The cardiovascular and endocrine responses to voluntary and forced diving in trained and untrained rats

Paul F. McCulloch, Karyn M. DiNovo, and Tiffany M. Connolly
Department of Physiology, Midwestern University, Downers Grove, Illinois

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McCulloch PF, DiNovo KM, Connolly TM. The cardiovascular and endocrine responses to voluntary and forced diving in trained and untrained rats. Am J Physiol Regul Integr Comp Physiol 298: R224–R234, 2010. First published November 18, 2009; doi:10.1152/ajpregu.00592.2009.—The mammalian diving response, consisting of apnea, bradycardia, and increased total peripheral resistance, can be modified by conscious awareness, fear, and anticipation. We wondered whether swim and dive training in rats would 1) affect the magnitude of the cardiovascular responses during voluntary and forced diving, and 2) whether this training would reduce or eliminate any stress due to diving. Results indicate Sprague-Dawley rats have a substantial diving response. Immediately upon submersion, heart rate (HR) decreased by 78%, from 453 ± 12 to 101 ± 8 beats per minute (bpm), and mean arterial pressure (MAP) decreased 25%, from 143 ± 1 to 107 ± 5 mmHg. Approximately 4.5 s after submergence, MAP had increased to a maximum 174 ± 3 mmHg. Blood corticosterone levels indicate trained rats find diving no more stressful than being held by a human, while untrained rats find swimming and diving very stressful. Forced diving is stressful to both trained and untrained rats. The magnitude of bradycardia was similar during both voluntary and forced diving, while the increase in MAP was greater during forced diving. The diving response of laboratory rats, therefore, appears to be dissimilar from that of other animals, as most birds and mammals show intensification of diving bradycardia during forced diving compared with voluntary diving. Rats may exhibit an accentuated antagonism between the parasympathetic and sympathetic branches of the autonomic nervous system, such that in the autonomic control of HR, parasympathetic activity overpowers sympathetic activity. Additionally, laboratory rats may lack the ability to modify the degree of parasympathetic outflow to the heart during an intense cardiorespiratory response (i.e., the diving response). Implanted biotelemetry transmitters; underwater maze; habituation; swimming; cardiorespiratory control system

Address for reprint requests and other correspondence: P. F. McCulloch, Dept. of Physiology, Midwestern Univ., 555-31st St., Downers Grove, IL 60515 USA (e-mail: pmccul@midwestern.edu).

The classical mammalian diving response consists of apnea, a decrease in heart rate with a reduction in cardiac output, and an increase in total peripheral resistance. The sympathetically mediated peripheral vasoconstriction is selective, such that during submersion there is a redistribution of oxygenated blood away from organs that can temporarily withstand hypoxia and respire anaerobically, while blood flow is maintained to tissues dependent on a constant supply of oxygen. In contrast to physically restrained forced-dived animals in the wild are usually active and remain underwater for only relatively short periods (6, 20). In this situation, the circulatory changes are often quite different, and while changes in heart rate are qualitatively similar, they are usually less intense than during forced diving. Although the initiation of the diving response is reflexly generated, experiments on both restrained and unrestrained diving animals have indicated that the cardiovascular responses to diving are very labile (3, 6). The intense bradycardia observed during forced submersion is not always seen in unrestrained animals. Perception of a stressful situation in the laboratory, or anticipation of a long duration dive in the wild, apparently precipitates maximal cardiovascular adjustments during the period of submersion. Conscious awareness, fear, and anticipation may modify the cardiovascular responses to those which are appropriate for a given situation (6).

There are many rodent experimental protocols, such as the forced swim test (2, 6, 31) and pedestal in the water protocol (12, 55), which utilize water either as a stressful or aversive stimulus. As a consequence, rats are often considered to be water phobic, even though they can exhibit swimming activity at birth and adult swimming behavior at 12 days of age (8, 49, 53). However, the behavior of rats when exposed to water will depend upon previous knowledge of their environment (5), and rats will often dive underwater during the exploratory phase of the forced swim test (7, 18, 28). Since rats exhibit the classical cardiorespiratory responses to diving (34, 41, 42), we wondered whether gradually training rats in the voluntary and forced diving procedures (32, 35) would affect the magnitude of the cardiovascular responses seen during diving. In addition, we wondered whether this dive training would reduce or eliminate any potential stress component due to water exposure in rats. We used implantable blood pressure transmitters to record the cardiovascular responses to swimming and diving (23, 24), and blood corticosterone concentrations as an indicator of stress (1, 31).

The following research had two objectives. Our first objective was to determine whether in rats the cardiovascular and endocrine responses during forced diving are more intense compared with the responses during voluntary diving. On the basis of this first objective, our first hypothesis was that the cardiovascular responses during forced diving will be greater in magnitude compared with the cardiovascular responses during voluntary diving. Our second hypothesis was that the amount of corticosterone produced will be greater during forced diving than during voluntary diving. The second objective of this research was to determine whether repetitive dive training would decrease both the magnitude of the cardiovascular response and amount of corticosterone produced during diving. On the basis of this second objective, our third hypothesis was that training rats in the voluntary and forced-dive procedures will decrease the magnitude of the cardiovascular responses to diving compared with untrained rats. Our fourth hypothesis was that training rats in the voluntary and forced dive procedures will decrease the amount of corticosterone produced during voluntary and forced diving compared with untrained rats.
METHODS

The Midwestern University Institutional Animal Care and Use Committee approved all experiments. Male Sprague-Dawley rats \( (n = 34) \) were purchased from a commercial vendor (Harlan, Indianapolis, IN). Rats were caged in threes or fours, except after surgery, when they were caged singly. All rats were allowed food and water ad libitum and were housed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals (12:12-h light-dark cycle; lights on from 0600 to 1800). To avoid conspecific communication via either ultrasonic vocalizations or urinary pheromonal signals (14, 19, 46, 47), rats were transported to an experimental room for all training and handling sessions, blood pressure and heart rate recordings, and all blood draws. Two experimental protocols were used to investigate the swimming, voluntary diving, and forced diving responses from rats. The first experiment used implanted transmitters to measure the heart rate and blood pressure from swimming and diving rats \( (n = 12) \), while the second experiment measured corticosterone levels in swimming and diving rats \( (n = 22) \). After experiments had ended, all rats were euthanized with an anesthetic overdose (Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA).

