Gravity-Related Paradoxes in Plants: Plant Neurobiology Provides the Means for Their Resolution

František Baluška, Peter W. Barlow, Dieter Volkmann, Stefano Mancuso

ABSTRACT: The plant body is shaped by gravity. Shoots grow up and roots grow down. This simple and obvious link between gravity and plant form is still not understood and continues to attract the attention of experimental plant biologists. Nevertheless, it is now generally accepted that sedimenting amyloplasts act as statoliths in those cells of both root and shoot which are specialized for gravisensing. Moreover, auxin is also evidently involved in the gravistimulated differential growth (gravitropism) of roots and shoots. But what is missing from a full explanation of the plant graviresponse is knowledge of the signal perception and transduction pathways, from the sedimenting statoliths to the motoric response of organ bending.

Recently, the new approach of plant neurobiology was introduced to plant sciences. It focusses on neuronal molecules, vesicle trafficking, integrated signaling and electrophysiology. In conjunction with the concepts developed in biosemiotics, plant neurobiology might bring fresh views to many of the old issues plant growth to environmental signals. It emerges that auxin acts as a neurotransmitter, being secreted at plant synapses and inducing electrical signals which then induce motoric responses. A hypothesis is proposed whereby the plant synapses themselves are considered to be gravisensitive; they might also be involved in memory phenomena and signaling integration. The transition zone of the root apex not only initiates the gravitropic bending but also acts as some kind of 'command centre' which integrates all sensory inputs into adaptive motoric responses, and may also store information in the form of a plant memory. This new neurobiological view of plant gravitational biology not only explains the close relationships between the gravity vector and polar auxin transport but also integrates the Němec–Haberlandt statolith-starch theory with the Cholodny–Went auxin transport theory.

1. Introduction

All biological organisms are embedded within a physical environment which shapes both their organization and behaviour.[1] In order to survive, all biological systems continuously retrieve information from their environment and use it to adapt their mode of growth and, hence, increase their fitness.[2] In humans and animals, neurons transform sensory information obtained from the environment into electrical impulses which are then translated into biological signals that induce motoric responses.[1] Similarly in plants, gravity, as well as other diverse variables within the physical environment, are continuously monitored via specialized cells such as root cap statocytes and root

transition zone cells. Any deviation of a plant organ and its cells from a certain selected, and presumably optimal, angle (the liminal angle) is sensed and leads to motoric growth responses such as the gravibending of a root apex. Just as the perception of gravity in mammals leads to a continual adjustment of posture so, in plants, graviperception continually helps plant axes to maintain the liminal angle within their living space.[2] If adjustments to the predominant direction of growth are not possible, then new forms of the plant are developed to accommodate their new relationship with the gravity vector.[3] At the subcellular level, reorientation of plant organs within a gravitational field induces rapid transients of cytoplasmic calcium[4] and electric signals,[5-8] both of which precede the gravitropic motoric response. Moreover, auxin transport is closely related to these gravity-induced calcium spikes.[4] Auxin emerges as plant neurotransmitter,[9-12] and its cell-cell transport is essential for root gravitropism. It is quite obvious that our rudimentary knowledge on the mechanistic and molecular basis of both gravisensing and gravitropism is largely due to our ignorance of the neurobiological aspects of plant life.[11,13] This is surprising when we consider the century-old tradition of studies on plant neurobiology.[11,14,15]

2. Differences between root and shoot gravitropisms

All plant organs are able to perceive weak gravity forces and respond to them in a predictable manner, aligning their cells and whole organs according to the gravity vector. Charles Darwin was one of the first who studied plant movements and he characterized the bending of plant organs in relation to gravity as 'gravitropism'.[16] Both gravitropism and phototropism, being adaptive motoric responses, are universal plant responses to the physical environmental parameters of gravity and light, respectively (for the most recent review, see 17). Despite the overall similarity of root and shoot gravi-behaviour, profound differences emerge upon detailed scrutiny.

First, root apices grow downwards whereas shoot apices grow upwards in response to the gravity vector. This is generally considered in a simplistic, or teleological way as roots are evidently underground organs and shoots are aboveground organs. However, the case is not so simple when we take into consideration the rhizoids and protonemata of *Characean* algae, which also grow downwards and upwards, respectively.[18] These two tip-growing cell-types show similar cytoarchitectures,[19,20] and their high gravisensitivity is related to the presence of sedimenting intracellular vesicles filled with barium crystals.[18] Interestingly in this respect, tip-growing root hairs and pollen tubes lack any sedimentable structures and are not responsive to gravity. The significant difference between rhizoids and protonemata of the *Characeae* is that, in the protonemata, the statoliths sediment closer to the tip than they do in the rhizoids. In the first-mentioned cell, the statoliths displace

the 'Spitzenkörper', a body which acts as a vesicle supply center, but they do not show this feature in rhizoids.[19,20] But even armed with this knowledge, the mechanistic link between the sedimentation of statoliths and the downward growth of the rhizoids and the upward movement of the protonemata remains elusive.[21] We should also keep in mind that *Chara* cells, similarly like most plant cells, are inherently excitable.[22]

Second, although the sedimenting starch-based amyloplasts are found in most gravisensitive cells of both shoots and roots, recent studies have revealed that their sedimentation is not inherently linked with shoot gravitropism.[23-25] Mutants defective in shoot gravitropism, but with normal root gravitropism, show normal sedimentation of starch-based amyloplasts in the shoot.[24,25] The feature of the *grv2* mutant of *Arabidopsis thaliana* provides strong genetically based evidence of profound differences between shoot and root gravitropism because *GRV2* is single-copy gene.[25]

Third, studies of the role of filamentous actin (F-actin) in gravitropism through the use of actin-depolymerising drug latrunculin B show that, whereas latrunculin stimulates gravitropism of both roots and shoots, the statoliths in roots sediment normally but in shoots sedimentation is inhibited.[26] These findings lead to the surprising conclusion that the stimulating effect of latrunculin B treatments on gravitropism is not related to sedimentation of the amyloplast-based statoliths. Depolymerization of F-actin via latrunculin B treatments must target some other processes essential for both gravisensing and gravitropism.

