Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability

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Received 5 February 2004; received in revised form 28 April 2004; accepted 10 May 2004

Abstract

We studied visual discrimination learning in a group of Nigerian dwarf goats using a computer-based learning device which was integrated in the animals’ home pen. We conducted three consecutive learning tasks (T1, T2 and T3), each of which lasted for 13 days. In each task, a different set of four visual stimuli was presented on a computer screen in a four-choice design. Predefined sequences of stimulus combinations were presented in a pseudorandom order. Animals were rewarded with drinking water when they chose the positive stimulus by pressing a button next to it. Noninvasive measurements of goats’ heartbeat intervals were carried out on the first and the last 2 days of each learning task. We analysed heart rate (HR) and heart rate variability (HRV) of resting animals to study sustained physiological effects related to general learning challenge rather than acute excitement during an actual learning session. The number of trials to reach the learning criterion was 1000 in T1, when visual stimuli were presented to the goats for the first time, but decreased to 210 in T2 and 240 in T3, respectively. A stable plateau of correct choices between 70% and 80% was reached on Day 10 in T1, on Day 8 in T2 and on Day 6 in T3. We found a significant influence of the task and of the interaction between task and day on learning success. Whereas HR increased throughout T1, this relationship was inverted in T2 and T3, indicating different effects on the HR depending on how familiar goats were with the learning task. We found a significant influence of the task and the interaction between task and time within the task on HRV parameters, indicating changes of vagal activity at the heart. The results suggest that changes in HR related to learning were predominantly caused by a withdrawal of vagal activity at the heart. With regard to nonlinear processes in heartbeat regulation, increased deterministic shares of HRV indicated that the animals did not really relax until the end of T3. Comparing changes of HR and HRV in T3 and in a subsequent postexperiment (PE), we assume a positive effect of such cognitive challenges once the task had been learned by the animals.

Keywords: Dwarf goats; Visual discrimination; Learning; Heart rate; Heart rate variability; Polyvagal theory

1. Introduction

The number of studies which use operant learning techniques in farm animals has been rapidly increasing [1,33,34]. In recent years, research focused on general aspects of cognitive abilities in farm animals, and also investigated factors which influence learning [13,15,22,43]. Increasing knowledge about animal cognition also inspires further research on how we treat and house animals. Boring husbandry conditions may be psychologically underdemanding and thereby cause suffering in farm animals [50]. One way to overcome the problems related to boredom is to engage animals to learn specific tasks to get a reward (e.g., food or water) and by this, at the same time, give the animals the opportunity to control at least some part of their environment. Such techniques have been used increasingly in zoo management since a number of years [7], but are still rare in farm animal husbandry. Basic research on learning often addresses how stress can interfere with learning [8,9,27,28]. However, learning may act as a stressor in itself. The latter is of particular interest with regard to the use of cognitive challenging tasks in animal husbandry in the future and the related issue of farm animal welfare.

This study had a dual purpose: to investigate visual discrimination learning in Nigerian dwarf goats (Capra hircus) and to assess sustained physiological effects associated with learning. We studied learning behaviour apply-
ing a fully automated learning device which was integrated into the animals’ home pen. To consider long-term effects related to learning, we analysed noninvasively measured heart rate (HR) and heart rate variability (HRV) of the animals at the beginning and the end of the learning tasks, i.e., with low and high learning success. HR reflects the net effects of both branches of the autonomic nervous system on the heart. Until now, almost any fluctuation of HR in farm animals in reaction to a stressor has been predominantly discussed in relation to changes of the sympathetic tone \([6,29]\), without considering parasympathetic influences. In contrast, Porges \([35]\) has postulated that the sinoatral node is predominantly under parasympathetic control, especially under resting conditions. There is increasing evidence that changes of vagal activity as indicated by fluctuations of HRV play an important role in regulation of HR in reaction to emotional or psychological stress, especially in the long run \([24,32]\).

