The Open Access Journal of Science and Technology
Vol. 3 (2015), Article ID 101127, 6 pages
doi:10.11131/2015/101127

AgiAl Publishing House
http://www.agialpress.com/

Research Article

Oyster Electrophysiology: Electrocardiogram Signal Recognition and Interpretation

Frederico M. Batista[1,2], Deborah M. Power[2], and Adrian P. Harrison[3]

[1]*Instituto Português do Mar e da Atmosfera (IPMA), Divisão de Aquicultura e Valorização, Estação Experimental de Moluscicultura de Tavira, Avenida 5 de Outubro, 8700-305 Olhão, Portugal*
[2]*CCMAR, Universidade do Algarve, Campus de Gambelas, Faro, Portugal*
[3]*IKVH, Faculty of Health & Medical Sciences, Copenhagen University, Copenhagen, Denmark*

Corresponding Author: Adrian P. Harrison; email:adh@sund.ku.dk

Received 7 August 2014; Accepted 29 August 2014

Academic Editors: Vassilis Paschalis and Salah A. M. Said

Abstract. After 100 years of published recording traces pertaining to the oyster electrocardiogram (ECG), we revisited the original experiments of Eiger (1913), using state-of-the-art electrophysiology recorders. Our aim was to confirm that a recordable ECG, similar to that of higher vertebrates, is present in the oyster heart. Portuguese oysters *Crassostrea angulata*, collected from the Guadiana estuary, Portugal, were used. The oysters were drilled through the right valve to reveal the pericardium. Gold and silver electrodes were placed through the hole and electrophysiological recordings were obtained. Stimulation of the oyster heart was performed *in vivo* and *in vitro* using a constant current power supply. Placement of electrodes around the heart revealed a trace that very closely matched the published ECG of Eiger (1913). However, we were unable to confirm that the recording was an ECG of the oyster heart. Moreover, measurements on isolated oyster hearts revealed a low conductivity (0.10 S m^{-1}). We did, however, record a depolarization signal from what we believe to be the visceral ganglia, and this preceded contractions of the oyster heart. Our findings indicate that so-called ECGs, previously recorded by [2] in *Ostrea edulis*, but also the "ECG" recorded by [4] in *C. virginica* from oyster hearts, are in fact an artifact arising from relative movement of the recording electrodes, giving rise to a baseline shift that mimics in some ways the P and QRS features of a typical ECG. Nevertheless, such recordings provide information pertaining to heart rate and are not without importance.

Keywords: oysters, heart, ECG, heart rate

1. Introduction

A little over one hundred and twenty years ago Sir Horace Darwin, son of the naturalist Charles Darwin, founded the Cambridge Scientific Instrument Company (1885) and began producing and selling devices that were capable of measuring the electrical signals in the heart—the so called electrocardiogram (ECG). However, it was not until the beginning of 1901 that Willem Einthoven, a Dutch medic and physiologist, completed a series of prototypes of a string galvanometer that enabled the first practical ECG´s to be recorded, for which he received the Nobel Prize in Medicine in 1924.

Prior to this, though, and inspired by the terminology used by Einthoven to describe an ECG by assigning the letters P, Q, R, S, and T to the various deflections of the signal, a young German scientist M. Eiger embarked on a huge project—that of establishing the basis for the ECG, a work that resulted in him measuring the ECG of a wide array of diverse species, among them oysters, and publishing his seminal work in 1913 entitled "Die physiologischen Grundlagen der Elektrokardiographie" in Pflügers Archiv für die gesamte Physiologie des Menschen und der Tiere. In this work, Eiger reveals an ECG taken from an oyster heart (European flat oyster *Ostrea edulis*), which appears to show the P-wave and QRS complex, that is to say, atrial depolarization and ventricular depolarization, respectively. In subsequent years an illustrated diphasic contraction with auricular contraction shown as a slight positive wave, preceding a depolarization, identified as being ventricular contraction was demonstrated in another oyster (American oyster C*rassostrea virginica*) by [11]. The notion that an oyster heart had an ECG and that this could be measured was now established, and such ECGs as those illustrated by [2, 11] have been shown in several publications, ever since.

