A prototype electronic board to investigate Galvanotaxis phenomena

Isabella Zironi, Luca Boccioletti, Gastone Castellani, Alessandro Gabrielli
Physics and Astronomy Department, University of Bologna, Italy
Interdepartmental Centre “L. Galvani” for integrated studies of bioinformatics, biophysics and biocomplexity (CIG)
National Institute for Nuclear Physics (INFN)

Luigi Galvani  Alessandro Volta
Galvanotaxis or electrotaxis is the movement of a cell subjected to currents induced by a continuous electric field (dcEF)

Time-lapse images of mouse fibroblast cells

Electrolysis of water:
\[ 2 \text{H}_2\text{O} \rightarrow 2 \text{H}_2 + \text{O}_2 \]
At pH 7 \( E = +1.229 \text{ V} \)

Huang et al., 2013
PLoS One, 8:e59447
How a cell can move?

A network of intracellular filamentous actin (green) is the major cytoskeletal component that is involved in cell motility and contraction. Fibronectin (red) is an important extracellular matrix (ECM) protein.

1. elongation of cortical actin filaments that connect to adaptor proteins and ‘push’ the membrane in the direction of movement;
2. formation of adhesive contacts through integrins, which interact with several signaling proteins and structural components of adhesions;
3. ‘focalized proteolysis’, involving cleavage of extracellular matrix (ECM) components by proteases;
4. contraction driven by binding of myosin II to actin filaments;
5. disassembly of focal contacts and detachment of the rear margin of the cell.
Polarization of ions inside the cell in the presence of external electric field

- Galvanotaxis should involve the same cytoskeletal rearrangements.
- For dcEFs of 10-100 mV/mm (the endogenous electric fields normally present in nature) applied to a cell 10 µm across, the cathodal side depolarizes by ~5 mV whereas the membrane facing the anode hyperpolarizes by the same amount.
- One link between the galvanotactic stimulus (the dcEF) and the fundamental mechanisms of cell motility is probably Ca²⁺.
- It is seen as inhibiting these channels or reducing the concentrations of Ca²⁺ ions in the medium, the galvanotactic effect appreciably decreases.

A cell exposed to a dcEF

The membrane towards the anode is hyperpolarized and attracts Ca²⁺. Consequently, this side of the cell contracts, thereby propelling the cell towards the cathode (Mycielska M.E. and Djamgoz M.B.A., 2004).
Functional roles of endogenous electric fields

- Is the first and most subtle hitherto detectable general biological information system.
- Physiological EF direct many cell- and molecular biological processes such as: embryogenesis, wound healing, regeneration. They are also guiding cues for cell migration, cell differentiation and proliferation.
- Calcium wave is the first information cues that determine domains like anterior/posterior or left/right in the very early embryo.
- The proton extrusion outwardly directed by an EF at the negative pole is large enough to induce the directed growth of nerves of the spinal cord in the amputated xenopus tail.
- In vitro experiments revealed that many cell types prefer to migrate to the cathode. Both speed and direction of the movement are voltage dependent.
- Several studies have shown that cell transformation also modifies responses to dcEFs. For example, under identical experimental conditions, primary human lens epithelial cells migrate towards the cathode, whereas their transformed counterparts migrate towards the anode.
The prototype

Microcontroller AT91SAM3X8E
Operating Voltage 3.3 V
Digital I/O Pins 54 (12 PWM)
Analog Outputs Pins 2 (DAC)
Flash Memory 51 kB

SRAM 96 kB
Clock Speed 84 MHz
Length 101.52 mm
Width 53.3 mm
Weight 36 g

6 voltage digital outputs (0 – 3.3 V)
1 electronic current output (5 µA – 5 mA)
2 output waveform generator, each channels produce 4 different waveform independently: sinusoidal, square, triangle and sawtooth at freq. 1 – 170 Hz
Time-lapse images of tumoral cell line obtained from a human brain cancer.
The EF exerts a force on each electrically charged object
Conclusions

• The main objective of this work is the design and development of an electronic platform capable to create and control Galvanotactic events in a scientific and easy reproducible manner.

• A recent study on Galvanotaxis it arose as the cells are sensitive even to sinusoidally oscillating field with a period of magnitude of minutes. This board allows the modulations of analog output signals instead of using the Pulse-Width-Modulation (PWM).

• Preliminary results on galvanotaxis phenomena, in particular on the effects of electric fields on cell movements.

• To date, the actual mechanism behind the Galvanotaxis remains unclear although several theories have been proposed about but is clear that have an important role in physiological processes of medical interest: embryogenesis, angiogenesis, wound healing and metastasis.

• Has been recently postulated that a specific potassium transmembrane channels couples with polyamines to sense weak extracellular electric fields.
KCNJ15/Kir4.2 couples with polyamines to sense weak extracellular electric fields in galvanotaxis.

Nakajima K(1), Zhu K(1,)(2), Sun YH(1), Hegyi B(3), Zeng Q(2), Murphy CJ(4)(5), Small JV(6), Chen-Izu Y(3), Izumiya Y(1,)(7), Penninger JM(6), Zhao M(1,)(5).

Weak electric fields guide cell migration, known as galvanotaxis/electrotaxis. The sensor(s) cells use to detect the fields remain elusive. Here we perform a large-scale screen using an RNAi library targeting ion transporters in human cells. We identify 18 genes that show either defective or increased galvanotaxis after knockdown. Knockdown of the KCNJ15 gene (encoding inwardly rectifying K(+) channel Kir4.2) specifically abolishes galvanotaxis, without affecting basal motility and directional migration in a monolayer scratch assay. Depletion of cytoplasmic polyamines, highly positively charged small molecules that regulate Kir4.2 function, completely inhibits galvanotaxis, whereas increase of intracellular polyamines enhances galvanotaxis in a Kir4.2-dependent manner. Expression of a polyamine-binding defective mutant of KCNJ15 significantly decreases galvanotaxis. Knockdown or inhibition of KCNJ15 prevents phosphatidylinositol 3,4,5-triphosphate (PIP3) from distributing to the leading edge. Taken together these data suggest a previously unknown two-molecule sensing mechanism in which KCNJ15/Kir4.2 couples with polyamines in sensing weak electric fields.