2. Animals, materials and methods

2.1. Animals and housing

We tested visual discrimination learning in 12 male Nigerian dwarf goats from 15 to 22 weeks of age. During the learning experiment, goats were kept in an indoor pen \((4 \times 3 \text{ m})\) with straw as litter. The pen contained the learning device, a wooden pyramid to climb on, and a hayrack. Hay was offered ad libitum, and concentrate was offered at a total amount of 300 g/day/animal. Drinking water was delivered in the learning box as described below. All animals wore a collar with a responder for individual recognition in the learning box (Urban, Germany). All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agricultural of Mecklenburg-Vorpommern, Germany.

2.2. The learning device

The computer-controlled learning device was installed in a box \((850 \times 750 \times 300 \text{ mm})\) and was active 24 h/day. Only one animal could enter the box at the same time. The learning device consisted of a 15-in. TFT screen protected by a transparent acrylic pane in front, a water bowl fixed 20 cm below the screen to deliver drinking water, and an aerial for individual identification of the animals (Fig. 1a). A cross was fixed on the acrylic pane to separate the pane and the screen behind it into four similar sectors. In the outer corner of each sector, a plastic button was mounted on the pane. The buttons could be pressed by the goats with their nose-bridge (Fig. 1b) to get 30 ml of drinking water. The custom-written software controlling the device allowed simultaneous presentation of four visual stimuli on the screen, one in each sector. The programme controlled the presentation of predefined sequences of stimulus combinations and the delivery of water depending on the animals’ actions at the buttons. In addition, it recorded the times individuals entered into the box and left it as well as all button presses during a visit.

2.3. The shaping procedure

Goat kids were grouped together after weaning at an age of 6 weeks. Over a period of 8 weeks, they were then shaped stepwise to press buttons to get their drinking water. In the first stage, water was offered ad libitum in a slightly backward-sloped bowl, so that during drinking, the animals’ nosebridges inevitably touched a button mounted at the back board. In the next stage, the animals had to press different buttons to get a small amount of water. At first, either button triggered water delivery, thereafter only one button worked at a time (position changed daily but pseudorandomly). When the goats reliably pressed the buttons to get drinking water, the learning experiment started.

2.4. The learning experiment

We carried out three consecutive visual discrimination tasks (T1, T2 and T3), each of which lasted for 13 days. When a goat entered the box, its responder was identified, and four stimuli were immediately presented on a white computer screen, with one stimulus in each sector. The
stimuli were black shapes on a white background. One stimulus was rewarded (S+) and three were unrewarded (S−). Positive and negative stimuli contained equal amounts of blackness. The three S− were identical to each other in T1, but different in T2 and T3 (Fig. 2a). The stimuli used within a task were the same for all goats.

To get drinking water as a reward, goats had to choose S+ by pressing the related button. Each trial was followed by an intertrial interval (ITI) lasting 6 s (black screen), before the same four stimuli were presented on different positions determined by a pseudorandom series. In T1, only four different combinations were possible, but in T2 and T3, we used 24 different combinations. The sequence of patterns were the same for all goats. The controlling software ensured that position preferences were counteracted: when an animal showed a position preference, the S+ was not offered on that position anymore for a number of trials, until the preference was no longer observable. To check for spontaneous preferences for a single stimulus, we conducted a one-day pretest (PT) using the same stimuli and the same sequence of patterns as in the following tasks, but with all buttons rewarded. After T3, a postexperiment (PE) followed for 10 days. During PE, we presented a white screen, and all buttons were rewarded. The PE served to study effects caused through removing a previously provided cognitive challenge which the animals had learned. Fig. 2b shows an overview of the time schedule for the whole experiment. We surveyed the number of individual correct choices per day every evening to ensure that all animals got sufficient daily water.

2.5. Heartbeat measurements

The telemetric system Polar S810 (Polar Elektro Oy, Finland) was used for noninvasive measurement of heartbeats (interbeat interval, IBI) in the goats. The system consists of a chest belt with integrated two electrodes and a radio transmitter for wireless data transmission, a wristwatch-like receiver plus data logger, an interface for downloading the data to a PC, and corresponding software (Polar Precision Performance SW 4.00.023). The Polar system has been used recently with success to monitor HR and HRV in humans as well as in pigs, cattle and horses [2,32,47]. The goats were habituated to the equipment for a total of 5 days in the last 2 weeks of the shaping period. All goats were shaved on the left side and electrode gel was used for better electrode-to-skin contact. The chest belt was additionally protected by a stretch belt.

