UNDERSTANDING THE WELFARE OF RATS LIVING IN STANDARD VERSUS SEMI-NATURALISTIC LABORATORY ENVIRONMENTS

by

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Abstract

Rats are one of the most commonly used animals in research. Differences in rat housing lead to differences in brain, behaviour, physiology and health. These differences can also affect rat welfare and the validity of data obtained from these animals. Few studies have assessed the consequences of housing rats in standard laboratory cages compared to more complex, naturalistic environments; fewer still have assessed these consequences in females, or after more than a few weeks of differential housing. The aim of my thesis was to assess the sustained welfare consequences of housing female rats in standard versus semi-naturalistic laboratory conditions. The psychological well-being of animals is central to the concept of animal welfare, so Chapter 2 provides a review of the scientific methods of assessing affective states in animals, how these methods have been applied to rats, and what the results can tell us about rats' experience of various emotional states. Chapter 3 investigated rats' propensity to engage in behaviours that are not possible in standard laboratory cages: burrowing, climbing and standing upright. Results indicated that burrowing and standing upright may be especially important to rats. Chapter 4 assessed the sustained affective consequences of standard versus semi-naturalistic housing using an anticipatory behaviour test. Results indicated that standard-housed rats were experiencing poorer welfare than the semi-naturalistic-housed rats. These studies were not designed to test differences in health between the two housing conditions, but given the very limited amount of research on the long-term health effects of differential housing in rats, Chapter 5 documented differences in body weight and development of naturally-occurring tumours. Standard-housed rats were much heavier than semi-naturalistic-housed rats, but there were no differences in the rate of tumour development. Collectively, these results indicate that, compared to the semi-naturalistic housing assessed in this thesis, standard laboratory housing for rats compromises rat welfare by 1) preventing the performance of important natural behaviours; 2) leading to negative affective states; and 3) leading to overweight animals predisposed to developing other health issues. Implications for rat welfare and

the quality of the science obtained from standard-housed rats are discussed, and recommendations are provided.

Preface

All of the work presented here was conducted at the Animal Welfare Program at the University of British Columbia, Vancouver campus. All projects and associated methods were approved by the University of British Columbia's Animal Care Committee (protocol number: A12-0179).

A version of Chapter 2 has been published: Makowska, I.J., Weary, D.M. 2013. Assessing the emotions of laboratory rats. Applied Animal Behaviour Science 148: 1-12. I.J. Makowska developed the main ideas for this paper. D.M. Weary helped to interpret material and edit drafts.

A version of Chapter 3 has been submitted for publication: Makowska, I.J., Weary, D.M. The importance of burrowing, climbing and standing upright for laboratory rats (*Rattus norvegicus)*. I.J. Makowska developed and researched the main ideas for this paper. D.M. Weary supervised, helped to develop ideas, and edited drafts.

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The first pages of these chapters have similar information in the footnotes.

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For my rats 

1. Introduction

1.1. Background

Societal views on the acceptability of using animals in science are polarized (European Commission, 2010; Gallup Poll, 2010; Ipsos MORI, 2010). However, most individuals would agree that if animals are used, then we have a moral obligation to minimize harm as much as possible, and even to strive to give the animals 'a life worth living' (CCAC, 2014; Hubrecht, 2014). Indeed, it is societal concerns about the quality of life of animals that gave rise to the science of animal welfare (Fraser et al., 1997).

Good laboratory animal welfare is important not only from a moral perspective, but also from a scientific perspective. Increasingly, the claim is being made that data from animals whose welfare is compromised lack validity (Bayne and Würbel, 2014; Garner, 2005; Olsson et al., 2003; Poole, 1997; Sherwin, 2004). Animals experiencing poor welfare – for example, animals who are chronically stressed – are not normal and therefore introduce abnormal results into experiments. If data obtained from these animals are not valid, then this negates the purpose for which they were used in the first place.

Rats (*Rattus norvegicus*) have historically been and continue to be an important research animal. The breeding of rats for the purpose of experimentation began in the 1840s in Europe, making them the first mammalian species to be domesticated primarily for scientific purposes (Lindsey, 1979; Richter, 1959). Rats are currently the third most commonly used animals in research, surpassed in numbers only by mice and fish (CCAC, 2015; Home Office, 2014). However, recent advances in rat genomics have enabled researchers to create genetically modified rat strains, something that until now has been primarily done in mice; because rats are better models than

mice for many complex disorders common in humans, it is anticipated that the number of rats will catch up and even surpass the number of laboratory mice currently used ([Editorial], 2010; Abbott, 2009).

1.2. Rat housing

1.2.1. Laboratory cages in North America

Of the many factors that comprise a laboratory animal's life, housing is one of the most pervasive. This may be especially true for laboratory rats and other nocturnal rodents, who for the most part spend their waking hours in this cage undisturbed (except in relatively uncommon cases when nocturnal animals are housed under a reversed light cycle). For the most part, the behaviour and experiences of laboratory animals are largely dictated by the features and characteristics of their cage, making this cage an important factor affecting their welfare.

Some of the earliest records on laboratory rat housing date back to the 1920s. In those early days of experimentation on the rat, a popular type of enclosure was a glass jar with a perforated metal lid (Brewer, 1980). Other types of housing at that time were 'homemade' cages constructed from wood or metal by individual researchers (Hessler, 1999). For example, a researcher at John Hopkins University made wooden cages measuring 48 x 61 x 42 cm (W x L x H) and filled them with bedding; a researcher at the Connecticut Agricultural Experiment Station created cages consisting of a sheet-steel pan covered with a cylindrical wire top that was 23 x 20 cm (diameter x H); and a researcher at the Bussey Institution constructed cages consisting of a 40 x 35 cm sheet-metal pan fitted with a sloped wire-mesh top that was 10 cm high at one end and 21 cm high at the other end (Ferry, 1920; Greenman and Duhring, 1923).

Wooden cages were inexpensive and preferred by the animals because they were warmer and offered a darker environment (Hessler, 1999). However, not only did rats gnaw on them,

wooden cages were also difficult to sanitize, and could stay permanently wet if washed frequently. For these reasons, wood was gradually phased out and replaced with metal. Wire-bottom cages suspended over a litter pan became wide-spread in the 1950s due to their ease of cleaning (Eaton and Cabell, 1961; Eaton, 1949; Hessler, 1999). Large-scale commercial plastic cages were introduced in 1953, but were seldom used until the 1970s (Hessler, 1999).

The first set of widely accepted guidelines for the care and use of laboratory animals in America was published in 1963 by the U.S. Department of Health, Education and Welfare (ILAR, 1963). These guidelines stated that cages "should be designed with the animals' physical comfort as a primary consideration" but that the design should also "facilitate effective sanitary maintenance and technical servicing". In the fifth revision of the guidelines published in 1996, the wording was amended to say that "cages should be constructed with materials that balance the needs of the animal with the ability to provide for sanitation" (ILAR, 1996). It is not until these 1996 guidelines that wire-bottom cages were discouraged, citing decade-old research that had found an association between wire-bottom caging and damage to plantar nerves of rats' hind feet.

Spread of disease within rodent colonies had always been an important concern. To help limit the transmission of pathogens, researchers in the late 1950s began placing various types of filters on the cage lids, and eventually developed individually ventilated cages in the mid-1960s. The push towards increasing animal housing density in the mid-1970s led to the development of pressurized ventilated caging systems, which came into prominent use in the early 1990s (Hessler, 1999).

Animal 'comfort' or 'needs' were often cited as important criteria guiding cage design, but the meaning of these terms did not seem to extend much beyond 'free of disease'. Consequently, emphasis always seemed to be placed on easy and effective cage sanitation – with one interesting exception.

In 1906, The Wistar Institute of Anatomy and Biology in Philadelphia began studying the rat to uncover the conditions necessary for producing healthy, vigorous animals (Lindsey, 1979). This Institute is credited for laying down the foundation on which the rat became established as an important laboratory animal. Through an "intimate acquaintance with the habits of this little animal", researchers at The Institute strived to uncover "the means of making it contented and happy" (Greenman and Duhring, 1923). Some of the Institute's earliest findings were that "confining a rat to the limited quarters of a cage necessarily restricts its activities, modifies its mental processes, and influences its growth and development", and that "fear and lack of exercise are factors which react unfavourably upon the growing rat" (Greenman and Duhring, 1923). To compensate, "as far as is possible, for the disadvantages of cage life", researchers at the Wistar Institute designed two types of wooden cages that were to be used in combination: a *dormer* cage (from the French dormir, to sleep) and an *exercising* cage. Both cages measured 41 x 82 x 22 cm (W x L x H) and housed up to ten rats. These cages were divided into two compartments by means of a partition with a circular opening; the purpose of the division was to segregate space so it could be used for different activities, and to offer rats the opportunity to cross to the adjacent compartment if they became frightened. It was noted that "this simple shifting of location appear[ed] to satisfy the animal that it has protected itself". The cage was furnished with bedding and nesting material ('wood wool' was preferred) in which rats could form burrows and build nests. The exercising cage was similar, except that it communicated with a large, 53-cm diameter running wheel (Greenman and Duhring, 1931, 1923).

Today, cages made of polycarbonate are the industry standard (Hessler, 1999). Following the animal care guidelines of various countries, cages manufactured by leading laboratory caging companies are 18-20 cm tall and offer 825 – 920 cm^2 floor area (e.g., 22 x 38 cm or 20 x 48 cm). At Canadian universities, these cages typically house two or three rats and contain contact bedding and

some type of shelter, although current Canadian guidelines do not actually stipulate that a shelter should be provided (CCAC, 1993). The most common type of shelter given to rats is a short tunnel or piece of PVC pipe open on both ends (Patterson-Kane, 2003), although some scientific evidence as well as my own experience suggest that rats have little use for these pipes and prefer instead shelters with at least one closed end (Bradshaw and Poling, 1991; Chmiel and Noonan, 1996). Housing rats without a shelter appears to be fairly common in the United States, perhaps particularly in toxicology testing (King-Herbert et al., 2012; Turner and Burne, 2014).

1.2.2. Effects of housing on rodent brain, behaviour, physiology, and health

Concerns for animal welfare and the quality of scientific data have prompted researchers to investigate differences in brain, behaviour, physiology and health between animals housed in simple versus more complex environments. These more complex environments are often said to be environmentally 'enriched'. While I believe that the term 'environmental enrichment' should strictly refer to features that actually enrich the animals' lives in a meaningful way (Newberry, 1995), the term is often used for anything that is added to the cage beyond bedding, even if these items may merely fulfill the animals' basic needs (e.g., nesting material for mice or shelters for rats).

Studies comparing rodents housed in simple versus 'enriched' environments are numerous; several review papers have also reviewed the effects of more complex environments on brain, behaviour, physiology, cognition, health and emotion (Fox et al., 2006; Girbovan and Plamondon, 2013; Laviola et al., 2008; Simpson and Kelly, 2011; Würbel, 2001). My aim here is not to provide another comprehensive review of this literature, but to describe the general findings.

Rodents housed in two different environments are almost always different in several of the parameters tested in a particular study. These differences are often, but not always, beneficial for the animals housed in the more complex environment. Regarding the animals' brains, more complex conditions generally improve learning, memory, and problem-solving skills (reviewed by Simpson

and Kelly, 2011; Würbel, 2001). These improvements presumably result from some combination of an increased number of neurons, synapses, dendritic branches, and better brain plasticity.

Environmentally 'enriched' animals also typically behave differently in common behavioural tests of emotionality (reviewed by Fox et al., 2006; Girbovan and Plamondon, 2013; Simpson and Kelly, 2011). 'Enriched' rats tested in an Open Field test are usually better able to adapt to a new environment and explore it more freely. In the Elevated Plus Maze, they spend more time on, and make more entries into, the open arms; in the Social Interaction test, they engage in more social play; in the Forced Swim test, they spend more time swimming, climbing, and diving, and less time immobile; and in all of these tests they tend to show less defecation and freezing. These differences in behaviour are believed to indicate lower levels of anxiety and depressive-like states. Environmental 'enrichment' also reduces the incidence of abnormal behaviours, such as stereotypies (functionless, repetitive behaviours) and barbering (over-grooming resulting in the loss of fur or whiskers; reviewed in Garner, 2005; Mason et al., 2007). Abnormal behaviours are generally seen as indicating poor welfare, and are thought to arise because of persistent negative internal experiences or the inability to perform highly motivated natural behaviours (Duncan and Fraser, 1997).

Data on baseline physiological measures of stress are less clear, with rodents tested shortly after being placed in an 'enriched' environment sometimes showing an increase, a decrease, or no change from their 'pre-enrichment' baseline (Girbovan and Plamondon, 2013; Simpson and Kelly, 2011). Physiological methods usually consist of measuring serum concentrations of cortisol or ACTH, both of which are released when the HPA axis is activated. However, physiological measures of stress are not necessarily reflective of the quality of an experience (positive or negative), but of arousal (see next Chapter, section 2.4). Arousal shortly after being placed in a new environment should not be surprising, and not necessarily viewed as something negative. While data are mixed

on the effects of 'enrichment' on baseline physiological measures of stress, evidence is strong that animals from a more complex environment respond better to challenges. For example, 'enriched' animals consistently show lower increases in acetylcholine following a stressful event such as restraint or a saline injection.

Finally, there is evidence that animals housed in more complex environments are healthier (for a more detailed review on the effects of a complex environment on animal health, please see Chapter 5). Fox et al. (2006) reviewed evidence that environmentally 'enriched' animals have better immune systems, with higher natural killer cell activity and buffered immune system reactivity to stress. More complex environments are also beneficial in neurodegenerative diseases such as schizophrenia, Huntington's, Parkinson's, and Alzheimer's (reviewed by Laviola et al., 2008; Nithianantharajah and Hannan, 2006). In addition, a few studies have linked an 'enriched' environment to lower rates of cancer: in one study, environmentally 'enriched' mice had reduced tumour growth and increased remission in melanoma and colon cancers (Cao et al., 2010), and in another study, mammary tumour burden was 84 times lower, and tumours were less likely to be malignant, in environmentally 'enriched' female rats compared to age-matched controls (Hermes et al., 2009).

1.2.3. Gaps in the literature

Despite a multitude of studies investigating the effects of environment on laboratory rodents, more research is needed to fully understand this topic (Laviola et al., 2008; Simpson and Kelly, 2011). For example, relatively little is known about the consequences of environmental 'enrichment' on animal emotion (Coke-Murphy et al., 2014).

Of particular interest to me is the small amount of research on the effects of housing rats under commonly used standard laboratory conditions (e.g., rats housed in pairs with a piece of PVC

pipe). The difference might be subtle, but the majority of studies reviewed in the previous sub-section have focused on the general effects of an 'enriched' environment, as opposed to the effects of a standard environment; consequently, the modifications were modest and the control group was usually housed in a very basic environment: a single rat in a cage with no furnishings. Indeed, 'enrichment' in these studies has sometimes consisted of adding one or more companions, and/or adding a shelter – basically amounting to what would now be considered fairly standard housing in Canadian laboratories.

In addition, studies on the differences between simple and more complex environments, including those assessing differences in welfare, have primarily been performed in mice; and of those conducted in rats, the majority has looked at males housed in these environments for only a few weeks. Simpson and Kelly (2011) conducted a literature review on the effects of housing rats in environmentally 'enriched' environments. Of the 361 relevant articles published between 1960–2009, 60% had tested males only, and 70% had tested the animals only 1-8 weeks after placing them in that environment.

1.3. Thesis aims

My general aim for this thesis was to assess the sustained welfare consequences of housing laboratory rats in a common Canadian laboratory environment, which consists of a pair of rats housed in a standard-sized cage (purchased from a major cage manufacturer) containing an open-ended piece of PVC pipe.

The literature on 'environmental enrichment' is somewhat messy; what people mean by 'enrichment' varies greatly between studies, and not many systems/items have been validated systematically for their potential to enrich rat lives and improve welfare (Newberry, 1995; Patterson-Kane, 2004). In order to avoid idiosyncratic differences resulting from specific,

commercially-available 'enrichment' devices, I have opted to compare standard-housed rats to rats housed in a system more closely resembling their 'natural' environment; i.e. the environment they have evolved in as a species. The main constraint was creating several replicates of this 'semi-naturalistic' environment within a laboratory; for this reason, a modest-sized cage was unavoidable. To select the features of this cage, I have largely relied on two books describing the behaviour of wild and domesticated rats: *The Ecology and Sociology of the Norway Rat* (Calhoun, 1963) and *The Rat: A Study in Behavior* (Barnett, 1975).

Learning about rats' natural behaviours made it apparent that a burrowing substrate was important. What this substrate should consist of, how deep it should be, and how to manage it in an artificial setting were largely informed by the studies of Boice (1977) and Pisano and Storer (1948). Wild rats live in large colonies subdivided into smaller populations sharing a burrow. Small groups consisting of five to ten females typically associate with one male; males do not live in close-knit groups with each other (Calhoun, 1963). For this reason, I chose to house five females in each cage (no male was included due to the laboratory animal facility's regulations against mixing sexes in one room). Furthermore, cages and husbandry procedures were designed to allow rats to climb, perch, run, hide, explore, and have access to a varied diet.

The psychological well-being of animals is central to the concept of animal welfare (Fraser et al., 1997), and I deemed that a solid understanding of rats' ability to experience positive and negative emotions was an important starting point to the study of rat welfare. Consequently, the aim of Chapter 2 was to review the scientific methods of assessing affective states in animals, how these methods have been applied to rats, and what the results can tell us about rats' experience of various emotional states. The aim of Chapter 3 was to assess laboratory rats' propensity to engage in behaviours that are not possible in a standard laboratory cage, and to gauge the potential welfare consequences of denying rats these activities. The aim of Chapter 4 was to use anticipatory

behaviour to assess the affective state experienced by rats housed in a standard versus semi-naturalistic environment for more than one year. My set-up and experiments were not designed to test differences in health, but given that this is an important research area and that there is so little long-term research in animals, I have described some of these differences here. The aim of Chapter 5 was thus to document the unintended health consequences of nearly two years of living in a standard versus a semi-naturalistic laboratory environment.

2. Assessing the Emotions of Laboratory Rats[1]

2.1. Introduction

Rats are one of the most commonly used animals in research, currently third behind mice and fish (CCAC, 2015; Home Office, 2014). Rats have been used extensively in the field of psychology as a model organism to study the basis of several mental processes, including emotion (Carmichael, 1950). While the focus has been on how results from rats apply to humans, the process has yielded a large amount of information on rats themselves. Knowledge of rats' ability to experience emotions including pain is important as this helps to inform and motivate concerns for welfare (Broida et al., 1993; Knight et al., 2009). The aim of this chapter is to bring together information on rat emotion from a number of disciplines and over several decades, synthesizing this information and making it easily accessible. But first, I describe and critique the methods used to infer the existence of different emotional states in rats.

2.2. What are emotions?

There is no clear agreement on how to define emotion (one survey describes 92 different definitions; Kleinginna and Kleinginna, 1981), but the general consensus is that emotion is a construct referring to four different, imperfectly related phenomena: (1) a change in brain activity to certain stimuli, (2) a change in cognitive processes, (3) a preparedness for, or display of, a behavioural response, and (4) a consciously detected change in feeling (Kagan, 2007).

The first three components (changes in brain activity, in cognitive processing, and in behaviour) can be recorded, but the fourth component (the conscious detection of a change in feeling) is difficult to investigate scientifically (e.g., Cohen and Dennett, 2011; Dehaene and

[1] A version of this chapter has been published: Makowska, I.J., Weary, D.M. 2013. Assessing the emotions of laboratory rats. Applied Animal Behaviour Science 148: 1-12.

Changeux, 2011). Although I consider it likely, I cannot definitively know whether animals 'feel' emotions even if they display changes in brain activity, cognitive processing and behaviour. In this chapter, I will discuss emotions without making conclusions as to whether they experience those emotions consciously.

The study of emotion has taken two approaches: the 'discrete emotions' approach that is focused on studying discrete emotions such as anger or fear (e.g., Panksepp, 1998; Plutchik, 1982), and the 'dimensional' approach that plots emotions in two-dimensional space along an 'affective valence' axis and an 'arousal' axis (Fig. 2.1; e.g., Russell, 1980; Stanley and Meyer, 2009). Valence refers to the positive or negative aspect of emotion and ranges from pleasant to unpleasant, while arousal refers to the activation or intensity of an emotion and ranges from high to low. Mendl et al. (2010) proposed a framework that integrates these two approaches and allows for the representation in two-dimensional space of affective states resulting from the interaction between discrete emotions, which they define are short-term responses to specific stimuli, and longer-term background mood.

