White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/246301/

Version: Published Version

Article:
Freestone, N.S., Corns, L.F., Rouse, J.L. et al. (2025) How to obtain physiologically relevant cardiovascular data with students using chick embryo ventricular cardiomyocytes. Advances in Physiology Education, 49 (4). pp. 1026-1033. ISSN: 1043-4046

https://doi.org/10.1152/advan.00215.2024

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial (CC BY-NC) licence. This licence allows you to remix, tweak, and build upon this work non-commercially, and any new works must also acknowledge the authors and be non-commercial. You don't have to license any derivative works on the same terms. More information and the full terms of the licence here:
https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Adv Physiol Educ 49: 1026–1033, 2025.
First published September 8, 2025; doi:10.1152/advan.00215.2024

american physiological society

ADVANCES IN PHYSIOLOGY EDUCATION

SOURCEBOOK OF LABORATORY ACTIVITIES IN PHYSIOLOGY

How to obtain physiologically relevant cardiovascular data with students using chick embryo ventricular cardiomyocytes

Nicholas S. Freestone,[1] Laura F. Corns,[2] James L. Rouse,[3] and Aidan Seeley[4]

[1]*School of Life Sciences, Pharmacy and Chemistry, Kingston University, London, United Kingdom;* [2]*School of Biosciences, University of Sheffield, Sheffield, United Kingdom;* [3]*School of Biology, University of Leeds, Leeds, United Kingdom; and* [4]*Swansea University Medical School, Swansea University, Swansea, United Kingdom*

Abstract

The chick embryo ventricular cardiomyocyte model provides students easy access to experiments involving fundamental features of cardiac cell physiology and pharmacology. With standard physiology teaching laboratories and basic cell culture equipment, spontaneously beating colonies of electrically connected cardiomyocytes can be obtained by the students themselves. Students learn aseptic techniques and cell culture alongside experiments illustrating, at the simplest level of experimentation, how beating rate can be altered physiologically or pharmacologically. In the typical course of the type of experiments presented here, students first observe the effect of temperature (beating rates decline to a third going from 37°C to room temperature; e.g., to 40 from 130 beats/min) and media change (beating rates increase up to 50%) before moving on to the pharmacological characterization of various receptors in these cells. Most obviously, in the cardiac cell context, this involves drugs acting on β-adrenoceptor subtypes. Students can obtain predictable dose-dependent increases in beating rates (up to maximal 100% increases in beating rate; from ∼100 to 200 beats/min typically) with the addition of stimulatory β-adrenoceptor agonists (e.g., isoproterenol) but also observe dose-dependent decreases in beating rate with β_3-adrenoceptor agonists (reducing beating rate by up to a third). Consequently, "classical" log dose-response curves can be obtained in the "real world," enhancing student understanding of fundamental mechanisms of drug action. Although these experiments focus on physiological and pharmacological techniques, the model can be extended to encompass biochemical or molecular biological studies in terms of intracellular signaling systems activated and protein expression patterns.

NEW & NOTEWORTHY Many in today's societies see the use of animals for experimentation and education as unnecessary and even immoral. There is nevertheless a need to investigate the fundamental physiological principles underlying life itself, and students need to be trained in these principles for the wider benefit of humanity and the planet. This article provides an ethical alternative to the traditional models used in the study of cardiac physiology to train the next generation of physiologists.

chick embryo cardiomyocytes; humane experimental technique; partial replacement; 3Rs

INTRODUCTION

There is a shift in how universities worldwide approach the teaching of physiology laboratory practical skills, particularly those involving animal models. This is driven by the interaction of financial, logistical, and ethical considerations. One key issue is the imperative to apply the principles of humane experimental technique, as framed by the 3Rs principles: replacement, reduction, and refinement. These principles aim to minimize the use of animals in research and education by promoting alternatives, minimizing the number of animals used, and improving welfare when animal use is unavoidable. The use of animal models in undergraduate physiology cardiovascular experiments in particular traditionally involves mammalian species, but lower-order vertebrates, such as chick embryos, can serve as a more humane option, aligning with a partial replacement aspect of the 3Rs while still offering students valuable hands-on experience with the cardiovascular system. Chick embryos are logistically simpler to house and maintain in laboratory settings than mammals and may offer relevant training in techniques and principles central to cardiovascular physiology. These may include heart development, angiogenesis, development of physiological responses, and pharmacological characterization of cardiac receptors to name but a few. Using chick embryos reduces costs associated with housing, maintenance, and husbandry of mammals, and while cost should never be a factor from an ethical perspective, this may present an attractive alternative in some educational settings. This is especially important to ensure that students are given the opportunity to obtain hands-on laboratory experience in increasingly resource-constrained environments where students may not routinely be exposed to practical work of this nature. The model discussed here can be adjusted to suit

Correspondence: N. S. Freestone (n.freestone@kingston.ac.uk).
Submitted 31 October 2024 / Revised 16 December 2024 / Accepted 27 August 2025

1043-4046/25 Copyright © 2025 The Authors. Licensed under Creative Commons Attribution CC-BY-NC 4.0.
Published by the American Physiological Society.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

varying levels of student expertise, laboratory resource, and time constraints, making it a flexible alternative for generating data via hands-on experimentation.