Swimming and Dive Training

Selected rats were trained gradually to swim and dive through a maze, as has been described previously (35). The maze consisted of a water-filled Plexiglas tank \( (100 \times 60 \times 15 \text{ cm}) \) divided into five \( 100 \times 10 \text{ cm} \) channels (see Ref. 35 for tank details). The water in the tank was about 10 cm deep, and it was maintained at \( 31 \pm 2^\circ \text{C} \) to reduce thermal stress through evaporative cooling (29) and minimize fluctuations in corticosterone levels induced by changes in water temperature (1). A raised platform was placed in one corner of the tank, in which the rats could climb out of the water. At the beginning of training, the rats were placed a few centimeters away from the platform. During each training session, the distance to the platform was gradually increased. After 3 wk of training, the rats were able to successfully swim a 3-m length of the maze, taking 10–20 s to complete the maze. No external reward (i.e., food) was used during the training protocol.

After swim training was completed, the rats were trained gradually to voluntarily dive under water, using the same swim training tank. The rats were placed in a starting chamber located in the opposite corner to the raised platform. The only exit from the starting chamber was through an underwater opening leading to the swim channels. When each rat was ready, it initiated a voluntary underwater dive to exit the chamber. Once the rats had accomplished exiting the starting chamber, a horizontal Plexiglas cover was placed just under the surface of water in the channel leading from the starting chamber. This increased the distance of the underwater swim. This cover was gradually extended with each training session. After 3 wk of training, the rats could dive 3 m under water, taking 15–20 s to dive through the maze.

After voluntary dive training had been completed, the rats were trained gradually to undergo forced dives. Initially, the rats were placed in and acclimated to a Plexiglas rodent restraint device (18 cm length and 6.5 cm internal diameter). Next, the restraint device was placed in 1 cm of water in the middle of the Plexiglas tank. With each training session the depth of the water was increased, until the rat and the restraint device were completely submerged and held under water for 10 s. This training was completed within about 2 wk.

Training sessions were held in the morning and afternoon 5 days/wk, and consisted of three trials each session. With the 6 daily trials, the rats would be in the water for a total of \( \sim 2 \text{ min/day} \). Rats initiated all swimming and voluntary diving trials, and swim at their own speed. No attempt was made to increase the velocity or effort at which the rats swam or dived underwater. Before each trial, each rat was gently held for 1 min. After each trial, when the rat had reached the raised platform, the rat was left on the platform for 1 min, and then the rat was held and dried off with a towel for 30 s.

Heart Rate and Blood Pressure Measurements

Rats \( (n = 12) \) were divided into two groups: trained \( (n = 6) \), and untrained \( (n = 6) \). The trained group received training in swimming and diving twice daily for 5 days/wk, but the untrained group did not. After the trained group had completed their swim and voluntary dive training (after 6 wk of training; weight \( \sim 331 \pm 5 \text{ g} \) ), they were implanted with biotelemetric transmitters [model PA-C40; Data Sciences International (DSI), St. Paul, MN]. Using the same procedures, we also implanted the adult rats \( (weight \sim 391 \pm 46 \text{ g}) \) in the untrained group with DSI transmitters.

For rats undergoing transmitter implantation surgery, the surgical instructions provided by DSI were followed. Rats were anesthetized with isoflurane (5% vol initial induction, and 2–3% when used with a nose cone). Under aseptic conditions, a longitudinal incision was made along the ventral midline to expose the abdominal contents. The transmitter catheter tip was inserted into the descending abdominal aorta and held in place with tissue cement. Sterile nylon sutures were used to both close the abdominal muscle layer and anchor the transmitter within the peritoneal cavity. The incision was closed with metal wound clips, and the rats were given a postsurgical analgesic (ketoprofen, 3–5 mg/kg sc). The rats were then placed in their cages under a heat lamp until anesthetic recovery, at which time they were returned to the animal facility. If necessary, a supplemental analgesic injection (ketoprofen, 3–5 mg/kg sc) was given 24 h after surgery. The wound clips were removed a week after surgery, at which time the rats were allowed to return to the maze for swim and dive trials.

The telemetry system consisted of the implantable transmitters, a telemetry receiver [either wand receiver (RLA3000) or rodent cage receiver (RPC-1)] and calibrated pressure adapter. Decoded electronic data were fed through an A/D converter [Micro 1401; Cambridge Electronic Design (CED), Cambridge UK] to a computer where the signals were stored and analyzed using Spike 2 (CED). Pulsatile arterial blood pressure signals were used to determine mean arterial pressure (MAP) and heart rate (HR). MAP and HR were measured from 10- to 30-s long continuous data tracings, while the rats remained alone in their cages for 30 min (control), while they were being gently held on a towel by one of the experimenters for 5 min (Handled group), 30 s before swimming and diving (preswimming or prediving, respectively), and during swimming. For voluntary and forced diving, the initial diving HR (taken from the first cardiac interval immediately after submergence), the average diving HR (calculated by dividing the number of heart beats while under water by the dive duration), and the lowest diving HR (taken from the longest cardiac interval while under water) were determined. Because the telemetric signal was occasionally lost due to the movement of the rats through the maze and/or the attenuation of the signal by the water, calculation of the average dive MAP was not possible. Consequently, the initial diving MAP and highest diving MAP were calculated from a single pulse pressure, using MAP = diastolic pressure + (systolic pressure – diastolic pressure)/3.

