university of groningen

Silke Vogel (S3633829)

Research Project Behaviour and Neurosciences (IIa)

The role of estradiol and cholecystokinine in hunger and satiety in female rats

Supervisors: Prof. Dr. G. (Gertjan van Dijk) and Christy Donata

March 17, 2023

Abstract

Obesity is a chronic disorder that is mainly caused by a high-fat diet. Overeating of high-fat foods is caused by the low satiety factor of a high-fat diet. Satiety and hunger are important feelings that regulate our food intake. Behaviour and social context also play an important role in eating behaviour. In this research the influence of a high-fat and a low-fat diet on the behaviour of female Wistar rats is investigated. A behavioural analysis is performed. The rat that took the first sip out of the burette, the rat that drank the longest and the rat that presented the most dominant behaviour was scored. To conclude, there is no clear correlation between the different diets and dominant behaviour. There is however a negative correlation between the rat that takes the first sip and the weight gain of the rat. Female dominance should be tested more by using different stimuli to trigger dominance in future studies. Moreover, female dominance behaviour is an understudied topic that should be researched more in the future.

Introduction

The public health system is under a rising pressure due to the increasing prevalence of obesity. This chronic disorder is an unnatural buildup of bodyfat and causes detrimental effects on our health (Agnha & Agnha, 2017). Key environmental and cultural causes of obesity are the development of an inactive lifestyle and the constant access to low-priced and energy-dense food (usually high in fat). The commercial promotion of unhealthy food and the ignorance about this topic of the society as a whole, causes this serious health care problem to not be resolved (Prentice, 2001).

The onset of obesity is promoted by eating a high-fat diet. There is even a direct relationship between the amount of dietary fat and the degree of obesity. A high-fat diet is low in satiety and high in caloric density, which causes overeating (Golay & Bobbioni, 1997). An understanding of the feelings controlling the amount of food we eat is essential in trying to tackle this health care crisis. On one hand, the feeling of being full and not wanting to eat is induced by the secretion of leptin. On the other hand, the feeling of hunger and seeking food is promoted by ghrelin. Energy homeostasis is achieved by interaction between those hormones, that is processed in the hypothalamus to a feeling of hunger or satiety (Yeung and Tadi, 2022). Another important hormone for a feeling of satiety is cholecystokinin (CCK). An additional effect of CCK is the optimization of digestion and the absorption of nutrients. A diminished sensitivity to CCK is associated with Diet-induced obesity (DIO) and chronic intake of energy-dense food leads to a dysfunctional CCK system (Cawthon & De La Serre, 2021). The female sex hormone estradiol stimulates the effect of CCK and therefore the satiation pathway. This causes a decrease in meal size and food intake when female rats have an increase in the amount of estradiol, when the female is in estrus (Geary, 2001). The estrus phase is part of the estrous cycle, which refers to the reproductive cycle of the female rat. The cycle consists of four phases, namely proestrus, estrus, metestrus and diestrus and it lasts for 4 to 5 days (Ajayi & Akhigbe, 2020).

Besides hormones that control satiety and hunger, there are also behavioural aspects that influence eating behaviour. Social context has a strong effect on eating behaviour (Higgs & Thomas, 2016). Social hierarchies are inevitable in basically all animal groups. Both physical and emotional wellbeing are affected by social ranking within these groups (Fulenwider et al., 2022). However, most research is done on male hierarchical behaviour and social dominance. This behaviour is studied in males across many different species. Nonetheless, female aggressive behaviour is widely understudied and most of the studies that focus on female aggressive behaviour target on maternal defense of offspring. Nevertheless, females do show aggressive behavior towards other females like fighting,

chasing and mounting. The function of female-female mounting in rodents could also be interpreted as sexual or masculinized behaviour. Research has shown that not all females execute mounting behaviour, but when females mount other females it is likely the females initiating the mounting are dominant over the other female. Also sniffing is also a sign of dominance, in which the dominant rat is sniffing the subordinate rat (Wesson, 2013). The estrus state does not have a significant effect on aggressive behaviour, besides the elongated time spent in estrus by dominant females compared to recessive females. Social dominance behaviour will be expected to arise when resources like food, water, territory or access to mates cause competition between individuals (Williamson et al., 2019).

In order to gain insight into the dominance between two rats, a stimulus should trigger both rats. Sucrose water is an effective stimulus for Wistar rats, that is not influenced by sex or food/water deprivation (Fonseca-Rodrigues et al., 2022). In this experiment the rats are put on two different diets, a High-fat (HF) diet and a Low-fat (LF) diet. This research focusses on examining to what extend a specific diet can influence the dominance behaviour between female Wistar rats. One burette with a limited amount of sucrose water will be attached in the cage and the behaviour will be analyzed. By looking at past research, the expectations are that the rats on the HF diet will exhibit more hierarchical behaviour. Since these rats have a satiety level set higher than the rats on a LF diet, which makes the HF rats more eager to drink from the sucrose water. However, by looking at the rat that took the first sip, the rat that drank the longest and the rat that presented the most dominant behaviour there was no clear difference between the HF and LF rats determined.

Materials & methods

Weighing rats, food hoppers & water bottles

Materials:

- Female Wistar rats (x20)
- 10 cages (80x55x50)
- Scales (x3)
- Food hoppers (x20)
- Water bottles (x20)
- High-fat food
- Low-fat food
- Lab journal
- Table on wheels

Methods:

The first thing done in the morning was weighing the rats, food hoppers and water bottles. This was done by the four people that were present in the morning. The water bottles and the food hoppers were taken out of the cage and put on the table with wheels. Subsequently, two people took the table into the next room, where a scale was at hand. All the water bottles and food hoppers were weighed and if necessary the bottles and food hoppers were filled. After weighing the refilled bottles and hoppers, all the data was noted in the lab journal and all the bottles and food hoppers were put back on the table. The table was rolled back into the animal facility. At the same time, the other two people were weighing the rats on the scales available in the animal facility, by taking the rats one by one and putting them on the scales.

Estrus practicum

Materials:

- Female Wistar rats (x20)
- 10 cages (80x55x50)
- 500 mL bottle with demi water
- Superfrost slides
- Lighter
- Spirit burners (x2)
- Shelves for slides
- Multiple Öses
- Gloves
- Tissue
- Filter paper
- Permanent markers (x2)
- Microscopes
- Microscope paper
- Filtered Giemsa
- 0.05M Tris-base buffer pH 7.0-7.4
- Burette 25 mL
- ≥ 250 mL Beaker

- 500 mL bottle with 70% alcohol
- Timer
- Coloring container (x2)
- Measuring cylinder (250 mL)
- Pipette balloon
- Coloring tray
- Magnetic mixer
- Magnetic stirrer (x2)

Methods:

The vaginal smears were done according to the manual 'Estrous practicum' on page 2. The vaginal smears dried for one day in the animal facility and were taken up to the lab the next day. Then, the staining of the slides with the vaginal smears was done such as described in the manual 'Estrus cell staining'. After the staining, the slides dried for a day. The next day, the stained slides were assessed under the microscope one by one according to the manual 'Estrous practicum' on page 3.

Dominance assay

Materials:

- Female Wistar rats (x20)
- 10 cages (80x55x50)
- Timers (x2)
- Separation wedges (x10)
- Mixing plate
- 10% sucrose water
- Burets (x10)
- Little attachment anchor for every cage
- Pen (x2)
- An analysis form

Methods:

First of all, the sucrose water (10%) was made by mixing sugar and water on a mixing plate.

Day 1-6:

Ten burets were filled with 10 mL of the previously made sucrose water. At 12:30 the separation wedges were put in every cage and at 13:00 the lights were turned off. On day 1 the observations started at 13:10. However on Day 2 till Day 6, the experiment started at around 14:15 after the food preference experiment was finished. Two people observed one cage at a time with both a timer in hand. Each person was assigned one color of rat to observe in every cage, one observed the blue rat and the other observed the red rat. This meant timing every time that rat drank from the burette and observing the behaviour of this rat. The separation wedge was taken out of the cage and the burette with sucrose water was attached to the top of the cage with a little attachment anchor. The rat that took the first sip was written down on the form. The behaviour was observed during 10 minutes and all the observations were written down on the form. Soon after, at the other cages the same procedure of timing and observing was followed.

Day 7 & 8:

At 12:30 the separation wedges were put in every cage and at 13:00 the lights were turned off. On Day 7, the experiment started at around 14:15 after the food preference experiment. However, on Day 8 the observations started at 13:10. The separation wedge was taken out of the cage in two cages, one above the other, at the same time. One person watched the top cage and one person watched the bottom cage. Subsequently, two burets were dipped in the sucrose water and attached to the cage. The rat that took the first sip was written down on the form for both cages. During 10 minutes, the behaviour was observed and written down on the form for two cages at a time. Later, at the other cages the same procedure of timing and observing was followed.

