Good welfare or a nightmare?

Resting behaviour at night in dogs over an acclimatisation period after intake at the shelter.


Research project Veterinary Medicine University Utrecht 28-07-2017

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Abstract

Due to prolonged periods of stay of dogs in shelters, considered a stressful environment for dogs, monitoring of dog welfare at shelters becomes more important to determine whether dogs are able to adapt to the shelter environment. By entering the shelter, dogs can show a stress response, due to several stressors such as novel surroundings. In humans and rats it is known that stressors influence their sleep patterns. This study explored whether stressors encountered in a shelter environment affect resting behaviour of shelter dogs during a 14-day acclimatisation period after intake at the shelter. To examine whether there was a change in resting behaviour during this period, resting behaviour was observed in nine shelter dogs at the first, second and thirteenth night after intake. Stress related behaviour and urinary cortisol/creatinine ratios were measured in addition, to get an indication of the change in stress responses and therefore whether shelter dogs were able to adapt to their new environment. Subsequently a possible correlation between cortisol and rest was calculated. Results suggest that there was an increase of 11% in total time rest behaviour during the acclimatisation period, although this was not significant, due to large individual differences. Mean duration per rest bout was significantly doubled from night 1 to night 13. Urinary C/Cr ratio decreased significant during the acclimatisation period. Of all stress related behaviours, mouth licking, nosing, grooming and jerking were showed by nearly all dogs every night, however no significant change over the nights was found. These results suggest that dogs were able to adapt to the shelter environment and that resting behaviour increased during adaptation. However, the individual sleep patterns and the unclear relation between stressors encountered in a shelter environment and rest have to be considered.

Contents

Introduction

In 2014, 10,472 dogs entered an animal shelter in the Netherlands, which is 20% less than four years before (Heijst *et al*. 2015). Despite this decrease in the amount of dogs entering shelters, currently the mean length of stay of dogs in shelters has increased to more than three months, reported by Pel (2016), whereas in 2014 79% of the dogs stayed in the shelter for less than three months (Heijst *et al*. 2015). Mainly dogs with health or behaviour problems spend prolonged periods of time in a shelter. To rehome these dogs 'with special needs', rescue charities need to invest 2.4 million euro's per year (Pel 2016). As these dogs spend prolonged periods of time in a shelter, it is important to assess the impact of shelter housing and the showed stress response by shelter dogs.

Dogs often show a stress response after intake at a shelter, due to several stressors such as novel and unpredictable surroundings (Tuber *et al*. 1996; Beerda *et al*. 1998), separation from any familiar contacts (Tuber *et al*. 1996; Hennessy *et al*. 1997), confinement in a limited space (Hetts *et al*. 1992), unexpected loud noises (Coppola *et al*. 2010; Sales *et al*. 1997) and new odors from other (aggressive) dogs (Hennessy *et al*. 1998; Beerda *et al*. 1997). During a stress response, the hypothalamic-pituitary-adrenal (HPA) system is activated, resulting in an elevated level of cortisol. The HPA axis is known as "the body's primary stress-responsive physiological system" (Hennessy 2013), and it is highly responsive to stressors that dogs are confronted by in a shelter environment, as reviewed by Protopopova (2016) and Hennessy (2013). According to Hennessy *et al*. (1997) the level of cortisol in shelter dogs increases during the first three days after intake at the shelter and declines thereafter, while Stephen and Ledger (2006) found a cortisol level peak on day 17 before it started to decline. Cortisol levels can be measured in blood plasma, saliva, hairs, faeces and urine. The advantage of urine samples over the other matrices is that it can be collected non-invasively and that it can reflect cortisol levels over the previous time period up to 24 hours (Hiby et al. 2006; Beerda, Schilder, Bernadina, et al. 1999; Rooney et al. 2007; Schatz & Palme 2001). Because the absolute cortisol measured in urine is influenced by the urine concentration, cortisol is expressed as a cortisol to creatinine (C/Cr) ratio. Besides the physiological response to stressors, a stress response in dogs can also be expressed behaviourally, such as by paw-lifting, self-grooming, panting and barking, as reviewed by Protopopova (2016). By measuring urinary C/Cr ratios together with behavioural observations, more information on the origin of the stressors and the valence of the response can be gathered (Hiby *et al*. 2006).

It is known that exposure to stressors for a prolonged period of time, may influence the well-being and state of health of an animal, i.e. such as illness and depression (Hennessy *et al*. 1997). To prevent chronic stress and its consequences, a dog must be able to adapt to its new environment in order to prevent welfare problems (Ohl & Van Der Staay 2012).

In humans and rats it is known that stressors influence the quality and quantity of rest. In humans, stress interferes with the normal sleep cycle and a lack of sleep itself can be a stressor too (Kashani *et al*. 2012; Banks & Dinges 2007). Emotional stress, like worrying about going to work the next morning, results in changed sleep stage architecture, as reviewed by Kim and Dimsdale (2007). In rats the sleep-wake cycle is also sensitive to stress, but the effect of stressors on the sleep wake cycle differs for each individual, which depends on A) the type of stress (acute or chronic) and B) on the normal period of sleep of each individual without experiencing stress (Bouyer *et al*. 1997). Nevertheless, Abou-Ismail *et al*. (2008) showed in his study that rats with experimental disturbed sleep displayed signs of reduced welfare and that therefore sleep can give an indication of the state of welfare. As stressors are shown to influence sleep in humans and rats, the question arises whether stressors encountered in a shelter environment influence resting behaviour in dogs.

In general, dogs have a sleep cycle that exists of slow-wave sleep (SWS) (or quiet sleep) followed by Rapid Eye Movement (REM) sleep and ends with wakefulness (Langford & Cockram 2010). Dogs sleep approximately 60-80% of the night during 8 hours, depending on the housing conditions they sleep in (Adams & Johnson 1993). According to Adams and Johnson (1993), dogs in shelters (long-term and short-term stay) were asleep at night for 80% of the time, while dogs housed in groups and enriched kennels slept for 60-71% of the night and 30-37% during day time (Zanghi et al. 2012). Adams & Johnson (1993) suggest that domestic dogs living in various urban habitats have 23 sleep-wake cycles during 8 hours of sleep at night with an average of 16 minutes asleep and 5 minutes awake. Besides enrichment, other daily influences on changes in sleep routine are adjustments in work routine (Adams & Johnson 1994), change in feeding regimen, novel/adjustments in housing conditions (Adams & Johnson 1993; Hetts *et al*. 1992; Hubrecht *et al*. 1992), the amount of activity during the day (Zanghi *et al*. 2012), social interaction with humans and other dogs during the day and age (Takeuchi & Harada 2002; Zanghi *et al*. 2012).

Therefore, the available literature seems to show that sleep in dogs is sensitive to their environment, as previously shown in humans and rats. Moreover, Ruckebusch (1975) found changes in hypnograms of cattle during various environmental stressors and saw that the hypnograms re-established during a month. Therefore Ruckebusch (1975) suggested that rest and sleep behaviour could be a welfare indicator to other species, for example dogs, to estimate the stage of adaptation to a novel environment.

Recently, Owczarczak-Garstecka and Burman (2016) have evaluated sleep and resting behaviour as a welfare indicator in shelter dogs. They investigated whether an increased stress response (assessed by looking at stress related behaviour) affects the average sleep time of shelter dogs. Their observations began after the dogs had an acclimatisation period of at least 10 days in the shelter (range: 10 days – 2 months) and they did not measure a physiological parameter in addition to behavioural measures.

For now, no studies have looked at the disturbance and potential recovery of sleep and rest in dogs due to stressors encountered in a shelter environment during an acclimatisation period starting right after the intake at the shelter. Therefore, in this study resting behaviour of dogs will be assessed/observed and compared between the first two nights after intake at the shelter and in night 13 (after a 14-days acclimatisation period). The second night is taken into account to investigate whether resting behaviour changes between the first two nights already. To get an indication whether shelter dogs are able to adapt to the shelter environment, indicators of a stress response in dogs were also measured, including urinary C/Cr ratios and stress related behaviours.

Based on previous findings in humans, rats and cattle, an increase of resting behaviour in shelter dogs between the first two nights after intake at the shelter and night 13 is expected. A difference of resting behaviour between the first and second night Cortisol is expected to respectively decrease, as well the duration and rate of observed stress related behaviours. Furthermore, a correlation between cortisol and resting behaviour is expected to be found.

Materials and Methods

Subjects and housing

The study took place between June and October 2016 at the largest animal shelter of the Netherlands; Dierenopvang Amsterdam (DOA). Dogs selected for this study had to be at least one year old (range: 1-13 years old, mean age: 6.2 ± 3.7 years), safe to handle, healthy (diseases that could influence cortisol levels and/or behaviour). Dogs were strays or relinquished to the shelter by their owner. All dogs were examined by a veterinarian at the shelter within 48 hours after intake. The shelter dogs (n=9) that were participating in this study are described in table 1.

Table 1 Dogs participating in the study
Shown is a description (gender (M=male, F=female), age, breed and reason for being in the shelter) of each dog participating in this study.