In addition to HR and MAP measurements, the time between being placed in the starting chamber and initiation of voluntary dives was recorded, and the number of fecal pellets, which in rats are produced during stressful situations (4), were counted during swimming, voluntary diving, and forced diving.

Corticosterone Measurements

Rats \( (n = 22) \) were randomly divided into four groups: Naïve \( (n = 6) \), Handled \( (n = 4) \), Trained \( (n = 6) \), and Untrained \( (n = 6) \). The Trained group received training in swimming and diving (see above), but the remaining three groups did not receive any swim or dive training. Because each training session involved human handling and
lasted ~5 min, two control groups were used. The Handled group was gently held for 5 min, twice a day for 5 days a week for 8 wk, while the Naïve group was untouched and remained in its cage for the entire duration of the experiment, except for blood draws. While the Trained group underwent twice daily training sessions to become familiar with the water tank and the swimming and diving protocols, the Untrained group only encountered the water tank on blood draw days.

For each blood draw, a 30-gauge needle and heparinized syringe were used to remove 0.1-ml blood samples from the tail vein. All blood draws were scheduled during the late morning and early afternoon to correspond with the timing of training sessions. Also, endogenous production of corticosterone follows a circadian rhythm and is lowest during morning hours (18, 43), which was confirmed by preliminary experiments. For each blood draw, one experimenter gently restrained the rat in a towel while exposing the rat’s tail, and another experimenter (a trained phlebotomist) drew blood from the tail vein. The drawn blood was placed into EDTA tubes then centrifuged at 14,000 rpm for 2 min to separate red blood cells and platelets from plasma. Immediately following the centrifugation, the plasma supernatant was removed and placed into a microcentrifuge tube and stored at −4°C. For sample analysis, the samples were thawed for 1 h before use. A double-antibody corticosterone kit (cat. No 07-12103; MP Biomedicals, Cleveland, OH) using iodine125 determined corticosterone concentrations. All samples were run through a gamma counter (Packard Cobra Auto Gamma Counter), and sample counts were compared with a standard curve to determine corticosterone concentrations.

After completion of swim training (end of week 3), each Trained rat was individually placed in the middle of the water tank and swim around the tank until they found the raised platform. After a 15-s rest on the platform, the rat was gently returned to the middle of the tank to swim again. This continued for 5 min, after which the rat was returned to its cage. Plasma corticosterone peaks 15–30 min after a 2-min restraint stress (9), and ~10 min after swim stress in 22°C water (13). Using our swimming and diving protocols, we found that preliminary experiments indicated 15 min was sufficient time to allow for the production and release of corticosterone into the circulation. Therefore, a blood draw was taken 15 min after the 5 min of swimming. The same swimming and blood draw procedures were followed for the Untrained group, even though they had not received any swim training. Blood draws were also taken from the Handled and Naïve groups at this time.

After the Trained group completed voluntary dive training (end of week 6), a blood draw was taken 15 min after 3 voluntary dives. The same diving and blood draw procedures were followed for the Untrained group. Because the Untrained group had not received any dive training, they were unable to complete the same distance successfully negotiated by the Trained group. Therefore, it was necessary to shorten the distance the Untrained rats swim under water. Consequently, the underwater dive length was 30–90 cm for the Untrained group and 3 m for the Trained group. Blood draws were also taken from the Handled and Naïve groups at this time.

After the Trained group completed forced dive training (end of week 8), a blood draw was taken 15 min after three forced dives. The same forced diving and blood draw procedures were followed for the Untrained group. Blood draws were also taken from the Handled and Naïve groups at this time.

Statistics and Presentation

All data were statistically analyzed using SigmaStat (SPSS, Chicago IL), with significance set at \( P < 0.05 \); HR, MAP, and fecal pellet production data were analyzed with two-way repeated-measures (RM) ANOVA procedures, using the primary factors of training (untrained and trained) and activity (control, handled and swim/dive positioning; preswimming/diving, initial diving, mean diving, and lowest/highest diving; or swimming, voluntary diving, and forced diving). Activity data within training were analyzed using one-way RM ANOVA procedures. Corticosterone data were analyzed with a two-way RM ANOVA procedure, using the primary factors of time (at weeks 3, 6, and 8) and human interaction (naïve, handled, trained, and untrained). Human interaction data within time were analyzed using one-way RM ANOVA procedures. If significant \( P \) values were found, Tukey’s post hoc all pairwise multiple-comparison procedures determined which groups were significantly different. Additionally, linear regressions and \( t \)-tests were used as indicated below. Values are presented as means ± SE. Figures were created using SigmaPlot (SPSS) and CorelDraw (Corel, Ottawa, Canada). In the digital images presented in Fig. 2, CorelDraw was used to remove electronic noise from the tracings when the telemetric signal was lost.

RESULTS

Behaviors

Upon their introduction to water, all rats would initially appear unsteady as they first encountered the sensation of floating, and would paddle about in an uncoordinated fashion while trying to find a way out of the water. However, in subsequent trials, the rats were much more coordinated, as they swam toward the raised platform. The Trained group quickly learned the task of swimming through the channels to the platform. Once on the platform and during the 1-min wait before their next trial, most rats thoroughly explored the platform. Some rats would reenter the water to swim, and/or submerge their head underwater while still sitting on the platform. During voluntary diving, each dive was initiated soon after (10.7 ± 2.3 s) the rat had been placed in the starting chamber. Average voluntary dive duration was 11.7 ± 0.9 s. During the forced dive training sessions, it became increasingly more difficult to get the rats to enter the Plexiglas restraint device. The unwillingness to enter the restraint device increased with subsequent training sessions as the depth of the water surrounding the restraint device was increased. Struggling in the restraint device was common, especially while underwater.