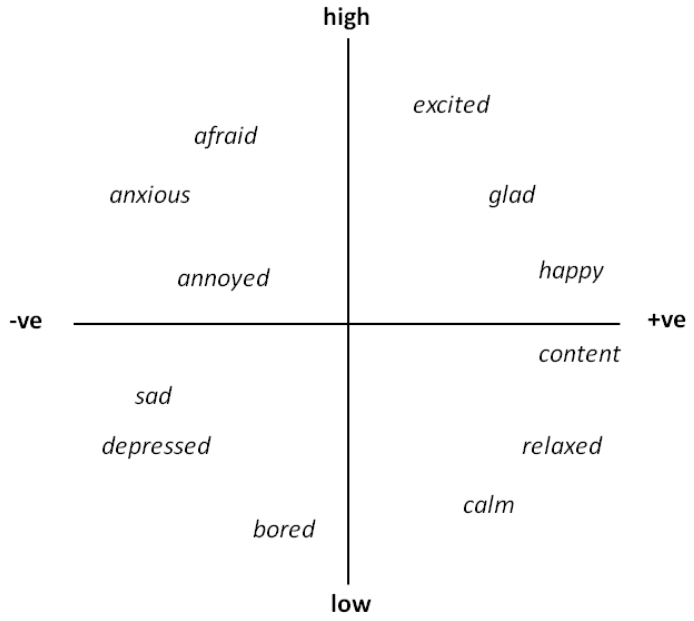

Figure 2.1. Bi-dimensional representation of emotion. Possible locations of discrete emotions along the valence (-ve to +ve) and arousal (high to low) scales. Adapted from Mendl et al. (2010) and Russell (1980).

2.3. Scientific methods of assessing emotions in animals

Traditionally, scientists often relied upon physiological measures (e.g., hormone levels, heart rate, etc.) to draw inferences about emotional states in animals, but these measures are more related to arousal than affective valence. For example, elevated cortisol levels are associated with both negative stimuli such as restraint, and positive stimuli such as mating (Moberg, 2000). Behavioural measures and changes in cognitive processing appear to be more related to affective valence. For example, a negative judgement bias is indicative of depression (a low arousal negative affective state) or anxiety (a high arousal negative affective state; Mendl et al., 2010).

Although both arousal and valence ultimately contribute to the overall emotion, valence may be of more practical use to animal welfare science. Knowing that an animal is in a negative affective state without knowing whether the state is high arousal (e.g. fearful) or low arousal (e.g. depressed) may matter more than knowing that an animal is highly aroused without knowing whether the arousal has positive valence (e.g., excited) or negative valence (e.g., fearful).

I therefore focus below on methods used to measure changes in behaviour and cognitive processing rather than changes in physiology, distinguishing between methods more suitable for assessing shorter-term discrete emotions associated with a specific stimulus ('discrete emotions') versus those for the assessment of more pervasive mood states ('mood'). I focus on methods that have been validated as indicators of affect and will be mentioned in section 2.4.

2.3.1. Discrete emotions

2.3.1.1 Place conditioning

Conditioned place preference and conditioned place aversion, collectively known as 'place conditioning', are used to determine whether stimuli are rewarding or aversive to animals. In this paradigm, an environment is paired with a stimulus, and later the animal's willingness to re-enter or

avoid that particular environment is used to infer whether the animal found the stimulus rewarding or aversive (Carlezon Jr., 2003). The amount of time animals spend in the environment compared to baseline and compared to occasions when other stimuli are offered are taken as indications of the strength of the affect induced by the stimulus (i.e. arousal).

Place conditioning has mostly been used to assess the rewarding or aversive properties of drugs (e.g., amphetamines) but is now increasingly used in animal welfare science to examine the affective state induced by various stimuli. For example, this method is increasingly being used to assess the emotional component of pain (Tzschentke, 2007).

One advantage of place conditioning is that it tests animals in a 'sober' state; when tested, animals are not affected by the stimulus under study (Bardo and Bevins, 2000). This is important because some stimuli may have direct effects on behaviour including locomotion (e.g., induction with inhaled anaesthetics causes ataxia) and cognitive processing (e.g., pain impairs rat cognitive function: Moriarty et al., 2011), thereby influencing the choice irrespective of the rewarding properties of the stimuli. One drawback of place conditioning is that motivation to explore novel environments may influence and confound behaviour on testing day (Bardo and Bevins, 2000). During conditioning, one environment is paired with a stimulus but it is possible that the stimulus is impairing familiarization with that environment (e.g., inhaled anaesthetics impair rat memory: Alkire and Gorski, 2004). If this happens, the environment that had been paired with the stimulus during conditioning may seem relatively more novel for reasons other than positive or negative associations with that environment.

2.3.1.2. Motivation tests with an operant

In operant tests, animals are trained to work (i.e. perform a specific behavioural response) to access a resource; the more work they are willing to perform the higher their motivation to access that resource is inferred to be. Motivation tests with an operant differ from simpler

preference tests because they allow us to quantify the strength of motivation for a resource, instead of merely showing which of a set of resources is preferred.

Motivation testing with an operant has been used extensively in both farm and laboratory animal welfare science (see Patterson-Kane et al., 2008 for a review). In rats, the work to be performed often consists of pressing a lever, turning a wheel, or pushing or lifting a weighted barrier (Manser et al., 1996).

This type of testing assesses motivation rather than affect, and evidence suggests that the neural substrates of 'wanting' (motivation) and 'liking' (affect) are different (Berridge, 1996; Berridge and Robinson, 2003). 'Wanting' involves mesotelencephalic dopamine systems, while 'liking' involves neurotransmitter systems such as opioid and GABA systems. However, several emotional theorists suggest that affect motivates most behaviour with the goal of maximizing pleasure or minimizing displeasure (e.g. Cabanac, 1992; Fraser and Duncan, 1998; Spruijt et al., 2001), and as such, these operant tests could be good indicators of conditions associated with a positive shift in affective valence. However, these tests cannot differentiate between resources that animals 'want' because they induce positive affect versus those that decrease negative affect. For example, if animals work to access an enriched cage, is it because the enriched cage induces positive affect (e.g. from playing with toys) or reduces negative affect (e.g., alleviate boredom) or both? The resource (e.g., enriched cage) being a positive affect-inducer or negative affect-reducer is also not fixed but will depend on the context (e.g., animal's existing housing conditions). In humans, there is evidence that enjoyment and preference for a resource tends to grow with familiarity (Zajonc, 1971). If this was shown also to be true in other animals, then recording the amount of work performed over time could help differentiate between resources animals 'want' because they induce positive affect versus those they 'want' because they alleviate negative affect. The rationale is that work for a resource that induces positive affect will increase over time as familiarity and

enjoyment increase, while work for a resource that strictly decreases negative affect will be more stable over time. Such an experiment would have to include adequate controls for the effects of habituation.

A final drawback of operant testing is that operant tasks themselves can be rewarding. Contrafreeloading is the phenomenon in which animals choose to work for a resource that is freely available (e.g., Inglis et al., 1997), perhaps because of long-term fitness advantages associated with investing some effort into exploration in addition to exploitation of existing resources. One way to control for the inherently rewarding properties of performing an operant is to establish the amount of work animals are willing to perform to access a resource they can also access freely; for example, rats may be asked to press a lever to access rat chow that is also freely available in their cage. This would provide a baseline against which other resources can be judged.

2.3.1.3. Embodied emotion

In humans, many discrete emotions are associated with distinct, universal facial expressions that vary little across cultures (Darwin, 1872; Ekman and Oster, 1979). Until recently, rodents were generally believed to lack facial expressions, but more recent studies have shown otherwise. For example, rats display highly consistent facial expressions in response to flavours that they 'like' and those they 'dislike', and these expressions are homologous in a variety of mammalian species including humans (Berridge and Robinson, 2003; Grill and Norgren, 1978). Sotocinal et al. (2011) found that rats display facial expressions of pain, and that these are broadly similar to those expressed by humans and mice in pain (Langford et al., 2010). This 'Rat Grimace Scale' was validated by verifying that expressions identified as being indicative of pain were attenuated by the administration of morphine in a dose-dependent manner.

The Rat Grimace Scale is said to be usable in real time on freely moving animals, but because this scale was developed and validated using still photographs captured from video, the

validity and accuracy of real time assessment has yet to be determined. Capturing frames is labour intensive, but the development of the Rodent Face Finder® software may aid in the process (Sotocinal et al., 2011). As a prey species rats are likely motivated to avoid displays of weakness, suggesting that overt behavioural signs including facial expressions may only emerge when pain is severe. I suggest that the study of facial expressions in rats (and indeed other species) is still in its infancy, and that more homologies useful in the assessment of emotions will be uncovered in the years to come.

2.3.1.4. Vocalizations

Vocalizations have been linked to the expression of affective state in several species including rats (e.g., Knutson et al., 2002; Manteuffel et al., 2004). Rats emit a variety of ultrasonic vocalizations that mainly fall in the range of 20-32 kHz and 35-70 kHz, and these calls have been termed collectively as '22-kHz calls' and '50-kHz calls'. Fifty-kHz calls are believed to indicate positive affect akin to the excitement seen during high arousal and anticipation of a reward, while 22-kHz calls are believed to indicate negative affect associated with anticipation of punishment (Knutson et al., 1999).

Fifty-kHz calls are emitted in, and are predictive of, naturalistic contexts that elicit approach behaviours; emission of these calls has been behaviourally associated with locomotor activity, rearing and exploration (Fu and Brudzynski, 1994). Twenty-two-kHz calls are emitted during, and are predictive of, the onset of avoidance behaviours; emission of these calls has been behaviourally associated with tense, motionless crouching, freezing and flight (Brudzynski et al., 1993). Twenty-two-kHz calls may be more indicative of anxiety rather than fear and can be attenuated by the administration of an anxiolytic (Jelen et al., 2003; Nobre and Brandão, 2004).

These two types of calls are for the most part accepted as indicating positive and negative affect, but some discrepancies remain. For example, 50-kHz calls generally associated with positive

affect have also been recorded during presumably negative events, such as carbon dioxide euthanasia (Niel and Weary, 2006) and aggressive resident-intruder interactions (Takahashi et al., 1983). Some evidence suggests that 50-kHz calls may be used as signals to establish or keep contact with other rats (Wöhr and Schwarting, 2007; Wöhr et al., 2008), suggesting that these calls may also be emitted as social signals. Calls labelled as '22-kHz' or '50-kHz' actually vary considerably in frequency, length, and shape, and may consist of several subpopulations (e.g., Brudzynski et al., 1993). Closer study may help differentiate between the different types of calls, rendering them a more reliable indicator of affect.

2.3.2. Mood

2.3.2.1. Drug self-selection

In drug self-selection studies, animals are given the choice between consuming food or water that does or does not contain a specific drug. The inference is that a drug will be reinforcing only to animals who suffer from the condition the drug is targeting, so only affected animals, but not healthy controls, will consume more of the drug. For example, rats in pain given the option between drinking water with or without an analgesic tend to drink more of the medicated water than healthy controls, and the amount of drug consumed mirrors the intensity of pain (Colpaert et al., 1982, 1980).

To my knowledge, drug self-selection studies have mostly been performed with analgesics, but one study on mice found preferential intake of an anxiolytic (Sherwin and Olsson, 2004). I suggest that there is scope to extend this approach to other classes of drugs, including self-selection of antidepressants as a method of assessing whether animals in certain environments are depressed. One limitation of this approach is that the palatability of the drug may also affect intake. Animals must also be given sufficient forced exposure to each condition (e.g., water with and

without the drug) to be able to form an association, and must be able to discriminate between the two conditions during the choice phase to be able to display their preference.

2.3.2.2. Anticipatory behaviour

Anticipatory behaviour was first described as "a state of agitation which continues so long as [a desired] stimulus is absent" (Craig, 1918, p.91). In rats, anticipatory behaviour is characterized by an increased level of activity resulting from frequent and abrupt behavioural transitions between short fragments of behaviour (Spruijt et al., 2001; van der Harst et al., 2003b). Anticipatory behaviour can be observed in the interval between presentation of a conditioned stimulus (e.g., light or tone) and the arrival of the unconditioned stimulus (e.g., food reward). It has been argued that the hyperactivity in anticipation of the arrival of a reward reflects the activation of reward centres in the brain, and that the level of activation depends in part on the incentive value of the reward (Koob, 1996; Spruijt et al., 2001). Thus, the presence of anticipatory behaviour before the arrival of an unconditioned stimulus is used to infer that the stimulus is rewarding to the animal, and the magnitude of the response gives clues as to the strength of the reward. Rats display no hyperactivity (and sometimes, a decrease in activity; e.g., Carrive, 2000) before the presentation of an aversive stimulus (van der Harst et al., 2003b).

The display of anticipatory behaviour may also be used to draw inferences regarding an existing affective state. In general, negative experiences affect reward sensitivity. More specifically, it seems that most negative events increase sensitivity to rewards (Piazza et al., 1990), but negative events resulting in depression diminish sensitivity to rewards (Cabib and Puglisi-Allegra, 1996). Therefore, deprived rats tend to exhibit more anticipatory behaviour than controls before access to a reward, while depressed rats display less anticipatory behaviour than controls. Anticipatory behaviour in presumably depressed animals can be re-established by treatment with an antidepressant (Von Frijtag et al., 2002). These results suggest that anticipatory behaviour can be

used to determine existing affective state, and even differentiate between animals in a strongly valenced negative state versus those in a milder negative state.

One limitation is that the 'reward' used to elicit anticipatory behaviour must be perceived as such; a lack of anticipatory behaviour could either indicate that animals are depressed or that the 'reward' is perceived as neutral or aversive (see above), irrespective of existing affective state. Furthermore, due to the curvilinear nature of the relationship between affective state and anticipatory behaviour, a lack of anticipatory behaviour could also be associated with a very positive state (Boissy et al., 2007). Testing after the administration of an antidepressant may help confirm whether an animal failing to exhibit an anticipatory response is in a very positive versus anhedonic state.

2.3.2.3. Cognitive bias

Cognitive bias may also be used to draw inferences regarding affective state in animals. This approach borrows from research in humans showing that people in a negative affective state tend to judge ambiguous stimuli negatively (e.g., MacLeod and Byrne, 1996). Animal welfare researchers have used this phenomenon to test whether particular manipulations induce positive or negative affective states in several animal species including rats (see Mendl et al., 2009 for a review). Manipulations are usually environmental (e.g., standard vs. enriched housing) but genetically 'helpless' rats used as a model of depression also judge ambiguous stimuli more negatively than genetically 'normal' rats (Enkel et al., 2010; Richter et al., 2012).

In cognitive bias testing, animals are trained to perform one response when exposed to a particular stimulus to obtain a positive outcome (e.g., press left lever after hearing a high frequency tone to receive a food reward) and to perform another response when exposed to a different stimulus to avoid a negative outcome (e.g., press right lever after hearing a low frequency tone to avoid being exposed to noise). Animals are then tested with ambiguous stimuli (e.g., intermediate

frequency tone); those in a negative affective state are expected to interpret the stimuli negatively (e.g., by pressing the right lever, a pessimistic judgment bias) and those in a positive state are expected to interpret the stimuli positively (e.g., by pressing the left lever, an optimistic judgment bias).

Studies have reported different types of judgment biases in rats. One study reported bias only at probes nearest to the positive training cue (Harding et al., 2004); two studies reported bias only at probes nearest to the negative training cue (Burman et al., 2008; Enkel et al., 2010); and one study reported bias at all probe locations (Richter et al., 2012). If we assume that an ambiguous cue is most strongly associated with the training cue it is closest to, then an ambiguous cue closest to the positive training cue should lead to a positive response and therefore a negative response would indicate a decreased expectation of positive events. Similarly, biases closest to the negative cue may reflect an increased expectation of negative events. In humans, the former is associated with depression while the latter is associated with anxiety (MacLeod and Salaminiou, 2001; MacLeod et al., 1997), so biases at different probe locations may reflect different emotional states (Burman et al., 2008).

2.3.2.4. Startle potentiation

The use of the startle reflex as a measure of affective state in animals is also borrowed from human psychology, where reflexes have been shown to vary according to the subject's emotional state (Bowdich and Warren, 1890; Ison and Hoffman, 1983; Sechenov, 1965). Specifically, defensive reflexes (e.g., startle following a sudden loud noise) are enhanced if the subject is already in a negative emotional state, and attenuated if the subject is in a positive emotional state. Similarly, appetitive reflexes (e.g., salivation following a sucrose probe) are enhanced if the subject is already in a positive emotional state, and attenuated if the subject is in a negative emotional state (Lang et

al., 1990). A common use of this method has been to measure the magnitude of the startle reflex following a loud noise in fear-conditioned versus control rats.

The advantage of the startle reflex in drawing inferences regarding affective valence is that elicitation, recording and quantification are relatively simple. However, care must be taken to ensure that the magnitude of startle is not influenced by other extraneous factors. In rats and other species, presentation of a prestimulus just before the probe (e.g., Blumenthal and Gescheider, 1987; Hoffman and Ison, 1980) attenuates the magnitude of the startle response, as does habituation (e.g., Valsamis and Schmid, 2011). In humans, attenuation of the startle response when in a positive affective state is much less reliable than an exaggeration in the startle response when in a negative affective state (see Grillon and Baas, 2003), so this tool may be more useful in assessing negative rather than positive affective states.

2.4. Current knowledge of rat emotions

In the previous section I described methods that have been validated (to the extent that true validation of methods assessing subjective states is possible) for rats as indicating certain affective states. In this section I will give examples of how these methods have been applied to rats and what the results can tell us about their experience of various emotional states. In addition to the methods described in the previous section, I will also give examples of spontaneous behavioural responses, explaining how these may also be used to draw inferences regarding emotional states.

2.4.1. Negative emotions with high arousal

Fear and anxiety are considered to be high arousal negative states, and much psychological research has focused on understanding their development and response to drugs. The traditional psychological view is that fear is associated with an actual aversive stimulus, while anxiety is

associated with the anticipation of an aversive stimulus (Blanchard et al., 1997). Fear and anxiety share common neural substrates (e.g., Bandler and Depaulis, 1992; Panksepp, 1998) and are often investigated together. These neural substrates are similar across all mammals and much research on fear has been done on rats (Bandler and Depaulis, 1992; Ledoux, 2002).

Because fear and anxiety are believed to have evolved as a means to protect animals from danger (e.g., Blanchard and Blanchard, 1990), the underlying assumption is that behaviours associated with danger are driven by fear. Panksepp (1998) has shown that activation of brain regions located along specific pathways situated in deep, subcortical limbic regions common to all mammals elicits distinct emotional reactions. Among others, Panksepp identified a FEAR pathway (Panksepp always capitalizes this term to signal that it refers to a specific circuit in the brain rather than the emotion 'fear') which causes humans and other animals including rats to freeze or flee. In humans, stimulation of this system also leads to verbal reports of intense anxiety. Rats show conditioned place-aversion to the location where the stimulation took place, and when given the opportunity will turn off stimulation of this pathway (Panksepp, 2005; Sacks and Panksepp, 1987).

The bulk of fear research on rats has focused on characterizing behaviours elicited by natural predators (innate fear) or signals predicting electric shock (conditioned fear). When confronted with a natural predator such as a cat or a dog, or an object impregnated with their odour, rats typically react by fleeing if an escape route is available (Blanchard and Blanchard, 1971; Blanchard et al., 1975). If there is no escape route, rats react by freezing, orienting towards the threat, vocalizing in the 22-kHz range, and may bare teeth, bite, and attack if the predator is very close (Blanchard and Blanchard, 1990). For up to 24 hours after the predator or the predator scent is removed (Blanchard and Blanchard, 1989), rats continue to show reduced mobility and exhibit so-called 'risk assessment' behaviours that are believed to indicate anxiety (Blanchard and Blanchard, 1990; Molewijk et al., 1995). These risk assessment behaviours consist of rats poking their head into

the area where they encountered the predator, approaching the area with a flattened back, and a stretched attention posture (Blanchard and Blanchard, 1990; Kaesermann, 1986; Poel, 1979). Risk assessment behaviours are diminished by the administration of anxiolytic drugs (for a review, see Blanchard et al., 1993).

In fear conditioning studies, an initially neutral stimulus (conditioned stimulus; usually a light or tone) is paired with an aversive stimulus (unconditioned stimulus; usually a mild electric shock). Animals quickly learn than the conditioned stimulus precedes the unconditioned stimulus, and react to the conditioned stimulus alone. In these studies, rats are usually confined to a cage, and just as they would react to a natural predator when no escape route is available, they exhibit freezing. Rats develop conditioned place aversion to locations where they receive mild electric shock (Ferguson et al., 2004; Panksepp, 1996). Rats also exhibit a stronger startle reflex if they are startled after the presentation of the conditioned stimulus (Steiner et al., 2011), and this reflex is attenuated after administration of an anxiolytic (Steiner et al., 2012). Rats receiving non-contingent foot shock (the unpredictability of which likely causes anxiety; Grillon et al., 2004) self-administer more cocaine (Goeders and Guerin, 1994) and anxiolytics (Cook and Davidson, 1973) than rats receiving no shock or rats who could control shock delivery. Housing rats individually in small, opaque cages also likely causes a negative affective state akin to anxiety. Alexander et al. (1978) have shown that rats housed in such conditions drank more water containing morphine than rats housed socially in a large, enriched enclosure; morphine is believed to have anxiolytic effects (see Gray, 1987).