Objectives and Overview

Experiments using chick embryo (*Gallus gallus domesticus*) ventricular cardiomyocytes are discussed in this article. This first involves the isolation of single ventricular cardiomyocytes from 7-day-old chick embryo hearts and their culture over 2–4 days. This results in the formation of colonies of spontaneously beating cells. Students then get an introduction to aseptic technique and cell culture and have visible evidence of their mastery of the technique when they obtain viable, spontaneously beating colonies of electrically connected cardiac cells. Once such cells have been obtained, a variety of different experimental approaches can be applied. In this article the focus is on using the model for the pharmacological characterization of different cardiac receptor populations and associated intracellular signaling pathways. At its simplest level, the model allows the student to visualize the beating rate of the cells under a standard laboratory inverted microscope and apply various interventions to change the beating rate.

Background

Fertilized eggs from chickens can be used to obtain enzymatically isolated single cardiac cells. These can be grown in culture to produce spontaneously beating colonies of ventricular cells. Students are thus presented with an easy way to visualize how heart rate may be altered by a number of physiological, pharmacological, or biochemical factors (1). Such simple experiments can be extended into biochemical and molecular biological investigations into intracellular signaling pathways and the regulation of various other cell processes. Chickens are a good model for illustrating human heart function because they possess a four-chambered heart with in-flow and out-flow tracts and are thus more similar to human hearts than other nonmammalian organisms.

In the chicken, as in mammals, heart rate and rhythm are regulated intrinsically by the spontaneous depolarization of cells in the sinoatrial node and extrinsically by the autonomic nervous system (ANS) and endogenous hormones. Pacemaker currents, similar to those in humans (I_f, the funny current), arising from the chick's heart itself in the sinoatrial node, have been described at early stages of embryo development (2). Additionally, the regulation of intracellular free calcium concentrations, crucial for modulating cardiac force and rate of contraction, has been found to be dependent on a calcium-induced calcium release process similar to that found in humans (3). This arises in chicks on the second day of embryonic development (3).

Autonomic regulation of the heart serves to enable an organism to rapidly respond to changing metabolic requirements and provide as much O_2 and nutrients as the body needs under different circumstances. Two branches of the ANS, the sympathetic nervous system and the parasympathetic nervous system, regulate cardiac activity. The sympathetic nervous system releases norepinephrine (noradrenaline), which causes epinephrine (adrenaline) release from the adrenal gland. Epinephrine and norepinephrine can bind to a number of receptors on cardiac cells to modulate function, primarily by affecting the concentration of intracellular calcium, subsequent to mobilization of an intracellular signaling molecule, cAMP. Most consequentially, this involves β_1 and β_2 G_s-coupled receptors, involving stimulatory G proteins, as well as β_3-adrenergic receptors, which are G_i-coupled receptors in cardiac cells, involving inhibitory G proteins. β_1- and β_2-adrenergic receptors mediate increases in heart rate (positive chronotropy) by acting on the pacemaker cells as well as causing increases in cardiac force (positive inotropy), cardiac conduction (positive dromotropy), and relaxation rate (positive lusitropy) by acting on atrial and ventricular cardiomyocytes. The role of the cardiac β_3-adrenergic receptor was only fully delineated in the 1980s by Gauthier and colleagues and results in negative inotropic and chronotropic responses (see, e.g., Ref. 4 for a review of this topic).

On the other hand, the parasympathetic nervous system releases acetylcholine (ACh) at its nerve terminals. At the heart, this primarily involves innervation by the vagus nerve. Release of ACh by the vagus nerve results in activation of cardiac muscarinic ACh receptors of the M_2 subtype, which are G_i-linked receptors. This activation results in a decrease in pacemaker cell activity (a negative chronotropic effect) and decreases in cardiac force (negative inotropic effect) and conduction rate (negative dromotropic effect).

Apart from the endogenous neurotransmitters mentioned above, various exogenous drugs can be added to cardiac systems to modulate function experimentally as well as in clinical settings. For example, digoxin (which increases intracellular calcium concentrations), β-adrenergic agonists such as isoproterenol, epinephrine, dobutamine, and dopamine, and phosphodiesterase inhibitors such as milrinone, which increases the intracellular concentration of signaling molecules activated by β_1- and β_2-adrenergic receptor stimulation and augments cardiac function, can all be employed in the laboratory setting.

Other factors that might affect the beating rate of cultured cardiac cells include temperature. In isolated chick embryo cardiomyocytes cardiac function is optimal at 37°C. Thermal stress results in alterations in cardiac function and especially spontaneous beating rate in these cells. For example, decreasing temperature from 37°C to 34°C has been reported to result in decreases of 22% in beating rate due to an effect on pacemaker ion currents (5).

The chick embryo expresses adrenergic receptors by day 5 that respond dose-dependently to increases in epinephrine with increases in beating rate due to elevation in intracellular cAMP and calcium concentrations (6). Most important for the utility of this model in a teaching context is the fact that it has been shown that the chick embryo expresses no pain receptors until embryonic day 13 (7).