Rats from the Untrained group, which for the transmitter-implanted rats were adults when first introduced to water, would initially swim in an uncoordinated fashion while trying to get out of the water. During subsequent swimming trials, the Untrained group of rats appeared more coordinated than initially, but never appeared to swim as smoothly as rats in the Trained group. During voluntary diving, untrained rats took longer to initiate their dives (137.0 ± 18.7 s; maximum of 9 min), and the three trials took much longer to complete than they did for trained rats. Average voluntary dive duration was 5.4 ± 0.6 s, which was shorter than for trained rats (\( t \)-test; \( P < 0.001 \)). Often, while waiting to initiate a voluntary dive, untrained rats would vocalize and exhibit teeth chattering. During the forced diving, it was easier to get the Untrained group into the restraint device than it was the Trained group.

Rats in the Untrained group produced more fecal pellets (0.58 ± 0.02 pellets per swim/dive) than did rats in the Trained group (0.09 ± 0.01 pellets per swim/dive; \( P = 0.002 \)). In untrained rats, voluntary diving produced more fecal pellets (1.05 ± 0.08 pellets per dive; \( P = 0.006 \)) than did swimming (0.31 ± 0.04 pellets per swim) or forced diving (0.38 ± 0.02 pellets per dive). In trained rats, forced diving produced more fecal pellets (0.28 ± 0.03 pellets per dive; \( P = 0.006 \)) than did...
swimming (0 pellets per swim) or voluntary diving (0 pellets per dive).

The Handled group generally would explore its surroundings while being gently held on a towel. No scratching or biting occurred, and no fecal pellets were produced by these rats during the handling procedure.

Heart Rate and Blood Pressure

When alone in their cages in a quiet room containing no other animals, control HR and MAP of both untrained and trained rats were ~400 bpm and 114 mmHg, respectively (Table 1). Control HR and MAP were not different between untrained and trained rats \((P = 0.803 \text{ for HR, } P = 0.529 \text{ for MAP})\). However, during this 30-min period when the rats were left alone in their cages in a quiet room, both HR and MAP showed linear decreases with time \([HR = -1.506(time) + 435, r^2 = 0.94; \text{MAP} = -0.346(time) + 121, r^2 = 0.95; \text{Fig. 1}]\). The regressions for HR and MAP were not different between untrained and trained rats \((P = 0.696 \text{ for HR, } P = 0.383 \text{ for MAP})\).

When being held on a towel by one of the experimenters (Handled group), all rats explored their environment but made no attempt to escape off the towel. In untrained rats with limited experience being held on a towel, HR increased by 9% \((P < 0.001)\), while MAP increased by an additional 4% \((P = 0.826 \text{ for MAP})\). When rats in the Trained group were experienced being held during every training session, HR increased by 11% \((P < 0.001)\), and MAP increased by 14% \((P < 0.001)\), compared with control values (Table 1). The Handled group’s HR and MAP were not different between untrained and trained rats \((P = 0.323 \text{ for HR, } P = 0.826 \text{ for MAP})\).

When rats in the Untrained group were being positioned in the maze to swim or dive (swimming/voluntary diving/forced diving positioning), HR increased by an additional 7% \((P = 0.002)\), and MAP did not increase further \((P = 0.999)\), compared with Handled values (Table 1). When rats in the Trained group were being positioned to swim or dive, HR did not increase \((P = 0.354)\), while MAP increased by an additional 4% \((P = 0.002)\), compared with values in the Handled group (Table 1). While being positioned to swim or dive, HR and MAP were not different between untrained and trained rats \((P = 0.489 \text{ for HR, } P = 0.120 \text{ for MAP})\).

Swimming. For both untrained and trained rats, swimming caused little change in either HR or MAP (Fig. 2A). For untrained rats, swimming HR and MAP were 489 \pm 13 bpm and 130 \pm 3 mmHg, respectively, which were not different from preswimming HR and MAP \((P = 1.00 \text{ for HR, } P = 0.401 \text{ for MAP})\). For trained rats, swimming HR and MAP were 490 \pm 10 bpm and 134 \pm 2 mmHg, respectively, which were not different from preswimming HR and MAP \((P = 0.999 \text{ for HR, } P = 0.931 \text{ for MAP})\). Neither swimming HR nor MAP were different between untrained and trained rats \((P = 0.982 \text{ for HR, } P = 0.331 \text{ for MAP})\).

Voluntary diving. For both Untrained and Trained groups of rats, voluntary diving caused an immediate and substantial decrease in HR (Fig. 2B). For untrained rats, HR decreased by 74%, from 469 \pm 17 bpm prediving to a mean voluntary diving HR of 124 \pm 8 bpm \((P < 0.001)\). Of the 345 \pm 13 bpm decrease in HR, 88% \((305 \pm 20 \text{ bpm})\) occurred during the first cardiac interval. The lowest voluntary diving HR was 88 \pm 8 bpm. For trained rats, HR decreased by 78%, from 453 \pm 12 bpm prediving to a mean voluntary diving HR of 101 \pm 8 bpm \((P < 0.001)\). Of the 352 \pm 10 bpm decrease in HR, 89% \((312 \pm 12 \text{ bpm})\) occurred during the first cardiac interval. The lowest voluntary diving HR was 78 \pm 8 bpm.

For both Untrained and Trained groups of rats, voluntary diving caused an immediate decrease in MAP followed by a slower onset increase in MAP (Fig. 2B). For untrained rats, MAP initially decreased 18.6%, from 140 \pm 4 mmHg prediving to an initial diving MAP of 114 \pm 4 mmHg \((P < 0.001)\). While under water, MAP then increased to a maximum of 172 \pm 4 mmHg, which was a 23% increase compared with prediving MAP \((P < 0.001)\). The mean time to the highest voluntary diving MAP was 3.7 \pm 0.3 s after the start of the dive. For trained rats, MAP initially decreased 25%, from 143 \pm 1 mmHg prediving to an initial diving MAP of 107 \pm 5 mmHg \((P < 0.001)\). While under water, MAP then increased to a maximum of 174 \pm 3 mmHg, which was a 22% increase compared with prediving MAP \((P < 0.001)\). The mean time to the highest voluntary diving MAP was 4.5 \pm 0.4 s after the start of the dive.