2.4.2. Negative emotions with low arousal

Rats are inquisitive (Small, 1899), but standard laboratory conditions offer rats few stimuli to explore and few opportunities to perform behaviours other than sleeping and reaching for food

and water. Several authors have argued that such environments may engender boredom or helplessness (van Rooijen, 1991; Wemelsfelder, 1990). Boredom can be defined as "the unpleasantness of monotony, [...] and the seeking of stimulation" (White, 1959), while helplessness can result from the long-term experience of lack of control over the environment and the inability to change the aversive situation (Fox, 1986).

A multitude of studies have shown that rats will work to access cages that provide extra stimulation (e.g., Bradshaw and Poling, 1991; Collier and Hirsch, 1971; Denny, 1975; Iversen, 1998; Patterson-Kane et al., 2001). Rats reared in isolation display less anticipatory behaviour before access to sucrose than do pair-housed rats (Van den Berg et al., 1999; Von Frijtag et al., 2000), indicating that they are likely in a state of depression. Compared to controls, rats housed under conditions where aversive events occurred on an unpredictable schedule (e.g., reversal of the light/dark cycle, damp bedding, tilting of the cage) showed negative cognitive bias indicating a negative affective state. Because bias occurred at the ambiguous probe closest to the positive training cue, this negative state was likely depression (Harding et al., 2004).

2.4.3. Positive emotions

Positive states are largely under-studied in animals (Boissy et al., 2007) and specific discrete emotions are not well qualified; however, the high-arousal emotion experienced in anticipation of a reward can be referred to as excitement, while the emotion experienced during access to the reward may be referred to as joy. Lower arousal and often longer-lasting mood states may be described as contentment or happiness.

Play has often been linked to the experience of positive emotions (Fraser and Duncan, 1998; Špinka et al., 2001) and is sometimes used to infer positive welfare (for a review, see Held and Špinka, 2011). Juvenile rats readily engage in rough-and-tumble play with other rats; rat play

consists of 'pouncing', where one rat approaches the other and attempts to nose or rub the other's nape of the neck, and 'play pinning', where one rat lies on its back while the other stands over it (Vanderschuren et al., 1997).

Rats also seem to enjoy being 'tickled' in a manner similar to the way we would tickle a child (for a review, see Panksepp, 2007). Rats will seek out hands that have tickled them much more than hands that have petted them an equal amount of time (rats use their sense of smell to distinguish between individual humans; Panksepp, 2007), and will learn to press a lever for a tickling reward (Burgdorf and Panksepp, 2001). When being tickled and during social play, rats emit 50-kHz calls that may be indicative of positive affect (e.g., Burgdorf et al., 2008; Panksepp, 2007). Panksepp argues that 50-kHz vocalizations during play and tickling are analogous to laughter in human infants. In addition to neural and functional homologies, Panksepp reports that just as infants will not laugh unless they feel safe and comfortable, tickled rats will not emit 50-kHz vocalizations in the presence of predatory or stress odours (e.g., cats) or in laboratories where they are frequently punished (e.g., fear learning). This last finding also indicates that these calls are not simply an automatic response to physical stimulation but are dependent on affective state (Panksepp and Burgdorf, 1999).

Several contexts are believed to induce positive affect in rats, and these include nonagonistic encounters with conspecifics as well as engaging in sexual behaviour. Rats vocalize in the 50-kHz range in anticipation of, as well as during, social contact with other rats (Brudzynski and Pniak, 2002); they will work for contact with other rats (Patterson-Kane et al., 2004, 2002); and they show anticipatory behaviour before contact with other rats (Van den Berg et al., 1999). Rats also emit 50-kHz calls during appetitive aspects of sexual behaviour (Barfield et al., 1979; McGinnis and Vakulenko, 2003); they develop conditioned place preference for locations where they engaged in sexual behaviour (Hughes et al., 1990); they will work for the opportunity to engage in sexual

behaviour (reviewed by Pfaus et al., 2001); and they show anticipatory behaviour before an opportunity to engage in sexual behaviour (Mendelson and Pfaus, 1989; van der Harst et al., 2003b).

Electrical stimulation of rewarding brain centres as well as administration of drugs that lead to the release of dopamine (e.g., morphine and amphetamines) are also associated with positive affect in rats. Rats vocalize in the 50-kHz range during and in anticipation of electrical stimulation of rewarding brain centres (Burgdorf et al., 2000). Rats also emit 50-kHz calls during the unconditioned administration of amphetamine or morphine (Burgdorf et al., 2001; Thompson et al., 2006) and in locations where they have previously received morphine or amphetamine (Knutson et al., 1999); they show conditioned place preference for locations where they received amphetamine or morphine (Bardo et al., 1995); and they exhibit anticipatory behaviour before administration of morphine (Hinson and Siegel, 1983).

2.4.4. Pain

Pain is not usually considered to be an emotion, but because the emotional aspect of pain is suggested to be fundamental to the pain experience (Le Bars and Cadden, 2005), I have chosen to include it in this chapter. Indeed, the International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".

Rats are the most common model for the study of pain in humans, and have been for more than four decades (Mogil, 2009). Studies observing involuntary reflex responses to pain are historically common, but these do not allow for strong inferences regarding the emotional components of pain. In a review article on pain, Vierck and colleagues (2008) argue that reflex responses engage spinal and supraspinal circuits that are separate from circuits transmitting pain from the periphery to cerebral structures mediating sensory, emotional and motivational reactions

to pain. Consequently, the authors argue, behavioural responses to pain may be investigated, but the focus should be on behaviours that are cortically mediated, as they are more likely to engage the structures involved in processing the emotional and motivational reactions to pain. Indeed, while behavioural measurements of pain in animals may not be definitive indicators of the emotional experience of pain, they are becoming increasingly popular and are currently the prevailing method in the study of pain and analgesia (Mogil, 2009).

Bennett and Xie (1988) showed that rats with a ligature around one of their common sciatic nerves exhibited pain behaviour like that seen in humans. These rats walked with a limp suggesting a reluctance to place weight on the affected paw, they often raised the affected paw from the floor and held it in a protected position next to the flank ('guarding behaviour'), they slept or rested on the side opposite to the affected paw, with the affected paw in the guarded position, and developed overgrown claws on the affected side. Studies using brain imaging techniques such as PET scans or fMRI have shown that these behaviours are cortically mediated, and that key cortical regions involved in the perception of pain in humans are also activated in the rat. For example, Neto et al. (Neto et al., 1999) have shown that rats afflicted with arthritis in one hind limb displayed 'guarding behaviour' of the inflamed paw and responded with agitation, struggle, and vocalization during the 20 minutes they were subjected to additional mechanical stimulation. These rats were shown to exhibit brain activity in many structures, including thalamic, limbic and cortical regions. Other studies have shown similar results (e.g., Mao et al., 1993; Paulson et al., 2000; Porro et al., 1999).

Although several key cortical regions are involved in the perception of pain in humans, each is believed to process a different component of the pain experience; for example, some regions are involved in the detection of pain and others in the encoding of pain intensity (Woo et al., 2015). The unpleasantness of pain in humans has been shown to be mediated by a region in the frontal cortex known as the anterior cingulate cortex (ACC; Rainville et al., 1997; Tölle et al., 1999). The ACC also

appears to be involved in the affective component of pain in rats: studies using place conditioning have shown that rats developed conditioned place aversion for locations where they received a noxious stimulus (indicating a negative association with these locations; e.g., Lei et al., 2004) and that destruction of neurons originating from the ACC reduced this place aversion (Gao et al., 2004; Johansen et al., 2001).

Using the drug self-selection method, Colpaert et al. (1982, 1980) showed that arthritic rats consumed more water containing an analgesic (a nonsteroidal anti-inflammatory, i.e. NSAID in the first study, and an opioid in the second study) versus sweetened water than did healthy controls, and that the amount of drugged water consumed mirrored the inflammatory process as assessed by the diameter of paws and joints. They further confirmed that pain, and not the rewarding or addictive action of the drugs, was the stimulus for self-selection (Colpaert et al., 2001). Similarly, Mickley et al. (2006) showed that rats who had undergone surgery drank more water containing an analgesic than did control rats, and that animals who had experienced the surgery drank enough medicated water to raise pain thresholds on a hot-plate test. This suggests that arthritic rats learn to choose water from one bottle over another, and that this choice is likely driven by the relief from pain afforded by the medicated water.

In humans, chronic pain is associated with a wide range of conditions that affect quality of life, such as anxiety, depression, appetite suppression, attentional deficits and sleep disruption. All of these conditions have also been recorded in rats subjected to pain (see Mogil, 2009).

2.5. Implications and conclusion

Current methods of assessing emotions in animals provide compelling evidence that rats indeed experience a range of positive and negative emotions, although we may never know whether rats and other animals experience emotions consciously. I conclude that the 'likes' and

‘wants’ of rats should be taken into account when deciding how to house and care for them. Existing standards in many laboratories, where rats are housed singly without access to a shelter and without opportunities to play or socialize with conspecifics, are likely to profoundly affect their welfare.

3. The Importance of Burrowing, Climbing and Standing Upright for Laboratory Rats[2]

3.1. Introduction

The breeding of rats (*Rattus norvegicus*) for the purpose of experimentation began in the 1840s in Europe, making rats the first mammalian species to be domesticated primarily for scientific purposes (Richter, 1959). The foundation for laboratory rat husbandry was laid down by researchers at The Wistar Institute of Anatomy and Biology in Philadelphia who, beginning in 1906, conducted research into "the means of making [rats] contented and happy" (Greenman & Duhring, 1923, p. 3) to enable them to develop appropriate housing and ancillary equipment (Lindsey, 1979). As a result of their research, the cages designed by The Wistar Institute contained, among other things, a substrate that allowed rats to burrow, and a large, 53-cm diameter running wheel (Greenman and Duhring, 1931, 1923).

The Wistar Institute's early cage was chiefly designed with rats' welfare in mind, but other models prioritized low costs and ease of cleaning (Greenman and Duhring, 1931; Scharmann, 1991). Today's standard laboratory cages offer rats little opportunity to perform many behaviours that are part of their repertoire in the wild, such as burrowing and climbing. Standard cages also prevent rats from standing upright: current regulations in the European Union (EP and Council of the European Union, 2010), the United States (NRC, 2011) and Canada (CCAC, 1993) mandate a minimum cage height of 18-20 cm, but rats stand at a height of about 22 cm by 2.5 months of age and 26-30 cm by the time they are fully grown (Büttner, 1993; Pullen, 1976).

In the wild, rats construct and live inside burrows that they expand and modify frequently (Calhoun, 1963; Pisano and Storer, 1948). Rats are also adept climbers and use this behaviour to

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escape from predators or to forage (Barnett, 1975; Huck and Price, 1976). Norway rats have been observed to climb up trees, thicket and dry stalks to forage for berries and grain (Hill et al., 1983; Pisano and Storer, 1948). They stand upright as they explore and socialize with other rats (Grant and Mackintosh, 1963).

Despite more than 150 years of captive breeding, laboratory rats who are placed in a more naturalistic environment still perform these and other behaviours from their wild ancestors' repertoire (Boice, 1977; Modlińska et al., 2015; Peplow, 2004). Domestication does not seem to have eliminated any behaviours, although in some cases it may have altered the quality and thresholds needed to initiate them (Price, 1999; Timberlake and Silva, 1995). For example, when given the opportunity, laboratory rats readily burrow and climb, but burrows tend to be less complex (Price, 1977; Stryjek et al., 2012) and climbing bouts are shorter and less frequent (Huck and Price, 1976) in domesticated laboratory rats versus wild Norway rats.

Although it is known that laboratory rats readily engage in burrowing, climbing and upright standing, there is little information regarding how important these behaviours are to rats. Some information could be gained by investigating rats' propensity to perform these behaviours and how these change over the course of the animals' development. Rats, like many animals including humans, spend less time in ambulatory activity and exploration and more time resting as they age (e.g., Casadesus, Shukitt-Hale, & Joseph, 2001; Goettl, Wemlinger, Colvin, Neff, & Hadjiconstantinou, 2001; Martin, Fuchs, Bender, & Harting, 1986; Soffié, Buhot, & Poucet, 1992; Spangler et al., 1994). Arguably, more weakly motivated activities will be traded for rest as animals age, while strongly motivated activities will continue to be performed. However, a special case should be made for activities that require a high degree of physical aptitude: aging is associated with loss of muscle strength, coordination and balance, so the performance of more physically challenging activities may decline because of physical inability rather than low motivation.

Animals must decide how much time to allocate to different behaviours, and if the total daily active time (i.e. 'total income') decreases, then the 'cost' of performing any individual behaviour increases (Dawkins, 1988). According to this perspective, behaviours that are important to an individual will continue to be performed even if the cost is high; such behaviours are said to show 'inelastic demand' (Dawkins, 1988, 1983; McFarland and Houston, 1981).

To my knowledge, no study has investigated the frequency, duration or distribution across time of burrowing behaviour in wild or domesticated rats. With respect to climbing, one study reported that male laboratory rats aged 7-8 months climbed an average of 0.2 times and for 0.7 s, and females climbed 1.1 times and for 27.4 s, when placed into an unfamiliar enclosure for 15 min during the light phase of the light-dark cycle (Huck and Price, 1976). The propensity to climb likely differs in a novel versus home environment, and in the light versus the dark phase, so drawing any conclusions about the importance of this behaviour in rats' daily life based on these results is difficult. Finally, two studies investigated upright standing in the rat. The first recorded the amount of time 6-month old rats spent in upright standing over the course of five days, and found that rats spent on average 5-14% of daily active time standing taller than 22 cm, and 3-6% time standing taller than 27 cm (Büttner, 1993). No information was given on the frequency or temporal distribution of upright standing. The second tested the proportion of time large males spent in cages that were 16.8 cm versus 23 cm high, and found no preference for one cage over the other (Galef and Durlach, 1993). However, even a height of 23 cm would not allow a large rat to stand fully upright, so this study tested rats' preference for increased vertical space rather than the ability to stand upright. Secondly, if individual bouts of upright standing were brief, then even if rats used this cage frequently to stand (but for short periods of time), this may not have necessarily translated into more frequent use. Indeed, in a barren cage, rats may prefer the lower cage for resting and the taller cage for exploring and stretching.

The first aim of this study was to describe the daily frequency, duration and distribution throughout the day of burrowing (excavation of burrows), climbing and upright standing in laboratory rats reared in semi-naturalistic cages. This was done at three different ages (3, 8 and 13 months old) to capture developmental changes as rats age. Previous work has shown that at 3 months of age rats are at their most active (Hitchcock, 1925; Richter, 1922; Slonaker, 1907), and that by 8 months of age they have become socially mature, a stage associated with changes in behaviour (Adams and Boice, 1983).

The second aim of this study was to record the frequency of lateral stretching in laboratory rats reared in standard versus semi-naturalistic cages. Stretching – formally referred to as pandiculation – occurs in similar form and context across a wide range of species (Baenninger, 1997; Fraser, 1989). Stretching seems to be a corrective response to stiffness or positional stress (Bertolucci, 2011; Fraser, 1989). Rats reared in standard laboratory cages have less freedom of movement and are less active than rats housed in larger and more complex cages (Spangenberg et al., 2011), and are unable to stand upright. I hypothesized that if the restrictions imposed by standard caging resulted in physical stiffness and positional stress, then standard-housed rats would stretch more frequently than rats housed in a more behaviourally permissive environment.

3.2. Materials and methods

3.2.1. Animals and housing

Forty-two, 22- to 23-day-old female Sprague-Dawley rats were purchased from Charles River Laboratories Canada. As soon as they arrived, they were systematically assigned to either semi-naturalistic cages (6 cages each housing five rats) or standard cages (6 cages each housing two rats). In assigning rats to housing treatment, I alternated between semi-naturalistic and standard

cages, and within each cage alternated between rats huddled at the back of the shipping box and those who reared at the front.

All cages were in one room. Rats were housed under reversed lighting, with lights off from 11:00 – 23:00 h. Mean (± SD) room temperature and humidity were 23.9 ± 0°C and 44.5 ± 10.6% during data collection at 3 months of age; 24.0 ± 0°C and 20 ± 0% at 8 months of age; and 21.6 ± 0°C and 66.5 ± 2.1% at 13 months of age.

Semi-naturalistic cages (Fig. 3.1; Critter Nation™ double unit with stand, MidWest Homes for Pets, Muncie, IN, USA) were made of horizontal galvanized wire bars to enable climbing, and measured 91 x 64 x 125 cm (L x W x H) The lower portion of each cage was lined with Plexiglas so that the bottom 30 cm of the cage could be filled with a mixture of black earth, compost, and sphagnum peat moss (3-in-1 Landscape Soil, Premier LiteWay, Rivière-du-Loup, QC, Canada). This soil substrate was watered every few days to prevent it from drying out and causing burrows to collapse (Boice, 1977). Burrow construction and maintenance caused soil to fall outside of the cage, so fresh soil was added as needed to maintain levels. Rats had *ad libitum* access to rat chow (LabDiet® 5012, PMI® Nutrition International, LLC, Brentwood, MO, USA) and tap water, but their diet was supplemented several times per week with unsweetened cereal, nuts or seeds.

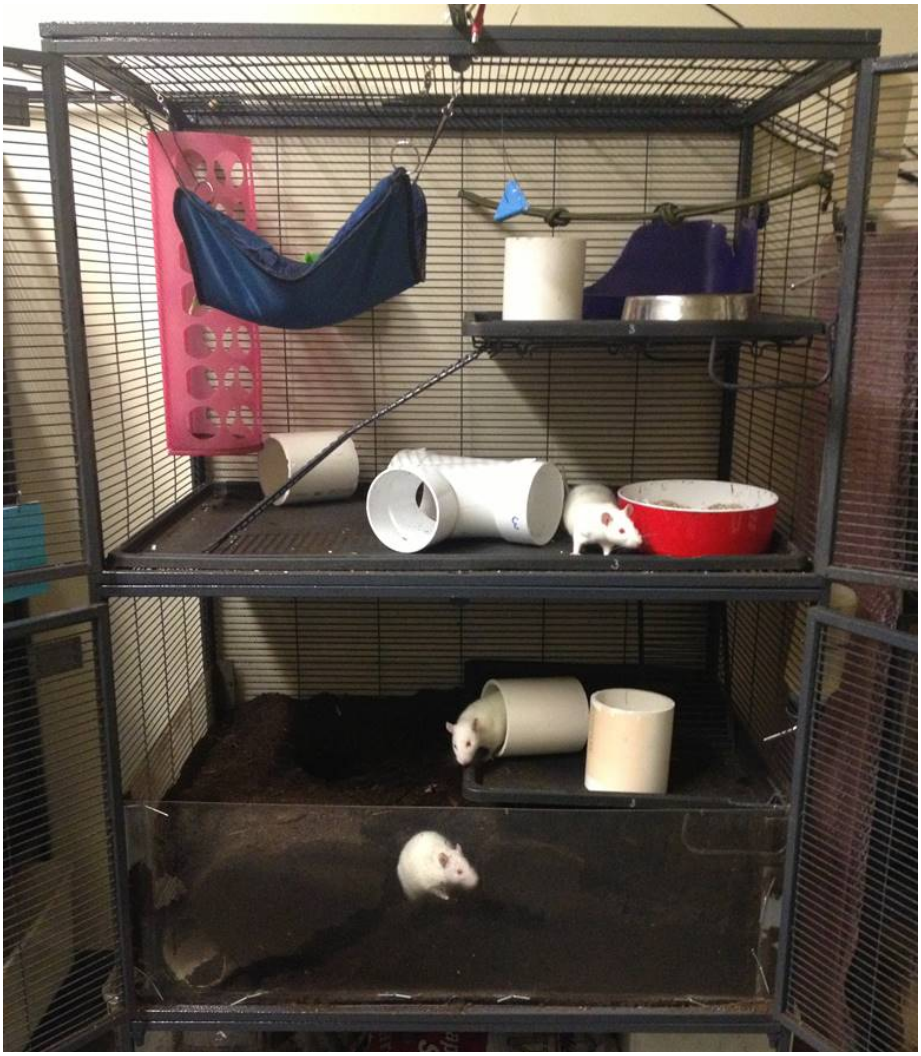

Figure 3.1. Photograph of a semi-naturalistic cage. Cages were split into four levels connected by ramps. Each cage was furnished with litter boxes, several PVC pipes, a climbing structure, a hammock, and a horizontal rope across the top floor. The bottom level was filled with soil substrate.

Standard cages were made of polycarbonate and measured 45 x 24 x 20 cm (L x W x H). Each cage contained aspen chip bedding (Northeastern Products Corp., Warrensburg, NY, USA), one PVC pipe measuring approximately 18 x 10 cm (L x diameter), and two pieces of brown paper towel. Rats had *ad libitum* access to rat chow and tap water.