Baseline recordings of IBIs (tachogram) were conducted on 2 days during the last week of shaping. Throughout the learning experiment, we recorded IBIs in two periods: on the first 2 days (beginning, B) and the last 2 days (end, E) of T1, T2, T3 and PE, respectively. On the first day, we recorded IBIs in six goats from 0800 to 1100 h and in the other six animals from 1300 to 1600 h. On the second day, we switched the times, so we got a tachogram of 3 hours for each individual once in the morning, and once in the afternoon within each 2-day period. To minimize influences on IBIs caused by different levels of physical activity, and to study long-term effects on HR related to learning, we only considered parts of the tachogram when the animals were lying down since at least 5 min and seemed calm and undisturbed. To this end, we videotaped the behaviour of the goats during the measuring periods. By doing so, we did not obtain HR measurements at fixed times since the last drinking episode. However, more important for this approach, we ensured that HR data were derived from calm and unexcited animals. In all cases, at least 15 min passed by since the last drinking episode. Automatic correction of the tachograms was carried out using the correction routines included in the Polar software. Finally, only parts of the tachogram which lasted between 10 and 20 min and which had a corrected fault rate of less than 10% were included in the analysis.

All calculations of the tachograms were carried out in the program MULTIDAT [31]. HR (bpm) and several HRV parameters were calculated as mean of gliding 5 min windows which moved over the data set with a temporal shift of 150 s. In the time domain, we quantified the following: (1) The root mean square of successive differences of IBIs (RMSSD, ms). The RMSSD reflects alternations in the vago-sympathetic balance that are vagally

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**Fig. 2.** (a) Visual stimuli used in the three learning tasks. The correct stimulus within each task is placed in the upper left corner in this example. (b) Time schedule of the different periods of the learning experiment (PT1–3 = pretest; T1–3 = learning tasks; PE = postexperiment; for details, see text).
mediated. (23) The standard deviation of all IBIs (SDNN, ms). The SDNN is a more complex parameter reflecting vagal as well as sympathetic influences on HR. (3) The ratio between RMSDD and SDNN, which is a global indicator for general changes of the vago-sympathetic balance of the organism [45].

For the quantification of nonlinear processes of the HRV, we used the recurrence quantification analysis (RQA) [49]. Using this method, we quantified the percentage of recurrent points in the recurrence plot forming upward diagonal lines, i.e., deterministic sequences of recurrence (DET).

2.6. Statistical analyses

Button presses in PT1–PT3 were analysed for spontaneous preferences for a stimulus using the two-tailed Mann–Whitney test. We tested the number of choices of S+ in each sector against the mean number of choices of the other three stimuli in the same sector summarised over all animals. Learning success in T1–T3 was analysed for each individual on the basis of button presses in reaction to pattern presentations. We calculated success rate on the basis of blocks of 50 trials and on a daily basis, respectively. Because of the four-choice design (level of chance 25%), the learning criterion was calculated as 46% of correct choices (P < .001; according to the Binomial test with P0 = .25 and n = 50) which is much lower compared to the learning criterion of 72% in similar studies applying the common two-choice design. An influence of the task on the number of trials to reach the learning criterion was tested applying one-way ANOVA, followed by Tukey’s post hoc test. Data on daily success rates were tested by a general (mixed model) ANOVA with learning task (T1–T3) as fixed factor, test day (1–13) as repeated factor and corresponding interaction terms.

Because HR and HRV parameters did not differ significantly between Day 1 and Day 2 of the two-day measuring periods, nor between morning and afternoon (paired t test; P < .05), we considered data on these 2 days as measurement repetitions. Effects of the learning task and the period (B or E) on HR and HRV were tested by a general (mixed model) ANOVA as above, with the fixed factor learning task (T1–T3), the repeated factor period (B, E) and the corresponding interaction terms. For post hoc tests between tasks on single days with regard to success rate and between the periods with regard to HR and HRV, the Tukey–Cramer correction was used, to ensure a multiple type I error probability of less than .05. Comparisons of HR and HRV parameters between shaping (as control) and each learning task (T1–T3) were carried out separately for B and E using the Dunnett–HSU test. All models were calculated with the procedure MIXED in SAS (SAS Systems, Release 8.2, SAS Institute, Cary, NC).

