HABITUATION OF THE CARDIAC RESPONSE TO INVOLUNTARY DIVING IN DIVING AND DABBLING DUCKS

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SUMMARY

1. Bradycardia in response to forced submergence was habituated in dabbling (*Anas platyrhynchos*, Linnaeus) and diving (*Aythya americana*, Eyton) ducks by repetitively submerging the animals, each day for several days, for periods of 40 and 20 s, respectively. The onset of pronounced bradycardia was delayed with each successive trial, until little or no bradycardia occurred during submergence. Diving bradycardia is driven by chemoreceptors in the dabbler and caused by stimulation of narial receptors in the diver.

2. Mean arterial blood pressure in dives was unchanged from pre-dive levels in both naive and trained dabbling ducks. Pa_{O_2}, Pa_{CO_2} and pHa at the end of a dive were similar before and after habituation training.

3. Bradycardia occurred in dives by habituated dabbling ducks if the animal breathed 15 % O_2 before submergence. The ventilatory responses to breathing high and low levels of oxygen were unaffected by habituation training.

4. The changes in blood gases during dives by naive and habituated dabbling ducks were the same: therefore, in the absence of a demonstrated decrement in receptor chemosensitivity or efferent potency, the locus of habituation must reside in the central nervous system.

INTRODUCTION

The purely reflexogenic aspects of the response of an animal to submersion are readily examined in the laboratory. However, it is important also to consider the influence of higher levels of the central nervous system (CNS). The most evanescent interference, according to the literature, arises from the animal's state of arousal when restrained. It appears that the level of CNS arousal is capable of either maximizing the development of the diving response or completely abolishing some aspects of it (Irving, Scholander & Grinnell, 1941; Folkow, Nilsson & Yonce, 1967). The discrepancy between cardiac responses of animals forcibly submerged in the laboratory and animals voluntarily diving in the wild, as well as the variability between successive voluntary dives, may be accounted for by this CNS arousal factor (Jones *et al.* 1973; Butler & Jones, 1982; Blix, 1985; Kanwisher & Gabrielsen, 1984).

Simple forms of vertebrate learning have recently been considered as having a crucial influence on the higher nervous centres controlling cardiovascular function (Galosy, Clarke, Vasko & Crawford, 1981; Cohen & Randall, 1984; Engel & Schneiderman, 1984). With regard to diving, it has been demonstrated in seals that both the rate and degree of bradycardia can be increased by classical and operant conditioning (Ridgway, Carder & Clark, 1975), and this may account for anticipation or enhancement of some of the responses. Habituation of cardiac responses to forced submersion has also been demonstrated in dabbling ducks (Gabbott & Jones, 1985; Gabrielsen, 1985). Higher levels of the CNS may also be involved in forced dives if, as Gabrielsen (1985) claimed, the initial diving bradycardia is part of an orientating response (OR).

The present study was done to examine the function of cardiac habituation to repeated forced dives in both dabbling and diving ducks. In the former, arterial chemoreceptor stimulation is crucial to development of diving bradycardia (Jones & Purves, 1970; Jones, Milsom & Gabbott, 1982), whereas in diving ducks, cardiac adjustments to forced submersion are brought about by stimulation of nasal receptors when the head enters the water (Furilla & Jones, 1986). Dabbling ducks were subjected to more extensive investigation than divers because chemoreceptor output in dives can be remotely manipulated by changing oxygen levels in the gas breathed before diving. Therefore, effects of changes in chemoreceptor output can be studied without direct interference by the experimenter. Hence, we attempted to delimit how habituation affected afferent or efferent neural pathways by studying breathing and cardiac regulation in naive and habituated animals.

MATERIALS AND METHODS

Fifteen adult domestic Pekin ducks (*Anas platyrhynchos*; eight female, seven male) and three adult redhead ducks (*Aythya americana*; two female, one male) were used in these experiments.

Five sets of experiments were carried out: (a) habituation of the cardiac response to diving in diving and dabbling ducks; (b) effect of habituation on blood variables; (c) effect of hyperoxia and hypoxia on the habituated response; (d) effect of habituation on the oxygen breathing test.