Similar to humans, the heart in chick embryos is a four-chambered pump with two superior atria and two inferior ventricles comprised of the venous, deoxygenated circulatory system on the right side and the arterial, oxygenated circulatory system on the left side. There are some dissimilarities, however: chick hearts do not possess papillary muscles and tendinous cords, and the atria and ventricle inner walls are smoother, resulting in less friction during the propulsion of

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

blood, which explains the high beating rate of up to 400 times per minute exhibited by chick hearts in vivo.

In many physiology-oriented undergraduate degree programs across the world cardiac physiology has traditionally depended on the Langendorff-perfused whole heart preparation (most often of the rat) to illustrate various features of cardiac function, including interventions that produce inotropic and chronotropic changes. More advanced studies, usually, but not exclusively, at postgraduate level, have used isolated cardiac cells from various species (rat, mouse, guinea pig, rabbit) to look at single-cell contractility and intracellular signaling systems involved in regulation of cardiac function. More recently, advances in gene technology have allowed the insertion of specific genes into cardiac tissue, so that specific proteins crucial for cardiac contractility can be examined in more detail. Many United Kingdom higher education institutions have reduced and sometimes ceased their experimental use of mammals, because of financial and ethical constraints. There is still a need, however, for students to learn the necessary skills to conduct experiments on living tissue. In particular, the future of clinically orientated research seems to still rest on our ability to use animals to develop new medicines and test the safety of these new therapies.

Learning Objectives

After completing this activity, the student will be able to:

1) Employ aseptic technique in a cell culture context and understand how to maintain sterility when generating and experimenting on primary cell cultures. This learning objective would be successfully met when the students can routinely obtain contamination-free, spontaneously beating cardiac cells.
2) Design experiments to assess the impact of a range of chronotropic interventions in spontaneously beating cardiac myocytes. This learning objective would be successfully met when students can demonstrate that beating rates can be increased as well as decreased, depending on the intervention deployed.
3) Evaluate and analyze data relating to change in the activity of spontaneously beating ventricular myocytes in culture. This learning objective would be successfully met when students can demonstrate appropriate graphical plotting of the data (e.g., log dose-response curves or bar charts) and statistical analysis of the chronotropic changes seen under the different conditions employed.

Activity Level

This activity is suitable for students at different levels of their university education. I have had students in the laboratory from the following backgrounds:

- School-age summer internship students (aged 17–18 yr) working on shorter projects for 2 mo.
- Most often, though, this type of work is undertaken by final-year undergraduate project students in the general bioscience disciplines and taught Master's student programs.
- However, given the adaptability of the level of investigations that can be used with this model, Master's by Research students and first-year PhD students can also employ this model as a method for providing an early opportunity for them to learn new skills and gain knowledge of a research environment, while at the same time generating potentially meaningful and novel data.

Prerequisite Student Knowledge or Skills

Before doing this activity, students should have a basic understanding of:

1) Cardiac physiology
2) The role of cardiac myocytes in cardiac contraction
3) Excitation-contraction coupling
4) Calcium-induced calcium release.

Students should know how to:

1) Use a standard laboratory micropipette
2) Set up and obtain good images through a standard laboratory inverted microscope
3) Undertake calculations to be able to determine final concentrations of drugs added to cells from concentrated stock solutions
4) Make up simple solutions.

Time Required

Isolation of viable single ventricular myocytes takes ~3 h when isolating hearts from 12–18 eggs. Experiments on the subsequently formed spontaneously beating colonies of cells can be performed after 2–4 days in culture. The cells obtained can be reused after drug addition by simply removing the drug-containing medium and washing the cells twice with fresh medium. Consequently, in terms of time required, during the 2–4 days delineating the cell's viability the supply of cells is inexhaustible and students can obtain data for days at a time if they have the inclination. Consequently, deriving the data to construct a typical dose-response curve to one drug might be obtained in 2 wk of laboratory work.

METHODS

Equipment and Supplies

United Kingdom-specific suppliers have been used here, but most of the equipment cited below is routinely available in many countries.

- Fertilized chicken eggs (can be obtained commercially or via a local farmer; here Henry Stewart and Co Ltd, Fakenham, UK, a commercial supplier, was used)
- 70% alcohol solutions to sterilize laminar flow work area and dissecting equipment
- An egg incubator (Brinsea Octagon Advance, Brinsea Products, Weston-super-Mare, UK; alternatively, hand-turning of eggs in a suitably warm and humid environment will suffice)
- A water bath (Thermo Fisher Scientific, type DMU12, Abingdon, UK)
- An inverted microscope (Brunel Microscopes Ltd., Chippenham, UK)