Statistical test

To see how significant the results are a multiple linear regression test was used in spss. This multiple linear regression made use of a specific dependent variable, which was diet in this experiment. Since diet was used to see if there was any difference in dominant behaviour. This dependent variable was set against different independent variables, which were the three parameters in which the dominant behaviour was assessed. The correlation and significance between the dependent and independent variables were concluded by looking at the results from this statistical test.

Results

During the experiment, 20 female Wistar rats were researched over the course of 4 weeks. There were ten cages in which the 20 rats were co-housed in pairs. The rats were numbered from 1 through 20, all the even numbered rats were marked with a red dye and the uneven numbers with a blue dye. Half of the cages were on a high-fat diet (HF) and the other half was on a low-fat diet (LF).

Dominance assay

In table 1 the results of the first sip from the sucrose water burette are presented. The rows represent the cages, the top five rows are the cages on a HF diet and the bottom rows are on the LF diet. The columns represent all the experiment days (1-8) and the last column summarizes the data per cage over the eight days. The color of the rat that took the first sip of the burette was noted per cage for every experiment day. On day 1, cage 2 has a 0-score due to the fact there was no activity of the rats towards the burette. On day 2, the same situation occurred in cage 3. On day 5, the blue and red rat started drinking from the burette at the same time in cage 9, so it was unclear which rat took the first sip. In cages 6 & 8, the blue rat took the first sip every time. Both of these cages are HF, so this could mean there is a more distinct distribution of hierarchy between the HF rats. However, some results from the first sip table in LF rats are just as distributed as the HF rats so no clear conclusion can be drawn from just looking at the first sip.

Table 1 First sip. The rat that takes the first sip is indicated per cage (horizontally) for the 8 experiment days (vertically) in the table below. The last column sums up the amount of first per rat. Blue/red indicates an unclear first sip and 0 implies no sips.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Total:
Cage 2 (HF)	0	Red	Blue	Red	Red	Blue	Blue	Blue	3x RED, 4x BLUE
Cage 6 (HF)	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	8x BLUE
Cage 8 (HF)	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	8x BLUE
Cage 9 (HF)	Blue	Blue	Blue	Red	Blue/Red	Red	Blue	Blue	2x RED, 5x BLUE
Cage 11 (HF)	Red	Blue	Red	Red	Red	Blue	Blue	Red	5x RED, 3x BLUE
Cage 3 (LF)	Red	0	Blue	Red	Red	Red	Red	Blue	5x RED, 2x BLUE
Cage 5 (LF)	Blue	Red	Red	Red	Red	Red	Red	Red	7x RED, 1x BLUE
Cage 12 (LF)	Red	Red	Red	Red	Blue	Blue	Red	Blue	5x RED, 3x BLUE
Cage 14 (LF)	Blue	Red	Blue	Red	Red	Red	Red	Blue	5x RED, 3x BLUE
Cage 15 (LF)	Blue	Red	Blue	Blue	Blue	Blue	Blue	Blue	1x RED, 7x BLUE

In Figure 1A, the drinking time in cage 6 is displayed. The drinking time is expressed in percentages, to be able to see a difference in drinking time between rat 13 and rat 14. The columns represent the six days this was examined and the last column is the average distribution of the drinking time. The blue color indicates rat 13 and the red color rat 14. In cage 6 there is a clear distribution, rat 13 is drinking nearly 80% of the time. The last two experiment days did not include tracking the drinking time, since there was just a very small amount of sucrose water in the burette due to the quick dipping of the tip in the sucrose water. The drinking time-figures of the other cages are in Appendix A (Figures 1B-1E).

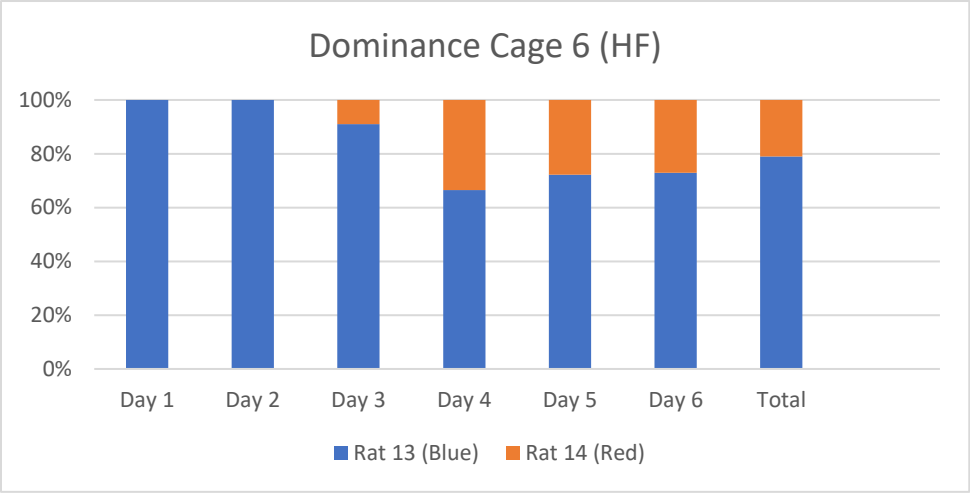


Figure 1A Drinking time rat 13 (blue) and rat 14 (red) in cage 6. The percentage of time spent drinking for rat 13, indicated by the blue color, and rat 14, indicated by the red color, from day 1 to day 6.

In Table 2, the drinking time in percentage is shown for all the HF and LF cages (summary for all figures in Appendix A), with the averages for both diets. The rat that spent the most time drinking was labeled the 'Dominant rat' for this experiment and the rat spent less time drinking the 'Recessive rat'. The first five rows are the HF cages, followed by the averages over all the HF cages for the dominant and recessive rats. The last five rows are the LF cages, with the averages for the dominant and recessive rats. Just like the results of the first sip, there is no clear conclusion that can be drawn from these outcomes. Some HF cages are very unevenly distributed, insinuating that one rat is more dominant than the other. Nonetheless, the outcomes of the LF cages are comparable. Accordingly, the averages of both the HF and LF rats are very close together. Therefore, no clear conclusion can be drawn by looking at the difference between HF and LF rats in drinking time.

Table 2 Drinking time in percentage. For every cage, the proportional percentage of time spent drinking for the dominant and subordinate rat. First 5 rows are the HF rats and bottom rows the LF rats, both summed up by the average underneath both the HF and LF group.

-	Dominant rat	Recessive rat
Cage 2 (HF)	56,4	43,6
Cage 6 (HF)	79,4	20,6
Cage 8 (HF)	52,2	47,8
Cage 9 (HF)	60,4	39,6
Cage 11 (HF)	53,4	46,4
Average (HF)	***60,36***	***39,6***
Cage 3 (LF)	64,8	35,2

Cage 5 (LF)	58,9	41,1
Cage 12 (LF)	64,4	35,6
Cage 14 (LF)	50,8	49,2
Cage 15 (LF)	60,1	39,9
Average (LF)	***59,8***	***40,2***

In Table 3, the form used to score the behavioural analysis for cage 6 is shown. This format was used for all cages. The parameters examined each day were energetic, sniffing, fighting and other odd behaviour. By taking all the measurements on these behavioural elements, a dominance between the rats was determined each day. This is shown in the table by color coding the day the same as the most dominant rat. On day 2 there was no clear dominance, so this day is neutral.

Table 3 Behavioural analysis cage 6 (rat 13 & rat 14). The form used to score the different kinds of behaviour (vertical) for every experiment day (horizontal). The color coding of the days indicates the overall dominant rat of that day, a black coding resembles a neutral outcome of the behavioural analysis.

Days	Energetic	Sniffing	Fighting	Odd behaviour ('mating')
Day 1 (14-02-2023)	Yes, both rats explore the cage.	No.	No, the red rat avoids the blue rat. No pushing behavior. Only very subtle interactions.	The red rat waits for the blue rat. The entire pipette is empty and the blue rat drank the whole pipette.
Day 2 (16-02-2023)	Yes.	Yes, both sniffed each other once.	No.	•
Day 3 (21-02-2023)	Yes.	Yes, both sniffed each other.	Rat blue won fight.	2x: Red tried to mate blue. Red doesn't seem to like sucrose or know how to drink it.
Day 4 (23-02-2023)	Yes, very active.	Yes, blue sniffed red.	Winning of fights (5x blue, 6x red): 1. Blue 2. Red 3. Red	Buret empty very fast.