Dog	Gender	Age (years)	Breed	Relinquishment or stray
Yui	M	7	Pekinese Shih Tzu mix	Relinquished
Lola	F	5	American Bulldog	Relinquished
Jerry	M	10	Poodle mix	Relinquished
Joop	M	13	Jack Russel	Relinquished
Senna	F	8	German Shepherd	Relinquished
Buddy	M	1	American Staffordshire terrier	Stray
Kyra	F	4	American Staffordshire terrier	Relinquished
Bella	F	5	Shepherd mix	Relinquished
Chico	M	3	American Staffordshire terrier	Stray/Relinquished

Dogs were housed individually, in a kennel divided in a glass fronted inside kennel and a bar-fronted outside kennel ± 5 m^2 each. All other sides were closed walls. The inside kennel was glass fronted to reduce noise and smells. The pens were separated by a plastic flap which is closed during cleaning only (time differs per dog). A soft dog bed was provided inside next to the plastic flap.

Routines were the similar during most days. Dogs were fed wet or dry food twice a day and water was available unlimited. Every afternoon food enrichment was given to the dogs. Dogs had access to a playing field once or twice a day, together with other dogs where possible. Dogs were walked at least every other day and some of them got additional training to reduce potential behavioural problems. Kennels were closed between 19:00 p.m. and 7:00 a.m.

Behaviour observations

Observations of the dogs began right after intake at the shelter at night 1, 2 and 13. Two night vision camera's (PRO 2-bullet camera system 2B03P, BASCOM cameras, Nieuwegein, The Netherlands) were placed in front of the kennel, aiming that the inside and outside pen were recorded by one camera each. Positioning of the cameras took place at the day before night 1, so dogs could habituate to this new object. Video recordings were made of each dog at the first, second and thirteenth night in the shelter, resulting in continuous video recordings of the time period between 00:00- 04:00 a.m.. As the kennels are closed between 19:00 p.m. and 7:00 a.m., and video observations were made in different seasons, this period was considered a quiet and dark phase in which the dogs would be able to rest with the least disturbances Observer XT (Noldus Information Technology) was used to observe the videos. All nights were observed randomly (random.org).

Resting behaviour

Each observed night, the activity and resting behaviour of the dog was scored according to the ethogram in table 2. In this study, rest defined as recumbent head down because of the following reasons:

- First of all, because of the absence of Electroencephalography (EEG) data, as this was considered too invasive and impractical in a shelter situation, it was difficult to observe whether the shelter dogs were asleep and to quantify brain activity corresponding to sleep stages, among which REM sleep. Therefore here, rest was observed behaviourally.
- Second, the position of the eyes was not taken into account when defining rest, because the eyes where not visible on video all of the time (eyes were behind bars or the dog bed). When the eyes were visible during the observations, it was scored whether the eyes were open or closed, however this data was only used to calculate a cut off for resting behaviour, as described below.

Table 2 Ethogram of resting behaviour. All behaviours were measured for duration and rate.

Recumbency			Description
Head not visible			Dog is lying with its torso on the ground, with either its head up/down and eyes closed/open[1,2*]
Head up	Eyes not visible		Dog is lying with its torso on the ground, with its head up, eyes not visible[1,2*]
	Eyes open		Dog is lying with its torso on the ground, with its head up, eyes are open[1,2*]
	Eyes closed		Dog is lying with its torso on the ground, with its head up, eyes are closed[1,2*]
Head down	Eyes not visible		Dog is lying with its torso on the ground, with its head down, eyes not visible[1,2*]
	Eyes open		Dog is lying with its torso on the ground, with its head down, eyes are open[1,2*]
	Eyes closed	In which no obvious REM sleep is seen	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[3]. No movement is seen.
		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. Time between movements is 30 seconds at the most. Can be accompanied by vocalisation like whining, yelping and muffled barking[3]

1. (Titulaer et al. 2013), 2. (Schipper et al. 2008), 3. (Adams & Johnson 1993)
* Means edited

Stress related behaviour

To get an indication of the stress response and the adaptation to the shelter environment, stress-related behaviours and behaviours observed in positive contexts, such as play were observed during the same night period as when resting behaviour was observed. The ethogram for stress-related behaviour was based on previous ethograms from different welfare related behaviour studies in dogs. Attachment A shows the ethogram with references used for this study, with 30 stress related behaviours and 2 positive context related behaviours. Duration (d) and/or amount of display (rate (r)) were scored per behaviour, see attachment A.

Cortisol

Cortisol:creatinine ratios were measured in morning urine of the dogs. Naturally voided urine was collected in the morning after the observed nights between 8:45- 10:00 a.m., except for one sample: at 11:20 a.m. A ladle was used for collection and the urine was transferred, while using pipettes, to polypropylene vials. The vials were immediately frozen in the shelter at -20 °C and stored within two weeks at -80 °C at the Faculty of Veterinary Medicine, Utrecht University. The samples were analysed by the University Veterinary Diagnostic Laboratory (UVDL). Urinary cortisol concentrations were established using a Radio-Immuno-Assay (RIA) with an antibody developed by the UVDL. Creatinine was measured by a kinetic colour test (Jaffé method) on Beckman Coulter AU analysers and so cortisol to creatinine ratios were calculated.

Data analysis

By calculating the total duration, mean duration and total number of rest bouts, an indirect indication of **resting behaviour** was calculated for each night per dog. Definitions of these variables are given in table 3. By calculating the percentage of the total duration rest over the absolute observed time, comparisons with previous studies are easier to make. Also, due to the video software, seconds to minutes of video recordings were missing, therefore percentage of the total duration is a better measure than total duration itself.

Table 3 Definitions of variables used to measure resting behaviour

Variable	Definition
Total duration spent resting	Total duration of rest during the 4 hours of observed video
Percentage total duration of rest	Total duration of rest divided by the total observation duration
Mean duration per rest bout	Total duration of rest divided by the total number of rest bouts
Total number rest bouts	Sum of all rest bouts

The means per night ± standard deviations (SD) for these four variables for each dog individually were calculated by the Observer XT. All data were further analysed in SPSS Statistics 24. Resting behaviour was selected as the dependent and nights as the independent variable. To determine whether there is a difference in resting behaviour between the two nights after the intake and a night after 14 days, repeated measurement ANOVA was used if assumptions were met. Wilcoxon signed rank test with a corrected α for multiple comparisons was used if assumptions for repeated measurement ANOVA were not met.

A high number of alternations between recumbent head up and head down within a short time period could not to indicate rest, but rather unrest. Therefore, inaccuracies in the measurement and interpretation of resting behaviour could take place. To not include this 'unrest' in measures of rest, in previous literature authors have defined dogs to be at rest or at sleep after a certain time frame in a resting position. For example, Owczarczak-Garstecka and Burman (2016) defined sleep as being in a resting position (with eyes closed) for more than two minutes. Clarke and Fraser (2016) studied rest using an accelerometer and they defined the dog as "resting" when the dog was at least one minute in head-down recumbency. In both studies, no explanation was found why they choose this length of time for their definitions of sleep or rest. To remove short bouts of recumbent with head down from the data, which can be seen as unrest, two options can be performed. The first option is to observe when the dogs close their eyes, assuming that at that moment dogs trust their environment and have found their rest. However, this was found to be impossible in this study as eyes were often not visible on the camera due to kennel bars, dog beds etc. Therefore the second option, a cut off in merged data of recumbent head down (a sum of eyes open, closed and not visible) is applied, based on the average duration (latency) until the dogs closed their eyes on moments that the eyes were visible on the camera. Applying this time as a cut off at all the resting bouts of the merged data, the ‘unrest’ data (short resting bouts) were removed.

The latency from ‘recumbency head down eyes open’ to ‘recumbency head down eyes closed’ , following behaviour other than recumbent (see figure 1), was calculated in Observer XT for all video data in which this was observable, for each night of four dogs. This could not be calculated for more dogs due to limited time. The average of all these latencies was calculated, as an indication of when dogs on average would close their eyes when recumbent. All rest bouts with shorter durations than average were removed by the Observer XT in all nights for all dogs. Both data with and without this cut-off were analysed as mentioned above.

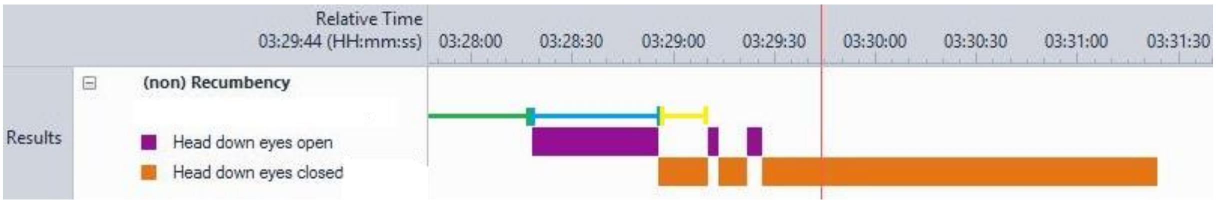


Figure 1 Example of the visualization of data in Observer XT whereas the green line is behaviour other than recumbency, the blue line is recumbency with eyes open, and yellow line is recumbency eyes closed. The duration of ‘Recumbency eyes open’ only was noticed when ‘other behaviour’ was displayed before ‘recumbency eyes open’ and ‘recumbency eyes closed’ was shown subsequently.