3. Results

Two goats did not reach the learning criterion in T1 and T2, one of them failed in T3 as well. Nevertheless, these animals got their drinking water, too. They drank remaining water from successful learners or they displaced other animals from the learning box to steal their water. These animals were excluded from all further analysis. Therefore, all calculations were done with a sample size of 10.

The number of trials per day and individual was similar in PT1–PT3 and in PE, when all buttons were rewarded. It ranged between 34 and 44 presses. Analysis of spontaneous preferences for a single stimulus in PT1–PT3 revealed a tendency for a preference of S+ in T2 and T3 (P = .057 and P = .056). In T1, the mean number of trials per day per individual increased to 250 during the first 3 days and decreased thereafter to a stable level of about 80 trials per day. In T2 and T3, the number of trials was about 100 on Day 1 and decreased continuously to 60 trials per day at the end of both of these tasks. The mean number of trials to reach the learning criterion in two consecutive blocks of 50 trials was 1000 in T1, 210 in T2 and 240 in T3. There was a significant influence of the task on the number of trials to reach the learning criterion (ANOVA, df = 2, F = 26.97, P < .001). Post hoc tests revealed differences between T1 and T2 and between T1 and T3 (P < .001).

Fig. 3 presents the least square means (LSM) of the percentage of correct choices in T1–T3 on a daily basis. There was a significant influence of task, test day and of the interaction between task and test day on success rate (P < .001). Multiple post hoc tests showed that mean success rate in T1 was significantly lower compared with T2 and T3, respectively, through Days 1–9 (P < .05). However, no such differences were found between T2 and T3. In T1, mean success rate started below the level of chance. The rise of the learning curve was slow during the
first 3 days, and much steeper between Days 4 and 8. The learning criterion was not reached until Day 7. In contrast, the learning criterion was already reached on Day 1 in T2 and in T3. However, the following rise of the learning curve was not as steep as in T1.

Fig. 4 shows the LSM of the HR in all measuring periods. There was a significant influence of task \( (P<.001) \) as well as of the interaction between task and period \( (P<.001) \) on HR. Whereas HR was lower in B compared with E in T1, this ratio was inverted in T2 and T3 (all \( P<.05 \)). The overall level of HR was lower in T3 compared with T2 (ANOVA, \( df=1, F=10.29, P<.002 \)). In PE, HR again was lower in B compared with E \( (P<.05) \). Compared with Shape, HR was higher during T2-B and lower during T3-E and PE-B \( (all \ P<.05) \).

Fig. 5 summarizes the LSM of three linear and one nonlinear HRV parameters in all measuring periods. There was a significant influence of task as well as of the interaction between task and period on RMSSD, the ratio of RMSSD/SDNN and DET \( (P<.05) \), but not on SDNN. Therefore, multiple post hoc tests were carried out only for the first three parameters. Significant differences between B and E within a task were found in T3 and in PE only \( (P<.05) \). There were significant differences between Shape and T2-B for RMSSD/SDNN and between Shape and PE-B for RMSSD, RMSSD/SDNN and DET \( (all \ P<.05) \).
4. Discussion

4.1. The experimental design

In contrast to previous visual discrimination experiments in goats [4,5,44] as well as in other farm animals [13,16,21], we investigated learning behaviour applying a group learning design and integrated the learning device into the animals’ home pen. By doing so, additional excitement or artificial arousal in the run-up to the learning sessions were avoided. Moreover, learning success in an experimental compartment and under human supervision does not guarantee that animals will be able to recall what they have learned adequately in their normal home pen, because of the different context [46]. The approach applied here seems well appropriate to study learning abilities of a larger number of animals under normal husbandry conditions. For similar reasons, we preferred the four-choice design over the common two-choice design, as in normal life animals have frequently to choose between more than two stimuli simultaneously. Drinking water was assumed to be an adequate reward because it was consistently requested by the animals but not overused when quasi ad libitum available with high learning success. This was affirmed by identical levels of daily water consumption in PT1–PT3, in PE and at the end of T1–T3. Nevertheless, a drawback of the experimental design was that goats could apply strategies different from visual discrimination learning to get rewarded. However, we did not find any indications that individuals who drank remaining water from successful learners had a specific influence on learning behaviour of other individuals.