Forced diving. For both Untrained and Trained groups of rats, forced diving caused an immediate and substantial decrease in HR (Fig. 2C). For untrained rats, HR decreased by 76%, from 453 \pm 10 bpm prediving to a mean forced diving HR of 110 \pm 5 bpm \((P < 0.001)\). Of the 343 \pm 7 bpm decrease in HR, 89% \((306 \pm 13 \text{ bpm})\) occurred during the first cardiac interval. The lowest forced diving HR was 79 \pm 6 bpm. For trained rats, HR decreased by 76%, from 439 \pm 9 bpm prediving to a mean forced diving HR of 107 \pm 9 bpm \((P < 0.001)\). Of the 332 \pm 7 bpm decrease in HR, 86% \((287 \pm 9 \text{ bpm})\) occurred during the first cardiac interval. The lowest forced diving HR was 78 \pm 7 bpm.

For both Untrained and Trained groups of rats, forced diving caused an immediate decrease in MAP followed by a slower onset increase in MAP (Fig. 2C). For untrained rats, MAP initially decreased only 3%, from 128 \pm 4 mmHg prediving to an initial diving MAP of 124 \pm 3 mmHg \((P = 0.891)\). While under water, MAP then increased to a maximum of 191 \pm 4 mmHg, which was a 49% increase compared with prediving MAP \((P < 0.001)\). The mean time to the highest forced diving MAP was 5.4 \pm 0.6 s after the start of the dive. For trained rats,

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Values are expressed as means ± SE. *Significantly greater than Control group. †Significantly greater than Handled group. Heart rate (HR) and mean arterial pressure (MAP) values are not significantly different when comparing untrained to trained rats.
MAP initially decreased 12%, from 135 ± 2 mmHg prediving to an initial diving MAP of 119 ± 5 mmHg (P = 0.022). While under water, MAP then increased to a maximum of 189 ± 4 mmHg, which was a 40% increase compared with prediving MAP (P < 0.001). The mean time to the highest forced diving MAP was 4.4 ± 0.4 s after the start of the dive.

Effect of dive type. Although both voluntary and forced diving caused significant decreases in HR compared with prediving, the type of dive (voluntary or forced) did not produce differences in initial diving HR (P = 0.828; Fig. 3A), lowest diving HR (P = 0.212; Fig. 3C), or decrease in HR (P = 0.195; Fig. 3D). For mean diving HR, there were no significant effects of dive type (P = 0.353; Fig. 3B), although there was an interaction effect (P = 0.047), such that within untrained rats, the mean forced diving HR was lower than the mean voluntary diving HR (P = 0.046). Forced dives did, however, produce increases in initial diving MAP (P = 0.002; Fig. 3E) and highest diving MAP (P < 0.001; Fig. 3F), compared with voluntary dives.

Effect of training. Training rats for voluntary or forced diving did not produce differences in initial diving HR (P = 0.892; Fig. 3A), mean diving HR (P = 0.212; Fig. 3B), lowest diving HR (P = 0.525; Fig. 3C) or decrease in HR (P = 0.852; Fig. 3D). Dive training produced no differences in either initial diving MAP (P = 0.316; Fig. 3E) or highest diving MAP (P = 0.946; Fig. 3F) compared with no dive training.

Corticosterone

The amount of human interaction encountered by the rats had a significant effect on blood corticosterone concentration (P < 0.001; Fig. 4). When the three blood draws (from weeks 3, 6, and 8) were averaged, naïve rats left in their cages had the lowest corticosterone concentration (32.2 ± 16.62 ng/ml), whereas handling the rats for 10 min/day caused only a slight increase in corticosterone concentration (90.3 ± 20.2 ng/ml; P = 0.155). Rats that experienced both daily handling and swim and dive training (trained rats) had a corticosterone concentration (165.3 ± 13.4 ng/ml) greater than both naïve (P < 0.001) and handled rats (P = 0.029). Rats that were neither handled daily nor received any swim or dive training but still experienced swimming, voluntary diving, and forced diving (untrained rats) had a corticosterone concentration (232.9 ± 13.4 ng/ml) that was greater than all three other groups (P < 0.001 for each pairwise comparison).

At the end of week 3, when the Trained group had completed its swim training, the corticosterone concentrations from the two groups that did not undergo swimming (naïve and handled) were less than that in the two groups that did undergo swimming (trained and untrained; Fig. 4). The corticosterone concentration from untrained rats was greater than the corticosterone concentrations from naïve (P = 0.008) and handled rats (P = 0.020) but was not different from trained rats (P = 0.348). Untrained rats had a corticosterone concentration 45% greater than that from trained rats.

At the end of week 6, when the Trained group had completed its voluntary dive training, the corticosterone concentrations from the two groups that did not undergo voluntary diving (naïve and handled) were less than the 2 groups that did undergo voluntary diving (trained and untrained; Fig. 4). However, the corticosterone concentration from trained rats was not different from handled rats (P = 0.001), although it was greater than naïve rats (P = 0.008). The corticosterone concentration from the Untrained group was greater than all three of the other groups (P < 0.001 for naïve, P = 0.004 for handled, and P = 0.001 for trained). Untrained rats had a corticosterone concentration 95% greater than that from trained rats.