3.2.2. Data collection

Cages were filmed continuously with infrared security monitoring cameras (Swann SWDVK-162608; resolution: 480 TVL) mounted to face each cage. Each age period was defined as lasting 2 weeks from the day rats turned the target age; for example, the period '3 months' was defined to be when rats were 3 to 3.5 months old. At each age period, I identified a subset of days when there were minimal husbandry procedures (e.g., no cage cleaning) or other disturbances (e.g., no adding soil). From each subset, I randomly selected 2 days for analysis; half the cages at each age period were scored on each of these two randomly selected days. At 3 months of age, only four cages were scored instead of six because of missing video files. Lateral stretching in standard cages was scored only at 13 months of age.

Within each age period, I randomly selected one semi-naturalistic cage to be scored continuously for the full 24-h period. I then sampled the resulting 24-h data at different intervals and durations to determine a sampling method that predicted the actual frequency and duration of each behaviour with >80% accuracy in at least two of the three cages sampled, and used this sampling method to score the remaining cages (see Results below). I could not assume that the frequency and temporal distribution of lateral stretching would be the same in the standard cages (indeed, I hypothesized that it would be different) so I also scored three randomly-selected standard cages for a full 24-h period to determine a sampling method that would predict the frequency of lateral stretching in standard cages with the same accuracy criteria as used for the semi-naturalistic cages (i.e. >80% accuracy in at least two of the three cages), and used this sampling method to score the remaining three standard cages.

I recorded the start and end time of each occurrence of the target behaviours (Table 3.1; Altmann, 1974). Rats were unmarked and could not be identified as individuals; therefore, frequencies and durations were scored collectively for the whole cage, and this total was divided by

the number of rats in the cage to obtain mean values per rat. All semi-naturalistic cages housed five rats at 3 months of age, but by 8 months, two rats had been removed from the study for health reasons, so two semi-naturalistic cages housed four rats instead of five. By 13 months of age, one standard-housed rat was removed from the study for health reasons, so one cage housed one rat and the others housed two rats each.

Table 3.1. Behaviours scored, and their definitions

Behaviour	Definition
Burrowing	Rat is displacing soil using fore legs and/or kicking out with the hind legs
Climbing	Rat is suspended with all paws in contact with a vertical surface or the cage ceiling
Upright standing	Rat is upright; hind legs are fully extended and fore paws are either unsupported (rare) or resting on a vertical surface (common); back is either straight or slightly arched
Lateral stretching	Rat is parallel to the ground with the body elongated and back slightly arched; head and tail are angled upwards; hind legs and sometimes one fore leg are outstretched; rat is often yawning

All four behaviours were scored in semi-naturalistic cages; lateral stretching was also scored in standard cages (the other three behaviours were not possible in the standard cage).

Burrowing frequently occurs in bouts, with rats repeatedly digging their way into the burrow (and out of view) and reappearing some moments later pushing soil out with the fore paws; once at the surface, they quickly turn around and repeat the sequence (Pisano & Storer, 1948; personal observations of rats working on tunnels formed along the Plexiglas wall). Therefore, scoring each time the rat was burrowing at the soil surface as a separate event overestimated the frequency and underestimated the duration of burrowing. For a more accurate estimate of the frequency and duration of burrowing, rats were scored as engaged in one burrowing event if they burrowed their way into a burrow *and* were still burrowing when they re-emerged (pushing soil out

of the burrow with their fore paws). On rare occasions, rats burrowing their way into a tunnel did not emerge for several minutes. In these rare cases, the burrowing event was considered finished if a rat failed to re-emerge within 4 min. This criterion was based on observations of rats who could be seen burrowing inside tunnels built along the Plexiglas wall at the front of the cage.

Two experienced observers scored behaviours in the semi-naturalistic cages, each scoring half of the cages at each age period. To determine inter-observer reliability for burrowing, both observers scored a set of 12 randomly selected 2-h clips; for climbing, upright standing and lateral stretching in the semi-naturalistic cages, both observers scored a set of 30 randomly selected 5-min clips (see sub-section on sampling method in the results section for rationale). Only one observer scored lateral stretching in the standard cages, so reliability was not tested for this behaviour in this housing system.

3.2.3. Statistical analysis

To determine inter-observer reliability, a Pearson correlation coefficient (SAS v.9.4) was calculated on the 12 (burrowing) or 30 (climbing, upright standing, lateral stretching in the semi-naturalistic cage) pairs of data. After performing visual inspection of residuals to verify normality and homogeneity of variances, the effect of age on the frequency and duration of each behaviour was calculated using a mixed model that included age as a repeated measure. The frequency of lateral stretching between semi-naturalistic and standard cages at 13 months of age was compared using an independent samples t-test with the Satterthwaite variance estimator method for unequal variances.

3.3. Results

3.3.1. Sampling method

The sampling method found to work well for burrowing was a rate of 66% and consisted of scoring continuously for 2 h, every 3 h during the 24-h period. The sampling method that worked well for all other behaviours in the semi-naturalistic cages was a rate of 33% and consisted of scoring continuously for 5 min, every 15 min during the 24-h period. Accuracies (estimated with the sampling method relative to what was measured in the full 24-h period) provided by this sampling method are presented in Table 3.2.

Table 3.2. Accuracies[1] (%) of the sampling methods used in the semi-naturalistic cages

	Frequency			Duration		
	Age (months)			Age (months)		
Behaviour	**3**	**8**	**13**	**3**	**8**	**13**
Burrowing	116	110	95	105	113	119
Climbing	102	97	104	107	117	117
Upright standing	97	100	107	92	111	112
Lateral stretching	88	70	88	84	64	88

[1]Values represent the estimated frequency and duration relative to the full 24-h sample; accuracy = estimated value / sampled value x 100.

The sampling method that suited lateral stretching in the standard cages was a rate of 75% and consisted of scoring continuously for 3 h, every 4 h during the 24-h period (Table 3.3).

Table 3.3. Accuracies[1] (%) of the sampling methods used in the standard cages

	Frequency			Duration		
	Cage			Cage		
Behaviour	**1**	**2**	**3**	**1**	**2**	**3**
Lateral stretching	95	108	99	99	109	105

[1]Values represent the estimated frequency and duration relative to the full 24-h sample; accuracy = estimated value / sampled value x 100.

3.3.2. Inter-observer reliability

Pearson correlation coefficients were 0.98 for the frequency and 0.99 for the duration of burrowing; 0.96 for the frequency and 0.90 for the duration of climbing; 0.99 for the frequency and 0.97 for the duration of upright standing; and 0.81 for the frequency of lateral stretching in the semi-naturalistic cages. While both observers recorded a very similar frequency of upright standing in each 5-min sample, when specific occurrences recorded by both observers were compared, each observer missed approximately 15% of the observations from the combined total. This means that while the frequencies provided by each observer did not differ, both observers tended to underestimate the total number of events of standing upright by at least 15%. Similarly, the frequency of climbing was underestimated by 10-15%.

3.3.3. Main study

Burrowing, climbing, upright standing and lateral stretching were performed in every cage and at every age (Fig. 3.2). The frequency ($F_{2,8}$=0.41, p=0.6799) and duration ($F_{2,8}$=0.63, p=0.5563) of burrowing did not vary with age. Climbing and upright standing declined with age in both frequency

(climbing: $F_{2,8}$=30.49, p=0.0002; upright standing: $F_{2,8}$=20.52, p=0.0007) and duration (climbing: $F_{2,8}$=24.98, p=0.0004; upright standing: $F_{2,8}$=6.30, p=0.0228). All behaviours were expressed consistently throughout the 24-h period, but at much higher frequencies during the dark phase (Fig. 3.3).

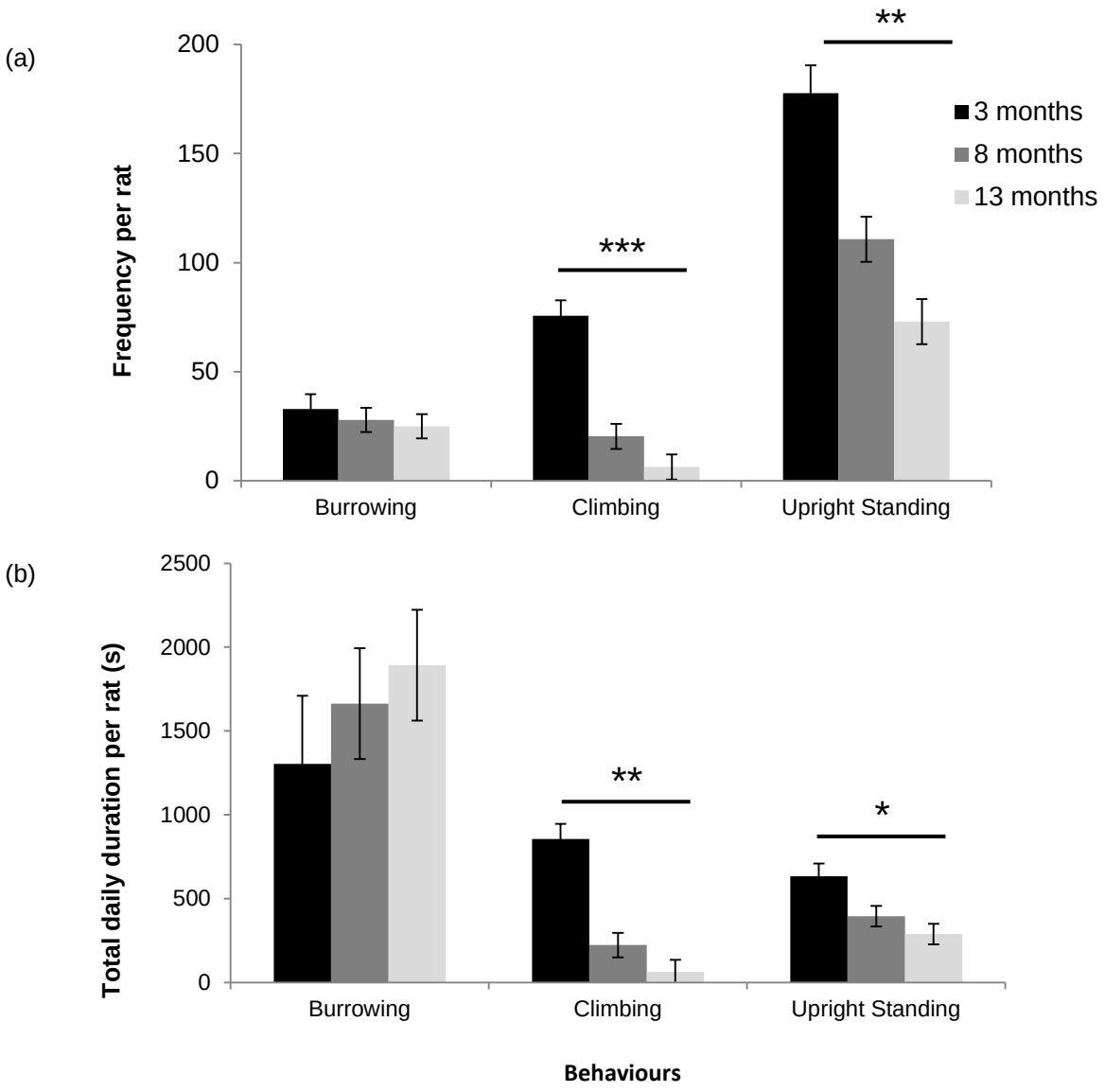

Figure 3.2. LS mean ± SEM frequency (a) and duration (b) of burrowing, climbing and upright standing per day per rat at 3, 8 and 13 months of age in semi-naturalistic cages. Data are based on four cages housing five rats at 3 months; and six cages housing five (n=4) or four (n=2) rats at 8 and 13 months.

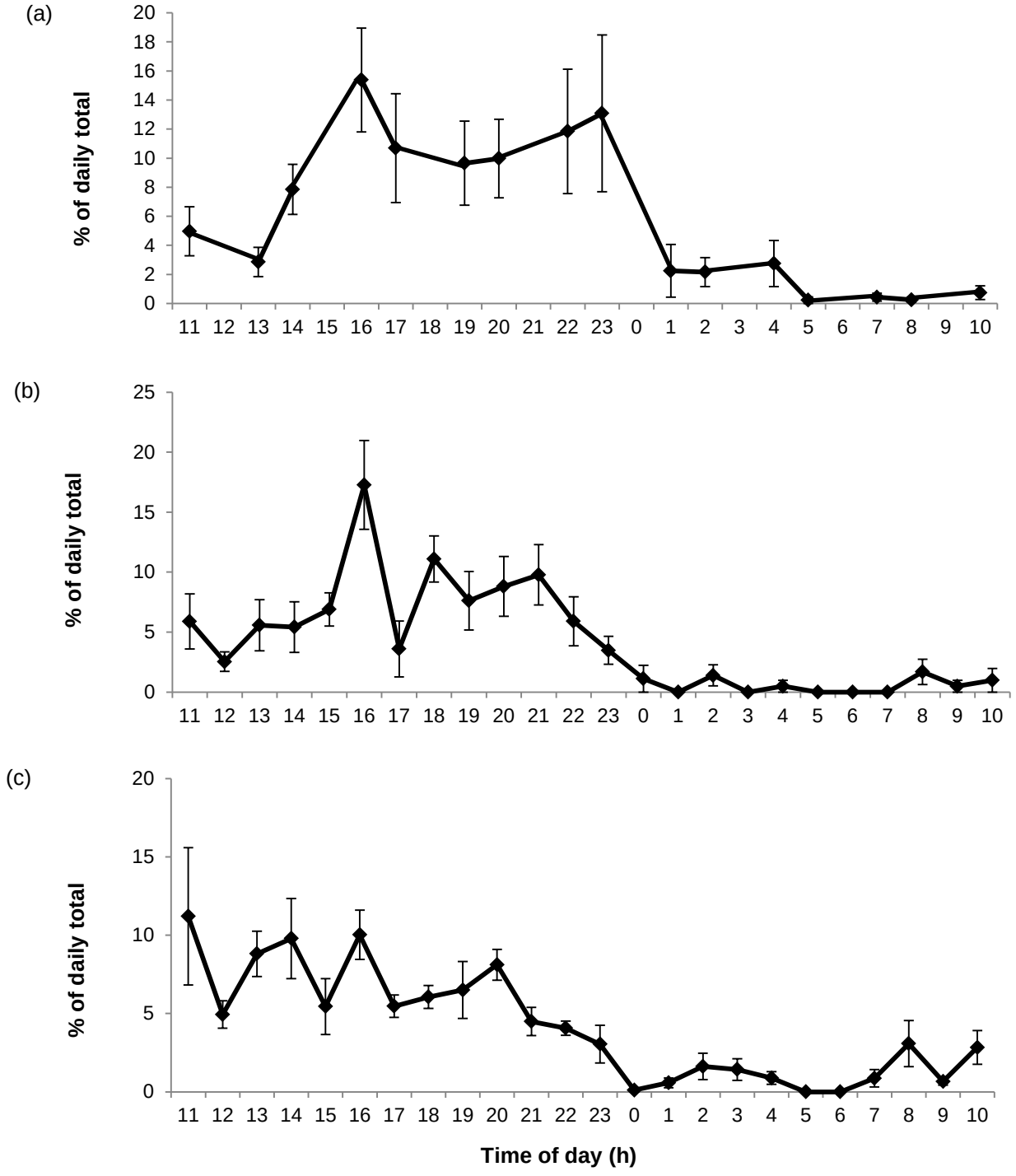

Figure 3.3. Representative sample illustrating the distribution of burrowing (a), climbing (b) and upright standing (c) throughout the day at 8 months of age. The dark period was from 11 h to 23 h. Data represent mean ± SE based on values obtained from six cages housing five (n=4) or four (n=2) rats.

The duration of burrowing bouts varied from approximately 1 s to 13 min; approximately 25% of bouts lasted longer than 1 min (Table 3.4). Approximately 20-30% of climbing bouts lasted 1 to 2 s, with maximum bout duration of 3 min in 3-month old rats, declining to 40 s in 13-month old rats. The majority of bouts of upright standing were brief, with approximately 70% of bouts lasting just 1 to 3 s.

Table 3.4. Range and median duration (s) of all bouts of burrowing, climbing and upright standing

	3 months old		8 months old		13 months old	
Behaviour	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Burrowing	1-435	16	1-693	26	1-967	27
Climbing	1-166	5	1-97	5	1-39	6
Upright standing	1-127	2	1-137	2	1-32	2

Rats burrowed even though several tunnels were already present, and the conformation of tunnel entrances as seen from the soil surface changed on an almost-daily basis. Frequently, rats who were engaged in other activities in the upper levels of the cage would suddenly run down to the soil to begin burrowing, often bounding in and out of the burrow during and after a burrowing bout.

Many climbing events seemed to occur as a means of moving from one location to another. In these instances, rats chose to climb rather than to take a longer route via ramps. Other climbing bouts seemed to serve an exploratory function: rats would often start out rearing, then would jump up and climb the cage wall as high as it allowed; after some seconds in this suspended position, they would climb down near to where they started. The observers noted a few instances of rats using the cage ceiling as ‘monkey bars’, i.e. swinging from the ceiling by their fore limbs.

Most occurrences of upright standing seemed to serve an exploratory function: rats’ heads were angled upwards with indications that they were sniffing (e.g., slight up-down head

movements). Rats also appeared to stand upright to stretch; on these occasions, the back was arched, the fore limbs were at a >90° with regard to the body (as opposed to the fore limbs being at an angle approximating 90° during other occurrences of standing upright) and rats usually threw their head back and yawned. When scoring upright standing, observers noted if the event appeared to serve the purpose of exploration or stretching. However, the reliability of differentiating these two types of upright standing was poor so I do not report the results. However, 'exploratory' bouts of upright standing appeared to be 5-10 times more frequent than upright stretching.

Rats housed in the semi-naturalistic environment performed lateral stretching (LS means ± SEM) 9.2 ± 3.18 times a day at 3 months; 13.0 ± 2.59 times a day at 8 months; and 6.4 ± 2.59 times a day at 13 months. This difference was not statistically significant ($F_{2,8}$=1.65, p=0.2513). Duration of lateral stretching also did not vary with age ($F_{2,8}$=1.82, p=0.2225; 14.50 ± 5.42 s per day at 3 months; 23.50 ± 4.42 s at 8 months; and 12.00 ± 4.42 s at 13 months). Thirteen-month old standard-housed rats performed lateral stretching much more frequently (mean ± SE = 52.8 ± 10.02 times per day) than age-matched rats housed in the semi-naturalistic condition ($t_{5.3128}$=-4.56, p=0.0052; these are corrected Satterthwaite degrees of freedom).

3.4. Discussion

Rats' propensity to burrow remained constant throughout this study at an average frequency of 30 times per day for a total of 20-30 min per day. That rats maintained stable burrowing levels, despite becoming progressively less active, may indicate that rats' demand for burrowing is inelastic (Dawkins, 1988), suggesting that burrowing is particularly important to rats. Burrowing leads to the formation of a burrow, which is crucial for rat survival in the wild. Burrows offer shelter from predators, from light, and from the elements, and rats use burrows extensively for sleeping, eating and storing food, and raising their young (Calhoun, 1963; Pisano and Storer,

1948). Burrows can also be advantageous in a laboratory (Patterson-Kane, 2003). Retreating into a burrow allows rats to withdraw from perceived threats, such as unfamiliar humans or loud noises (Blanchard and Blanchard, 1990); to shelter them from light, which is aversive to rats and leads to retinal atrophy and blindness at levels commonly used in laboratories (Schlingmann et al., 1993a, 1993b); and to regulate ambient temperature, which in most laboratories is likely below rats' thermoneutral zone (Poole and Stephenson, 1977).

This study did not examine whether it is burrowing *per se* that is important to rats, or the functional consequences of burrowing (i.e. having a burrow). Laboratory gerbils, who are prone to developing stereotypic digging, dig much less if an adequate artificial burrow is provided (Wiedenmayer, 1997). In my study, cages were furnished with artificial shelters in the form of PVC pipes, but there is evidence that these open-ended pipes are not regarded as satisfactory shelters (Bradshaw and Poling, 1991; Chmiel and Noonan, 1996); therefore, burrowing in the presence of these shelters may not be evidence that burrowing *per se* is important. In laboratory mice, burrowing appears to be important regardless of its functional consequences. In one study, mice continued to work to gain access to burrowing substrate despite increasing cost, and burrowed equally whether the burrows they previously built were left intact or destroyed (Sherwin et al., 2004). As noted earlier, rats in this study also constructed new tunnels even when several others were already present, suggesting that burrowing *per se* may also be important to rats. In addition, some rats' demeanour as they burrowed – running towards the soil, bounding in and out of the soil as they burrowed, and bounding away after a bout – suggests that engaging in this activity was reinforcing.

