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Carrier training cats reduces stress on transport to a veterinary practice

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ABSTRACT

Transport to and visiting a veterinary practice frequently is stressful for cats and their owners. Handling a fearful cat can be a challenge for the veterinary team and stress can influence physiological parameters. This study investigated whether carrier training reduced stress during a 10 min transport by car and increased compliance during the veterinary examination. A blinded randomized controlled trial with a paired sample design (training group (TG): N = 11; control group (CG): N = 11) consisting of two visits to a sham veterinary practice was carried out. After all cats had completed the first visit, the training group received 28 sessions of positive reinforcement based carrier training over a period of six weeks. Thereafter, all cats underwent the second veterinary visit. Stress levels during the car ride were assessed on video recordings using a modified version of the Cat Stress Score (CSS) (inter-rater reliability: $r_s = 0.81$, $p < 0.02$) and behaviour analyses with Interact® Software (intra-rater reliability: $r_s > 0.90$, $p < 0.01$). To evaluate physiological reactions, ear temperature readings were taken before and after transport. The change in parameters between visit 1 and 2 (calculated by subtracting result of visit 1 from visit 2) was used to test for differences between groups. Trained cats showed a significant reduction in CSS during the car ride (mean ± SD: TG: − 0.60 ± 0.37, CG − 0.23 ± 0.25; p = 0.007), an increase in searching for food rewards (TG: 33 ± 24 s; CG: 6 ± 7 s; p = 0.001), lip licking (median (range): TG: 18 (7–65), CG: 0 (−3 to 24); p < 0.001), changes in body posture (TG: 13 (−2 to 46), CG: 1 (−5 to 13); p = 0.01) and sitting (TG: 233 ± 146s, CG: − 4 ± 35 s; p < 0.001). The veterinary examination was significantly shorter in trained cats (TG: − 42 ± 31 (−101 to −2) s, CG: −12 ± 24 (−50 to 31) s; p = 0.010). Significant differences in the response patterns of ear temperature further indicated lower stress in the TG (e.g. Δ ear_temp: GLMM: main effect group: p = 0.022; group*experiment: p = 0.019). Training proved to be effective in reducing stress during the car ride and led to a shorter veterinary examination. Owners should be encouraged and instructed to carrier train their cats to reduce stress around veterinary visits.

1. Introduction

A visit to a veterinary practice contains potential stressors that can compromise the cat's welfare. Such stressors include confinement to a carrier (Graham and Brown, 1996), transport, encounters with strangers and other animals, loud noises, unfamiliar smells, handling and a new environment (Carlstead et al., 1993; Carney et al., 2012; Stella et al., 2013). Owners may avoid veterinary visits for reasons of difficulties with getting their cats into the carrier and signs of stress and fear during transport and at the veterinary practice (Volk et al., 2011; Mariti et al., 2016). When feeling threatened cats may either try to escape, freeze or act aggressively to protect themselves (Bowen and Heath, 2005). Dealing with a fearful cat in the veterinary practice will increase the risk of injury to veterinary staff and owners (Drobatz and Smith, 2003), make procedures more time consuming (Carney et al., 2012) and require more staff to restrain the cat. Negative experiences during the veterinary visit will increase the fear on subsequent visits (Yin, 2009; Rodan, 2010) and thus make handling even more difficult. In addition, the release of stress hormones can lead to elevated blood glucose levels (Rand et al., 2002), lymphocytosis, neutrophilia and hypokalaemia (Griffith et al., 2000). They prepare the body for the fight or flight response and can increase heart and respiration rate, elevated blood pressure and hyperthermia (Rodan, 2010). Stress responses make it difficult to interpret clinical signs of disease and can diminish the validity of findings (Rodan, 2010). Awareness of these problems has grown amongst professionals and has been implemented in many guidelines to address this (Vogt et al., 2010; Rodan et al., 2011; Carney et al., 2012; Arhant et al., 2017). Guidelines now recommend that

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owners should accustom their cats to the carrier and get them used to car rides as an important step in making the veterinary visit less stressful. Positive reinforcement training methods have been successful in improving animal welfare, facilitating veterinary and husbandry procedures (Young et al., 2004) and decreasing physiological and behavioural aspects of acute stress in a variety of species such as nyalas (Grandin et al., 1995), bongos (Phillips et al., 1998), snow leopards (Broder et al., 2008), baboons (O'Brien et al., 2008) and chimpanzees (Lambeth et al., 2006). In cats, acclimation protocols to temporary restrictions within a respiration chamber (Gooding et al., 2012) and training for different methods of blood collection (Lockhart et al., 2013) were effective in reducing stress. A behavioural protocol for conditioning laboratory cats to handling and transport has been described by Gruen et al. (2013). Although they gained promising insights, the positive effects of training on fear reduction, stress responses and ease of handling were based on subjective observations only. Stress assessment usually includes observing the animal's behaviour, ideally in combination with physiological measures (Young et al., 2012). The Cat Stress Score (Kessler and Turner, 1997) has been widely used to assess stress in cats based on behavioural and postural attributes (Dybdall et al., 2007; Pereira et al., 2016; Stevens et al., 2016; Pankratz et al., 2017). Non-invasive methods to assess short-term physiological stress responses in cats used in other studies are measurements of salivary cortisol (Siegford et al., 2003), faecal cortisol metabolites (Palme et al., 2001) and ear temperature (Mazzotti and Boere, 2009). The aim of our study was to investigate the effect of carrier training on reducing stress during transport to a veterinary practice with objective behavioural and physiological measures. We hypothesized that trained cats would be less stressed during a transport by car than non-trained cats, that they would be easier to place into the carrier and be more compliant during the veterinary examination.

2. Animals, materials and methods

2.1. Animals

The study population consisted of 22 cats (13 male, 9 female; all neutered) aged between 8 months to 4.3 years (mean ± SD: 2.7 ± 1.1 years). The cats weighed between 3.11 and 5.28 kg (4.04 ± 0.66 kg) and were of the domestic shorthair (N = 21) and long hair mixed breed (N = 1). Each cat was identifiable through a subdermal microchip implant. All cats were up to date with their vaccinations and free of chronic health problems. The cats were declared as healthy by veterinary examination prior to the start of the study.

2.2. Housing

The cats lived at the Institute of Animal Nutrition and Functional Plant Compounds of the University of Veterinary Medicine Vienna (Vetmeduni, Vienna, Austria). They were housed in pairs or in groups of four to six cats in rooms sized between 14–29 m^2. All but one room had adjacent fenced outdoor areas (56–70 m^2) that were always accessible via a cat flap. Two cats were kept in an indoor only room. All rooms were equipped with shelves, elevated resting places, scratching posts, toys and hiding opportunities. Each room contained one water bowl and several litter trays. Daily caretaking routine included feeding a commercial cat food (Whiskas® wet food, Mars Austria OG, Breitenbrunn, Austria) twice a day, administering fresh drinking water, basic cleaning of the rooms and scooping the litter trays. Once a week the rooms were thoroughly cleaned by wiping the floors and washing the litter trays. Caretakers socially interacted with the cats daily. During the study period one main caregiver, several students of the animal care assistant school and two researchers (LP and JR) took care of the cats.