4.2. Learning performance

Although the motor pattern of button presses was well shaped, the presentation of visual stimuli on the computer screen was new to the goats in T1. Normally, introducing a new type of stimuli is part of the shaping process [16,37]. Therefore, we assume that the slow increase of success rate during the first 3 days of T1 revealed the time the animals needed to from an association between the visual stimuli on the screen, the related buttons and the reward. Moreover, a success rate below the level of chance indicated persistent field preferences during this time. Although such side or button preferences are often the strategy of choice at the beginning of discriminatory learning tasks [10,51], this phenomenon has been discussed only marginally in many studies. Without counteracting field preferences, it takes animals much longer to learn similar discriminatory tasks like in this study. The steep increase of the learning curve beginning from Day 4 in T1 and accompanied by a sharp decrease of button presses indicate that the animals then locked on the stimuli as meaningful indicators of the reward, and real discriminatory learning started. The number of trials to reach the learning criterion was higher in T1 compared with similar experiments in other farm animals [15,37,51]. This was probably caused by the fusion between shaping to the screen presentation and visual discrimination learning. When comparing the number of trials with the performance of Spanish goats, which needed between 1000 and 2300 trials for shaping of the task plus learning the S+ [5], performance in our study was much higher. Probably, this was due to the higher salience of S+ contrasting to three identical S−.

Visual learning performance in T2 and T3 was higher than previously reported for goats [4,5], but fits recent findings in horses [15,38], which performed classic two-choice discrimination tasks using similar visual stimuli. This may indicate (a) higher learning performance of goats applying the four-choice design compared with the two-choice design and (b) their performance in simple visual discrimination tasks to be not much different to that of horses. Almost identical learning curves in T2 and T3 revealed similar abilities of goats to discriminate closed shapes and open letters. In contrast to learning experiments with a fixed number of sessions and trials per day, with our experimental design we included trials during which the animals probably did not spend much attention to the task or just played with the buttons which they may have perceived as an environmental enrichment. As a consequence, learning curves approached an asymptote which did not exceed 80% success rates in any of the three tasks.

Our results do not indicate that goats are able to use previously learned information to increase learning performance in subsequent tasks. This ‘learning-to-learn’ phenomenon [17] has recently been shown for horses [15,16,38]. However, in these studies, animals were confronted with a larger number of consecutive combinations of stimuli before they started to establish a “learning set”.

4.3. Changes of the HR related to visual discrimination learning

Mental effort during learning and the restricted delivery of the reward in a learning experiment may act as stressors [3,42]. Whereas mental effort acts as an acute stressor during learning, the latter probably triggers long-term effects. Hitherto, indicating such long-term effects by changes of the HR have been discussed controversially [30,35,39], probably because it is difficult to separate them from actual short-term effects. With the approach to analyse HR in resting and calm animals, we tried to exclude short-term effects caused by changing physical activity, excitement or acute mental effort, but instead intended to reveal the long-term effects of learning on HR.

