Plant neurobiology

Intelligent Plants or Stupid Studies

In recent years, some researchers have been trying to establish 'plant neurobiology' as a serious research field. One point of criticism so far, however, has been that they use rather inappropriate and exaggerated terminology. But what about the quality of their results?

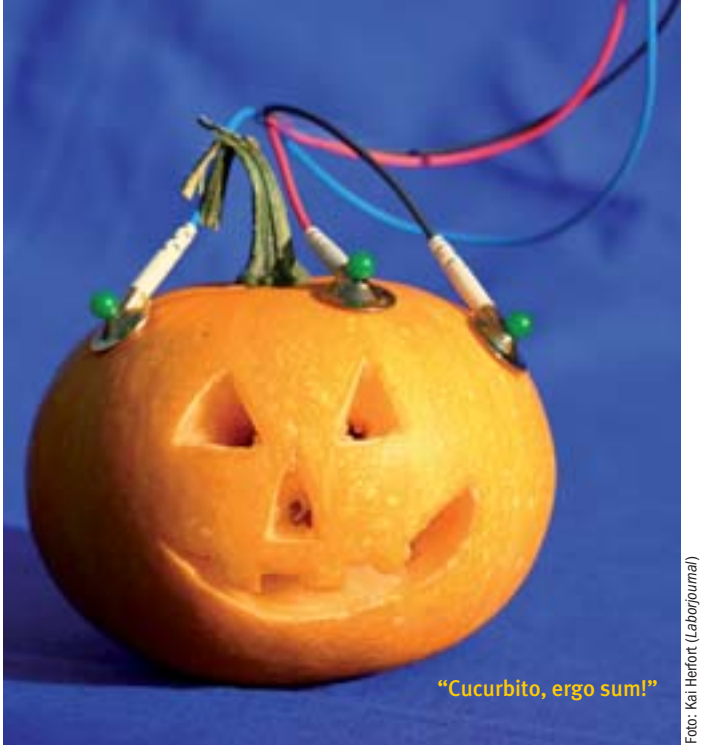
"Cucurbito, ergo sum!"

Foto: Kai Herfort (*Laborjoumal*)

In March 2009, an article appeared in the *Proceedings of the National Academy of Science USA* (*PNAS*) with the innocent title "Spatiotemporal dynamics of the electrical network activity in the root apex"(vol. 106(10):4048-53). The authors, Elisa Masi *et al.*, wrote about measuring electrical signals at the root tips of maize with a 60-channel electrode set-up. Using this system, they claim to have seen not only spontaneous electrical activity but also artificially triggered electrical spikes, which were locally transmitted.

Masi *et al.* conclude from their findings that these wandering electrical waves occurring in plants were similar to those found in excitable tissues in animals. The authors suggest that the cells in the root tips assimilate internal and external signals in order to adapt to their environment.

According to the discussion, "Our finding that cells in the transition zone show synchronised oscillation agrees with other data suggesting that this region of the growing root apex is some kind of sensory zone, specialized for integration of diverse sensory input formation that enable the growing apex to continuously monitor diverse environmental parameters and to mount appropriate adaptive output responses."

That is a general definition of brain function; in other words, plants have a brain and this brain is made up of root tip cells.

The sponsor of this sensational discovery – if it is one – is the brain researcher Emilio Bizzi from MIT, who is the president of the American Academy of Arts and Sciences. The main author of Masi *et al.* is Stefano Mancuso from Florence University. Frantisek Baluska from the Institute for Cellular and Molecular Botany at Bonn University also signed the publication. Mancuso and Baluska are members of the "steering committee" of the Society for Plant Neurobiology.


Plant brain?
No, plant tumour!

Lab Times' German sister journal *Laborjournal* already reported on Baluska's and Mancuso's ideas in 2005 (*LJ* 10/2005: 22-26). Baluska assured us then that, as in animals, plants have synapses in which the plant hormone auxin works as a neurotransmitter and he speculated that the root tips contain similar structures to those in animal brain tissue. These theories were met with resistance, even then. "That is absolute rubbish", said Gerhard Thiel, a membrane biophysicist from Darmstadt, Germany, to the *Laborjournal* reporter.