2.3. Study design and allocation to groups

A blinded randomized controlled trial with a paired sample design was used to conduct the study. The experiment consisted of two transports per cat to a sham veterinary practice where a veterinary examination was performed (test 1 and test 2). A total of 22 cats were assigned to either the training group (N = 11) or the control group (N = 11). The groups were balanced for age, gender, experience of recently being neutered and shyness with humans according to the caretaker report. During the first test (January 2015) baseline values of all measurements were collected. In the subsequent six week period the training group received a standardised carrier training. The second veterinary visit was conducted in March 2015. The study protocol was approved by the institutional ethics and animal welfare committee and the national authority according to §§ 26ff of Animal Experiments Act 2012 (Tierversuchsgesetz 2012 – TVG 2012, GZ 68.205/0216-WF/V/3b/2014).

2.4. Selection criteria

Cats' reaction to strangers was tested via an approach test prior to the study to ensure that we would be able to work with them (Arhant and Troxler, 2017). Additionally to be selected, each cat had to be willing to take treats off the hand of an unfamiliar person, tolerate being stroked once from head to tail along the back (dorsum) and being lifted up. For the safety of the cats and the people involved in the study the following exclusion criteria were set: systemic illness, injury, a severe stress or panic reaction, severe aggressive behaviour towards the handler or if the duration of placing the cat into the carrier exceeded 10 min. No cat had to be excluded based on these criteria.

2.5. Experimental procedure

The visits to the sham veterinary practice were conducted between 9:30 and 15:00. The cats were fed at last 1.5 h before the start of the test to minimise the risk of motion sickness. Only one cat per room was tested per day to avoid influences on behaviour caused by changes in daily routine or possible emissions of pheromones and unfamiliar odours by the tested cat. No cats were tested on weekly cleaning days. During the tests, researcher LP represented the "owner" of the cats and stayed with them throughout the transport and visit. Researcher NM was driving the car and conducting the veterinary examination as the "vet". The same car was used for the training sessions and during the tests. Two identical hard plastic cat carriers Model Atlas 30 open (Ferplast®, Castelgomberto, Italy; outside measurements: length 58 × width 37 × height 32 cm) were used for the training and the tests. This carrier has an easily removable top part, an additional door on the top and slit shaped openings on the sides and the back of the top part. During the experimental procedure the two carriers were used on a rotating basis. They were cleaned after each cat with an enzymatic detergent (Biodor® Pet Animal, Düren-Hoven, Germany) to minimise the influence of odours and to remove potential contamination with body excretions. For each cat a new towel was provided as padding in the bottom of the carrier. The towel had been in the cats' housing for at least 12 h to take up the familiar scent.

For the actual test a single cat was carried on the "owner's" arm into the training room, released on the floor and given an acclimatization period of 5 min. Thereafter the carrier was placed on the floor and presented to the cat with the front door opened. A period of 3 min was given to allow the cat to enter the carrier without interference. If the cat did not enter the carrier by itself, the "owner" attempted to place the cat gently into the carrier by these methods in subsequent order: 1. through front door; 2. through door on the top; 3. placement in bottom half of disassembled carrier, then reassemble carrier; 4. usage of a large towel to facilitate placing the cat through front or upper door. Once the cat was inside the carrier it received three treats. When the cat was in

the carrier a camera (Digital HD Video Recorder, Sony®, Tokyo, Japan) was installed on a special retainer and the cat's behaviour within the carrier was recorded through the front door for 3 min. Thereafter the cat (within the carrier) was brought to the nearby parked car. The carrier was placed on the back seat, with the front door facing driving direction, and secured with the seatbelt. A 10 min car ride on the estate of the University of Veterinary Medicine took place. The "owner" tossed three treats into the carrier shortly after the car ride commenced and then one treat per minute during the car ride. If treats were not consumed for several times in a row then no more treats were offered. To minimise uncontrollable external stressors a sham veterinary practice had been set up. In a building on the campus of the Vetmeduni two adjacent rooms were adapted as examination room (5 m × 3.48 m × 3 m) and as waiting room (5 m × 1.9 m × 3 m). The impression of a real veterinary practice was created by providing scents of other animals and disinfecting agents. Upon arrival at the veterinary practice a period of 5 min was spent in the waiting room. The carrier was placed on a chair and the "owner" took a seat next to it. The cat's behaviour during this time was recorded and one treat per minute was given. Subsequently, the "vet" asked the "owner" to proceed to the examination room. The carrier was placed on the examination table and the camera was dismounted. The front door was opened to allow the cat to exit within 3 min. If the cat did not leave the carrier within 3 min the top part of the carrier was removed. The bottom half of the carrier was accessible for all cats throughout the veterinary examination. A standardised veterinary examination in a predetermined order was carried out: evaluation of conjunctival colour by gently pulling right then left lower eye lid ventrally (2 s per eye), evaluation of oral mucosa by lifting upper lip (1 s), measurement of capillary refill time (3 s), inspection of distal part of external ear canal by gently pulling left and then right pinna dorsally (2 s per ear), auscultation of the heart sounds (first left side 60 s, then right side 15 s), auscultation of the lung sounds (first left, then right side; each 15 s), palpation of femoral pulse on right inner thigh (15 s), abdominal palpation (30 s) and measurement of rectal temperature with a digital thermometer (Versican®, Scala Electronic GmbH, Stahnsdorf, Germany). Cats were handled friendly by speaking in a soft and low voice, moving slowly and smoothly and by avoiding direct eye contact or leaning over the cat. Only minimal restraint was used by gently preventing the cats from moving away from the examiner. After the veterinary examination the fully assembled carrier was presented to the cat on the examination table. A period of 3 min was given to allow the cat to enter through the front door voluntarily. The same methods as at the beginning of the test were applied to place the cat into the carrier if necessary. If the cat was already sitting in the bottom half by the end of the examination the top half and front door were added. Treats were given once the cat was confined to the carrier. Finally, the cat was driven home on the shortest route and returned into the training room. The carrier was placed on the floor and the front door was opened to allow the cat to leave the carrier. After leaving the carrier the cat was returned to its housing.

2.6. Training

After completion of the first test the TG commenced their training. Positive reinforcement based training was used to teach the cats to willingly enter and stay in a cat carrier and to get used to car rides. Food was used as a primary reinforcer. Depending on the cat's preference, cat biscuits (Kitekat Knusperfit®, Whiskas® Knuspertaschen; Mars Austria OG, Breitenbrunn, Austria), meat sticks (Molly Fleischsticks®, SPAR Österreichische Warenhandels-AG, Salzburg, Austria), meat paste (Landhof® Kalbsleberwurst, Landhof GesmbH & Co KG, Linz, Austria) or canned tuna (Almo nature® Atlantikthunfisch, Almo Nature USA, Inc., Miami, USA) were given. The training was divided into seven phases (see Table 1) which were implemented consecutively. To proceed into the next phase, the aim or a maximum of six repetitions had to be achieved. Due to restrictions in time available the number of training sessions was limited to 28 per cat. The training was conducted as part of a diploma thesis (Rost, 2016) by researcher JR over the six week period between the first and the second test. Training sessions were held in the training room. The number of training sessions required to complete one phase ranged from two to six. On average a training unit lasted 8 min (range 3–16) and on average 4 treats were given per minute (range 1–10). Three cats completed the training protocol. Six cats reached phase 7 and two cats reached phase 6 without achieving the

Table 1
Training protocol.

