The effect of a hiding box on stress levels and body weight in Dutch shelter cats; a randomized controlled trial

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Abstract: While staying in an animal shelter, cats may suffer from chronic stress which impairs their health and welfare. Offering hiding opportunities can significantly reduce behavioural stress in cats, but confirmation with physical parameters is needed. Therefore, the aim of this study was to determine the effect of a hiding box on behavioural stress levels (scored by means of the Cat-Stress-Score) and a physical parameter as body weight in newly arrived cats in a Dutch animal shelter during the first 12 days in quarantine situations.

Twenty three cats between 1 and 10 years of age were randomly divided between the experimental (N = 12) and control group (N = 11) with and without a hiding box. Stress levels were assessed on days 1, 2, 3, 5, 7, 9 and 12 according to the non-invasive Cat-Stress-Score (CSS). Body weights were measured on days 0, 7 and 12. Finally, adoption rates and length of stay (LOS) were determined.

Major findings of the study are: (1) the mean Cat-Stress-Score decreased with time for all cats, but cats with a hiding box however showed a significant faster decrease in the CSS, reaching a lower CSS-steady state seven days earlier than the control group; (2) nearly all cats in both groups lost significant body weight during the first two weeks; (3) hiding boxes did not significantly influence weight loss; (4) no differences were found in the adoption rates and the LOS between both groups.

Hiding enrichment reduces behavioural stress in shelter cats during quarantine situations and can therefore be a relatively simple aid to shelter adaptation. It offers no prevention however against feline weight loss, which indicates a serious health risk for shelter cats.

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Additional data availability information:
The effect of a hiding box on stress levels and body weight
in Dutch shelter cats; a randomized controlled trial

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While staying in an animal shelter, cats may suffer from chronic stress which impairs their health and welfare. Offering hiding opportunities can significantly reduce behavioural stress in cats, but confirmation with physical parameters is needed. Therefore, the aim of this study was to determine the effect of a hiding box on behavioural stress levels (scored by means of the Cat-Stress-Score) and a physical parameter as body weight in newly arrived cats in a Dutch animal shelter during the first 12 days in quarantine situations.

Twenty three cats between 1 and 10 years of age were randomly divided between the experimental (N = 12) and control group (N = 11) with and without a hiding box. Stress levels were assessed on days 1, 2, 3, 5, 7, 9 and 12 according to the non-invasive Cat-Stress-Score (CSS). Body weights were measured on days 0, 7 and 12. Finally, adoption rates and length of stay (LOS) were determined.

Major findings of the study are: (1) the mean Cat-Stress-Score decreased with time for all cats, but cats with a hiding box however showed a significant faster decrease in the CSS, reaching a lower CSS-steady state seven days earlier than the control group; (2) nearly all cats in both groups lost significant body weight during the first two weeks; (3) hiding boxes did not significantly influence weight loss; (4) no differences were found in the adoption rates and the LOS between both groups.

Hiding enrichment reduces behavioural stress in shelter cats during quarantine situations and can therefore be a relatively simple aid to shelter adaptation. It offers no prevention however against feline weight loss, which indicates a serious health risk for shelter cats.
Introduction

About 200 animal shelters in the Netherlands take in and rehome 27,000 stray and relinquished cats annually [1]. A shelter life is often associated with many stressors. Cats entering a shelter are introduced to a foreign environment with unfamiliar animals, people, sounds and smells. During these first days many of these cats struggle to adapt to these prolonged or repeated stressors and thus show stress responses [2-4]. This may elicit clinical signs like hiding behaviour, defecating and urinating outside the litter box, decreased grooming or over-grooming behaviour and a loss of appetite [2,5-8]. Stress-induced longterm high cortisol levels can reduce the efficacy of the immune system against infectious diseases [1,5,6,8,9], and chronic stress can therefore harm a cat’s health as well [5,7,10,11].

When in a state of stress, the majority of cats will stop eating. Tanaka et al. found that stress elicited a decrease in food intake, negatively correlated with stress scores [12,13]. This stress response can have grave impact on cats: severe body weight losses in only a short period of time can induce feline hepatic lipidosis [5,14,15].

Several studies show that stressed cats display increased alert resting behaviour behind their litter box in an environment without hiding opportunities [10,16,17]. This is interpreted as alternative hiding behaviour for it offers some concealment [10,16]. Real concealment can be offered by providing a hiding box to shelter cats. A study of Kry and Casey [17] demonstrated a decrease in stress, measured by the Cat-Stress-Score (CSS), when shelter cats were offered hiding boxes. Weight loss during quarantine is another phenomenon in shelter cats associated with stress [12]. However, little research has been done on the preventive effect of a hiding box on this stress induced weight loss.

A previous study conducted by Vinke et al. has been the first step to scientific evidence about the effect of a hiding box on stress levels of newly arrived cats in a Dutch animal shelter during the first 14 days in quarantine situations. The results show that cats with a hiding box recovered at least 4 days earlier from stress than cats without a hiding box [10]. The present study was designed with more frequent CSS scoring between day 5 and 12, to gain more insight in the feline recovery to stress and to relate these behavioural stress levels to a physical parameter as body weight.
The primary aim of this study was to determine the effect of a hiding box on behavioural stress levels and on body weight of newly arrived cats in a Dutch animal shelter during the first 12 days in quarantine. The additional aim was to compare the Length of Stay (LOS) of cats in both study groups. It was hypothesized that a hiding box would significantly reduce stress levels of newly arrived cats compared to the non-hiding box group, reflected in a lower CSS, less weight loss and a shorter LOS.

Materials and methods

The study was approved by the Animal Welfare Body Utrecht, after assessing the present study. It was concluded that the study does not meet the definition of an animal experiment as defined in the Dutch Experiments on Animals Act and Directive 2010/63/EU because the animals encountered no discomfort.

Animal shelter

This study was performed at a Dutch animal shelter (Stichting Dierentehuis Arnhem en omstreken), a medium size animal shelter with an open intake of around 700 cats per year [31]. Cat housing is situated in five separate quarantine units, an isolation ward and an adoption unit, providing a maximum shelter capacity of 90 cats in total. Dutch legislation mandates animal shelters to have quarantine and isolation wards and a legal stray holding period of 14 days. New animals were quarantined at intake for at least 2 weeks, as is legally required [19]. For this study an informed consent was obtained from the shelter staff. In order to relate this study to daily shelter management, the original shelter protocols about the intake of new animals, daily animal care and hygiene were generally accepted, and substantial adjustments were avoided.

Animals

For this study 23 European short hair cats, 11 males and 12 females, were selected out of the cats entering the shelter between the 4th of November and the 30th of December 2015. At intake cats entering the shelter were examined by the shelter staff for gender, breed and age and received a treatment against
ecto- and endoparasites (Stronghold® and Milbemax®). As all the cats came in as strays, age was estimated in years. Within 5 days after intake the shelter veterinarian performed a physical health check. During this veterinary check (during the morning hours) the cats were microchipped and vaccinated with an attenuated vaccin (Versifel CVR®) against feline panleukopenia virus (FPV), feline herpes virus (FHV-1) and feline calici virus (FCV). Intact cats were spayed or neutered after Day 14. Inclusion criteria for this study were based on breed (European shorthair cats), health status and age (between 1 and 10 years of age). When new cats showed no clinical signs of illness, obvious heat, pregnancy or signs of nursing during the physical examination at intake, they were included in this study. As it is not generally accepted practice in Dutch animal shelters to screen apparently healthy cats through diagnostic testing (e.g. FIV/FeLV) at shelter intake, apart from the physical examination, no additional information was available on the feline health status of the cats in this study. All cats were observed for at least 12 days after intake. During the study two cats participating in this study, left the shelter before their last observation day: from the Hiding box group one cat went to a foster home, from the Control group one cat was released within a trap-neuter-relaese (TNR) program. Data of both cats were excluded from this study. Two other cats were not included in data for the length of stay, but were included in data for the Cat-Stress-Score, body weight and the adoption rate. After the 12 days observation period, one of these cats (nr. 8, control group) proved to be infected with FeLV and was euthanized a few days after the quarantine period of 14 days, while another cat (nr. 19, control group), because of its semi feral behaviour, was also released through the TNR program. Because shelters often take in these non-clinical but infected cats and stray cats being poorly socialized (and even rehome them), this study has included these two cats in three of the four measured parameters. The reason for selecting cats between 1 and 10 years of age was to avoid inclusion of juveniles with related specific behaviour and elderly cats with increased chances for subclinical disease which might influence their behaviour [18,20]. As previous studies [17,18] found no gender related significant differences in stress behaviour, both male and female cats were included in the present study. The 23 cats were randomly assigned to one of the two groups with and without access to a hiding box.
**Housing conditions**

The cat housing in the two adjacent quarantine wards consisted of cages (L x W x H: either 84 x 95 x 80 cm or 69 x 91 x 87 cm) in which the cats were individually housed. Every cage was furnished with a food and water bowl, bedding of towels, a litter box and a perching shelf at 28 cm above the cage floor. The cages of the experimental group contained a hiding box that was placed at the right side at the back of the cage. To avoid place preference for towels as bedding, the towels were covering the entire floor of the cage including the shelf and the inside of the hiding box.

Cardboard boxes were used as hiding box and measured 44 x 31 x 26 cm (L x W x H). These boxes had two entrances (WxH 0.16 x 0.20 m) [10]. Hiding boxes were never reused.

Access to the cats in the quarantine wards was restricted to the caretakers and the observer. Natural daylight was provided through windows in both quarantine wards, combined with fluorescent lighting between 08:00 AM and 5:00 PM. Daily temperatures in the quarantine wards ranged from 16.0 to 19.8 °C. In the quarantine wards no dog vocalizations could be heard.

**Daily animal care**

The shelter staff cleaned the cages daily between 09:00 and 12:15 AM by removing waste and applying a spot cleaning method [21]. During this procedure cats remained in their cages. Litter boxes were daily cleaned with hot water and dried with clean paper towels. Cages were disinfected between cats or when indicated (e.g. diarrhea) with a chlorine disinfectant containing sodium dichloroisocyanurate (Halacid®).

Food was provided once daily between 9:30 and 10:00 AM and comprised of around 50g per day Adult Royal Canin® dry cat food (SC 365D) with a metabolizable energy content (ME) of 4066 kcal/kg (16.995 MJ/kg). Fresh water was provided ad libitum. Cats kept their own litter box for the time of this study.

**Behavioural observations**

Cats were given an habituation period of 24 hours after shelter intake (= Day 0), before behavioural assessment was performed [17]. Behavioural data were collected on days 1, 2, 3, 5, 7, 9 and 12 between 12:30 and 5:15 PM, during which interactions with caretakers were avoided.
Each cat was observed for 20 minutes per day by using video-recording. Outside the cage a video camera (H.264 DVR) was mounted on a tripod at cage height. For new observations the combination camera-tripod had to be readjusted to the new cat cage. Video recordings were viewed in real-time in an adjacent room and stored for subsequent analysis (Fig 1). Only one camera was used for recording.

**Fig 1. Diagram of the experimental set up, observer and both camera positions in the quarantine wards in the animal shelter.**

**Cat-Stress-Score (CSS)**

Kessler and Turner [18,22] developed a 7-level cat stress score (CSS) which has been used in several studies to estimate stress levels in confined cats [3,10,17,18,]. This scoring system assesses the level of feline stress based on the posture of body elements (e.g. belly, legs, tail, head, eyes, pupils, ears, whiskers) and behaviour (vocalization and activity) as described in the ethogram of the UK Cat Behaviour Working Group [22]. The CSS ranges from 1 (fully relaxed) to 7 (terrorized).

One observer (LS) assessed the CSS score per cat on Day 1, 2, 3, 5, 7, 9 and 12. Intra-observer variation was minimized by observational training using (video) images of pre-described feline behaviours from previous experiments with shelter cats.

After the video camera had been positioned, the scan sampling started after 2 minutes in which the cat habituated to the novel situation. Thereafter the cat was scored according to the Scan Sampling method, in which four scores (= four samplings) were made during the observation time (the 1st observation at 5 min, the 2nd at 10 min, the 3rd at 15 min and the 4th at 20 min) [23]. Imperceptible posture and behavioural elements were noted as missing values.

Each of the elements of the Cat-Stress-Scores was scored separately. The scores of the four samplings were averaged to assign an overall CSS for each separate cat per day.

**Body weight**
During the study every cat was weighted on Day 0, 7 and 12 by using an electronic scale (accuracy ± 10 g). The standardized shelter feeding regime consisted of approximately 50g per cat per day of Adult Royal Canin (RC)® dry cat food, which equals 203.3 kcal or 849.8 kJ per cat per day.

To secure adequate nutrition for the cats in this study, the daily caloric feline requirements (FEDIAF guidelines (80 kcal [335 kJ] ME per kg0.67)) were determined per individual cat [24].

Adoption rates and length of stay (LOS)

In order to determine the effect of a hiding box in quarantine situations on the subsequent adoption success, the adoption dates of the cats in this study were noted. Adoption rates ( = # cats adopted / all cats in this study) and the length of stay (LOS: number of days between the shelter intake of a cat and its day of adoption) was determined per cat. The LOS included the mandatory quarantine period of two weeks and only included adopted cats, excluding cats which were euthanized or returned to their outdoor environment after finishing this study.

Statistical analyses

A randomised controlled trial (RCT) design was used [25]. Data were stored in Microsoft Excel 2010 files (Microsoft Corp, Redmond, Wash.). Two statistical software programs were used for analysis of the data:

- SPSS (IBM Corp, Armonk, NY version 25) for the two-sample T-test and chi-square test.
- R (version 3.3.0) for the linear mixed regression models [28].

For the statistical analysis of effect of time and hiding box on the CSS (model ‘CSS-Time-Box’) a linear mixed regression model [27] was assumed, with the CSS as the outcome, while Time after arrival, the availability of a hiding box and the interaction between both were used as explanatory factors. CatID was used as the random effect to take the correlation between observations within cat into account. An AR1 correlation between the time points was added as well as a variance model to allow different variances for the separate time points. A maximum likelihood-based method was used to calculate the
Akaike’s Information Criterion (AIC) to select the best model using a backward selection approach (smaller is better).

For the statistical analysis of effect of time and hiding box on the body weight, a linear mixed regression model [27] was used to analyse the weight as the outcome and Time after arrival, the availability of a hiding box and the interaction between both as explanatory factors. Although keeping the box in the linear mixed model resulted in a worse fit of the model, the availability of the box nevertheless was added in coherence with our primary aim. Also in this model CatID was used for the random effect.

The validity of both models was confirmed by a visual inspection of the residuals for normality and constancy of variance.

Per experimental group the number of adopted cats was analyzed using chi-square test, while the length of stay (LOS) was analyzed using the two-sample T-test. The assumptions for these variables for equal variance (Levene’s test) and for normal distribution (Shapiro-Wilk’s test) were met.

We reported the estimated effects of the availability of a hiding box according the reporting guidelines for randomized controlled trials (www.reflect-statement.org).

Results

Characteristics of the study population

The experimental group consisted of 12 cats (6 males and 6 females) of which the estimated age ranged between 1 and 7 years (mean: 3.3 years, SD: 2.2). The control group consisted of 11 cats (5 males and 6 females) with estimated ages between 1 to 10 years (mean: 4.9 years, SD: 3.1, with n=10: due to her semi feral behaviour no age could be estimated of cat nr 19).

The cats in this study are presented in the appendix with their ID, experimental group, gender, age, bodyweight at intake (kg) and the quarantine wards they went after intake.

Daily Cat-Stress-Score (CSS): behavioural assessment
The time-dependent reduction of the individual CSS per cat in both groups is visualized in Fig 2.

**Fig 2. Course of the Cat-Stress-Score in time of individual cats from the control group and the experimental group.**

Cats from the hiding box group reached a steady state sooner (at day 2) than cats from the control group (at day 9). The model results for the mean CSS are presented in Table 1. The estimated means of the CSS of the hiding box group (mean CSS = 2.7) and the control (mean CSS = 3.1) at Day 1 are similar as their difference is not significant (-0.4, 95% CI: -0.97 to +0.12). At all other days the mean CSS of the hiding box group is significantly lower than the mean CSS in the control group, largest at day 2 (-0.99, 95%CI: -1.38 to -0.61) and decreasing in difference between the groups on day 12 (-0.33, 95%CI: -0.57 to -0.08).

### Table 1. Results of the model for the Cat-Stress-Score with 95% confidence interval, influenced by Day and availability of a hiding box and interaction between both.

| Research group | Time (days after Intake) | Estimate | Lower bound 95% CI | Upper bound 95% CI |
|----------------|--------------------------|----------|---------------------|---------------------|
| Control        | Day 1                    | 3.13²    | 2.74                | 3.53                |
| Control        | Day 2                    | -0.11³   | -0.43               | 0.20                |
| Control        | Day 3                    | -0.54³   | -0.91               | -0.17               |
| Control        | Day 5                    | -0.76³   | -1.15               | -0.37               |
| Control        | Day 7                    | -0.82³   | -1.21               | -0.42               |
| Control        | Day 9                    | -0.92³   | -1.32               | -0.53               |
| Control        | Day 12                   | -0.91³   | -1.34               | -0.49               |
| Hiding box     | Day 1                    | -0.43⁴   | -0.97               | 0.12                |
| Hiding box     | Day 2                    | -0.99⁴   | -1.38               | -0.61               |
| Hiding box     | Day 3                    | -0.51⁴   | -0.79               | -0.23               |
| Hiding box     | Day 5                    | -0.25⁴   | -0.47               | -0.03               |
| Hiding box     | Day 7                    | -0.23⁴   | -0.40               | -0.05               |
| Hiding box     | Day 9                    | -0.12⁴   | -0.24               | -0.01               |
| Hiding box     | Day 12                   | -0.33⁴   | -0.57               | -0.08               |

Legend:

1 CI = Confidence Interval
2 Mean CSS in cats in Control group at day 1.
3 Difference between mean CSS at specified day in Control group compared to mean CSS at day 1 of same cats.
4 Difference between mean CSS at specified day in cats of group with Hiding box compared to mean CSS of cats in group Control group at same day.
Body weight

For the comparison of both experimental groups, the absolute body weight was used. At intake the control cats were on average 300 grams heavier than those in the experimental group. This initial weight difference between both groups reduced to 210 grams at Day 7 and Day 12: cats in the control group lost overall 7.7% of their initial body weight, while cats with a hiding box lost 6.3% of their initial body weight during those 12 days (Table 2). The initial weight and weight reduction between the groups however proved not to be significant.

Table 2. Results of the model for Body weight with a 95% confidence interval, influenced by Day and availability of a hiding box and interaction between both.

| Research group | Time (days after Intake) | Estimate (kg) | Lower bound 95% CI$^1$ (kg) | Upper bound 95% CI$^1$ (kg) |
|----------------|--------------------------|---------------|-------------------------------|-------------------------------|
| Control        | Day 0                    | 4,39$^2$      | 3,77                          | 5,01                          |
| Control        | Day 7                    | -0,25$^3$     | -0,35                         | -0,15                         |
| Control        | Day 12                   | -0,32$^3$     | -0,42                         | -0,22                         |
| Hiding box     | Day 0                    | -0,30$^4$     | -1,16                         | 0,56                          |
| Hiding box     | Day 7                    | -0,21$^4$     | -1,07                         | 0,65                          |

Legend:
$^1$ CI = Confidence Interval
$^2$ the mean Body weight of cats in the Control group at Day 0.
$^3$ Difference between the mean Body weight of cats in the Control group at the specified Day compared to the mean Body weight of the same cats at Day 0.
$^4$ Difference between the mean Body weight of cats in the Hiding box group and the mean body weight of cats in the Control group at the specified Day.

The individual proportional decrease in body weight is visualized in Fig 3. All cats except one lost weight during both weeks. When weight loss at Day 12 was calculated as a percentage of initial body weight at intake, it was found that 7 of the 23 (35%) cats lost ≤ 5% of their body weight, whereas 15 of the 23 (65%) cats lost 5% or more of their weight. The maximum body weight loss was found in cat nr. 8 (control group) which lost 19% of its initial weight in 12 days and was diagnosed with an infection of FeLV a few days after completing this study.
Adoption rates and length of stay (LOS)

Of the 23 shelter cats in this study, 21 were rehomed after the observation period was finished. In the control group 9 out of 11 cats were adopted (82%), in the experimental group 12 out of 12 (100%). No significant difference was found in the adoption rate between the two groups (p = 0.55).

As we defined LOS as the number of days between the shelter intake of a cat and its day of adoption, 2 cats were not included in this data set, for they were not adopted.

The mean LOS for the control group (n = 9) was 24.1 days (SD 5.4, range 15-30 days) and for the hiding box group (n= 12) was 22.9 days (SD = 4.4, range 16-30 days). No difference in the mean LOS was found between control and the hiding box group (p-value = 0.58).

Discussion

The aim of the present study was to determine the effect of a hiding box on behavioural stress levels and body weight in shelter cats during the first 12 days in quarantine. While in a previous study cats were monitored on days 1 through 5 and the 14th day [10], this new study added more insight about the differences in CSS between Day 5 and Day 12.

The most important findings of this study are:

- The mean Cat-Stress-Score decreased with time for all cats, but cats with a hiding box however showed a significant faster decrease in the CSS and recovered from stress seven days earlier than the control group.

- Nearly all cats lost significant body weight during the first two weeks. On average, cats with hiding boxes lost 40 grams less of their initial body weight compared with cats without a box, although this difference was not significant.

- The mean adoption rates and the LOS of cats with and without hiding boxes were equal.
Cat-Stress-Score (CSS): behavioural assessment validation

In this study, cats with a hiding box showed a significant faster decrease of behavioral stress compared to the control group, which was most prominent during the first observation days. These results were in line with earlier findings of Vinke et al. [10] and with a study of Gourkow and Fraser, in which the mean CSS of cats, housed in single barren cages without positive human-cat interaction, was higher compared to the other groups and only reached a similar CSS on Day 9 [13]. The findings of the present study complete the results obtained by Vinke et al., where the hiding box group recovered at least four days earlier. By increasing the number of observational days during the first 12 days, the current research provides more details in reaching the CSS-steady state, indicating that hiding boxes accelerate the recovery of behavioral stress by seven days. This is important, because the hiding box clearly helps the shelter cat to adapt more quickly in a stressful new environment. This in order to prevent the development of chronic stress [17].

Body weight

This study shows a significant decrease in feline body weight during the first 12 days in an animal shelter. Approximately a third of the cats lost less than 5% body weight during the first 12 days, while two-third lost over 5%. These results agree with previous findings of Tanaka et al., in which 57% of their cat population showed 5% or more weight loss during their shelter stay [12]. When otherwise healthy cats lose weight unintentionally, it is a dramatic indicator of a health risk. Weight loss can be caused by insufficient nutritional management (the shelter offers inadequate quantity and/or quality of food) and also by a decrease in feline appetite by a physical stress response. Although food intake was not registered in the present study, it was observed that some cats were completely anorectic, especially during the first days. For the shelter this was the reason to standardize the feeding schedule of 50 g dry cat food per cat per day. According to the FEDIAF guidelines [24] for daily caloric feline requirements, during this study cats over 4.01 kg might have been offered an inadequate amount of food. With an individual requirement of 80 kcal (335 kJ) ME per kg^{0.67}, 50 g dry cat food per day will
meet maintenance energy requirements of cats up to a body weight of 4.01 kg. Cats weighing over 4.01 kg, need more Adult RC food daily. Of the 23 cats, 13 (57%) cats weighed more than 4.01 kg. The heaviest cat weighed 6.41 kg at intake and hence required at least 68.4 grams of catfood per day. During the daily observation however, cats rarely finished their food rations during these first two weeks. An inadequate quantity of food was therefore not considered to be the cause of the observed body weight loss.

The effect of stress however on the body weight of shelter cats was first shown by Tanaka et al. [12], who found a negative correlation between food intake and stress scores of cats. The conclusion was that cats, admitted to an animal shelter were likely to lose weight while in the shelter. These results are consistent with our findings, indicating that a decrease of feline appetite caused by a physical stress response, is most likely responsible for the weight loss.

Although the provided commercial food in this study was of a high quality, there is less understanding of the role of palatability of food for shelter cats in relation to weight loss. The only cat in this study which gained weight, received medication for cystitis (meloxicam 0.05 mg/kg) mixed with canned food. This gives an indication of the importance of palatability of food for shelter cats.

Although the analysis of the effect of time and the presence of a hiding box on the body weight suggested that there was a difference between the two groups in body weight losses, as cats with hiding boxes showed approximately 40 grams less weight loss in comparison with the control group, this difference was not significant. For the individual cat, however, this could be biologically relevant, for weight loss due to feline anorexia has a serious impact on a cat’s health, increasing the risks of hepatic steatosis [5,14,15]. A significant difference in body weight between cats with and without hiding facilities, however, was not identified. Therefore more research is necessary to monitor these cats for a longer period of time, to register the process of adaptation to the new environment in correlation to the weight losses and to experiment with ways to prevent or reduce body weight losses in shelter cats (i.e. highly valued palatable food items might overcome the fear motivation and stimulate consumption despite the challenging environment).

Apart from stress, progressive weight loss can also be a sign of serious medical problems [32]. One of the cats from the control group showed a weight loss close to 20% in 12 days and was eventually
diagnosed with FeLV. Shelters could use weight loss during quarantine time as an early warning sign for serious declines in physical conditions, but this asks for a daily weighing as a standard procedure.

**Adoption rates and length of stay (LOS)**

Sometimes shelter staff expresses their worries about using hiding boxes, for boxes might decrease the visibility of cats to potential adopters and therefore slow down adoption rates (personal communications). Kry and Casey however showed that providing shelter cats with hiding enrichment, did not decrease the likelihood of those cats being adopted [17]. Also our study did not reveal differences in the adoption rates and the LOS of both groups. The hiding enrichment itself, however, could not have influenced the adopters’ choices based on the (in)visibility of the cat, for hiding boxes were only present in 12 of the 23 cages during the first 12 days of quarantine time, while no hiding boxes were available in the adoption area.

**Finally, Stressors versus Signals of Safety?**

Historically, the science of stress emphasizes the role of stressors in evoking stress responses. Stress reduction is caused by reducing the impact of a stressor (in number, strength, duration, etc) on the behavioural and physiological responses of the individual in question by adapting to it [30]. Stressed cats are likely to reduce their feed intake and subsequently loose body weight. Cats in shelters [12], in laboratories [16], in boarding facilities and even privately owned cats [5] show this general stress response to stressors in challenging situations. Because previous research had shown a robust effect of the hiding box on the behavioural stress response [10] reflecting adaption, expectations were that hiding opportunities would also aid in the reduction of weight loss. While this study proved again a significant decrease of the behavioural stress response when shelter cats were offered a hiding opportunity, the effects on body weight were minor. These results challenge our point of focus on stress in shelter animals: a shelter environment offers numerous stressors for which feline hiding behaviour appears not sufficient enough to induce a fast adaptation, for more than 90% of the cats with and without hiding opportunities lost body weight during the first 12 days after intake. New theories on human stress
response mechanisms might shed some light on the feline stress response in these complex shelter environments and contribute to more practical tools for stress reduction. According to Brosschot [33], who introduced the Generalized Unsafety Theory of Stress (GUTS), ‘the stress response of the body is always “on” and it stays on as long as there is no obvious safety.’ This default response can only be inhibited when ‘signals of safety’ are perceived by the animal. We therefore should not look for the causation of a stress response but rather ask ourselves ‘what stops the stress response?’ When present results are reviewed in the light of this GUTS, the hiding enrichment itself caused a decrease in feline behavioural stress scores, but did not provide an adequate signal of safety (SOS) to prevent weight loss in most cats. Changing the food presentation (e.g. food offered inside hiding boxes) might give shelter cats more safety signals. This GUTS approach asks for a comparison of the effect of distinct SOS’s (like hiding materials, food presentations, enriched feeding, feline pheromones, human contact, increased cage space, solitary housing, etc) and for the reinforcing effects of combining these signals on the majority of shelter cats. In addition to focusing on reduction of numerous stressors in the shelter environment, we should also search for signals of safety which are strong enough to inhibit the stress response and thus create a situation which the majority of animals can perceive as safe.

Limitations

Limitations of this randomized controlled trial included the small sample size.

Conclusion

Providing hiding boxes can be a relatively simple way for cats to self-manage stress and to adapt faster to the shelter environment. The majority of the shelter cats however loose (considerable) weight during the quarantine time in an animal shelter. Providing them with hiding enrichment during that period, gives no prevention against this weight loss. Neither do hiding boxes have effect on the adoption rates and the length of stay of both groups. However, instead of keeping focus on identifying and reducing stressors in a very challenging environment like an animal shelter, an additional approach could be found in the application of ‘signals
of safety’ (SOS), strong enough to inhibit the stress response and thus create a situation which animals
can perceive as safe.

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Author contributions

Van der Leij conceived the study and Vinke assisted with the study design. Selman assisted with data
collection and data maintenance. Vernooij, Selman and Van der Leij analyzed the data and drafted the
manuscript. Vinke supervised the draft and submission.

Conflicts of interest

The authors declare that no conflict of interest exists in which any author or authors’ institution has a
financial or other relationship with other people or organizations that may inappropriately influence the
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Supporting information

S1 Fig 1. Diagram of the experimental set up, observer and both camera positions in the quarantine wards in the animal shelter.

S2 Fig 2. Course of the Cat-Stress-Score in time of individual cats from the control group and the experimental group.

S3 Fig 3. The proportional change (%) in body weight in individual cats from the control group and the experimental group.

S1 Appendix. Baseline characteristics of treatment cohorts in a randomized field trial comparing quarantine cat housing with and without hiding opportunities in one Dutch animal shelter.
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Supporting Information
S1_Appendix base line.bmp