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Electrophysiological characterization of a novel human neuroectodermal cell line

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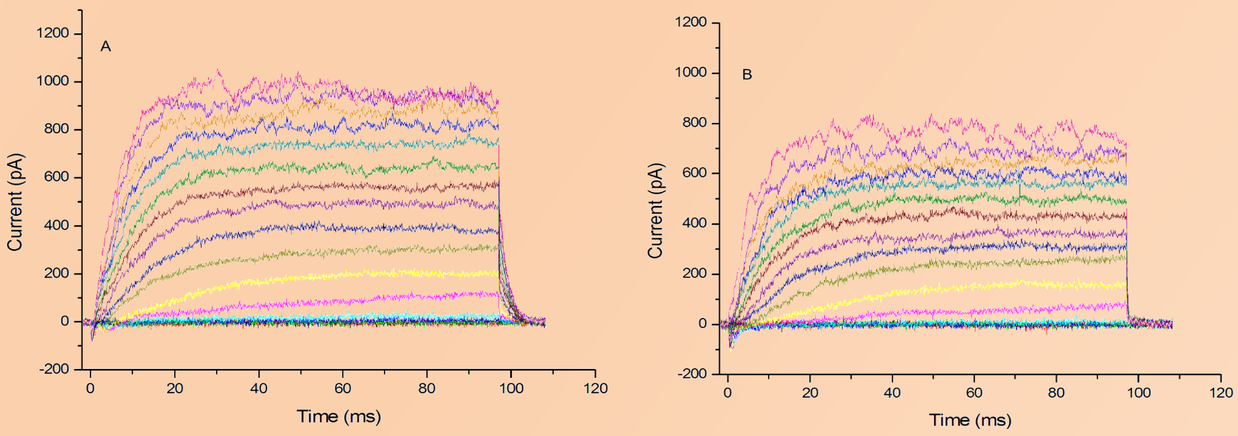
Introduction

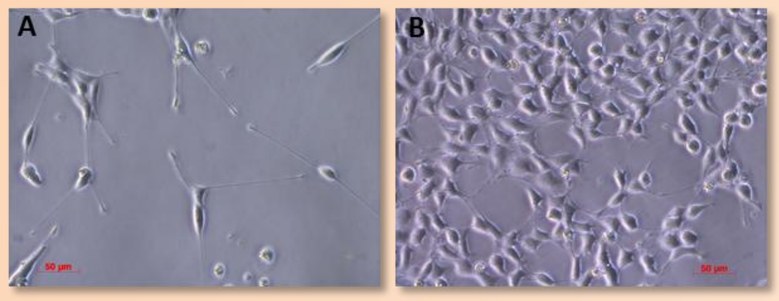
The electrophysiological properties of a novel human neuroectodermal cell line (TB) have been studied by whole cell patch-clamp. The cell line has been established from a cerebrospinal fluid (CSF) specimen of a patient with clinical diagnosis of primary leptomeningeal melanomatosis and has been previously characterized by immunological and ultrastructural analysis [1]. The cells are able to differentiate morphologically if treated with retinoic acid (RA) [1].

Results

In both untreated (A) and treated (B) cells a negligible inward current can be appreciated.

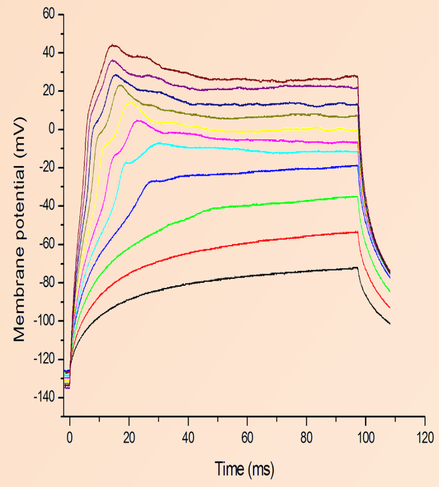


Examples of current recordings at V_H -70mV



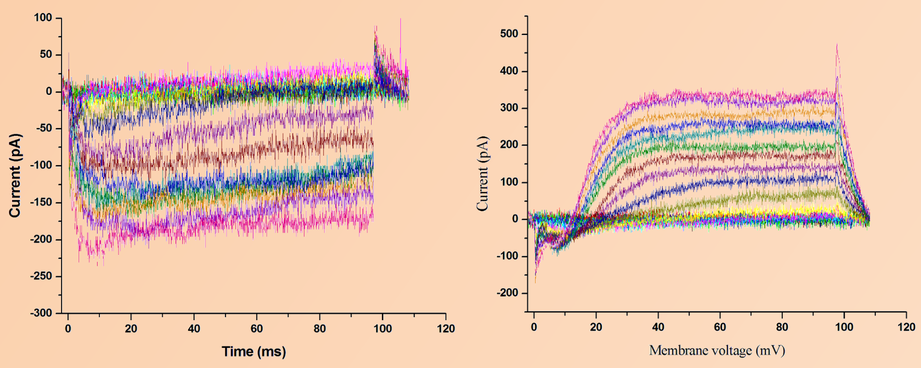
TB cells after 7 days RA-treatment (A) and without treatment (B) .