Phases 1–7	Training methods	Aim of each phase (A) Rate of reinforcement (R)
1 Bottom half of carrier	• presentation of bottom half of carrier prepared with food • reward approach, stepping into bottom half with front feet, then completely entering it • once cat enters willingly reward stay in carrier • lure with treats if cat is not initiating desired behaviour • presentation of carrier bottom becomes cue to enter	A: voluntarily enter bottom half stay there for 1 min R: two treats/min
2 Complete carrier	• presentation of carrier prepared with food without front door • reward approach and entering carrier • lure with treats if cat is not initiating desired behaviour • initially reward stay in carrier, later calmly sitting or lying • front door added but left open in end stages of phase	A: voluntarily enter carrier stay there for 30 s R: two treats/min A: four out of five attempts to enter within 15 s of presentation
3 Stay in carrier	• build positive association with front door by giving treats while door is moved slightly • toss treats into carrier through front door, increase time of door being closed	A: stay calmly in carrier for 3 min R: one treat/min
4 Being carried	• reward being lifted up within the carrier for a short moment • extend time being lifted up • progress to slightly swaying carrier, repeat lifting up and walking a few steps • increase time being carried • reward sitting calmly if possible	A: stay calmly while being carried around for 1 min R: two treats/min
5 Stationary car engine off	• familiarisation with way to car and car • offering very tasty food (tuna) in car	A: stay in stationary car with engine off for 2 min R: one treat/min
6 Stationary car engine on	• familiarising cat with sounds of engine, car stationary • offering very tasty food (tuna)	A: stay in stationary car with engine on for 2 min R: one treat/30 s
7 Car ride	• short car rides (50–90 s) initially • reward calm behaviour or counterconditioning fear with food, petting and verbal praise • increase duration of car ride 2–4 fold if cat stays relaxed	A: 2 min car ride R: one treat/min

aim of the phase by the end of the training period. For familiarisation with researcher LP and the training room all cats received additional familiarisation sessions during the training period. Two individual 5–10 min sessions were held where LP offered treats, petted the cat and offered the cat to play with a fishing toy.

2.7. Behavioural measurements

2.7.1. Cat stress score

The Cat Stress Score (Kessler and Turner, 1997) allows classification of stress by observation. Based on behavioural and postural attributes the stress level is assigned between score 1 "fully relaxed" and score 7 "terrorised". The rating scheme from Dybdall et al. (2007) was slightly modified and used for analyses. Instead of a global score per time period, the categories 'body', 'belly', 'legs', 'tail', 'head', 'eyes', 'pupils', 'ears', 'whiskers' and 'activity' were rated individually. Each category had its own matrix (see supplementary material: Cat Stress Score Matrix) in which the body postures, facial expressions, behaviours and their corresponding scores were listed. Further alterations of this scheme were made by removing shaking in the category 'body' and removing ventilation in the category 'belly' as they could not be evaluated due to motions during the car ride. Purring (category 'vocalisation') was removed as it was not always audible on the videos. Panting was shown by a number of cats and therefore was added to the category 'belly' assigned with a score of five. The category 'pupils' was rated but due to different lighting conditions caused by seasonal changes during the first and second test pupil size was strongly influenced and was therefore excluded from the final analyses. For each time period a mean score of all categories was calculated. The cat's behaviour within the carrier in the training room, during the car ride and in the waiting room was recorded. The video recording before transport (duration in training room: 3 min) was rated during three time periods: minute 0:00–1:00, 1:01–2:00 and 2:01–3:00, the recording during the transport (duration of car ride: 10 min) at minutes 0:30–1:30, 2:30–3:30, 4:30–5:30, 6:30–7:30 and 8:30–9:30 (five time periods) and after transport (duration in waiting room: 5 min) at minutes 0:00–1:00, 2:00–3:00 and 4:00–5:00 (three time periods). The obtained mean scores were averaged to one score per location (training room; car ride; waiting room). Each time period was watched several times with the focus set on different categories, e.g. body region, legs, tail. A behaviour needed to be seen at least three times, or if less than three times, for 20 s continuously to be coded as present. To avoid missing values the video sequences before or after each time period were watched if it was difficult to allocate a score due to the positon of the animal (e.g. face averted from camera, legs not visible on the recordings). The tail was scored as close to the body if it was not visible at all. If a behaviour or body posture was ambiguous, e.g. ears between half back (score 2) and erected to back (score 4), the higher score was assigned. Behaviour coding of the video recordings was conducted by two observers blinded to the treatment. A total of 12 min video material (12 time periods) was observed to determine the inter-rater reliability ($r_s = 0.81$, $p < 0.02$) and the intra-rater reliability of each observer (both $r_s = 0.97$, $p < 0.001$). The observations were done on one minute episodes of different cats and time periods were equally chosen between the first and second test.

2.7.2. Continuous behavioural analysis during car ride

A more detailed behavioural analysis of the 10 min video recording of the car ride was performed with Interact®14 software (Mangold International GmbH, Arnstorf, Germany). Frequency and duration of the cats' behaviours in the categories body posture, activities, vocalisation and face were evaluated by one trained observer (researcher LP, intra-rater reliability $r_s > 0.90$, $p = 0.01$) by continuous sampling. A detailed description of all behaviours coded is provided in the supplementary material (The ethogram of behavioural responses rated with Interact® during the car ride).

Because the face or the front legs were not always visible for evaluation over the whole 10 min period, a rate per second was calculated for the frequencies (fr) of lip licking and panting, and the durations (dur) of panting and lying ventrally with front legs bent or extended. Hereto the following formula was applied: (frequency or duration of variable (seconds)/total duration face or front legs visible) × 600 (seconds). Results adjusted by this formula are marked with [c]. Purring was not always audible on the video recordings and therefore was rated only by direct observation during the car ride by researcher LP.