Finally, there is a dramatic difference between the speed with which shoots and roots accomplish their gravitropism. Whereas it takes 6 days to complete gravitropism of maize shoots[27,28] it takes 2 hours to conclude gravitropism of the roots.[29,30] The nature of the extremely rapid graviresponse of roots is currently unknown. though its significance for root biology is clear. It is somehow related to a root-specific organ known as the root cap which covers the whole root apex. The shoot apex lacks such an organ. In contrast to the root apex, the shoot apex lacks clear demarcation of growth zones.[31] Growing root apices are actively searching for plant food (nutrients and water) to nourish the whole plant. All this implicates the root apex as the 'head-like' anterior pole of the plant body while the shoot apex, being specialized for the development of sexual organs, acts as the posterior pole.[13]

3. Root Cap

Growing root apices show several other directed growth responses including hydro-tropism, oxytropism and electrotropism. Evidently, roots monitor a wide spectrum of physical parameters, and then integrate the signals obtained in order to perform

appropriate and often complex growth manoeuvres to cope with the immediate environmental circumstances. The more acute sensitivity of root apices to various types of signals already mentioned, when compared to shoot apices, is related to their root caps.[32] With few exceptions, these small organelles cover the root apex and are specialized for sensing and interacting with the physical parameters of root environment.[33] Intriguingly, root cap statocytes, grouped together within a mechanosensitive root cap, resemble in many respects the vestibular organs of lower animal.[34] This fits nicely with the above-mentioned plant neurobiological perspective in which the root apex represents the anterior pole of plant body.[13] Root apices are always actively seeking nutrients and avoiding dangerous regions of the soil which would jeopardise growth of the whole plant – as does the head of a lower animal.

Charles Darwin and his son Francis were well aware of the unique properties of the root apex with respect of its screening of environmental parameters and of initiating rapid root bendings in order to obtain water and inorganic nutrients.[16] Moreover, they realized that root apices not only receive information about the environment but also integrate this information. In their book, 'The Power of Movements in Plants', the Darwins likened the root apex to the brain of a lower animals: "it is hardly an exaggeration to say that the tip … acts like the brain of one of the lower animals; the brain being seated within the anterior end of the body, receiving impressions from the sense organs, and directing the several movements".[16:573]

4. Statoliths

Maybe it was the sentence above from the Darwins' book which stimulated, 20 years later, Bohuslav Němec (Figure 1) to postulate that the central part of a root cap acts as a vestibular organ for root apices.[35] Němec, having been trained as zoologist, realized that the polarized cells of the cap, known as statocytes, are specialized for sensing of gravity via their sedimenting starch-filled plastids. Almost simultaneously with Bohuslav Němec, Gottlieb Haberlandt proposed a 'statolith theory' for sedimenting starch-based amyloplasts in shoot endodermal cells.[36]

Ever since those early days in which starch grains were postulated to be plant statoliths whose sedimentation underlies the exquisite gravisensitivity of growing root apices, numerous studies have been published which confirm this concept as one of the basic tenents of gravitropism. However, as so often happens in science, after its initial enthusiastic reception, the Němec–Haberlandt theory was slowly abandoned, only to be resurrected after more than sixty years when it was shown that surgical removal of the maize root cap did not compromise root growth but that such decapped roots lost almost completely their gravisensitivity.[37] Later, a genetic approach to cap

ablation finally confirmed that starch-based statoliths act as plant statoliths for both roots and shoots.[24,38,39] In the mean time, the technique of magnetophoresis, which allows the manipulation of statolith positions within statocytes, had also provided strong experimental evidence for the status of sedimenting starch-filled amyloplasts as gravity-perceptive statoliths.[40-42] Still mysterious, nevertheless, is how the signal perceived by and transduced from statolith movement is relayed to the processes of differential cell growth.

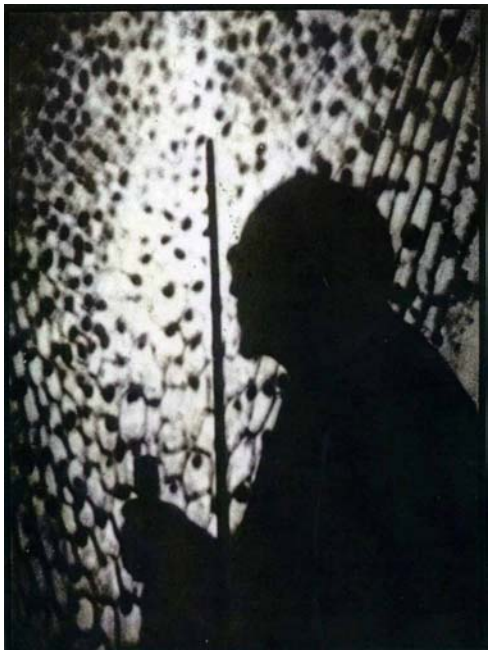

Figure 1: Bohumil Němec, at the age of 91, delivering a lecture entitled 'Georeceptors in plants' at the 10th International Botanical Congress in Edinburgh, 1964. The theme of his talk was that the primary stimulus of gravitation consists in a heavier or lighter pressure on the external cytoplasmic layer (cf. Fig. 2). Němec died two years later, having witnessed a modern revival of his statolith theory proposed in 1901.