			4. Blue 5. Red 6. Blue 7. Blue 8. Red 9. Red 10. Red 11. Blue	
Day 5 (28-02-2023)	Yes.	Yes.	Yes, 7 fights: Red won 5x Blue won 2x	Leaking buret Red was digging holes a lot.
Day 6 (2-03-2023)	Not really.	Yes, red sniffs blue.	Yes; Red won 4x Blue won 4x	
Day 7 (7-03-2023)	Not really.	Red sniffs blue.	Yes; Red won 1x Blue won 1x	Start drinking water together.
Day 8 (9-03-2023)	•	•	•	Red mating on blue 2x.

The amount of times the red or the blue rat was labeled the most dominant, is summarized for every cage over the eight experiment days in Table 4. The rat that is according to the observations the most dominant, is labeled the 'Dominant rat'. So, the rat that is the least amount of times dominant is the 'Recessive rat'. The average is shown for the recessive rats and the dominant rats for both HF and LF. There are a few cases where there is an equal distribution of observed dominance in a cage (cage 8, 9 and 5), in which both the dominant and recessive rat were dominant for 50% of the time.

Table 4 Behavioural observations in percentage. The percentage of a rat being awarded the most dominant per cage over the eight experiment days.

-	Dominant rat	Recessive rat
Cage 2 (HF)	71,4	28,6
Cage 6 (HF)	85,7	14,3
Cage 8 (HF)	50	50
Cage 9 (HF)	50	50

Cage 11 (HF)	83,3	16,7
Average (HF)	***68,08***	***31,92***
Cage 3 (LF)	83,3	16,7
Cage 5 (LF)	50	50
Cage 12 (LF)	71,4	28,6
Cage 14 (LF)	87,5	12,5
Cage 15 (LF)	62,5	37,5
Average (LF)	***70,94***	***29,06***

All the data collected for the first sip, drinking time and the behavioural analysis is summarized per cage in Appendix B (table I-X). In 6 cages there was an alignment between the rat that took the most first sips, the rat spending the most time drinking and the rat that presents the most dominant behaviour. In the HF cages 2, 8 & 9 was the blue rat the obvious dominant female by looking at all those previously mentioned factors. The same alignment exists in the LF cages 5, 12 & 14. So, both the LF and HF cages have the same amount of cages with a clear dominance. Therefore there is still no obvious difference in dominance between the two diets.

Statistical analysis

Statistical analysis is done to find a correlation between diet and dominance behaviour. In Figure 2, the outcome of the statistical test Multiple linear regression is shown. difDiet is the dependent variable and is set against the different parameters (displayed under 'Model'). The standardized coefficients Beta is the strength of the effect each individual parameter has on the dependent variable. So, all the parameters have a negative effect except for drinking time which has a positive coefficient. Furthermore, in the last column the Sig. is given which has to be below 0.05 in order to be of significance to the dependent variable diet. All the independent variables have a significance that is much higher than 0.05, so the outcome of this test is not significant.

Table 5 Multiple linear regression with diet as dependent variable. This table shows if there is a significant correlation between dominance behaviour and diet.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	1,338	,544		2,460	,026
	firstsip	,000	,005	-,022	-,069	,946
	percentdrinking	-,023	,017	-,589	-1,353	,196
	behavior	-,001	,005	-,028	-,111	,913
	drinkingtime	,002	,001	,742	1,866	,082

a. Dependent Variable: diet

Table 6 Weight change from 03-02-2023 to 10-03-2023 in HF rats. The start weight and the end weight are stated per rat. The last column is the weight difference between the start and end weight.

Rat	Diet	Start weight	End weight	Weight difference
1	HF	279	303	24
2	HF	234	258	24
5	HF	240	256	16
6	HF	265	325	60
7	HF	286	314	28
8	HF	265	281	16
13	HF	254	275	21
14	HF	219	244	25
15	HF	262	290	28
16	HF	245	282	37

The weight change can also influence the dominance behaviour between rats. In table 6 and 7, the weight per rat is displayed. The weight change of the HF rats are shown in table 5. Furthermore, the weight change of the LF rats are shown in table 6. The start weight, end weight and weight difference in both tables are expressed in grams.

Table 7 Weight change from 03-02-2023 to 10-03-2023 in LF rats. The start weight and the end weight are stated per rat. The last column is the weight difference between the start and end weight.

Rat	Diet	Start weight	End weight	Weight difference
3	LF	237	248	11
4	LF	256	269	13
9	LF	273	272	-1
10	LF	261	257	-4
11	LF	228	248	20
12	LF	261	256	-5
17	LF	250	248	-2
18	LF	287	310	23
19	LF	248	270	22
20	LF	285	294	9

The weight change is now taken into account for the Multiple linear regression test. This statistical test is used to see if there is a linear relationship between weight difference and dominance behaviour. Weight difference (in figure: deltabw) is the dependent variable and is set against the independent variables (firstsip, drinkingtime, percentdrinking and behaviour). The firstsip parameter is the only independent variable with a negative Standardized Coefficient Beta, the rest of the independent variables are positive. The outcome is significant if the Sig. is under 0.05, which is the case for the independent variable firstsip. This means that a heavier rat is less likely to take the first sip, since there is a negative correlation between gaining weight and taking the first sip.

Table 8 Linear regression with change in body weight as dependent variable. This table shows if there is a significant correlation between dominance behaviour and weight difference.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-8,546	11,861		-,721	,482
	firstsip	-,278	,114	-,650	-2,432	,028
	drinkingtime	,049	,028	,583	1,743	,102
	percentdrinking	,124	,371	,122	,333	,744
	behavior	,013	,113	,025	,118	,908

a. Dependent Variable: deltabw

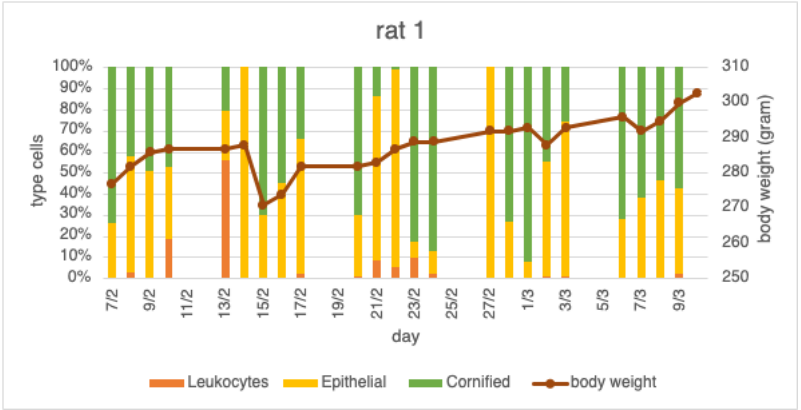


Figure 2A : Estrous cycle illustrated through cell count from vaginal smears. The graph illustrates the days the vaginal smears were taken on the x-axis and the quantity of cells counted in percentage on the y-axis. Leukocytes, epithelial and cornified cells were counted since these cells can show the phase of the cycle.

Lastly, the estrous cycle is taken into account. In Figure 2A, the counting in percentage of cells (vertical) is set against the date the vaginal smear was taken (horizontal) for rat 1. The estrous cycle for the other 19 rats are in Appendix C (Figure 2B-2T). The vaginal smears of the HF rats are color coded with yellow, orange and green. The cells in the LF rats are colored grey, blue and orange in the

figure. The leukocytes, epithelial cells and cornified cells are counted and calculated in percentages. Di-estrus phase is characterized by an abundance of leukocytes, the pro-estrus phase by epithelial cells and estrus phase by cornified cells. The effect the estrous cycle can have on dominance is a possible elongated cycle of a dominant female, with extra time spent in estrus. The results from the assessment of the vaginal smears did not indicate an elongated cycle in any of the tested rats.

Discussion

There is no significant data on dominance behaviour having a correlation to HF and LF diet, so no clear conclusion can be drawn from the multiple linear regression test. However, there is a significant negative correlation between the first sip and the weight gain of the rats. This means the heavier rat is less likely to have taken the first sip, which contradicts the general belief that the heavier animal would be the most dominant and would be able to take the first sip (Fulenwider et al., 2022).

For future research another stimulus could be considered since sugar water might be a too intense stimulus. The research of Samaha (2021) has shown that sugar water is more rewarding than cocaine in the short term. By picking a different stimulus to test dominance, the possible excessive effect of sucrose water on the rat is diminished. Hierarchical behaviour is expected to arise when resources like food, water, territory or access to mates cause competition between individuals (Williamson et al., 2019). Besides changing up the stimulus, there is a general shortage of research done on female rodents and dominance behaviour. So future studies should redirect the focus on male dominance towards female hierarchical behaviour studies (Fulenwider et al., 2022).

