Journal of
Clinical Neurology and Neurosurgery

Research Article Open Access

# Sciatic Nerve Cryo-Conservation and Superconductor-Like Behavior

**Hanini A[1], Azzouz A[2], Bouslama Z[3], Saili L[3], Rejeb A[3], Ben Salem M[4], Sakly M[1], Tliba S[5] and Abdelmelek H[1]***

[1]*Laboratory of Integrated Physiology, Faculty of Sciences, University of Carthage, Jarzouna, Bizerte 7021, Tunisia*
[2]*Laboratory of Ecology of Terrestrial and Aquatic Systems, Faculty of Sciences, Badji Mokhtar University, PO Box 12, 23000 Sidi Amar, Annaba, Algeria*
[3]*Service of Pathological Anatomy National School of Veterinary Medicine of Sidi Thabet 2020 Sidi Thabet, Tunisia*
[4]*Laboratory L3M, Faculty of Science of Bizerte 7021 Jarzouna, Tunisia*
[5]*Laboratory of Biological Engineering of Cancers, Faculty of Medicine, University Abderrahmane Mira, Aboudaou, Bejaia, 06000, Algeria*

## Abstract

The purpose of the present study was to determine the ability of nerve cryopreservation approaches, slow freezing, to preserve electric properties of sciatic nerves and histological structure. In the present manuscript, the electric resistivity (R) at different temperatures (T) was studied in frog sciatic nerve in two approaches i) nerve freezing between 300 K to 200 K and ii) nerve warming from 200 K to 300 K. Firstly, when the electrical contacts were leaned into frog sciatic nerve we note a semi-conductor behavior and a striking decrease of nerve resistivity at 252 K showing a super conductor-like behavior. Secondly, when electrical contacts were embedded to the nerve we note a metallic behavior and a fall of resistivity was shown at 252 K indicating a superconductor-like behavior. In addition, findings illustrate the importance of electric and histological studies to examine cryo-sensitivity of nerve.

**Keywords:** Sciatic nerve; Cryo-conservation; Resistivity; Superconductor-like behaviour; Schwann cells

## Introduction

Nowadays, research on cryo-conservation has been directed at different species and scarce investigations report frog sciatic nerve studies. Our laboratories are keen to bring attention to these understudied species especially electric properties of nerve at low temperature [1] characterize their fundamental nervous system biology and, most importantly, develop approaches for preserving their nerves integrity. Because at least many frog species remain rare as *Rana temporaria*, there appears to be a conservation opportunity — preserve nervous tissue for different applications [2]. To date, nervous tissue from various mammals has been cryo-preserved using slow freezing, whereas frog nerve is well understood in physiology and ecology [3] especially cold acclimation. We choose the frog as a model because there were limited studies in electric properties at low temperature and we expected that lessons learned would be applicable for future studies in cryo-preservation of nervous system of other species. Success was determined by evaluating the influence of freezing on nerve electric properties and the integrity of its structure in frog [2]. Physiological studies have shown neurochemical and electrical changes in nervous system during globe temperature variations that could be associated with adaptive mechanisms; leading to evolution of species from poikilotherm to homeotherm [2]. Besides the action of monoamines other electrical properties such as action potential or superconductor-like behaviour play a key role in the optimization of nerve network. Previous study by Mbainaibeye et al. [4] revealed different electrical responses of frog sciatic nerves during the decrease of temperature and propose wavelet models.

The present study aims firstly to evaluate the influence of freezing and warming on electrical properties of nerve and secondly to study the Schwann cells number evolution in order to examine cryo-conservation sensitivity of frog sciatic nerve.