Because only several **stress related behaviours** were shown by the dogs and due to limited time, only stress related behaviours that were shown by most dogs (minimal of n=5) during every night were analysed. For duration behaviours percentage of total duration and total number were calculated by the Observer XT. For rate behaviours the total number per night was analysed. For all behaviours selected, averages (±SD) were calculated and repeated measurement ANOVA was used to determine differences in duration and/or rate of each behaviour between the observed nights.

Urinary cortisol concentrations were expressed as ratios to urinary creatinine concentrations. To get an indication of the differences and changes over the three nights in cortisol, repeated measurement ANOVA was used. To determine whether cortisol levels were correlated to resting behaviour (without and with cut-off), Spearman rank correlation was used as data was not normally distributed. Statistical significance was set to $\alpha < 0.05$.

Results

All data of the nine observed dogs was complete, no data was missing.

Change resting behaviour over acclimatisation period without cut off

For each dog descriptive statistics for each variable of rest was calculated and graphs with individual data are shown in attachment B. Averages of all dogs per night are shown in table 4 which summarizes results of the variables.

On average, dogs spent 75% (±11.8), 80% (±18.3) and 85% (±17.6) of the time resting between 00:00-04:00 a.m. on respectively night 1, 2 and 13 (figure 2). This increase in percentage reflected in total an increase of approximately 25 minutes in a resting position during the observed four hours from night 1 to night 13 (table 4). An average rest bout lasted for 3.92 minutes (±1.87) on the first night, 6.27 minutes (±3.05) on the second night and 7.63 (±5.12) minutes on night 13 (figure 3). Dogs took 54 (±22), 36 (±17) and 39 (±28) rest bouts on night 1, 2 and 13 respectively during the four analysed hours on average (figure 4). However, repeated measurement ANOVA showed that there was no statistically significant effect of the acclimatisation period on any of the variables without the cut-off of short resting bouts.

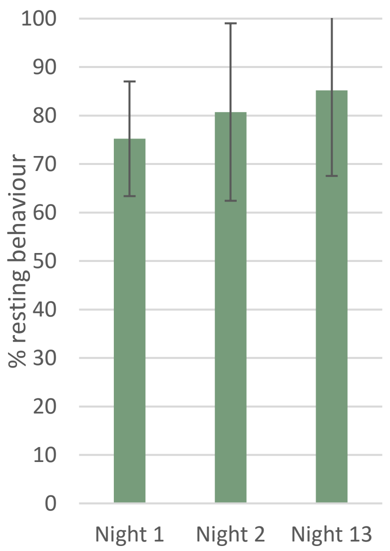


Figure 2. Mean percentage resting (Y-axis) behaviour per night (X-axis) (±SD) without cut off

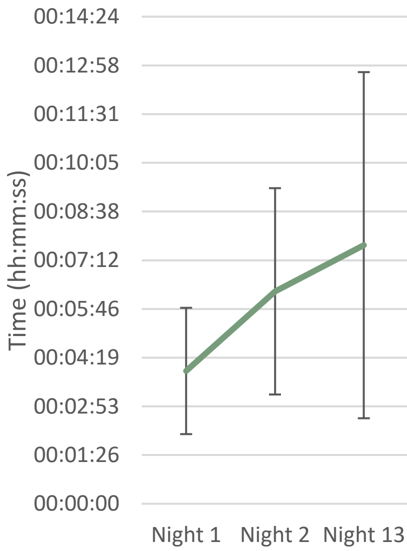


Figure 3. Average of mean duration per rest bout (Y-axis) per night (X-axis) (±SD) without cut off

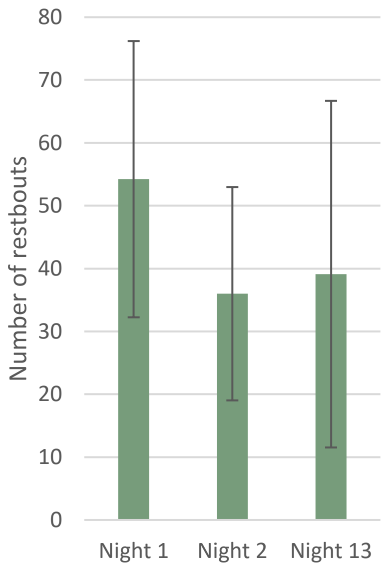
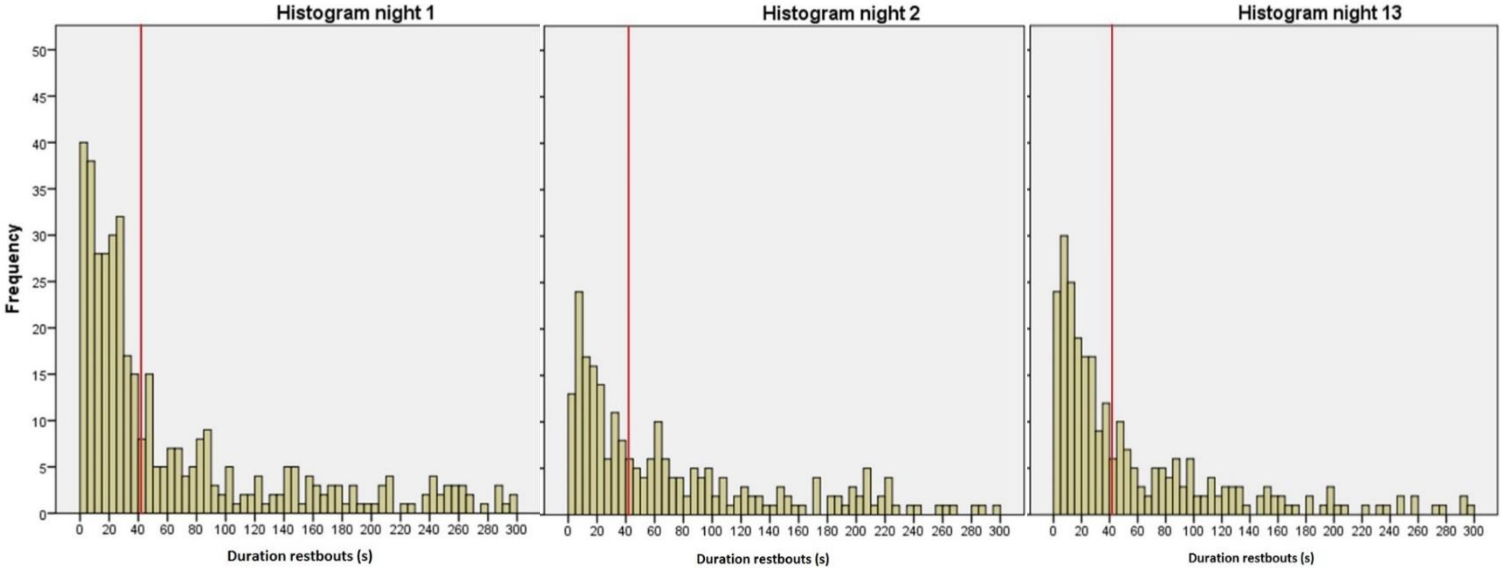


Figure 4. Mean total number rest bouts (Y-axis) per night (X-axis) (±SD) without cut off

Figure 5 Frequency histograms of all resting bouts of all dogs. The histogram shows how frequent a rest bout duration between e.g. 0 and 5 seconds was displayed during one night by all dogs together. From left to right night 1, 2 and 13. On the X-axis a sum of all rest bouts durations seen per night, categorized per five seconds. On the Y-axis the frequency of the occurrence per categorized duration of a rest bout. Only the rest bout durations up to 300 seconds are shown in this figure.



Resting behaviour measures with cut off

The average latency from 'recumbency head down eyes open' to 'recumbency head down eyes closed', following behaviour other than recumbent , as described in the Materials and Methods section, was 42.3 (±50.8) seconds. This timeframe was used as a cut off to remove all the 'unrest' short resting bouts of the merged data. Frequency histograms were made to show the impact of the cut off on the removal of data, indicated with the red line (figure 5).

