Using stimulation of the diving reflex in humans to teach integrative physiology

Julia K. Choate, Kate M. Denton, Roger G. Evans, and Yvonne Hodgson

Department of Physiology, School of Biomedical Sciences, Monash University, Melbourne, Victoria, Australia

Submitted 4 November 2013; accepted in final form 5 September 2014

Choate JK, Denton KM, Evans RG, Hodgson Y. Using stimulation of the diving reflex in humans to teach integrative physiology. Adv Physiol Educ 38: 355–365, 2014; doi:10.1152/advan.00125.2013.—During underwater submersion, the body responds by conserving O2 and prioritizing blood flow to the brain and heart. These physiological adjustments, which involve the nervous, cardiovascular, and respiratory systems, are known as the diving response and provide an ideal example of integrative physiology. The diving reflex can be stimulated in the practical laboratory setting using breath holding and facial immersion in water. Our undergraduate physiology students complete a laboratory class in which they investigate the effects of stimulating the diving reflex on cardiovascular variables, which are recorded and calculated with a Finapres finger cuff. These variables include heart rate, cardiac output, stroke volume, total peripheral resistance, and arterial pressures (mean, diastolic, and systolic). Components of the diving reflex are stimulated by 1) facial immersion in cold water (15°C), 2) breathing with a snorkel in cold water (15°C), 3) facial immersion in warm water (30°C), and 4) breath holding in air. Statistical analysis of the data generated for each of these four maneuvers allows the students to consider the factors that contribute to the diving response, such as the temperature of the water and the location of the sensory receptors that initiate the response. In addition to providing specific details about the equipment, protocols, and learning outcomes, this report describes how we assess this practical exercise and summarizes some common student misunderstandings of the essential physiological concepts underlying the diving response.

diving; bradycardia; heart rate; total peripheral resistance; reflex control of arterial pressure

This report describes how undergraduate physiology students can record, measure, and analyze changes in cardiovascular variables during stimulation of the diving reflex in a 3-h practical laboratory class. It describes the equipment and methodology used, summarizes the physiological concepts that could be taught before and after this practical class, and provides learning objectives, a sample assessment question, a class data set, and suggestions for using the practical as an inquiry-based laboratory class.

Background

The diving response, also referred to as the diving reflex, was initially described for the porpoise in 1941 (22). In mammals, it is a sequence of physiological adjustments that involve the nervous, cardiovascular, and respiratory systems acting in a manner that promotes O2 conservation [through decreased heart rate (HR) and cardiac output (CO)] and the diversion of blood flow to vital organs such as the brain and heart during underwater submersion (7, 17). In an undergraduate physiology laboratory class, the diving reflex can be stimulated experimentally by engaging students in breath holding exercises, in combination with facial immersion in water (20). Variables such as water temperature and the area of facial stimulation (e.g., intranasal, forehead, and/or cheek) can be altered to investigate the different triggers of the diving reflex and to observe specific cardiovascular adjustments (8, 35). The physiology practical exercise reported in this article uses the Finapres finger cuff to record cardiovascular variables (14, 21) and is based on the “simulated human diving” teaching laboratory exercise previously described by Hiebert and Burch (20), in which only the effects on HR were examined. The Finapres finger cuff, together with Beatscope software, allows continuous and simultaneous recording and calculation of multiple cardiovascular variables. These include HR, arterial blood pressure (BP) [diastolic BP (DBP), systolic BP (SBP), and mean arterial pressure (MAP)], CO, stroke volume (SV), and total peripheral resistance (TPR; 14, 21). The unique aspect of the diving reflex is that it involves the simultaneous effects of both sympathetic and parasympathetic nerve activation on the cardiovascular system to alter HR, CO, arterial pressure, and TPR (17, 28). This profile of responses contrasts, for example, with the arterial baroreceptor reflex, which involves reciprocal changes in sympathetic and parasympathetic activity. By comparing these reflexes, students can learn that the autonomic responses to physiological challenges can be patterned in different ways.

Learning Objectives

After completing this practical laboratory class and writing the associated scientific report, students should be able to:

1. Collect, analyze, and plot the cardiovascular data resulting from the maneuvers in which components of the diving reflex are stimulated.

2. Discuss the cardiovascular responses (for CO, HR, TPR, MAP, DBP, SBP, and SV) observed when components of the diving reflex are stimulated by facial immersion in cold water, breathing with a snorkel in cold water, facial immersion in warm water, or breath holding in air (apnea).

3. Describe the neuronal pathways that mediate the cardiovascular responses observed when the diving reflex is stimulated and explain the physiological mechanisms underlying these.

4. Communicate the background, results obtained (including statistical analysis), and conclusions drawn from the practical exercise in the form of a scientific paper.

Activity Level

This activity is suitable for undergraduate science, biomedical science, or medical and allied health students, but it could...
also be used in a simplified form (in which only HR and/or BP are measured) for high school students of biology. We run this practical class for a third-year physiology science class (Clinical and Experimental Cardiovascular Physiology course) of ~300 students (in groups of ~40 students) of 19–28 yr old.