2.7.3. Compliance during placement into the carrier and during veterinary examination

Data regarding the compliance during placement into the carrier and the veterinary examination were obtained by direct observation. It was noted whether a cat entered the carrier voluntarily or if they needed to be placed into it. The ease of placing the cat into the carrier was categorised as: no resistance; splay out paws or claws against entrance of the carrier; trying to escape; mild aggression (hiss, growl or trying to scratch lightly); severe aggression (biting; scratching severely); impossible to place into carrier. The latency for the placement into the carrier was measured from presentation of the carrier until confinement of the cat inside the fully assembled carrier with doors closed. In the examination room the latency to exit the carrier was measured from the opening of the front door until the cat had all four paws outside of the carrier. A maximum of 3 min was given to exit the carrier voluntarily, thereafter the carrier was disassembled. It was noted if cats left the carrier voluntarily. The cats' location during the veterinary examination was allocated to one of the following three categories: only or mainly in the bottom half of the carrier; equally in bottom half of the carrier and on the examination table; or mainly or only on the examination table. The potential presence of aggressive behaviour during the veterinary examination was categorised as: no aggression; mild aggression (trying to scratch lightly); and severe aggression (trying to bite or scratch severely). Escape attempts and hiding during the veterinary examination were recorded. The steps reached in the veterinary examination were noted. Reasons for termination of the examination before completion were declared as repeated struggling (more than three times during the same step of exam), prolonged intense struggling (more than 2 s during same step of exam) or severe aggression (trying to bite or scratch severely). The duration of the veterinary examination was measured from the first step (conjunctival colour) until completion or the last step reached.

2.8. Physiological measurements

2.8.1. Pilot study: salivary cortisol

To ensure the usability of salivary cortisol as a measure of stress in cats a pilot study was conducted 10 days prior to the actual study. Its aim was to investigate the method of collecting the saliva with a rod-shaped swab (Salivette®, Sarstedt AG & Co, Nürnbrecht, Germany) and the influence of different food and time of feeding on salivary cortisol. All cats (N = 22) were familiarised with the sampling procedure before the pilot study (3–5 sessions per cat). For the pilot study 18 cats were randomly chosen and assigned to three groups that received either cheese, tofu or cat treats (meat stick). Three salivary samples were collected per cat: one before any food was given and two post-consumption (immediately and 20 min later). The samples were frozen shortly after collection until further analyses. After defrosting, the samples were centrifuged (Centrifuge GS 6KR, Beckman Coulter GmbH, Krefeld, Germany) for 10 min with 2500*g*. Only 23 out of the total of 54 samples contained sufficient saliva (50 µl) for further analysis. The salivary cortisol measurement was therefore excluded from the study.

2.8.2. Ear temperature

Ear temperature measurements were taken by researcher LP with the Instant Ear Thermometer for Pets (Pet-Temp®, Home Use Model PT-

300, Advanced Monitors, San Diego, U.S.A.) according to the manufacturer's instructions. The thermometer measures the tympanic membrane temperature, referred to as ear temperature within this article. The first measurement was taken in the cats' housing immediately before the beginning of the test. The second one was taken after the car ride, shortly before the veterinary examination was conducted. For each cat, two valid readings (> 36.9 °C) per ear were taken. Only the higher of the two readings of each ear was entered for analysis. To demonstrate asymmetrical thermal responses, the difference of the right ear temperature minus the left ear temperature (Δ ear_temp) was calculated for each point of measurement (before and after transport) for both tests.

2.8.3. Somatic signs of stress and fear during transport

The incidence of excessive salivation, vomiting, urination, defecation and anal gland secretion during transport was documented.

2.8.4. Physiological data from veterinary examination

Heart rate, respiration rate and rectal temperature were assessed during the veterinary examination (see Section 2.5. Experimental procedure).

2.9. Statistical analyses

All data except ear temperature were analysed with SPSS Statistics Version 21. Descriptive results are provided in Tables 2–6. To analyse the effects of training on behavioural and physiological measures, the differences between the first and the second test were calculated for the measures CSS, searching for food rewards in the carrier, lip licking, changes of body posture, sitting, lying ventrally legs bent or extended, eating, no vocalisation, moving, lying ventrally crouched on paws, latency to be placed into carrier, latency to exit carrier, duration of the veterinary examination and respiration rate, heart rate, and rectal temperature during the veterinary examination. They will be referred to as delta (Δ) of the respective variable, e.g. Δ CSS. The deltas were calculated by subtracting the results of test 1 from test 2, therefore a negative Δ indicating a reduction and a positive Δ an increase in values in the second test. A Mann-Whitney-U-test was used to test the deltas for differences between the training and control group. To find a balance between type 1 and type 2 errors, we corrected for multiple testing using the Bonferroni method in four clusters of tests. The goal was to reach an alpha level of 0.05 in each of the clusters. Cluster 1 – effect of training on the cats' CSS during the stay in the carrier (3 tests): p – value considered to be significant is $p \leq 0.016$. Cluster 2 – effect of training on continuous behaviour during the car ride (11 tests): p – value considered to be significant is $p \leq 0.0045$. Cluster 3–effect of training on latency to enter or exit the carrier and on the duration of the veterinary examination (3 tests): p – value considered to be significant is $p \leq 0.016$. Cluster 4–effect of training on physiological data collected during the veterinary examination (3 tests): p – value considered to be significant is $p \leq 0.016$. The intra- and inter-rater reliability for the CSS and the Interact® analyses was determined with Spearmańs rank correlation coefficient.

The ear temperature was analysed with linear mixed models, using the "lme" function from the package "nlme" (Pinheiro et al., 2013) in the statistics environment R version 3.0.3 (R Core Team, 2014). Separate models were calculated for right ear temperature, left ear temperature and Δ ear_temp. Group (TG, CG), test (1, 2), point in time (before transport, after transport) and all interactions were included as fixed factors. The individual cat was included as a random factor. Model assumptions were checked by graphical inspection. The data presented includes outliers > 3 SD. Models excluding these outliers were calculated but did not differ with regard to significant differences from the models including the complete data.

3. Results

3.1. Behavioural data

3.1.1. Cat stress score

Overall, our main measure the CSS, ranged between 3.14 ("weakly tense", according to the classification of Kessler and Turner, 1997) and 5.31 ("fearful, stiff") (Table 2). The average CSS of all cats during confinement in the carrier in the training room, during the car ride and in the waiting room was "very tense" (mean ± SD: 4.29 ± 0.46). The ΔCSS during the car ride indicates that both groups had a lowered mean CSS during the second test. However, the reduction of the ΔCSS in the TG was significantly larger than in the CG ($p = 0.007$, Table 2). Hardly any changes of ΔCSS were found between the groups and tests during the stay in the carrier in the training room ($p = 0.75$) and in the waiting room ($p = 0.40$).

3.1.2. Cat behaviour during car ride

Descriptive statistics of continuously coded behaviours obtained from the video of the 10 min car ride are presented in Table 3. The effects of training were assessed by comparing the TG and the CG regarding their change in behaviour between the first and second visit to the sham veterinary practice. The deltas of the respective behaviours are either presented as mean ± SD (range) in seconds for durations (dur) or as median (range) for frequencies (fr). Searching for food rewards in the carrier, lip licking and the number of body posture changes increased significantly in the TG: Δ searching for food rewards in the $carrier_{dur}$ (TG: 33 ± 24 (0 to 89) s, CG: 6 ± 7 (−2 to 19) s; U = 13.5, $p = 0.001$), Δ lip $licking_{fr}$ (TG: 18 (7 to 65), CG: 0 (−3 to 24); U = 7, $p < 0.001$) and Δ changes of $body\ posture_{fr}$ (TG: 13 (−2 to 46), CG: 1 (−5 to 13); U = 12.5, $p = 0.01$). Further, a significant change was found for Δ $sitting_{dur}$ (TG: 233 ± 146 (0 to 538) s, CG: −4 ± 35 (−83

Table 2
Mean (SD) and range for Cat Stress Score (CSS) and Δ CSS obtained in the training room, during the car ride and in the waiting room for training and control group during test 1 and test 2. P-value considered to be significant after correction for multiple testing is $p \leq 0.016$.

	Δ CSS			CSS			
				Test 1		Test 2	
	Training Group	Control Group		Training Group	Control Group	Training Group	Control Group
	Mean ± SD (Range)		U p	Mean ± SD (Range)			
Training room	−0.05 ± 0.48 (−0.76 to 0.65)	−0.16 ± 0.42 (−0.91 to 0.45)	U = 55 p = 0.75	4.01 ± 0.45 (3.33–4.78)	4.37 ± 0.28 (3.71–4.75)	3.96 ± 0.57 (3.14–4.76)	4.21 ± 0.42 (3.44–4.85)
Car ride	−0.60 ± 0.37 (−1.11 to 0.18)	−0.23 ± 0.25 (−0.64 to 0.20)	U = 20.5 p = 0.007	4.41 ± 0.28 (3.64–4.68)	4.54 ± 0.36 (3.89–5.03)	3.81 ± 0.31 (3.43–4.57)	4.31 ± 0.43 (3.64–4.82)
Waiting room	0.09 ± 0.33 (−0.50 to 0.59)	0.19 ± 0.34 (−0.49 to 0.61)	U = 47 p = 0.40	4.30 ± 0.48 (3.60–4.93)	4.52 ± 0.34 (3.82–5.02)	4.39 ± 0.34 (3.83–5.11)	4.71 ± 0.47 (3.87–5.31)

Table 3
Behavioural codes, median (range) of frequencies and mean (SD) and range of durations assessed with Interact® for training and control group during test 1 and test 2 on the 10 min video of the car ride.

	Frequency (per 10 minutes), Median (Range)				Duration (seconds) Mean ± SD (Range)			
	Test 1		Test 2		Test 1		Test 2	
Coded behaviour	Training Group	Control Group	Training Group	Control Group	Training Group	Control Group	Training Group	Control Group
Searching for food rewards	0 (0–19)	0 (0–3)	9 (0–38)	2 (0–14)	11 ± 27 (0–88)	2 ± 3 (0–11)	44 ± 41 (0–127)	7 ± 9 (0–29)
Eating	0 (0–19)	0 (0–1)	7 (2–14)	0 (0–13)				
Lip licking[c]	14 (0–28)	4 (0–21)	40 (13–70)	9 (0–24)				
Meowing plaintively	18 (0–52)	16 (0–80)	9 (0–34)	1 (0–115)				
No vocalisation					577 ± 29 (506–600)	576 ± 40 (467–600)	592 ± 9 (576–600)	588 ± 18 (553–600)
Standing	0 (0–3)	0 (0–1)	1 (0–17)	0 (0–1)	3 ± 8 (0–25)	0 ± 1 (0–4)	24 ± 58 (0–198)	0 ± 0 (0–1)
Sitting	0 (0–11)	0 (0–8)	11 (0–25)	0 (0–10)	21 ± 44 (0–145)	28 ± 72 (0–241)	254 ± 161 (0–538)	24 ± 50 (0–158)
Lying ventrally paws crouched	1 (0–5)	1 (0–7)	8 (1–23)	2 (0–11)	83 ± 87 (0–234)	73 ± 177 (0–598)	157 ± 129 (27–440)	65 ± 93 (0–282)
Lying ventrally front legs bent or extended[c]	1 (0–9)	1 (1–3)	1 (0–4)	1 (1–3)	412 ± 185 (0–600)	417 ± 224 (3–600)	156 ± 188 (0–556)	506 ± 124 (229–600)
Moving	1 (0–5)	0 (0–3)	0 (0–3)	0 (0–4)	8 ± 12 (0–41)	4 ± 8 (0–24)	3 ± 5 (0–15)	2 ± 4 (0–11)
Changes of body position	7 (1–33)	3 (1–22)	23 (2–54)	4 (1–25)				

[c] Results adjusted by this formula: (frequency or duration (seconds)/total duration face or front legs visible) × 600 (seconds) to correct for legs or face being not visible for evaluation over the whole 10 min period.

Table 4
Descriptive data of seldom behaviours during the car ride expressed by only a few cats of the training and control group during test 1 and test 2. Frequencies and durations are based only on data from cats displaying the behaviour. Obtained values are presented for the respective number of cats (N) and percentage per group (%).

	Number of cats (N) Percentage of cats per group (%) Frequencies $_{fr}$ [Median (Range)] Durations $_{dur}$ [Mean ± SD (Range) in s]			
	Test 1		Test 2	
Behaviour	Training Group	Control Group	Training Group	Control Group
Milk treading $_{fr}$	N = 1 9% 1	N = 0 0% –	N = 3 27% 1 (1–12)	N = 0 0% –
Rub body or face against carrier $_{fr}$	N = 2 18% 1(1–1)	N = 0 0% –	N = 6 55% 4 (1–28)	N = 1 9% 1 (1–1)
Grooming $_{fr}$	N = 1 9% 1	N = 0 0% –	N = 2 18% 2 (1–2)	N = 0 0% –
Purring (assessment sheet)	N = 0 0% –	N = 0 0% –	N = 3 27% –	N = 0 0% –
Hiding $_{fr,dur}$	N = 3 27% 5 (1–7) 273 ± 267 (57–572) s	N = 2 18% 6 (5–6) 74 ± 44 (43–106) s	N = 0 0% – – –	N = 4 36% 6 (1–8) 19 ± 12 (3–30) s
Panting[c] $_{fr,dur}$	N = 2 18% 7 (6–8) 29 ± 13 (19–38) s	N = 4 36% 7 (5–9) 89 ± 96 (25–229) s	N = 0 0% – – –	N = 2 18% 2 (2–2) 26 ± 15 (15–37) s

[c] Data corrected for time being visible.

to 71) s; U = 6, p < 0.001). Sitting increased on average about 4 min in the TG. Consequently, Δ lying ventrally legs bent or extended$_{dur}$ (TG: −256 ± 288 (−523 to 556) s, CG: 89 ± 177 (−101 to 522) s; U = 11, p = 0.001) decreased about 4 min in the TG. Lying ventrally legs bent or extended in contrast increased in the CG, but with only ~1.5 min during the 10 min car ride. No significant differences between groups were found for other behaviours: Δ meowing plaintively$_{fr}$ (U = 47.5, p = 0.4), Δ no vocalisation$_{dur}$ (U = 54.5, p = 0.7), Δ standing $_{dur}$ (U = 43, p = 0.3), Δ moving$_{dur}$ (U = 48, p = 0.4) and Δ lying ventrally crouched on paws$_{dur}$ (U = 46, p = 0.4). Although the frequency of eating did not differ significantly (Δ eating$_{fr}$: U = 39.5, p = 0.2), a greater percentage of cats in the TG started to eat in the second test. During the first test, eight cats in the TG (73%) and nine cats in the CG (82%) did not eat at all. During the second test all cats in the TG ate (100%) whereas in the CG only four out of the eleven cats ate (36%).

Some behaviours were rarely observed and seen only in very few cats. Therefore they could not be tested for significant differences (descriptive statistics are presented in Table 4). However, most of them strongly reflected positive or negative emotional states worth mentioning. Behaviours expressing positive emotional states such as milk treading and rubbing the face against the carrier were predominantly seen in the TG (Table 4). Ambiguous behaviours such as purring and grooming were only seen in the TG, purring only in test 2. In contrast, behaviours indicating fear during the car ride such as hiding and panting were completely eliminated in the TG during the second test but persisted in the CG.

3.1.3. Placing cat into the carrier and behaviour during veterinary examination

There was neither a significant difference between groups for Δ latency to be placed into the carrier (TG: − 4 ± 109 (−179 to 177) s, CG: −14 ± 94 (−169 to 151) s; U = 57, p = 0.9) nor Δ latency to exit the carrier (TG: 17 ± 85 (−115 to 161) s, CG: −11 ± 60 (−132 to 73) s; U = 42, p = 0.2). Behaviours observed during placement into the carrier and during the veterinary exam are presented in Table 5. After disassembling the carrier on the exam table, the majority of the cats chose to stay in the bottom half for the entire or most of the veterinary examination. The main finding was a significant reduction in Δ duration of the veterinary examination in the TG (TG: − 42 ± 31 (−101 to −2) s, CG: −12 ± 24 (−50 to 31) s; U = 22.5, p = 0.010). In most cats the veterinary examination could be completed and the only reason for premature termination was trying to take the rectal temperature. Fearful behaviours such as escape attempts and hiding were expressed in fewer cats during the second test (Table 5).

Table 5
Behaviours expressed by cats during placement into the carrier and during the veterinary examination presented for the training and control group during test 1 and test 2. Obtained values are presented for the respective number of cats (N) and percentage per group (%).

	Number of cats, Percentage per group (%)			
	Test 1		Test 2	
	Training Group	Control Group	Training Group	Control Group
Entering carrier – start of test:				
– voluntarily	7 (64%)	4 (36%)	7 (64%)	3 (27%)
Being placed into the carrier:				
– through front door	4 (36%)	7 (64%)	4 (36%)	6 (55%)
– through upper door	0 (0%)	0 (0%)	0 (0%)	2 (18%)
– resistance with paws during placement	0 (0%)	1 (9%)	0 (0%)	1 (9%)
Veterinary examination:				
Use of carrier:				
– left carrier by themselves within 180 s	9 (82%)	6 (55%)	7 (64%)	7 (64%)
– stayed in bottom half for entire or most of the time during the examination	10 (91%)	9 (82%)	11 (100%)	9 (82%)
– spent at least half of the time on the examination table	1 (9%)	2 (18%)	0 (0%)	2 (18%)
– cats already in bottom half by the end of the examination	10 (91%)	10 (91%)	10 (91%)	8 (73%)
– if not entering carrier themselves	1 (9%)	0 (0%)	1 (9%)	2 (18%)
– being placed through the front door	0 (0%)	1 (9%)	0 (0%)	1 (9%)
Compliance & behaviour:				
– veterinary examination completed	6 (55%)	9 (82%)	9 (82%)	10 (91%)
– premature termination while taking the rectal temperature	5 (45%)	2 (18%)	2 (18%)	1 (9%)
– escape attempts	5 (45%)	8 (73%)	1 (9%)	4 (36%)
– hiding	3 (27%)	1 (9%)	0 (0%)	0 (0%)
– mild aggression	2 (18%)	0 (0%)	0 (0%)	0 (0%)

3.2. Physiological data

3.2.1. Ear temperature

In both tests the temperature of the right and left ear significantly increased during the car ride in both groups (Fig. 1A and B; GLMM: main effect of the point in time: right ear: $Chi^2 = 16.40$, $p \leq 0.001$; left ear: $Chi^2 = 14.09$, $p \leq 0.001$). The temperature of the right ear was influenced by treatment group ($Chi^2 = 6.94$, $p = 0.008$) and test ($Chi^2 = 7.66$, $p = 0.005$) as well by the interaction of treatment group and test ($Chi^2 = 6.44$, $p = 0.011$). The boxplot shows that the training group's right ear temperature before the car ride was lower in the first test than in the second. However, the increase of the right ear temperature during the car ride was much higher during the first test than in the second. In contrast, the right ear temperature of the control group had similar baseline values before the car ride and with the ear temperature increasing similarly in both tests. Furthermore a significant main effect of treatment group on the Δear_temp ($Chi^2 = 5.27$, $p = 0.022$) and a significant interaction of treatment group and test ($Chi^2 = 5.46$, $p = 0.019$) indicate a different development of the Δear_temp between the training and control group during the first and the second test (Fig. 1C).

3.2.2. Somatic signs of stress and fear during the car ride

Somatic reactions presumably induced by stress or fear were rarely found. In the first test two cats in the TG urinated in the carrier and one TG cat vomited during the second test. Defecation, anal gland secretion and excessive salivation never occurred.

3.2.3. Physiological data from the veterinary examination

Physiological data is presented in Table 6. The average respiration rates were above the reference values (24–36 breaths/min, Lappin, 2013) for a clinical setting. Heart rates were within normal limits (120–180 beats/min, Lappin, 2013) and no cat showed hyperthermia (rectal body temperature ≥ 39.2, Lappin, 2013). No significant differences were found between treatment groups and tests for the physiological data collected during the veterinary examination (Table 6).

4. Discussion

Carrier training reduced stress in cats during a car ride to a sham veterinary practice, which was shown here by reduced behavioural signs of stress and a modified pattern of cats' ear temperature. Cats that had received carrier training took less time at the veterinary examination than the control group. By using low stress handling techniques, it was easy to place even untrained cats quickly into the carrier. The majority of cats were compliant during the examination. Our results support recommendations of feline behavioural guidelines on reduction of stress in the veterinary practice by carrier training cats and applying techniques of low stress handling (Anseeuw et al., 2006; Yin, 2009; Rodan et al., 2011; Lindell, 2015).

4.1. Behaviour during confinement in the carrier before, during and after the car ride

Training has been demonstrated to reduce stress in cats during confinement in a respiration chamber (Gooding et al., 2012) and during blood collection procedures (Lockhart et al., 2013).

We could successfully apply a training protocol that diminished behavioural signs of stress during the car ride. In practice most cats score between 2–4 on the 7-point CSS (Bradshaw et al., 2012), whereby a CSS up to 3 indicates low stress levels (Kessler and Turner, 1997). Confinement to a carrier and transport by car are potentially stressful situations. Therefore, it seems plausible that despite training no cat scored under 3. The seven-level CSS (Kessler and Turner, 1997) is a good instrument to differentiate between relaxed and stressed cats, but several attempts have been made to improve its sensitivity to assess subtle differences in stress levels (Dybdall et al., 2007; Bradshaw et al., 2012). Bradshaw et al. (2012) have added half points between CSS 2–4 to increase the sensitivity of the scoring system. Based on our experience we consider half points as the smallest detectable unit of the CSS. Consequently, the average reduction of about 0.6 points in the TG during the car ride is a valuable result in our opinion.

Combining the CSS with behavioural analyses was very useful to further differentiate stress levels. Despite that the frequency of eating did not change between tests, cats in the TG showed more lip licking, spent more time searching for food rewards and although not shown to

Table 6
Mean (SD) and range of descriptive data and Δs of physiological parameters of the training and control group obtained during the veterinary examination (test 1 and test 2). Obtained values are presented for the respective number of cats (N) and percentage per group (%). P-value considered to be significant after correction for multiple testing is $p \leq 0.016$.

		Δ			Test 1		Test 2	
		Training Group	Control Group	U p	Training Group	Control Group	Training Group	Control Group
Respiration rate (breaths/min)	N (%)				11 (100%)	11 (100%)	11 (100%)	11 (100%)
	Mean ± SD (Range)	−0.73 ± 23.99 (−32 to 36)	−1.45 ± 20.88 (−44 to 36)	U = 58.5 p = 0.89	67 ± 26 (36–108)	70 ± 23 (40–116)	66 ± 17 (44–96)	69 ± 24 (44–128)
Heart rate (beats/min)	N (%)				11 (100%)	9 (82%)	11 (100%)	11 (100%)
	Mean ± SD (Range)	9.45 ± 44.73 (−64 to 96)	−5.33 ± 22.45 (−32 to 44)	U = 36.5 p = 0.33	153 ± 25 (112–200)	175 ± 16 (114–200)	162 ± 24 (128–208)	171 ± 21 (136–208)
Rectal temperature (°C)	N (%)				6 (55%)	9 (82%)	9 (82%)	10 (91%)
	Mean ± SD (Range)	−0.02 ± 0.38 (−0.60 to 0.30)	−0.36 ± 0.43 (−1.10 to 0.10)	U = 13 p = 0.18	38.0 ± 0.5 (37.1–38.5)	38.3 ± 0.4 (37.8–38.8)	37.9 ± 0.5 (36.9–38.3)	38.0 ± 0.5 (37.3–38.9)

be significant, we observed all cats to start eating during the second test. In contrast, more than half of the CG cats did not eat at all during the second test. Stress and low palatability can lead to refusal of food (Tanaka et al., 2012; Mariti et al., 2016). Lip licking is a hedonic and likewise aversive taste reactivity pattern to food (Van den Bos et al., 2000). Although speculative, the TG seemed to be more interested in the food and temporary refusal to eat might have been due to expectation of more palatable food (canned tuna was given during training). Immobility, inhibition of behaviours (Rehnberg et al., 2015) and crouched body postures can be indicators of stress. Cats in the training group spent more time sitting, which is a body posture that is not usually expressed when being very fearful or stressed (Kessler and Turner, 1997). They also were more active as indicated by more changes in body postures. Although this could be interpreted as restlessness and be a sign of stress, the lower CSS and increased searching for food rewards more likely indicate a rise in exploratory behaviour. Positive behaviours such as milk treading, purring and scent marking the carrier by rubbing against it (Vitale Shreve and Udell, 2017) were only seen in the TG whereas behaviours indicative of stress or fear, such as hiding (Landsberg et al., 2012) and panting (Morton and Griffiths, 1985), were eliminated.

No effects of training on behavioural signs of stress before the car ride (training room) were found. The last training sessions mainly occurred on the way to or in the car. Therefore, the car was the most recent training location. On the way to the car some cats encountered potentially fearful situations such as unfamiliar people and noises. For this reason this was the most difficult training part and some cats were unwilling to enter the carrier in later training sessions. This reluctance might account for the similar latencies to enter the carrier between groups during the second test. Another possible explanation is that cats in the control group were less familiar with the training room and perceived the carrier as a hiding opportunity. However, by using low stress handling methods and choosing a suitable carrier model (Rodan et al., 2011) all cats could be easily placed in the carrier, in which their score indicated low to moderate stress levels. Despite creating a relatively cat friendly environment (Carney et al., 2012) the highest CSS values were found in the waiting room. This was the only setting not included in the training and therefore the waiting room was the most unfamiliar room. As the waiting room was the last setting, the accumulation of stressors may have here resulted in the elevation of stress levels (Notari, 2009).

4.2. Training protocol

Our training protocol adhered to a strict regime to enable standardizing for the purpose of this study. As a consequence the cats' individual needs may have been neglected in some cases. Cats with shy personalities and with lower interest in food rewards have lower gains in learning (Kogan et al., 2017). Even so they were forwarded to the next phase despite not achieving the goal. Cats received 4–5 training sessions per week, whereas fewer training sessions might have been favourable (Meyer and Ladewig, 2008). To improve training success, cats should be given enough time to achieve the training goal. Only highly palatable food should be used and exposure to potentially fearful situations should be avoided or reduced by covering the carrier with a towel. In addition, counterconditioning to specific fear-inducing stimuli should be considered and all settings (e.g. including the veterinariańs waiting room) should be implemented in the training protocol.

4.3. Veterinary examination

Accessing the cats for the examination was easy in both groups and most cats chose to exit the carrier themselves within 3 min. Cats remaining in the carrier could be reached by removing the top half of the carrier. Many cats used the bottom half to retreat during the examination. Our findings should encourage veterinary personal to work

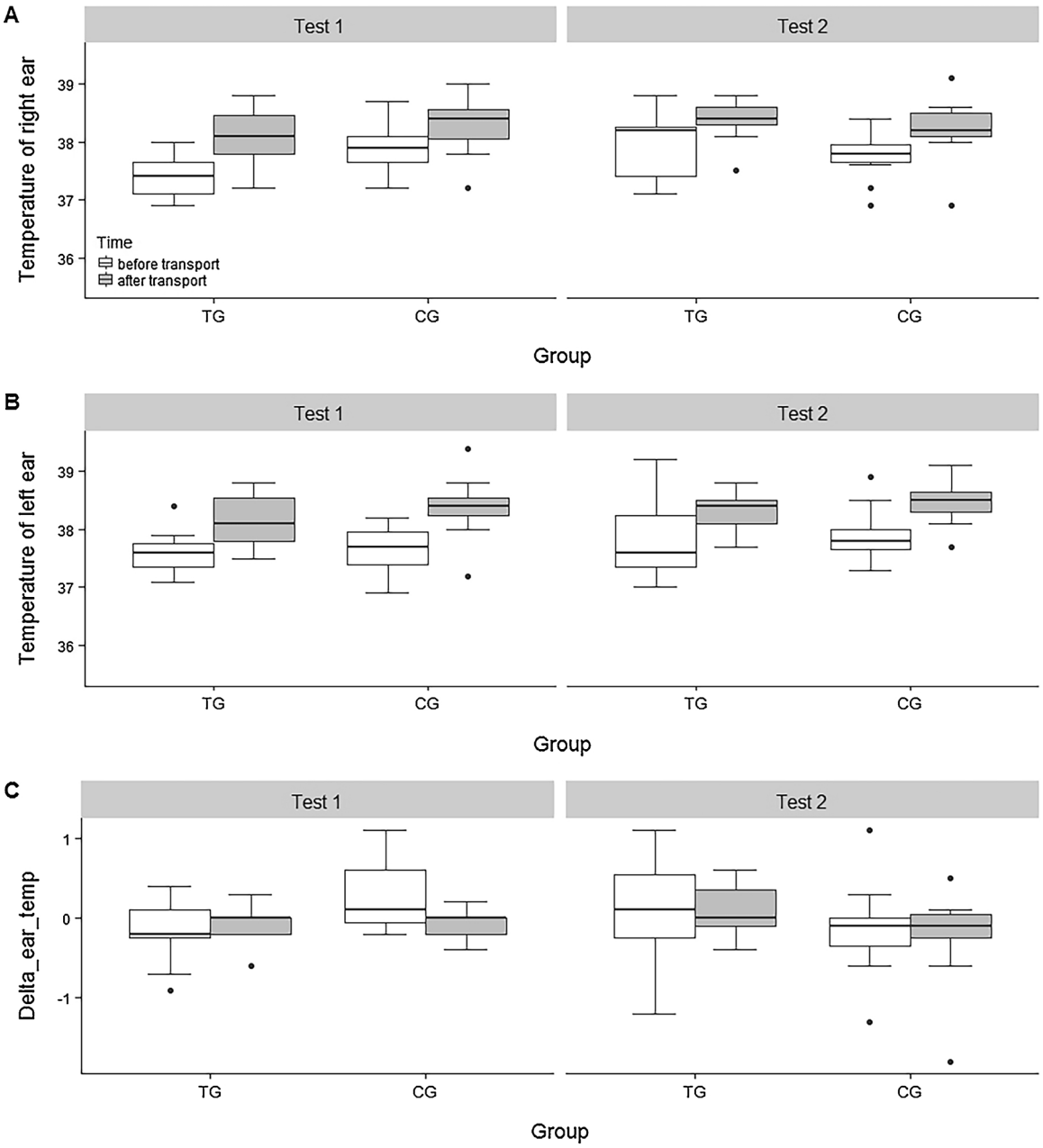

Fig. 1. Boxplots of the temperature of the right ear (A), the left ear (B) and difference between the right ear temperature minus left ear temperature, "Δ_ear_temp" (C) for training group (TG) and control group (GC) before and after the transport to a veterinary practice during test 1 and 2.
A) Significant main effect of the point in time ($p \leq 0.001$), main effect of group ($p = 0.008$), experiment ($p = 0.005$), and interaction of group and experiment ($p = 0.011$).
B) Significant main effect of the point in time: $p \leq 0.001$.
C) Significant: main effect of group ($p = 0.022$) and interaction of group and experiment ($p = 0.019$).

"slowly" with cats and to provide them with a safe place to retreat (Anseeuw et al., 2006; Rodan et al., 2011). In general, cats were very compliant during the veterinary examination and the examination could be performed within a maximum of 6.4 min. Familiarity with handling and procedures might have caused a reduction in fear-induced behaviours in both groups during the second visit (Gourkow and Fraser, 2006; Nibblett et al., 2015). Taking the rectal temperature was the sole cause for premature terminations. It was the most invasive procedure and most cats do not like their caudal body regions being touched (Soennichsen and Chamove, 2002; Ellis et al., 2015). However, the number of cats resisting it decreased from six to two in the TG during the second visit. This may have contributed to the significantly shorter duration of the examination. To prevent a fear response, measurement of the ear temperature instead of the rectal temperature should be considered in apparently healthy cats (Anseeuw et al., 2006). No cat resisted strongly against the ear temperature being taken, but a good technique with sufficient experience is required to reach agreement between the ear and rectal temperature (Sousa et al., 2011; Smith et al., 2015).

4.4. Physiological parameters

A rise in body temperature is a common measure of stress (Broom and Johnson, 1993). In our study the ear temperature rose about 0.5–1 °C during transport. The response patterns of Δear_temp and the right ear in the TG changed in the second test whereas in the CG the

reaction pattern remained similar in both tests. Activation of the right brain hemisphere is associated with withdrawal from a stimulus, emergency responses and control of the stress response (Rogers, 2010). Although interpretation is not straight forward (Propper and Brunyé, 2013), studies involving a stressful event found relationships between stress and the right tympanic membrane temperature. For example, the right ear was warmer in cats with higher cortisol levels after a visit to a veterinary hospital including transport (Mazzotti and Boere, 2009). Similarly, right tympanic ear temperatures increased in chimpanzees after exposure to videos with negative emotional content, whereas the left tympanic ear temperature remained largely unchanged (Parr and Hopkins, 2000). The differences between the left and right ear temperature in this study, with a less marked increase in the right ear, further supports the effectiveness of training in stress reduction during transport. However, a much larger variability of Δear_temp and the right ear temperature before the second transport was found in the TG. This raises the question whether individual cats perceived the training as mental enrichment or even as stressful. However, no cat showed hyperthermia (measured by rectal temperature) during the veterinary examination. This is in contrast with other studies (Quimby et al., 2011; Nibblett et al., 2015) which evaluated stress during a veterinary examination, whereby the home was compared to a clinical setting. In Nibblett's study low stress handling was applied as well but cats were confined for longer periods (average 45 min) before the examination than in ours (average 20 min). Prolonged confinement (> 25 min) may increase fear and anxiety (Pereira et al., 2016) and thus lead to hyperthermia. Elevated respiration rates were found in all cats throughout both tests. This appears to be a normal finding with cats exposed to a novel environment (Pankratz et al., 2017).

5. Conclusion

Carrier training plays an important role in creating less stressful veterinary visits for both, owners and their cats. We could demonstrate that trained cats showed reduced behavioural signs of stress during the car ride. A suitable carrier model allowed easy placement in and out of the carrier and most cats chose to retreat to its bottom half during the veterinary examination. Besides ear temperature measures, there was no difference in physiological parameters between groups. Elevated respiration rates were evident in both groups. All cats were fairly compliant and easy to handle. However, in trained cats the duration of the veterinary examination was decreased. As a consequence, owners should be encouraged to carrier train their cats but training will only be successful if there are no negative experiences during the trip. Continuous training will be needed to maintain beneficial effects, especially after unpleasant procedures. The veterinary team has to create a cat friendly environment and use low stress handling techniques to minimise the risk of stressful experiences.

Conflict of interest

All authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

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