5. Actin cytoskeleton

In the 1990's, a hypothesis was proposed by Andreas Sievers and his co-workers in which sedimented amyloplasts were postulated to push upon actin filaments anchored at the plasma membrane, preferably at stretch-sensitive channels which thereby would be activated.[43,44] However, the hypothesis was not supported by later observations which showed that root cap statocytes are actually devoid of prominent F-actin elements[29,45,46] and, moreover, that depolymerization of F-actin does not compromise gravisensitivity but, in fact, increases it in all plant cells so far tested. These data thus support a converse view, namely that plant statocytes are sensitive to gravity because their actin cytoskeleton is actually less robust and as well as extremely dynamic.[29,44,45] Obviously, these features preclude any actomyosin-based control over larger organelles such as amyloplasts. In fact, the plastid surfaces are associated with a unique population of myosins which seem unable to control statolith positioning.[45] So, in accordance with the finding that depolymerization of F-actin stimulates root gravitropism, there is also stimulation due to the inhibition of myosins.[47] Intriguingly, even decapped maize roots regained their ability to perform root gravitropisms if they are caused to become devoid of both F-actin and myosin activities.[30] These findings correspond well to previous observations which suggested that, besides the root cap, there are some other tissues of the root apex which are graviresponsive and can initiate root gravitropism.[48]

6. Endocytosis, vesicle trafficking, and auxin transport

If it is not the actin cytoskeleton, then which cellular structures are the gravireceptor and gravitransducer? Because gravitropism is very rapid in root apices, the transducer, at least, might be expected to be in close proximity to the growth machinery. Recently gathered data indicate that the vesicle trafficking apparatus might be the elusive structure, and that it is affected by the sedimenting starch-based amyloplasts in such a way that an asymmetric growth response initiates gravitropism. Moreover, the vesicle trafficking apparatus is closely associated with the actin cytoskeleton since endocytosis, exocytosis, as well as vesicle movements are all processes dependent on F-actin and myosin.[49-53] Furthermore, several gravitropism-defective mutants are deficient in those proteins which are specifically related to vesicle trafficking.[24,25,54]

The *Arabidopsis* gene *GRV2* encodes a protein similar to the DnaJ-domain protein RME-8[25] which functions in endocytosis and vesicle trafficking in animal cells;[55,56] a similar function is therefore expected of it in plant cells. Interestingly, another plant DnaJ-domain

protein, ARG1, is implicated in plant gravisensing as *arg1* mutant plants of *Arabidopsis* are defective in their response to gravity.[54] Moreover, ARG1 localizes to vesicles that recycle the auxin transporter PIN2 which normally drives the basipetal polar auxin transport essential for root gravitropism.[57] PIN2 localizes to specific plant endosomes which are characterized by the sorting nexin, AtSNX1. Gravistimulation promotes accumulation of PIN2 in endosomes of cells at the upper part of gravistimulated roots[58] whereas PIN2 accumulates at the cell periphery in the lower part of gravistimulated roots.[58-60]

Recent localization of auxin within endosomes of root apex cells,[12] as well as the rapid and powerful inhibition of polar auxin transport with inhibitors of secretion, such as brefeldin A and monensin, indicate that auxin is secreted from root cells via a vesicle trafficking apparatus.[9-12] Possible roles for endocytosis and endosomes in gravisensing and gravitropism are inferred from the latest data obtained from *Characean* rhizoids using high-pressure freeze fixation and 3D dual-axis electron tomography. Distinct 'endocytic sites' with associated clathrin-coated vesicles have been visualized at domains to which statoliths sediment, and another population of clathrin-coated vesicles was found in the Spitzenkörper.[21] This vesicular body has been revealed as an endosomal compartment in fungal tip-growing cells using FM4-64 labelling.[61]

The hypothesis of starch-based amyloplasts acting as statoliths, as well as that proposing a role for auxin in the bending of plant organs – the Cholodny-Went theory – were often criticized, though in recent years they have found renewed support.[17] Advances in our molecular and cellular understanding of polar auxin transport have identified clearly that endocytosis, endosomes and vesicle trafficking are all crucial players in those process[12,51,60] that shape the plant body in accordance with sensory information received from light and gravity.[11,12,62] A further crucial finding is that lateral transport of auxin across a gravistimulated plant organ[63] drives its gravitropism.[30,62] Nevertheless, the big question remains as to why auxin transport follows the gravity vector. A possible answer to this question can be provided by invoking putative gravisensitive auxin secretion domains[12] which we have termed 'plant synapses' – as acting in accordance with the gravity vector (see Fig.1 in 10). We shall now discuss evidence for that view.