Prerequisite Student Knowledge or Skills

Prerequisite student knowledge. Before doing this activity, students should have a basic understanding of the human cardiovascular, respiratory, and autonomic systems. The specific physiological concepts required are listed below:

- CO is determined by the rate at which the heart contracts (HR) and the volume of blood ejected with each cardiac contraction (SV).
- MAP of the systemic circulation is altered by changes in CO and TPR.
- The peripheral nervous system consists of cranial, spinal, somatic motor, and autonomic nerves.
- Short-term control of cardiac function (SV and HR) and TPR, and thus MAP, is in part achieved by the central nervous system processing incoming (afferent) neuronal information from a range of sensory receptors. These sensory receptors include arterial baroreceptors, chemoreceptors, and atrial and pulmonary volume receptors. Changes in the activity of afferent nerves alters the outgoing (efferent, autonomic) neuronal activation from the cardiovascular centers in the medulla oblongata and thus the activity of postganglionic autonomic nerves that innervate effector organs (the heart) and tissues (blood vessels).
- Increased activation of parasympathetic nerves (in the vagus) and decreased activation of sympathetic nerves innervating the sinoatrial node slows HR by decreasing the rate of the spontaneous generation of cardiac pacemaker action potentials.
- Differential activation of sympathetic nerves innervating arterioles leads to regional enhancement of the contraction of vascular smooth muscle (i.e., arteriolar constriction).
- Breathing requires the activation of the diaphragm, intercostal, and accessory thoracic skeletal muscles via somatic motor neurons projecting from the respiratory center in the medulla oblongata of the brain stem.
- Apnea is the cessation of breathing, which can be voluntarily or reflexly induced.
- Arterial chemoreceptors respond to changes in \( P_{O_2} \) (\( P_{O_2} \)) and \( P_{CO_2} \) (\( P_{CO_2} \)). Central chemoreceptors are activated by acidification of blood (pH), which occurs when \( P_{CO_2} \) increases. Apart from effects on respiratory drive, activation of arterial and central chemoreceptors also alters cardiovascular function through activation of the autonomic nervous system.

Prerequisite student skills. Students should know how to do the following:

- Use the recording software (start and stop recording, add comments to the recording trace, save the file, and extract and enter the data into an Excel spreadsheet).
- Calculate the mean and SD and plot data using the appropriate software [e.g., Excel and Graphpad Prism (18)].
- Calculate (for each variable) the percent change from the baseline values in response to each test condition.

- Perform statistical analysis of these data. [For instructors: this component could be simplified if the statistical analysis was performed and provided to the students, but this is something that students can easily complete if they are provided with the appropriate instructions].
- Interpret the results of the statistical analysis of class data (i.e., what does \( P = 0.01 \) mean?).
- Source and cite research articles.

Time Required

The entire experimental protocol, which is composed of four maneuvers in which components of the diving reflex are stimulated, can be completed during a 3-h practical class.

METHODS

Each of our practical classes have ~40 students, and the practical is run 8 times (we had 317 students in 2013). Students work in 10 groups of 4 students/group, where each group member is assigned one of the following team roles: 1) a voluntary experimental subject who completes the four experimental maneuvers; 2) a computer operator who obtains the recordings of the data; 3) a data manager who extracts and calculates the values of the specific cardiovascular variables from the record of the data; or 4) a team manager who manages the experimental protocol, including acting as a time keeper, and coordinates the other group members.

Equipment and Supplies

The photograph in Fig. 1 shows how some of the equipment for this practical class is organized on the laboratory bench. Each student group is provided with the following equipment and supplies:

- A Finapres finger cuff recording system, Finometer MIDI, with Beatscope software (14, 21)
- A 10-liter plastic basin containing ~7.5 liters of tap water at room temperature
- A thermometer
- A timer (in min:s)
- A plastic nose clip
- A snorkel (no mask) or a plastic breathing tube (vinyl, internal diameter: 10 mm) (a snorkel is preferable to a plastic breathing tube)

Fig. 1. An experimental subject immersing his face in 7.5 liters of water (15°C) placed in a 10-liter plastic basin. The subject breathes through a plastic tube (held out of the water with their left hand). The Finapres finger cuff is placed on the index finger of the right hand. A time keeper taps the subject on the shoulder every 10 s and states the time out loud. A cloth towel is positioned nearby for drying the face once the experimental maneuver is complete.
Ice and warm tap water

Publications/Ethical-Policies/Animal-and-Human-Research. At Monash University, Human Research Ethics approval is not required for undergraduate teaching involving the participation of students. How- 

Step 1. Allocate the team roles for each member of your group (i.e., voluntary experimental subject, computer operator, data manager, and team manager). (For instructors: in our classes, we do not specify the sex of the subject, principally so that each group of 4 students can select the person most capable of performing the protocol. However, 

The use of human subjects for these practical laboratory experiments may require ethical approval from your institution’s human research committee. Adopters of this activity are responsible for obtaining permission for human or animal research from their home institution. For a summary of Guiding Principles for Research Involving Animals and Human Beings, please see www.the-aps.org/mm/ publications/Ethical-Policies/Animal-and-Human-Research. At Monash University, Human Research Ethics approval is not required for undergraduate teaching involving the participation of students. However, students are told that their participation as experimental subjects in the diving reflex protocol is voluntary and that they can withdraw at any time.

