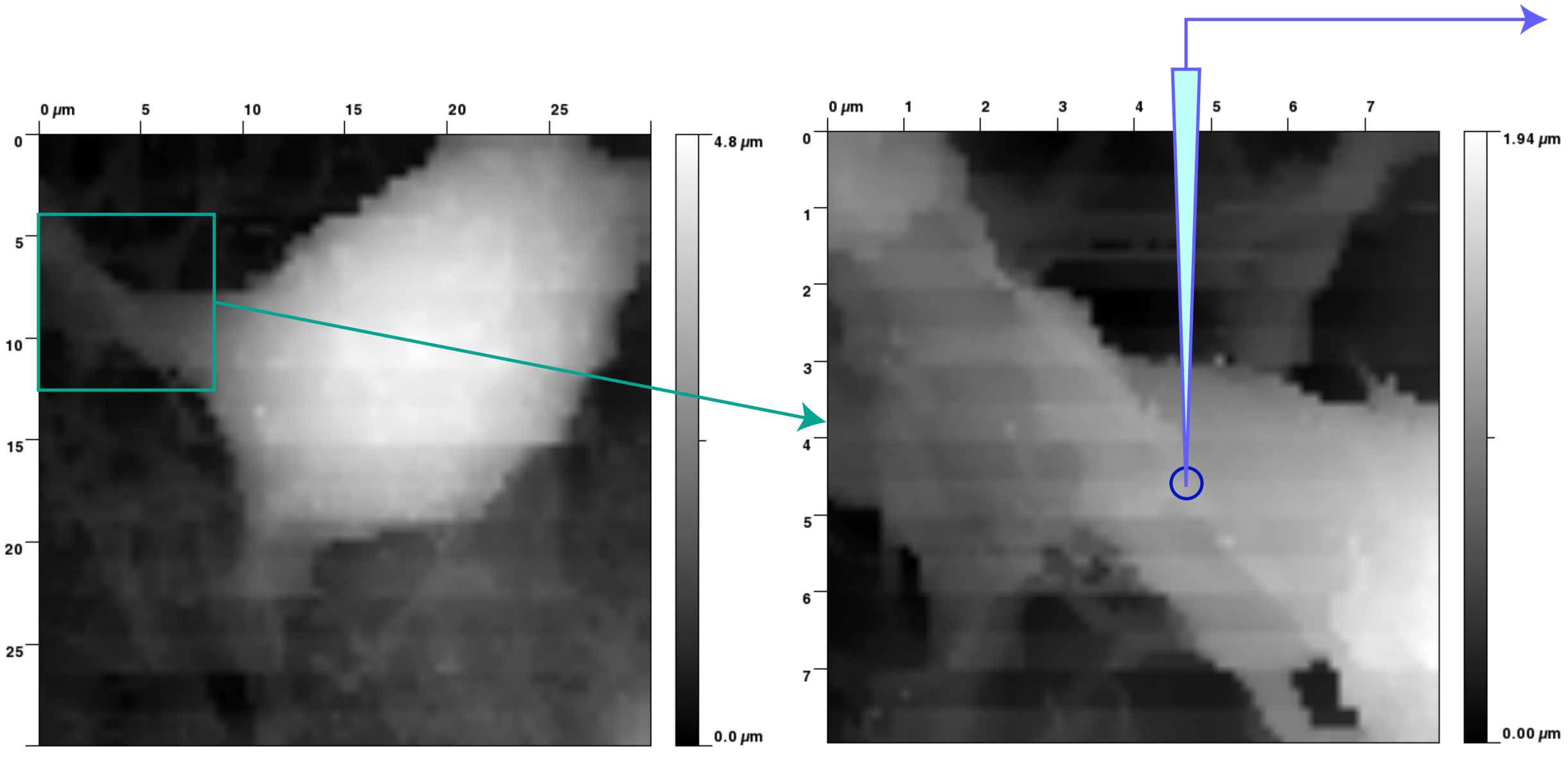


...DPI has also been demonstrated in the “vibrodissociated” or “nerve-bouton” preparation, in which only the boutons from presynaptic cells remain, while the dendrites and soma are absent. Thus, DPI cannot depend on activity in those regions.

Nevertheless, Christie & Jahr’s evidence is problematic, and we return to the need to prove NMDAR presence in the terminals. How can this be done?

