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Minireview

Electrophysiology of growth control and acupuncture

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Abstract

Bioelectric fields have been shown to interact with morphogens and guide growth control. The morphogenetic singularity theory published a decade ago suggests that organizing centers have high density of gap junctions and high electrical conductance. They are the singular points in morphogen gradient and bioelectric field. A growth control system originates from a network of organizing centers containing under-differentiated cells and retains its regulatory functions after embryogenesis. The formation and maintenance of all the physiological systems are directly dependent on the activity of the growth control system. The evolutionary origin of the growth control system is likely to have preceded all the other physiological systems. Its genetic blueprint might have served as a template from which the newer systems evolved. The growth control signal transduction is embedded in the activity of the function-based physiological systems. The regulation of most physiological processes is through growth control mechanisms such as hypertrophy, hyperplasia, atrophy, and apoptosis. Acupuncture points, which also have high electrical conductance and high density of gap junctions, originate from organizing centers. This theory can explain the distribution and non-specific activation of organizing centers and many research results in acupuncture. In several 'prospective blind trials', recent research results have supported its corollary on the role of singularity and separatrix in morphogenesis, the predictions on the high electric conductance and the high density of gap junctions at the organizing centers. These advances have broad implications in biomedical sciences.

Keywords: Organizing center; Gap junction; Morphogenesis; Growth control; Developmental biology; Acupuncture; Electrophysiology

The role of electric fields in morphogenesis

Several lines of evidence suggests that the bioelectric field guides morphogenesis and cell division [1,2,3,4]. The growth and migration of a variety of cells are sensitive to electric fields of physiological strength [5]. Somite fibroblasts migrate to the negative pole in a volt-

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age gradient as small as 7 V/m [6]. Asymmetric calcium influx is crucial in the migration which can be blocked or even reversed by certain calcium channel blockers and ionophores [7]. In most cases, there is enhanced cell growth toward the cathode and reduced cell growth toward the anode in electric fields of physiological strength [8,9]. Fast growing cells tend to have more negative polarity than other cells. This polarity is due to the increased negative membrane potential generated by the mitochondria at a high rate of energy metabolism [10]. Imposed electric fields can cause polarization of mouse blastomeres [11]. The anterior-posterior polarity [12] and dorsal-ventral polarity [13] in lower animal morphogenesis can be completely or partially reversed when the polarity of the imposed electric field is opposite to that of the intrinsic electric field. The electric signals and chemical signals are likely to enhance each other in growth control. For example, growth factors and extracellular calcium are required for electric field-induced directional migration of human keratinocytes [14]. Electric field-induced cell growth and differentiation can be blocked by calcium channel blocker or protein kinase C inhibitor [15]. Electric field in turn can induce the expression of transforming growth factor—beta1 [16] and c-fos, [15] as well as cause asymmetric distribution of growth factor receptors and other membrane proteins [1]. Morphogens such as retinoic acid are usually charged molecules or ions and can form a more stable concentration gradient over long range guided by electric field than by reaction-diffusion only. The effect of morphogens or growth factors on ion channels and pumps can modify the electric field.

The electrophysiology of organizing centers

In development, the fate of a larger region is frequently controlled by a small group of cells, which is termed an organizing center [17]. Organizing centers such as blastopore, zone of polarizing activity and apical ectodermal ridge have been well studied since the era of Spemann. The morphogenetic singularity theory published a decade ago [18] predicted several common properties of organizing centers which have been confirmed:

1. Organizing centers have high electrical conductance and current density: The amphibian blastopore, a classic organizing center, has high electrical conductance and current density [19] (Figure 1) Similar phenomena have also been observed in higher vertebrates [20]. Change in electric activity at the organizing centers correlates with signal transduction and can precede morphologic change [21,22]. For example, in amphibians, an outward current can be detected at the site of a future limb bud (an organizing center) several days before the first cell growth [23]. Disruption of the intrinsic electric field at the organizing center can cause malformation [19].
2. Organizing centers have high density of gap junctions: The high electrical conductance at organizing centers is further supported by the finding of high density of gap junctions at the sites of organizing centers [24,25,26,27]. It is well established that gap junctions facilitate intercellular communication and increase electrical conductivity. Gap junctional communication has been shown to play a crucial role in morphogenesis [28].
3. At the macroscopic level, organizing centers are singular points in the morphogen gradient and electric field [18]: A singular point is a point of discontinuity as defined in mathematics. It indicates a point of abrupt transition from one state to another. An organizing center which has high density of gap junctions and high electrical conduc-

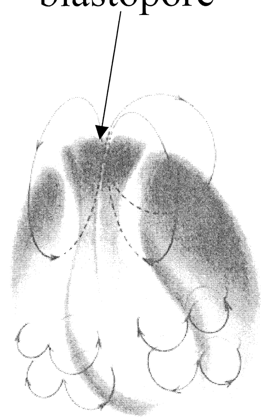

Fig. 1. Ionic currents traversing an embryo (Shi & Borgens. *Dev Dynamics* 1996;202:102. Courtesy of John Wiley & Sons). The blastopore, a classic organizing center, has high electric conductance and current density. A steady blastopore current persists after early embryogenesis. The electric fields polarize the embryo and serve as cues for morphogenesis. These results confirmed the predictions made in 1989. (Shang. *Am J Chin Med* 1989;17:119–27.)

tance will also have locally maximum electrical current density—a converging point of surface current. It is a singular point of abrupt change in electric current flow. Small perturbations around singular points can have decisive effects on a system. As James Maxwell observed: "*Every existence above certain rank has its singular points . . . At these points, influence whose physical magnitude is too small to be taken account of by a finite being, may produce results of the greatest importance.*" [29] As expected from the nature of singular points, organizing centers can be activated by various nonspecific stimuli such as mechanical injury and injection of nonspecific chemicals [17,30].

4. Based on the phase gradient model in developmental biology [18,31], organizing centers tend to locate at the extreme points of curvature on the body surface such as the locally most convex points (e.g. the apical ectodermal ridge and other growth tips) or concave points (e.g. the zone of polarizing activity).

The growth control system after embryogenesis

According to the morphogenetic singularity theory, a growth control system originates from a network of organizing centers. In ontogeny, the development of organizing centers precedes the development of other physiological systems. The formation and maintenance of all the physiological systems are directly dependent on the activity of the growth control system. Growth control is a primary function for all multi-cellular organisms. As the individual embryonic development recapitulates the evolution of the species, (Ontogeny recapitulates phylogeny) the evolutionary origin of the growth control system is likely to have preceded all the other physiological systems. Its genetic blueprint might have served as a template from which the newer systems evolved. Consequently, it overlaps and interacts with other systems but is not simply part of the nervous system, immune system or circulatory system. The growth control signal transduction is embedded in the activity of the function-based physiological systems. The regulation of many neural, circulatory and immune processes is through growth control mechanisms such as hypertrophy, hyperplasia, atrophy, apoptosis with shared messenger molecules and common signal transduction pathways involving growth control genes such as proto-oncogenes [32,33,34,35,36]. Many "non-excitable" cells have shown electrochemical oscillation, coupling, long range intercellular communication [37,38] and can participate in the signal transduction of growth control. The gap junction genes can behave as tumor suppressor genes both in culture and in animal tests in restoring growth regulatory properties to metastatic cancer cells [39]. According to the morphogenetic singularity theory [18], the network of organizing centers retains its regulatory function through high levels of intercellular communication correlated with relatively low levels of cell differentiation after embryonic development. This prediction is consistent with the finding that the high electrical conductance persists at the organizing centers after early embryogenesis [4].