7. Plant synapses as gravisensing domains secreting auxin

Recently, we have proposed that cellular end-poles represent subcellular domains specialized for cell-cell communication via vesicle trafficking. These end-poles are the 'plant synapses'.[10] Endocytosis and vesicle recycling would be part of an ideal system for gravitransduction, the primary graviperception occurring via the mass of

protoplasm in which statoliths (if present) augment this mass and, hence, increase the speed of gravireaction. Endocytosis is inhibited by an increased tension of the plasma membrane.[64] This mechanical stress is then relieved by vesicle fusion (exocytosis).[64] The next step in this gravisensing scenario is that the cytoplasm, under the influence of gravity, pushes upon the lower plasma membrane (Figure 2) thereby increasing cytoplasmic density.[10] This might then have the effect of inhibiting endocytosis and promoting exocytosis. An opposite situation occurs at the upper plasma membrane: here, cytoplasm is pulled away from the membrane which then has a lower tension, and these features then promote endocytosis and inhibit exocytosis (Figure 2). Thus, the plant synapse with its vesicles, cytoplasm and plasma membrane represents an acute 'flip-flop' type of gravisensor.[10] This is an extremely suitable system within the context of plant gravisensing as the system would now be free to act without the necessity for the participation of sedimentable larger organelles such as amyloplasts.[10] This type of direct gravisensing via the cytoplasm is very likely to be evolutionarily older than a type which relies upon sedimenting statoliths.[65,66] Gravisensing by utilising membrane and vesicle properties must ultimately be in harmony with the idea that synaptic end-poles are oriented in such a way that the gravity vector can redistribute synaptic cytoplasmic masses at upper and lower end-poles. Often the gravity vector is acting perpendicular to end poles, as when the root is growing vertically downwards and displaying positive orthogravitropism. Any perturbation of the root and, hence, of end-pole orientation also, leads to a disturbance of the usual cytoplasmic and synaptic gravisensing which can be immediately corrected.[10] Gravisensing and gravitropism are two autocorrecting processes designed to maintain a specific plant morphology.[2] It seems that the active maintenance of plant synapses perpendicular to the gravity vector is the driving force behind the plant gravitropism.

Importantly, the synapse concept also explains how gravity perception can be memorized[67-72] for several hours, or even days, as well as how information concerning the gravity vector is integrated with information from other sensory inputs such as light, electric fields, humidity, touch, oxygen.[7,68,73-77] All these processes optimize decision-making about the future behavior and navigation of plant organs. The Darwins already proposed this as occuring within the brain-like plant root apex! [13,16,94]

Evidently, there are cells within the plant which are gravisensitive but do not contain any sedimentable organelles. To explain this conundrum, some authors have proposed that the mass of the whole plant protoplast is a sedimenting structure.[80-82] When the cell is vertically oriented, the protoplasmic mass is directed upon the lower end-pole[78-82] and stretched at the upper pole.[10] Then, in addition to vesicle trafficking, actomyosin-driven cytoplasmic streaming may be proposed as another candidate for the elusive gravisensing process which is in a position to act also as the necessary motor

for the differential growth of gravitropism. The giant internodal cells of the *Characeae* do not have any obvious sedimentable organelles, whereas the cytoplasmic streaming in these cells is gravity-sensitive.[80-82] When in a horizontal orientation, the *Characean* cytoplasm streams along each internal side (upper and lower flanks) of the cell at the same rate. Repositioning of the cells into vertical position causes 10 % faster downward streaming than upward streaming.[80,82] Intriguingly, the cytoplasmic streaming in these cells is organized by their end-poles, walls which correspond to the synaptic end-poles of root apical cells.[9,12] If intact end-poles (synapses) are essential for the gravity-sensitive streaming,[80,82] then maybe it is not the cytoplasmic streaming itself, but the vesicle trafficking activity (synaptic activity or strength) which is of greater importance, though perhaps there is also an influence from the small extra pushing force offered by the stream on either side (upper and lower root parts) of the end-pole/synapse which can capture and transduce information concerning gravity and its vector. [10]

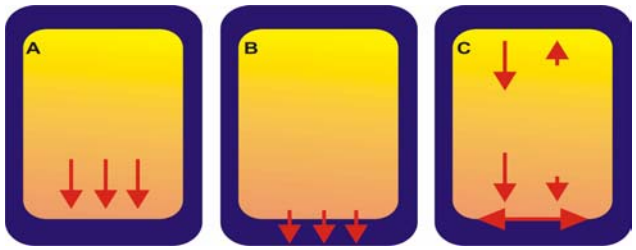

Figure 2: A gravisensing plant cell experiences the load of its protoplast,[80-82] as well as of its sedimented statoliths (not shown in this simplified scheme) upon both the lower plasma membrane (A) and the plasma membrane–cell wall interface (B). The cytoplasm pushes upon the lower plasma membrane (A) which increases in density, a feature which might interfere with local vesicular and organellar trafficking. Moreover, the settling of the whole protoplast might activate putative sensors at the plasma membrane–cell wall interface (B). The lower plasma membrane, now under high stretch stress (the horizontal arrow in C), shows inhibited endocytosis and a promotion of exocytosis (vertical arrows near the lower plasma membrane). An opposite situation is experienced at the upper plasma membrane which has lower tension (when compared with side portions of the plasma membrane), and this will promote endocytosis and inhibit exocytosis (vertical arrows near the lower plasma membrane). Plant cells in roots and shoots are polarized, having tubular shapes which are maintained by cell walls and plasma membrane-associated cytoskeleton. A protoplast devoid of these supportive structures would immediately lose this shape and become pear-shaped due to the gravity-dependent settling of the whole protoplasm within the lower portion of the cell.[10]

Root cap statocytes are highly polar cells with end-poles which transport auxin via the activities of PIN3 auxin efflux carrier.[83] In downward-growing root apices, PIN3 localizes to any lower, apical-most, statocyte synapse. In horizontal, gravistimulated root apices, PIN3 rapidly redistributes to the new lower surface. This is brought about by the continued preferential downward movements of recycling vesicles to what previously was a lateral cell wall but which now creates a new lower cell periphery, becoming perpendicular to the gravity vector. A similar scenario is plausible in order to explain gravity-regulated auxin-secreting synapses in the root meristem as well as in the transition zone. This would then explain reports that cells of the transition zone of growing root apices are also able to initiate root gravitropism independently of the root cap and its statocytes.[30,48,84] Relevant here is that plant synapses continuously monitor their positions with respect to the gravity vector and work, by means of their vesicle trafickings, to keep these positions perpendicular to the gravity vector.