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

- A dissecting microscope (Steddy B from Medline Scientific Ltd, Oxford, UK), although with practice the dissection can be undertaken without a microscope.
- A Class II laminar flow hood (LabCaire, Clevedon, UK)
- A CO_2 incubator (Heraeus Heracell 150i from Thermo Fisher Scientific, Abingdon, UK)
- Hemocytometer for cell counting (Sigma-Aldrich, catalog no. Z359629, Gillingham, UK)
- Trypan blue to assess viability of obtained cells (Sigma-Aldrich, catalog no. T8154)
- Gilson Micropipettors (10, 100, 1,000 μL volumes)
- Standard laboratory sterile plasticware: 35-mm sterile cell culture dishes, sterile plastic Pasteur pipettes, sterile pipette tips, sterile plastic Falcon centrifuge tubes (10 mL and 50 mL volume), 2-μm microfilters, and 10-mL sterile plastic syringes for solution sterilization
- Dissection instruments: forceps, tweezers, microdissecting scissors (e.g., Castroviejo microdissecting spring scissors), and single-edge razor blades.
- Trypsin (Worthington catalog no. LS003707, TRL3, 260 U/mgP)
- Sterile Dulbecco's phosphate-buffered saline (DPBS; Sigma-Aldrich, catalog no. P4593) without calcium and magnesium
- Sterile fetal calf serum (Sigma-Aldrich catalog no. F7524)
- Sterile M199 cell culture solution (Sigma-Aldrich, catalog no. M453)
- Antibiotic/antimycotic (Sigma-Aldrich, catalog no. A5955)
- Cardioactive drugs targeting β-adrenoceptors (Tocris Bioscience, Abingdon, UK). To obtain the maximal positive chronotropic changes, isoproterenol (ISO) is used. To show that there are negative chronotropic changes mediated by cardiac β_3-adrenoceptors, BRL-37344 is used.

Human and Animal Subjects

Legislation relevant to experimentation with chick embryos is covered by the Animals (Scientific Procedures) Act, 1986 in the United Kingdom. At the age used in the experiments described here, the chick embryos are not covered by this Act. The Act only applies to vertebrate embryos two-thirds into their gestation or incubation period. Similarly, in the United States the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, the US Animal Welfare Act, and Animal Welfare Act Regulations (AWRs) all have specific requirements regarding proposed animal experimentation. These regulations will be brought to bear locally via an institution's Institutional Animal Care and Use Committee (IACUC). In the United States context, embryos younger than embryonic day 15 are deemed to experience no pain and therefore no protocol is needed to be submitted to the IACUC.

Instructions

Under aseptic conditions, chick embryonic ventricular cells can be isolated and cultured according to the methods previously described (8, 9) with slight modifications.

1. 12–18 fertilized Dekalb White chick eggs (53–63 g) are placed in an automatic egg incubator (Maino MPS 24 and Brinsea Octagon 10) for 7 days at 37.8°C and 87% humidity.
2. After this incubation period, chick hearts are isolated under sterile conditions from the 7-day-old embryos.
3. Using a standard laboratory dissecting microscope, remove the hearts from each of the embryos. In practice, at this stage of development the chick embryo only has two clearly identifiable organs that could possibly be dissected from the trunk of the body. These are the heart and the liver. The heart is easily located as it lies underneath one of the forelimbs of the embryo. Identifying and isolating the heart in this way, it is a relatively easy task to pinch and pull the heart away from the main body with a pair of forceps. Figure 1 shows the extraction of the embryo from the egg and the morphology of an isolated heart.
4. Collect all the dissected hearts in the amniotic fluid that surrounds each embryo and add each heart to amniotic fluid in a separate petri dish for storage while dissecting more embryos.
5. Separate the ventricles from the atria and associated vasculature. Figure 2 shows the clear difference in appearance of atrial and ventricular chambers under a standard laboratory dissecting microscope. Clamp the heart at the atrial chambers with a pair of curved forceps and cut the ventricles away from the atria and associated blood vessels with curved Castroviejo microdissecting scissors. The ventricles obtained are then minced with single-edged razor blades in DPBS solution.
6. Enzymatically digest the ventricular chunks by shaking them manually for 7 min in a sterile 10-mL Falcon tube in a 37°C water bath containing 0.005% trypsin solution.
7. The supernatant obtained from this ventricular tissue digest (containing individual cardiac myocytes) is then pipetted out into a growth medium solution made up of 90% M199 solution, 10% FBS, and 0.1% antibiotic/antimycotic solution of penicillin, streptomycin, and amphotericin B.
8. The remaining ventricular tissue fragments are manually shaken for another 7-min period at 37°C in a fresh enzyme solution. This process of 7-min enzyme dissociations and cell supernatant removal into growth medium is continued until all the ventricular chunks have been digested. Typically this involves two or three separate digestions.
9. Cells obtained by these enzymatic digestions are then spun at 216 *g* for 5 min in a standard laboratory centrifuge. The purpose of this centrifugation is to remove the traces of enzyme-containing solution left with the cells.
10. The supernatant from the centrifuged tubes is discarded and the cell pellets collected and pooled in fresh growth medium solution.
11. A small aliquot of homogeneous cell solution is then mixed with trypan blue in a 1-to-1 ratio and cells counted with a hemocytometer under a light microscope so that cell concentration (cells/mL) and total number of cells obtained is determined.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