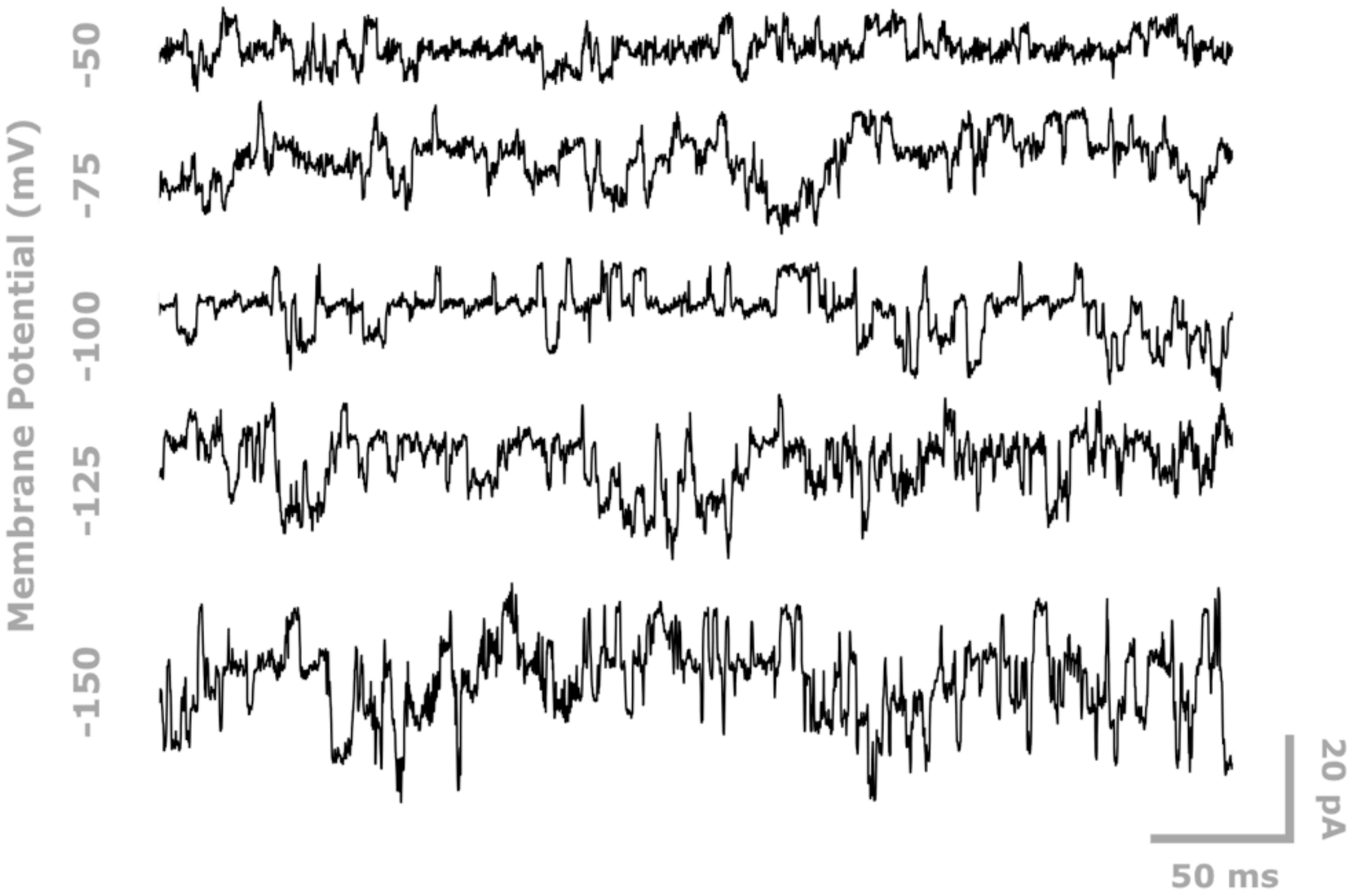
Well, electrophysiological recording could provide evidence of functional channels, but because we are interested in the very precise localisation of these channels the recordings would need to be taken from very specific, known sub-cellular structures, which is not how patch clamping normally works.

Enter the ‘smart patch’