Instructions

Step 4. Place ~7.5 liters of tap water into the plastic basin. Adjust the temperature of this water to 15°C by adding warm tap water or ice to the water. Please note that the room temperature during the experimental protocol is maintained at 21 ± 1°C.

Step 5. Fit the Finapres finger cuff onto the index finger of the dominant hand of the experimental subject. Calibrate the finger cuff and fix the height indicator into place on the subject’s chest at the level of the heart. Enter the demographic information about the experimental subject into the computer (name, age, weight, and sex), using Beatscope software. This software is set up to capture recordings and calculations of the following cardiovascular variables: HR, CO, MAP, DBP, and SBP. [For instructors: in our practical class, students calculate SV and TPR (the Finapres system can do this, but performing these calculations will help students understand the relationships among MAP, CO, HR, SV, and TPR). A description of the Finapres technology can be found in a previous teaching publication (21).] Assign an appropriate name to this file and continuously record cardiovascular variables for the experimental protocols. You will be adding comments to this recording as you complete the protocols.

Step 6. With the subject in the relaxed position (elbows on bench with the basin in front of them), record cardiovascular variables for at least 5 min. Label this as “test run” on the computer recording. The experimental protocol in which components of the diving reflex are stimulated can now be performed (see step 2 and Fig. 2). Before commencing the four maneuvers, complete a test run for facial immersion in water so that the subject can become familiar with the experience. This is an important step as it will reduce the effects of stress on the cardiovascular recordings when the actual experiment is carried out. The subject puts their face in the water for ~5 s, with the water covering the forehead, eyes, and nose (see Fig. 1). Before facial immersion in water, the subject should take a moderate inspiration, exhalation against closed airways, leading to increased thoracic pressure and reduced venous return (37)]. After this trial facial immersion, the
subject rests for 5 min to allow values to return to a steady baseline before proceeding with maneuver 1.

Step 7. To commence maneuver 1, record cardiovascular variables for at least 5 min to obtain baseline values. The experimental subject then places their face into the cold water (15°C) for 30 s. Label the computer recording as “stimulated diving reflex maneuver 1 - cold water.” This represents the control maneuver for the experiment.

Step 8. A time keeper taps the subject on the shoulder every 10 s and states the time out loud, allowing the subject to keep track of the time and to remain calm (see Fig. 1).

Step 9. The subject removes their face from the water. Use a cloth or paper towel to dry the subject’s face. Label the computer recording as “recovery” and continue recording for 5 min or until the cardiovascular variables stabilize.

Step 10. Repeat steps 7–9 for maneuver 2 (breathing with a snorkel in cold water), maneuver 3 (facial immersion in warm water), and maneuver 4 (breath holding in air). For maneuver 3, the water temperature in the basin should be increased to 30°C by the addition of warm water; for maneuver 4, the subject holds their breath for 30 s (with their head out of water but still leaning over the basin). Again, for maneuver 4, a Valsalva maneuver should not be performed. The subject rests for at least 5 min between each of the maneuvers in which they stimulate components of the diving reflex (see Fig. 2).

Step 11. Once the four experimental maneuvers are completed, stop the recording and save the file. The data manager in the team can now extract values for each of the cardiovascular variables (HR, CO, MAP, DBP, and SBP) at 1, 3, and 5 min during the relevant baseline period and at 0, 15 and 30 s after commencing each of the four maneuvers in the protocol. The experimental team should work together to calculate SV and TPR and enter all of the cardiovascular variables into a class spreadsheet (using Excel) in preparation for plotting the data and performing statistical analyses. A sample data collection sheet for maneuver 1 is shown in Table 1. [For instructors: the class data set should be scrutinized at this stage to look for data calculation or entry errors. In addition, occasionally subjects show a marked stress response (increased HR) for each condition except the breath holding condition (i.e., maneuver 1).]

Steps 12. Using the class data, average the first two time points during the baseline period (1 and 3 min) and the time points 15 and 30 s after commencing the experimental maneuver for each experimental subject and for the four maneuvers in the protocol. We do not use the values at the 5-min mark of the baseline period or the values immediately after commencing the experimental maneuver (0 s) because we find that most students display an anticipatory response, which can dilute the effect. The rationale for this is explained to the students. Determine the mean and SD for each cardiovascular variable for the class data [For instructors: it is best to use SD because it provides a measure of dispersion that, unlike SE, is not dependent of the number of observations on which it is based.]

Step 13. We use Graphpad Prism for statistical analysis. There are two steps to the statistical analysis (steps a and b).