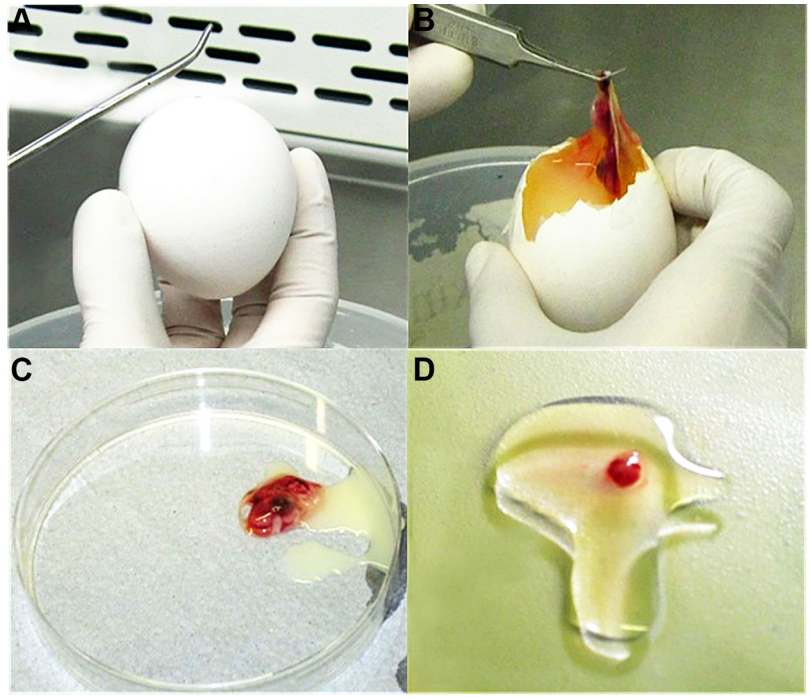


Figure 1. Extracting 7-day-old chick embryos from fertile chick eggs. *A*: eggs were cracked open on the blunt side of the egg to easily locate the embryo. *B*: the embryo was gently removed from the egg. *C*: the embryo was gently placed onto a sterile petri dish. *D*: with a light dissecting microscope, the ventricular tissue was separated from the atria and placed in Dulbecco's phosphate-buffered saline (DPBS) solution to be minced into smaller chunks.

12. Cells can then be plated to the desired concentration (usually ~1 million cells/mL) in 35-mm sterile culture dishes and are maintained in a sterile incubator (37.5°C and 95% O_2-5% CO_2) for 48–96 h to allow for cell proliferation and initiation of spontaneous contractile activity before drug exposure.
13. The plating concentration of the cells of ~1 million cells/mL will result in discrete clusters of electrically connected cells separated from other cell clusters. This means that each petri dish may yield a number of experimental observations. Plating at a higher density will result in a monolayer of electrically connected cells being formed, meaning that each dish will yield only one dataset.
14. After 48 h of incubation, the cultured chick embryonic ventricular cells can be aseptically replenished with fresh prewarmed growth medium. After any such changes of medium, the cells are left for at least 20 min in the CO_2 incubator to reequilibrate and adjust to their new environmental conditions.
15. Culture dishes containing the cultured ventricular cells are then placed on a heated microscope stage of an inverted microscope at 37°C and the spontaneous basal beating rates of the chick cardiomyocytes counted by visual observation of the cells contracting down the eyepiece of the microscope.
16. Upon consistent beating of the cultured cells, various cardioactive agents are aseptically introduced to the single wells and any changes in spontaneous beating rate recorded. In the experiments described here ISO was used to obtain dose-dependent positive chronotropic changes (10–40 nmol/L) and BRL-37344 (100–400 nmol/L) was used to dose-dependently obtain negative chronotropic changes.

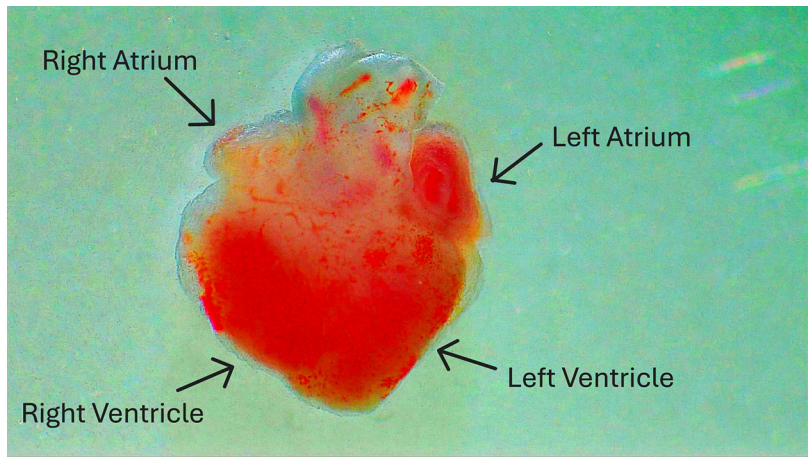


Figure 2. Chick embryo heart under the dissecting microscope. Forceps are used to clamp the tissue at the boundary between the atria and ventricles and the ventricles cut free from the rest of the tissue with curved microdissecting scissors.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

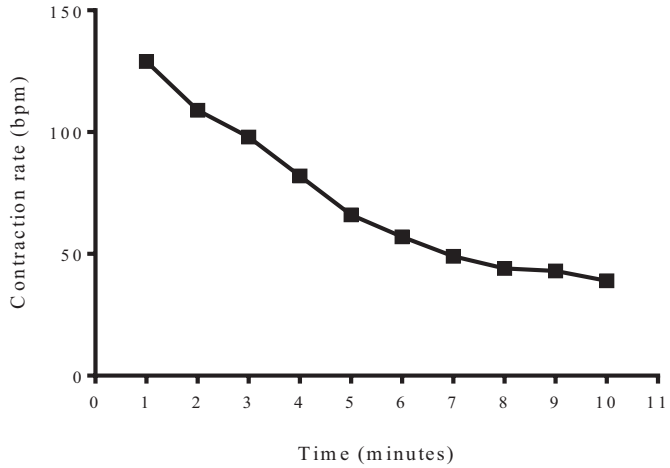


Figure 3. The effect of room temperature on the mean contraction rate of chick embryonic ventricular cardiomyocytes in 1 particular set of experiments. Data are presented as means ($n = 5$). Spontaneous cardiac myocyte contraction decreased with time spent on the microscope at room temperature, but the contraction rate was more stable and consistent at 37°C on the heating mantle of the microscope. bpm, Beats per minute.