Working towards the goal of achieving or maintaining safety (e.g., by building and maintaining a burrow) may itself be rewarding, independently of having safety. Work in human psychology has shown that central to our sense of well-being is how successful and unsuccessful we

are in our pursuit of approach and avoidance goals (Strauman et al., 2015). Approach goals/motivations can be divided into two types: *promotion* motivation, which aims to attain gains (e.g., securing rewards), and *prevention* motivation, which aims to attain nonlosses (e.g., securing and maintaining safety; Higgins, 1997). Individuals can be high on one or both of these motivations, and the strength of these motivations is stable across time (Higgins et al., 1997). Recent work by Franks and colleagues (2013; 2012; 2014) has shown that these principles also apply to rats and cotton-top tamarins. For example, rats were given the opportunity to actively maintain darkness, and to contain a manageable threat. Those rats who performed these two tasks the most frequently (i.e. those who showed strongest prevention motivation) were also those who had lowest indicators of chronic stress (Franks et al., 2014). In a broader sense, there are additional benefits to *taking action* and succeeding in achieving and maintaining safety. According to this view, the provision of an adequate burrow may help prevent negative affective states caused by exposure in the open, but the building and maintaining of the burrow – actively working towards safety – may be enjoyable in itself, and therefore provide opportunities for positive affect.

Climbing decreased steadily with age. Three-month old rats climbed, on average, 75 times per day for a cumulative duration of about 15 min, compared to 6 times per day for a total of about 1 min for 13-month old rats. Maximum climbing bout duration declined from the order of minutes at 3 months of age, down to the order of seconds at 13 months of age. Climbing may have declined in part because of rats' tendency to explore less as they age (Goodrick, 1971; Soffié et al., 1992; Willig et al., 1987), but I speculate that declining physical ability played a larger role. Climbing requires muscle strength and coordination, both of which deteriorate with age (Goettl et al., 2001). Indeed, one study investigated rats' performance while climbing down a wire mesh pole as a function of age. The authors found distinct differences between rats from the various age groups. Younger rats held onto the pole cautiously and climbed down gradually, sometimes turning around

to return to the top and repeating their descent. In contrast, older rats often slid down the pole or even fell, never making their way down in a coordinated, systematic manner, and never climbing back up (Wallace et al., 1980).

Young rats climbed frequently and consistently during the dark period. While climbing *per se* may not be a highly motivated behaviour, its performance does add to the limited behavioural repertoire of a captive rat and as such may be beneficial to rat well-being. Because climbing behaviour decreases as rats age, the ability to perform climbing may be more important to young rats than it is to older rats. In addition, Huck and Price (1976) have shown that both wild and domestic female rats climb more than males, so the opportunity to climb may be more important to females.

Standing upright was by far the most commonly expressed behaviour of the four measured here, with average frequencies of 180 times per day in the 3-month old rats, declining to 75 times per day at 13 months. The total daily duration of standing upright averaged about 10 min per day in young rats, with the large majority of events lasting 1 to 3 s. It is worth noting that by our definition, standing upright was only scored when a rat's back was completely straight (or slightly arched due to over extension) and hind limbs extended. Rats frequently stood at a height that was taller than the 18-20 cm allowed by a standard cage, but with their backs minimally curved or hind limbs not fully extended, so these occurrences were not recorded because they did not meet my criteria for upright standing.

As with climbing, the frequency and duration of upright standing decreased as rats aged. Because most occurrences of upright standing were likely exploratory, this behaviour may have decreased as a function of lower exploratory behaviour associated with aging (Goodrick, 1971; Soffié et al., 1992; Willig et al., 1987). Standing upright does not require particular physical prowess, so declining physical fitness is less likely to have been an important cause for lower expression.

Upright standing was widely expressed even in older rats. It has been suggested that an animals' species-specific forms of kinesis (including stretching and straightening of the back and extending of the limbs) is one of eight systems of behaviour forming the broad basis of animal health and behavioural needs (Fraser, 1988). According to this view, rats' ability to stand upright is an inherent component of their welfare. According to public opinion, housing animals in enclosures that restrict freedom of movement and ability to fully extend limbs is unacceptable (Benard and de Cock Buning, 2013; Boogaard et al., 2011; Vanhonacker et al., 2008). Mounting public opposition has led to regulatory changes in the way many farm animals are housed. The European Union has now banned battery cages for chickens, and several US states (Arizona, California, Colorado, Maine, Michigan, and Oregon) have enacted legislation prohibiting the housing of animals without the ability to stand up or extend their limbs without touching the sides of their enclosure (National Agricultural Law Center, 2015). These results suggest that public opinion would also be against the use of caging for rats that prevents upright standing, but future research could specifically address public attitudes regarding this practice.

Stretching is a peri-somnolent phenomenon (occurring before or after sleep) but is also expressed in response to stiffness caused by extended periods of immobility, positional stress, and sub-optimal movements (Bertolucci, 2011; Fraser, 1989). Rats housed in the semi-naturalistic cages maintained a consistent daily frequency and duration of lateral stretching as they became older, suggesting that there may be a stable, optimal level of stretching in freely moving rats in this housing system, and that lower activity levels when rats were older (e.g., lower rates of climbing and standing upright) were nonetheless not low enough to cause positional stress or stiffness that would have required compensation through increased stretching.

Lateral stretching in standard cages was only scored in 13-month old rats, partly because video scoring was extremely time-consuming, and partly because evidence from semi-naturalistic-

housed rats suggested that levels of stretching were relatively stable across time. Thirteen-month old standard-housed rats stretched approximately eight times more often than 13-month old rats housed in the semi-naturalistic cages, at a mean frequency of 53 versus six times per day. Rats in the semi-naturalistic cages may have also stretched inside their burrows and out of view. Our observations suggest that semi-naturalistic-housed rats also stretched in the upright position (see discussion above on upright standing), and this upright stretching may have contributed to lower rates of lateral stretching. The fact that semi-naturalistic-housed rats stretched in an upright position when both lateral and upright stretching were possible indicates that there may be advantages to upright stretching. The much higher frequency of lateral stretching in standard-housed rats suggests that standard-housed rats were using this behaviour to compensate for their inability to stand upright and perhaps also their generally reduced levels of mobility.

3.5. Conclusion

Laboratory rats reared in an environment that allowed them to burrow, climb and stand upright performed these behaviours regularly throughout the day and well into adulthood. Burrowing and upright standing appeared to be especially important to rats given the frequency and consistency with which these behaviours were performed. Rats housed in standard laboratory cages were unable to perform these behaviours. Perhaps in compensation for the inability to stand upright, the standard-housed rats engaged in more lateral stretches. This stretching might also be a corrective response to stiffness and positional stress associated with restricted movements in standard cages. These findings suggest that current standard laboratory cages interfere with important natural behaviours, and this likely compromises rat welfare. Providing rats with burrowing substrate and increasing cage height are recommended.

4. Differences in Anticipatory Behaviour between Standard- and Semi-Naturalistic-Housed Rats[3]

4.1. Introduction

In the wild, Norway rats engage in a host of behaviours, from foraging and building burrows to traveling several kilometers per day patrolling their territory (Barnett, 1975; Davis et al., 1948). In a laboratory, the descendants of the Norway rat are typically kept in relatively small cages with few stimuli to explore and few opportunities to perform behaviours other than sleeping, eating and drinking. Research has shown that laboratory rats prefer larger and more complex environments (Bradshaw and Poling, 1991; Collier and Hirsch, 1971; Denny, 1975; Iversen, 1998; Patterson-Kane et al., 2002, 2001), and when they are placed in a semi-natural environment, they display a behavioural repertoire similar to that of their wild relatives (Boice, 1981; Peplow, 2004; Stryjek et al., 2012).

Indeed, in Chapter 3 I have shown that laboratory rats are motivated to burrow, climb and stand upright. I have also shown that that the inability to stand upright, coupled with generally low levels of activity, likely results in stiffness or positional stress. Here, I explore the affective consequences of housing rats in this more restrictive, standard laboratory environment.

The physiological (Fox et al., 2006), neurological (van Praag et al., 2000) and health (Katsnelson, 2010; Nithianantharajah and Hannan, 2006) effects of current laboratory housing standards are well established (see Chapter 1), but fewer studies have addressed the sustained emotional impact of a standard cage environment. An animal's emotional well-being is central to its welfare (Fraser et al., 1997) and new advances in animal welfare science have given rise to a variety

[3] A version of this chapter has been published: Makowska, I.J., Weary, D.M. 2016. Differences in anticipatory behaviour between rats (*Rattus norvegicus*) housed in standard versus semi-naturalistic laboratory environments. PLoS ONE 11: e0147595.

of methods of studying affective states in animals (Makowska and Weary, 2013). Some studies have assessed the affective consequences of housing rats in an 'enriched' environment (what this involves varies widely across studies), but the control group is often housed in conditions that can be considered worse (Abou-Ismail and Mahboub, 2011; Alexander et al., 1978; Harding et al., 2004) or better (Abou-Ismail et al., 2010; van der Harst et al., 2003a) than the common Canadian standard system that houses two rats with a piece of PVC pipe. Moreover, few studies have evaluated females, and fewer still have evaluated animals reared in standard versus enriched systems for longer than a few weeks.

As reviewed in Chapter 2, one method of assessing affective states in animals consists of looking at their anticipatory behaviour – that is, the behaviour exhibited in the interval between a signal of the impending arrival of a reward and the arrival of that reward. Research has suggested that the level of anticipatory behaviour, usually measured as total behavioural frequency, displayed by an animal is influenced by the animal's underlying affective state, suggesting that differences in anticipatory behaviour can be used to make inferences about animal welfare (Van der Harst and Spruijt, 2007; Watters, 2014). It has been shown that 'impoverished' animals exhibit a stronger anticipatory response than 'normal' animals, and that severely depressed (i.e. anhedonic) animals fail to show an anticipatory response altogether. For example, male rats housed in standard laboratory cages exhibited more anticipation before access to a sucrose solution than rats housed in enriched cages (van der Harst et al., 2003a). Male rats subjected to a long-term severe stressor (social defeat followed by months of isolation) did not display an anticipatory response before access to sucrose (Von Frijtag et al., 2000), but treatment with an antidepressant restored the anticipatory response (Von Frijtag et al., 2002). Thus there appears to be a curvilinear relationship between affective state and anticipatory behaviour: poor welfare is associated with increased anticipation, but in extreme cases, anhedonia may reverse the more typical relationship. When two

groups of animals differ in their level of anticipation, testing after administration of a mood-enhancing drug (e.g. antidepressant) offers a way to explore the nature of the affective state experienced by each group.

It is well documented in humans and other animals that exposure to stressors affects sensitivity to rewards at a behavioural and neurophysiological level (Cabib and Puglisi-Allegra, 1996; Goeders, 2002; Piazza et al., 1990). Individuals deprived of essential stimuli are more sensitive not only to the particular stimuli they are deprived of, but to all rewarding and aversive stimuli (Ahmed et al., 1995; Van den Berg et al., 1999). Spruijt et al. (2001) proposed that differences in the level of anticipation reflect differences in reward sensitivity, which in turn are related to an animal's subjective evaluation of his or her internal state and environment (Van der Harst and Spruijt, 2007). Reward sensitivity is mediated by the opioid and dopaminergic systems, and the display of anticipatory behaviour is the result of the release of endorphins and dopamine. In general, the release of endorphins or dopamine causes increased locomotor activity, and this change in behaviour can facilitate the finding of resources (Pijnenburg et al., 1976; Spruijt et al., 2001). Indeed, Spruijt et al. (2001) argue that the 'characteristic' behavioural pattern exhibited in anticipation of a reward resembles the behavioural pattern induced by the injection of a low dose of opioids.

However, there seem to be no accounts in the literature describing what 'characteristic' anticipatory behaviour looks like. When it was first written about, anticipatory behaviour was described generally as a "state of agitation" that manifests externally as "restlessness" or "activity" (Craig, 1918). More recently, anticipatory behaviour has been characterized as an increased level of activity resulting from frequent and abrupt transitions between short fragments of behaviour (Spruijt et al., 2001; van der Harst et al., 2003b). One study on rats reported that the most frequent behavioural categories exhibited by standard-housed rats anticipating a reward were exploration,

locomotion and arousal (van der Harst et al., 2003b). Descriptions of individual behaviours, and descriptions for rats housed in non-standard cages, do not seem to have been published.

The primary aim of this study was to use anticipatory behaviour to assess the affective state experienced by female rats a) reared and housed long-term in standard laboratory cages versus a semi-naturalistic environment, and b) before and after treatment with an antidepressant or an anxiolytic. A secondary aim was to add to the literature on anticipatory behaviour by describing and comparing the frequency and duration of individual elements of anticipatory behaviour displayed by rats reared in these two systems.

Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) need to be taken for several weeks before they are clinically effective (Duman and Aghajanian, 2012), but ketamine given at low doses is effective within hours (e.g. review in humans: aan het Rot et al., 2012; studies in rats: (Carrier and Kabbaj, 2013; Cryan and O'Leary, 2010; Tizabi et al., 2012). For this reason ketamine was the antidepressant selected for this study. Traditional anxiolytics cause sedation, which would not be appropriate when studying behavioural activation. Therefore, I used α-S1 tryptic casein, a non-sedating, naturally derived protein used in veterinary medicine to treat anxiety in cats, dogs and horses. This protein is effective in rats, with anxiolytic effects similar to those seen with a medium dose of a benzodiazepine (Messaoudi et al., 2009; Miclo et al., 2001; Violle et al., 2006). Doses selected for this study were within the range known to be effective in similar studies but without causing behavioural activation or suppression.

4.2. Materials and methods

4.2.1. Animals and housing

Forty-two, 22-to 23-day-old female Sprague-Dawley rats were purchased from Charles River Laboratories Canada. As soon as they arrived, they were systematically assigned to either semi-

naturalistic cages (6 cages each housing five rats) or standard cages (6 cages each housing two rats). In assigning rats to housing treatment, I alternated between semi-naturalistic and standard cages, and within each cage alternated between rats huddled at the back of the shipping box and those who reared at the front.

All cages were in one room, and cage type was symmetrically distributed across the room. Rats were housed under a reversed light cycle, with lights off from 10:00 – 22:00 h so that all testing was performed during rats' active period. During training and testing, the room was illuminated with a low pressure sodium light (Master SOX-E 18W, Royal Philips, Amsterdam, the Netherlands) that emits yellow-orange light visible to humans but likely not to rodents (McLennan and Taylor-Jeffs, 2004). Temperature and humidity were kept at (mean ± SD) 23.3 ± 0.8 °C and 36 ± 15%, respectively.

Rats were marked once by means of a spot applied to their coat with a permanent nontoxic animal marker (Stoelting Co., Wood Dale, IL, USA) for individual identification. Rats took part in the year-long, observational study described in Chapter 3 before being used here. Training for the experiments described here began when rats were 14 months old, and testing began when rats were 19 months. Rats were 21 months old at the end of the last experiment and weighed 672 ± 69 g (n = 7) in the standard cages and 577 ± 72 g (n = 20) in the semi-naturalistic cages. Morbidity and mortality were expected given the long-term nature of treatments. Rats developing health problems, which consisted of a tumour in approximately three quarters of cases of morbidity in each housing condition, were removed from the study. At the beginning of testing there remained eight standard-housed rats (four cages each housing two rats), and 20 semi-naturalistic-housed rats (six cages each housing two (n = 2), three (n = 2) or five (n = 2) rats).

Standard cages were made of polycarbonate and measured 45 x 24 x 20 cm (L x W x H); they were fitted with a wire lid and a filter top (Ancare Corp., Bellmore, NY, USA) to minimize the

transmission of smells and sounds from the semi-naturalistic cages. Each cage contained aspen chip bedding (Northeastern Products Corp., Warrensburg, NY, USA), a piece of PVC pipe (approximately 18-cm in length and 10-cm in diameter) and two pieces of brown paper towel. Once behavioural training and testing began, rats were housed in a standard cage that had an 8-cm diameter opening drilled into one end; this opening was covered from the outside with a piece of Plexiglas held with industrial strength Velcro. This opening allowed us to connect the standard cage to a testing cage without having to handle the rats before testing. Rats had *ad libitum* access to rat chow (LabDiet® 5012, PMI® Nutrition International, LLC, Brentwood, MO, USA) and tap water. Cages were cleaned and rebedded twice a week by the facility's animal care technician.

The semi-naturalistic cages (Critter Nation™ double unit with stand, MidWest Homes for Pets, Muncie, IN, USA) measured 91 x 64 x 125 cm (L x W x H). They were made of horizontal galvanized wire bars that allowed climbing, and offered four levels (lined with removable plastic inserts) connected by ramps. The lower portion of each cage was lined with Plexiglas, which allowed us to fill the bottom 30-cm of the cage with a mixture of black earth, compost, and sphagnum peat moss (3-in-1 Landscape Soil, Premier LiteWay, Rivière-du-Loup, QC, Canada). This soil substrate was watered every few days to prevent it from drying out and causing burrows to collapse (Boice, 1977). Burrow construction and maintenance caused soil to fall outside the cage, so fresh soil was added as needed to maintain levels. Each cage contained two litter boxes (filled with aspen chip bedding), several pieces of PVC tubing, a hammock, a lava rock, and a horizontal rope across the top floor. On occasion, rats were also provided with timothy hay or strips of paper that they could access by pulling through the wire bars or removing from a PVC tube; rats typically used these items to line their hammock. The top shelf was lined with polar fleece blankets that rats could burrow into.

Semi-naturalistic-housed rats also had *ad libitum* access to rat chow and tap water, but their diet was supplemented three to five times per week with various types of unsweetened cereal,

nuts, seeds or oats usually provided in a large bowl and mixed with clean aspen chip bedding, so that rats had to sort through the wood chips to find the treats. Once a week, the PVC tubes and plastic inserts lining each level were removed and disinfected (Quatricide® PV, Pharmacal Research Laboratories, Inc., Waterbury, CT, USA), litter boxes were changed, and fleece blankets were laundered. Plastic inserts were wiped down (Mohawk FloorCare Essentials, CHEMSPEC, Baltimore, MD, USA) every second day between washing. These tasks were performed by a laboratory assistant, and occasionally by me.

All rats approached my hand when it was placed in their cage. However, unlike the standard-housed rats who were handled twice a week during cage changing, rats housed in the semi-naturalistic environment were rarely handled because they always chose to retreat into a burrow rather than to be picked up. For this reason, experiments were designed to avoid handling rats before testing.

4.2.2. Pilot Study: Individual anticipatory behaviour

In the Pilot Study, anticipation of a sweet food reward (Honey Nut Cheerios®, General Mills Canada Corporation, Mississauga, ON, Canada) was tested individually in an arena that was similar in size for rats from both housing conditions; there were no drug interventions.

4.2.2.1. Testing apparatus

The testing apparatus consisted of the rats' home cage connected to a treat cage via a short tunnel (Fig. 4.1). To enable us to test rats individually and in the same space as standard-housed rats without having to handle them, the testing apparatus for rats housed in the semi-naturalistic environment also included an inverted standard cage that was placed inside the semi-naturalistic cage, on the bottom shelf. All testing equipment was cleaned between cages.

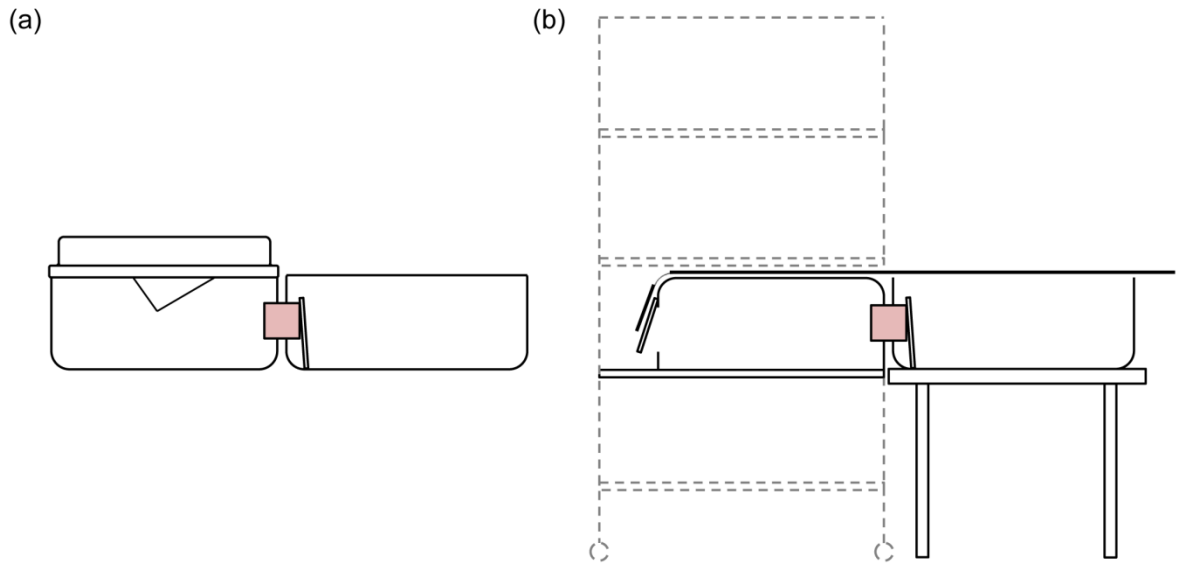

Figure 4.1. Testing apparatus used in the Pilot Study for rats housed in standard (a) and semi-naturalistic (b) cages. In both cases, the treat cage (48 x 38 x 20 cm) is on the right and is connected to the home cage via a red transparent tunnel (7.6 cm diameter x 7.7 cm long). Tunnel exit into the treat cage was blocked with a piece of Plexiglas during 60 s cue-reward interval. For semi-naturalistic-housed rats, an inverted standard cage was placed inside the home cage. One end of this inverted cage connected to the red tunnel while the other end had a hole (10 cm diameter) covered from the outside with an oversized piece of Plexiglas ('flap door') on hinges. A rope system allowed me to open the flap door when a rat approached, allowing her to enter. This way, rats could not enter unless let in by me, but once inside, they could exit by pushing on the flap door from the inside. Only one rat was allowed inside at a time.