Paired *t*-tests were used to test for significant differences between complete data sets obtained from the same animals, i.e. before and after habituation training. Otherwise, data were analysed for significant differences using one-way or two-way analysis of variance. The 95 % confidence level was set as the fiducial limit of significance in all tests. Values are given in the text and tables as the mean ± S.E.M. and n = number of observations in N animals.

General protocol

The animal's body was secured firmly, without restriction of breathing movements, to a padded platform. The head was positioned in a padded brace allowing no movement. To prevent cranial oedema, the platform was inclined at 25° to keep the

animal's head level with the rest of the body. The beak was kept partially open by means of a 2-cm section of soft, large-bore tubing taped in position to allow water to drain completely out of the mouth. The entire apparatus was situated within a light-tight chamber which prevented the animal from seeing the trainer. The diving bucket was operated remotely by a lever. Raising the bucket immersed the duck's head in water to a level above its eyes; at the end of the dive the bucket was lowered. A large-diameter hose was attached to a drain in the bottom of the bucket so that it could be emptied and filled without disturbing the animal.

Heart rate (HR) was determined from the electrocardiogram (ECG) which was obtained from three electrodes: one inserted subcutaneously in the abdominal wall adjacent to the left leg; a second inserted subcutaneously in the right side of the chest; and a third 'grounding' electrode attached to the web of the right foot by means of an alligator clip. These ECG leads were amalgamated into a cable which led out of the chamber through a small opening in the wall.

Habituation of the cardiac response to diving in diving and dabbling ducks

For Pekin ducks, the daily training schedule consisted of 15 trials of 40-s head immersions with 5- to 6-min intervals between immersions. Each session was preceded by a 20- to 30-min quiet period to allow the animal to settle down. In the case of two animals, the immersion time for the first 9 days of training was 60 s, after which the usual 40-s trials commenced. In the case of one animal, an initial 7-day training schedule of 40-s trials was followed by a further 3 days of 60-s trials.

ECG was recorded on a chart recorder and a four-channel tape recorder using an FM converter (A. R. Vetter Co., Rebersburg, PA, USA). Pre-dive heart rate (HR) was determined over the 10-s period just before immersion, and dive HR from the final 10 s of the dive, before the diving bucket was lowered.

The redhead ducks were secured to a level platform in a manner similar to that used for the Pekin ducks. However, their heads were left unrestrained. The trials were accomplished by firmly but gently forcing their heads into a beaker of water. The training protocol involved 20-s trials with 5 min between each trial and an average of 30 trials a day. ECG was recorded as described for the Pekin ducks. Pre-dive HR and end-dive HR were also determined.

Effect of habituation on blood variables

To obtain blood samples and monitor blood pressure, one of the brachial arteries was cannulated. Under local anaesthesia (2 % Xylocaine), a 2-cm section of the artery was exposed in the region adjacent to the humerus and the flared end of a 50-cm length of PE 90 tubing was inserted 7·5–8·0 cm into the artery so that the tip lay within the ascending aorta. After the cannula had been well secured, the skin was sutured closed. Animals were allowed at least 1 day to recover from the effects of surgery.

During experiments the trailing length of cannula extended through the chamber wall and was connected to a port on a three-way stopcock. A pressure transducer was connected to a second port and the remaining port was used for withdrawing blood

samples. Pre-dive blood samples were taken a few seconds before immersion and end-dive blood samples were taken within the last 7 s of the dive. Each sample was then immediately analysed on an IL-813 blood gas analyser (Instrumentation Laboratories, Lexington, MA, USA). Blood gas analyses were done on a maximum of six 0·5-ml samples from each animal each day and haematocrit was closely monitored over the training period. Between sessions, the cannula was flushed with heparinized saline (100 i.u. ml^{-1}), sealed off and coiled into a small loop that could be tucked under the bird's wing which was then taped down to protect the cannula.