However, exactly 100 years after the first published ECG for an oyster heart by [2], which only presented the P and QRS complexes and not the T wave, we question whether an ECG is at all possible for the heart of an osmoconformer. This query is perhaps not without foundation since detailed structural evidence of the oyster heart by [6] revealed that the wall of the heart shows evidence of myocardial cells but that they are extremely scarce. Moreover, work by [12] aimed at assessing the electrophysiology of both oysters and other bivalves showed that in the Pacific oyster (*C. gigas*) the auricles and ventricles possess different intrinsic rates of contraction. These findings led the authors to conclude that stretch was the principal mediating factor in the coordination of auricular and ventricle contraction in the oyster heart. They also went on to state that evidence for a conduction pathway, as found in higher vertebrates, was not to be found in the oyster heart.

It is with these issues in mind that we have chosen to revisit the experiments of [2, 11], using state-of-the-art electrophysiology equipment. The hypotheses tested were 1) that oyster hearts have a conduction pathway similar to that of higher vertebrates and 2) a recordable ECG with the full P, QRS, and T complexes should occur.

2. Materials and Methods

2.1. Animals. In the current study we used oysters Portuguese oyster *C. angulata* collected from the Guadiana estuary, Portugal. The oysters were all apparently healthy and were in good condition. All the animals used in these experiments were handled humanely.

2.2. Recording equipment. In the current study, an ADInstruments PowerLab (4/25T—Oxford, United Kingdom) recording at 40,000 samples per second, in conjunction with 24 carat gold electrodes, as well as sterling silver electrodes was used to record electrical signals from the oyster pericardium—both around the exposed heart and in direct contact with the general cardiac tissue.

The signals were recorded onto a MacBook Air and analyzed using Chart v5.5.6 software (ADInstruments Ltd—Oxford, United Kingdom) in terms of their frequency (Hz), amplitude (mV), and baseline drift (Δ mSec). Input impedance was 200-MΩ differential.

The recording electrodes were hand—made from jewelry grade gold and silver wires, soldered to cables that enabled them to be inserted into the amplifier head—stage (MLA2540 5-Lead Shielded Bio Amp Cable) and sealed with heat—shrink plastic. All cables were shielded to the tip.

2.3. Preparation. The oysters were acclimated to the experimental conditions in a quiet and shaded laboratory for a period of 30 minutes. A small window was drilled through the right valve to reveal the edge of the adductor muscle and the pericardium (see Figure 1). The oysters were then allowed to rest and equilibrate once again in a glass petri dish filled with fresh sea water for a further 30 minutes.

Once the oysters began to open and filter sea water (approx. 20 minutes after preparation), gold or silver electrodes were placed on/and around the pericardium (see Figure 1) and electrical recordings were taken.

Upon completion of the experiment, any viable oysters were sealed using an adhesive pad (Patafix Pro Power—UHU, Baden, Germany) and returned to the research centre at IPMA, Tavira, Portugal.

2.4. Electrical stimulation. Direct stimulation of the oyster hearts *ex vivo* or *in vivo* was achieved through two silver chloride electrodes connected to a constant current isolated stimulator (DS3—Digitimer Ltd, Hertfordshire, UK). The stimulator was connected to the PowerLab (4/25T) and could be controlled *via* the computer to deliver stimuli in the range 0–90 V and 0–32 mA with pulse durations of between 10 μsec and 2000 msec.

2.5. Histology. Oyster hearts were fixed in Davidson solution, processed, and embedded in wax and sections stained using H&E before analysis using a light microscope.

2.6. Conductivity. The conductivity of the oyster heart was measured using a power unit that enabled both a controlled voltage and current. A voltage of 1.720 volts was applied to a known resistor and the current, which had been set roughly to 200 mA, was then measured as being precisely 177 mA.