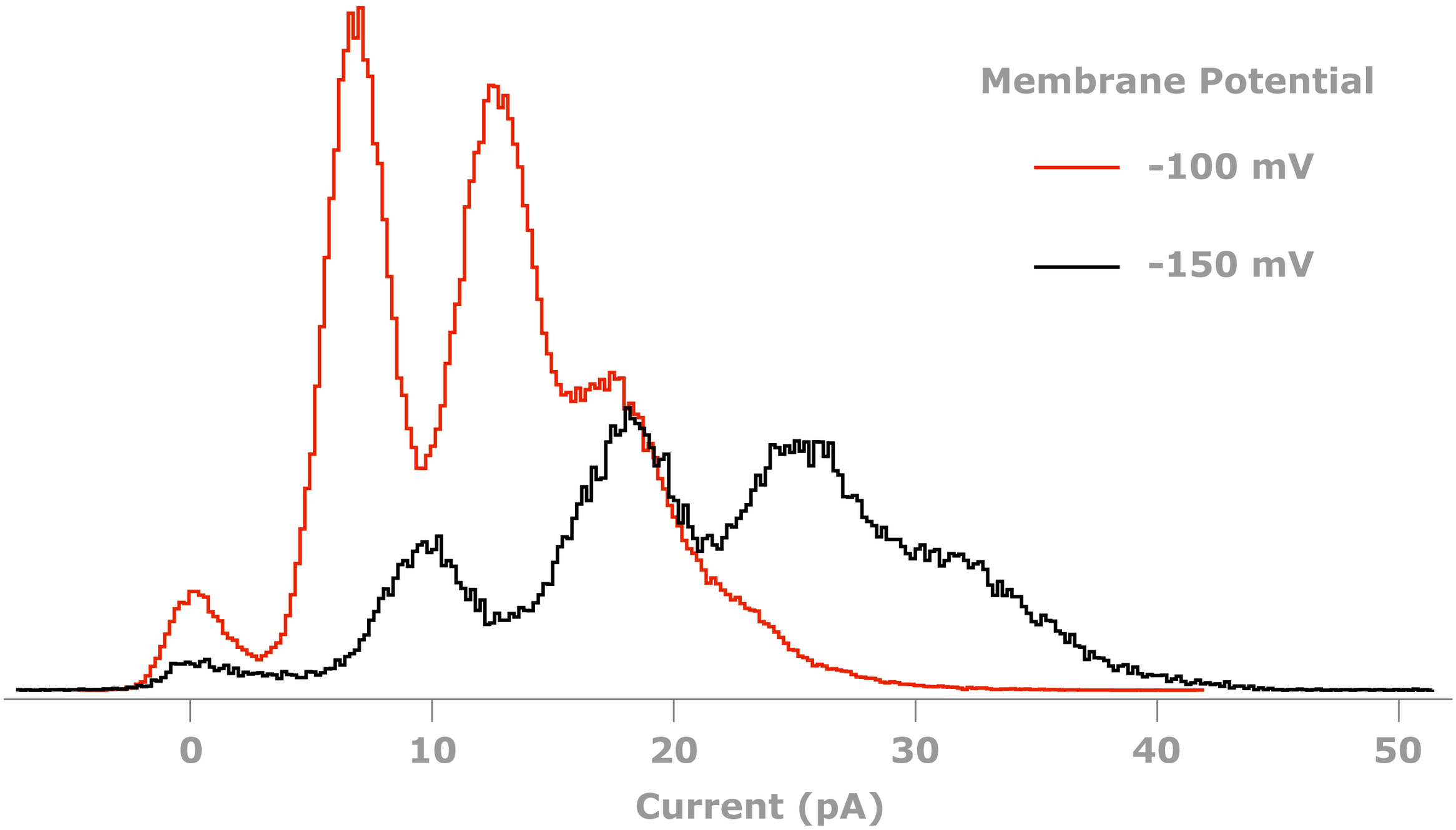


The scanning pipette used in ion conductance microscopy can be reused as patch electrode for single channel recording, and precisely positioned within the context of a known scanned topography. This should potentially allow the mapping of localised ion channel distribution.

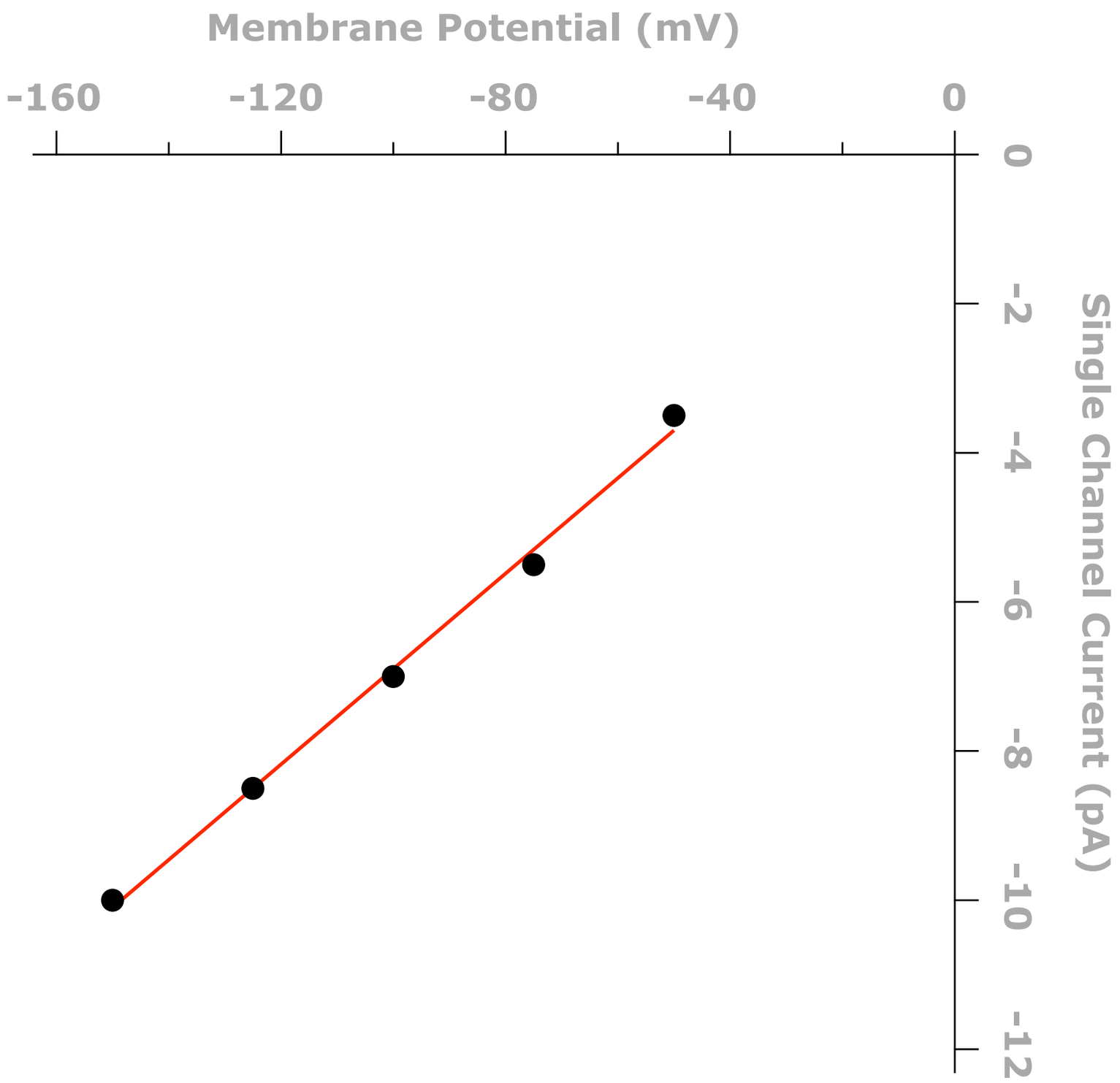
We can identify NMDARs in control conditions