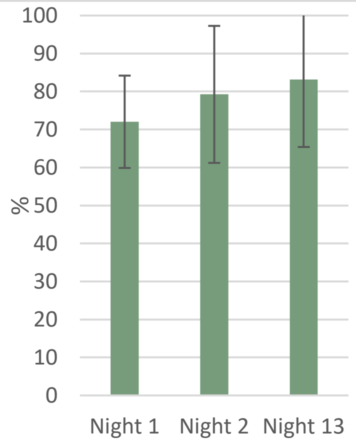


Figure 6. Mean percentage resting behaviour per night with cut off (±SD)

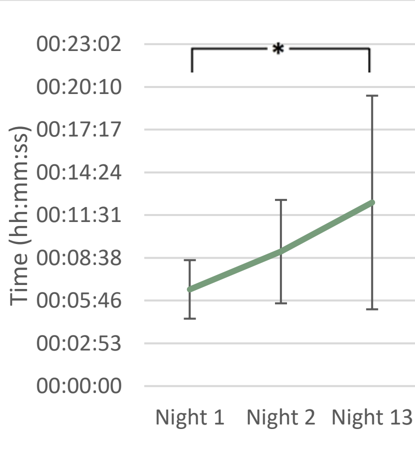


Figure 7. Average of mean duration restbout per rest bout per night with cut off (±SD).
*** P<0,05**

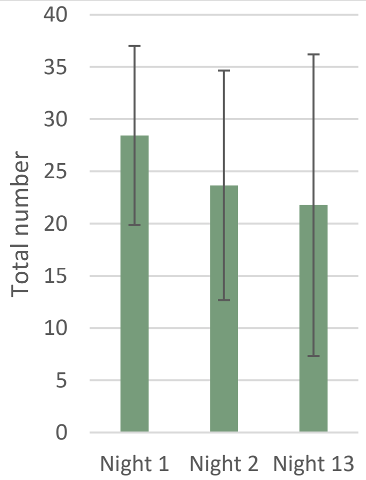


Figure 8. Mean total number restbouts per night with cut off (±SD)

Descriptive statistics for each variable was recalculated with cut off of these shorter resting bouts. Graphs of individual data are shown in attachment B. On average, dogs spent 72% (±12.2), 79% (±18.0) and 83% (±17.8) resting during the four hours on respectively night 1, 2 and 13 (figure 6). Therefore, on average, an increase of +27 minutes of resting was displayed by dogs during the 4 hours observation. The average rest bout lasted for 6.52 (±1.97) minutes on the first night, 9.07 (±3.48) minutes on the second night and 12.37 (±7.20) minutes on night 13 (figure 7). Dogs took 28

(±9), 24 (±11) and 22 (±14) rest bouts on average on night 1, 2 and 13 respectively during the four analysed hours (figure 8). To get an overview of the changes in averages with and without the cut off, averages are shown in table 4.

Repeated measurement ANOVA showed that the average of mean duration per rest bout differed statistically significantly between nights ($F(2, 16) = 3.696$, $p = 0.048$). Post hoc tests using the Tukey LSD revealed that the average rest bout at night 13 (12.37 ± 07.20 minutes) was statistically significantly higher than at the first night (6.52 ± 1.97, Tukey LSD post hoc, $p = 0.028$). There was no significant effect on any other variable. For the variable Total Number, assumptions for an ANOVA were not met, Wilcoxon signed rank Test did not show a significant effect.

Table 4 Resting behaviour per night without and with cut off

	Night	Average without cut off ±SD	New average with cut off ±SD
Total duration spent resting (hh:mm:ss)	1	03:00:20 ±00:28:21	02:52:43 ±00:29:07
	2	03:13:34 ±00:43:54	03:10:00 ±00:43:15
	13	03:24:59 ±00:42:07	03:20:05 ±00:42:22
Percentage total duration of rest (%)	1	75 ±11.8	72 ±12.2
	2	80 ±18.3	79 ±18.0
	13	85 ±17.7	83 ±17.8
Total number rest bouts (n)	1	54 ±22	28 ±9
	2	36 ±17	24 ±11
	13	39 ±28	22 ±14
Mean duration per rest bout (mm:ss)	1	03:55 ±01:52	06:31 ±01:58
	2	06:16 ±03:03	09:04 ±03:29
	13	07:38 ±05:07	12:22 ±07:12

Cortisol and resting behaviour

Urinary cortisol measures of each dog individually are shown in a spaghetti plot graph in attachment C. A statistically significant decrease of C/Cr ratio over the nights ($F(2, 14) = 4.446$, $p = 0.032$) was found, with C/Cr ratio at night 13 being lower (2.74 ± 1.16) than at the first night (4.80 ± 2.36, Tukey LSD post hoc, $p=0.020$) (figure 9). There was no significant difference in C/Cr ratios between night 2 (4.24 ± 2.30) and any other night.

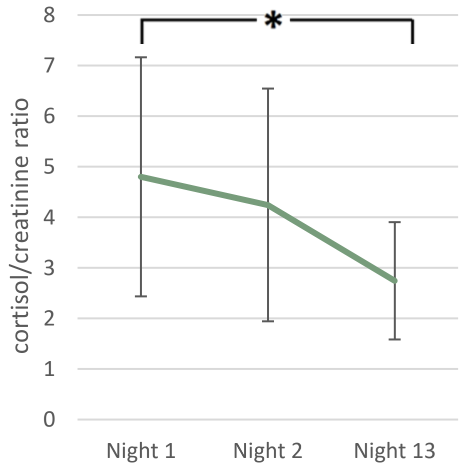


Figure 9. Mean cortisol/creatinine ratio per night (+/- SD). *, P<0,05

There were no significant correlations between cortisol and any of the following resting-behaviour measures:

- total duration and percentage of total duration (night 1: $r=0.071$, $p=0.867$, night 2: $r=-0.636$, $p=0.066$, night 13: $r=-0.383$, $p=0.308$),
- total number of rest bouts (night 1: $r=0.180$, $p=0.670$, night 2: $r=-0.475$, $p=0.197$, night 13: $r=-0.250$, $p=0.516$)
- mean duration of a rest bout (night 1: $r=-0.310$, $p=0.456$, night 2: $r=-0.017$, $p=0.966$, night 13: $r=0.033$, $p=0.932$)

Stress related behaviours

No repetitive stress related behaviours were observed in the first two weeks, except for pacing (at night one, showed three times in total by 2 dogs) and circling (at night 2 once, showed by one dog).

Pacing, circling, licking lips, yawn, nosing, smacking, vocalisations, startle, grooming, tail-wagging, paw-lifting, body-shake, jerk, sneeze, stretch, pawing at door and play bow were seen during the observations for at least once during the three nights by one dog. Due to the small amount of observations of most behaviours, results of the tests regarding the effect of time over nights on the behaviours could not be considered to be reliable and therefore are not mentioned here. Some behaviours were notable enough to analyse them statistically, because nearly all dogs showed it almost every night. Nosing decreased in rate (21 (±20.7), 9 (±5.2), 6 (±8.4)) and percentage (1.0 (±1.1), 0.5 (±0.3), 0.4 (±0.5)) on night 1, 2 and 13 respectively. Grooming increased in rate between night 1 (10 ±9.6) and night 2 (12±8.1) and decreased at night 13 (11 ±7.6), however, the duration of displayed grooming decreased between night 1 (7.8% ±9.3) and night 2 (2.6 ±2.7) and increased slightly to night 13 (3.9 ±4.3). Licking lips was displayed 27 times (±15.0) at the first night, 15 times (±17.4) at night 2 and at night 13 18 times (±23.0). Dogs displayed a decrease of jerking at night 1 (55 ±43.0), night 2 (36 ±51.9) and night 13 (13 ±12.7). However, for, nosing, grooming, licking lips and jerking, repeated measurement ANOVA found no significant differences between the nights ($p \geq 0.187$) (figure 10 and 11).

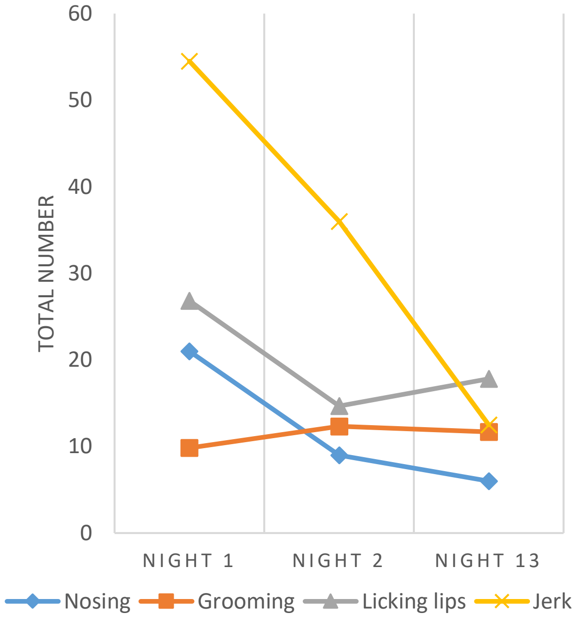


Figure 10. Total number of several behaviours displayed per behaviour over four hours of observation per night

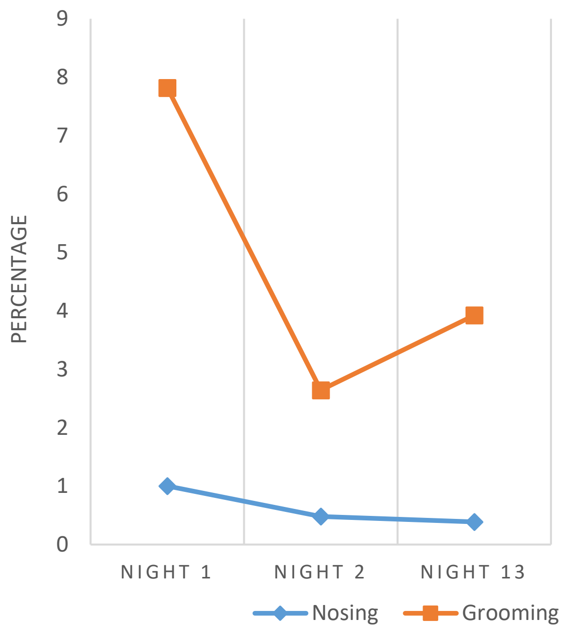


Figure 11. Percentage of displayed behaviours nosing and grooming per night over four hours of observation

Discussion

This study observed whether resting behaviour of shelter dogs could be disturbed and potential recovered due to stressors encountered in a shelter environment over an acclimatisation period starting right after the intake at the shelter. To get an indication of whether the observed dogs were able to adapt to the shelter environment, C/Cr ratios and stress related behaviours were evaluated as a measure of the stress response.