## Material and Methods

Animals were sacrificed using light anaesthesia. Sciatic nerve samples (n=6) were harvested from frogs (*Rana esculenta;* 20 g) with light anaesthesia (Halothane 2.50% in air). The proximal segments of the sciatic nerves (1 cm) were harvested in order to study resistivity and histological study. Animals were cared for, under the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculté des Sciences de Bizerte, Tunisia). Sciatic nerves were conserved in Ringer-buffer during 1 to 5 min. Ringer-buffer composition is: $NaHCO_3$ solution at 1%, $CaCl_2$ at 1%, KCl at 1% and NaCl at 0.60%. Then, the electrical resistivity variations of the sciatic nerve with varying temperature were investigated by employing the four-probe technique which is the most common method of determining the critical temperature (Tc) of a superconductor, Tc onset is temperature at which resistivity starts decreasing. In the present experiment we studied the effect of the decrease and the increase of temperature on nerve resistivity. The temperature variation was achieved using a Helium exchange gas filled cryostat (closed cycle refrigerator). Temperature was measured using a calibrated Si-diode sensor with an accuracy of 0.10 K and was varied from 300 K to 200 K. Wires are attached to a material via two methods: Wires were leaned in frog and especially inserted into the nerves of frog. The two external wires (the distance between the current wires: 8 mm) were used as current leads and the other two as the voltage leads (the distance between the current wires: 2 mm) to record potential differences. Through two of these points a voltage is applied, we used a variable current with very low frequency (36 Hz). The value of the current used for the resistivity measurements was 20 μA (3; 4) and if the material is conductive, a current will flow. Then, if any resistance exists in the material, a voltage will appear across the other two points in accordance with Ohm's law. When the material enters a superconductor state, its resistance drops to zero and no voltage appears across the second set of points [1].

### Tissue preparation

The animals were sacrificed using light anaesthesia and their sciatic nerves were immediately harvested and fixed in 10% neutral formalin

***Corresponding author:** Hafedh Abdelmelek , Laboratory of Integrated Physiology, Faculty of Sciences, University of Carthage, Jarzouna, Bizerte 7021, Tunisia, Tel: (216) 93006057; E-mail: Hafedh.Abdelmelek@fsb.rnu.tn

**Received** September 09, 2017; **Accepted** September 21, 2017; **Published** October 05, 2017

**Citation:** Hanini A, Azzouz A, Bouslama Z, Saili L, Rejeb A, et al. (2017) Sciatic Nerve Cryo-Conservation and Superconductor-Like Behavior. J Clin Neurol Neurosurg 1: 101.

for at least 48 h; after that the samples are ready to be placed in a dehydration machine (Leika TP1020) [5].

**Dehydration and lightening:** The automatic circulation step was performed using automate. Samples were transferred in different baths of alcohol, toluene and paraffin for 24 h.

Firstly, the dehydration: This operation aims to take out or extract water from tissues by soaking samples in seven successive baths of alcohol (ethanol) in increasing concentration, one alcohol bath 80°C (1h00), three alcohol baths 95°C (1st bath four 1 h, 2nd and 3rd bath four 1 h 30 min) and three alcohol baths 100°C (1st bath four 1 h, 2nd and 3rd bath four 1 h 30 min)

Secondly, enlightenment through xylene: This will replace or remove alcohol and allow rapid penetration of paraffin into the tissue. Xylene is a solvent that replaces the dehydration agent (alcohol) this step takes place in three successive baths of xylene (1st bath four 2 h, 2nd bath four 2 h 30 min and 3rd bath four 1 h 30 min)

The final step of the automatic circulation frequently used two paraffin baths to 57°C to impregnate the samples (1st and 2nd bath four 2 h).

## Inclusion

Then the samples are coated in paraffin blocs. The blocks preparation was with paraffin distributor and cold plate (-15°C) which allows the sample orientation. After that, the cutting is carried out according to usual methods using a microtome to a thickness of 3 µ.

## Coloration

**Four steps:** Dew axing by passage through one xylene bath, rehydration by successive passage in alcohol baths at 100°, 90°, 80° and water, routine coloration with haematoxylin-eosin (H-E), rapid dehydration by successive passage in alcohol baths at 80°, 90°, 100° and one bath of xylene.

Finally, reading histological sections and images taken with automatic image analyser microscope (Leica Qwin) (9; 18)

The calculation method of the topography of Schwann cells in different species was performed according to the model showed in Figure 2 [5].

## Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) the one-way ANOVA for comparison between groups. Values for (*) $p \leq 0.01$, (**) $p \leq 0.001$, (***) $p \leq 0.0001$ were considered statistically significant. The data are shown as a mean ± Standard Error of the Mean (SEM).

# Results

In frog, when the gold contacts were leaned to the nerve, the R-T curve show an increase of the sciatic nerve resistivity for 252 K<T<300 K, showing a semi-conductor-like behaviour and at 252 K we observe a markedly fall of resistivity without reaching the zero point, indicating a superconductor-like behaviour. Then, at temperatures lower than 241 K, the resistivity of the nerve remains constant. Secondly, if we change the model and gold contacts embedded into the frog sciatic nerve, a linear decrease of the sciatic nerve resistivity is observed for 243 K<T<300 K revealing metallic behaviour and an abrupt rise of conductivity is shown below 232 K, reporting a superconductor-like behaviour. Then, the resistivity of the nerve remains constant and close to one tenth of its ambient temperature value and all the measurement were reproducible during freezing and warming of sciatic nerve (Figures 1 and 2).