We have RECENTLY been able to do this, occasionally, with a following wind and the moon in the right phase, and this is one such recording. Although noisy, the traces show several clear conductance levels.



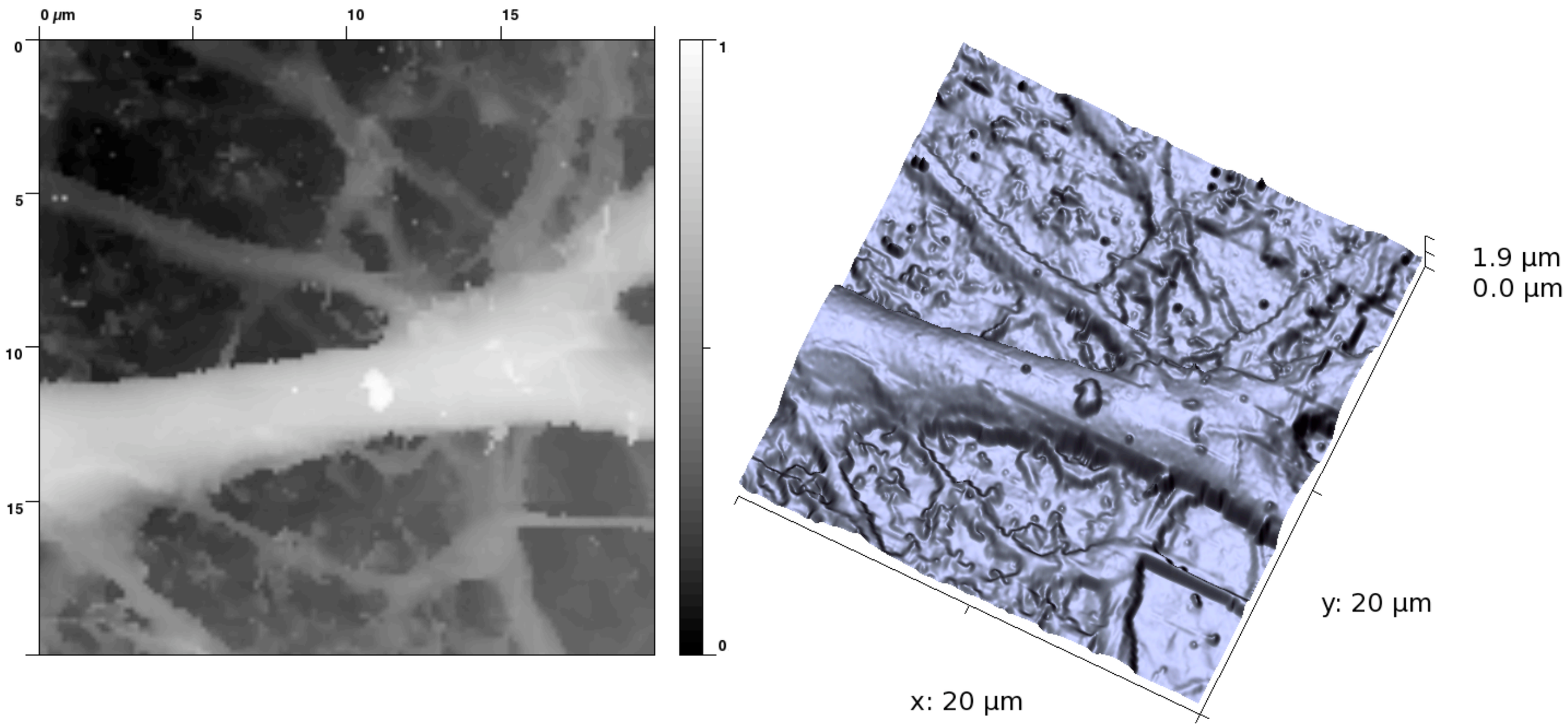
A histogram of current levels shows clear, fairly evenly-spaced peaks.