Firstly, an increase in the amount of resting behaviour was expected. Total amount of rest increased with 11% (approximately 27 minutes over 4 hours of observation) from night 1 to night 13. Although it was a biological measurable difference, this difference was not statistically significant. Despite the total number of resting bouts was decreased with six rest bouts, this could not be confirmed statistically as the difference was not found to be significant. The mean duration of a rest bout, however, was significantly doubled in two weeks from 6 minutes and 31 seconds to 12 minutes and 22 seconds after removal of shortest rest bouts indicative of 'unrest'. Overall, resting behaviour measures suggests that resting behaviour may improve/re-establish from the first night to night 13, and therefore that the dogs have more rest after an acclimatisation period. Second, a decrease in C/Cr ratios and less stress related behaviours were expected after an acclimatisation period compared to the period after intake at the shelter. Indeed, the C/Cr ratio decreased significantly between the first night compared to night 13. This decrease in C/Cr ratio could indicate that on average, dogs were able to adapt to the shelter environment during their first two week stay, as the stress response declines. However, no significant decline was found for stress related behaviours during the two weeks. Third, a correlation between C/Cr ratios and resting behaviour was expected. However, no correlations between any of the resting behaviour variables were found. In summary, results imply that dogs may have been able to adapt to the environment as expected and that resting behaviour has increased.

Distinction between rest and unrest

In previous studies, a distinction between rest and unrest had been made by taking a certain time frame in a resting position, however, no arguments were given on why they choose this length of time for their definitions of sleep or rest (Owczarczak-Garstecka & Burman 2016; Clarke & Fraser 2016). In the present study, it was decided to get an indication of the average time that dogs would close their eyes when recumbent, by looking at the latency from 'recumbency head down eyes open' to 'recumbency head down eyes closed' , following behaviour other than recumbent, to make a 'cut off' between short resting bouts that may be indicative of unrest. Due to time pressure the cut off was calculated based on the data of only a few dogs (n=4), and averaged over all nights. Moreover, by calculating a cut off, it makes it harder to compare the results with other studies. In future research, it is recommended to calculate a cut off per dog per night, given the high standard deviation of the cut off ((±50.8) over an average of 42.3 seconds).

Individual differences

Looking at the means, standard deviations and individual data of the measured variables in this study, high individual differences between responses of the dogs were observed (attachment B). First, it is interesting to observe that standard deviations increased over the nights for all variables of resting behaviour, suggesting that the difference between individuals become larger after acclimatisation. It seems that at night 1 less individual differences were measured, which may indicate that many dogs had a disturbed rest that night. Whereas at night 13, a larger difference between individuals was visible, which may indicate that some dogs were able to adapt and other dogs were not. It is possible that different adaptability patterns can be seen. For example, looking at the percentage of total resting behaviour in attachment B, patterns are visible where resting behaviour increases over the nights, but decreasing patterns of resting over the nights are also visible of other dogs e.g. between night 2 and 13. As mentioned in the introduction, it has been found that sleep in rats was influenced by stressors. However, the effect on sleep in these rats differed individually, depending on which type of stress the rat seems to experiences (acute or chronic) and the individual sleep pattern when no stress response was measured (Bouyer et al. 1997). This could clarify the individual patterns and differences found in the present study. This may imply that dogs have individual resting patterns, like rats do. Moreover, dogs are shown to have individual coping styles, as mentioned in Rooney *et al.* (2007) and Koolhaas *et al.* (1999). Which type of coping style/stress response to stressors is shown by dogs depends, among other things, on a dog's past such as if a dog was submitted as a stray or relinquished by its owners (Rooney *et al*. 2007). In the present study, the dogs named Chico (stray) and Lola (relinquished) seem examples for this theory of individuality , as Chico's C/Cr ratios are low and stable since the first night and his amount of rest is high and stable over the nights, whereas Lola seems to show a high stress response and little resting behaviour at the beginning (night 1) but it seems that she was able to adapt over the nights (C/Cr ratios decreased, percentage resting behaviour increased) (Attachment B+C). The many individual differences imply that maybe it is better to focus on resting patterns than to measure rest on average of the whole group, to get reliable and better results. Individual results could be more informative on which dog needs, for example, a quieter sleeping place or more attention and training during the day. Furthermore, dogs who do not show an increase in resting behaviour at night 13 and seem to be (abnormally) restless at night, may be high-risk dogs for welfare problems after adoption when not matched to a suitable (quiet) environment.

Looking at attachment B at the dog named Chico, a first impression could be that he seems to be "relaxed" because of its exceptional long resting periods mainly at night 13. However, long resting periods could also be a sign of depression (Stephen & Ledger 2005), as increased inactivity has been described to be an expression of depression (Rochlitz et al. 1998; Dalm et al. 2009; Meagher et al. 2013; Stephen & Ledger 2005). In cats (Rochlitz *et al*. 1998), rats (Dalm *et al*. 2009) and mink (Meagher *et al*. 2013) increased inactivity has been found to be sign of reduced welfare, however other studies with mice suggest that inactivity is associated with adaption to the environment (Würbel *et al*. 1998). The difference between inactivity caused by reduced welfare and caused by relaxation could be the position of recumbency (Meagher et al. 2013). Animals who show a stress response, will be in a position that makes it easier to run away, they hide behind obstacles and/or they are alert and check the environment constantly, resulting in shorter sleeping and rest bouts (Meagher et al. 2013; Lima et al. 2005). Regarding the results of the present study, it is difficult to distinguish normal rest, (abnormal) unrest and signs of abnormal rest by looking at the recumbent position only, as discussed next. Moreover, during observing the video tapes, it was noticed that even though the dog was in a 'recumbency head down' position, the position was tense. Despite this, the dog was coded as resting, following the ethogram. Thus, if resting behaviour is observed as a welfare indicator, the results need to be interpret carefully.

Moreover, in this study resting behaviour is defined and observed by using the recumbent-head-down position, without looking at the position of the eyes (open/closed) as the eyes were often not visible on camera. However, observing the eyes in addition to recumbent behaviour can provide much more information, for example when dogs close their eyes, this may suggest they trust the environment at that point, to take their rest (Owczarczak-Garstecka & Burman 2016; Lima et al. 2005). Also, Meagher *et al.* (2013) suggest that lying awake (eyes open) may indicate a negative, 'boredom-like state'. Therefore, it is recommended to observe the position of the eyes in further research to be better able to distinguish between rest and unrest, and maybe even sleep.

Additionally, in order to be able to distinguish between rest or sleep and unrest and to examine the correlation with stressors encountered in a shelter environment, physical parameters like heart frequency and respiration rate can be assessed, knowing that these parameters are influenced by stressors (Palestrini et al. 2005). Other studies, which investigated whether an accelerometer could be used to measure resting behaviour/activity, suggest that this device could give an indication when the dog is relaxed or restless (Clarke & Fraser 2016). Using the accelerometer could reduce the time needed for observing resting behaviour in dogs and therefore a bigger sample size of dogs could be measured. Summarizing, further research is needed to distinguish when a dog is ‘relaxed’ or restless, by assessing the position of eyes, heart and respiratory measures and/or accelerometers in addition to observed resting behaviour. Therefore interpretations of measurements of rest will be more reliable.

Effect of activity on resting behaviour

This study only observed resting behaviour during the night, however activity and resting during the day can effect resting behaviour during the night (Owczarczak-Garstecka & Burman 2016). If resting behaviour during daytime is observed in addition to night time, this can provide more information on whether the dog is tired at night as a consequence of high activity during the day (which could be part of a stress response) or whether the dog is calm and resting (Jones et al. 2014). In future research also resting behaviour during the day needs to be observed to understand the relation between resting behaviour and stress related behaviour during the day and night.

Also activity and sleep deprivation during the night can increase resting behaviour at the following night, as sleep is homeostatically regulated and the intention to sleep increases as a function of prior wakefulness (Huber et al. 2007). Dogs may sleep more the following night. For example, in a dog named Lola (see attachment B), total resting time was exceptionally increased in the second night compared to the first, although it decreases from night 2 to night 13. A reason for this increase in rest on night 2 could be that she was exhausted due to sleep deprivation at night 1. Sleep deprivation in rats was compensated by more intensive slow wave sleep and a lower number of sleep bouts (Meerlo *et al*. 2001). This matches with Lola’s rest pattern over the days: a decrease of rest fragmentations and longer rest bout durations were seen at night 2 compared to night 1.