Separatrix - Boundary

At early stages of embryogenesis, gap junction-mediated intercellular communication is usually diffusely distributed which results in the entire embryo becoming linked as a syncytium. As development progresses, gap junctions become restricted at discrete boundaries, leading to the subdivision of the embryo into communication compartment domains [40]. These boundaries are also major pathways of bioelectric currents. A separatrix defined in mathematics is a trajectory or boundary between different spatial domains [41] (Figure 2) and often connects singular points. Separatrices can be folds on the surface or boundaries between different structures [18,42]. Consistent with the under-differentiation of the growth control network, it has been observed that the most apical part of folds in embryos remain undifferentiated in morphogenesis [43]. This is also true of the organizing centers such as zone of polarizing activity [44] and apical ectodermal ridge [45].

A hierarchy of under-differentiated cells?

According to the morphogenetic singularity theory, the under-differentiated, inter-connected cellular network is related to both internal and external structures. Its distribution is a result of morphogenesis. The primary tumor distribution pattern of a certain cell type reflects the distribution of its normal counterpart. For example, the distribution of primary pheochromocytoma reflects the distribution of normal sympathetic ganglion cells. The germ cell is one of the least differentiated cells. The germ cell tumors [46,47] have a midline and para-axial distribution pattern which spans from the sacrococcygeal region through anterior mediastinum, tongue, nasopharynx, to pineal gland. It appears to concentrate at seven locations: sacrococcygeal region, gonads, retroperitoneum, thymus [48], thyroid [49], suprasellar region, and pineal gland [50]. The pattern suggests the existence of under-differentiated cells which may be highly interconnected in a normal state and provide important regulatory functions [51]. It is likely that there is a hierarchy in the degree of cell differentiation and function in the growth control system with the germ cell system as the least differentiated and constituting the central core of the regulatory system. The more peripheral separatrices and organizing centers are more differentiated and further down in the hierarchy.

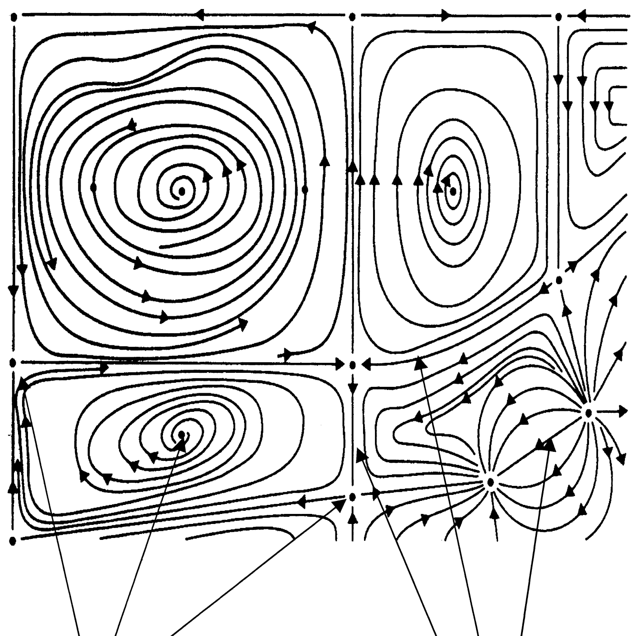

Fig. 2. Illustration of singular points and separatrices (modified after Figure 2.1 in: Mesterton-Gibbons. *A Concrete Approach to Mathematical Modelling.* Wiley; 1995:47. Courtesy of John Wiley & Sons). This also approximates the pattern of organizing centers and boundaries—the singular points and separatrices of electric field on body surface.