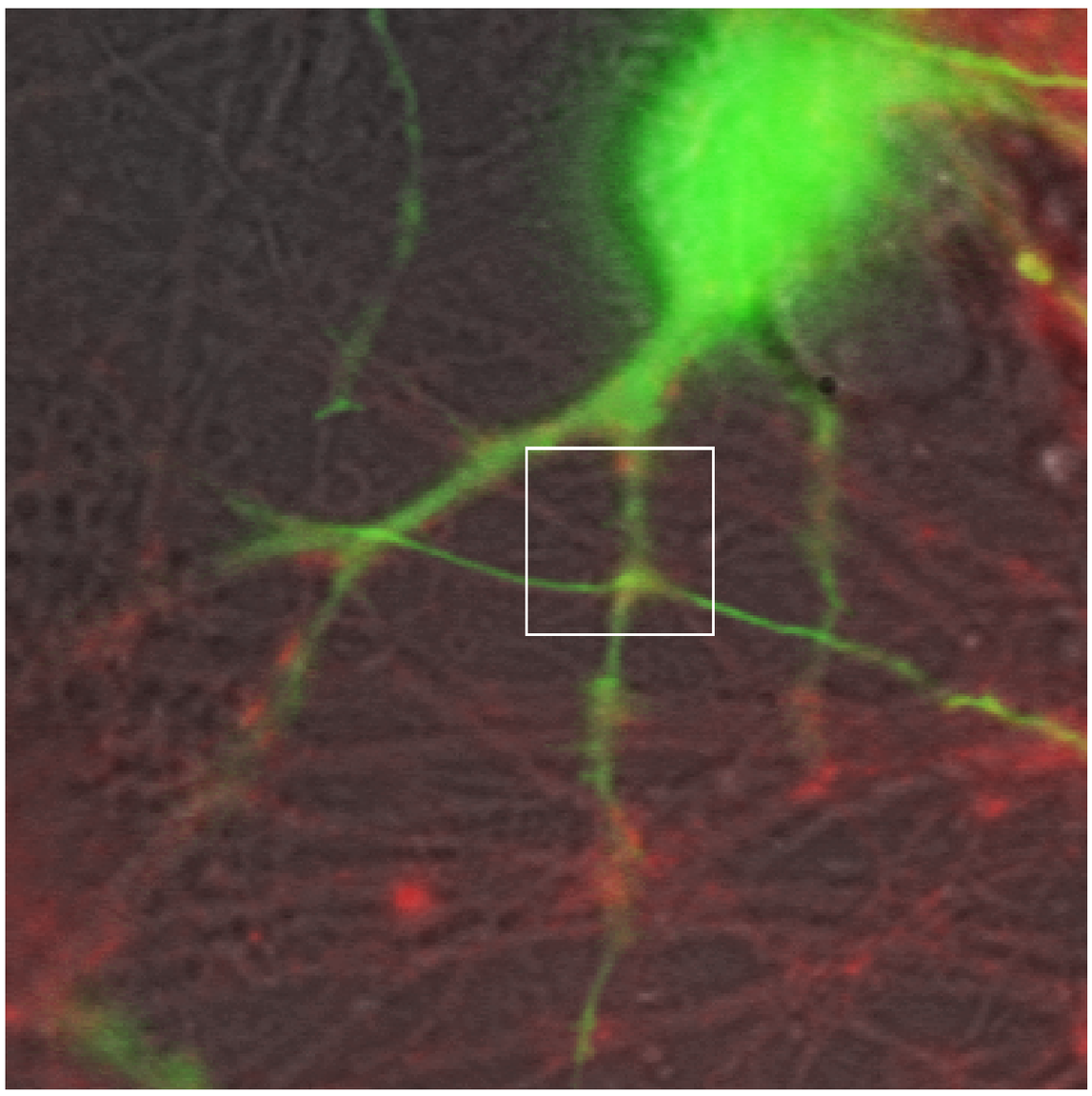
We can make a plot of these levels at different voltages to determine the channels' "slope conductance", which is in reasonable accord with previously-determined values for NMDARs.

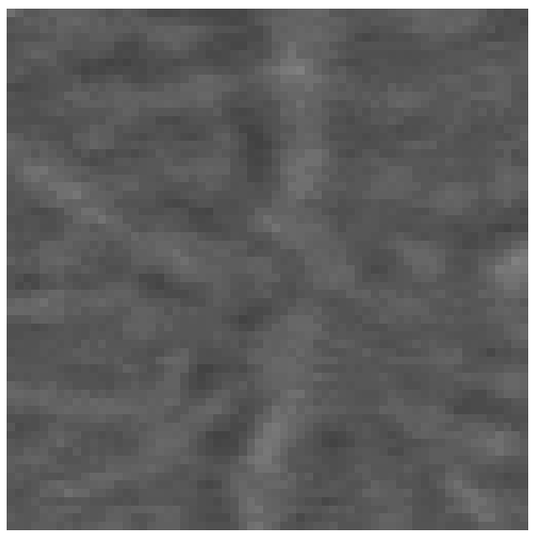
Note again that this is in control conditions, recording from somewhere we already know there are NMDARs. Even here our hit rate is not always what we would wish it to be. Nevertheless, it is possible.