At the end of week 8, when the Trained group had completed its forced dive training, the corticosterone concentrations from the two groups that did not undergo forced diving (naïve and handled) were less than the two groups that did undergo forced diving (trained and untrained; Fig. 4). The corticosterone concentration from trained rats was greater than the corticosterone concentrations from naïve (P < 0.001) and handled rats (P = 0.001). The corticosterone concentration from untrained rats was also greater than the corticosterone concentrations from naïve (P < 0.001) and handled rats (P < 0.001) but was not greater than that from trained rats (P = 0.824). Untrained rats had a corticosterone concentration only 10% greater than that from trained rats.

Over the 8-wk duration of the experiment, plasma corticosterone concentration from the three blood draws did not change in either naïve (P = 0.361; Fig. 4) or handled rats (P = 0.834; Fig. 4). In trained rats, plasma corticosterone concen-
tion was greater after forced diving than after voluntary diving ($P = 0.023$; Fig. 4). Corticosterone concentration did not change in untrained rats ($P = 0.872$).

**DISCUSSION**

The results from the present experiments indicate that rats have a substantial diving response that includes an immediate and substantial bradycardia, and a slower onset peripheral vasoconstriction that causes an increase in MAP. Blood corticosterone levels indicate rats trained in swimming and voluntary dive procedures find diving no more stressful than being held by a human, but rats not trained in the procedures find swimming and diving very stressful. Forced diving is stressful to both trained and untrained animals, although compared with
voluntary diving, forced diving increases the amount of corticosterone produced in trained rats but not in untrained rats. Thus, the trained rats may have habituated to voluntary diving but not to forced diving. Training rats in the voluntary and forced dive procedures does not decrease the magnitude of the cardiovascular responses to diving, as there were no differences in the cardiovascular responses to voluntary and forced diving between trained and untrained rats. However, the in-

Fig. 3. HR and MAP during both voluntary (Vol) and forced (For) dives for untrained and trained rats. A: HR calculated from the first cardiac interval after submergence. B: average HR during the submerged period. C: HR calculated from the longest cardiac interval during submergence. D: the decrease in HR during the dive. E: MAP calculated from the first pulse pressure after submergence. F: highest MAP calculated from a single pulse pressure during submergence. Values are expressed as means ± SE. † HR is significantly less than voluntary diving in untrained rats; * MAP from forced dives is significantly greater than for voluntary dives.

Fig. 4. Corticosterone concentrations from rats left in their cages (naïve), rats handled for 10 min/day (handled), rats trained to swim and dive (trained), and rats receiving no swim or dive training (untrained). Left: corticosterone was collected after trained rats had completed their swim training. Center: corticosterone was collected after trained rats had completed their voluntary dive training. Right: corticosterone was collected after trained rats had completed their forced dive training. * Value is significantly greater than Naïve. † Value is significantly greater than Handled; ‡ indicates value is significantly greater than Trained; § indicates in Trained rats forced dive corticosterone is significantly greater than voluntary dive corticosterone.

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crease in MAP was greater in forced dives than in voluntary dives. The diving response of rats, therefore, appears to be dissimilar from other animals, as most birds and mammals show intensification of the diving bradycardia during forced diving as compared with voluntary diving. Rats may exhibit an accentuated antagonism between the parasympathetic and sympathetic branches of the autonomic nervous system, such that in the autonomic control of HR, parasympathetic activity overpowers sympathetic activity. Additionally, rats may lack the ability to modify the degree of parasympathetic outflow to the heart during an intense cardiorespiratory response (i.e., the diving response).

Voluntary and Forced Diving in Rats

We refer to “voluntary dives” as dives that were initiated by rats through their own volition. This is in contrast to the terminology employed by Butler and Jones (6) who defined “voluntary” or “natural” dives as those performed by unrestrained animals in the wild or in a simulated habitat. Butler and Jones (6) might consider the voluntary dives in our trained rats as “trained” dives, since these rats received daily swim and dive training, and the voluntary dives in our untrained rats as “escape” dives, since these rats received no training. However, in the present context, we prefer to retain the term “voluntary” for these dives to facilitate comparison between rats that did and did not receive daily swim and dive training but that initiated their own dives voluntarily.

In the present research, voluntary underwater submergence in rats resulted in an immediate 75% decrease in HR and 20% decrease in MAP. Almost 90% of the ~350 bpm decrease in HR occurred during the dive’s initial cardiac interval, which suggests limited, if any, chemoreceptor contribution to the initiation of this response (34). Following the initial decrease in arterial pressure and ~4 s after the start of the dive, MAP increased to a peak that was ~25% greater than prediving MAP. The classical mammalian diving response, consisting of apnea, parasympathetically mediated bradycardia, and a sympathetically mediated selective increase in peripheral vascular tone (6), has previously been observed in rats instrumented with trailing arterial cannulas during conscious voluntarily diving (34). The present research confirms and extends these findings through the use of implantable pressure transmitters that minimize stress artifacts (23, 24) and can provide more accurate pressure measurements (25). The cardiovascular responses of rats during voluntary diving are therefore similar, both qualitatively and quantitatively, to the responses from other small mammals, such as muskrat (10, 17, 30, 33), mink (54), and beaver (52) undergoing voluntary underwater submergence. Similar cardiorespiratory responses have also been observed in voluntarily diving rats that were trapped under water (42) and in anesthetized rats during simulated diving with nasal stimulation (36, 48).