8. Transition zone as 'command centre': integration of sensory signals into adaptive motoric responses via polar auxin transport

Transition zone cells are not only sensoric but they are also plastic in their behavior. The distal portion of the zone includes cells which are still competent for cell division and which can, if necessary, regenerate a complete new meristem. On the other hand, cells of the proximal part of the transition zone have begun to achieve competence for rapid cell elongation, requiring only an appropriate signal to do so. Such an event occurs at the onset of root gravitropism when cells of the proximal part of the transition zone starts to elongate rapidly on the upper part of a gravistimulated root apex while the corresponding cells of the lower part postpone their transition to rapid cell elongation.[85] A similar delay in the onset of rapid cell elongation, associated with a lengthening of the transition zone, can be induced by an increase of extracellular calcium or by adding root cap mucilage to the external flanks of the transition zone.[86] This latter finding indicates that the root cap can directly influence cell fate in the transition zone via the amount of mucilage synthesized within different time periods. Mucilage production is one factor that can be modulated by the environment of the root cap[33] during its exploration of new niches in the soil.

Gravitropic bending of the root apex is initiated within the transition zone[86,87] and is much more rapid when compared with the slow gravitropic bendings which shoots accomplish via their elongation region. Although gravitropic bending of root apices is initiated within the transition zone,[88] touch can induce another bending in the elongation zone,[76] making the bending of root apices rather complex. The tight co-ordination of two bendings accomplished simultaneously in two different root

zones provokes serpentine, or S-like, shapes of the growing root apices. It is interesting that electrotropism of roots is accomplished via bending in the more basal elongation region.[74]

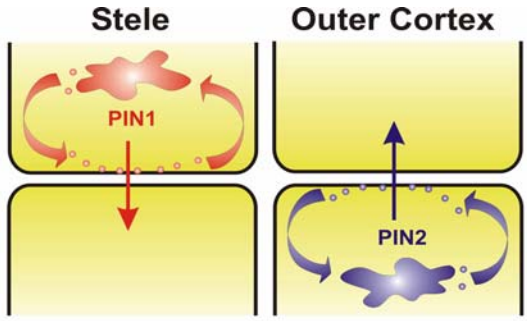

Figure 3: Auxin-secreting synapses in root apex cells. In the stele, acropetal auxin transport is driven by the efflux carrier PIN1, whereas epidermal and outer cortex cells support basipetal auxin transport driven by the efflux carrier PIN2. Both these polar auxin transport processes are dependent on endosomes which recycle both these efflux cariers at the presynaptic areas. PIN1 is recycled via endosomes marked with GNOM ARF-GEF while PIN2 is recycled via endosomes marked with sorting nexin SNX1. Localization of PIN2 is gravity sensitive. The PIN2-driven basipetal transport is essential for the gravitropism of root apices.[57-59]

Active plant synapses secreting auxin can be expected to drive rapid root bendings in the transition zone. Therefore, it is not surprising to find that up to five efflux carriers of the PIN family (PINs 1, 2, 3, 4, 7) drive auxin transport in root apices[89,90] whereas only one PIN protein (PIN1) is sufficient to satisfy the requirements of the morphologically more complex shoot apices (Figure 4, for recent review see 91). Interestingly, the basipetal auxin flow at the root periphery driven by an epidermis-outer cortex-based PIN2 efflux carrier joins, at the basal limit of the transition zone, with the acropetal auxin flow, which is driven preferentially by the PIN1 efflux carier,[89,92] located in the root stele. Other efflux carriers, PIN3, PIN4 and PIN7 are responsible for lateral redistribution of auxin across the root cap (PIN3) and the root apex (PIN4 and PIN7), setting up complex loops of the auxin transport throughout the interior of the root apex (Figures 3 and 4).[89,90] As for the morphologically more complex shoot apices, PIN1 is sufficient, as already mentioned (Fig. 4).

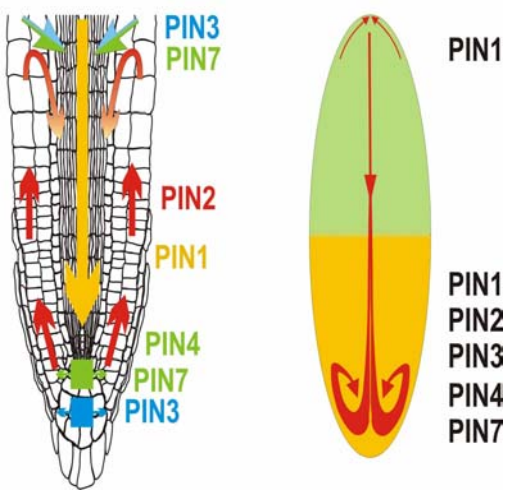

Figure 4: At the left, a root apex of Arabidopsis is depicted with a complex looping flow-pattern of auxin driven by five different efflux carriers. Yellow arrow indicates the acropetal flow driven by PIN1, the other arrows indicate basipetal auxin flows driven by PIN2, PIN3, PIN4, and PIN7. At the right, a highly schematized auxin flow (in red) throughout the whole plant body. Note that a single efflux carrier (PIN1) is sufficient to drive the polar auxin transport within the morphologically more complex shoot apex. Shoot portion is in green, root portion in yellow.