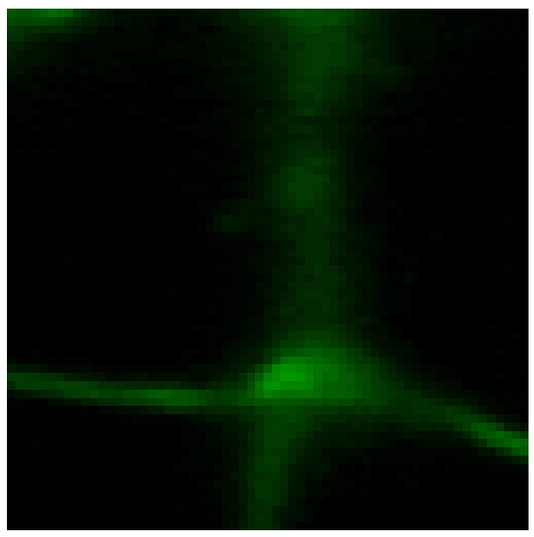
Spot the bouton?

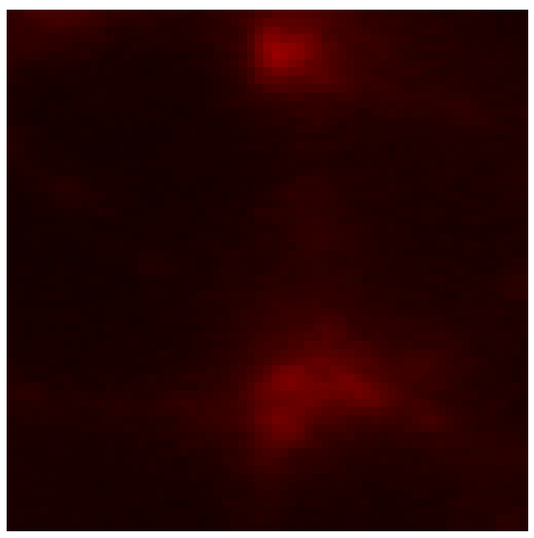


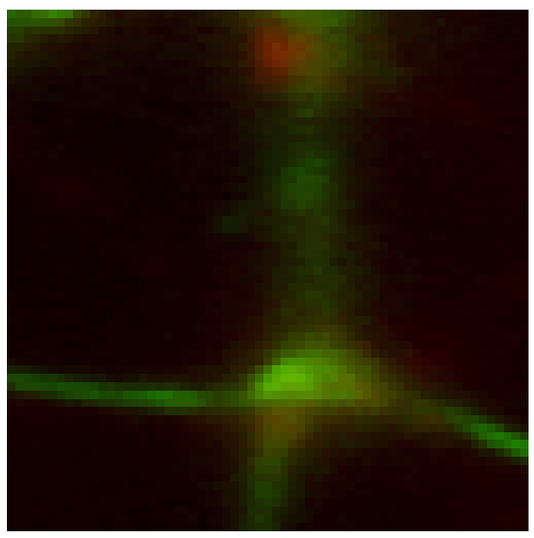
That's only half the problem, though, since we need to localise channels to the specific boutons in question. Which means we need to find those boutons. Topography alone does not provide sufficient information, so we must bring additional weapons to bear.