The research question to what extent a specific diet influences the dominance behaviour between female Wistar rats can not be answered by looking at the results of the research. There is not an obvious distinction between rats on a HF and rats on a LF diet in regard to dominance behaviour. However, there were interesting insights on female dominance behaviour and in some cages correlation between the data showing a clear dominant female. Since there is a lack of data on female dominance behaviour, this research conducts as an incitement to look more into female dominance behaviour.

References

Agha, M., & Agha, R. (2017). The rising prevalence of obesity: part A: impact on public health. *International journal of surgery*, *2*(7), 17. https://doi.org/10.1097/ij9.0000000000000017

Ajayi, A. F., & Akhigbe, R. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertility Research and Practice*, *6*(1). https://doi.org/10.1186/s40738-020-00074-3

Cawthon, C. R., & De La Serre, C. B. (2021). The critical role of CCK in the regulation of food intake and diet-induced obesity. *Peptides*, *138*, 170492. https://doi.org/10.1016/j.peptides.2020.170492

Fonseca-Rodrigues, D., Gonçalves, J. C. S., Laranjeira, I., Almeida, A., & Pinto-Ribeiro, F. (2022). Sucrose intake and preference by Wistar Han rats are not influenced by sex or food/water deprivation. *Pharmacology, Biochemistry and Behavior*, *216*, 173387. https://doi.org/10.1016/j.pbb.2022.173387

Geary, N. (2001). Estradiol, CCK and satiation. *Peptides*, *22*(8), 1251–1263. https://doi.org/10.1016/s0196-9781(01)00449-1

Golay, A., & Bobbioni, E. (1997). The role of dietary fat in obesity. *International Journal of Obesity*, *21 Suppl 3*, S2-11.

Higgs, S., & Thomas, J. M. (2016). Social influences on eating. *Current opinion in behavioral sciences*, *9*, 1–6. https://doi.org/10.1016/j.cobeha.2015.10.005

Prentice, A. M. (2001). Overeating: The Health Risks. *Obesity Research*, *9*(S11), 234S-238S. https://doi.org/10.1038/oby.2001.124

Samaha, A. (2021). Sugar now or cocaine later? *Neuropsychopharmacology*, *46*(2), 271–272. https://doi.org/10.1038/s41386-020-00836-z

Wesson, D. W. (2013). Sniffing Behavior Communicates Social Hierarchy. *Current Biology*, *23*(7), 575–580. https://doi.org/10.1016/j.cub.2013.02.012

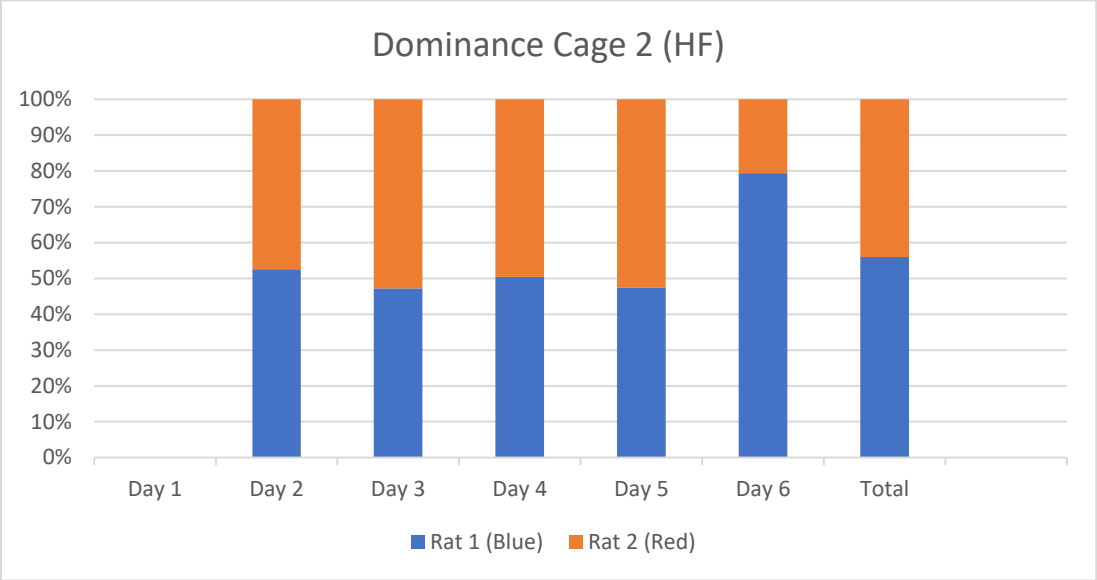
Williamson, C. M., Lee, W. Y., Decasien, A. R., Lanham, A., Romeo, R. D., & Curley, J. P. (2019). Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-43747-w

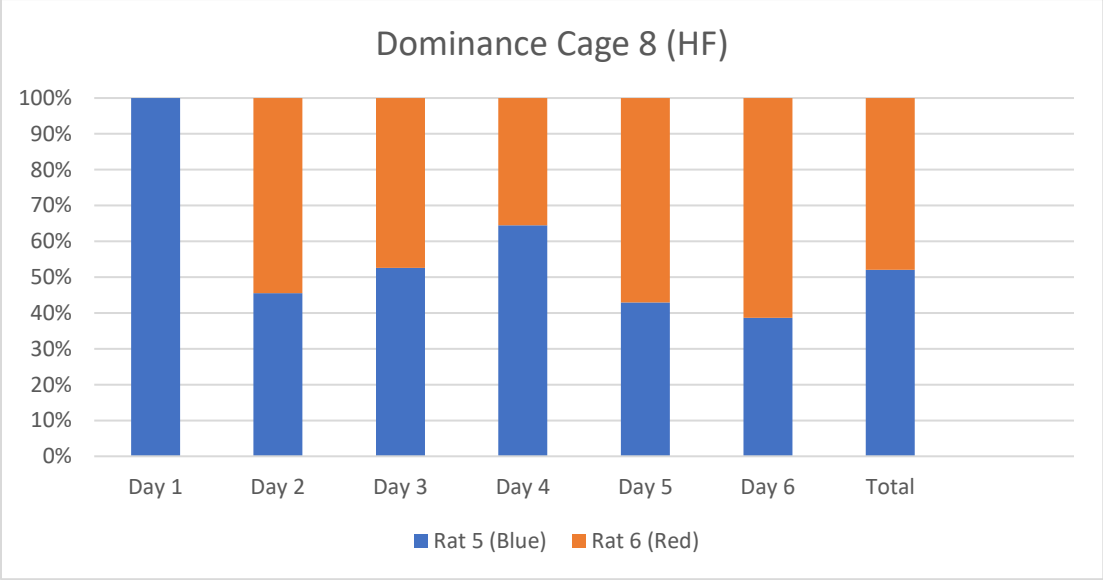
Yeung, A. Y., & Tadi, P. (2022). Physiology, Obesity Neurohormonal Appetite And Satiety Control. In *StatPearls*. StatPearls Publishing.