17. Any of a multitude of drugs or other physiological intervention (e.g., temperature, ion concentration, pH) can be applied to these cells. Equally noteworthy is the fact that any drugs applied can be washed out by removing the drug-containing medium and replacing with fresh medium and the cells returned to their basal beating rate. Consequently, within the 2–4 day time limit for the viability of these cells, the number of experiments that can be performed is only limited to the number of hours academic staff and laboratory time are available.

RESULTS AND DISCUSSION

Troubleshooting

Maintaining aseptic conditions and sterility in the cell preparations is not usually a problem, but once infection appears in any of the cell preparations all cells should be disposed of and thorough cleaning protocols employed to get rid of the source of infection. Most CO_2 incubators have automatic self-sterilization programs that can be used for this.

Calculation of final concentrations of drugs is often a source of error in the student work. This should be countered by an introductory exercise involving different calculations of drug volume and concentration (essentially a focus on $C_1V_1 = C_2V_2$) and the mole concept.

Counting rapidly beating colonies of cells under the microscope may also present a logistical challenge to students. This can be countered by using student pairs to validate each set of observations.

Safety Considerations

Although the usual COSHH regulations pertaining in the United Kingdom apply to these experiments, perhaps of more concern is the need to maintain sterility. Students should then be extensively instructed in aseptic techniques to minimize bacterial contamination of their cell cultures. If sufficient care is taken, this usually is not a concern when running these experiments. Dissecting equipment is a potential risk of injury to students undertaking these experiments, with razor blades and microdissecting scissors being used. Safe use of these implements is initially demonstrated by the academic member of staff before student usage. To further minimize any risks, single-edged razor blades are used in the confines of the sterile environment afforded by the laminar flow hood and laboratory gloves worn during the procedure both for sterility reasons and to provide some physical protection against potential cuts and puncture wounds. In practice, over many years with students of varying experience and digital competence, the microdissecting scissors and razor blades have never caused an injury.

Expected Results

After the 48- to 96-h incubation, the chick ventricular cardiomyocytes in culture formed colonies having spontaneous rhythmic contractions. The cardiac cell monolayers of 7-day-old chick embryo had an average constant beating rate of 90.30 ± 0.62 beats/min ($n = 253$) for data aggregated over a number of different student projects. Experiments showed that upon 10-min exposures to room temperature (23°C), the spontaneous contraction rate of the chick cell decreased from the first minute to the last minute (129 beats/min to 39 beats/min) (Fig. 3). However, upon the use of a heating mantle set to 37°C on the stage of the inverted microscope, the spontaneous contraction rate was more consistent during this time. Figure 4 shows that simply replacing the medium that has bathed the cells during their incubation period with fresh medium results in pronounced changes to the spontaneous beating rate of the cardiac cells.

The results shown above give an indication of the viability of each cell preparation. The responses to ISO (Fig. 5) and BRL-37344 (Fig. 6) are especially pertinent as they show that drug addition can result in both positive and negative chronotropic changes (4) and that results obtained are not an artifact of the experimental setup.

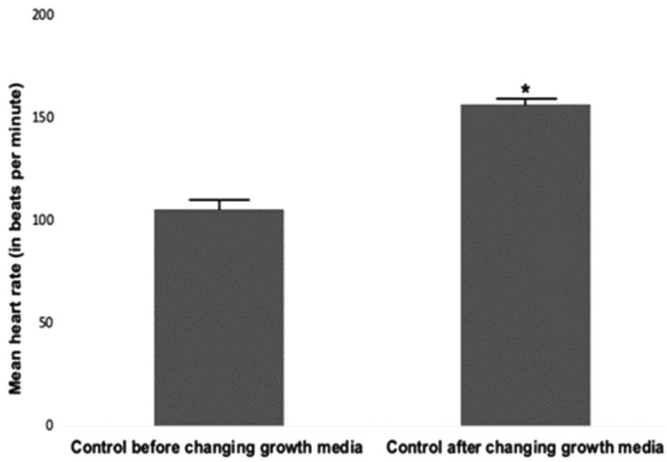


Figure 4. The typical effect that changing growth medium had on heart rate (HR) of control chick embryonic ventricular cardiomyocytes. This represents data obtained from 1 particular set of experiments. Data presented as means ± SE of $n = 128$ beating groups of cardiac myocytes for each condition. *$P < 0.05$.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