4.2.2.2. Testing procedure

The procedure for standard cages was as follows: the filter top, water bottle, wire lid and PVC pipe were removed, and the rat not being tested was gently picked up and placed in a holding cage containing familiar bedding. The home cage lid and filter top were placed back, the piece of Plexiglas covering the hole drilled into one side of the home cage was removed, and the cage was joined to the treat cage via the tunnel. The tunnel exit was blocked with a piece of Plexiglas. I

delivered the conditioned stimulus (three 'beep' sounds from a Timex® Triathlon digital stopwatch) and stood to the left of the home cage. After 60 s, the Plexiglas barrier was removed and the rat could access 14 reward items in the treat cage. The rat in the holding cage remained there while her companion ran the trial; after the first rat completed her trial, roles were reversed. Order of testing alternated between trials.

The procedure for semi-naturalistic cages was as follows: a door at the front of the cage was opened and the inverted standard cage was placed on the bottom shelf. One end of this inverted cage had a flap door (see Fig. 4.1) and the other end was connected to the treat cage via the tunnel; the tunnel exit was blocked with a piece of Plexiglas. As soon as a rat approached the flap door, the door was pulled open and the rat could enter the inverted cage. Only one rat was allowed to enter the cage at a time. I immediately delivered the conditioned stimulus and stood to the left of the home cage. After 60 s, the Plexiglas barrier was removed and the rat could access the reward items in the treat cage. Once a rat ate all the reward items, the flap door was opened and the rat exited.

Testing alternated between standard and semi-naturalistic cages. Cages were tested in the same order every day, and each cage was tested once a day at the same time each day to control for daily rhythmic differences in activity.

Rats from both housing treatments had to be trained daily over several weeks to cross the tunnel and retrieve treats from the treat cage. Anticipatory behaviour training took place once a day, as lag time between sound cue and access to the treat cage was gradually increased from 0 to 60 s. Rats were trained to the behavioural criterion of 60 s, and this took 7-14 days depending on the individual. Training and testing were performed in the animals' housing room.

4.2.2.3. Data collection

All rats were initially trained to perform this experiment, but not all were willing to participate. One standard-housed rat never acclimated to the attached treat cage and was excluded from the study due to persistent burying of the tunnel leading to the treat cage. Only 12/20 semi-naturalistic-housed rats entered the inverted cage, and of those only five were willing to remain for the required 60 s. Therefore, sample size for the Pilot Study was seven standard-housed rats from four cages and five semi-naturalistic-housed rats from five cages.

All trials were recorded directly onto a laptop using a high definition webcam (Microsoft® LifeCam Studio 1425, Redmond, WA, USA; 30 frames/s). Standard-housed rats all reached the 60 s criterion on the same day, but data were only collected on this first day for those rats in each pair who ran the trial first (i.e. were not placed in the holding cage before their trial) and on the second day for the remaining standard-housed rats, when order of testing was reversed. For consistency, data were also collected on the first or second day a semi-naturalistic-housed rat reached the 60 s criterion, except for one semi-naturalistic-housed rat whose initial videos were poor quality, so the third day was scored instead. Therefore, data were collected on the first day rats reached the 60 s criterion for four standard- and three semi-naturalistic-housed rats, on the second day for three standard- and one semi-naturalistic-housed rat, and on the third day for one semi-naturalistic-housed rat.

Videos were scored using The Observer XT 9.0 (v.9.0.436, Noldus Information Technology, Wageningen, the Netherlands). Behaviours were scored using an ethogram adapted from Draper (1967) and van der Harst et al. (2003a; Table 4.1). During data collection I noticed that rats would spend long periods of time rearing, but this rearing was not static. Indeed, rats would often shift positions – their front paws would go from leaning on one wall to leaning on another – without touching the ground in between. I believe that these shifts in position while already rearing reflect

behavioural activity and should be captured in the measure of total behavioural frequency, and therefore scored them as ‘rear-move’, versus ‘rear-only’ for the initial rear. In addition, I was interested in the location of rats’ focus when they were sitting; therefore, the behaviour ‘sit’ was further qualified as ‘sit-treat’ in which rats sat facing the location where the treat would appear (in this case, sitting with their head in the tunnel), and ‘sit-only’ in which rats sat facing any other direction.

Table 4.1. Ethogram used in the Pilot Study and in Experiments 1 and 2

Behaviour	Qualifier	Description
Agonistic behaviour*		Two or more rats engaged in offensive or defensive behaviour; pinning or being pinned down, pawing at each other, gripping skin
Alert		Head raised suddenly, body and head held still, body appears tense
Bite		Biting on the wire lid or wire bars
Climb		Rat is suspended vertically with all four paws on a vertical surface
Dig		Rapid, successive movements of the front and/or back paws while displacing bedding or dirt
Drink		Rapid licking at the spout of the water bottle
Eat		Rat is pawing at the food hopper in an attempt to grab rat chow, or eating something she picked up
Groom self		Maintenance behaviours; includes face washing, coat cleaning, and scratching
Groom (social)*		Licking or nibbling of fur by or of a conspecific
Jump		Rat bends down before springing up, with four paws momentarily in the air at once
Lie down		Rat's abdomen is resting on a flat surface; body is not supported by the paws
Mounting		Placing of forequarters over the hindquarters of a conspecific, or inspecting/submitting to anogenital inspection (lordosis)
Rear		Upper body is raised, with front paws either unsupported or resting on a vertical surface
	Rear-only	Rat rears after performing some other behaviour
	Rear-move	Starting in a rear position, rat moves both front paws into a new position
Shake		Quick shake of entire body
Sit		All paws and hind quarters on the ground, no forward locomotion; rat may be looking around or pivot without moving hind paws
	Sit-only	Rat sits facing any direction other than the location of upcoming treat
	Sit-treat	Rat sits facing the location of upcoming treat (Pilot Study: head in the red tunnel; Exp. 1 & 2: me)
Sniff (non-social)		Sniffing air, ground or object; air: head raised and slightly pointing upwards with minor up-down movements; ground or object: nose contacting the ground or object
Sniff (social)*		Rat's nose contacts another rat; excludes anogenital inspection
Stretch		Rat elongates her limbs and abdomen and arches her back
Sway		Rat is standing still except for slow left-right movements of the head
Turn		While remaining in the sitting position, rat turns around to face a different direction
Urination		Rat lifts her hind quarters and the base of her tail, holds still for a few seconds
Walk		Forward locomotion, often includes sniffing of the ground; all four paws are moving
Yawn		Rat briefly opens mouth wide
Out of sight		Rat is partially or fully out of view, precluding observation

Asterisks denote social behaviours, which were only scored in Experiments 1 and 2.

4.2.2.4. Statistical analysis

Data were analysed using the SAS software (v.9.3). Because visual inspection of residuals revealed that data were not normally distributed and not amenable to transformation, and because parametric statistics are non-robust for small sample sizes, I used the non-parametric Mann-Whitney U test to compare total behavioural frequency and frequency and duration of individual behaviours between standard and semi-naturalistic cages. Most behaviours never occurred or occurred very rarely, so only rear-only, rear-move, sit, sit-only, sit-treat and walk were analysed statistically. For comparisons of duration, rear-only and rear-move were combined into a single category called 'rear'. For data presentation purposes, durations were converted into percent trial time. All *p* values are two-tailed.

4.2.3. Experiments 1 and 2: cage-level anticipatory behaviour with drug treatment

Results from the Pilot Study revealed that a major change in methods was required; the testing procedure in the Pilot Study resulted in a small sample size (many rats avoided the testing apparatus; see Data Collection section above), biased sampling (only the boldest individuals were likely included) and a different relationship with the testing apparatus for rats from the two housing conditions (inclusion of the inverted cage inside the semi-naturalistic cages). To avoid these problems, in Experiments 1 and 2 anticipation of a sweet food reward (slice of ripe banana, 3-mm thick) was tested directly in the home cage (and therefore in the presence of cage mates). Each rat was tested at baseline, under the influence of an antidepressant (Exp. 1) or an anxiolytic (Exp. 2), and after return to baseline (Exp. 1).

4.2.3.1. Testing procedure

I delivered a sound cue (three 'beep' sounds from a digital stopwatch) and stood motionless to the left of the home cage. After 300 s, each rat was given one slice of banana. For standard cages, the filter top was removed for the duration of the trial.

Cages were tested in the same order every day, alternating between standard and semi-naturalistic cages. Each cage was tested twice per day (one morning and one afternoon trial) at the same time of day. Rats were tested twice in each condition to give them the opportunity to learn through experience the new incentive value of the reward once they were in a new (drug-induced) motivational state (Dickinson and Balleine, 2002). Although all rats in the cage participated in the anticipatory task at every trial, each day data were collected only from one rat per cage per drug treatment (baseline vs. on drug vs. back to baseline). The order in which individuals from each cage were observed was determined at random.

In Experiment 1, the drug intervention was an antidepressant (ketamine hydrochloride, Bioniche Animal Health Canada Inc., Belleville, ON, Canada; 42 mg/kg). In Experiment 2, the drug intervention was a nutritional supplement with anxiolytic properties (α-S1 tryptic casein, Vétoquinol N.-A. Inc., Lavaltrie, QC, Canada; 15 mg/kg). Drugs were delivered in a treat: the appropriate amount of drug was mixed with one teaspoon of peanut butter with honey (Kraft Canada Inc., Don Mills, ON, Canada) and sandwiched between two crackers (Ritz Munchables Buttery Thins, Christie Brown & Co., Mississauga, ON, Canada). All rats received a peanut butter cracker sandwich at the same time, but only the target rat from each cage received a sandwich laced with the drug. This rat was observed closely to ensure that in no case was the sandwich hoarded or stolen by a cage-mate.

In Experiment 1, rats were trained over 11 days to associate the sound cue with arrival of a slice of banana in this context (some rats had already learned the association between this sound cue and the delivery of a treat in the Pilot Study); lag time between sound cue and reward was

gradually increased from 5 to 300 s, usually in 30 s increments. After training was complete, all cages were tested daily until data collection was complete. Three weeks after the completion of Experiment 1, rats were re-trained on the anticipatory task over three trials. Then, each cage was tested daily until data collection was complete. Training and testing were performed in the animals' housing room. Timelines for testing in these two experiments are presented in Tables 4.2 and 4.3.

Table 4.2. Timeline for Experiment 1

Day	AM trial	+ 3 h	PM trial	+ 1 h	sandwich
1	baseline		baseline		regular
2	(not recorded)		(not recorded)		antidepressant
3	on drug		on drug		regular
4-10	(not recorded)		(not recorded)		regular
11	back to baseline		back to baseline		regular

Rats were tested twice daily and given a regular or drugged peanut butter cracker sandwich at the end of the day. Baseline activity was collected on what counted as Day 1 for a particular rat; activity on the drug was collected on Day 3; and return to baseline was collected on Day 11.

Table 4.3. Timeline for Experiment 2

Day	AM trial	+ 2 h	sandwich	+ 1 h	PM trial	+ 1 h	sandwich
1	(not recorded)		regular		baseline		anxiolytic
2	(not recorded)		anxiolytic		on drug		regular

Rats were tested twice daily and given a regular or drugged peanut butter cracker sandwich between the two daily trials and at the end of the day. Baseline activity was collected on what counted as Day 1 for a particular rat, and activity on the drug was collected on Day 2.

4.2.3.2. Data collection

In Experiment 1, all but two rats from the semi-naturalistic housing condition were tested; these two rats were excluded because one did not eat the ketamine sandwich and the other developed a health problem. Therefore, sample size for Experiment 1 was eight rats from four standard cages, and 18 rats from six semi-naturalistic cages. One standard-housed rat had to be euthanized for humane reasons after her data collection was complete, so the 'back to baseline' data was collected only from her now singly housed cage-mate after she had become single-housed. This single-housed rat was included in the analysis because her results were within the range of results obtained from her when she was pair-housed.

In Experiment 2, only two rats per cage were tested (for cages housing more than two rats, subjects were chosen at random), but three rats from the semi-naturalistic housing condition were excluded from analysis because consumption of the entire sandwich (i.e. full drug dose) could not be confirmed. Therefore, sample size for Experiment 2 was seven rats from four standard cages, and nine rats from five semi-naturalistic cages.

Trials were video recorded with a high definition camcorder (Canon HD10, Japan; 25 frames per s) and scored using The Observer XT 9.0. The same ethogram was used as in the Pilot Study but with the addition of several social behaviours (Table 4.1). In these experiments, sitting orientation (sit-only versus sit-treat) was not obvious in the standard cages, so standard-housed rats were simply scored as 'sit' without further qualification. Scoring was from video with the scorer blind to drug treatment and time of day, but not housing condition (the latter was impossible given that testing was in the home cage).

4.2.3.3. Statistical analysis

Residuals were examined to verify normality and homogeneity of variances. In Experiment 1, paired sample t-tests revealed no differences in the total frequency, or frequency or duration of individual behaviours, between the morning and afternoon trials, so data were averaged to obtain one value per rat per day. Most behaviours occurred rarely, so the effect of drug treatment, cage type, and their interaction on the frequency and duration of these behaviours were not analysed statistically. The effect of drug treatment, cage type, and their interaction on total behavioural frequency, as well as frequency and duration of rear-only, rear-move, sit, walk and groom self were analysed using a mixed model in SAS (v.9.3) following St-Pierre (2007). For tests of duration, rear-only and rear-move were analysed as a single category called 'rear'. The model included rat and cage as random effects, drug treatment as a repeated measure, and the Kenward-Roger degrees of freedom approximation to account for unbalanced data (i.e. different number of rats per cage and cages per housing treatment). I also included a contrast statement in the mixed model to compare baseline vs. back to baseline (Exp. 1) and baseline vs. drug (Exp. 1 and 2). Cage was the statistical unit for tests of cage type, and rat was the statistical unit for tests of drug treatment and the interaction of drug treatment and cage type. To account for periods when rats were out of sight, I computed mean frequencies and durations per minute. For data presentation purposes, mean durations per min were converted into percent time. All *p* values are two-tailed.

4.3. Results

4.3.1. Pilot Study

The total frequency of behaviours was higher in the standard treatment compared to the semi-naturalistic treatment (Fig 4.2; Z = 2.327; p = 0.02). Standard-housed rats performed rear-only and rear-move more frequently (Z = 2.3771; p = 0.0175; Z = 2.0494; p = 0.0404, respectively) and also spent more time rearing (Z = 2.327; p = 0.02). Standard-housed rats also performed sit-only (facing away from the tube) more frequently (Z = -2.1268; p = 0.0334) and spent less time sitting (Z = -2.327; p = 0.02) and performing sit-treat (with their head in the tube; Z = -2.327; p = 0.02). Standard-housed rats spent more time walking (Z = 2.0821; p = 0.0373).

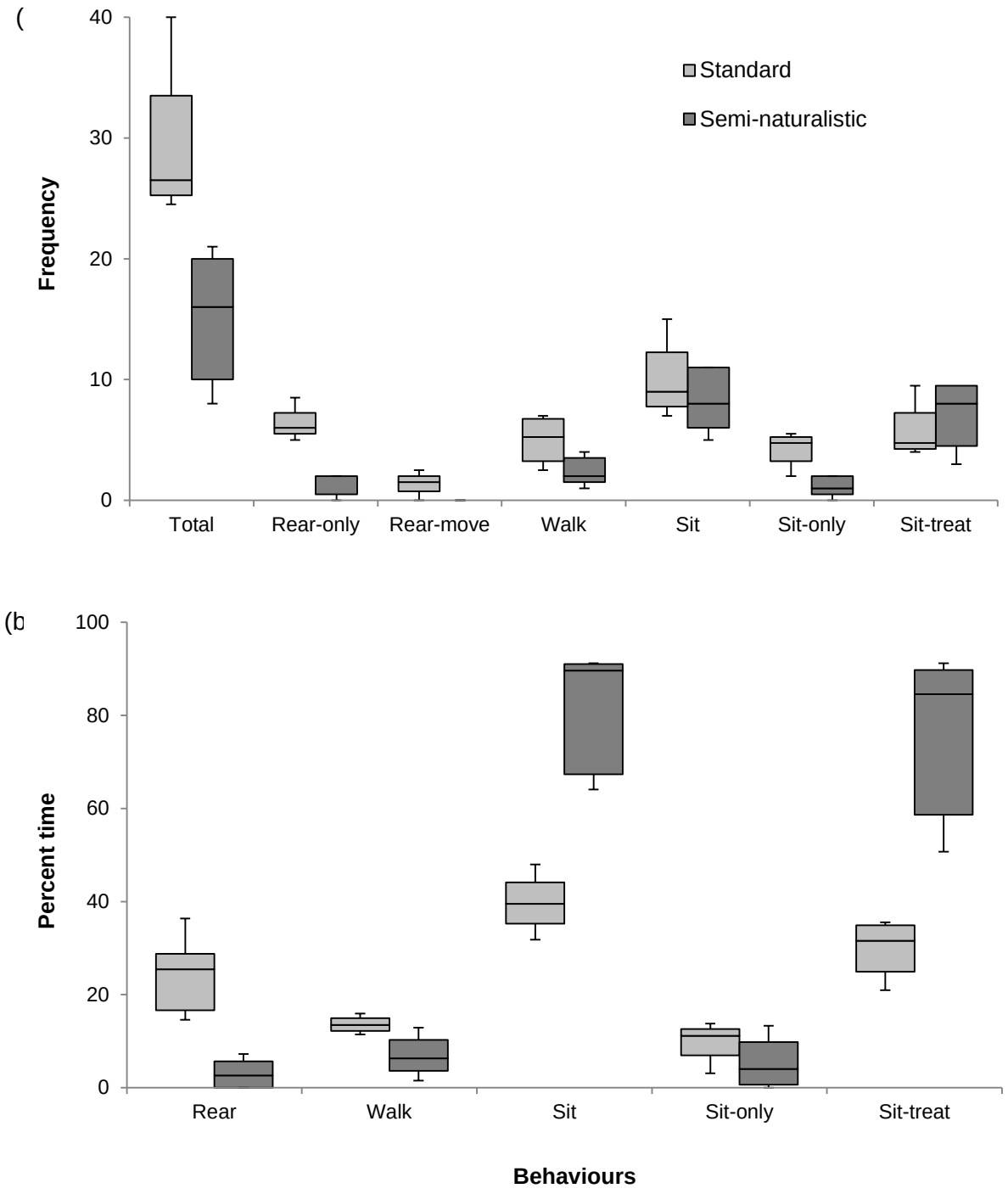

Figure 4.2. Frequency (a) and percent time (b) of behavioural elements displayed in the Pilot Study. Data presented as medians with 1st and 3rd quartiles as lower and upper limits of the box, and whiskers as lowest and highest data values; n=4 standard cages and n=5 semi-naturalistic cages; *p<0.05.

4.3.2. Experiments 1 and 2

Drug treatment and the interaction of drug treatment and cage type had no effect on the total behavioural frequency nor the frequency or duration of any individual behaviours.

In both experiments, the total frequency of behaviours was higher in standard-housed compared to semi-naturalistic-housed rats (Fig 4.3; Exp. 1: $F = 11.49$; $p = 0.0066$; Exp. 2: $F = 23.2$; $p = 0.0006$). In Experiment 1, standard-housed rats performed rear-only ($F = 13.89$, $p = 0.0036$), rear-move ($F = 19.60$; $p = 0.0033$) and walk ($F = 18.67$, $p = 0.0002$) more frequently than semi-naturalistic-housed rats. In Experiment 2, standard-housed rats performed rear-only ($F = 24.01$; $p = 0.002$) and rear-move ($F = 22.31$; $p = 0.0003$) more frequently, and tended to walk more frequently ($F = 5.53$; $p = 0.0504$). In both experiments, standard-housed rats spent more time rearing (Exp. 1: $F = 44.51$, $p = 0.0002$; Exp. 2: $F = 18.77$; $p = 0.0117$) and walking (Exp. 1: $F = 15.64$, $p = 0.0006$; Exp. 2: $F=6.32$, $p = 0.0389$) and less time sitting (Exp. 1: $F = 26.37$, $p < 0.0001$; Exp. 2: $F = 14.47$, $p = 0.0019$) compared to semi-naturalistic-housed rats.