In contrast to voluntarily initiated dives, dives in which animals are restrained and submerge are “forced” dives (6). In the present research, forced underwater submergence in rats resulted in an immediate 76% decrease in HR and 3–12% decrease in MAP. This decrease in arterial pressure was then followed ~5 s later by the highest diving MAP that was 40–49% greater than prediving MAP. Although diving MAPs were greater during forced diving than during voluntary diving, the HR responses in rats to forced diving were not significantly different from voluntary diving. The initial diving HR, mean diving HR, and lowest diving HR, as well as the absolute decrease in HR, were similar between the two types of dives. The diving response of rats, therefore, appears to be dissimilar from other animals, as most birds and mammals show intensification of the diving bradycardia during forced diving as compared with voluntary diving (6, 20). For instance, in muskrats diving HRs during natural, escape, and forced dives are 115, 95, and 60 bpm, respectively (33). Therefore, on the basis of these results, our first hypothesis stating that the cardiovascular responses during forced diving will be greater in magnitude compared with the cardiovascular responses during voluntary diving is partially rejected. In rats, there is not an increase in the magnitude of the parasympathetically produced bradycardia during forced diving compared with voluntary diving, but there is an increased sympathetic response that produces greater MAP during forced diving.

When trained in voluntary diving procedures, rats find voluntary diving no more stressful, at least in terms of corticosterone production, than swimming. In contrast, even after 2 wk of daily training, trained rats showed a significant increase in plasma corticosterone during forced diving compared with voluntary diving. However, untrained rats had high plasma corticosterone levels that were unchanged between swimming, voluntary diving, and forced diving. Thus, our second hypothesis stating that the amount of corticosterone produced will be greater during forced diving than during voluntary diving is partially accepted. Forced diving increases the amount of corticosterone produced in trained rats but not in untrained rats.

Training

The rats trained in the swim and dive protocols were not “exercise” trained (21, 22). During each training session the trained rats were handled for a maximum of 10 min during their three trials. Of that 10-min period, the rats were only in the water for ~1 min. The rats were not made to swim or dive against a current (21, 22, 50), and they progressed through the maze at their own pace. Additionally, there were no differences between preswimming and swimming HR, as occurs in swim-exercised rats (22).

Daily human handling was not a particularly stressful event for rats. Plasma corticosterone levels were not significantly different between rats that were handled daily (handled) and rats that were never handled (naive). However, although both HR and MAP increased in rats being handled by a human vs. rats left alone in their cages, there were no differences in HR or MAP between trained and untrained rats when being handled. Thus, repetitive training does not attenuate the cardiovascular responses caused by human handling. Also, rats were easily trained to swim and voluntarily dive through the maze, as has been found previously (32, 35). However, corticosterone levels were not significantly different in the trained rats during swimming and voluntary diving than in the handled rats, suggesting that swimming and voluntary diving is no more stressful to trained rats than is human handling alone.

During voluntary diving, there were no significant differences in the prediving and diving HRs and BPs between trained and untrained rats. However, plasma corticosterone...
was lower in trained rats vs. untrained rats during voluntary diving. Thus, it appears that repetitive daily training decreases the stressfulness associated with voluntary diving, although it has no effect on diving HR or MAP. Additionally, during forced diving, there were no significant differences in the prediving and diving HRs and BPs between trained and untrained rats. However, in trained rats, forced-dive plasma corticosterone increased compared with voluntary dives, such that the plasma corticosterone in both the trained and untrained rats were not significantly different during forced dives. This suggests that rats find forced diving stressful, even if they have been trained in the forced-dive procedures. Our results also suggest that swimming and diving can be stressful to rats if they have not been trained to perform these behaviors. Therefore, on the basis of these results, our third hypothesis stating that training rats in the voluntary and forced dive procedures will decrease the magnitude of the cardiovascular responses to diving compared with untrained rats is rejected. There were no differences in the cardiovascular responses to voluntary and forced diving between trained and untrained rats. Our fourth hypothesis stating that training rats in the voluntary and forced-dive procedures will decrease the amount of corticosterone produced during voluntary and forced diving compared with untrained rats is partially accepted. Repetitive dive training reduces the amount of corticosterone produced during voluntary dives but does not reduce the amount of corticosterone produced during forced dives.

Habituation is a decrease in the responsiveness to a repeatedly presented stimulus (39). Habituation is more likely to occur with less intense stimulation and may not even occur if the stimulation is sufficiently intense (39). From our results with the trained animals, habituation, as determined by corticosterone levels, occurred during voluntary dive training but not during forced dive training. This suggests that to rats, diving per se is not an extremely stressful event and is something to which they can habituate. In contrast, forced diving appears to be a stimulus of sufficient intensity to which habituation does not occur in rats, although habituation to facial immersion does occur in harbor seals (15). The trained rats became increasingly reluctant to enter the restraining device with subsequent training sessions, which might have contributed to the increased plasma corticosterone levels during forced diving in the trained rats. Therefore, it appears that the restraint of a rodent Plexiglas holder, as mild as it may seem, could be the stressful component of forced diving (40, 46), rather than the water submersion. We feel that ultimately this influence is neurally or hormonally mediated is debatable. However, blood corticosterone levels were increased in rats handled daily vs. rats left alone in their cages, suggesting a hormonal mechanism may be involved in the suprabulbar modification of HR.

Atypical Diving Responses in Rats

The fact that the diving bradycardia in rats was not different between voluntary and forced dives is an interesting finding. This suggests that rats, at least domesticated Sprague-Dawley rats, are unlike feral animals in regard to cardiovascular control mechanisms. There are several possible explanations as to why the voluntary and forced dive responses of domesticated laboratory rats are atypical compared with most other animals that have been studied (6, 20). These possibilities include 1) rats may be unable to modify their HR through descending suprabulbar influences; 2) rats may be unable to distinguish between voluntary and forced diving scenarios; and 3) rats may lack the ability to modify the degree of parasympathetic outflow to the heart during the initiation of an intense cardiorespiratory response (i.e., the diving response).

Rats may be unable to modify their HR through descending suprabulbar influences. This seems an unlikely possibility to explain the lack of differential responses to voluntary and forced diving in rats. Human presence and handling have the effect of increasing both HR and MAP, and the withdrawal of human presence causes a decrease in both HR and MAP. Thus, suprabulbar influences can affect HR in rats, although whether this influence is neurally or hormonally mediated is debatable.