STEP A. Perform repeated-measures ANOVA with factors of treatment (the four experimental maneuvers) and time (before and during the stimulations of the diving reflex) and their interaction followed by Student’s paired t-tests with a Bonferroni correction to account for multiple comparisons. [For instructors: ask your students to explain the characteristics of this data set that make it appropriate for use of repeated-measures ANOVA (i.e., all four maneuvers were performed on the same experimental subject) and the critical differences between repeated-measures ANOVA and conventional ANOVA (i.e., the potential inclusion of both between-subjects and within-subjects factors and the potential for use of corrections to protect against the increased risk of type I error arising from compound asymmetry). Conventionally, post hoc tests are only performed if the results of preliminary ANOVA shows some level of statistical significance (conventionally, $P \leq 0.05$). Students should be made aware of these scientific conventions, the reasoning that underlies them, and what a P value actually represents].

Post hoc analysis allows comparison of the baseline values for each protocol, to determine if resting values have changed across time (acclimatization). [For instructors: this reinforces the importance of well-controlled experiments and introduces the concept of acclimatization to experimental conditions across a study. This information may help the students to explain why expected reductions in the magnitude of the responses might not be observed].

Post hoc analysis also determines which variables significantly changed from baseline in response to each protocol, to establish if the maneuvers used to stimulate the diving reflex had effects (i.e., facial immersion in cold water, breathing with a snorkel in cold water, facial immersion in warm water, and breath holding in air).

STEP B. Perform repeated-measures one-way ANOVA, using the data expressed as percent changes from baseline, to determine how altering the test conditions (i.e., maneuvers 2–4) changes the response compared with facial immersion in cold water (i.e., maneuver 1, the control). [For instructors: encourage students to consider the variability in the data and why expressing data as percent changes reduces this variability].

If the results of ANOVA indicate significant differences between mean values, use Dunnett’s post hoc test to determine whether the magnitudes of the effects of each of the partial stimuli (maneuvers 2–4) differed from those to facial immersion in cold water (maneuver 1, the control). [For instructors: you may choose to advise students to reject the null hypothesis, i.e., that the magnitudes of the responses to each of the partial stimuli (maneuvers 2–4) did not differ from that to facial immersion in cold water (maneuver 1) if $P$ values from the Dunnett’s test are $\leq 0.05$ (or some other critical value you might choose). Alternatively, you

---

### Table 1. A sample data collection sheet showing the formatting and data for a single experimental subject that has undergone facial immersion in cold water (i.e., maneuver 1)

<table>
<thead>
<tr>
<th></th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>SV, ml</th>
<th>CO, l/min</th>
<th>TPR mmHg·l·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>129</td>
<td>69</td>
<td>88</td>
<td>80</td>
<td>84</td>
<td>6.6</td>
<td>14</td>
</tr>
<tr>
<td>3 min</td>
<td>132</td>
<td>71</td>
<td>88</td>
<td>73</td>
<td>83</td>
<td>6.1</td>
<td>14</td>
</tr>
<tr>
<td>5 min</td>
<td>132</td>
<td>73</td>
<td>89</td>
<td>86</td>
<td>83</td>
<td>7.0</td>
<td>14</td>
</tr>
<tr>
<td>Maneuver 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 s</td>
<td>146</td>
<td>79</td>
<td>99</td>
<td>95</td>
<td>80</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>15 s</td>
<td>168</td>
<td>91</td>
<td>110</td>
<td>66</td>
<td>81</td>
<td>5.3</td>
<td>22</td>
</tr>
<tr>
<td>30 s</td>
<td>168</td>
<td>92</td>
<td>114</td>
<td>68</td>
<td>84</td>
<td>5.7</td>
<td>23</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance.
could use this as an opportunity to discuss the meaning of DBP, and systolic blood pressure (SBP) [ arterial pressure [mean arterial pressure (MAP), diastolic blood pressure (DBP), and systolic blood pressure (SBP)]].

Fig. 3. A Finapres recording showing the effects of facial immersion in cold water (15°C) on cardiac output (CO), heart rate [HR; in beats/min (bpm)] and arterial pressure [mean arterial pressure (MAP), diastolic blood pressure (DBP), and systolic blood pressure (SBP)].

![Cardiac Output and Arterial Pressure](image)

Time (hours:minutes:seconds)

Safety Considerations

Appropriate Occupational Health and Safety procedures are followed in the laboratory. Students who have cardiovascular disease, a previous history of fainting, are pregnant, have any kind of infection, or are taking any medication that could alter cardiovascular responses should be excluded as experimental subjects.

Care must be taken to prevent the spread of infection. Between practical classes (and thus experimental subjects), the snorkel and plastic basin are cleaned with Sonidet (a bacteriostatic cleaning fluid designed for the medical field) and the liquid antiseptic Dettol. In addition, the benches are wiped down with 70% (vol/vol) ethanol and Dettol, and the inner lining of the Finapres finger cuff is cleaned with alcohol swabs.