The same cables and power settings were then applied to an isolated oyster heart, and the voltage drop across the heart

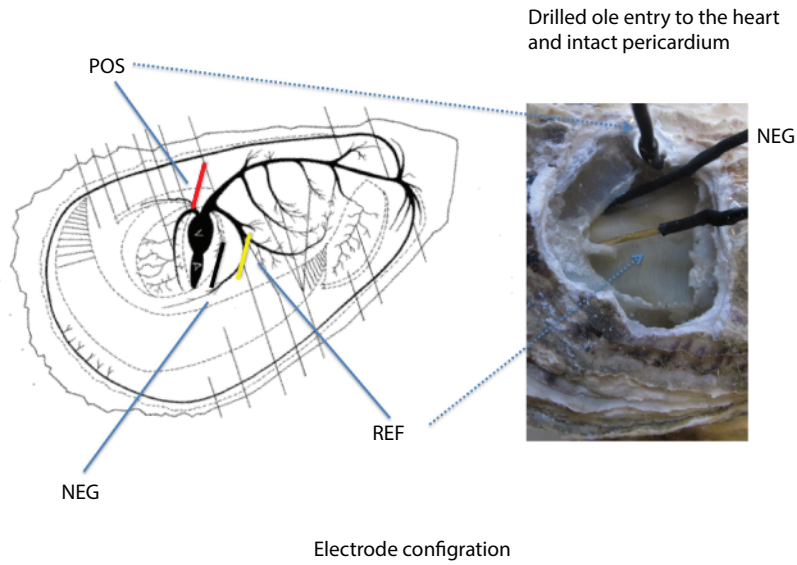


Figure 1: A schematic showing the relative placement of the silver POS and NEG electrodes as well as the gold REF electrode. This setup was found to not only give a clear recording of the depolarization signal, but also enable a recording artifact that coincided with the rise and fall of the pericardium that followed auricular contraction and ventricular contraction, respectively. The oyster we sealed after the experiment and released once the opening in the shell had been repaired. See Materials and Methods for details.

used with the current to determine the resistance of the heart according to Ohm´s law (V/I = R). The conductance, which is the reciprocal of the resistance, was then calculated (C = 1/R).

3. Results

3.1. Pericardial recordings. When recordings of oyster heart electrophysiology were attempted from the pericardium, a very small yet distinct signal was detected. A reference (gold; REF) electrode was placed on the visceral mass but with very close proximity to the heart. In this location the reference electrode was found to be very stable and not to move at all during the recordings. A negative electrode (silver; NEG) was placed on the surface of the pericardium alongside the auricles, whilst a positive electrode (silver; POS) was placed on the pericardium at the top of the ventricle (see Figure 1).

Initial recordings carried out in a similar fashion to those by [2] resulted in a trace that resembles that published one hundred years ago. It apparently showed the P and QRS complexes, but just like Eiger´s trace, it was devoid of a T-wave.

Further studies were carried out to validate the results in which a modified electrode configuration was used, resulting in an optimized trace recording. Using this approach it was possible to record a clear depolarization signal from what we believe to be the visceral ganglia (9×10^{-6} V in amplitude and 20 mSec in duration). The depolarization signal was very constant and regular and could be detected in a number of oysters using this specific electrode configuration (see Figure 3). Surprisingly this trace did not conform to that recorded using the [2] setup. To identify the potential cause of divergence a high- power dissecting microscope was used to observe the contraction of the heart in relation to the electrical signals being recorded. This depolarization signal preceded any sign of contraction in the auricles or the ventricle of the oyster heart. In this way we were able to follow the sequence of events and describe the contractions of the heart in relation to the electrical signals we recorded (see Figure 4).