Effects of hyperoxia and hypoxia on the habituated response

The open end of a clear polythene bag was tightly attached to the top of the diving bucket. The duck's head was inserted into the bag through a hole in the base which was then loosely secured around the animal's neck. Gases of various oxygen content entered at water level and left *via* the loose-fitting collar around the neck. In this way, at flow rates of $10 l min^{-1}$, it was impossible for the animal to breathe anything but the inflowing gases. Air of various oxygen contents was obtained by mixing flows of pure oxygen and nitrogen gas using flowmeters, and the percentage composition was checked with a Centronic 200 MGA gas analyser (Croydon, England). To reduce the possibility that the noise associated with switching gases might have served as a conditioned stimulus to the animal, a valve system was devised which reduced noise due to flow variations when gas mixtures were altered.

To examine the effect of breathing hyperoxic or hypoxic gas on the habituated diving response, five ducks were subjected to sufficient trials for a substantial level of cardiac habituation. The oxygen content of the air was then altered so that the ducks were breathing gas of 10, 15 or 100 % oxygen for at least 3 min before immersion. Ducks were allowed to surface into air and the next dive followed in sequence. Dives following exposure to altered oxygen levels were never done consecutively.

Effect of habituation on oxygen breathing tests

The ventilatory response to low and high oxygen levels was measured before and after habituation in three animals. In these tests, the animals were placed within a temperature-controlled body plethysmograph (Shimizu & Jones, 1987). Pure oxygen, air or hypoxic air (10 % O_2, balance N_2) was administered at flow rates of $6 l min^{-1}$ through a face mask attached to the front of the plethysmograph. Breathing was monitored with a Fleisch pneumotachograph attached to a port in the plethysmograph. The pressure drop across the pneumotachograph during breathing was recorded with a Validyne DP103 pressure transducer (CA, USA) and the airflow signal was fed into a Gould Integrator (Gould Inc., OH, USA) to provide tidal volume (V_T). From the change in V_T and respiratory frequency (f), minute ventilation ($\dot{V}_E$) was calculated once during air breathing and again after 30 s of breathing 100 % O_2 or 10 % O_2. In order to submerge the animal, its face mask was removed and its head immersed into a beaker of water. Two tests of the breathing response were run on each animal before and after a sufficient period of diving trials to establish a habituated response.

RESULTS

Habituation of the cardiac response to diving in diving and dabbling ducks

Naive or non-habituated ducks exhibited a fall in heart rate to about 30 % of pre-dive levels by the end of a 40-s dive (Fig. 1). The point at which obvious diving bradycardia occurred was gradually delayed with an increase in the number of trials (Fig. 1). The rate and amount of habituation varied among animals and all showed some degree of spontaneous recovery overnight. Recovery was never complete, and successive blocks of trials produced a rising 'saw-tooth' curve of the diminishing cardiac response with increasing trials (Fig. 2A). With repeated training sessions, potentiation of habituation resulted in the virtual elimination of the cardiac response, and in four animals, the HR response to immersion was transformed to a sustained submersion tachycardia (Fig. 2B).

One animal, allowed to rest for 48 h after sufficient training to abolish submersion bradycardia, showed good retention of the effects of training and habituated rapidly in subsequent sessions. Recovery to naive levels of diving bradycardia was complete, however, in two ducks tested after 1 month of rest from training, since test dives were indistinguishable from pre-habituation dives.
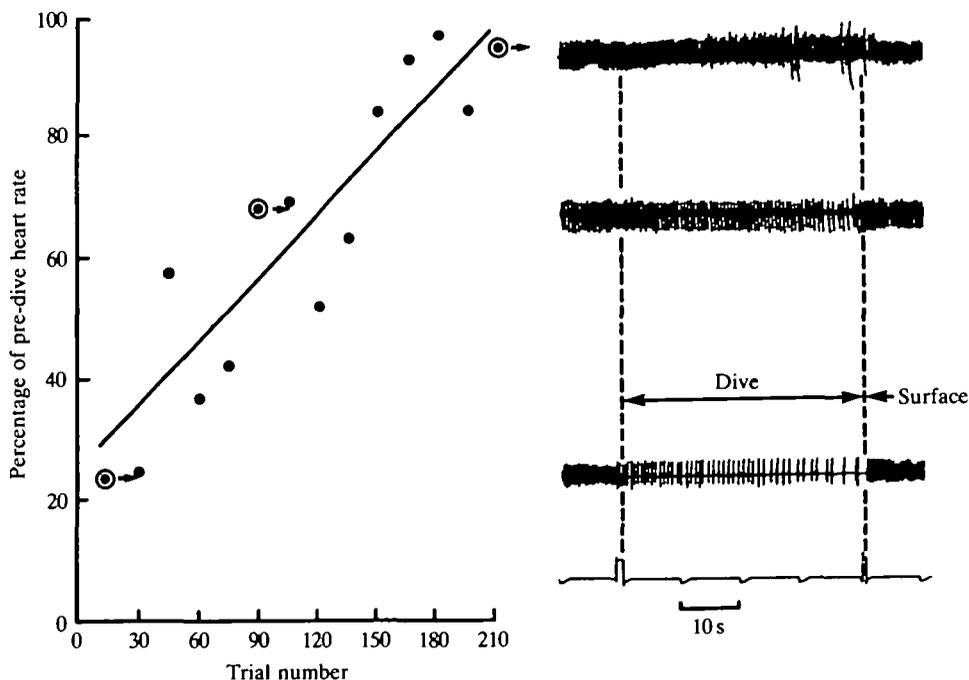