RESULTS

Effects of Stimulating the Diving Reflex on Cardiovascular Variables

The raw data trace shown in Fig. 3 shows that facial immersion in cold water (maneuver 1) causes HR and CO to decrease and arterial pressures (MAP, DBP, and SBP) to increase. These changes do not develop immediately but become more pronounced across the 30 s of the maneuver. Also shown in this experimental recording is an initial increase in HR (and thus CO), which is presumed to be an anticipatory response involving “central command activation” (11). Finally, during the recovery phase, the cardiovascular variables return to baseline fairly rapidly.

Table 2. Effects of the stimulation of the diving reflex on SV, SBP, and DBP

<table>
<thead>
<tr>
<th>Stimulated Diving Protocol</th>
<th>Percent Change in SV</th>
<th>Percent Change in SBP</th>
<th>Percent Change in DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial immersion in cold water (maneuver 1)</td>
<td>-6 (19)</td>
<td>27 (14)†††</td>
<td>40 (23)†††</td>
</tr>
<tr>
<td>Breathing with a snorkel in cold water (maneuver 2)</td>
<td>1 (13)</td>
<td>7 (7)**</td>
<td>10 (11)**</td>
</tr>
<tr>
<td>Facial immersion in warm water (maneuver 3)</td>
<td>14 (26)</td>
<td>10 (7)*††</td>
<td>15 (13)*†</td>
</tr>
<tr>
<td>Breath holding in air (maneuver 4)</td>
<td>4 (16)</td>
<td>4 (10)**</td>
<td>7 (16)*</td>
</tr>
</tbody>
</table>

Data are expressed as means (SD) of percent changes from baseline values for each maneuver, *P ≤ 0.05 and **P < 0.01 for maneuvers 2–4 vs. maneuver 1 (control group); †P ≤ 0.05, ††P < 0.01, and †††P < 0.001 compared with baseline values.

Troubleshooting

Some students who have limited swimming or snorkeling experience may find facial immersion in water a difficult task to accomplish. Furthermore, unfamiliarity with the maneuvers can contribute to excessive anticipatory anxiety (i.e., elevated HR). At the beginning of the class, a teacher or practical class technician should demonstrate the four different maneuvers for stimulating the diving reflex to the class. Also, as described above, the subject should complete a test run of the first maneuver; otherwise, the difference between maneuvers 1 and 2 could be due to a training effect.

We always discuss the concepts of sample size and variability with the students. The class data presented in this report represent 10 experimental subjects from a single practical class (see Table 2 and Fig. 4). To generate a data set with sufficient power to detect responses to the various experimental stimuli as well as differences between the responses to maneuvers 2–4 compared with those to maneuver 1, data from 8–10 experimental subjects are required. For example, with a sample size of eight and a 10% SD (α = 0.05), a 15% mean difference in HR can be detected with 80% power. It is also useful to generate a larger data set over time, to demonstrate the expected responses more clearly. Data should be excluded from the analysis if the subject’s HR and CO progressively increased during the stimulated diving reflex and did not return to baseline during the recovery phase. These responses are presumed to result from the effects of stress and therefore do not reflect the diving reflex. In a class with 10 experimental subjects, we find that the data from 1 or 2 subjects can be removed without seriously affecting the statistical outcomes. Over the years, during the running of this class, we have removed an average of 1.3 (SD 0.8) subjects (n = 16 classes; range: 0–2 subjects) from a data set due to these stress effects.
HR, TPR, SBP, DBP, and MAP responses were all less than those to maneuver 1 (all $P \leq 0.05$). The fall in CO was attenuated in response to facial immersion in warm water and breath holding in air (both $P \leq 0.05$). However, the fall in CO was not significantly less in response to breathing with a snorkel in cold water compared with facial immersion in cold water ($P = 0.08$). The change in SV was similar for all maneuvers (Table 2).

Interpretation of Class Data

The averaged experimental data from 10 subjects from a single practical class are shown in Table 2 and Fig. 4. Facial immersion in cold water (maneuver 1) reduced HR and CO and increased TPR, MAP, SBP, and DBP ($P \leq 0.05$ for all variables). Significant changes in cardiovascular variables were observed in response to the other three maneuvers, in which components of the diving reflex were stimulated. Breathing with a snorkel in cold water (maneuver 2) reduced HR and CO and increased TPR, MAP, SBP, and DBP (all $P \leq 0.05$). Facial immersion in warm water (maneuver 3) reduced HR and increased TPR, MAP, SBP, and DBP (all $P \leq 0.05$), but the fall in CO did not reach statistical significance ($P = 0.2$). Breath holding in air (maneuver 4) caused a small, but statistically significant, fall in HR ($P \leq 0.05$) but did not affect any other variable. SV was not significantly affected by any of the maneuvers (Table 2).

Dunnett’s post hoc test was then performed to compare the effects of maneuvers 2–4 (i.e., the partial stimuli) with those of maneuver 1 (the control). The results provide evidence that the responses to maneuvers 2–4 were blunted compared with those to maneuver 1 (facial immersion in cold water). For maneuvers 2–4, HR, TPR, SBP, DBP, and MAP responses were all less than those to maneuver 1 (all $P \leq 0.05$). The fall in CO was attenuated in response to facial immersion in warm water and breath holding in air (both $P \leq 0.05$). However, the fall in CO was not significantly less in response to breathing with a snorkel in cold water compared with facial immersion in cold water ($P = 0.08$). The change in SV was similar for all maneuvers (Table 2).