Rats may be unable to distinguish between voluntary and forced diving scenarios. This possibility also seems unlikely to explain the lack of differential responses to voluntary and forced diving in rats. The initial diving and highest diving MAP were both significantly higher in forced dives compared with voluntary dives. Changes in MAP during diving are produced by increases in sympathetic output to peripheral vasculature, suggesting that there is a greater sympathetic outflow during forced dives than during voluntary dives. Also, in trained rats, both blood corticosterone concentrations and fecal pellet production were significantly greater during forced dives compared with voluntary dives. Thus, rats do have the ability to distinguish between voluntary and forced dive scenarios, even if diving HRs were not significantly different between voluntary and forced dives.

Rats may lack the ability to modify the degree of parasympathetic outflow to the heart during the initiation of an intense cardiorespiratory reflex. The immediate time course of the HR decrease during both voluntary and forced dives suggests substantial vagal activation upon submersion. Muscarinic blockade with atropine eliminates diving bradycardia (30, 34, 51), while β-adrenergic blockade with nadolol contributes little to the development of diving bradycardia (50, 51). Thus, parasympathetic activation of the cardiac SA node is thought to be the primary efferent neural pathway for cardiac adjustments during diving (6).

Signore and Jones (50, 51) suggested that during diving, an accentuated antagonism exists between the parasympathetic and sympathetic branches of the autonomic nervous system. Accentuated antagonism means that in the autonomic control of HR, even relatively weak parasympathetic activity can overpower strong sympathetic stimulation (27). In addition, at high levels of vagal activity, changes in sympathetic activity...
will have only a negligible effect on HR (26). Thus, during diving in rats, the large parasympathetic output that causes HR to decrease by 75% within a single beat could block the chronotropic response to sympathetic stimulation of the heart.

Accentuated antagonism has been reported to affect diving bradycardia in muskrats (50, 51), harbor seals (11), and diving ducks (Aythya affinis; 37). However, even with the predominating effects of the parasympathetic nervous system, diving bradycardia can vary with physical circumstances and behavioral context, and likely involves a strong psychogenic component in muskrats (30, 33) and harbor seals (15, 16). Assuming then that the sympathetic nervous system has a limited ability to alter HR during diving, the variable diving bradycardia in muskrats observed during different diving conditions would be a result of variations in cardiac vagal outflow (50, 51). Thus during a forced dive, greater parasympathetic activity would then result in a more intense bradycardia.

Although feral animals like muskrats may have the ability to alter the degree of cardiac parasympathetic activity during different types of dives, this may not be the case for laboratory rats. If accentuated antagonism occurs in rats so that the sympathetic nervous system has a limited ability to alter HR during diving, then any change in HR during diving would be due to differential parasympathetic activity. Present results show that diving HRs, percent bradycardias, and absolute decreases in HR were similar between both voluntary and forced diving. The lack of differences between voluntary diving and forced diving HRs suggests there was no differential parasympathetic activity during these dives. Also, the magnitude of the bradycardia and decrease in HR suggests that the parasympathetic activity was near to its maximal output during both voluntary and forced dives. Thus rats may lack the ability to modify the degree of parasympathetic outflow to the heart during the initiation of an intense cardiorespiratory reflex such as the diving response.

The increase in MAP after its initial decrease upon submersion during both voluntary and forced diving presumably was due to sympathetically mediated peripheral vasoconstriction that increased total peripheral resistance during the dive. The slower (~4 s) time course of the pressure increase likely reflects the sympathetic nervous system’s inability to make rapid resistance changes to match the parasympathetically generated step-like decrease in cardiac output upon submersion (6). Also, MAP was higher in forced dives than in voluntary dives, which may indicate a greater sympathetic activity during forced dives. Thus, while rats may lack the ability to modify parasympathetic outflow to the heart during diving, they may have the ability to modify sympathetic outflow to peripheral vasculature depending upon the diving conditions. Dissociation of the parasympathetically mediated bradycardia and sympathetically mediated peripheral vasoconstriction during diving has been previously demonstrated (38, 50, 51). However, although cardiac sympathetic-parasympathetic autonomic coactivation may play a limited role in the development of the bradycardia during diving in rats, cardiac sympathetic activity may still have inotropic or dromotropic actions during diving (44, 45).

**Perspectives and Significance**

The present research using implantable radiotelemetric transmitters indicates that rats, like other small mammals, express the classical diving response during voluntary submersion. Implantable transmitters are less stressful to animals than other methods of blood pressure recording (24). Thus, the cardiovascular responses to diving that we measured were without the confounding influence of trailing arterial cannulas (34, 41, 42). Corticosterone measurements indicate swimming and voluntary diving are no more stressful to properly trained animals than human handling alone. However, in trained and untrained rats the cardiovascular responses to voluntary diving were similar, while the blood corticosterone levels were different. Thus, researchers should be aware that the endocrine and cardiovascular systems of rats can respond differently to a given situation and should be cautious about relying solely on cardiovascular responses when assessing stress levels in rats. Researchers also need to be aware that repetitive behavioral training can decrease the stressfulness of a particular situation, assuming the intensity of the situation is low enough to allow habituation. Thus, repetitive daily training reduced corticosterone production during voluntary diving but not during forced diving. The diving HRs of rats appear to be subject to an accentuated antagonism between the parasympathetic and sympathetic nervous systems. The near maximal parasympathetic activation that produces the diving bradycardia appears to be so great that the sympathetic nervous system has little influence on dive HR. Additionally, laboratory rats apparently lack the ability to vary the parasympathetic outflow during different types of diving situations. Thus, the magnitude of the diving bradycardia in rats was similar during both voluntary and forced dives. This makes the diving response of rats dissimilar to the diving response of most other animals and may highlight a difference in the cardiorespiratory control systems of feral animals, such as muskrats and purposefully bred domesticated rats.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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