Fig. 1. Reduction in degree of bradycardia attained in 40-s dives by a Pekin duck during repeated trials. Only heart rate (HR) at the end of sample dives are shown and the line through the points was fitted by eye. HR indicated by the circled points were taken from the ECG traces shown on the right. The initial dive is the bottom trace, the ninetieth dive the middle trace and the final dive the top trace. Time marker refers to all traces.

Although the pre-dive HR in most ducks decreased as training progressed, the difference in mean pre-dive values from all animals before and after habituation was not significant. The mean pre-dive rate for the first trial of all animals was $176 \cdot 9 \pm 40 \cdot 0$ beats min^{-1} and the mean pre-dive value after training was $142 \cdot 7 \pm 36 \cdot 0$ beats min^{-1} ($N = 9$). The latter value was calculated from the pre-dive HR of the first trial which demonstrated marked habituation (this was arbitrarily defined as a fall in HR to less than or equal to 80 % of pre-dive HR). Of the nine ducks used in this part of the study, six showed a decreased pre-dive HR with training, two showed an increase and one remained unchanged.

Habituation proceeded slowly if the immersion period was longer than 40 s. Two animals that commenced their training schedule, for several days, with 60-s immersion showed little attenuation of the cardiac response after 60 s under water. As soon as the immersion period was shortened to 40 s, habituation proceeded rapidly. In one animal, changing the training schedule from 40- to 80-s immersion periods abolished the previously habituated response.

Redhead ducks respond to submersion with an immediate and rapid drop in HR. The pre-dive HR in these animals was considerably lower than in Pekin ducks (ranging from 100 to 130 beats min^{-1}), and HR fell to 30 % of the pre-dive rate within the first 10 s of immersion. As with Pekin ducks, repetitive submersion caused a gradual reduction in the degree of bradycardia. All animals showed some
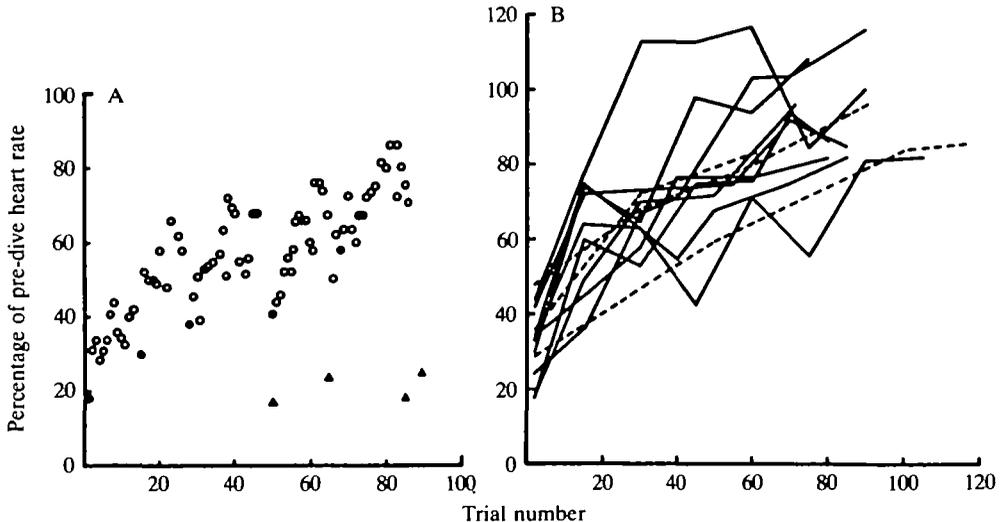