**Fig. 4.** Effects of facial immersion in cold water (CW; 15°C), breathing with a snorkel in cold water (T), facial immersion in warm water (WW; 30°C), and breath holding in air (A) on HR, CO, MAP, and total peripheral resistance (TPR). Data are expressed as mean percent changes from the corresponding values during the baseline period with SD ($n = 10$ students). $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$ compared with baseline values; $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$ for maneuvers 2–4 vs. maneuver 1 (facial immersion in cold water, control).

**Fig. 5.** A–D: schematic illustrating the sensory inputs and neuronal pathways involved in the cardiovascular and respiratory responses during the four maneuvers that stimulate components of the diving reflex. Solid lines represent neuronal excitation, and dashed lines represent neuronal inhibition. The thickness of the lines is indicative of the relative strength of the activation (i.e., thicker lines represent stronger activation). RVLM, rostral ventrolateral medulla; IMI, intermediolateral column; $P_aO_2$, arterial $O_2$; $P_aCO_2$, arterial $CO_2$. A: during facial immersion in cold water (maneuver 1, shown in gray), due to activation of temperature and touch-sensitive receptors on the face, apnea, and chemoreceptors, large falls in HR and CO are induced via parasympathetic activation, and increases in TPR due to sympathetic activation are observed. B: in response to breathing through a snorkel in cold water (maneuver 2, shown in green), in which the contribution of apnea and subsequent activation of chemoreflexes is removed, the integrated efferent output is reduced, resulting in an attenuation of the cardiovascular responses that make up the dive response. C: facial immersion in warm water (maneuver 3, shown in red), in which input from facial temperature-sensitive receptors is significantly reduced, also attenuates the signal driving the dive response, demonstrating the importance of water temperature in the initiation of the dive response. D: finally, if the only stimulus is apnea and the resultant activation of chemoreflexes, illustrated by breath holding in room air (maneuver 4, shown in blue), the integrated efferent output and cardiovascular responses are modest compared with those to facial immersion in cold water. Thus, on its own, apnea does not elicit a powerful dive response, but it does reinforce the impact of facial immersion in cold water, given the attenuated response to breathing through a snorkel in cold water.
The various sensory inputs that detect and trigger the response include:

- The various sensory inputs that detect and trigger the response
- The afferent pathways conveying information to the central nervous system
- Integration by the central nervous system
- The efferent pathways to the effector organs
- The corresponding responses of the effector organs

**Cardiovascular responses to stimulation of the diving reflex.** The cardiovascular responses induced by activation of the diving reflex include reductions in HR and CO and increases in TPR and MAP (2, 7, 17, 24, 28). All of these cardiovascular effects have been illustrated during facial immersion in cold water in our practical class and in experiments involving human divers (28).

Since there is little evidence of a change in SV during real diving or when the diving reflex is stimulated, the reduction in CO must result from the associated bradycardia (17, 28, 32). Although not observed in the class data set presented, in the initial classes that were run, we often observed a small, but significant, decrease in SV with breath holding in air, which would contribute to the reduction in CO. This small reduction in SV during breath holding was likely due to a Valsalva maneuver by the experimental subjects. That is, this maneuver increases intrathoracic and right atrial pressures, leading to decreased venous return and decreased SV, via the Frank-Starling mechanism (37). We now provide students with specific instructions at the beginning of the class about how to avoid performing a Valsalva maneuver. As shown in the presented class data, this has resulted in the SV remaining unaffected during stimulation of the diving reflex.

Students should come to the practical class with the knowledge that the changes in the cardiovascular variables recorded during each experimental maneuver result from the effects of sympathetic nerve activation on the arterioles and from changes in both sympathetic and parasympathetic nerve activation on the heart. To write their scientific report, they will need to investigate the neuronal pathways that underlie these autonomic effector responses.

**Neuronal pathways that underlie the cardiovascular responses to stimulation of the diving reflex.** Facial temperature and touch-sensitive receptors, chemoreceptors, baroreceptors, pulmonary stretch receptors, and atrial receptors project via afferent nerves to the nucleus tractus solitarius (NTS) in the medulla oblongata (17, 27). Activity in these afferent pathways, together with those arising from central chemoreceptors, can be altered when the diving reflex is stimulated or during real diving. As shown in the schematics in Fig. 5, from the NTS, neurons project within the medulla oblongata to the cardiac parasympathetic center (nucleus ambiguus), the vasomotor center [rostral ventrolateral medulla (RVLM)], and the respiratory center (17, 27). For sympathetic control of the cardiovascular system during the diving reflex, neurons in the NTS are activated. These neurons project via interneurons to the excitatory (glutamatergic) neurons in the RVLM. The RVLM controls sympathetic nerve activation via bulbospinal neurons that project to sympathetic preganglionic neurons via the intermediolateral column of the spinal cord (17, 27).
These experiments demonstrate the importance of the facial receptors and trigeminal afferents alone. that an attenuated response can be driven by facial temperature consequences (hypoxia and hypercapnia) contribute(s) to the). This supports the idea that the apnea and/or its SBP, and TPR, with a trend for a reduction in the fall in CO). We found that this stimulus caused smaller changes in cardiovascular variables (HR, MAP, DBP, SBP, and TPR, with a trend for a reduction in the fall in CO) than facial immersion in cold water with breath holding (maneuver 1). This supports the idea that the apnea and/or its consequences (hypoxia and hypercapnia) contribute(s) to the cardiovascular responses to facial immersion in cold water but that an attenuated response can be driven by facial temperature and touch-sensitive receptors alone.