Thus, it is not possible to correlate the complexity of auxin transport with requirements for growth and morphogenesis. However, because the activity of auxin seems to emerge more and more like a plant neurotransmitter secreted via plant synapses,[12] it can be expected that the complex pattern of its transport in growing root apices is somehow related to the sensitivity of these apices to their diverse environments and to their neuronal-like ability to integrate the diverse signals captured from these environments.[11] This is what would be expected of some kind of brain-like command centre.[13,16,93,94]

Recently, we discovered that growing root apices take up large amounts of gaseous oxygen in the distal portion of the transition zone (Stefano Mancuso, Sergio Mugnai, Dieter Volkmann, František Baluška, unpublished data). For both maize and *Arabidopsis* root apices, the peak oxygen influx is at exactly coincides with the location of the most active synaptic auxin transport,[12,47,95,96] and which is also the location most sensitive to the neuro-toxic element, aluminium.[97,98] Importantly, brefeldin A (BFA) exposure blocks this influx completely (Stefano Mancuso, Sergio Mugnai, Dieter Volkmann,

František Baluška, unpublished data). Employing microelectrodes specific to other ions has revealed that the transition zone is the most active part of the whole root apex with respect to ion uptake.[99] From the perspective of classical plant cell biology and physiology, these cells are regarded as almost 'dormant' on account of having ceased mitotic divisions and their slow growth.[31,86,93] However, as mentioned, they are fully competent to commence rapid cell elongation. Why, then, should the number of these cells be so high? The size of the transition zone matches well the size of the meristem in both maize[100] and Arabidopsis[31] root apices. Moreover, why should a peak of oxygen uptake be characteristic of these apparently inert cells? As yet, there is no answer for this conundrum by the route of classical plant biology.

Plant neurobiology, however, can easily accommodate all the above-mentioned aspects of the transition zone by suggesting that it is some kind 'processor' or 'command center' which, via synaptic activities, processes sensory information, stores memories, and takes existentialist decisions about future exploratory and adaptive root behavior. Central to this neurobiological view of the transition zone are the active plant synapses. It is these which we predict to be processing and storing information as well as taking decisions about motoric responses and growth of the root apices. Synaptic activity needs an enormous amount of ion-channel activity, endocytosis-driven vesicle trafficking, and cytoskeletal rearrangements. All these processes also require a huge ATP consumption as it is energetically very costly (as known from animal biology) to keep neurons active.[101,102] For example, it has been estimated that it requires 10^4 ATP molecules to transmit one bit of information at a chemical synapse.[103] In humans, the brain represents 2% of body mass but is responsible for 20% of the body's total oxygen consumption.[104] In an awake but resting state, about 80% of energy consumption is associated with vesicular cycling that is related to glutamate and GABA neurotransmitters,[104] and about 80-90% of total cortical glucose consumption is attributable to the energy requirements of glutamatergic neurotransmission.[105] Therefore, from the plant neurobiology perspective, it is logical that the transition zone cells should also have the highest oxygen requirement of the whole root apex. The transition zone, similarly like animal brain, is well supplied with both oxygen and sucrose. Phloem unloading of sucrose is accomplished at about 250 μm from the root apex.[106,107] This is exactly the site of the oxygen-consuming transition zone.[31,93,97] Moreover, many roots are liberally equipped with intercellular air channels through which oxygen can rapidly diffuse.

The transition zone is very active in nitric oxide (NO) production.[97] NO can have a direct impact, on synaptic communication in plants,[97] as it has in mammalian brains,[108,109] Moreover, NO protects neuronal cells from diverse stress factors such as oxygen deprivation[110,111] or neuro-toxic aluminium.[112] The peak of oxygen uptake not

only coincides with the peak of synaptic auxin transport[12,47,95,96] but is also extremely sensitive to gravistimulation. Repositioning a growing root apex from vertical to horizontal position induces extremely rapid changes (within a few seconds!) in oxygen uptake at the upper side of such a gravistimulated roots (Stefano Mancuso, Sergio Mugnai, Dieter Volkmann, František Baluška, unpublished data). And almost immediate oxygen and NO responses have been recorded during a brief periods of microgravity induced by parabolic flights (Stefano Mancuso, Sergio Mugnai, Boris Voigt, Andrej Hlavacka, Dieter Volkmann, František Baluška, unpublished data). All this suggests that whatever process lies downstream of the oxygen uptake, it is closely linked to the gravity sensing and processing which is accomplished within the transition zone.[30,48] Our preliminary data show that BFA, which inhibits vesicle trafficking, also inhibits the peak of oxygen uptake in the transition zone, but oxygen uptake is unaffected in the elongation region (Stefano Mancuso, Sergio Mugnai, Elisa Azzarello, Camilla Pandolfi, Andreas Sadler, Dieter Volkmann, František Baluška, unpublished data). This implicates synaptic activity, for BFA is an inhibitor of vesicle trafficking, and it is BFA-sensitive auxin transport which lies behind the oxygen uptake peak in the transition zone of growing root apices.