Fig. 2. Effect of habituation training on diving bradycardia expressed as a percentage of pre-dive heart rate (HR). (A) Decrement of end-dive bradycardia with increasing number of trials in a dabbling duck. ●, end-dive HR obtained for the first trial of each training session on each day including the naive animal; ○, end-dive HR for all other dives; ▲, end-dive obtained when the animal breathed 15 % oxygen before the dive. (B) Decrement of end-dive bradycardia in nine dabbling (solid lines) and three diving (dashed lines) ducks. The lines represent interpolation between the means of the HR of the last three trials of each training session except that they begin with mean HR of the first three trials of the first training session.

spontaneous recovery overnight, but it was insufficient to prevent them from achieving considerable habituation of the cardiac response after 60–120 trials, over 2–4 days of training (Fig. 2B).

Effect of habituation on blood variables

To assess the effects of habituation on blood pressure, arterial blood gas levels and pH, comparisons were made between measurements obtained from the first dive of the first training session and the first dive in which HR fell to less than or equal to 80 % of the pre-dive HR. All the animals habituated to this level within 4 or 5 days.

Despite the considerable bradycardia which developed in naive animals after a 40-s submersion, mean arterial pressure (MAP) remained unchanged from pre-dive levels. As training progressed and bradycardia diminished, MAP continued to match pre-dive levels (Fig. 3; Table 1). The mean end-dive MAP of five ducks before habituation was 158 ± 7 mmHg and after habituation was 157 ± 12 mmHg (Fig. 3) (1 mmHg = 133·3 Pa).

The direction of change in blood gas levels was the same before and after habituation. The training sessions had no effect on pre-dive Pa_{O_2}, and end-dive Pa_{O_2} was not significantly different between naive and habituated animals $(P < 0.05)$.
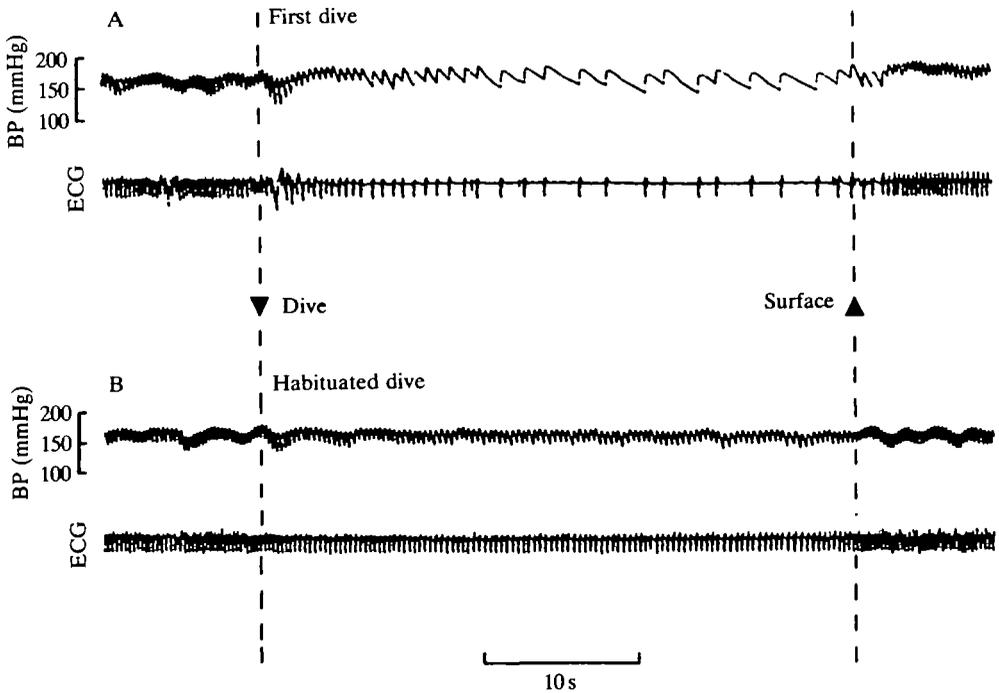