Growth control system and acupuncture

The fate of organizing centers in adults can be addressed by studying the electrical conductance and gap junctions. Interestingly, such studies have been done in acupuncture research for half a century. According to the Standard Acupuncture Nomenclature proposed by the World Health Organization [52], there are about 400 classic acupuncture points and 20 meridians/vessels connecting most of the points. Since the 1950s, it has been discovered and confirmed by researchers in many countries with refined techniques [53] that most acupuncture points correspond to high electrical conductance points on the body surface [54,55,56] and *vice versa* [57,58]. The high skin conductance of the meridian system is further supported by the finding of high density of gap junctions at the acupuncture points and meridians [59,60,61,62]. The high density of gap junction at the epithelia can increase both the cell to cell conductance and the cross-section area of the conducting tissue involved in the skin conductance measurement. The 'gap junction—embryonic epithelial signal transduction model' [59] proposed that the meridian system contains relatively under-differentiated epithelial cells connected by gap junctions which transmit signals and play a central role in mediating acupuncture effects. The *morphogenetic singularity theory* suggests that acupuncture points originate from the organizing centers. Both acupuncture points and organizing centers have high electrical conductance, current density, high density of gap junctions, and can be activated by nonspecific stimuli [18,63,64]. Like organizing centers, acupuncture points also tend to locate at the extreme points of surface curvature [18].

Acupuncture can speed up the wound healing process [65] and cause an exaggerated systemic wound healing and stress response [66,67]. The response can include excessive release of endorphins which stimulates epithelial cell growth [68] as well as analgesia. Other neurohumoral factors induced by acupuncture such as growth hormone [69], basic fibroblast growth factor [70], serotonin [71] and ACTH [72] also have growth-control effects [73]. In randomized controlled trials, acupuncture has shown efficacy in treating growth control related disorders including osteoarthritis [74], stroke [75,76] and low sperm quality [77]. Acupuncture has been shown to stimulate growth in plants which have no nervous system [78].

As the electrical conductance of organizing centers varies with morphogenesis, the conductance of acupuncture points also varies and correlates with physiological change [54] and pathogenesis [79,80]. The fact that the change in electric field precedes morphologic change [23] and manipulation of the electric field can affect the change [81] may shed light on the diagnosis [82] and treatment of many diseases.

In acupuncture, the often nonspecific perturbation at acupuncture points may not directly antagonize a pathological process but may indirectly adjust the process and restore normal function by activating the network of organizing centers in the organism. This is consistent with the "bi-directional normalizing effect" commonly observed in acupuncture. For example, same acupuncture at the acupuncture point ST36 suppresses hyperfunction and stimulates hypofunction of the gut motility [83]. Stimulation at PC6, ST36, or KI1 can relieve both hypotension and hypertension. Stimulation at PC6 decreases tachycardia and improves bradycardia [84]. The activation of the self-organizing activity is less likely to cause the side effects resulted from directly antagonizing a pathological process which often overlap with other normal and beneficial physiological processes. Therefore, proper use of acupuncture causes few side effects [85,86,87] as demonstrated in randomized controlled trials [88,89].

Summary & prospects

The morphogenetic singularity theory explains several phenomena in both developmental biology and acupuncture research. These include the distribution and non-specific activation of acupuncture points and organizing centers, the high electrical conductance of acupuncture points, the side effect profile of acupuncture, as well as the ontogeny, phylogeny and physiological function of the growth control system. Most of these have not been explained by any neurohumoral theory. In several 'prospective blind trials', researchers who were unaware of the theory, confirmed its corollary on the role of singularity and separatrix in morphogenesis [42], as well as its predictions of the high electrical conductance and high density of gap junctions at the organizing centers such as blastopore [4,19] and zone of polarizing activity [24,25,26]. Techniques involving the stimulation of the growth control system may activate the self-organizing activity of an organism and improve its structure and function at a more fundamental level than symptomatic relief. Development of these techniques may enable the diagnosis and treatment of a pathologic process at the early signal transduction stage prior to the anatomical or morphological change. Future directions include:

1. Mapping of the meridian system and the dynamics of its electric field with high resolution techniques such as the superconducting quantum interference device (SQUID).
2. Developing meridian system based techniques of early diagnosis (e.g. using electric impedance measurement or SQUID) and treatment. Electric impedance spectroscopy has been used in assessing the conductivity of gap junctions which correlates with tissue viability [90]. This technique can also be used to explore meridian system.
3. Delineating the cell differentiation and signal transduction in meridian system.
4. Mapping the body surface curvature through embryonic development with imaging techniques and correlating with the meridian system.

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