## Histological sections

Longitudinal sections of the sciatic nerves show the presence of nerve fibers with Schwann cells that is surrounding the myelin sheath of frog (Figure 3). Evaluation of Schwann cell number was performed using Image J software. The analysis of the data reported that the most

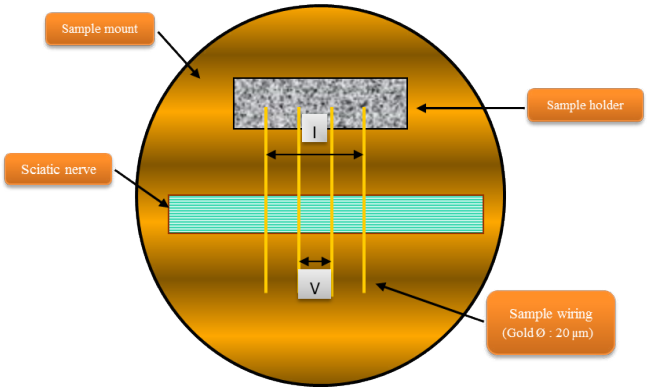


**Figure 1:** Four probe technique [3].

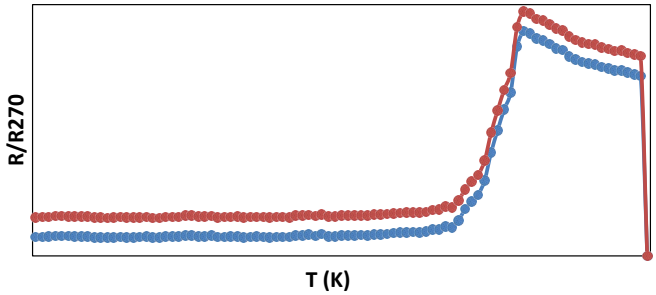


**Figure 2**: Evolution of the normalized electric resistance (R/Ra) of the frog sciatic nerve in terms of the temperature T (K) with external recording with the gold past.

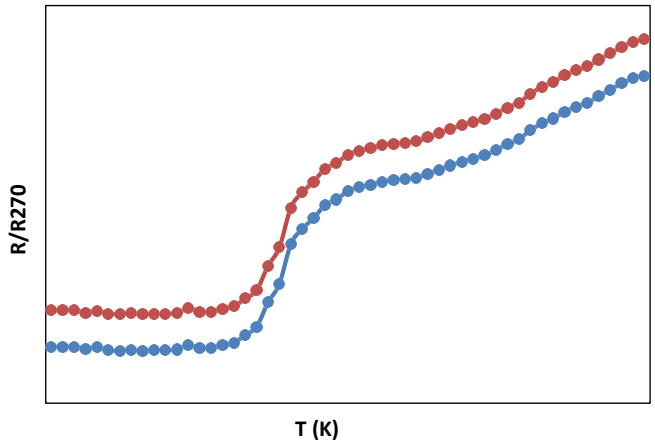


**Figure 3:** Evolution of the normalized electric resistance (R/Ra) of the frog sciatic nerve in terms of the temperature T (K) with internal recording with the gold past(A).

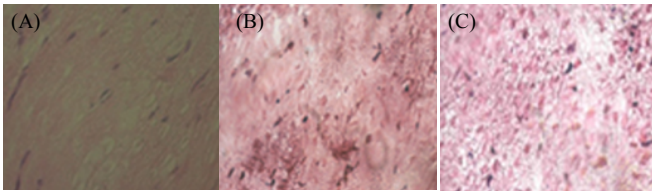


**Figure 4:** Longitudinal sections of the frog sciatic nerve (Control (A), Cryo-conserved (B) and warming (C)) (Hematoxylin-eosin x40).

important Schwann cells number was in control frog sciatic nerve compared to cryo-conserved sciatic nerve (168.40 ± 5.60 vs. 170.20 ± 6.32, p>0.01) and control group compared to warming group (165.82 ± 7.64 vs. 170.20 ± 6.32, p>0.01).