This depolarization signal, which most likely originates from the visceral ganglia, or another tissue not in the pericardium, seems to form the trigger for auricular contraction, forcing haemolymph into the ventricle which then becomes distended and forces the pericardium to rise. As the pericardium rises, it lifts the electrodes slightly in relation to the REF electrode that remained perfectly still, and in so doing it gave rise to a baseline shift (upward). We watched this happen under the microscope and pressed a trigger (remote switch) each time the pericardium was seen to rise, which placed a marker line on the pericardial recording trace. This marker line was found to coincide with the rise in the recording that immediately followed the depolarization signal (baseline shift upward). The ventricle, once full, began to contract and with it came a fall in the pericardium, again observed under the microscope and noted on the recording trace through the aid of a trigger (remote switch). This second marker line was found afterwards to coincide with the fall in the recording that immediately followed the postdepolarization signal rise (baseline shift downward). Thereafter the ventricle was observed to relax back to its

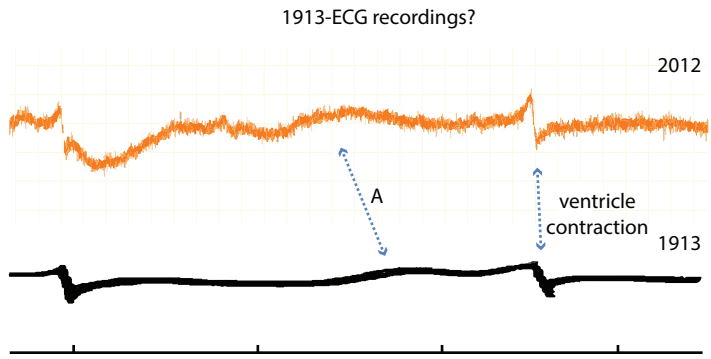


Figure 2: An illustration of the original [2] and Bureau's shellfish lab recording demonstrating what was believed to be an oyster ECG (lower panel—black). The authors identified a "gentle wave corresponding to auricular contraction A preceding by approximately one and half second the contraction of the ventricle". In our setup we were able to reconstruct just such a recording (upper panel—orange) and to show that the signal that was identified as being related to ventricular contraction actually precedes both auricular and ventricular contractions and is most likely the depolarization of the visceral nerve. Time intervals represent 1 second.

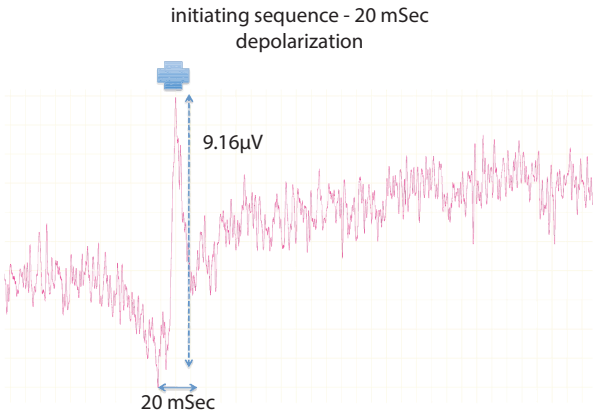


Figure 3: A typical depolarization signal taken from the visceral nerve as it enters the pericardium of the oyster. This signal was found to be very stable and constant, and to precede any contraction of the heart.

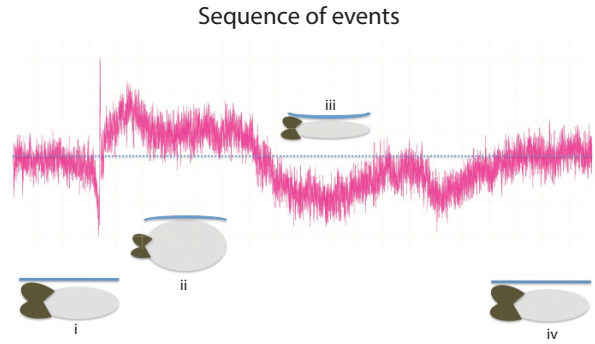


Figure 4: A typical sequence of events for a recording taken from the pericardium (A). The sequence starts with a depolarization signal (B)—as shown in Figure 3, which then gives rise to a baseline shift (upwards) as the ventricle fills. Once the ventricle begins to contract, a baseline shift (downwards) occurs, after which the ventricle and pericardium return to their resting state and the baseline returns to its starting point. In terms of notation: i) auricles and ventricle relaxed, ii) auricles contracted and ventricle distended, iii) ventricle contracted, and iv) auricles and ventricle relaxed.

resting state and the baseline was seen to return to its starting level (see Figure 4).