Incorrect interpretation...

When Mancuso and Baluska managed to publish their results in 2009 in the respected *PNAS*, it didn't make much of an impression on journalists, a folk usually susceptible to such sensational news. Maybe it was due to the conservative title that the papers weren't interested. Only one article addressing Baluska and Mancuso's trials, "The obscure plant brain" by Bernhard Epping, appeared in the German popular science magazine *Bild der Wissenschaft* (11/2009, page 30). Epping also seemed to have had his doubts and allowed the veteran plant electrophysiologist, Dietrich Gradmann, to voice his views opposing the results.

In the spring of 2009, Epping phoned Gradmann, pulling him out of retirement in his search for the opinion of an electrophysiologist on "plant neurobiology", only to discover that even he hadn't heard about Mancuso's work. However, after a good reading, Gradmann came to the conclusion that Mancuso had interpreted his results incorrectly and – even worse – they represented artefacts. Epping presented Gradmann's first conclusion in his article but did not go into detail on the latter.

Although Mancuso used the term 'plant brain'as a metaphor in his communication with Epping – he said, "Of course plants don't have a brain or nerves" – even the metaphor was too much for Gradmann. The quality of Mancuso's experiments eventually led Gradmann to write the following to the editors of the *PNAS*:

"Dear Editors, as an experienced plant electrophysiologist I'm horrified about the above article and its unprofessional reviewing. My blunt interpretation of the mysterious 'signals': The electrical contacts between the growing root tip and the rigid electrode array is frequently interrupted discontinuously and reformed continuously within some 10 ms.

Sincerely, Dietrich Gradmann"

The answer came the same day:

"Dear Dr. Gradmann, thank you for your comments. If you wish to contact the authors directly to discuss any questions you

might have, please feel free to do so. You can find the corresponding author's email address with the article.

Sincerely, Megan Miller, PNAS Editorial Office"

Gradmann answered:

"Dear Megan Miller, I did contact the corresponding author. However, my message was equally addressed to [the] editor and reviewers of this article. Please confirm, my message reached those persons.

Sincerely, Dietrich Gradmann"

On 05.11.2009 he was brushed off as follows:

"Dr. Gradmann, thank you for your feedback. We encourage you to contact the co-authors of the paper in addition to the corresponding author. Unfortunately we cannot reveal the review process of the paper.

Ryan Conley, PNAS Editorial Office"

One has to admit; at least it all went very quickly. In similar cases, other journals usually need weeks or even months as well as repeated enquiries until they deign to reply. Editors simply don't appreciate criticism about published articles.

... and even artefacts

The *PNAS* should, however, rethink their sponsoring system. Apparently, influential sponsors can push through dubious manuscripts without consulting competent reviewers.

Dietrich Gradmann wasn't going to resign himself to the fact that work like Mancuso's should undisputedly enter the annals of science. He believes that if such semi-scientific sorry efforts are published in a reputable journal, the misinformation could become virulent and channel resources to unfruitful fields of research. Therefore, Dietrich Gradmann explained his objections to Mancuso's *PNAS*-paper as follows:

"In 1992, David C. Wildon *et al.* published a paper entitled 'Electrical signalling and systemic proteinase inhibitor induction in the wounded plant' (*Nature* 360: 62-5). This work took the same line as Mancuso's and was much celebrated by strangers to this field but never confirmed by experts. Three dozen experts have, however, distanced themselves from the term 'plant neurobiology' in the article by Amedeo Alpi *et al.* 'Plant neurobiology: no brain, no gain?' (*Trends Plant Sci.* 2007, 12: 135-6).

"What's in a word?"

This critique has only influenced the authors of the above *PNAS*-article to a certain extent. In the current, phyto-electrophysiological context the key words synapse, nerve and brain are not literally used as they were previously (see *Trends Plant Sci.* 2005, 10(3)). However, Mancuso *et al.* do write, 'This behaviour is well known in cortical structures of animals...' in *PNAS* about their findings (page 4052, paragraph "Spatiotemporal Dynamics...", lines 6 and 7).