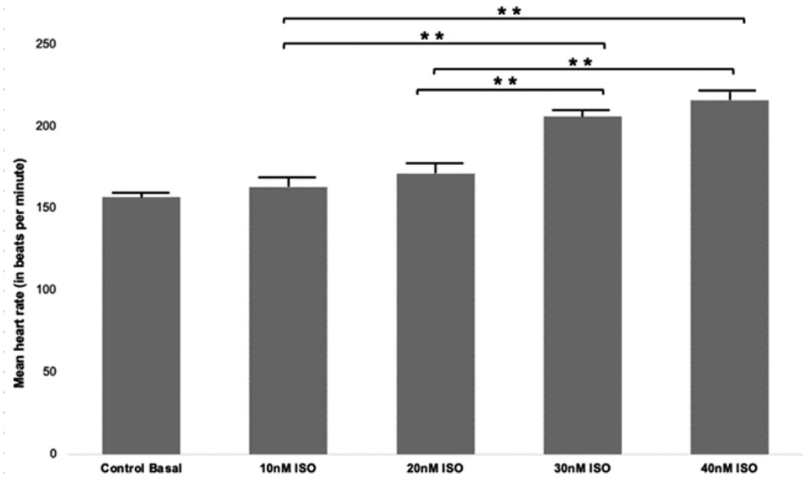


Figure 5. The typical positive chronotropic effect of isoproterenol (ISO) on chick embryonic ventricular cardiomyocyte heart rate (HR). Data presented as means ± SE of $n = 20$–128 beating groups of cardiomyocytes for each condition. ANOVA 1-way; $^{**}P < 0.01$, Tukey's post hoc test.

Misconceptions

Students see, when completing these experiments, that the cardiac cells do not need an external source of electrical stimulation to contract, and consequently students realize that the heart is myogenic, a characteristic of the vertebrate heart. Conceptually too, the fact that a particular β-adrenoceptor agonist can cause negative effects on the beating rate of the heart is also something students might not immediately appreciate.

Evaluation of Student Work

Student work in the method described here usually takes the form of dissertation/thesis or report write-ups that form the capstone project often undertaken by students at the end of their undergraduate degree programs. What is especially noteworthy about this technique is that it has been successfully used to induct a wide range of students, at different levels of study, into the techniques of primary cell culture and cardiovascular physiology, pharmacology, and biochemistry. Thus, the technique can be adapted to suit high school summer students, undergraduates, or postgraduate Master's project students. Here, results from students at a variety of such levels are presented. Pertinently, the level of evaluation of the results can be adjusted to the level of the student. At all levels the student work should contain graphical representation of the results in the form of bar charts or log dose-response plots. At Master's level, appropriate quantitative statistical analysis should be performed and relevant interpretation undertaken. At doctoral level, this model has been used to undertake preliminary investigations on the existence of a novel cardiac receptor now known as the low-affinity β_1-adrenoceptor, which is resistant to blockade by propranolol and stimulated by nonconventional partial agonists based on the β-blocker cyanopindolol (10).

Inquiry applications.

Given the flexible nature of the potential experiments that can be performed with this technique, students can, with guidance, decide for themselves what kind of experiments to perform. Therefore, this kind of project spans the facilitated to open inquiry types. The educator in the first instance teaches the students the basic techniques of obtaining cultures of spontaneously beating ventricular myocytes and the simple chronotropic changes that can be undertaken under a variety of conditions. This can involve, at the simplest level, monitoring changes in spontaneous beating rate after a change of physiological conditions or upon the addition of drugs of different kinds. Students at a more advanced level might then look into the biochemistry or molecular biology of the intracellular signaling systems and the receptor function involved in mediating these physiological and pharmacological changes.

Experience over a number of years shows that the educator has to demonstrate the technique three or four times and

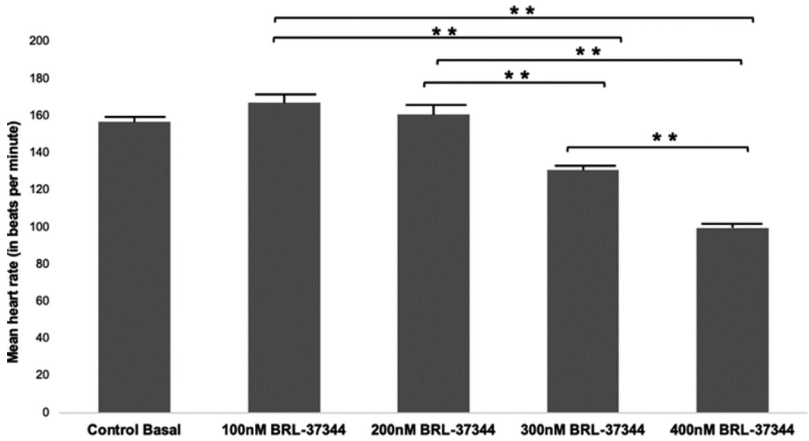


Figure 6. Negative chronotropic effect of BRL-37344 in chick embryonic ventricular cardiomyocytes. BRL-37344 is added in increasing concentrations to exert an increasing negative chronotropic effect on the heart rate (HR). Data presented as means ± SE of $n = 10$–128 beating groups of cardiomyocytes for each condition. ANOVA 1-way test; $^{**}P < 0.01$, Tukey's post hoc test.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.

then closely monitor the students performing the procedures themselves. After two or three solo attempts, students invariably reliably obtain sterile cultures of spontaneously beating cells. Once this has been achieved, students can be left to design and carry out their own experiments.