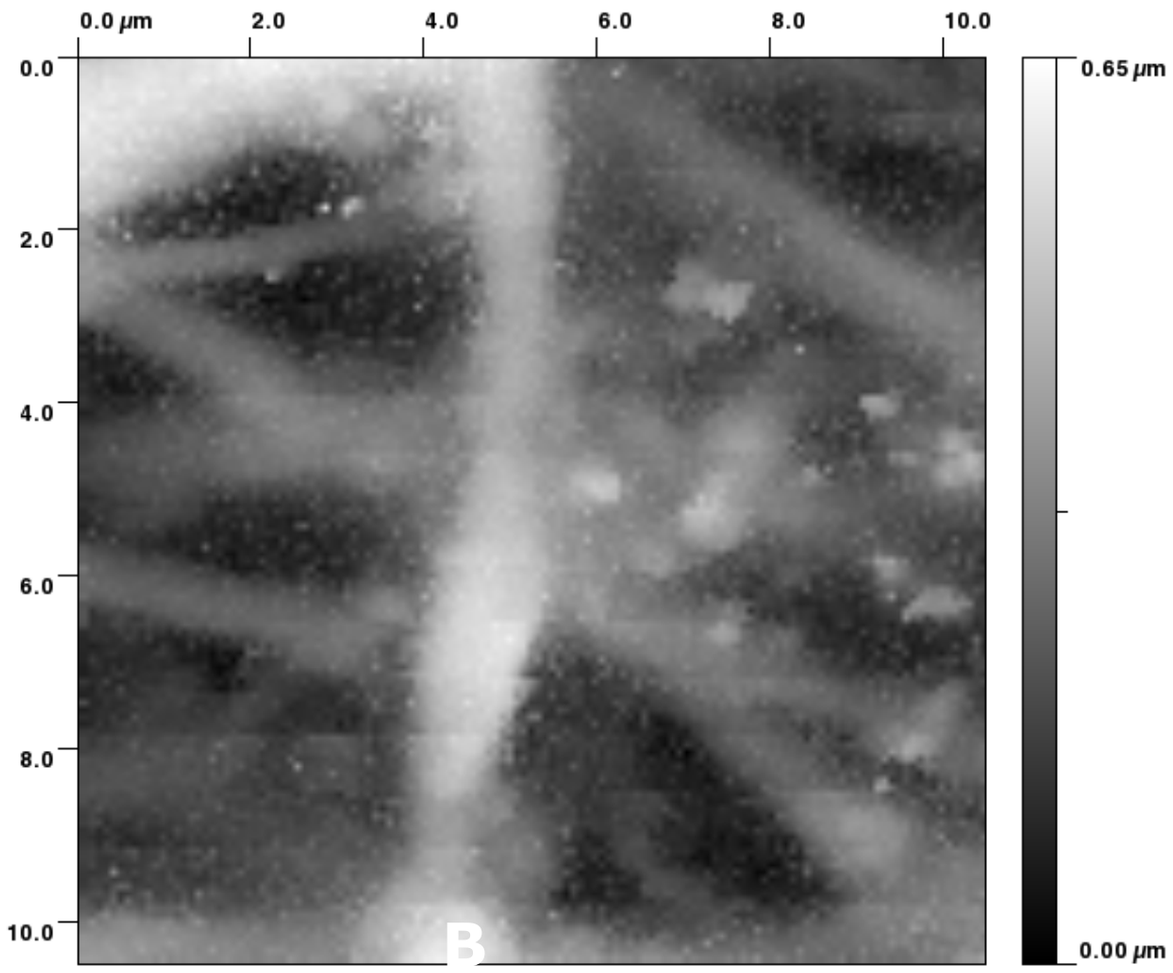


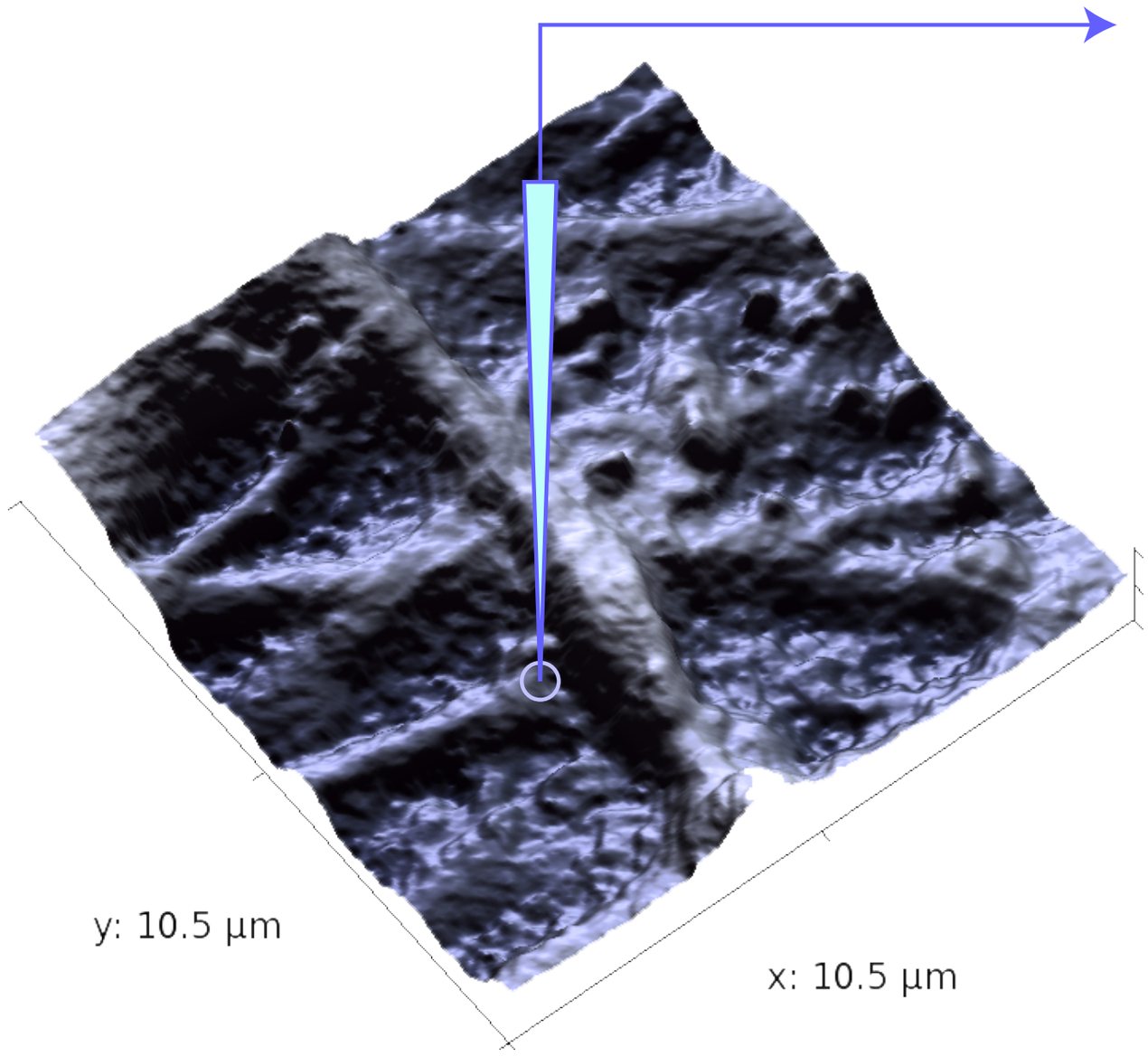




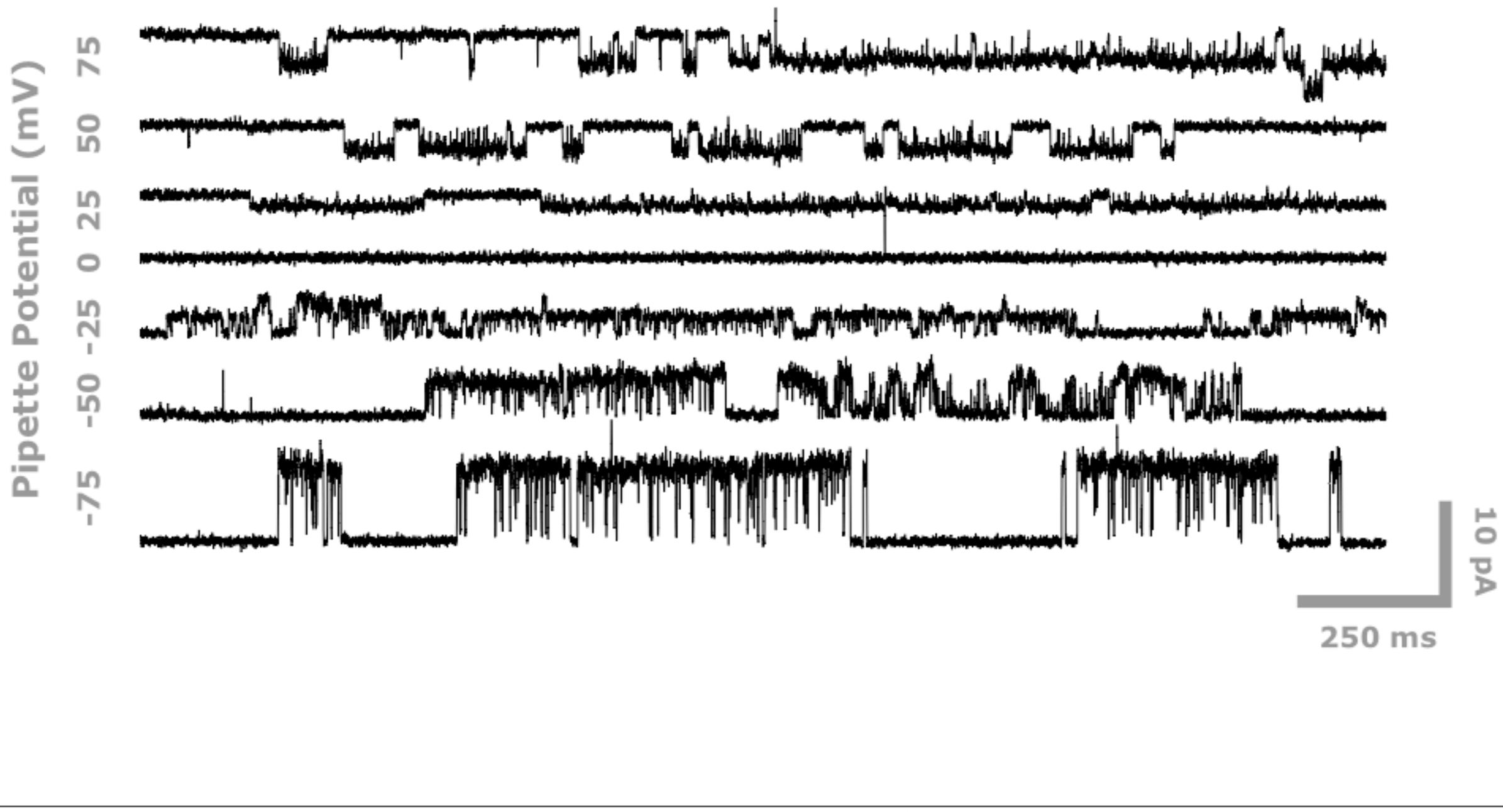
We employ two main fluorescence techniques, together, to locate boutons:
- transgenic mice express GFP in our interneurons, allowing us to identify the cells
- FM 4-64 stains synapses in an activity-dependent way

The imaging is still not always conclusive, but at least some of the time we can identify likely interneuron boutons where both markers coincide.





Using the fluorescence as a guide -- and assuming the corresponding feature can be identified in the topography, which is not guaranteed -- we can then manoeuvre the probe to the right place, lower it, and -- if we're very lucky -- patch.



This is far from a routine process, but we have managed it on a number of occasions, and here are some example recordings from one such putative bouton. Unfortunately, the channels we can see here are NOT NMDARs, but they demonstrate the possibility of localised recording.

Which is where the story ends, for now: the pieces are largely in place, but we haven't yet been able to put them all together. We hope to do so soon, although it's not entirely clear what kind of picture they'll make when we do...

Image: Dan Iggers

References:

Ian C Duguid & Trevor G Smart, “Retrograde activation of presynaptic NMDA receptors enhances GABA release at cerebellar interneuron-Purkinje cell synapses”, Nature Neuroscience 7(5):525-533, May 2004

Jason M Christie & Craig E Jahr, “Dendritic NMDA receptors activate axonal calcium channels”, Neuron 60(2):298-307, Oct 2008