This inward current was unable to produce spike activity when cells were recorded by current clamp method. In both in A and B groups, outward currents, although much higher than inward ones, never reached the consistency of the mature neurons.



Example of voltage recording at I_H corresponding to a V_H -120mV

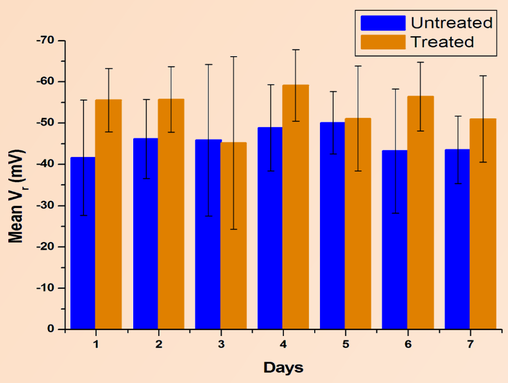
Recordings obtained by substituting KCl with CsCls showed that the main conductance producing the outward current was a K+ dependent.
However, residuals of different ions gave a contribute to the amplitude.



Examples of current recordings at V_H -70mV with CsCl

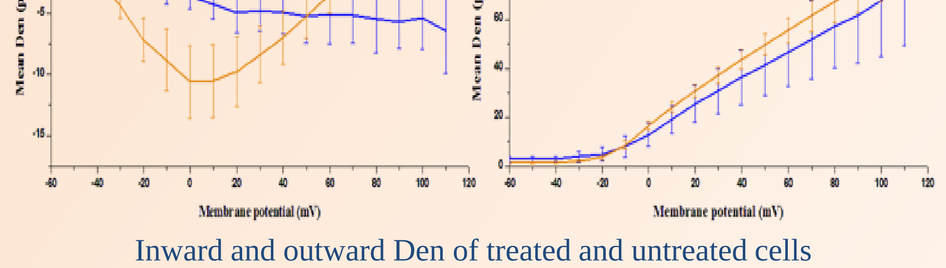
The Current/Voltage (I/V) relationships show that the treatment with RA produces a modification of the I/V curve. In fact, for untreated cells this relationship appears with a random shape while for treated cells it assumes the classical shape of those of the mature neurons.

The mean value of resting potential (V_r) does not change significantly ($p>0.05$) with respect to the days of treatment for both the treated and untreated cells but differences between groups were very significant (V_r = -45.6+/-3.1 mV and V_r = -53.5+/-4.6; $p<0.001$).



	Mean Diff.	DF	t-Value	P-Value
Vr untreated cell, Vr treated cell	8,983	69	4,246	<,0001

ANOVA tables for V_r untreated and treated cells and paired t-test (hypothesized difference = 0).



Inward and outward Den of treated and untreated cells

Discussion

Two considerations arise from the above results:

- the main significant difference induced after treating TB cell line with RA is a regulatory activity on the inward current that induces an I/V relationship of neuronal like type although the current density is not adequate to produce spikes;
- V_r increases with RA treatment. This could be a marker of an initial neuronal transformation [2].

Conclusion

Although morphological variations and positivity to neuronal markers after RA treatment are well documented [1], it seems that retinoic acid is not able to induce a time dependent functional variability in TB cells following a 7 days RA-treatment. The increase of inward current is not sufficient to produce spikes. However, RA-induced transformation prompts functional changes that, although not adequate to generate neuronal-like activity, go toward a neuronal phenotype.

[1] Sorrentino G., Monsurrò MR., Pettinato G., Vanni R., Zuddas A., Di Porzio U., Bonavita V. (1999). Establishment and characterization of a human neuroectodermal cell line (TB) from a cerebrospinal fluid specimen. *Brain Res. 827(1-2):205-9.*
[2] Santillo S., Schiano Moriello A. and Di Maio V. (2014). Electrophysiological variability in the SH-SY5Y cellular line. *General Physiology and Biophysics 33(1): 121-129. DOI: 10.4149/gpb_2013071 IF1.3.*