According to the coping/predictability concept by Stephens and Henry [18,19], the behavioural and physiological responses to a challenging situation mainly depend on its cognitive evaluation by an individual. Unpredictable or uncontrollable situations will activate the HPA axis, leading to depression of behaviour combined with reduced or, at
least, not increased HR [35,48]. The ability to cope with a stressor (controllability) is thought to be under control of the sympathetic nervous system and generally accompanied by an increase of HR. For the goats, T1 meant a situation for which they had no coping strategy until they established an association between the visual stimuli on the screen and the related buttons to get the reward. This was apparent from the learning performance below level of chance throughout Day 1 to Day 3. Therefore, low HR at the start of T1 may be interpreted as a physiological sign of a certain level of frustration in the goats because of the loss of control over drinking water. More sustained effects of learning on resting HR were observable at the end of T1. The learning curve reached an asymptotic level already at Day 10, but the animals still seemed to be challenged by the learning task 2 days later, as the increased level of HR indicated. Results in T2 and T3 were inverted to those in T1. Increased HR at the start of T2 and T3 indicated that the animals were challenged but, probably, not frustrated by the new tasks, as they actively dealt with the situation, following their experience in T1. They knew the task and showed a higher level of learning success right from the beginning of the tasks. We assume that decreased HR at the end of T2 and T3 together with high learning success indicated real relaxation of the animals as they reached a high level of control over the reward. Moreover, a general adaptation to the learning task was evident in T3 when the level of HR was lower compared with T2.

Removing the cognitive challenge by offering a white screen and rewarding all buttons in PE resulted again in an increase of HR. Gaining control over the situation and the reward in T2 and T3 which resulted in a reduction of HR, was probably disturbed in the PE when the learning task was removed. In summary, with regard to the modifications of HR throughout the learning experiment, we assume that the combination of the delivery of a special resource with a challenging cognitive task represented some kind of “positive stress” once the animals had understood the task and learned to recognize the S+. This would be in good agreement with effects of environmental enrichment suggested for zoo animals [7]. Positive effects of learning tasks, as some form of cognitive enrichment, for captive animals has just begun to be systematically investigated [26].

4.4. Autonomic control of HR

The analysis of HRV allows to differentiate which branch of the ANS mediates the effects of a challenging situation on the HR [14]. Whereas RMSSD has been broadly accepted as a reliable marker of vagally mediated variations of the HRV [23], it seems that SDNN is rather a marker of combined sympathetic and parasympathetic activity at the heart [20]. In this study, the RMSSD strongly mirrored the changes in HR. At the same time, changes of the SDNN were quite similar to the course of the RMSSD but less distinct. Studies in rats and horses have emphasized the role of sympathetic activation in reaction to psychological stress [40,41,47]. However, these investigations analysed HR of active animals when the vagal tone was already reduced. Studies in humans in resting conditions considering effects of mental workload on the cardiovascular system confirm a shift towards sympathetic dominance, but predominantly as a result of vagal withdrawal [25]. Results presented in this study from measurements on resting animals also suggest that long-term effects on HR related to visual discrimination learning were mainly caused by modifications of the vagal tone at the heart. These results are in good agreement to the Polyvagal theory [35,36], which considers a branch of the vagus nerve as the main mediator of emotional and psychological stress to the heart.

The HRV is an integrated signal which is influenced by a number of physiological control circuits which are neurologically interconnected. Therefore, HRV is affected by several feedback and feed-forward mechanisms. In recent years, a number of studies have shown that HRV contains nonlinear components in the sense of deterministic chaos [52]. For the analysis of heartbeat dynamics, RQA has been applied successfully in human medicine and in animal models to quantify such nonlinear processes [11,12,32]. Determinism, as calculated by RQA, is a variable for the regularity of the tachogram in an adequate multidimensional space which cannot be proven in the original time series. Seeing heartbeat regulation as a more stochastic process, a low level of DET in the HRV at the end of T3 indicated reduced correlation time of the heartbeat with a consequent decrease in the general predictability of the signal. In other words, the effect of controlling the learning device on cardiac dynamics is suggested to increase total cardiac flexibility. These results match our findings concerning HRV parameters in the time domain. Moreover, they are in good agreement with other studies, when challenging situations or an experimental withdrawal of vagal tone via the application of atropine caused an augmentation of RQA parameters of the HRV [12]. However, whereas changes of the HRV in the time domain mainly mirror vagal activity at the heart, the RQA delivers a more general statement about how seriously an organism is challenged by a specific situation.

Acknowledgements

The authors wish to thank K. Siebert, D. Sehland and U. Engel for care for the animals, data analysis and diverse technical assistance. We are also grateful to Kristin Hagen for commenting on a previous version of this paper.

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