Fig. 3. Chart recorder traces showing the effect of habituation training on arterial blood pressure (BP) and heart rate (ECG). (A) Recording from the first dive of the first day of training. (B) Recording from a dive on the fifth day of training. The dive starts at the downward-pointing arrowhead and ends at the upward-pointing arrowhead. Time bar refers to both sets of traces.

Table 1. *Effect of habituation on cardiovascular variables*

	n	Naive		Habituated	
		Pre-dive	Dive	Pre-dive	Dive
Pa_{O_2} (mmHg)	4	$95\cdot13 \pm 1\cdot42$	$55\cdot70 \pm 2\cdot99$	$93\cdot83 \pm 7\cdot30$	$56\cdot48 \pm 2\cdot75$
Pa_{CO_2} (mmHg)	4	$29\cdot15 \pm 2\cdot39$	$38\cdot50 \pm 2\cdot19$	$27\cdot80 \pm 4\cdot96$	$35\cdot33 \pm 5\cdot78$
pHa	4	$7\cdot44 \pm 0\cdot02$	$7\cdot40 \pm 0\cdot01$	$7\cdot45 \pm 0\cdot04$	$7\cdot41 \pm 0\cdot03$
Mean arterial pressure (mmHg)	5	$164\cdot4 \pm 5\cdot6$	$157\cdot5 \pm 7\cdot4$	$155\cdot9 \pm 3\cdot4$	$157\cdot2 \pm 12\cdot4$

Values are means ± S.E.

Naive values were obtained from the first dive of the first training session. Habituated values were obtained from the first dive to exhibit a fall in heart rate (HR) to less than or equal to 80 % of pre-dive HR ($n = N$).

During submersion before habituation, Pa_{CO_2} rose from $29\cdot2 \pm 2\cdot4$ mmHg to $38\cdot5 \pm 2\cdot2$ mmHg. After habituation, Pa_{CO_2} rose from $27\cdot8 \pm 5\cdot0$ mmHg to $35\cdot3 \pm 5\cdot8$ mmHg. The increase in end-dive pHa was only $0\cdot04$ pH units both before and after habituation (Table 1). The end-dive values of Pa_{CO_2} and pH were not significantly different between naive and habituated ducks.

The haematocrits of the ducks used in this study did not change over the training sessions and remained at 41 %. However, in two ducks sampled for blood gas analysis more frequently during training, haematocrit fell by 1–3 % over 6 days. This fall in haematocrit had no impact on the blood gas values of these two animals which showed the same trends as the other ducks.

Effect of hyperoxia and hypoxia on the habituated response

The mean fall in HR during submersion before habituation was to 30 % of pre-dive HR for the five animals studied (Fig. 4). After training, heart rate remained at $82\cdot5$ % of the pre-dive rate (Fig. 4). When these habituated animals were given air with low oxygen (15 % O_2) before forced submergence, HR fell to 31 % of the pre-dive level. If the oxygen content was lowered further (10 % O_2), pre-dive breathing increased and during submersion HR fell to 30 % of the pre-dive level. After breathing pure oxygen (100 % O_2), HR fell to 90 % of pre-dive rate in habituated animals, similar to the values for habituated animals breathing air (Fig. 4). The effect of a hypoxic test was not permanent as the very next trial with normal oxygen content (breathing room air) evoked the usual habituated response (Fig. 2A).