3.2. ECG. Even when the gain was increased to its maximum limit (2 μV range), no hint of an ECG was detectable using our electrode configuration. Moreover, the noise within the recording system was minimal and no filtering was needed. Finally, the application of a stepwise increase in electrical stimuli (20 mV to 2.5 V –1 Hz to 15 Hz) failed to elicit any contractile response in the oyster heart, both when stimulated *ex vivo* and *in vivo*.

3.3. Conductivity. The conductivity of the oyster heart was calculated according to Ohm's law. An electrical discharge of 1.720 V and 177 mA was applied across the oyster heart and a measurement of 0.004 V was recorded. Thus, the difference between the supplied and measured voltage is 1.720V–0.004V, which is 1.716 V. Using Ohm´s law, 1.716V divided by 0.177 A gives a value for R of 9.690 Ω. Thus since C = 1/R, the conductivity of the oyster heart is C = 0.10 (S m^{-1}). This value compares very well with the conductance of such mammalian tissues as liver, lung, muscle, and fat, which have been found to have values of 0.09, 0.11, 0.15–0.19, and 0.07 S m^{-1}, respectively. It is, however, very low compared to the conductance of mammalian heart which is typically between 0.4 and 0.6 S m^{-1} [3].

3.4. Histology. The prepared oyster hearts revealed a very loose structure with relatively few muscle fibers, being composed principally of interlocking connective tissue and a thick wall of epithelial cells at the periphery of the ventricle (see Figure 5).

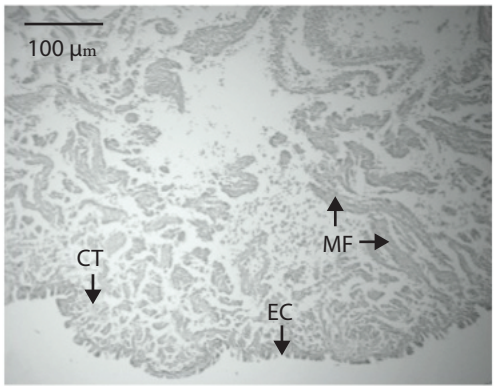


Figure 5: A typical light microscope section of a fixed oyster heart, stained with H&E to reveal epithelial cells (EC) and associated connective tissue (CT), as well as muscle fibers (MF) principally towards the center of each lobule. Note that as reported by [6] the wall of the oyster heart shows evidence of myocardial cells but that they are relatively scarce compared to the ventricular wall of a mammalian heart. Scale bar = 100 μm.

3.5. Discussion. This study has tested the hypotheses that 1) oyster hearts have a conduction pathway similar to that of higher vertebrates and 2) a recordable ECG with the full P, QRS, and T complexes should occur. The data obtained do not support the null hypothesis, and it is concluded that oyster hearts have a low conductance and do not exhibit a recordable full ECG.

These findings at first glance seem in some ways confusing as a recent study by [9] has shown that a number of isolated atrial cells grown in culture from *Crassostrea gigas* have both ionic currents and spontaneous contractile activity similar to that of vertebrate atrial cells. Yet, despite all this, when excised the heart ceased to contract in a coordinated fashion, which argues against an intrinsic electrical rhythm being the driving force behind cardiac contraction in the oyster. When the heart was isolated we noticed a form of contraction in 1 out of 6 cases, but this was not at the normal *in vivo* rhythm, nor was it found to be coordinated in nature. Furthermore, when one takes into consideration the fact that these organisms are osmoconformers, and that one might expect the pericardium to be exposed to high concentrations of NaCl, which for an electrical-based contractile organ may represent a challenge in terms of signal transmission, then perhaps this observation does make sense.