External influences

Next to internal influences on resting behaviour like individual coping styles and possible individual resting patters, it is important that external influences in the shelter such as noise, weather (thunderstorm) and vermin have to be taken into account while interpreting the results of a resting behaviour study in shelter dogs. In this study vermin was seen during video observations. No sound recordings were made to examine the noise during night.

Conclusion

In conclusion, the results of this study on urinary C/Cr ratios and behavioural observation of rest suggest that on average, dogs seem able to adapt to the shelter environment over a 14 day acclimatisation period and that the time spend in rest and the duration of single resting bouts increase during this acclimatisation period. However, the individual sleep patterns and the unclear relation between stressors encountered in a shelter environment and rest have to be considered when interpreting the results. Further research with individual monitoring, observing over 24 hours and additional measures such as heart rate, could help to exemplify the correlation between resting behaviour and stressors.

Acknowledgements

I am grateful to the department of Animal in Science and Society, at Utrecht University, for the place and time to fulfil my research project. I am thankful for the opportunity to learn more about welfare in other species too, besides the knowledge I have acquired due to my project. Thank you for the interesting discussions during the presentations and the good work environment. Special thanks to Claudia Vinke for her time and effort she put into the meetings and for examining my report. Last but not least, I really want to thank my project mentor Janneke van der Laan for her professional and pleasant support. Thank you for your trust, your patience and good formulated feedback. I'm not exaggerating, I was really lucky to have you as my supervisor!

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Attachments

Attachment A: Ethogram

Shown are descriptions of the stress related and behaviours in positive contexts scored on duration (d) or rate (r).

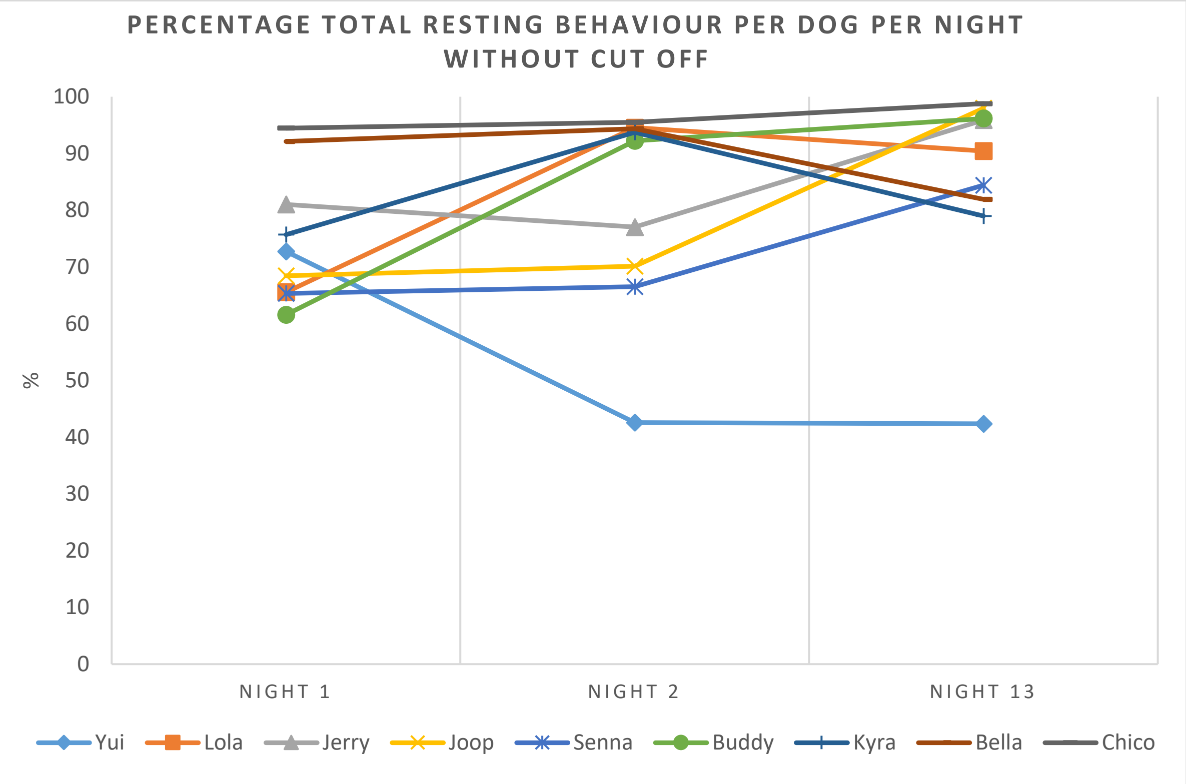
Stress related behaviours	**Description**
Pacing *(d)*	Dog repeatedly (> 3 times), consecutively paces around kennel in a fixed route[1,2,3]
Bouncing *(d)*	Dog repeatedly (> 3 times) jumps up kennel wall from one side to another[1,2,3]
Circling *(d)*	Dog repeatedly walks around in small circle (> 3 times)[1,2,3]
Tail chasing *(d)*	Dog chases its tail repeatedly (> 3 circles) for reasons other than discomfort or grooming [1,2,3]
Spinning *(d)*	Moving (repeatedly) in fast circular movements[8]
Jumping *(d)*	Repeatedly jumping with all four legs, falling down on the same place[7]
Auto-mutilation *(d)*	Repetitive, continuous licking or biting itself at the same place of the body, so intensely to cause self-indicted abrasions or even wounds[7*]
Chewing *(d)*	Repeatedly chews and bites at the bars of the kennel and bedding[1,2,3]
Licking lips *(r)*	Dog extrudes its tongue from its mouth and runs it over its lips- Excluding: following the ingestion of food[1,4,6,8]
Yawn *(r)*	Dog opens its jaws widely without vocalising -Mouth open wide with a deep inhalation or air[4,6,7,8] Excluding: while changing active to inactive patterns and vice versa
Panting *(d)*	Tongue outside mouth, quick breathing, heaving of the chest- Excluding: dog pants for reasons related to physical exertion or warm ambient temperature (< 25 ^{0}C) [1,2,3]
Nosing *(d)*	The nose is moved along objects and/or clear sniffing movements are exhibited[1,13]
Smacking *(r)*	Movement of the mouth without the tongue leaving the mouth, often followed by swallowing
Drooling*(r)*	Emitting saliva from the mouth[7]
Vocalisations *(r)*	Any vocalization, from high to low pitched and from long to short; growling, barking, howling or whining[1,2,4,5,6,9*]
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[1,2,10]
Grooming *(d)*	Licking, scratching or cleaning own body(parts)
Tail-wagging *(d)*	Repetitive wagging movements of the tail[1,2,]
Paw-lifting *(r)*	A forepaw is lifted off the ground and held there[1,2,11,12]
Digging *(d)*	Scratching with front-paws, on floor, wall or kennel bars[1,6] Excluding: scratching door*
Body-shake *(r)*	Rapid lateral rotation of the body in the standing position[1,2,9] Except after waking up
Coprophagy *(r)*	Feeding on (own) faeces[2,4,13]*
Jerk *(r)*	Single sudden, quick movement with body/head[4]*
Sneeze *(r)*	Rapid exhalation through the nose[4]
Tremble *(d)*	Visible body or body part shaking dog while dog is standing still or cowering[5]*
Shuffle *(d)*	Dog switches its weight from one foot to the other without changing position[6]