Effect of habituation on the oxygen breathing test

In naive animals, breathing pure oxygen decreased $\dot{V}E$ while breathing 10 % oxygen increased $\dot{V}E$ compared with breathing air. After training, each animal achieved a level of habituation in which only a 10 % drop in HR was elicited by submersion. When these animals were given the low- and high-oxygen tests, the changes in minute ventilation were of the same magnitude and direction as before. The ventilatory responses to high- and low-oxygen tests were significantly different
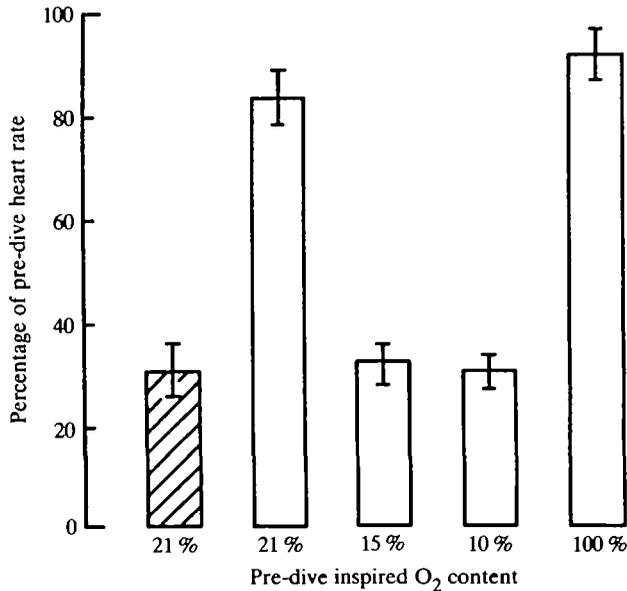
Fig. 4. Effect of various inspired oxygen contents on the cardiac response to submersion in habituated compared with untrained animals. The hatched bar represents values for untrained animals; open bars represent values for habituated animals. Values beneath each bar represent the inspired oxygen content ($N = 5$; $n = 10$).

Table 2. *Effect of habituation on the response to breathing low or high oxygen levels*

	Air	100 % O_2	10 % O_2
Before habituation	0.32 ± 0.02	0.22 ± 0.05	0.57 ± 0.03
After habituation	0.33 ± 0.04	0.23 ± 0.04	0.59 ± 0.06

After habituation values were obtained after five training sessions for each animal.
$N = 3$; $n = 6$.
Values are $\dot{V}E$ (l(BTPS) min^{-1} kg^{-1} ± S.E.).

from one another, but the values obtained before habituation training were not significantly different from those after habituation (Table 2).

DISCUSSION

This study confirms earlier findings (Gabbott & Jones, 1985; Gabrielsen, 1985) that repeated exposure to the stimuli evoked by submersion results in habituation of the normal or naive cardiac chronotropic response (bradycardia). The habituation is so pronounced that in spite of some spontaneous recovery between training sessions, bradycardia in many animals could be completely abolished after several sessions. The cardiac responses to submersion were suppressed, whether provoked by stimulation of 'narial type' receptors in diving ducks or chemoreceptors in dabbling

ducks. In some instances the heart rate response reversed during submersion, to give a diving tachycardia. Since chemoreceptor activation of the vagal deceleratory system is abolished by habituation, the tachycardia must result from further inhibition of the vagal system during submergence (a form of cardiac disinhibition) or direct sympathetic acceleratory effects.

In an earlier study by Rey (1971), habituation of diving bradycardia was not observed despite a training regime of one or two 60-s dives each day for up to 5 months. It is surprising that at the end of such an extensive training period, there was no obvious difference in diving bradycardia between trained animals and their untrained controls at any time in the dive. This study showed that stimulus intensity is a crucial factor for habituation. If dive times exceeded 40 s, or if the inspired oxygen content was only slightly reduced (Fig. 4), habituation of diving bradycardia was prevented or considerably reduced. However, retardation of onset of brady-cardia would be expected in Rey's (1971) ducks early in the dive even if heart rates after 60 s of submergence were unaffected. Obviously, one or two dives per day is not sufficient stimulus exposure for any habituation to occur.

The argument could be raised that training sessions served to familiarize the animals to an otherwise 'fearful' situation. When viewed in this light, the gradual reduction in the diving bradycardia could occur because the animals were less frightened. As accommodating as this interpretation sounds, it ignores the following observations: (a) naive dabbling ducks show little or no bradycardia during short periods of submergence after breathing pure oxygen (Furilla & Jones, 1986); (b) the same animals that habituate with 40-s trials will not habituate if the time is extended to 60 s, even if the number of training sessions is increased; (c) a fully habituated animal develops as profound a bradycardia as a naive animal after breathing air with a 15 % oxygen content before a dive.