To quote Schiller: what's in a word? But the danger lies in the associations that are derived from such terminology. For instance, Mancuso uses the word 'sleep' to describe the nocturnal lowering of leaves (nastic movement) and concludes that studying the sleep movement of plants could solve human sleeping problems.

Associations suggested by vague terminology are supported by abysmal experiments. On studying the *PNAS* article (vol. 106: 4048), one gets the impression that the authors are incapable of discerning between signals and artefacts. In detail:

1. The methods are inadequately described. Impedance, filtering und polarisability of the input circuit must be numerical-

ly named in an electrophysiological study. The reader is provided with none of these. It is, therefore, unsure as to whether the observances shown are due to the apparatus or the object. Furthermore, the 'representative' results (see the *PNAS* figure below 'Electrical Activity') are not supported by statistics, even the number of trials isn't provided for this example. This leads to the suspicion that the authors only showed the example, which best represented their preconceived concept.

Nothing but lost contacts?

2. The fundamental event shown in the *PNAS*-Fig.1H (image right 'MultiElectrode Array') does not in any way reflect an action potential. It comprises of an abrupt hyperpolarisation (from the given signal/noise-minimum 20 µV to ca. 200 µV), which decays mono-exponentially to the base level with a time constant of some 10 milliseconds. It is possible to record extracellular action potentials of more than 100 mV in seconds with ordinary sewing needles. In contrast, Mancuso's recordings show very small amplitudes. That is bad, but not critical, as we don't learn anything about the impedances of the object or the amplifier input. However, the time course is critical. Action potentials don't abruptly leap from the base level to the peak but produce a characteristic, smooth curve, which reflects increasing conductivities.

Furthermore, plant action potentials elapse in ranges of >1 second and not 100 times faster, as per Mancuso (see image right). Additionally, all known action potentials display an overshoot before returning to the resting voltage. This overshoot is missing in the fundamental events shown in the *PNAS* article.

A simple explanation for these individual events is that they are due to short interruptions in the contact between the electrodes and the preparation, and the subsequent reforming of this contact, within some 10 milliseconds because the output voltage from conventional electrometers will drift towards the saturation level after interrupted contact. The amplitude of these events is dependent on the completeness of the interruption. It seems to obey a simple, exponential distribution with occasional high amplitudes and frequent low amplitudes, whereas physiological events would be expected to scatter around a mean amplitude

3. The most important point: the presented pattern of single events (for example *PNAS* Fig. 2, bottom left hand side, left image) doesn't reflect a mysterious root intelligence but just a loose contact between the rigid electrode array and the moving surface of the root preparation. If the experimental procedure had been described in detail, then it would be possible to say whether this surface area reduced during the course of the observation due to wilting or increased due to continual growth. I presume the latter, as toxins disrupt growth (as shown in Mancuso's paper, *PNAS* 106; 4048-53, Fig. 6) but not evaporation.

The predominant pattern in *PNAS* Fig. 2 comprises of short episodes with almost simultaneous elementary events and longer gaps. This is to be expected, when the electrode array and the surface of the preparation don't move smoothly against each other but tend to 'stutter'. When the static friction of individual contacts is exceeded, these will then disconnect, whereby neighbouring points of contact are then more heavily stressed, which in turn gradually leads to the disconnection of many contacts. Then the electrode array and the surface of the preparation relax towards each other and new contacts are formed again. These will hold until the next burst of 'synchronised activity'.

"The paper must be withdrawn."

It is, therefore, easy to understand that the coupling between neighbouring contacts diminishes, depending on how far apart they are and that the contacts in the direction of growth break more frequently than those transverse thereof (see image 'electrical activity' bottom left hand side, middle and right image).

After pointing out this problem to Mr. Mancuso on 04.11.2009, he generously answered on the same day, 'Please, feel free to find all the evidences supporting your interpretation.' Apparently, Mr. Mancuso does not think much of the rules regarding the burden of proof. Should such bad work be permitted to have so much success? No responsible author, reviewer or editor of a scientific journal should have allowed this paper to go through. The *PNAS* paper (106:4048) must be withdrawn."

HUBERT REHM and DIETRICH GRADMANN

ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER