Assessing the welfare of shelter dogs by studying their sleep/rest patterns

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Abstract

An animal shelter can be a stressful environment for dogs. Inclusion in a shelter can lead to welfare impairment with short and long term effects. In order to monitor the level of well-being a change in sleep- or resting patterns could be used as an additional indicator.

This study examined sleep/rest quality and quantity and stress-related behaviour in a group of shelter dogs, in combination with urine cortisol: creatinine ratio (UCCR) and body weight.

The research group consisted of 32 dogs. In 5 dogs, changes in sleep/rest quality and quantity and in stress-related behaviour were examined using video images recorded the first night after intake in a shelter and again after 2 weeks. In the other 27 dogs only urine cortisol: creatinine ratio (UCCR) and body weight were examined. After intake at the shelter, dogs went through more and shorter sleep-wake cycles than laboratory dogs and dogs in domestic situations had done in a previous study. After two weeks in the shelter, dogs slept/rested more, had longer sleep/rest periods and showed fewer sleep disturbances than during the first 48 hours after admission to the shelter, although none of these differences were statistically significant. After two weeks the UCCR had dropped significantly ($p=0,001$), as had the body weight ($p<0,001$). Stress behaviours occurred less frequently, but this decrease in frequency was not significant.

Although no significant differences were found (possibly due to the small sample size) in sleep/rest patterns and stress-related behaviour in dogs between the first night after inclusion in the shelter and 2 weeks after that, all parameters point in the same direction, namely that animals on entering a shelter experience much stress at first but seem to adapt to life in the new environment within a couple of weeks. So, in conclusion, it looks as if monitoring sleep/rest patterns could be a possible indicator for animal welfare.

1. Introduction

In the Netherlands there are over 200 shelters, which together took in more than 10.400 dogs (*Canis lupus familiaris*) in 2014.[1] Unless a dog is reclaimed by its owner, it will remain in the shelter for at least two weeks, because during the first two weeks the municipality is legally responsible for the dogs[2] and they cannot be re-homed during this time. Often, however, dogs stay in the shelter for a longer period of time. In 2014 48% of the dogs stayed at the shelter for more than 4 weeks and 18% even longer than 12 weeks.[1] Long shelter stays are partly due to the fact that euthanasia of shelter dogs - except for medical reasons - is uncommon in the Netherlands, with only 395 dogs being euthanized in 2014.[1]

Since a kennel housing in a shelter can be stressful for dogs, it is therefore important and our responsibility, that the welfare of these animals is kept as optimal as possible.

There is no consensus in how to measure the welfare of an animal objectively.[3] Already, in 1965 the Brambell Committee suggested that (positive) animal welfare is secured when animals are kept free from hunger, thirst or inadequate food; thermal and physical discomfort; injuries or diseases; fear and chronic stress; and were free to display normal, species-specific behavioural patterns.[4] However, the absence of a negative status is not equal to a good welfare.

Nowadays the welfare of an individual is suggested to emphasise the importance of an animal's adaptive capacity. This has led to a modification of the five freedoms into the following definition of welfare (Ohl and Staay, 2012): 'An individual is in a state of positive welfare when it is able to actively adapt to its living conditions and achieve a state that it experiences as positive'.[3,5,6]

It has been shown that dogs may experience fear and anxiety immediately after admission to a shelter, although high individual variation does exist.[7] Shelter dogs may experience stress due to e.g. exposure to novel environments with unfamiliar people and other dogs, noise, separation from attachment figures, spatial and social limitations and unpredictable and uncontrollable situations.[8,9,10,11,12,13,7] For example, Tuber *et al.* (1996) found, that isolation in a novel environment activated the hypothalamic-pituitary-adrenal (HPA) system of dogs, as indicated by a significant elevation of plasma glucocorticoid levels.[14] The high levels of noise in a shelter are a contributing factor to the elevations in cortisol and compromise the animals' mental and physical states, according to a study of Coppola *et al.* (2010)[15] Sales *et al.* (1997) also found that dogs in kennels are exposed to high sound levels during the day and often even at night, which can be stressful and possibly cause damage to the hearing organs.[16]According to Adams et al. (1994), night time barking can cause agitation in other dogs.[17] Furthermore, Weiss (1972) showed that stressful events have greater impact when unpredictable and uncontrollable.[18]

At the moment a dog is exposed to acute stressors the primary stress-responsive system, the HPA axis, is activated preparing the individual to react and adapt to the stressor. The hypothalamus will release corticotrophin-releasing hormone (CRH) and anti-diuretic hormone (ADH), which stimulate the pituitary to produce ACTH (adrenocorticotropic hormone), which in turn triggers the zona fasciculata of the adrenal cortex to release cortisol in the blood. High levels of cortisol have a negative feedback loop on the production of CRH and ACTH and thus inhibit the stress response.[8,9,19] Cortisol is released in a pulsatile manner, in a circadian rhythm,[20,21] and there are various factors that could cause a rise in cortisol levels, such as physical activity[22] or low environmental temperature.[23] And a decrease in cortisol levels over time does not necessarily mean that a dog isn't stressed anymore, because it could also be a sign of dysregulation of the HPA-axis caused by a chronic state of stress.[9]

Exposure to acute and chronic stressors can give a variety of behavioural responses in dogs. Beerda *et al.* (1997) found, that dogs experiencing acute stress, vocalised and panted more than usual and displayed behaviour consistent with fear and submission, such as lip-licking and lowered postures.[24,25] Rooney *et al.* (2007) saw that dogs showed behavioural changes over the first 10 days in the shelter. Self-grooming and time spent inside the kennel increased over time, while locomotion, vocalisation, panting and paw-lifting decreased.[7]
Interpreting animal behaviour correctly can be very difficult. There are many individual variations possible and abnormal-looking behaviour is not always caused by stress and could easily be interpreted incorrectly.[25,26]

The chronic activation of the HPA axis can have several effects in dogs, such as stress-related behaviour and weight loss[27]. Dogs excrete the highest concentration of cortisol metabolites in their urine[28] and therefore urinary cortisol: creatinine ratio (UCCR) may be a non-invasive and valid measure of acute and chronic stress in dogs.[12,7,29,13]
Hennessy *et al.* (1997) found that the stress response in the form of cortisol after intake in a shelter was elevated during the first three days[10], while Stephen & Ledger (2006) found that the median cortisol concentrations remained elevated during the first 17 days, although there was a large degree of individual variation.[30]
A chronic stress situation can impair immune function by e.g. blocking cytokine production in macrophages, suppressing production and release of antibodies and lymphocytes and accelerating their removal from the circulation, which in turn will increase the animal's susceptibility to infections and diseases.[19,31,24] Although previous research did not give clear evidence of immunosuppression in long-term kennelled dogs[8], alertness is necessary, as infectious diseases frequently occur in animal shelters, caused for a large part by the high infection pressure.[32,33]
Reduction of stress, besides playing a part in preventing diseases and improving the well-being of shelter dogs, is also important for increasing the chances of adoption, as most people will rather

adopt a dog that appears to be well adapted to the shelter environment and interacts normally with them. Wells & Hepper (2000) found that dogs showing abnormal behaviour like excessive barking or dogs that stayed at the back of the kennel were less likely to be adopted.[34]

Urinary cortisol levels and behavioural responses are useful indicators of the level of stress in dogs, but it is not easy to draw valid conclusions from either of the two when studied by itself, so combining the results from the urine cortisol measurements and the behaviour studies could add value to the interpretation. But the study would become even more valuable if there were more methods to measure stress in shelter dogs.

A potential additional indicator of stress might be changes in sleeping patterns, as sleep quality and quantity have been found to be affected in high mental stress situations in humans.[35] Although the mechanism has not yet been completely understood, animals that are deprived of sleep for long enough show disturbances in physical and psychological functioning and eventually will die.[18] Sleeping deficiency in humans may cause cognitive impairment, sensory disturbances and behavioural disorders.[18]
In most mammals, the sleep cycle is divided into two stages; REM sleep and non-REM sleep (or slow-wave sleep [SWS]), wherein the non-REM sleep can be further divided into four stages. A typical sleep pattern is from light SWS – deep SWS – light SWS – REM sleep. The parasympathetic nervous system is dominant in slow-wave sleep and causes calm cardiovascular and respiratory patterns and relaxed muscles, but movements are possible. During REM sleep the sympathetic nervous system is dominant, causing an increase in blood pressure, heart- and respiratory rate, higher temperature, oxygen and glucose consumption by the brain and a generalised paralysis or loss of muscle tone, alternated by fasciculations and synchronous eye movements. This is the stage in which people have the most vivid dreams.[36,37]

Lucas *et al.* (1979) found that dogs examined in a laboratory setting had a typical cycle length of 20 minutes, 25% of which was taken up by REM sleep. The average total time spent asleep in a 24-hour period was 60%. The dogs had a propensity to sleep over a 16-hour interval from 13.00 – 05.00, but especially from 21.00 – 4.00, when it was dark.[38] Dogs living in various urban habitats, either restricted dogs living with people, dogs that were regularly at liberty and dogs that were residents at an animal laboratory, had 23 sleep-wake cycles during 8 hours. An average cycle consisted of 16 minutes of sleep and 5 minutes awake. In this study, there was one dog who was brought to a new environment. In this dog, REM sleep, observed as spasmodic movements of its muscles by video recordings, was not seen the first night after transfer to a new environment, but it occurred the next two nights, implicating that moving to a new and stressful environment could reduce the occurrence of active sleep. This might well be adaptive behaviour, as active sleep would make an animal less aware of its environment.[39]

As described above, lack of sleep is a potential risk factor for the well-being of an animal. Also, in humans, stress can cause disturbed sleep patterns, like decreases in SWS, REM sleep and sleep efficiency, as well as increases in awakenings.[35] In dogs, little is known about the effect of acute or chronic stress on sleeping patterns. For example, the effect of a new environment like a shelter, which has been recognised to be an acute stress situation for dogs, on the sleep quality and quantity of dogs has not been examined thoroughly so far. The parameter, however, might be a useful candidate to give insight into an individual's capacity to adapt to a new environment.

The aim of this study, therefore, is to examine the change in sleep/rest after the intake in the new shelter environment. Based on the results from the literature it is hypothesised that shelter dogs will:

1. show more and shorter sleep-wake cycles
2. have less total sleep/rest in the first 48 hours (quantity) in the shelter compared to a period of 48 hours after a common acclimatisation time of two weeks.
3. show more signs of a stress response i.e. elevated cortisol levels in the urine and/or stress-related behaviour during the first two nights in the shelter than after two weeks.
4. Lose weight after spending two weeks in the shelter.

In order to determine the sleep and resting patterns, 48 hours video recording were made for an afterwards determination of the sleep and rest quality (i.e. sleep disturbances and length of sleep-wake cycles) and sleep and rest quantity (i.e. total amount of sleep and sleep-wake cycles) of in total 32 shelter dogs, directly after entering the shelter and again after two weeks.
Adaptation to the stressful shelter environment was determined by comparing physical signs of stress (urine cortisol: creatinine ratio, body weight and stress-related behaviours) during the first 48 hours after entering the shelter and after two weeks. In the present research, the sleep and rest data and other behavioural patterns of five dogs are analysed and presented.

2. Materials and methods

2.1. Subjects and housing

The subjects used in this study were 32 dogs that were housed at the DOA (Dierenopvang Amsterdam), which is the largest shelter in the Netherlands. These 32 dogs were strays, abandoned or relinquished by their owners or confiscated by a prosecutor. They were of various breeds, ages (mean age ± SD: 4,3y ± 3,3y; min-max: 1-12y,), sizes and of both sexes (21 males, 11 females). Anxious and aggressive dogs were excluded from the study.
All dogs were routinely examined by the shelter's veterinarian within 48 hours after intake, to exclude dogs with physical disorders, like cystitis or conditions causing poor mobility or an over- or underproduction of cortisol. Also, dogs younger than 1 or older than 12 years were excluded from the study.
All dogs were housed individually in kennels with an indoor (glass-fronted) and an outdoor (bar-fronted) area, both measuring approximately $5m^{2.}$. The indoor area was provided with a plastic or fabric dog bed, a water bowl and one or more toys (e.g. a ball or a rope toy). These glass-fronted indoor kennels were well isolated to reduce noise in neighbouring kennels. The outdoor kennel contained a food and water bowl. The different areas were separated by a plastic flap door, although in some kennels this was absent, and all dogs could move freely between the inside and outside areas, except for the moment when the kennels were cleaned in the morning, between 8.30 and 12.30.
None of the experimental dogs were in view to a broader public and were only handled by the regular caretakers, volunteers and the researchers of this study. The shelter staff and volunteers worked each day from 8 am – 5 pm. The dogs were fed twice a day, with various brands of dry dog food and in various quantities, and received an additional food enrichment like a bone once a day. The aim was to put the dogs on a fenced playfield at least once a day, but this was not done every day and the amount of time spent outside was variable. During the test period, the dogs weren't walked, accept to collect urine and to let the housetrained dogs urinate.

2.2. Study design

Video records were made the first 48 hours after intake at the shelter and for 48 hours on day 12-14. Analysis of the videos occurred afterwards and consisted of determining sleep quality, sleep quantity and behavioural data using behavioural parameters (see paragraph 2.3). Urine was collected in the morning in order to determine UCCR ratios as a physiological parameter (see paragraph 2.4.1). On day 1 and 13 dogs were weighed, the change in body weight serving as a another physiological parameter (see paragraph 2.4.2). Table 1 contains an overview of the time schedule.

Table 1: Overall time schedule.

Day 1	Day 2	Day 3		Day 12	Day 13	Day 14
Urine sampling Weighing	Urine sampling	Urine sampling			Urine sampling Weighing	Urine sampling
48 hrs video recording					48 hrs video recording	

2.3. Video data

During the first 48 hours after entering the shelter and 48 hours on day 12-14, the dogs were continuously filmed by two night vision camera's (a PRO 2-bullet camera system 2B03P, BASCOM cameras, Nieuwegein, The Netherlands, was used in combination with 4 cameras, 2 corresponding BASCOM cameras and 2 IR colour submergible cameras M244485, CCTV system, Sony EFFIO E-system, ROC, Taiwan) which were positioned outside of the kennel; one facing the indoor area and the other one filming the outdoor area, so that the whole kennel could be seen.

Footage that was recorded at night, during a resting period between 00:00 and 04:00h, was observed afterwards. All observations were done by one observer using an ethogram which was created based on previous studies about sleep/rest patterns and stress-related behaviour (see Appendix 1). Sleep quality and quantity and stress-related behaviours were first recorded using the Observer data recording system (The Observer XT 12.0, Noldus, Wageningen, The Netherlands) and analysed afterwards. The order in which the videos were analysed, was randomly chosen using an online list randomiser.[40]

As the heads - and especially the eyes - of the animals were not always visible on the recordings, it was not always possible to determine with absolute certainty whether or not a dog was really sleeping. So the assumption was made that a dog was asleep / in a state of rest, when it was in recumbency with its head down. In the absence of an EEG the occurrence of REM-sleep had to be determined visually. This was only possible when the head and eyes were visible (see ethogram, Appendix 1).

<u>Sleep Quantity</u> was determined by measuring the percentage recumbency + head down compared to the duration of the observed period.

<u>Sleep Quality</u> was determined by 2 factors: 1. the average length of the sleep cycles (mean duration of recumbency + head down), in minutes and 2. the number of sleep disturbances (frequency of recumbency + head down) during the total observation period.

Stress-related behaviour observed in the course of this study - such as lip-licking, yawning, smacking and vocalisation - were also analysed, but because of the small number of dogs in the study these data were also combined for analysis.

2.4 Physiological signs of a stress response

2.4.1. Urine collection and hormone determination

Urine samples were collected in the morning between 8.40 and 11.30h on day 2, 3, 13 and 14. A mid-stream urine sample of naturally voided urine was collected by a ladle and immediately transferred to a plastic vial by using a plastic pipette. They were stored at -20°C within 42 minutes of being voided and stored at -80°C within 2 weeks until they were analysed by the Universitair Veterinair Diagnostisch Laboratorium (UVDL), Utrecht, The Netherlands. Urinary cortisol was measured (in nmol) using a radio-immuno assay (RIA), urinary creatinine was measured (in µmol) using spectrophotometry and the UCCRs were calculated.[41]

2.4.2. Body weight
The weight of the dogs was determined on day 1 or 2 and on day 13 or 14 (Scales: AllScales Europe VS 300kg/100gr).

2.5. Baseline measurement
In order to have some insight in the dog's home situation regarding sleeping patterns, stress-related behaviours and physiological signs of a stress response for every individual dog, a questionnaire was send to the former (pre-shelter) owners or from owners after the adoption. The results of the questionnaire was used as a 'baseline'. Post adoption owners of the dogs were used in case the dogs were strays or pre-shelter owners were not wanting/able to participate: they were contacted for the questionnaire more than 6 weeks after adoption. Urine was collected from all the dogs right after they entered the shelter. Subsequently, 6 weeks after adoption morning urine produced at their new homes, would be collected and analysed to compare these baseline cortisol levels with the cortisol levels measured during their stay in the shelter. The aim of the questionnaire was to gain an indication of sleep patterns, activity and stress-related behaviours of the dog in this home situation.

To find out whether this group of shelter dogs is a realistic representation of the dog population in the Netherlands a group of representative dogs in a domestic situation was enrolled in the study as comparisons for the shelter dogs. These dogs were matched to the shelter dogs regarding breed, gender and age category. This collection of data of the matched group is still ongoing and will not be presented in the present report.

2.6 Statistical analysis
The video data of 5 of a total of 32 dogs was analysed as a pilot as not all data could be analysed due to lack of time. However, the differences between UCCR levels (n1 - n2, n1 - n13 and n2 - n13) were calculated for all 32 dogs and also the difference in body weight between the dog's arrival at the shelter and 2 weeks after that.
A Shapiro-Wilk test showed that the results were distributed normally ($P > 0,05$). Due to the small sample size non-parametric tests were used.[42] For this study the Wilcoxon Signed Rank test was chosen. As there are 2 comparisons in each case (e.g. night 1 is compared to night 2 but also to night 13) a Bonferroni correction had to be applied, so statistical significance was set to $\alpha < 0,025$ instead of $\alpha < 0,05$. All data were analysed in SPSS (IBM SPSS Statistics 22, IBM Analytics, New York, USA). The results are presented as mean values ± SEM.

3. Results

3.1 Sleep/rest
In this study sleep/rest is defined as follows: a dog is assumed to be in a state of sleep or rest when it is in a lying position with its head down.

3.1.1 Quantity
Sleep quantity was defined as the percentage of the time that the dogs were in a state of sleep/rest. In night 13 this percentage was higher than in night 1 (92% vs. 72,1%; 3h 41m vs. 2h 53m). This difference was not significant ($p=0,043$) (see Table 2- sleep quantity).

3.1.2 Quality
Sleep quality was defined by the average length of a sleep/rest period and the number of sleep disturbances. The mean duration of a sleep/rest period in night 13 was longer than in night 1 (11m 35s vs. 5m 29s), but this difference was not significant ($p= 0,043$) (Table 2 – sleep quality).

The mean number of sleep disturbances dropped from 42,4 in night 1 to 22,2 in night 13 (Table 2). This difference was not significant (p=0,043). (See Table 2 – sleep quality).

Table 2: The effect of a two weeks stay in a shelter on sleep quantity and sleep quality in dogs.
The table shows sleep/rest quantity and quality (mean ± SEM, n=5) of dogs in a shelter during the first, second and thirteenth night between 00.00 and 04.00h.

	Night 1	Night 2	Night 13	Unit
Sleep quantity				
% in sleep/rest	72,1 ± 5,3	83,2 ± 5,7	92,0 ± 2	% of the observation time
Total duration in sleep/rest	02:52:41 ± 00:12:36	03:19:26 ± 00:13:33	03:40:33 ± 00:04:54	Hh:mm:ss / 4 hours
Sleep quality				
Length of sleep/rest	05:29 ± 01:37	08:16 ± 01:51	11:35 ± 02:17	Mm:ss/ 4 hours
Sleep disturbances	42,4 ± 10,9	32 ± 10	22,2 ± 4,3	Times/4hours

There were no significant differences between the various nights.

3.2 Behaviour

The following behaviour types that were defined in the ethogram (see Appendix 1) were not seen in any of the video recordings: all forms of repetitive stress behaviour, panting, drooling, startle, tail-wagging, paw-lifting, digging, chewing, coprophagy, jerk, sneeze, tremble, shuffle, catch flies, pawing at door, cower and play bounce.
The behaviours nosing, object investigation and environmental investigarion were not precisely defined in the ethogram and consequently got mixed up in the analysis, so these behaviours were not used for further analyses.

Table 3: The effect of a two weeks stay in a shelter on the behaviour of dogs.
The table shows the mean frequencies of stress related behaviour (mean ± SEM, n=5) of dogs in a shelter during the first, second and thirteenth night between 00.00 – 04.00h.

	Behaviour	Night 1	Night 2	Night 13	Unit
Oral stress behaviours	Lip licking	11,5 ± 2,9	6,0 ± 1,2	4,0 ± 1,4	Times/hour
	Yawning	1,1 ± 0,3	0,8 ± 0,4	0,5 ± 0,2	Times/hour
	Smacking	2,2 ± 1,3	1,2 ± 0,6	0,5 ± 0,4	Times/hour
	Vocalisation	4,5 ± 2,3	25,4 ± 25,4	3,4 ± 3,4	Times/hour
	All oral stress behaviours combined	18,9 ± 6,0	33,3 ± 25,5	8,4 ± 4,7	Times/hour
Other stress behaviours	Stretching	0,4 ± 0,2	0,5 ± 0,2	0,6 ± 0,1	Times/hour
	Body shake	0,4 ± 0,1	0,4 ± 0,2	0,6 ± 0,3	Times/hour
	Grooming	2,1 ± 0,9	1,4 ± 0,8	1,5 ± 0,7	Times/hour
Combined	All stress behaviours	21,3 ± 6,5	35,2 ± 25,6	10,8 ± 4,8	Times/hour

There were no significant differences between the various nights.

Oral stress behaviours occurred less frequently in night 13 than in night 1, but the difference was not significant. Combining the various types of oral stress behaviours also did not produce a significant difference between nights 1 and 13 (p= 0,22).
Combining all types of stress behaviour also showed a decrease in frequency between nights 1 and 13, but also this total was not statistically significant (p= 0,22) (Table 3).

3.3 Physiological parameter: UCCR

Figure 1 presents the UCCR results on day 2, 3 and 14. The results of the total group are presented (n=32) as well as the results of the pilot group of N=5 for which also the video analyses were completed. After 2 weeks in the shelter, a decrease can be seen in the mean UCCR in the group for which the video analysis was completed (n=5). This results, however, were not significant (p=0,1) In the complete group (n=32), the mean UCCR dropped from 8,57 ± 1,73 (x 10^6) after the first night to 4,96 ± 1,07 (x 10^6) on day 14. This decrease was significant (p=0,001).

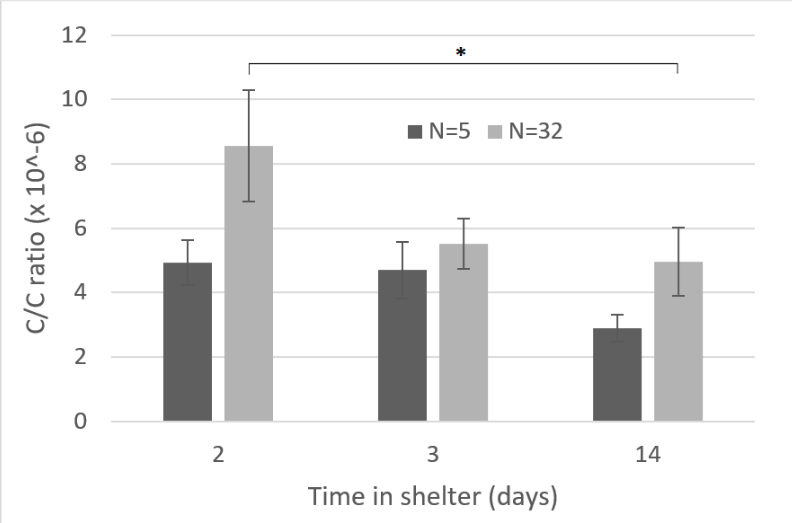

Figure 1: The effect of a two weeks stay in an animal shelter on the UCCR.
*The figure shows the mean UCCRs ± SEM on days 2, 3 and 14 for both N=5 and N=32 group. *: P<0,025.*

3.4 Physiological parameter: Body weight

In figure 2, the body weight is presented for the N=5 and N=32 group. In group N=5 there was a considerable - though not significant (p= 0,04) - decrease in mean body weight after 2 weeks in the shelter. However, for the total group (n= 32) mean body weight had dropped significantly from day 2 to day 14 (p< 0,001), being only 95,8% (± 0,77) of the initial value.

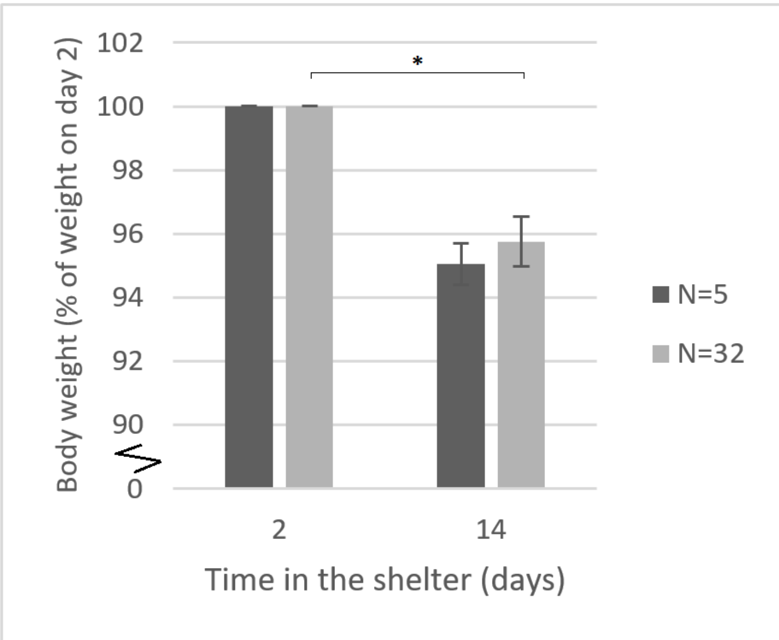

Figure 2: The effect of a 2 weeks stay in an animal shelter on the body weight.
The figure shows the mean body weight ± SEM on days 2 and 14 as a percentage of the weight on day 2, for both the N=5 and the N=32 group.
**: P<0,025.*

4. Discussion

In night 13, all five dogs in the pilot analysis slept/rested more, had longer sleep/rest cycles and showed fewer sleep disturbances (sleep cycles) than during the first 48 hours after admission to the shelter. Although all these differences were not significant they all refer to the same direction, namely the suggestion of recovery and stabilising of sleeping cycles after two weeks in the shelter. Stress behaviours also occurred less frequently after two weeks than just after admittance to the shelter. This was true for oral stress behaviours as well as for all stress behaviours combined, though the difference was not significant.
After two weeks UCCR decreased. This was not significant for the group N=5, but for the total group of 32 dogs it was. Body weights, however, decreased from intake to day 14, suggesting a previous stressful period whereby recovery cannot be seen within this short period of 14 days observation. Some of the results are discussed below in more detail.

Sleep/rest cycles are important indicators for restlessness during night and may be good indicators for recovery of stress during shelter stay. Studies of rats also show that stress can influence sleep patterns. Van Reeth *et al*. (2000) described that chronic stress in rats can lead to shorter sleep periods[43] and high cortisol levels in humans correlate positively with shorter sleep period.[44] Unfortunately only a few studies were done regarding the "normal" sleep pattern of dogs and in the current study the sleep patterns of shelter dogs were not compared to "normal" house dogs.
When we compare the current findings to the study by Adams & Johnson (1993), which described the sleep patterns of dogs in the laboratory as well as in domestic situations, the dogs in the current study had more sleep/rest cycles during the 4 hours of analysis (42,4 at night 1 versus 22,2 cycles at night 13) than the dogs in the study by Adams & Johnson had during 8 hours (23 cycles).[39] The length of the cycles was not measured exactly due to a different way of measuring sleep/rest using the ethogram, but the average duration of a sleep/rest period for the shelter dogs in this study was considerably shorter (5.5 min. at night 1 versus 11.5 min. at night 13) than the average 16 minutes in de study by Adams & Johnson[39] indicating that the dogs in this study experienced much more disturbances during the night time. The differences in the number of sleep/wake cycles and in the length of the cycles might be explained by the fact that a shelter is a very busy and stressful environment where sleep/rest gets disturbed more frequently than laboratory conditions or domestic situations. Herewith, the first hypothesis, whether shelter dogs have more and shorter sleep-wake cycles than dogs in domestic situations in previous studies, is supported by the findings in the present pilot.

The second hypothesis, whether shelter dogs will have less total sleep/rest in the first 48 hours in the shelter compared to two weeks later, is not supported by the results. There is a considerable increase of the amount of sleep/rest over time, but this is not significant. However, this lack of significance is probably due to the small sample size, which will be completed in the follow -up analyses of the complete dataset. In the present study the dogs were in sleep/rest for 72,1 - 92% (night 1 vs night 13), which is exactly the same as described by Owczarczak-Garstecka and Burman (2016). In that study, also conducted in a shelter, the percentage of sleep during the night was 71,6% and the percentage of rest was 20,5%, the sum of which is 92,1%.[45] These dogs had been staying in the shelter for at least 10 days, so that their situation was quite similar to night 13 of the current study. This increase in the sleeping percentage over time may appoint on less stress experiences after 2 weeks due to habituation, with a reparative sleep rebound after recovery. Also studies of rats describe that these animals often recover from acute stress by a reparative sleep rebound.[43]
In the study by Adams *et al*. (1993) the dogs slept 60% (unrestricted dogs), 70% (restricted dogs) or 80% (institution-housed dogs) during the night, which is a lot less than in the present study. This could be caused by the fact that the percentage of "rest" is not taken into account, but the difference could also be caused by the above-mentioned rebound effect.

The third hypothesis, whether shelter dogs will show more signs of a physiological stress response during the first two nights in the shelter than after two weeks, is partially supported by the results. The UCCR after the first night was indeed significantly higher than after 2 weeks in the shelter, if the whole group of 32 dogs was taken into account. This could indicate that the dogs experienced stress on entering the shelter, which later diminished because the dogs had adapted to life in the shelter. According to another theory, as already described in the introduction, the UCCR could have dropped as a result of HPA-axis dysregulation by chronic stress, as can be seen in e.g. pigs and cows, described by Mormède *et al*. (2007).[46] This is not very likely however because though the stress system might appear dysregulated and functioning at a baseline level, it is still more sensitive and will be acutely affected again when faced with new stressors.[46]

Regarding stress related behaviour the results do not support the hypothesis. In this study no significant difference in the frequency of stress related behavioural patterns was found between day 1 and day 13. According to Protopopova (2016), the presence of stereotypies or repetitive behaviour is the most reliable sign of poor welfare in captive animals[8] and that the length of time spent in a shelter has no effect on the rate of stereotypic behaviour. Neither stereotypies nor repetitive behaviour were observed in any of the dogs in the present study.

The last hypothesis, whether shelter dogs will lose weight after spending two weeks in the shelter, was supported by the results. After two weeks the dogs weighed significantly less than they did on arriving at the shelter. Rooney *et al.* (2009) explained that loss of weight or inability to gain weight could be a warning that a dog is experiencing stress, as the released cortisol and catecholamines can increase an individual's metabolism.[46,47] There can be different causes for losing weight, but when weight loss occurs in combination with the above-mentioned results it is logical to assume that it might well be caused by stress. Although there is no causal link, perhaps sleep deprivation could increase energy consumption.
Possibly, recovery from the stress of entering the shelter is already going on, but is not yet reflected in the body weight because gaining weight takes considerable time. Therefore, it could be interesting to keep track of the body weight a little longer to see whether this will increase over time.

The most important factor that could have influenced the results, is the small sample size because for only 5 dogs the night time footage was analysed. Although there seems to be a clear trend that shelter dogs slept more, longer and with less disturbances as they spent more time in the shelter, there was no significant proof. It is expected that a larger sample size will reveal significant differences.

Observational complications and possible disturbances
Due to the use of plastic dog baskets with raised edges dogs were not visible when in head down recumbency, while the head and eyes were important to see if the dogs were in REM sleep. Therefore it was not possible to analyse whether REM sleep was absent in all dogs their first night in the shelter as was seen in the study of Adams and Johnson[39] though it was seen in 2 of the 5 dogs during the first night and it was seen in all dogs at least once.

It is difficult to assess whether shelter dogs experience more sleep disturbances due to internal or external factors. Unfortunately no sounds were recorded, making it impossible to determine whether the dogs were woken up by the vocalisation of other dogs.
However, sometimes it was evident that a dog had been awakened by external factors. For example, the footage showed that a lot of mice and rats were trotting through the kennels which sometimes made the dogs very agitated or anxious, possibly causing dogs to wake up more often and fall asleep less quick.

There are several factors that could play a part in a dogs' capacity to deal with the shelter life, like breed and earlier confinement in a shelter. [48,49] Rooney *et al.* (2007) found that all shelter dogs had increased UCCR, but dogs that had never been in the shelter before had a significant higher increase in UCCR than those who had been.[7] In group N=5 no dogs had previously been in this shelter, however in group N=32 at least 3 dogs stayed at DOA or another shelter before, which could have caused lower UCCRs but also could have affected other parameters.

Further research
For further research, it would be interesting to watch the footage of the remaining 27 dogs, to be able to say with more accuracy what the difference is in sleeping/rest patterns and behavioural signs after intake in a shelter and two weeks later. It would be very interesting to include a control group consisting of dogs living in a domestic situation to compare the sleep/rest patterns with, using video footage. It is desirable to use flat pillows or blankets instead of baskets with raised edges, so the animals, especially their heads, will be better visible and studying them will be easier.

In the present study, no correlations between the various studies parameters were carried out because this was not very useful considering the small sample size and the non-significant outcomes. With a larger sample size with significant outcomes, this might be useful.

For shelter staff it is not very practical and too much time consuming to monitor the dogs' sleep using video cameras, therefore it would be useful if more research is being done on more feasible methods like the use of accelerometers to determine the dogs' activity. First, it must be verified that an accelerometer is an adequate tool to measure not only activity but also sleep, so this may be used in the future.

Conclusion
In this study differences in sleep/rest patterns, in the frequency of stress-related behaviours and also in other stress parameters were perceived between the first night after a dog's admission to the shelter and two weeks after that.
Although the differences in sleep/rest patterns and stress-related behaviours were not statistically significant, possibly due to the small sample size, they are in accordance with the changes in all other parameters, all suggesting that dogs entering a shelter suffer a considerable amount of stress at first, but seem to adapt to their new environment in a couple of weeks.

So it looks as if monitoring sleep/rest could be a valuable indicator for a dog's welfare.

More research is necessary to investigate this further and to find correlations between sleep/rest patterns and other stress parameters.

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Appendix 1
Ethogram of body positions and behaviours, used for analysing the night time video recordings of shelter dogs, between 00.00 and 04.00h.

Location (duration)			**Description**
Inside			Dog is in the inside part of its kennel[7]
Outside			Dog is in the outside part of its kennel[7]
Out of sight			Dog is out of sight, not in the range of the camera either on the play field, walking or at the vet[50*]
Recumbency (duration)			**Description**
Head not visible			Dog is lying with its torso on the ground, with either its head up/down and eyes closed/open[50,51*]
Head up	Eyes not visible		Dog is lying with its torso on the ground, with its head up, eyes not visible[50,51*]
	Eyes open		Dog is lying with its torso on the ground, with its head up, eyes are open[50,51*]
	Eyes closed		Dog is lying with its torso on the ground, with its head up, eyes are closed[50,51*]
Head down	Eyes not visible		Dog is lying with its torso on the ground, with its head down, eyes not visible[50,51*]
	Eyes open		Dog is lying with its torso on the ground, with its head down, eyes are open[50,51*]
	Eyes closed (sleep/rest)	REM sleep (active sleep)	Lying with its head down and neck muscles relaxed, but showing REM or spasmodic movements of its legs, paws, ears, tail, tongue or muzzle. Can be accompanied by vocalisation like whining, yelping and muffled barking[39]
		nREM sleep (quiet sleep)	Lying with its head on or between its forepaws, or on its side or back, with its neck muscles relaxed and completely still with its eyes closed[39]
Non-recumbency (duration)			**Description**
Stationary			Dog is still and not lying: sitting, standing[52*]
Movement			Dogs travels around in the enclosure without obviously investigating its environment; walking, trotting, pacing, running[13,50,51, 53,49*]
Repetitive stress behaviours (duration)			**Description**
Pacing			Dog repeatedly (> 3 times) paces around kennel in a fixed route[51,53,54]

Bouncing	Dog repeatedly (> 3 times) jumps up kennel wall from one side to another[51,53,54]
Circling	Dog repeatedly walks around in small circle (> 3 times) [51,53,54]
Tail chasing	Dog chases its tail repeatedly (> 3 times) [51,53,54]
Spinning	Moving (repeatedly) in fast circular movements[55]
Jumping	Repeatedly jumping with all fours, falling down on the same place[56]
Self-mutilation	Licking or biting itself continuously in the same place of the body, so intensely to cause abrasions or even wounds[56*]
Oral stress behaviours (Duration or rate)	**Description**
Licking lips (r)	Dog extrudes its tongue from its mouth and runs it over its lips- not following the ingestion of food[7,50,51,55]
Yawn (r)	Dog opens its jaws wide without vocalising -mouth open wide with a deep inhalation or air[7,50, 55,56]
Panting (d)	Dog had its tongue outside mouth, quickly breathing, heaving of the chest- dog pants for reasons unrelated to physical exertion or warm ambient temperature (< 25 ^{0}C)[51,53,54]
Nosing (d)	The nose is moved along objects and/or clear sniffing movements are exhibited[26,51]
Smacking (r)	Movement of the mouth without the tongue leaving the mouth, often followed by swallowing
Drooling(r)	Emitting saliva from the mouth[56]
Vocalisations (r)	Any vocalization, from high to low pitched and from long to short; growling, barking, howling or whining[7,51,53,50,57,58*]
Other stress behaviours (Duration or rate)	**Description**
Startle (r)	Legs flex briefly, and body and head quickly and briefly move back, usually in response to a sudden noise, or dog quickly moves back a few paces[51,53,49 1,2,10]
Tail-wagging (d)	Repetitive wagging movements of the tail[51,53,11]
Paw-lifting (r)	A forepaw is lifted off the ground and held there[51,53,12,13]

Digging (d)	Scratching with front-paws, on floor, wall or kennel bars[51,7]
Chewing (d)	Repeatedly chews and bites at the bars of the kennel and bedding[51,53,54]
Body-shake (r)	Rapid lateral rotation of the body in the standing position[51,53,58]
Coprophagy (r)	Feeding on faeces[53,50,26]
Jerk (r)	Sudden, quick movement with body/head[50]
Sneeze (r)	Rapid exhalation through the nose[50]
Tremble (d)	Visible shaking while dog is standing still or cowering[57]
Shuffle (d)	Dog switches its weight from one foot to the other without changing position[7]
Stretch (r)	Extending body and one or more front and/or hind-legs while remaining stationary[50,57]
Catch flies (r)	Trying to catch an imaginary fly with the mouth[56]
Pawing at door	One front paw makes contact with the cage door[57]
Cower (d)	Body in a lowered, crouched position[57]
Positive behaviours (duration)	**Description**
Object investigation	Object investigation or manipulation including playing with objects (excluding food bowl)[51]
Environmental investigation	Investigation of the environment[26]
Play bounce	Dog repeatedly displays play bow- lowered anterior part of body (lying on front-legs) and heightened posterior part of body (standing on hind legs)- posture (> 3 times) [51,53,54,50]