In a recent paper in the Journal of Physiology published by [5] entitled "Cell volume and membrane stretch independently control K^+ channel activity" it was shown that local membrane stretch induced by a negative hydrostatic pressure activates BK currents. The authors state that the BK channels respond directly to membrane stretch and that this mechanosensitivity is independent of Ca^{++} (this fits with others, e.g., [1, 8]). Thus it is conceivable that oysters may have the potential means for a hydrostatic driven system in which fluid returning to the oyster heart causes direct stretch of the membrane, activates BK channels, and sets up a local depolarization giving rise to contraction.

Indeed, there is evidence to suggest that the BK channel has a sensor (cytoplasmic C-terminus), which senses stretch—if this is deleted from BK channels from chick hearts then they lose their stretch sensitivity [10]. This chain of thought is all the more interesting as we observed in several isolated oyster hearts that had been excised between two and three hours that upon pinching the heart wall with a fine pair of forceps, they started to contract for between 3 and 11 beats before ceasing once again (data not shown).

However, despite many attempts with some very sensitive equipment, we were not able to detect an ECG in the oyster hearts, whether *ex vivo* or *in vivo*, and this compares favourably with the low conductance values we were able to measure. We were able to measure a movement artifact though, and by reversing the POS and NEG electrodes we were able to measure a signal that identically matches that of [2] both in terms of its time scale and its amplitude (see Figure 2). Whilst this is clearly not an ECG, as reported by [2], it does have its use as it serves to provide information pertaining to heart rate. The rate at which the oyster heart beats is of considerable importance at the scientific level as we endeavour to establish the drive for the contraction of the oyster heart and identify its regulation.

The histology of the heart [7] and observations from the present study taken on beating hearts *in situ*, which show a very loose structure, that when expanded become translucent support our proposal that the oyster heart is a stretch driven rather than electrical discharge driven tissue. This proposal is further supported by the detailed histology of [7] who revealed that the ventricular wall comprises a stratified prismatic epithelium with considerable dense and interlocking connective tissue, interspersed with a few thick layers of muscle fibers. These findings are also supported by our own histological analysis of the oyster heart (see Figure 5).

4. Conclusions

We conclude that modern sensitive techniques can be used relatively noninvasively to detect and record physiological processes in oysters. With regard to hypotheses 1 and 2, we reveal that oyster hearts do not have a conduction pathway like that of higher vertebrates, and, moreover, there is no recordable ECG with the full P, QRS, and T complexes. Thus, contrary to the general consensus, the heart in the oyster does not appear to be driven by an electrical discharge originating in the heart tissue itself. Rather, our findings appear to suggest that the pressure of fluid returning to the heart, inducing stretch of the auricular and ventricular walls may be the initiator of contraction, although we do not discount the possibility that cardiac contractions may be driven/regulated by electrical signals of extra-cardiac origin.

Acknowledgments

The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement n° 227799—ASSEMBLE. The authors wish to thank the skilled technical help of Teresa Santos and Helle Ruby.

References

[1] J. N. Barrett, K. L. Magleby, and B. S. Pallotta, Properties of single calcium-activated potassium channels in cultured rat muscle, *Journal of Physiology*, **331**, 211–230, (1982).

[2] M. Eiger, Die physiologischen Grundlagen der Elektrokardiographie, *Pflüger's Archiv für die Gesamte Physiologie des Menschen und der Tiere*, **151**, no. 1-3, 1–51, (1913).

[3] C. Gabriel, A. Peyman, and E. H. Grant, Electrical conductivity of tissue at frequencies below 1 MHz, *Physics in Medicine and Biology*, **54**, no. 16, 4863–4878, (2009).

[4] P. S. Galtsoff, *The American Oyster Crassostrea Virginica Gmelin*, **64**, Fishery Bulletin, US Department of the Interior, 1964.