Over the period of habituation, MAP remained unaltered in spite of the reduction in bradycardia. This suggests either that an equal and parallel reduction of the vascular constrictor response occurred, or that cardiac stroke volume was reduced in habituated animals. A Doppler flow probe was implanted around the ischiatic artery of one duck and the diving vasoconstriction was observed to habituate in parallel with cardiac habituation, indicating that stable MAP levels are the result of a compensa-tory reduction in vascular tone.

The efficacy of efferent neural cardiac control was confirmed in tests in which habituated animals breathed slightly hypoxic gas mixtures before submersion. Though the effect on ventilation before submersion was negligible, at least down to 15 % O_2, levels of bradycardia during dives were approximately the same as in naive ducks. The effect of a hypoxic trial was not permanent since the very next trial with normal air (21 % oxygen content) resulted in the return of the habituated response. This suggests that vagal output remains just as potent in habituated as in naive ducks.

Tests of receptor sensitivity are more difficult to accomplish. However, an attempt was made to detect a change in oxygen chemosensitivity before and after habituation

by comparing the effect on breathing of low and high oxygen levels. The changes in minute ventilation in response to either pure oxygen or 10 % oxygen appeared to be the same after 5 days of training sessions that resulted in cardiac habituation. Obviously these tests involve changes in an entirely different effector system from that in diving. Nevertheless, it is well established that carotid body chemoreceptors are the prime mediators of ventilatory change in response to the 'oxygen test' (Dejours, 1975) and are crucial for diving bradycardia (Jones & Purves, 1970). As with bradycardia during submersion of dabbling ducks, the removal of carotid body afferent activity also abolishes the ventilatory response to hypoxia (Bouverot, Flandrois, Pucinelli & Dejours, 1965; Jones & Purves, 1970; Bouverot & Leitner, 1972; Lillo & Jones, 1982). Consequently, it seems reasonable to suggest that receptor sensitivity, with respect to its role in diving, is probably unaltered by habituation.

During a dive, cardiovascular adjustments function to conserve oxygen; the elimination of these responses during submersion (i.e. habituation) ought to provoke greater oxygen depletion. However, the fall in Pa_{O_2} was the same in the habituated condition as in the control. This suggests that, at least within 40 s of submersion, the blood flow adjustments are not sufficiently developed to prevent oxygen loss. This is corroborated in experiments where the cardiovascular changes were eliminated pharmacologically by atropine and α-adrenoreceptor blockers, since the rate of oxygen depletion in the first 30 or so seconds of submersion was similar whether the cardiovascular responses were initiated or not (Butler & Jones, 1971; Bryan & Jones, 1980). It is only after this time, when the diving responses are fully developed, that the loss of oxygen from the blood is retarded.

In these experiments, the stimulus intensity for eliciting the cardiovascular responses to submersion appeared to be maintained in dabblers because blood gas variables were the same at the end of dives before and after habituation training. Furthermore, decrement in chemoreceptor sensitivity or efferent potency was not demonstrated in the habituated animals. Consequently, it seems that the locus of habituation is within the CNS. This is in agreement with evidence from other studies that support and have even demonstrated a CNS mechanism of habituation (Thompson & Spencer, 1966; Kandel, 1976). However, it remains unclear exactly what levels of the CNS are involved. Gabrielsen (1985) inferred from his data that higher levels of the CNS are involved because he suspected that the cardiovascular responses during forced diving are an orientating response (OR). He reached this conclusion primarily because the cardiac response is to decelerate and because an OR habituates rapidly. Both these criteria are questionable. Barry & Maltzman (1985) have criticized the popular misconception that HR deceleration necessarily charac-terizes an OR and, conversely, that HR acceleration characterizes a defensive reflex. The remaining criterion, the rapid rate of habituation, is equally open to criticism owing to the difficulty in defining relative rates of habituation. Obviously, further experiments will be required to prove at what level of the CNS habituation occurs in ducks.

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