A further point of interest is that root apices suffer from acute oxygen deficiency when experiencing the microgravity environment of the low-Earth orbital during spaceflight experiments.[113,114] Also, roots show unique responses to electricity in microgravity. Roots exposed to an electric field under microgravity condition stop growing,[7] whereas there is no effect on root growth if electric fields are imposed on Earth in a 1 g situation.[74]

Strong experimental evidence for the root-apex transition zone acting as the 'command centre' for the whole plant have been received recently by showing that the wounding of leaves by heat induces very rapid (within a few seconds) electrical responses specifically within the transition zone of *Vitis* root apices.[99]

9. Aluminium: a neuro-toxic substance for plants?

Aluminium is extremely toxic for cells of growing root apices, but less toxic for elongating and non-growing root cells as well as for most cells of above-ground plant organs. In roots, the toxicity is highest for cells of the root apex, but less so for the more proximal rapidly elongating cells. Sivaguru and Horst[98] identified the distal portion of the transition zone, cells which have ceased mitotic divisions but are still not growing rapidly,[86,92] as the most aluminium-sensitive part of the root. Importantly, aluminium inhibits the basipetal auxin transport[115] which is essential for the graviresponse of root apices.[116] Our recent data reveal that alumunium is not only internalized by endosomes but that it also inhibits endocytosis and the formation of the endosomal compartments which are induced by BFA.[97] In this respect, it is interesting that aluminium is internalized

preferentially in those cells which are the most aluminium-sensitive but not into cells of the elongation region.[97] Aluminium uptake into tobacco BY-2 cells was reported to depend on BFA-sensitive vesicle trafficking.[117] Moreover, in the course of a study of aluminium-tolerant tobacco mutants, the suggestion arose that endocytosis, vesicle trafficking, and auxin transport were interlinked.[118] Primary targets of aluminium are therefore probably related to some processes linked to endocytosis and vesicular trafficking, and which are especially active at plant synapses and that drive the polar transport of auxin,[12] a process important for root gravisensing. Relevant for the neurobiological view of root gravitropism are the reports that aluminium-induced depolymerization of microtubules and depolarization of the plasma membrane in cells of the transition zone are mediated by glutamate receptors.[120] This unique aluminium-sensitivity of the transition zone cells is reminiscent of the high sensitivity of human neurons to aluminium.[121]

10. Glutamate, acetylcholine and ATP: Plant neurotransmitters?

In agreement with the predictions made by plant neurobiology, but not easily explicable by the classical plant physiology, plant cells, including those involved in root gravitropism, have been found to use glutamate, acetylcholine, and ATP in the processes of cell-cell signaling. The use of these neurobiological molecules, acting in neurotransmitter-like mode, is still not accepted by mainstream plant sciences. Nevertheless, the amount of relevant data will soon reach the critical level, thus necessitating a re-evaluation of the data and the consequent development of new concepts.

The case is strong for glutamate as a plant neurotransmitter. The genome of Arabidopsis encodes for 20 members of the ionotropic glutamate receptors family.[122] They are used in the perception of touch and cold,[123] ionic stress responses,[124] carbon and nitrogen sensing,[125] ABA synthesis and water loss,[126] root branching control,[127] and defence against pathogens by triggering jasmonic acid biosynthesis.[126] Moreover, expression of the *GLR3.1* gene in rice is critical for the organization of the root apex in this species.[128] In mutant plants, the transition zone is almost missing, as evidenced by longitudinal sections which reveal only thin cells of normal cell length (see Figs. 1F, G in 128). The final widths of root cells are attained in the transition zone[85,86,92] where the highest aluminium toxicity, mediated via glutamate signalling, is shown.[97,98] As in the case of glutamate-based signalling in animal neurons, glutamate induces plasma membrane depolarizations and calcium transients in plant cells.[123,129,130]

Acetylcholine emerges as a transmitter of cell-cell communication in plants. There are numerous older reports concerning acetylcholine in plants which have been exposed to evironmental insults.[131] For instance, acetylcholine has been shown to affect

root growth, leaf movements, stomata movements, the response to water/salt stress, as well as root-shoot communication. Presumably, its actions relate to the membrane permeabilities for diverse ions.[131,132] Interestingly, acetylcholine is primarily produced in root apices, as it is here that much larger amounts of acetycholine have been determined when compared with other plant tissues and organs.[132] Acetylcholine is the only neurotransmitter which is inactivated by enzymatic activity. If the neuronal actions of acetylcholine in plants are real, then plant cells also should have acetylcholinesterase and thereby accomplish the enzymatic cleavage of acetylcholine at neuronal synapses. Indeed, acetylcholinesterase activities have been recorded in plants and, significantly, neostigmine bromide, an inhibitor of this enzyme in neurons, also inhibits plant cell acetylcholinesterase activity.[133] Neostigmine bromide has also been reported to inhibit shoot and root gravitropism in maize.[133,134] The recent cloning of the acetylcholinesterase gene in maize,[135] and the *in silico* analysis which has confirmed that acetylcholinesterase is present throughout the plant kingdom,[135] indicate that a role is urgently required for this enzyme and that a neurobiological context is the logical one.

Another well-known neurotransmitter, ATP,[136] has been shown to play signalling roles in diverse plant processes. For example, extracellular ATP depolarizes plasma membrane potential[137] and elevates cytoplasmic calcium levels.[138] Each of these can be coupled to downstream gene regulation,[139] implying that there could be a complete signaling pathway that centres around an ATP neurotransmitter. During the animal neurotransmission, ectoapyrases are responsible for rapid degradation of ATP in order to terminate the sensory-mediated signaling pathway.[140] Similarly in plants, extracellular apyrases emerge as being involved in sensory ATP signal transduction.[141] Moreover, in animals, extracellular ATP initiates signaling by binding to its purinoreceptors[142] and a similar scenario is emerging for plant cells too.[138,141]