Our analysis based on the calculation of Schwann cells number reveal that during freezing or warming the number and the density remained unchanged in the studied animals (Figures 4).

## Discussion

There is a need to develop nerve preservation protocols for diverse species especially frog nervous system. There are few studies reporting data related to frog sciatic nerve cryo-conservation and one exciting approach was to use the R-T methodology and histology. The present findings clearly extended the significance of cryo-approach selected to nerve tissue, specifically showing that slow freezing of sciatic nerve tissue preserve cellular integrity and function. Our hypothesis point to the presence of electric behavior proportional to nerve freezing and warming that could indicate that nervous system structural and functional properties will be preserved.

Previous results have demonstrated the existence of superconductor-like behaviour in sciatic nerve [1,2,4,6]. Our main interest lies in the electrical properties of large-scale nerve networks at low temperature during freezing or warming. Understanding these complex functions of nerves during freezing requires a multidisciplinary approach based on electrical and/or neurochemical properties of nervous system [2,7,8] especially sciatic nerve. According to our findings, the marked decrease of resistivity at low temperature in frog can be mediated by a mechanism having many similarities with inorganic and organic superconductors [1]. The analysis of Figures 2 and 3 showed a marked fall of resistivity in frog; indicating a superconductor-like behaviour associated to semi-conductor or metallic behaviour related to the gold contact use. Thus, in cryostat the decrease (freezing) or increase (warming) of temperature has a proportional effect on the sciatic nerve resistivity in frog. The present data point to the eventual integrity of structure but histological studies must be done in order to confirm this hypothesis. Interestingly, histological studies demonstrated a stability of Schwann cells during freezing or warming as shown in table 1. In fact, Abdelmelek et al. [1]

| | Control | Cryo-conserved | Warming |
|---|---|---|---|
| **Schwann cells number** | 170.20 ± 6.32 | 168.40 ± 5.60 | 165.82 ± 7.64 |

**Table 1:** Frog Schwann cells number analysis in three groups (control, cryo-conserved, and warming).

and Mbainaibeye et al. [4] reported that superconductor-like behaviour is related to the development of metabolic function, the development of myelin and the three-dimensional arrangement of proteins and ion channels. Interestingly, our data demonstrate that the distance between Schwann cells remained stable confirming the integrity of nerve [9].

## Conclusion

The present investigation has indicated that low temperatures induced a fall of nerve resistivity in frog, showing a superconductor-like behaviour. Data showed clearly a stability of Schwann cells number and disposition indicating the integrity of sciatic nerve.

### Conflict of Interest

The authors declare no competing financial interests.

### Acknowledgement

The authors are grateful to Bechir AZIB for the technical assistance, providing reagents and equipment.

### References

1. Abdelmelek H, El-May Ben Hamouda A, Ben Salem M, Pequignot JM, Sakly M (2003) Electrical conduction through nerve and DNA. Chin J Physiol 46: 137-141.
2. Abdelmelek H, Hanini A, Azzouz A, Sakly M (2017) Electrical and Neurochemical conduction through sciatic nerve at low temperature. Open Access J Neurol Neurosurg 4: OAJNN.MS.ID.555630.
3. Adam A, Friede RL (1988) The number of frog sciatic axons increases continually during body growth. Anatomy and Embryology 178: 537-541.
4. Mbainaibeye J, Ezzedine BB, Mohamed BS, Moshen S, Abdelmelek H (2012) Analysis and characterization of the electrical conductivity behavior of the sciatic nerve using wavelet transform and signal processing. IJECCE 4: 2278-4209.
5. Hanini A, Schmitt A, Kacem K, Chau F, Ammar S, et al. (2011) Evaluation of iron oxide nanoparticle biocompatibility. Int J Nanomed 6: 787-794.
6. Ahmed S, Malik S, Azeem MA, Noushad S (2012) Sciatic nerve conduction velocity and locomotory patterns in Frog, Uromastix and Rabbit. J Anim Plant Sci 22: 878-883.
7. Abdelmelek H, Cottet-Emard JM, Pequignot JM, Barré H (2000) Changes with age in spinal cord monoaminergic system responses to cold acclimatization. J Neural Transm 107: 1175-1185.
8. Schön JH, klocch, Batlogg B (2000) Superconductivity in molecular crystals induced by charge injection. Nature 406: 702-704.
9. Waxman SG (1977) Conduction in myelinated, unmyelinated and demyelinated fibers. Arch Neurol 34: 585-589.