Data analysis is usually undertaken by the students with input from the educator as to what techniques are most appropriate for the specific datasets gathered.

Thus, students learn desirable techniques of experimental design, statistical analysis, and plotting of graphical data in the course of their usual projects.

Wider educational applications.

The fundamentals of the pharmacological action of drugs can be explored via this technique as a problem-based learning exercise. Students could be provided with a battery of cardioactive drugs and be asked to prove which ones were full agonists, which partial agonists, competitive reversible antagonists, and inverse agonists, etc. Further digital and manipulative skill is required to isolate atrial cells, and these have been reported to respond differently to a variety of cardioactive agents compared to ventricular cells (11). Additionally, immature cells can be made to become hypertrophic via short-term incubation with agents such as phenylephrine. This manipulation opens up a whole battery of new experimental possibilities.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The authors thank all students who have mastered these techniques and obtained valid and reliable results that have contributed to the generation of new knowledge.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.S.F. conceived and designed research; N.S.F. performed experiments; N.S.F., L.F.C., J.L.R., and A.S. analyzed data; N.S.F., L.F.C., J.L.R., and A.S. interpreted results of experiments; N.S.F. prepared figures; N.S.F., L.F.C., J.L.R., and A.S. drafted manuscript; N.S.F., L.F.C., J.L.R., and A.S. edited and revised manuscript; N.S.F., L.F.C., J.L.R., and A.S. approved final version of manuscript.

REFERENCES

1. **Freestone NS**, **Sam CL.** Classical and novel pharmacological insights offered by the simple chick cardiomyocyte cell culture model: a valuable teaching aid and a primer for "real" research. *Adv Physiol Educ* 41: 163–169, 2017. doi:10.1152/advan.00178.2015.
2. **Vicente-Steijn R**, **Passier R**, **Wisse LJ**, **Schalij MJ**, **Poelmann RE**, **Gittenberger-de Groot AC**, **Jongbloed MR.** Funny current channel HCN4 delineates the developing cardiac conduction system in chicken heart. *Heart Rhythm* 8: 1254–1263, 2011. doi:10.1016/j.hrthm.2011.03.043.
3. **Komazaki S**, **Hiruma T.** Development of mechanisms regulating intracellular Ca^{2+} concentration in cardiac muscle cells of early chick embryos. *Dev Biol* 186: 177–184, 1997. doi:10.1006/dbio.1997.8595.
4. **Gauthier C**, **Leblais V**, **Moniotte S**, **Langin D**, **Balligand JL.** The negative inotropic action of catecholamines: role of beta3-adrenoceptors. *Can J Physiol Pharmacol* 78: 681–690, 2000. doi:10.1139/y00-045.
5. **Vostarek F**, **Svatunkova J**, **Sedmera D.** Acute temperature effects on function of the chick embryonic heart. *Acta Physiol (Oxf)* 217: 276–286, 2016. doi:10.1111/apha.12691.
6. **Lipshultz S**, **Shanfeld J**, **Chacko S.** Emergence of beta-adrenergic sensitivity in the developing chicken heart. *Proc Natl Acad Sci USA* 78: 288–292, 1981. doi:10.1073/pnas.78.1.288.
7. **Kollmansperger S**, **Anders M**, **Werner J**, **Saller AM**, **Weiss L**, **Süß SC**, **Reiser J**, **Schneider G**, **Schusser B**, **Baumgartner C**, **Fenzl T.** Nociception in chicken embryos, Part II: embryonal development of electroencephalic neuronal activity in ovo as a prerequisite for nociception. *Animals (Basel)* 13: 2839, 2023. doi:10.3390/ani13182839.
8. **DeHann RL.** Regulation of spontaneous activity and growth of embryonic chick heart cells in tissue culture. *Dev Biol* 16: 216–249, 1967. doi:10.1016/0012-1606(67)90025-5.
9. **Rabkin SW**, **Freestone N**, **Quamme GA.** Nifedipine does not alter the increased cystolic free magnesium during inhibition of mitochondrial function in isolated cardiac myocytes. *Gen Pharmacol* 25: 1483–1491, 1994. doi:10.1016/0306-3623(94)90178-3.
10. **Sarsero D**, **Molenaar P**, **Kaumann AJ**, **Freestone NS.** Putative beta 4-adrenoceptors in rat ventricle mediate increases in contractile force and cell Ca^{2+}: comparison with atrial receptors and relationship to (-)-[^{3}H]-CGP 12177 binding. *Br J Pharmacol* 128: 1445–1460, 1999. doi:10.1038/sj.bjp.0702936.
11. **Freestone NS**, **Ribaric S**, **Scheuermann M**, **Mauser U**, **Paul M**, **Vetter R.** Differential lusitropic responsiveness to β-adrenergic stimulation in rat atrial and ventricular cardiac myocytes. *Pflugers Arch* 441: 78–87, 2000. doi:10.1007/s004240000397.

Downloaded from journals.physiology.org/journal/advances at Univ of Sheffield (143.167.254.174) on October 5, 2026.