Extracellular ATP inhibits polar auxin transport and root gravitropism,[143] as well as inducing accumulation of superoxide via activation of NADPH oxidases.[141] Interestingly in this respect, auxin also induces ROS extracellularly[144] and increases cytoplasmic calcium,[145] as well as inducing electrical responses in roots.[146,147] Besides inhibiting the root graviresponse, extracellular ATP increases the sensitivity of root cells to exogenous auxin,[143] implying possible interactions between these two plant neurotransmitters. As the same secretory vesicles can release, in a quantal manner, auxin and ATP, this could surely increase the impact of these neurotransmitters on the postsynaptic domains of neighbouring cells. Moreover, inhibition of root gravitropism in maize via a sudden increase of extracellular ATP is linked to the inhibition of basipetal auxin transport in the root apex.[143]

11. Neurobiological behavior of plant roots

Growing plant roots show complex pattern of behavior, several aspects of which imply neuronal-like activities. First, and in contrast to shoots, the trajectory of growing roots is the result of their use of a large spectrum of environmental inputs. The result is that all information from the soil environment is integrated within the root apex, presumably at their synapses, allowing navigation of the root tips towards soil niches enriched with water and minerals. At the same time, roots avoid toxic elements such as aluminium,[148] and physical obstacles,[16,149] the high sensitivity to the latter being mediated by a type of negative feedback.[149] Second, roots can recognize self from non/self via physiologically-mediated processes.[150] Distribution of roots within soil is extremely plastic and roots can be territorial[151] and competitive[152] in their experience-driven, opportunistic, and search-avoidance behavior. Third, growing roots can cope with situations never experienced before. For instance, roots forced to grow into capillaries having a diameter slightly less than that of the root itself will rearrange their pattern of development, particularly that of the stele, so that they can enter the narrow space (P.W. Barlow unpublished). Intriguingly, roots forced to grow into slightly wider capillaries and oriented with the tip vertically upwards, also become thinner and, by some complicated 'gymnastic' movement, some of them turn back and grow down again along the gravity vector (František Baluška, Markus Schlicht, Dieter Volkmann, unpublished data). These phenomena are not yet understood at the cellular and sub-cellular levels, though it is obvious that neurobiological integration of signaling input[11] and plant synapses[10] could be relevant in this respect. It is no surprise that exogenous glutamate, the only amino acid from 21 tested, has been found to affect both root growth and root branching.[127] Importantly, only the L-glutamate isoform acts as relevant signal rgulating root system architecture.[127]

Plant roots, in their search for food and avoidance of toxic or harmful soil patches, resemble animals. Food-related behavior shaped the evolution of brains in animals, and this might also be the case for exploratory and invasive roots. Moreover, brain-power in animals is dependent oxygen availability.[153,154] Similarly, heterotrophic plant roots are often living in an environment in which oxygen levels drop below physiological levels. This necessitates a life style that can cope with these situations. Given the range of tropisms employed by roots – gravi-, thigmo-, oxy-, hydro-, electro- and magnetotropisms – there must be a continual monitoring and integrating of huge amounts of information and the consequent making of decisions about root growth direction and other activities. Obviously, roots are capable of perception of their physical environment as well as of spatial orientation,[149-152] and both are attributes of neural exploratory systems.[155]

12. Outlook

The concept of a gravisensing plant synapse[10] is highly attractive as it has power to explain several gravity-related paradoxes that are still unexplained by classical theories. First, plant neurobiology harmonizes the Němec–Haberlandt theory of plant statoliths with the Cholodny–Went theory of auxin transport via a vesicle trafficking apparatus. Sedimenting statoliths focus the load of the whole protoplast towards the lower synapse, amplifying the spatially-focused 'pushing' force which places a specific domain of the plasma membrane under a strong mechanical stress. Although this does not induce an especially active endocytosis, it does promote localized vesicle secretion; and addition to membraneous material relieves the local stress at the plasma membrane. Because these vesicles arriving at the synapse are filled with auxin,[12] gravisensing at the plant synapses is immediately related with the secretion of auxin downwards, along the gravity vector.

Second, the concept of gravisensing plant synapses has potential to explain the the plant memory phenomena whereby gravity-stimulated cells can memorize information concerning the direction of the gravity vector for hours, and even days, and then later retrieve this information.[67-72,156] The synaptic concept is also in a position to explain how this memorized gravity information can be integrated with informational inputs from other physical parameters of the environment such as light, humidity and oxygen (for blue light see 67,68; for more general reviews see 11).

Precise coordinations of body movements rely on sensory feedbacks.[155] This is true not only for animals but also for plant roots. The transition zone of the root apex emerges as a 'command centre' specialized for the integration of all sensory inputs and thereby the derivation of a coherent behavioral output in the form of a signal-mediated 'navigation' of root growth. Intriguingly, auxin emerges to have neuronal attributes such as neurotransmitter-like signaling[9-12] and obviously acts as an integrative information-processing molecule.[157] Such dynamic integration of diverse sensory stimuli is an inherent property of neurobiological systems[11,158] and is also typical of biological systems which adapt to their environment via conscious experiences.[158,159] This would also explain the capability of roots to cope with situations never experienced before. These require new and creative approaches, and not just passive reflex-like and simple behavior. Definitely, plants and especially roots do not act as automata driven only via robotized reflex-based circuits. The concept of plant neurobiology[11,160] is a natural 'fall-out' of many recent advances in plant and, especially, root biology. It can now claim specifically to study all areas of classical plant biology in a new way, thus endowing plants with an cognitive information-processing and even biosemiotic systems,[11,94,157,160,161] thereby bringing the plant and animal kingdoms into closer harmony.[94]

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