[5] S. Hammami, N. J. Willumsen, H. L. Olsen, F. J. Morera, R. Latorre, and D. A. Klaerke, Cell volume and membrane stretch independently control K^+ channel activity, *Journal of Physiology*, **587**, no. 10, 2225–2231, (2009).

[6] W. E. Hawkins, H. D. Howse, and T. G. Sarphie, Ultrastructure of the heart of the oyster Crassostrea virginica Gmelin, *J. Submicrosc. Cytol*, **12**, 359–374, (1980).

[7] M. Le Pennec and H. Grizel, *Atlas d'histologie et de cytologie des mollusques bivalves marins*, Ifremer, France, 2011, ISBN 978-2-84433-111-3.

[8] M. T. Kirber, R. W. Ordway, L. H. Clapp, J. V. Walsh Jr., and J. J. Singer, Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells, *FEBS Letters*, **297**, no. 1-2, 24–28, (1992).

[9] J. Pennec, H. Talarmin, M. Droguet, M. Giroux-Metgès, M. Gioux, and G. Dorange, Characterization of the voltage-activated currents in cultured atrial myocytes isolated from the heart of the common oyster Crassostrea gigas, *Journal of Experimental Biology*, **207**, no. 22, 3935–3944, (2004).

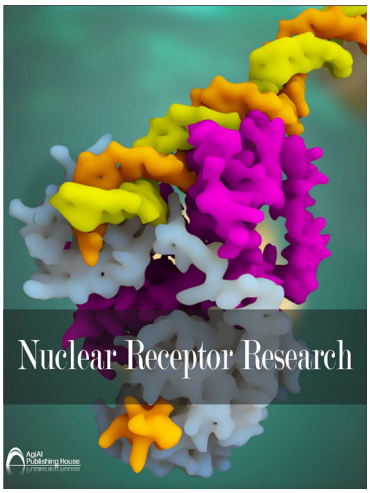
[10] Z. Qi, S. Chi, X. Su, K. Naruse, and M. Sokabe, Activation of a mechanosensitive BK channel by membrane stress created with amphipaths, *Molecular Membrane Biology*, **22**, no. 6, 519–527, (2005).

[11] I. R. Taylor and E. M. Walzl, The effect of direct current stimulation on the electrical, mechanical behavior of the heart of the oyster (Ostrea virginica), *Journal of Cellular, and Comparative Physiology*, **18**, no. 2, 278–281, (2005).

[12] U. Hiroyuki, Y. Hiroshi, and E. Arinobu, Coordination of activities between the auricle and ventricle in an oyster Crassostrea gigas, *Comparative Biochemistry and Physiology – Part A: Physiology*, **87**, no. 3, 689–694, (1987).

Nuclear Receptor Research

About the Journal



100% Free of Charge

Nuclear Receptor Research is a peer-reviewed open access journal that publishes high-quality, original research and review articles covering all aspects of research involving all members of the nuclear receptor superfamily.

The editorial board of *Nuclear Receptor Research* has over 70 scientists representing a wide-scope of interest and expertise in the field, from 20 countries around the world.

Nuclear Receptor Research has a fully automated Manuscript Management System (MMS) which makes submission and reviewing as well as tracking of manuscripts an easy, efficient and prompt process to the advantage of the authors. Published articles in *Nuclear Receptor Research* are available in different formats including full-text HTML, full-text PDF, full-text ePUB, full-text XML, and Mobi.

Free Advertising

100% Free of Charge

Advertise, in *Nuclear Receptor Research*, positions available in your laboratory, a position you are seeking, supplies and equipment as well as meetings and conferences related to the field of nuclear receptor research.

For more information about advertising in *Nuclear Receptor Research* please visit the journal website at: http://www.agialpress.com/journals/nrr/

For advertising in *Nuclear Receptor Research* please send your ad to nrr.ad@agialpress.com

Contact

Editor-in-Chief: badrm@umkc.edu
Editorial Office: nrr@agialpress.com

AgiAl Publishing House
http://www.agialpress.com/