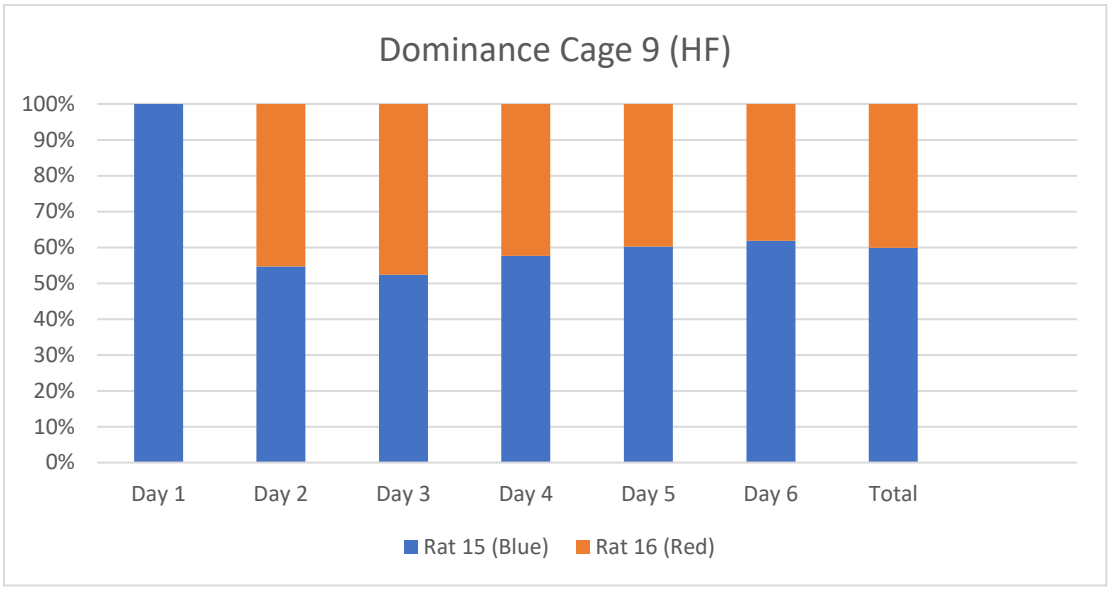
Appendices

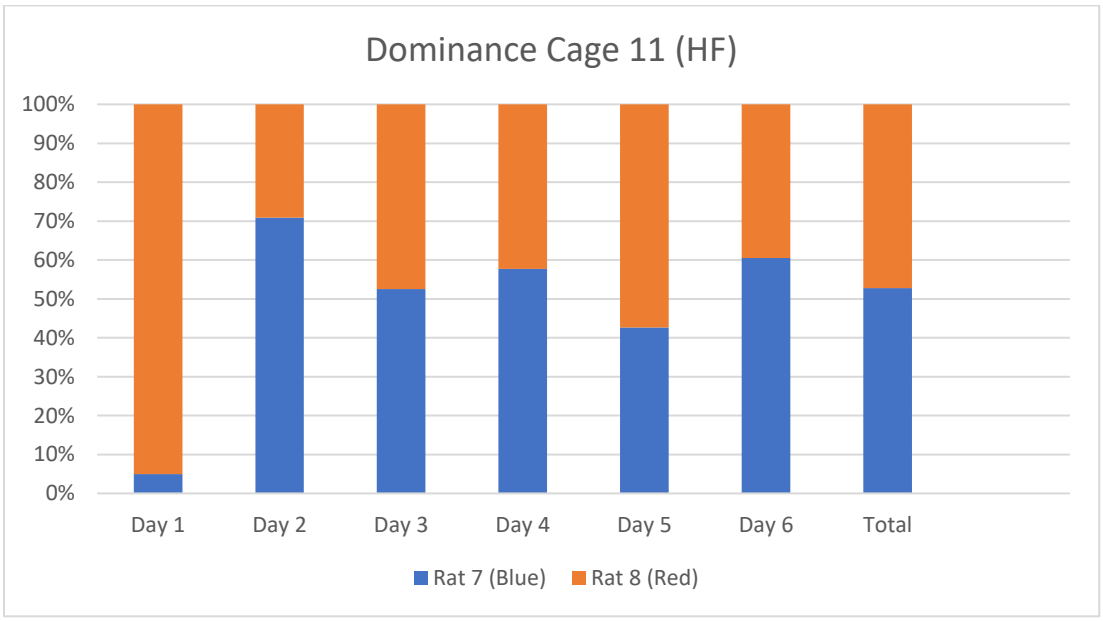
Appendix A

Figures 1B-1E (High-fat)

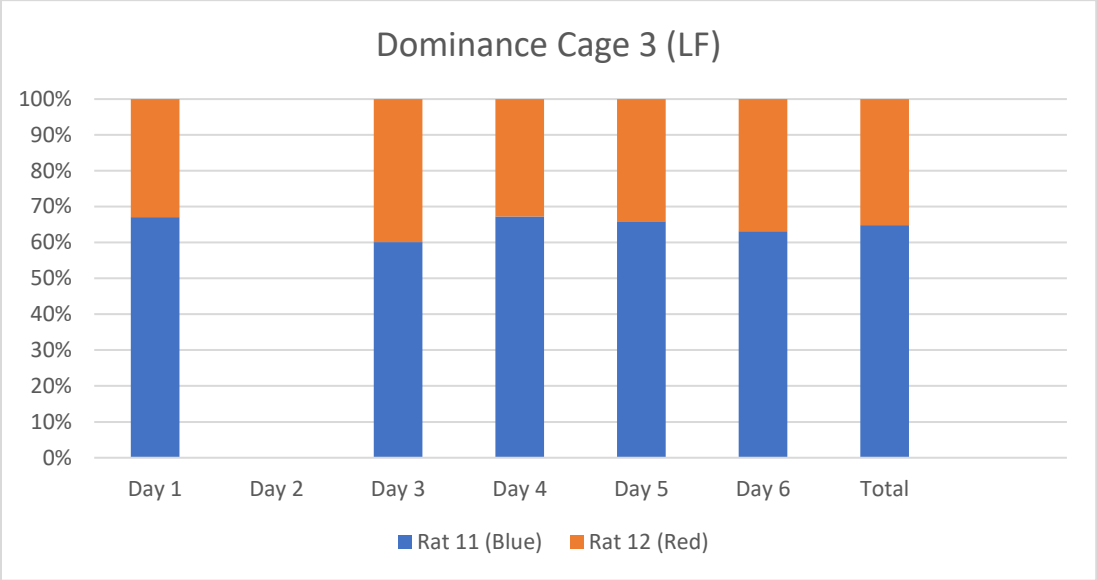




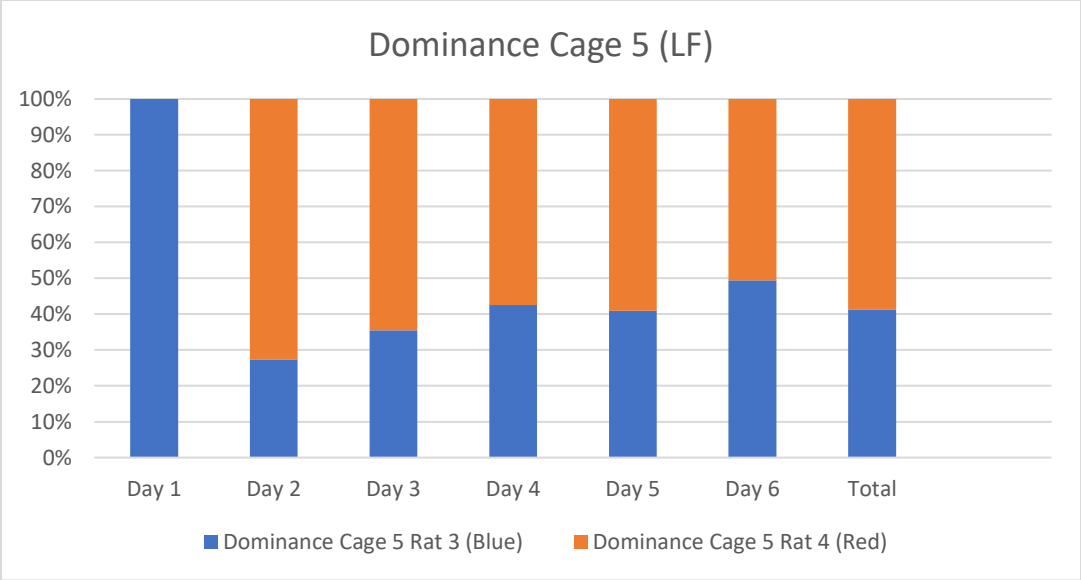

Dominance Cage 9 (HF)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Total
Rat 15 (Blue)
Rat 16 (Red)

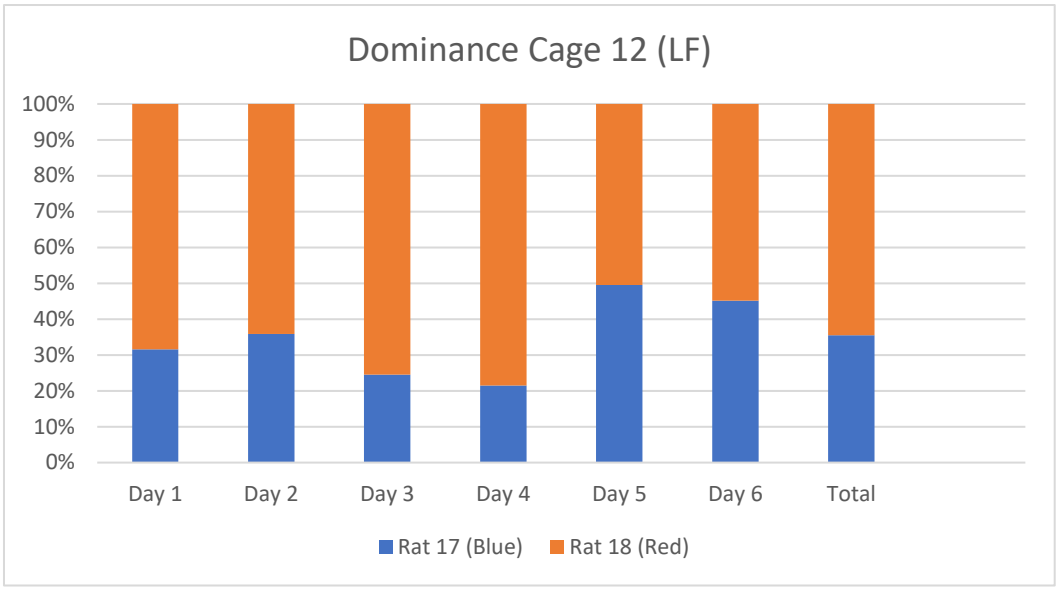

Dominance Cage 11 (HF)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Total
Rat 7 (Blue)
Rat 8 (Red)