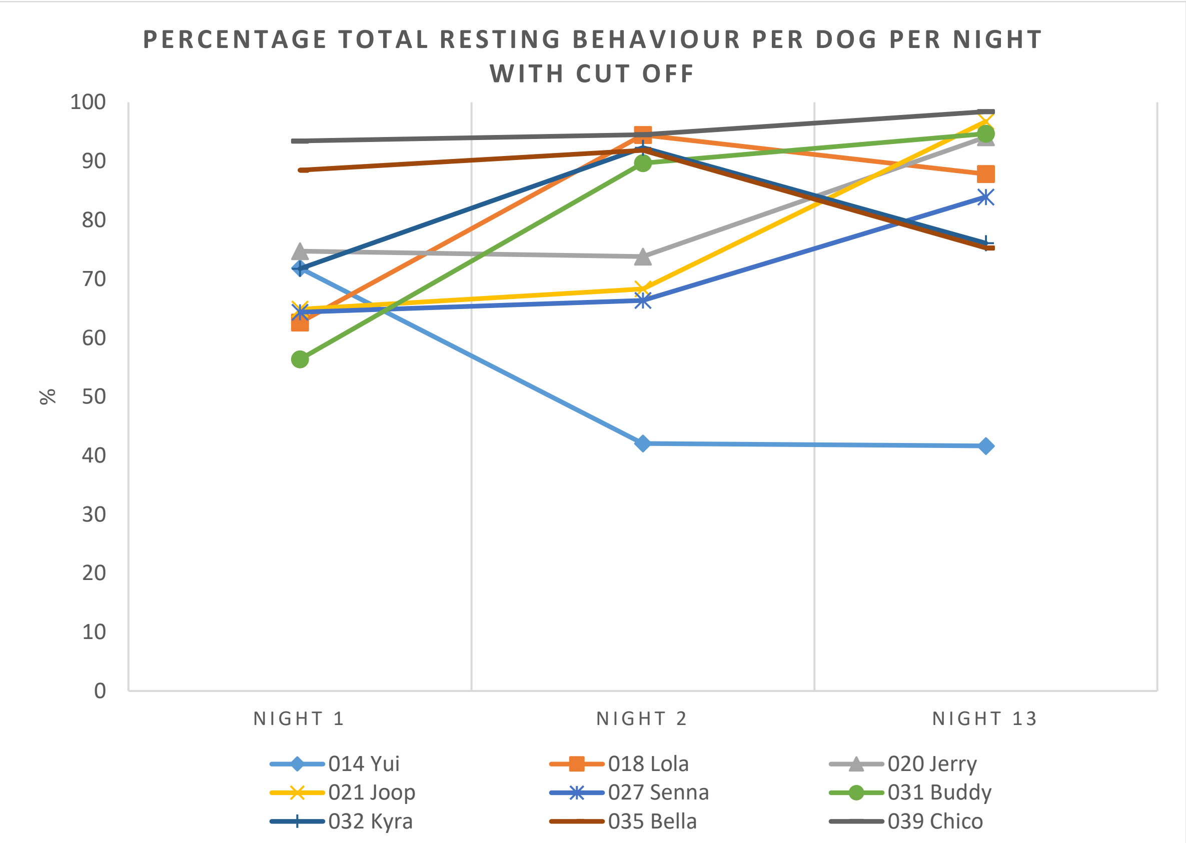
Stretch *(r)*	Extending body and one or more front and/or hind-legs while remaining stationary[4,5] Except after waking up.
Catch flies *(r)*	Trying to catch an imaginary fly with the mouth[7]
Pawing at door (*d*)	One front paw makes contact with the cage door[5]
Cower *(d)*	Body in a lowered, crouched position[5]
Positive behaviours	**Description**
Play with object *(d)*	Any vigorous or galloping gaited behaviour directed towards a toy or other object excluding food bowl, including chewing, biting, shaking it from side to side, batting it with a paw[2,16]. Destruction not included[9].
Play bow *(d)*	Lowered anterior part of body (lying on front-legs) and heightened posterior part of body (standing on hind legs). The play bow is associated with playful intentions [4]

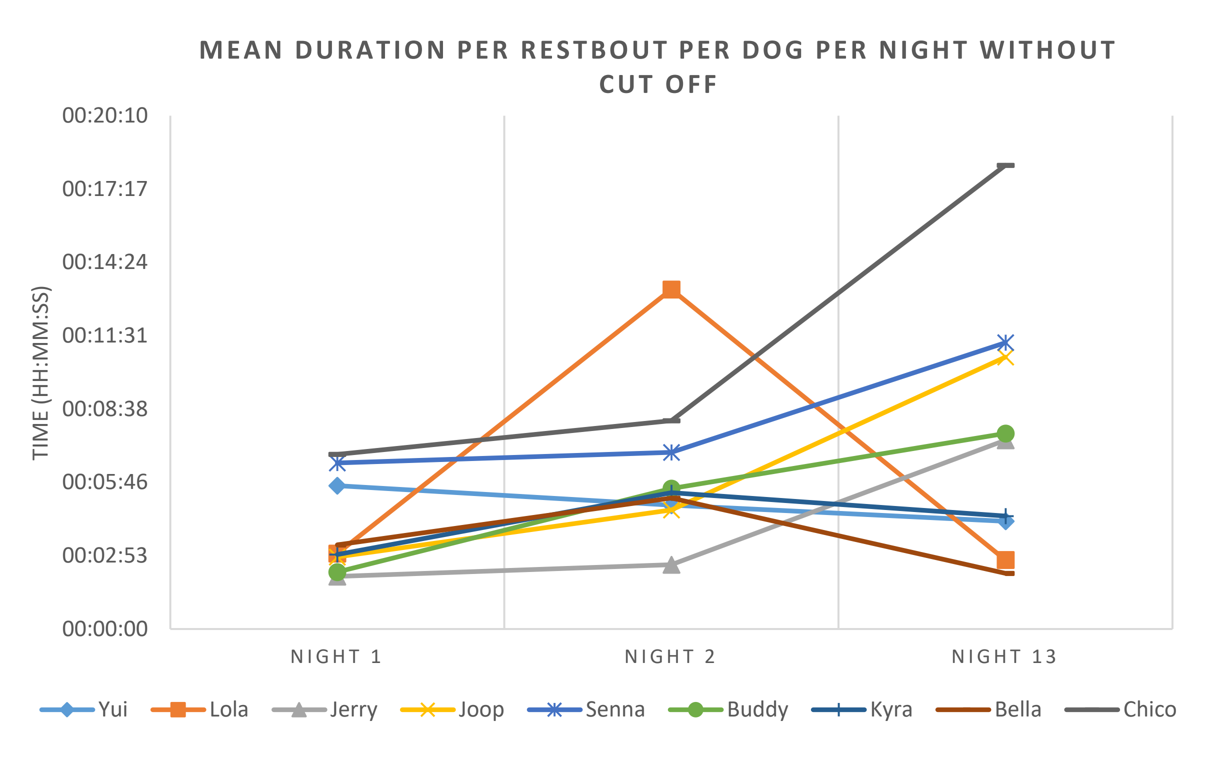
[1]Titulaer et al. 2013(Titulaer et al. 2013), [2](Kiddie & Collins 2014), [3](Stephen & Ledger 2005), [4](Schipper et al. 2008), [5](Protopopova et al. 2014), [6](Rooney et al. 2007), [7](de Palma et al. 2005), [8](Hewison et al. 2014), [9](Walker et al. 2014), [10](Hiby et al. 2006), [11](Beerda, Schilder, Van Hooff, et al. 1999), [12](Beerda et al. 2000), [13](Beerda et al. 1998), [14](Adams & Johnson 1993), [15](Tomkins et al. 2011), [16](Boissy et al. 2007)
* means edited

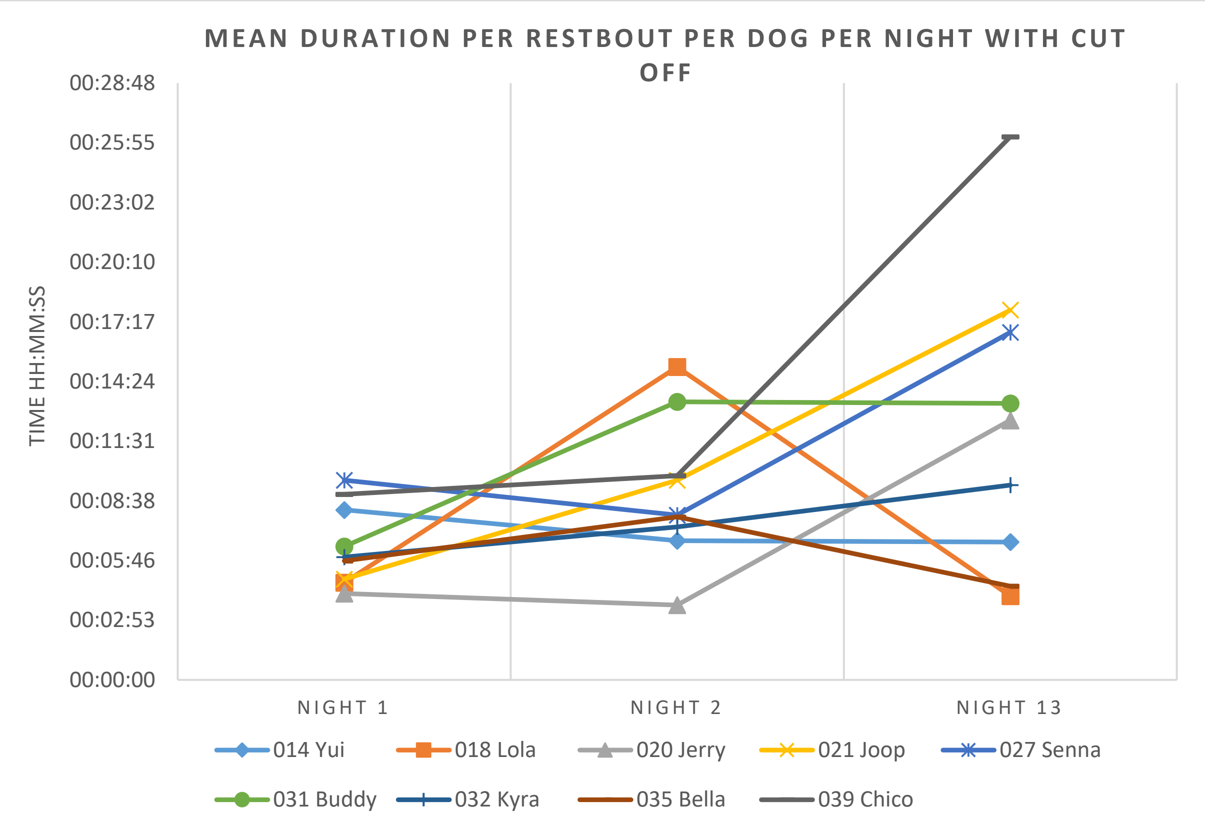
Attachment B: Individual differences in resting behaviour with and without cut off