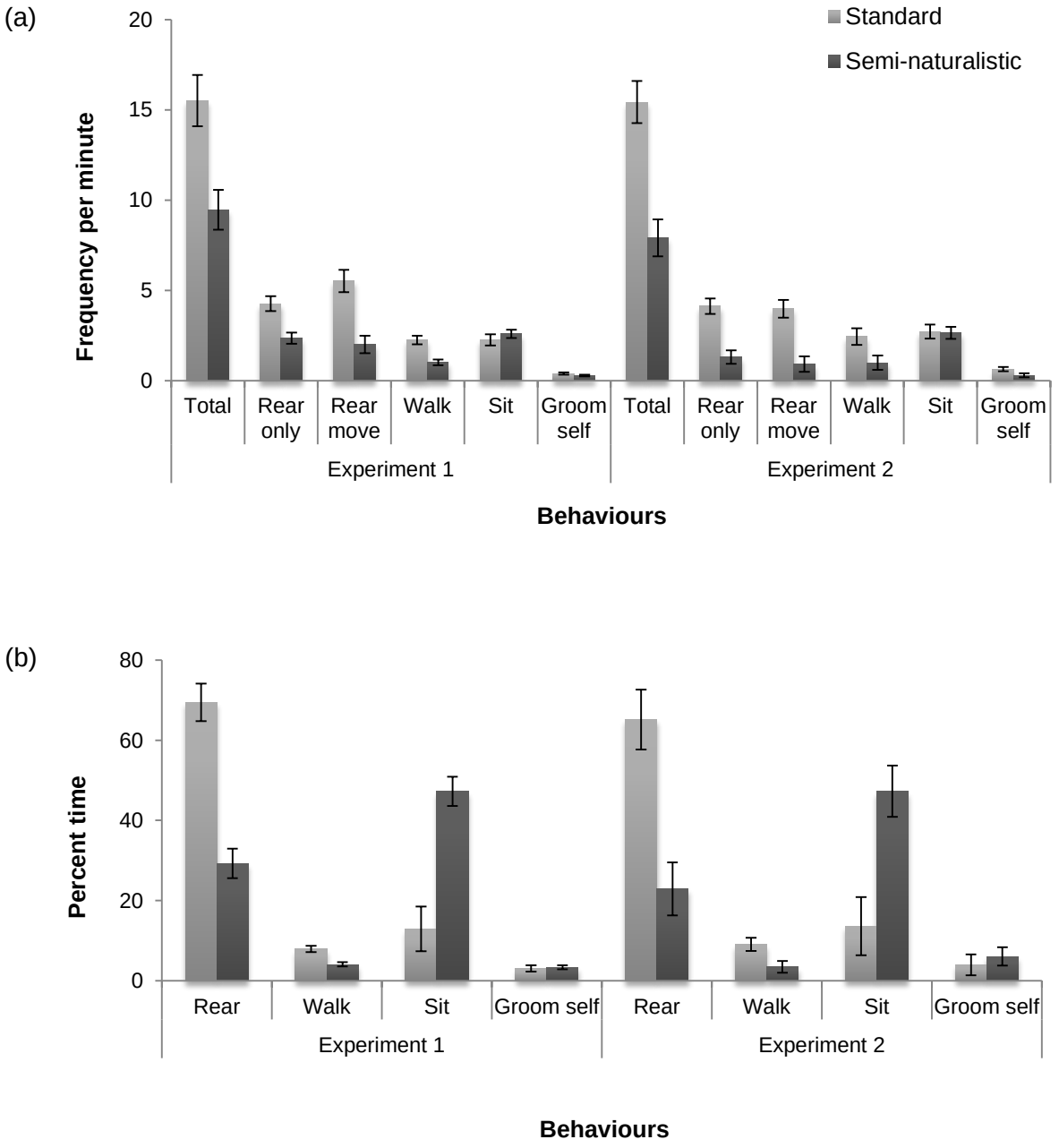

Figure 4.3. Frequency per min (a) and percent time (b) for behavioural elements displayed in Experiments 1 and 2. Bars represent LS means ± SEM. In Experiment 1, n=4 standard cages and n=6 semi-naturalistic cages, and in Experiment 2, n=4 standard cages and n=5 semi-naturalistic cages. Asterisks denote significant differences between the two housing conditions, where *p<0.05; **p<0.01 and ***p<0.001.

In general, standard-housed rats tended to rear on one side of the cage, shift positions several times while rearing, sit down briefly before walking over to the other side of the cage, and repeat the sequence. In contrast, rats in the semi-naturalistic environment typically ran to the location of the upcoming treat and sat down, occasionally rearing or sniffing the air before resuming the sitting position. The mean frequency and percent trial time per minute for all behaviours displayed during the anticipatory period are presented in Table 4.4. for Experiment 1 and Table 4.5. for Experiment 2.

Table 4.4. Frequency per min and percent time for behavioural elements in Experiment 1

	Standard			Semi-naturalistic		
	baseline	**antidepressant**	**back to baseline**	**baseline**	**antidepressant**	**back to baseline**
Frequency						
Agonistic behaviour	0.00±0.01	0.00±0.01	0.00±0.01	0.01±0.01	0.00±0.01	0.02±0.01
Alert	0.00±0.06	0. 25±0.06	0.00±0.06	0.06±0.04	0.11±0.04	0.1±0.04
Bite	0.60±0.42	0.11±0.42	0.27±0.42	0.46±0.28	0.46±0.28	0.55±0.28
Climb	n/a	n/a	n/a	0.08±0.05	0.19±0.05	0. 22±0.05
Dig	0.00±0.20	0.00±0.20	0.00±0.20	0.10±0.13	0.12±0.13	0.14±0.13
Drink	0.00±0.03	0.06±0.03	0.04±0.03	0.02±0.02	0.01±0.02	0.01±0.02
Eat	0.06±0.04	0.05±0.04	0.12±0.05	0.02±0.03	0.02±0.03	0.00±0.03
Groom self	0.31±0.11	0.45±0.11	0.43±0.11	0. 30±0.07	0.28±0.07	0.28±0.07
Groom (social)	0.00±0.04	0.03±0.04	0.00±0.04	0.03±0.03	0.03±0.03	0.06±0.03
Jump	n/a	n/a	n/a	0.04±0.20	0.43±0.20	0.04±0.20
Lie down	0.00±0.01	0.00±0.01	0.01±0.02	0.02±0.01	0.02±0.01	0.01±0.01
Mounting	0.00±0.01	0.04±0.01	0.00±0.01	0.00±0.01	0.01±0.01	0.01±0.01
Rear-only	4.01±0.52	3.78±0.52	5.03±0.55	2.36±0.38	2.43±0.38	2.26±0.38
Rear-move	5.81±0.75	4.83±0.75	5.93±0.79	2.26±0.57	2.07±0.57	1.71±0.57
Shake	0.00±0.01	0.04±0.01	0.00±0.01	0.01±0.01	0.01±0.01	0.01±0.01
Sit	1.88±0.41	2.29±0.41	2.60±0.44	2.65±0.30	2.85±0.30	2.27±0.30
Sit-only	n/a	n/a	n/a	0.48±0.19	0.60±0.19	0.25±0.19
Sit-treat	n/a	n/a	n/a	2.17±0.22	2.24±0.22	2.01±0.22
Sniff (non-social)	0.25±0.11	0.39±0.11	0.12±0.12	0.27±0.08	0.31±0.08	0.14±0.08
Sniff (social)	0.00±0.02	0.03±0.02	0.01±0.02	0.01±0.01	0.02±0.01	0.01±0.01
Stretch	0.00±0.01	0.00±0.01	0.01±0.01	0.00±0.00	0.01±0.00	0.00±0.00
Sway	0.00±0.01	0.01±0.01	0.00±0.01	0.00±0.00	0.00±0.00	0.01±0.00
Walk	2.34±0.33	1.93±0.33	2. 50±0.36	1.31±0.22	1.07±0.22	0.64±0.22
Yawn	0.03±0.02	0.00±0.02	0.00±0.02	0.02±0.01	0.01±0.01	0.00±0.01
Total	15.38±1.74	14.09±1.74	17.06±1.83	10.05±1.26	9.87±1.26	8.48±1.26
Percent time						
Agonistic behaviour	0.00±0.38	0.00±0.38	0.00±0.41	0.03±0.26	0.00±0.26	0.45±0.26
Alert	0.00±0.41	0.04±0.41	0.00±0.44	0.68±0.30	0.81±0.30	0.61±0.30
Bite	6.96±2.63	1.60±0.96	3.19±1.85	6.05±2.94	4.43±2.94	5.76±2.94
Climb	n/a	n/a	n/a	2.04±0.30	3.82±1.30	4.14±1.30
Dig	0.00±1.27	0.00±1.27	0.00±1.28	0.54±0. 54	1.27±1.27	1.28±1.28
Drink	0.00±0.69	0.74±0.69	0.10±0.74	0. 87±0.50	0.35±0.50	0.45±0.50
Eat	0.40±0.76	0.38±0. 76	0.71±0.82	0.90±0. 52	0.37±0.52	0.05±0.52
Groom self	3.08±1.17	3.66±1.17	2.38±1.25	2.88±0. 78	3.22±0.78	3.94±0.78
Groom (social)	0.00±1.20	0.18±1.20	0.00±1.29	1.69±0.80	0.57±0.80	1.02±0.80
Jump	n/a	n/a	n/a	0.68±0.30	0.26±0.30	0.03±0.30
Lying	0.00±2.04	0.00±2.04	0.04±2.20	2.87±1.36	0. 19±1.36	0.02±1.36
Mounting	0.00±0.24	0.00±0.24	0.00±0.26	0.00±0.16	0.33±0.16	0.00±0.16
Rear	74.03±5.62	65.78±5.62	68.58±5.85	29.13±4.25	29.52±4.25	29.14±4.25
Shake	0.00±0.02	0.07±0.02	0.00±0.02	0.00±0.01	0.00±0.01	0.00±0.01
Sit	6.34±7.08	17.28±7.08	15.17±7.46	44.35±4.72	48.11±4.72	49.33±4.72
Sit-only	n/a	n/a	n/a	11.75±4.38	10.33±4.38	5.73±4.38
Sit-treat	n/a	n/a	n/a	33.47±4.19	38.64±4.19	44.46±4.19
Sniff (non-social)	1.38±0.88	2.10±0.88	0.61±0.93	1.83±0.59	2.56±0.59	1.16±0.59
Sniff (social)	0.00±0.06	0.05±0.06	0.02±0.07	0.01±0.04	0.13±0.04	0.02±0.04
Stretch	0.00±0.03	0.00±0.03	0.09±0.03	0.00±0.02	0.02±0.02	0.00±0.02
Sway	0.00±0.04	0.03±0.04	0.00±0.05	0.00±0.03	0.00±0.03	0.05±0.03
Walk	7.51±0.65	7.84±0.91	8.42±1.26	5.38±0.78	4.21±0.78	2.73±0.78
Yawn	0.06±0.05	0.00±0.05	0.00±0.05	0.07±0.03	0.01±0.03	0.00±0.03

Data are displayed as LS means ± SEM. Individual rats were tested in their home cages in the presence of their cage-mates at baseline and under the influence of an antidepressant. The symbol n/a denotes behaviours that were not possible or not scored in that system; n=8 rats from four standard cages and n=18 rats from six semi-naturalistic cages.

Table 4.5. Frequency per min and percent time for behavioural elements in Experiment 2

	Standard		Semi-naturalistic	
	baseline	**anxiolytic**	**baseline**	**anxiolytic**
Frequency				
Agonistic behaviour	0.00±0.03	0.03±0.00	0.04±0.02	0.00±0.02
Alert	0.00±0.06	0.00±0.06	0.23±0.06	0.12±0.06
Bite	0.11±0.27	0.23±0.27	0.40±0.24	0.00±0.24
Climb	n/a	n/a	0.07±0.03	0.02±0.03
Dig	0.03±0.01	0.00±0.01	0.00±0.01	0.00±0.01
Eat	0.09±0.05	0.08±0.05	0.05±0.04	0.00±0.04
Groom self	0.71±0.17	0.60±0.17	0.35±0.15	0.25±0.15
Groom (social)	0.03±0.06	0.00±0.06	0.04±0.05	0.06±0.05
Jump	n/a	n/a	0.09±0.03	0.00±0.03
Lie down	0.03±0.07	0.00±0.07	0.04±0.06	0.13±0.06
Rear-only	3.77±0.59	4.50±0.59	1.67±0.52	0.95±0. 52
Rear-move	4.09±0.60	3. 87±0.60	0. 97±0.53	0.87±0.53
Shake	0.14±0.05	0.06±0.05	0.02±0.04	0.07±0.04
Sit	2.34±0. 60	3.10±0.60	2.58±0.53	2.71±0.53
Sit-only	n/a	n/a	0.54±0.45	0.21±0.45
Sit-treat	n/a	n/a	2.03±0.40	2.49±0.40
Sniff (non-social)	0.51±0. 27	1.29±0.27	0.99±0.24	0.65±0.24
Sniff (social)	0.06±0.04	0.06±0.04	0.07±0.03	0.00±0.03
Sway	0.03±0.02	0.00±0.03	0.04±0.03	0.00±0.03
Turn	0.03±0.04	0.06±0.04	0.02±0.04	0.16±0.04
Walk	2.26±0. 60	2.62±0.60	0.85±0.53	1.16±0.53
Yawn	0.00±0.04	0.00±0.04	0.00±0.04	0.07±0.04
Total	13.93±1.24	15.81±3.52	7.86±1.72	7.70±1.22
Percent time				
Agonistic behaviour	0.00±0.06	0.00±0.06	0.10±0.06	0.00±0.06
Alert	0.00±0.25	0.00±0.25	0.76±0.22	0.46±0.22
Bite	0.24±4.20	1.10±4.30	7.03±3.79	0.00±3.79
Climb	n/a	n/a	0.80±0.32	0.26±0.32
Dig	0.05±0.02	0.00±0.02	0.00±0.02	0.00±0.02
Eat	0.71±2.43	2.28±2.43	2.53±2.15	2.73±2.15
Groom self	4.23±3.02	3.71±3.02	5.17±2.66	6.97±2.66
Groom (social)	0.18±1.35	0.00±1.35	0.49±1.20	1.89±1.20
Jump	n/a	n/a	0.08±0.02	0.00±0.02
Lying	0.55±9.30	0.03±9.30	14.65±8.28	7.47±8.28
Rear	69.61±8.95	60.74±8.95	20.73±7.91	22.07±7.92
Shake	0.08±0.05	0.05±0.05	0.02±0.05	0.11±0.05
Sit	11.66±8.81	15.58±8.81	42.15±7.77	52.37±7.77
Sit-only	n/a	n/a	3.77±4.71	2.58±4.71
Sit-treat	n/a	n/a	38.11±7.27	49.53±7.27
Sniff (non-social)	1.95±1.19	5.26±1.19	3.757±1.05	1.07±1.05
Sniff (social)	0.21±0.12	0.09±0.12	0.19±0.10	0.00±0. 10
Sway	0. 63±0.39	0. 10±0.39	0.38±0.34	0.01±0.34
Turn	0.13±0. 21	0.15±0.21	0.04±0.18	0.49±0.18
Walk	8.46±2.07	9.69±2.07	3.13±1.83	3.81±1.83
Yawn	0.00±0. 10	0.00±0. 10	0.00±0.09	0.17±0.09

Data are displayed as LS means ± SEM. Individual rats were tested in their home cages in the presence of their cage-mates at baseline and under the influence of an antidepressant an anxiolytic. The symbol n/a denotes behaviours that were not possible or not scored in that system; n=7 rats from four standard cages and n=9 rats from five semi-naturalistic cages.

4.4. Discussion

These experiments assessed differences in anticipatory behaviour between female Sprague-Dawley rats reared and housed in common standard laboratory cages versus semi-naturalistic environments for more than one year. In all experiments, standard-housed rats were more active while anticipating a reward. Similarly, van der Harst et al. (2003a) showed that standard-housed male Wistar rats were more active in anticipation of a reward than enriched-housed rats. My results are consistent with the idea that standard-housed rats are more sensitive to rewards, and suggest that standard-housed rats were experiencing poorer welfare (see Van der Harst and Spruijt, 2007) than rats reared in the semi-naturalistic environment.

In Experiments 1 and 2, rats were tested in their home cages and in the presence of their cage-mates; these experiments were primarily designed to test within-rat differences in response to treatment with an antidepressant or anxiolytic. The amount of space and number of cage-mates differed between the two housing treatments; these factors may have encouraged higher activity in the semi-naturalistic cages where it was possible to perform a wider range of behaviours and to make use of a larger area (Spangenberg et al., 2011), and where there was greater probability of social modulation of behaviour. However, I actually found that rats in the semi-naturalistic environment were less active during the anticipatory period than were the standard-housed rats. This finding is consistent with my results from the Pilot Study, in which rats were tested individually and in a testing arena similar in size for both housing conditions. My results are not likely explained by cognitive differences caused by markedly different rearing environments, because van der Harst

et al. (2003a) observed similar differences after differentially housing post-pubescent rats for only two weeks. The confound between housing treatment and group size was intentional; I considered a larger group size to be an essential component of the semi-naturalistic environment (Barnett, 1975; Calhoun, 1963). Further work will be required to determine which specific differences in the housing systems are responsible for the various differences I described.

It would have been helpful to compare differences in the level of activity during the cue-reward interval for rats in each housing treatment before and after anticipatory training. This way, I could have assessed not only absolute differences between rats from different housing conditions, but also changes in each group from baseline. Unfortunately, because rats in the Pilot Study had to be trained extensively to cross the tunnel and enter the treat cage before they could be trained on the anticipatory behaviour task, at the beginning of anticipatory training they already knew to expect a reward in the treat cage. In reality, the purpose of anticipatory behaviour training was likely more to habituate rats to waiting for 60 s rather than to associate cue with reward. Connecting the treat cage (standard-housed rats) or entering the inverted cage (semi-naturalistic-housed rats) were also cues rats likely associated with the upcoming reward. The sound cue used in Experiments 1 and 2 was the same as in the Pilot Study; consequently, rats already associated the cue with a reward and therefore baseline pre-training levels in Experiments 1 and 2 were also unreliable.

This study also showed that the patterns of behaviour – *what* rats did in anticipation of the reward – were different between the two housing treatments. In Experiments 1 and 2 the standard-housed rats spent the most time rearing (Fig. 4.3; approximately 65-70% trial time vs. 20-30% for rats in the semi-naturalistic condition) while semi-naturalistic-housed rats spent the most time sitting (47% vs. 13% for standard-housed rats). However, 75-95% of sitting time in semi-naturalistic-housed rats was spent facing the location of the upcoming treat (see Table 4.3). This suggests that

semi-naturalistic-housed rats did anticipate the treat, even though they expressed their anticipation differently from standard-housed rats.

In Experiments 1 and 2, the test cage was different between the two housing treatments; standard-housed rats had to rear to better access smells from the room, while semi-naturalistic-housed rats' cage was entirely made of bars so room smells were accessible from the sitting position. This difference could explain why standard-housed rats primarily reared while semi-naturalistic-housed rats primarily sat. However, in the Pilot Study all rats were tested in an enclosed 'standard' cage (for standard-housed rats, the filter top was on during testing) so rats from both housing treatments could best access room smells through the short tunnel that led to the open treat cage. In this experiment rats from both housing treatments spent three to four times as much time sitting compared to Experiments 1 and 2, but standard-housed rats still spent less time sitting with their head in the tube (sit-treat) and more time walking and rearing. Therefore, differences in what rats did were likely not only due to environmental conditions during testing.

One interpretation for why rats from the two housing conditions behaved differently is that standard-housed rats are more impulsive. Other evidence suggests that rats reared in isolation are more impulsive than environmentally enriched rats. For example, in impulsive choice studies, isolated rats tend to choose smaller, but more immediate rewards, over larger, but delayed rewards (Kirkpatrick et al., 2013; Perry et al., 2008). A study by Wood and colleagues (2006) showed that isolated rats were more impulsive in an operant-shaping procedure in which they would gain access to sucrose by nose-poking a lit hole following a fixed intertrial interval (ITI). The authors found that isolated rats impulsively responded to the operant stimulus by initiating more pokes during the ITI, even though general activity levels were similar between the two groups. The authors argued that isolated rats were more impulsive because they were more sensitive to rewards: since rewards were more salient to them, they had a stronger impulse to seek them. In contrast, a study by Kirkpatrick

and colleagues (2013) found that isolated rats were less impulsive than enriched-housed rats, but also explained their results in terms of reward sensitivity. The task in Kirkpatrick et al.'s study was similar to the one just described (Wood et al., 2006), except that responding during the ITI caused the ITI to be reset. With an imposed cost to impulsive responding (longer ITI), isolated rats were actually less impulsive, requiring fewer responses per reward than enriched-housed rats. The authors speculated that isolated rats were more sensitive to rewards, leading them to be less impulsive because this was the most efficient strategy (i.e. in this way they earned the most rewards). Overall, the consensus in the literature seems to be that impulsivity is driven by reward sensitivity. Therefore, both how active rats are and what they do may reflect differences in reward sensitivity. An alternate explanation is that rats reared in restricted environments appear more impulsive because they have little experience with exerting control over, or receiving feedback from, their environment, and therefore failed to learn to inhibit or vary their behaviour in response to external cues (Sackett, 1970, as cited by Gluck and Pearce, 1977).