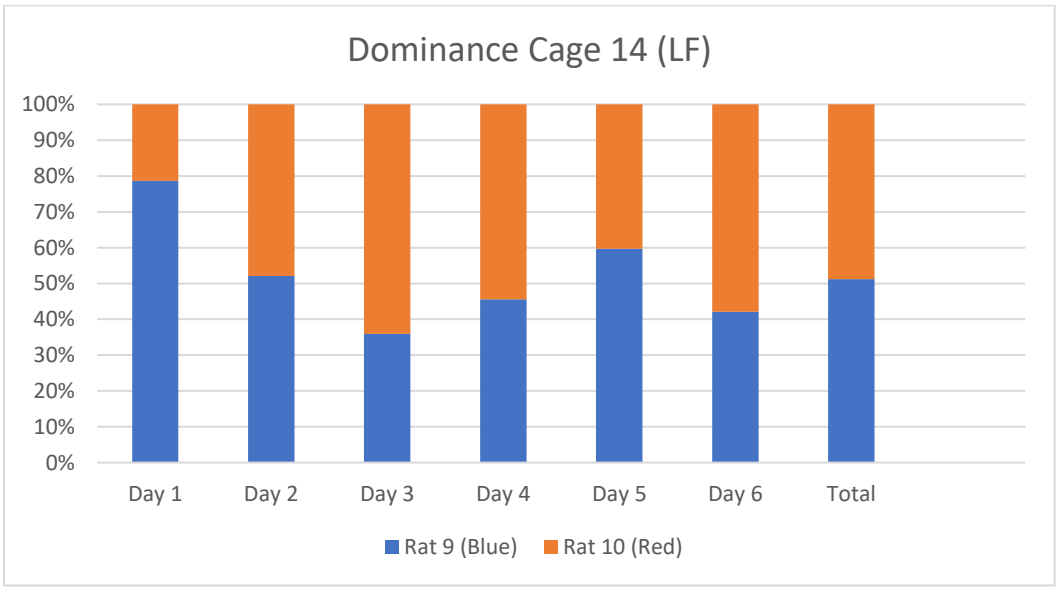
Figures 1F-1J (Low-fat)

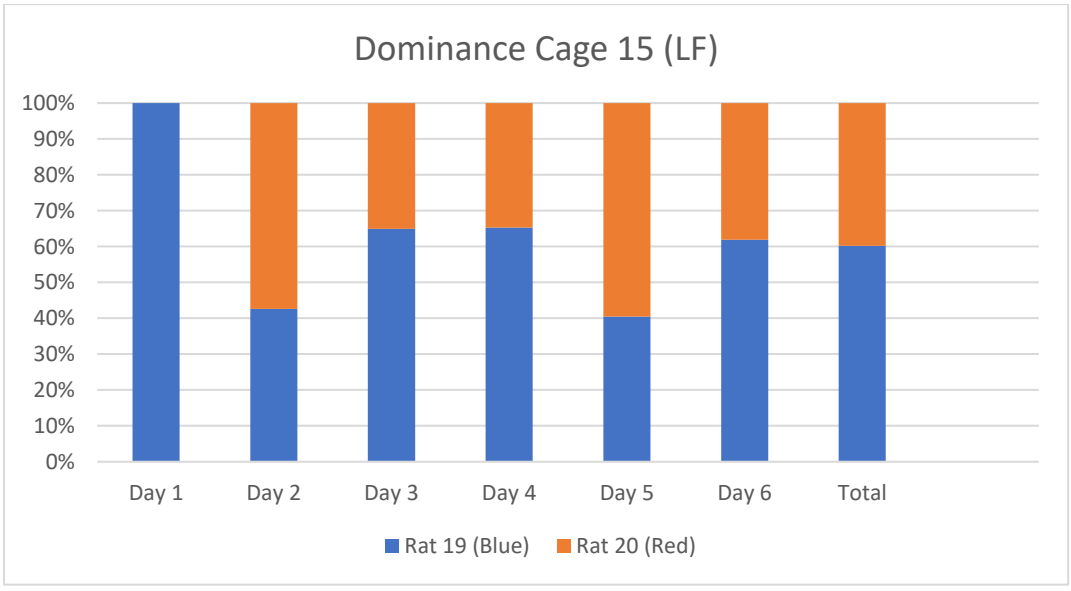


*Nothing at Day 2 implies there was no drinking at all.




Dominance Cage 12 (LF)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Total
Rat 17 (Blue)
Rat 18 (Red)


Dominance Cage 14 (LF)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Total
Rat 9 (Blue)
Rat 10 (Red)


Dominance Cage 15 (LF)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Total
Rat 19 (Blue)
Rat 20 (Red)

Appendix B

Table I - X

Results Cage 2 (HF):

Behavioral analysis:

- 1x Neutral
- 5x Blue
- 2x Red

First sip:

- Blue 4x
- Red 3x

Drinking time:

- Blue: 12:09
- Red: 09:33

Dominant: Blue

Results Cage 3 (LF):

Behavioral analysis:

- 2x Neutral
- 5x Blue
- 1x Red

First sip:

- Blue 2x
- Red 5x

Drinking time:

- Blue: 11:29
- Red: 06:13

Results Cage 5 (LF):

Behavioral analysis:

- 2x Neutral
- 3x Blue
- 3x Red

First sip:

- Blue 1x
- Red 7x

Drinking time:

- Blue: 09:21
- Red: 13:21

Dominant: Red

Results Cage 6 (HF):

Behavioral analysis:

- 1x Neutral
- 1x Blue
- 6x Red

First sip:

- Blue 8x

Drinking time:

- Blue: 12:24
- Red: 03:18

Results Cage 8 (HF):

Behavioral analysis:

- 2x Neutral
- 3x Blue
- 3x Red

First sip:

- Blue 8x

Drinking time:

- Blue: 11:40
- Red: 10:44

Dominant: Blue

Results Cage 9 (HF):

Behavioral analysis:

- 2x Neutral
- 3x Blue
- 3x Red

First sip:

- Blue 5x
- Red 2x

Drinking time:

- Blue: 11:20
- Red: 07:35

Dominant: Blue

Results Cage 11 (HF):

Behavioral analysis:

- 2x Neutral
- 5x Blue
- 1x Red

First sip:

- 3x Blue
- 5x Red

Drinking time:

- Blue: 12:10
- Red: 10:54

Results Cage 12 (LF):

Behavioral analysis:

- 1x Neutral
- 2x Blue
- 5x Red

First sip:

- Blue 3x
- Red 5x

Drinking time:

- Blue: 05:21
- Red: 09:43

Dominant: Red

Results Cage 14 (LF):

Behavioral analysis:

- 1x Blue
- 7x Red

First sip:

- Blue 3x
- Red 5x

Drinking time:

- Blue: 08:55
- Red: 08:29

Dominant: Red

Results Cage 15 (LF):

Behavioral analysis:

- 3x Blue
- 5x Red

First sip:

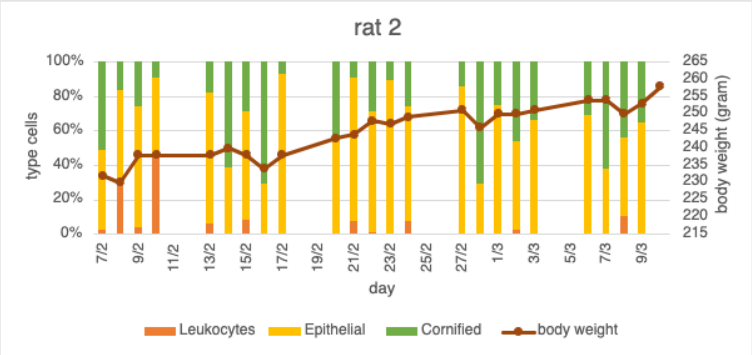
- Blue 7x
- Red 1x

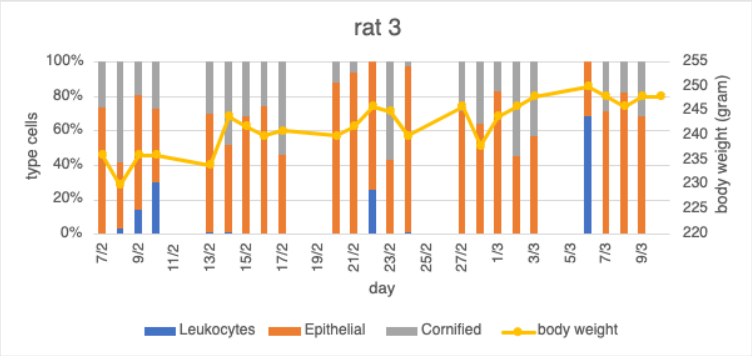
Drinking time:

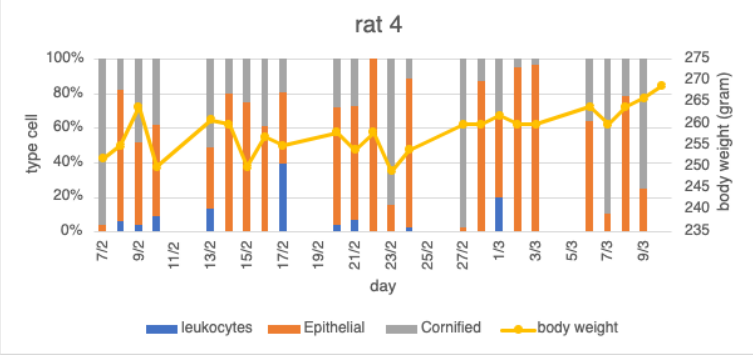
- Blue: 09:28
- Red: 06:16

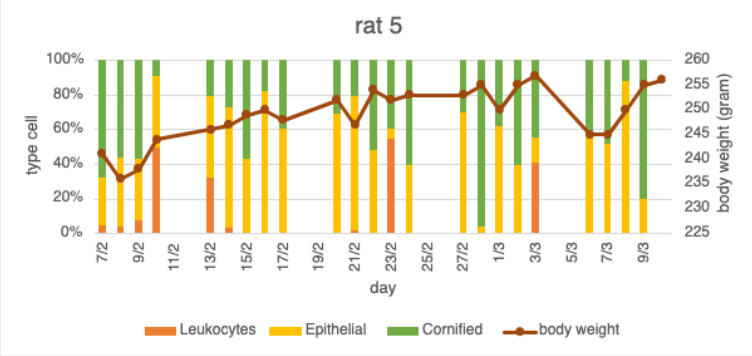
Appendix C

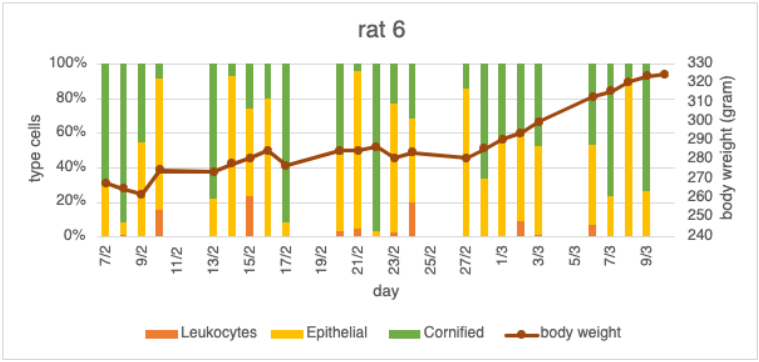
Estrous cycle (2B-2T)

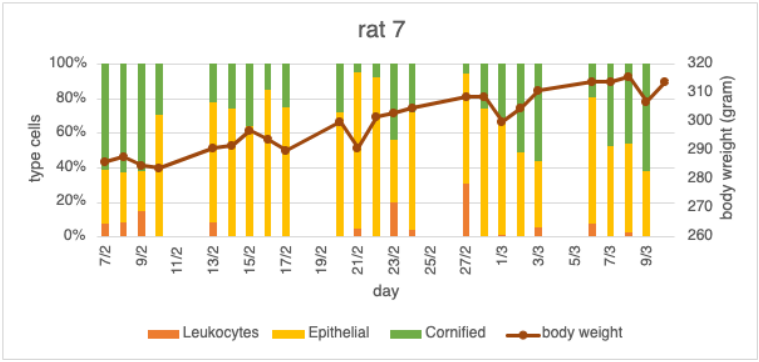


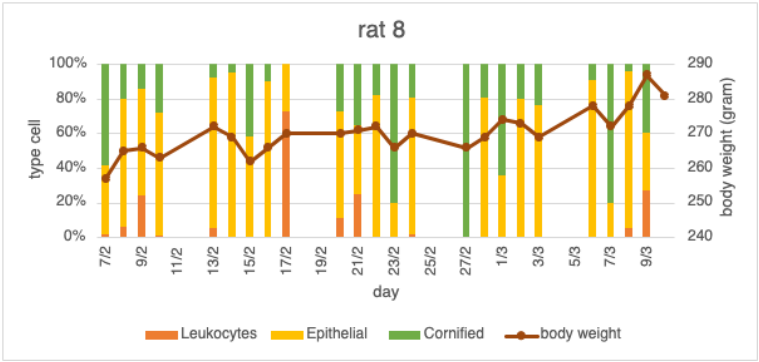


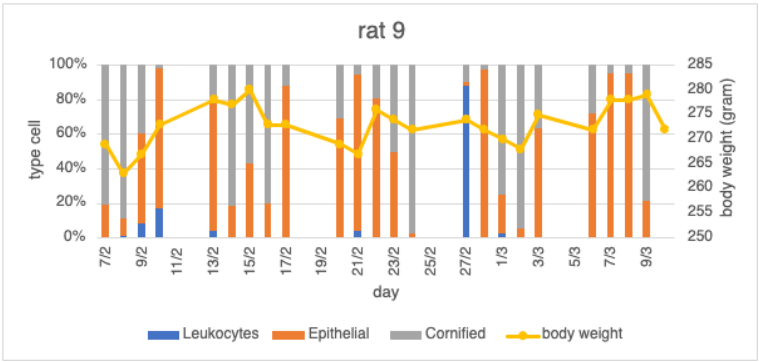


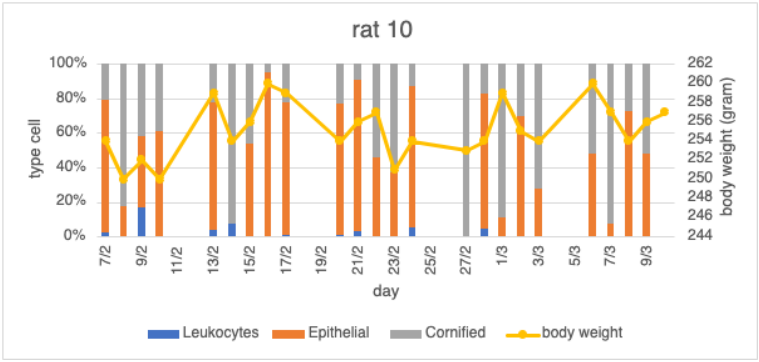


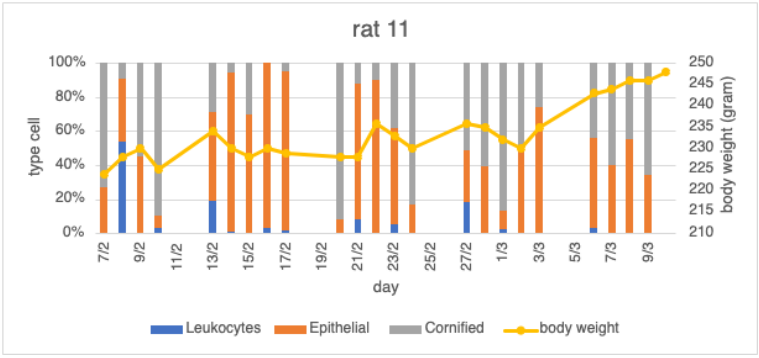

rat 6
type cells
body wreight (gram)
day
Leukocytes
Epithelial
Cornified
body weight

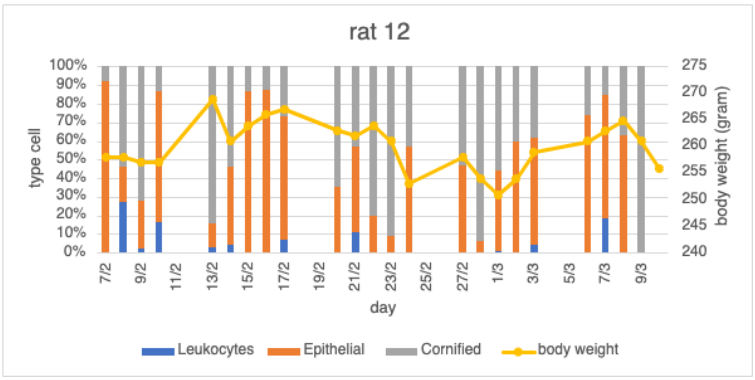

rat 7
type cells
body wreight (gram)
day
Leukocytes
Epithelial
Cornified
body weight