We found that facial immersion in warm water (maneuver 3) resulted in smaller changes in cardiovascular variables (HR, CO, MAP, DBP, SBP, and TPR) than facial immersion in cold water (maneuver 1). This suggests that water temperature is an important stimulus for driving the dive response (Fig. 5C) and is consistent with experimental evidence showing that there is greater activation of facial temperature-sensitive receptors when they are exposed to water at lower (10–15°C) than higher temperatures (17).

To extract the relative contributions of facial immersion in water and breath holding to the cardiovascular responses during the diving reflex, we compared the responses to breath holding during facial immersion in cold water (maneuver 1) versus breath holding in air (maneuver 4, i.e., without facial immersion). Our results show that breath holding in air only caused a minor fall in HR (~7 beats/min) compared with facial immersion in cold water (~27 beats/min), with no other detectable cardiovascular response (Fig. 4). This observation demonstrates that apnea alone is not a primary driving force for the dive reflex (Fig. 5D) but that apnea does reinforce the response to facial immersion in cold water (Fig. 5A).

In conclusion, these experiments demonstrate that humans, like aquatic animals, exhibit a dive response. It was established that facial immersion in cold water and apnea, together, produced the greatest dive response. Thus, exposure of the face to cold is a major stimulus. Furthermore, apnea on its own does not drive the dive response but is a secondary reinforcing stimulus, at least under the experimental conditions of facial immersion in cold water.

Evaluation of Student Work

Each student group prepares a written practical report in the format of a condensed scientific paper consisting of an Introduction (maximum: 200 words), Results (no limit), and a Discussion (maximum: 1,000 words). [For instructors: if you have a smaller class, it is feasible for students to submit individual reports.] They are provided with detailed instructions on the writing of a scientific report. The instructions cover both the approach they should take and the format of the scientific report. These have been developed over many years by various members of staff of the Faculties of Medicine and Science of Monash University. For the Introduction, students are directed to start broadly and then to finish with specific statements of the experimental aims and hypotheses. They are provided with detailed instructions on the presentation of their scientific results. For the Discussion, they are advised to start with their major findings, and the conclusions drawn from these, in the context of their aims and hypotheses. They should follow this with arguments that link the findings and conclusions. Specifically for the Discussion, the students are given the following subheadings to incorporate:

1. Main findings
2. Population characteristics (are the baseline values within the normal range for this population?)
3. Facial immersion in cold water (To describe the response and discuss, based on the literature, the afferent and efferent pathways involved and how these contribute to survival)
4. Each of the other test conditions (breathing with a snorkel in cold water, facial immersion in warm water, and breath holding in air) should be discussed in turn by subtraction, identify the different components of the reflex and their importance to the response to facial immersion in cold water
5. Limitations (a paragraph discussing the limitations of the study and how they might impact on the results)
6. Conclusion (a paragraph about the physiological significance of the experiment)

Students are provided with two starter references for their report: Hiebert and Burch (20) and Foster and Sheel (17). These instructions are guides to ensure that all major points are covered. Students are allowed latitude in the order in which the information is presented.

An essay question on the diving response is included on the end-of-semester examination. This is not compulsory (i.e., students are given a range of essay topics to choose from). In the past 3 yr, 10% (2012), 35% (2013), and 55% (2014) of the students answered the diving response question, with average marks of 75% (SD 21, 2012), 73% (SD 21, 2013), and 81% (SD 5, 2014). An example of a diving response essay question, for which 40 min is allowed for completion, is given below:

Describe the cardiovascular reflex responses to cold water face immersion. Include the critical physiological variables involved in the response:

1. The stimuli that elicit the response
2. The receptors and afferent and efferent pathways involved
3. The changes that occur in cardiovascular variables

If the conditions were altered as the subject immersed their face in warm water, while breathing through a snorkel, how do you think the responses would be altered? Why?

Misconceptions

Analysis of the students’ scientific reports and exam essays revealed some common misunderstandings of the diving response. These are summarized below, together with selected student quotes. Many of these misunderstandings reflect language skills rather than knowledge deficiencies. Furthermore, some students use jargon (especially under exam conditions) rather than scientific language.

Misconception 1. Misunderstanding of the anatomy and actions of the autonomic nervous system during the diving response:

Stimulation of chemoreceptors activates the sympathetic and parasympathetic nervous systems, which causes blood vessels to constrict.
This student did not understand that only sympathetic nerve activation alters arteriolar diameter.