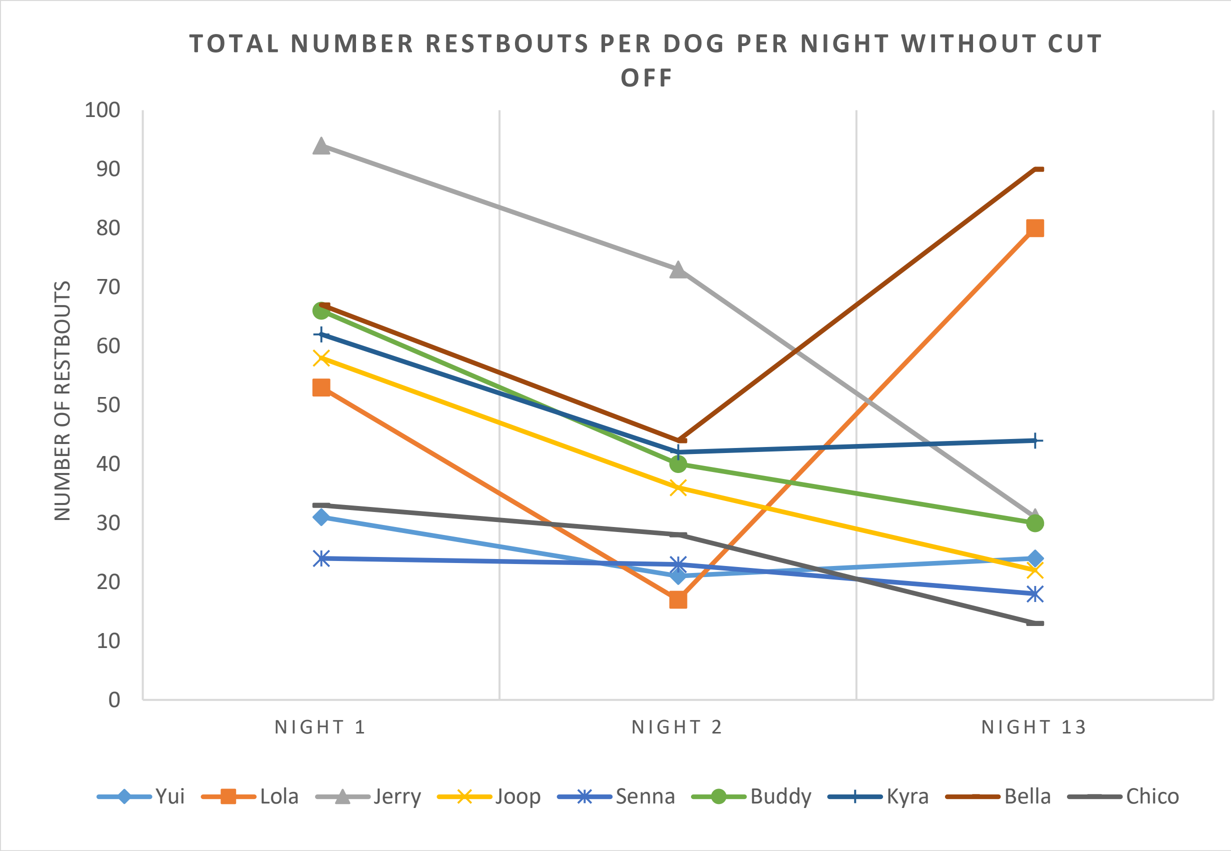
For each dog descriptive statistics for each variable (percentage total resting behaviour, mean duration per rest bout and total number of rest bouts) of rest was calculated and graphs with individual data are shown.

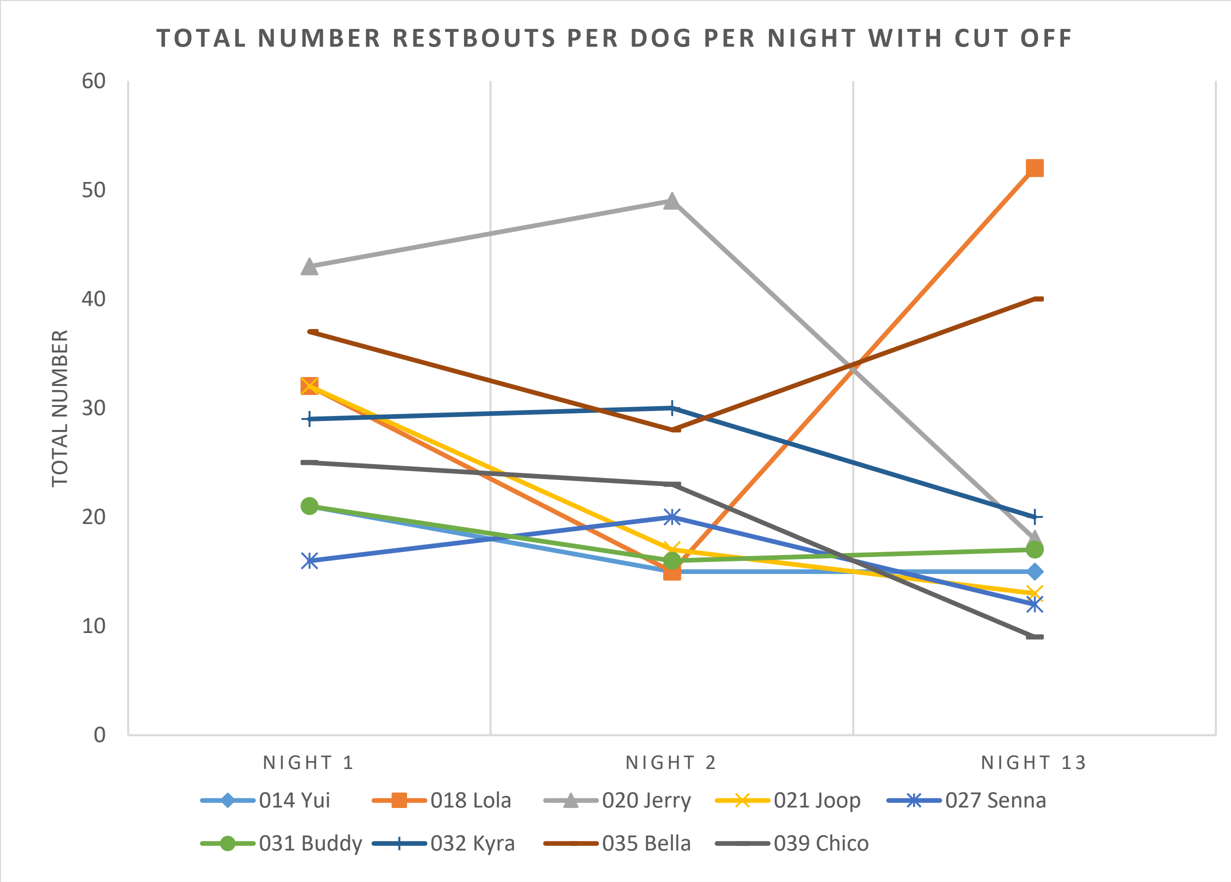





MEAN DURATION PER RESTBOUT PER DOG PER NIGHT WITHOUT CUT OFF
TIME (HH:MM:SS)
00:20:10
00:17:17
00:14:24
00:11:31
00:08:38
00:05:46
00:02:53
00:00:00
NIGHT 1
NIGHT 2
NIGHT 13
Yui
Lola
Jerry
Joop
Senna
Buddy
Kyra
Bella
Chico


MEAN DURATION PER RESTBOUT PER DOG PER NIGHT WITH CUT OFF
TIME HH:MM:SS
00:28:48
00:25:55
00:23:02
00:20:10
00:17:17
00:14:24
00:11:31
00:08:38
00:05:46
00:02:53
00:00:00
NIGHT 1
NIGHT 2
NIGHT 13
014 Yui
018 Lola
020 Jerry
021 Joop
027 Senna
031 Buddy
032 Kyra
035 Bella
039 Chico


TOTAL NUMBER RESTBOUTS PER DOG PER NIGHT WITHOUT CUT OFF
NUMBER OF RESTBOUTS
100
90
80
70
60
50
40
30
20
10
0
NIGHT 1
NIGHT 2
NIGHT 13
Yui
Lola
Jerry
Joop
Senna
Buddy
Kyra
Bella
Chico


TOTAL NUMBER RESTBOUTS PER DOG PER NIGHT WITH CUT OFF
TOTAL NUMBER
60
50
40
30
20
10
0
NIGHT 1
NIGHT 2
NIGHT 13
014 Yui
018 Lola
020 Jerry
021 Joop
027 Senna
031 Buddy
032 Kyra
035 Bella
039 Chico

Attachment C: Overview of the course of individual cortisol measures