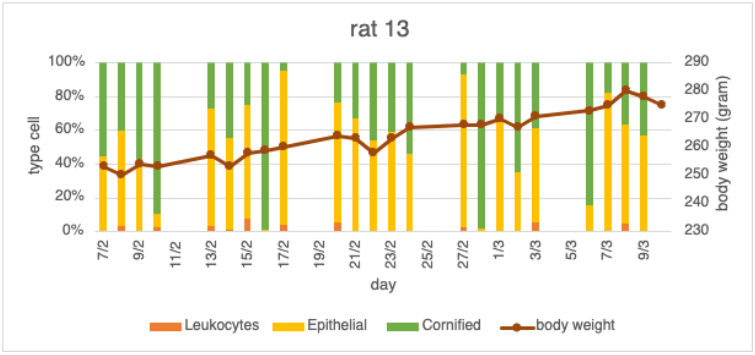

rat 8
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight

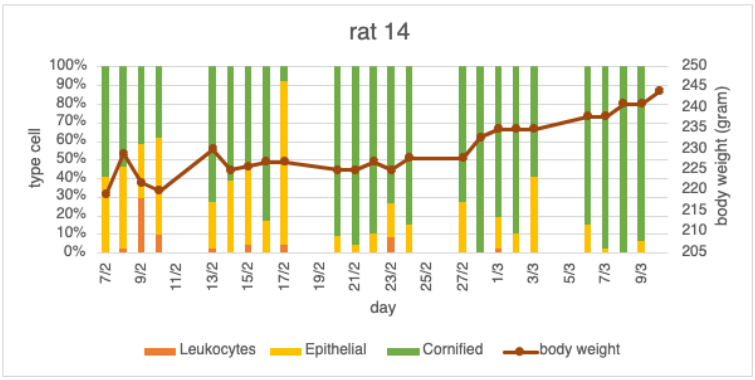

rat 9
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight

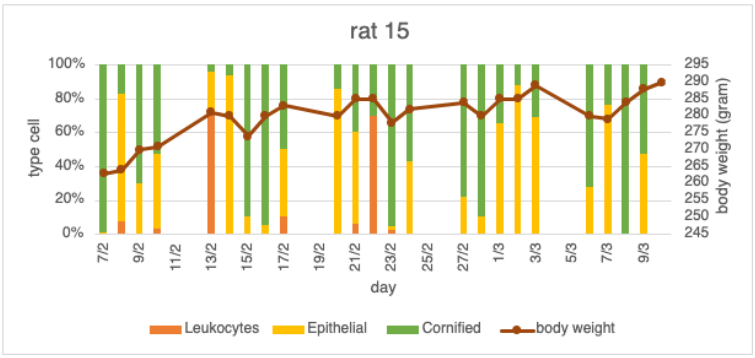

rat 10
type cell
100%
80%
60%
40%
20%
0%
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
262
260
258
256
254
252
250
248
246
244
body weight (gram)
Leukocytes
Epithelial
Cornified
body weight

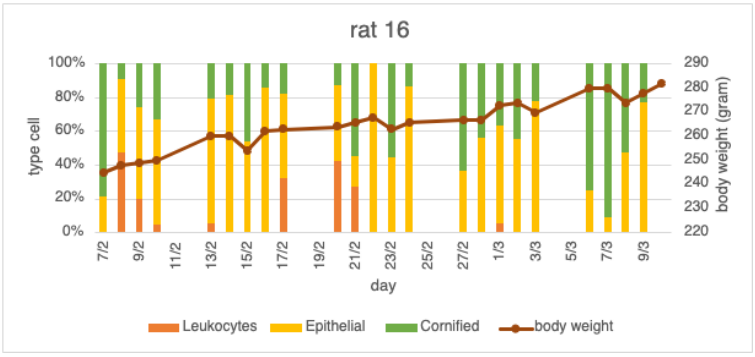

rat 11
type cell
100%
80%
60%
40%
20%
0%
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
250
245
240
235
230
225
220
215
210
body weight (gram)
Leukocytes
Epithelial
Cornified
body weight

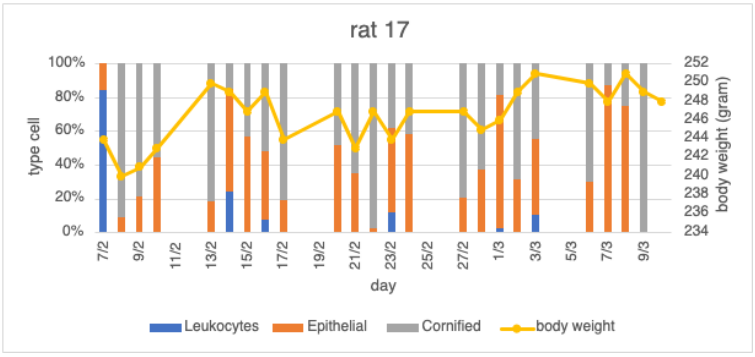

rat 12
type cell
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
275
270
265
260
255
250
245
240
body weight (gram)
Leukocytes
Epithelial
Cornified
body weight

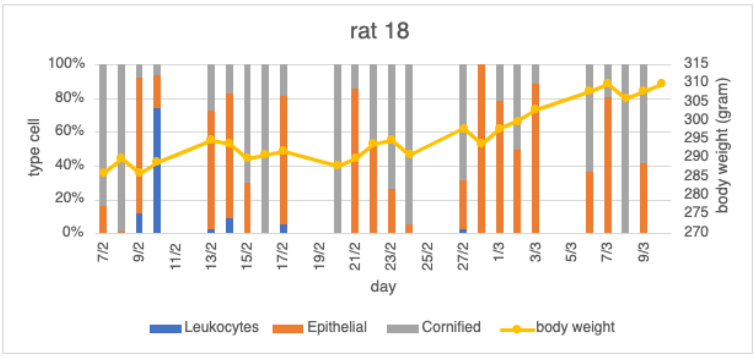

rat 13
type cell
100%
80%
60%
40%
20%
0%
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
290
280
270
260
250
240
230
body weight (gram)
Leukocytes
Epithelial
Cornified
body weight

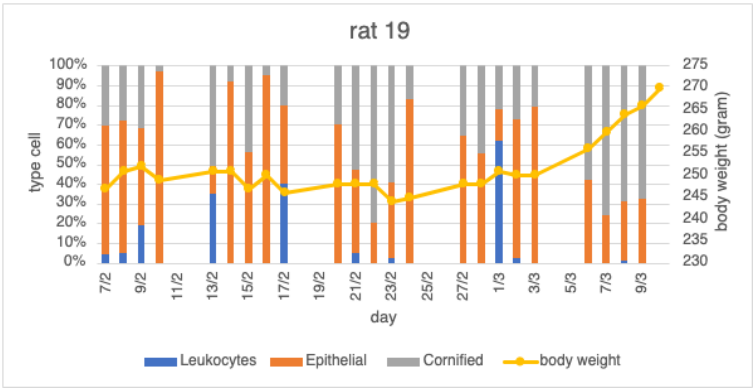

rat 14
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight

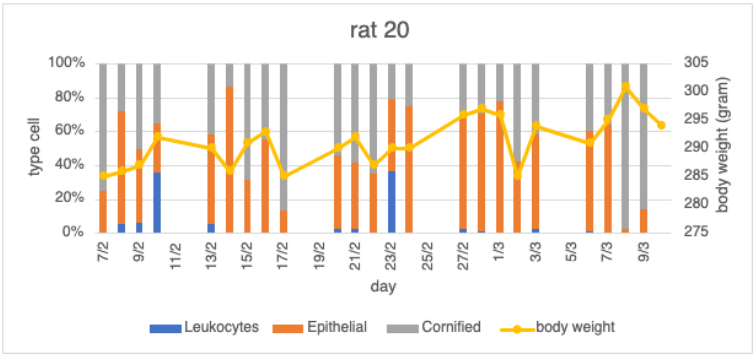

rat 15
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight


rat 16
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight


rat 17
type cell
body weight (gram)
day
Leukocytes
Epithelial
Cornified
body weight


rat 18
type cell
100%
80%
60%
40%
20%
0%
315
310
305
300
295
290
285
280
275
270
body weight (gram)
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
Leukocytes
Epithelial
Cornified
body weight


rat 19
type cell
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
275
270
265
260
255
250
245
240
235
230
body weight (gram)
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
Leukocytes
Epithelial
Cornified
body weight


rat 20
type cell
100%
80%
60%
40%
20%
0%
305
300
295
290
285
280
275
body weight (gram)
7/2
9/2
11/2
13/2
15/2
17/2
19/2
21/2
23/2
25/2
27/2
1/3
3/3
5/3
7/3
9/3
day
Leukocytes
Epithelial
Cornified
body weight