Trigeminal and mechanoreceptor stimulation increases sympathetic and parasympathetic input to the vagus nerve.

Only parasympathetic nerves are within the vagus.

Misconception 2. The involvement, locations, and functions of multiple types of sensory receptors (i.e., arterial chemoreceptors, facial temperature, and touch-sensitive receptors) during the diving simulations:

- The touch receptors on the forehead will elicit a cardiovascular response via cold water acting on the carotid arch

This student has confused receptor location and type; the temperature-sensitive receptors are on the face and the baroreceptors are in the aortic arch and carotid sinus.

Skin receptors on the face relay messages to the brain:

What are skin receptors?

These nerves detect the decrease in temperature and trigger the chemoreceptors to send information to the respiratory center.

This student had changes in temperature activating chemoreceptors.

Immersion your face in water causes the chemoreceptors on the face to become stimulated.

Arterial chemoreceptors are activated during apnea because of the evolving hypoxemia. This student might think that there are facial chemoreceptors or that facial chemoreceptors sense temperature.

Misconception 3. Some students did not seem to understand the locations and functions of the arterial baroreceptors. Baroreceptors respond to changes in arterial stretch/pressure by altering their rate of firing, not by changes in receptor number:

- The wetness of the cold water and pain causes the baroreceptors to respond...
- Arterial receptors are decreased.

Misconception 4. Some students do not understand the difference between apnea and hypoxia and that these two features of the diving reflex are detected by separate mechanisms. In the diving reflex, the voluntary apnea inhibits the medulla respiratory center from which arise inspiratory neurons that innervate the diaphragm and inspiratory intercostal muscles. Hypoxemia, which occurs some time after the apnea has been initiated, is detected via peripheral chemoreceptors in the carotid body. During prolonged apnea, arterial O2 levels decrease, whereas CO2 levels increase, and in a normal person, these increasing arterial CO2 levels will stimulate breathing after 1–2 min.

- When apnea (decreased oxygen) is present baroreceptors begin to fire.

Inquiry Applications

This practical class is a methods level inquiry class, for which students are provided with the experimental design and questions. It serves as an introduction to experimental design, data analysis, and the use of tests of statistical significance as well as in the use of the Finapres recording system. Subsequent to this practical class, we ask students to design their own experiments, using the Finapres technology. Thus, they rapidly move to a guided inquiry level. In one example of an inquiry level physiology practical class, we ask them to design and complete an experiment to determine the acute effects of “energy drinks” on the cardiovascular system.

This diving response practical class could easily become an open inquiry practical class, with students generating the questions and designing the experiments. For example, students could generate questions or experiments that are aimed at determining the specific sensory factors that contribute to the cardiovascular changes during the diving response. Some potential questions and experiments are summarized below:

Question 1: where are the sensory receptors on the face? Students could try exposing different parts of the face (i.e., the forehead, nose, or cheeks) to water (see Ref. 35). For example, a previous study (8) has demonstrated that nasal irrigation leads to bradycardia. They could also determine whether the diving response during facial immersion is attenuated if only one side of the face is exposed or if a nasal clip is worn.

To determine whether exposure of the forehead to cold water is essential for the diving response, students could compare facial immersion with and without a face mask.

Question 2: could the activation of skin receptors on non-facial parts of the body contribute to the diving response? Students could compare immersion of the face in water with immersion of some other body part, such as a hand, arm, or foot (20). Campbell et al. (8) compared immersion of the body with immersion of the torso (and not the face). This could be logistically tricky in the practical laboratory setting as a bathtub or swimming pool may be required! There is also the possibility that posture (i.e., leaning over the basin vs. lying down) could affect the response, so this could also be investigated.

Question 3: does training (swimming/diving/exercise) enhance the diving response and/or remove the anticipatory response? The bradycardia with breath holding is smaller in untrained than trained breath-hold divers, suggesting that there is a training effect (13, 36). Students could divide the practical class into swimmers/divers versus nonswimmers/divers and test the effect of swimming/diving experience on the diving response. They could also take into account the fitness level of the participant (i.e., trained athletes vs. students who do no regular exercise).

Question 4: are facial sensory receptors sensitive to temperature or (water) pressure? The temperature of the ambient air can also alter diving bradycardia (33). To dissect out the relative contributions of the pressure or temperature of the water on the diving response, students could alter the room temperature. If you are fortunate, you may have access to a temperature-controlled room. Students could also use “dry” face cloths of known temperatures.

ACKNOWLEDGMENTS

This practical laboratory class would not be possible without the technical support of the physiology practical laboratory staff (Scott Clarke, Rebecca Flower, Svetlana Stanojkovic, Kushani Weerakoon, Kavitha Yattukulan). The funding to purchase the Finapres systems was provided by the Office of the DVC Education.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS


REFERENCES

18. GraphPad Software. GraphPad Prism (online). http://www.graphpad.com/scientific-software/prism/ [18 September 2014].