The three most frequent behaviours displayed by standard-housed rats when they were tested in their home cage (Exp. 1 and 2) were rearing, sitting and walking, respectively. This result is somewhat consistent with van der Harst et al. (2003b) who tested anticipatory behaviour in the home cage of male standard-housed rats. The authors reported that the most frequent behavioural categories displayed by their rats were exploration, arousal and locomotion, where exploration included mobile and immobile exploration and rearing; arousal included running; and locomotion included walking, running and mobile exploration. I did not differentiate between walking and running (running was not possible in my standard cage) and between walking and mobile exploration. The main difference between my findings and theirs is that the second most frequent behaviour displayed by my rats was sitting, while in their study, resting (including sitting) was one of the least frequent behaviours. One factor that could account for the difference is that my rats were

tested in the presence of conspecifics, while van der Harst et al. (2003b) tested animals in the absence of conspecifics. In a separate study that recorded total behavioural frequency but not the frequency of individual behaviours, van der Harst et al. (2003a) found that rats were more active in the absence of conspecifics, although their rats were also being tested in a different context than they were trained in, so the effect of absence of conspecifics and novel context were confounded. My rats were also older than rats tested by van der Harst et al. (2003b), and propensity to sit may increase with age.

Experiments 1 and 2 were also designed to test whether rats were depressed or anxious, by testing their anticipatory behaviour at baseline versus under the influence of an antidepressant or an anxiolytic, respectively. My hypothesis was that if rats were experiencing one of these states, they would exhibit different behaviour when they were given the drug. I found no differences in how rats behaved before and after drug intervention, but regardless of drug treatment, standard-housed rats behaved differently from semi-naturalistic-housed rats. One potential explanation for the lack of difference was the lack of statistical power due to relatively small sample sizes. This explanation seems unlikely, as Experiments 1 and 2 were designed to provide sensitive, within-rat tests of the effect of drug (despite no differences being found) and a weak, between-cage test of cage type (despite many differences being found). Post-hoc power analysis indicated that unreasonably large sample sizes would be required to detect differences with the variance and treatment differences observed (e.g., between 79-1380 rats would have been needed to detect drug effects for rats in the semi-naturalistic condition).

It is also possible that the lack of drug effect suggests that: 1) standard-housed rats were not experiencing conditions analogous to depression or anxiety, even though they are more sensitive to rewards, or 2) my drug interventions were ineffective. In the former case, it is possible that while standard-housed rats were experiencing negative affect compared to semi-naturalistic-

housed rats, this negative affect was not analogous to depression or anxiety. Also, the very act of repeatedly announcing a reward is enriching and could have reversed behavioural and neurological effects of a restrictive environment (Kamal et al., 2010; Van der Harst et al., 2005). This may have been even truer in the case of the anxiolytic (Exp. 2) because it was tested second.

In the latter case, it is possible that higher doses were needed to successfully treat the severity of depression or anxiety rats were experiencing. For example, the dose of α-S1 tryptic casein given in this study was comparable to a moderate dose of a benzodiazepine (Violle et al., 2006), but perhaps doses comparable to a high dose of a benzodiazepine were needed. It is also possible that the negative affect experienced by standard-housed rats was not associated with a deficit in the receptors and/or neurons targeted by ketamine and α-S1 tryptic casein; the former likely targets glutamate receptors and GABAergic interneurons, while the latter targets $GABA_A$ receptors (Duman and Aghajanian, 2012; Miclo et al., 2001). Finally, it could also be that the drugs did improve affective state, but that these changes in affect do not influence anticipatory behaviour. Von Frijtag et al. (2002, 2000) found that treatment with antidepressants re-established anticipation of a reward in anhedonic rats. Treating anhedonia, in which rats are essentially de-sensitized and unable to interact with the environment, may be different from treating states in which rats are sensitized to rewards; this difference could explain why Von Frijtag et al. (2002, 2000) found that antidepressants modified anticipatory behaviour while I did not.

4.5. Conclusion

Standard-housed laboratory rats are more sensitive to rewards than rats housed in semi-naturalistic conditions, as reflected by the quantity and form of their anticipatory behaviour. This study adds to mounting evidence that standard laboratory housing for rats compromises rat

welfare, and provides further scientific support for recommendations that current minimum standards be raised.

5. The Effects of Living in a Standard versus Semi-Naturalistic Laboratory Environment on Rat Weight Gain and Tumour Growth

5.1. Introduction

The physical health of laboratory animals has always been a concern for scientists and caretakers (Greenman and Duhring, 1923; Hessler, 1999; ILAR, 1963). 'Basic health and functioning' is one component of animal welfare (Fraser et al., 1997), and for some (e.g., Broom, 1991; Moberg, 1985), the most important.

In humans, overweight and obesity are considered to be serious health problems and are associated with infertility and premature death resulting from type 2 diabetes, liver and gallbladder disease, coronary heart disease and stroke, pulmonary disease, osteoarthritis, and cancer (Brown et al., 2009; Kopelman, 2007; Must et al., 1999). Overweight rodents develop health issues similar to those of humans, and are used as models to study obesity and its complications (Jeong et al., 2015; Kanasaki and Koya, 2011; Lim et al., 2013).

Greater weight gain is seen in animals leading a sedentary lifestyle, irrespective of diet. For example, Spangenberg et al. (2005) housed newly weaned male Sprague-Dawley rats individually in standard cages, or in groups in large, structurally enriched pens. Both groups were fed standard laboratory rodent chow, and there were no differences in food or water intake between the two groups. After one month, standard-housed rats weighed significantly more than pen-housed rats, presumably because they were less active. Similarly, Augustsson et al. (2002) found that male Sprague-Dawley rats housed in standard cages in pairs gained significantly more weight than their counterparts who were housed in groups in large, structurally enriched pens, despite there being no differences in food intake between the two groups. Voluntary (Goodrick, 1980) and forced (Skalicky et al., 1996) exercise also result in lower body weight compared to sedentary controls.

Being overweight is a known risk factor for developing cancer in humans (Calle et al., 2003) and in rodents (Cleary et al., 2004; Keenan et al., 1999; Seilkop, 1995). Rat and mouse studies have found that body weight is proportional to tumour number, tumour size, and latency to first tumour (Rogers et al., 1999; Williams, 2013). A few studies have also found a link between tumour growth and rearing environment, irrespective of body weight. For example, Hermes et al. (2009) reared female Sprague-Dawley rats in isolation or in groups of five. Rats from both groups maintained similar body weights, but by 15 months of age, the socially isolated females had 135% more mammary tumours, and these tumours were more than 80 times larger and 3.3 times more likely to be malignant. Similarly, Cao et al. (2010) found larger and more rapidly developing melanoma and colon cancer tumours in male C57BL/6 mice housed in groups of five in standard laboratory cages versus in groups of 18-20 in large, structurally enriched enclosures. In this study the authors concluded that it was the 'enriched' environment that was responsible for differences in cancer development, because a separate group of mice who were housed in standard cages with access to a running wheel weighed less, had less body fat, and a better immune response than mice without access to a running wheel, and yet there were no differences in the weight of tumours between these two groups.

Sprague-Dawley rats are known to have a high incidence of naturally occurring tumours, but the majority of these tumours develop when rats are middle-aged (Davis et al., 1956). Due to time and facility constraints associated with long-term research, most studies investigate young animals with artificially-induced tumours (e.g., injection of a carcinogen or cancerous cells). Indeed, only one study appears to have investigated the relationship between rearing environment and incidence of naturally occurring tumours in rats (Hermes et al., 2009). One other study investigated the relationship between rearing environment and incidence of chemically induced tumours in rats (De la Roca-Chiapas et al., 2016). While the investigations described here were not designed to test

differences in health between standard- and semi-naturalistic-housed rats, data on rat weight and rat morbidity had been collected as part of routine animal monitoring. Given the paucity of research on the health effects of long-term differential housing of rats, the aim of this chapter was to describe differences in body weight, incidence of tumours, and their interaction in female Sprague-Dawley rats housed in standard versus semi-naturalistic cages from weaning until rats were 21 months old.

5.2. Materials and methods

5.2.1. Animals and housing

Forty-two female Sprague-Dawley rats were purchased from Charles River Laboratories Canada. They arrived at my facility when they were 22-23 days old and were immediately and systematically assigned to either standard or semi-naturalistic cages. In assigning rats to housing treatment, I alternated between semi-naturalistic and standard cages, and within each cage alternated between rats huddled at the back of the shipping box and those who reared at the front. Rats were housed under a 12 h light: 12 h dark reversed light cycle. Room temperature and humidity throughout rats' life in the laboratory averaged (mean ± SD) 23.3 ± 0.8 °C and 36 ± 15%, respectively.

There were six standard cages each housing two rats, and six semi-naturalistic cages each housing five rats. Standard cages measured 45 x 24 x 20 cm (L x W x H) and contained aspen chip bedding, a piece of PVC pipe (18 x 10 cm, L x diameter), and two pieces of brown paper towel. Semi-naturalistic cages measured 91 x 64 x 125 cm (L x W x H) and offered four levels connected by ramps. These cages were made of horizontal wire bars that allowed climbing, and contained several pieces of PVC tubing, a hammock, a lava rock, a horizontal rope across one floor, a climbing

structure, and soil for burrowing. More details on housing and husbandry are given in Chapters 3 and 4.

Rats from both housing conditions had *ad libitum* access to rat chow (4.15 kcal/g; LabDiet® 5012, PMI® Nutrition International, LLC, Brentwood, MO, USA) and tap water. In addition, the diet of semi-naturalistic-housed rats was supplemented three to five times per week with oats (3.89 kcal/g), sunflower seeds (5.85 kcal/g), peanuts (5.67 kcal/g), walnuts (6.54 kcal/g), Melba toast (3.9 kcal/g) or unsweetened cereal; namely, puffed rice (3.75 kcal/g), puffed wheat (5.45 kcal/g), or shredded wheat (3.62 kcal/g). The quantity given was approximately a third of a handful per rat on each occasion. These items were usually provided in a large bowl and mixed with clean aspen chip bedding, so that rats had to sort through the wood chips to find them.

Rats from both housing conditions were for the most part left undisturbed for their first year in the laboratory, except for regular husbandry as described in Chapter 4. At 14 months old, all rats began behavioural training and subsequently participated in an anticipatory behaviour experiment (see Chapter 4). All rats were given equal amounts of treats (Honey Nut Cheerios®, banana, or peanut butter crackers) during the experiment. Rats who became sick and required treatment were removed from the experimental colony.

5.2.2. Procedure

Rats from both housing conditions were weighed at 16, 20 and 21 months of age. Rats were weighed individually in a plastic basket on a laboratory weighing scale precise to the gram. Weight was recorded once the reading had stabilized for > 2 s.

All rats were visually inspected twice a day for signs of morbidity, including tumour growth. Visual inspection was performed once in the morning by the facility's animal health care technician when the lights were still on, and once in the afternoon by me or one of my laboratory assistants

after the lights were off and the rats were more active. Visual inspection during the dark phase of the light cycle was performed under red light from a headlamp (Black Diamond spot headlamp), or under yellow-orange light from a low pressure sodium lamp (Master SOX-E 18W, Royal Philips, Amsterdam, the Netherlands). Animals were also visually inspected once a month by the University's clinical veterinarian. When a health issue was noticed, its nature and the identity of the sick rat were recorded in the laboratory's log book. Rats who were euthanized at 21 months of age were palpated for tumours.

5.2.3. Statistical analysis

The effect of housing condition (standard versus semi-naturalistic) on rat weight was analysed using a mixed model (PROC MIXED) in SAS (v. 9.4) that included age (16, 20 and 21 months) as a repeated measure and cage number as a random effect. Cage was the statistical unit because rats within the same cage could not be considered independent. At each age, only rats who had not yet developed a tumour were included, because tumour weight would have inflated body weight values. This measure allows to compare body weight irrespective of tumour incidence and size.

Independent samples t-tests with the pooled variance estimator for equal variances were performed to compare the weight of rats at 16 months between individuals in each housing condition who went on to develop a tumour by 20 months of age versus those who remained healthy. The endpoint for tumour development of 20 months rather than 21 month was chosen to provide a more even balance between rats in the 'tumour' and 'tumour-free' categories.

Finally, survival analysis (PROC LIFETEST and PROC LIFEREG) was performed in SAS to compare the rate of tumour development (irrespective of weight) between the two housing conditions. Rats who became sick for reasons other than tumours were censored; i.e. they were

included in the sample until they became sick, taking into account that they had lived without a tumour until this age. For consistency, only tumours that were noticed through visual inspection before rats were euthanized were included in the analysis. A Fisher's exact test was also performed to compare the number of rats who were healthy versus those who had developed a tumour by the age of 21 months, including those whose tumours had been detected only through palpation post-mortem. This final analysis included all the rats reared in my laboratory, except those who had been removed for reasons other than tumours.

5.2.4. Ethical note

The University's clinical veterinarians were closely involved with my work, and were notified every time a new tumour was observed. In several cases, when a tumour had reached a size that could reasonably be expected to soon interfere with a rat's normal activities, one of the veterinarians performed surgery to remove the mass. These post-operative rats were removed from the experimental colony and placed in a separate room where other post-operative rats, as well as rats who had been previously used in a pilot study, were group-housed. When they were 21 months old, I euthanized the remaining rats with isoflurane anaesthesia followed by exposure to carbon dioxide. I did not consider rehoming these rats to be a humane alternative to euthanasia, given that they were older and poorly socialized.

5.3. Results

Rats in both housing conditions continued to gain weight throughout the study period (Fig. 5.1). At each weighing, standard-housed rats were approximately 80-140 g heavier than rats from the semi-naturalistic condition ($F_{1,9}$=20.57; p=0.0014).

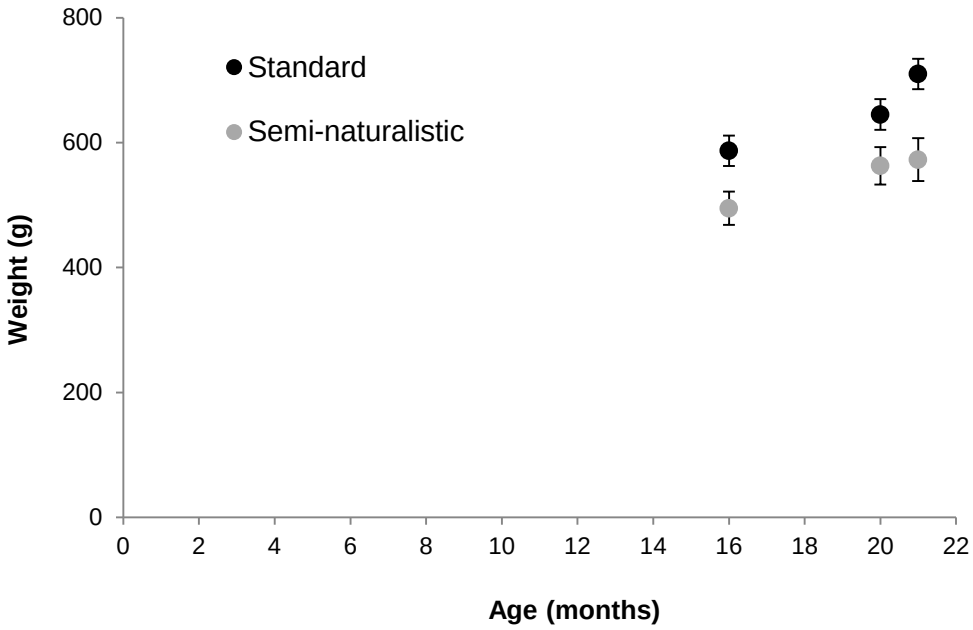

Figure 5.1. LS mean ± SEM rat body weight as a function of age. Samples at each age include only rats who were visually assessed as tumour-free at that time; at 16 months: n=6 standard cages (10 rats) and n=6 semi-naturalistic cages (28 rats); at 20 months: n=4 standard cages (5 rats) and n=6 semi-naturalistic cages (26 rats); at 21 months: n=3 standard cages (3 rats) and n=5 semi-naturalistic cages (11 rats).

There were no differences in body weight at 16 months between rats who went on to develop a tumour at 20 months versus those who did not (standard: 562 ± 36 g (n=4) vs. 599 ± 31 g (n=5), tumour vs. healthy; semi-naturalistic: 484 ± 24 g (n=9) vs. 497 ± 26 g (n=16), tumour vs. healthy).

There were also no differences in the latency to develop a visible tumour between rats from the two housing conditions (Fig. 5.3). The proportion of rats with visible or palpable tumours at 21 months of age also did not differ between standard- and semi-naturalistic-housed rats (7/10 versus 15/26, respectively).

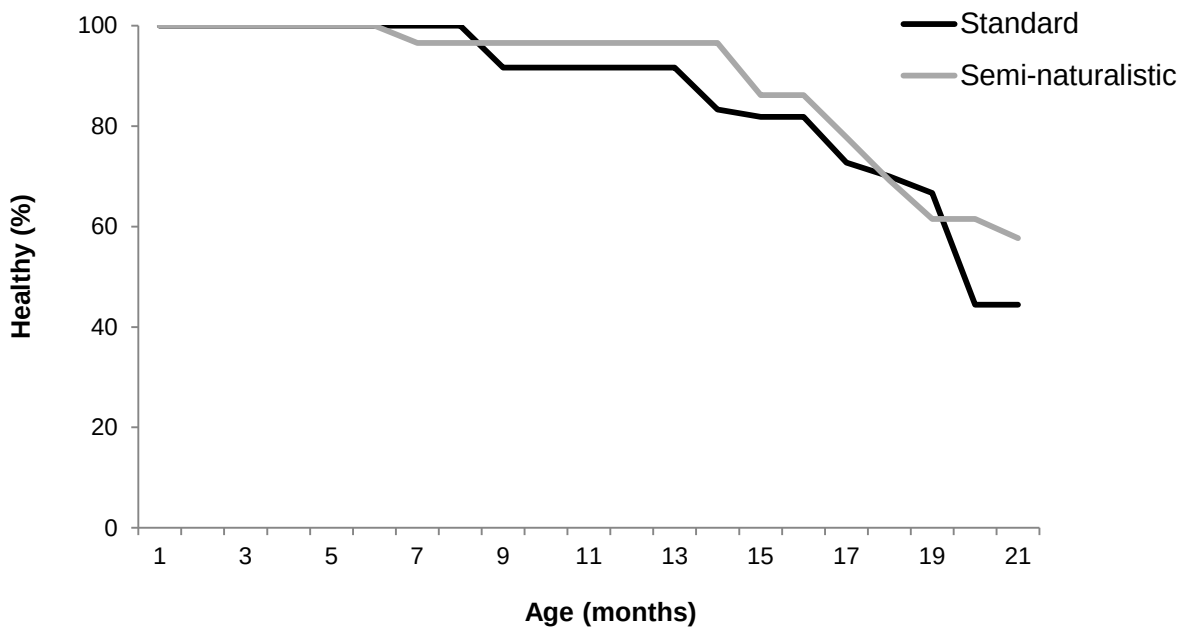

Figure 5.2. Percent healthy rats as a function of age. Analysis includes only rats whose tumours were detected visually. Rats who were removed from the colony for reasons other than the appearance of a tumour were censored.

5.4. Discussion

My studies were not designed to address health-related effects of standard versus semi-naturalistic housing; to do so properly would require much larger sample sizes, as well as more rigorous tumour detection methods and tumour analysis. The long-term health data that I collected during my studies are reported here in such a way as to facilitate future review papers or meta-analyses on this topic.

Standard-housed rats weighed considerably more than semi-naturalistic-housed rats at each of the three weighing periods. Rats were not weighed when they entered the study, so it is possible that by chance rats allocated to the standard treatment were heavier. However, any unintentional

difference would have been relatively small; according to the supplier of these rats (Charles River, 2016), weaned rats are supplied at fairly uniform weights (mean ± SD = 45 ± 5 g).

Spangenberg et al. (2005) and Augustsson et al. (2002) also compared body weights in standard- versus enriched-housed Sprague-Dawley rats, although these studies assessed only young males. Both studies found differences in body weight after 1 and 2 weeks, respectively, and these differences persisted until the conclusion of the studies 4 and 10 weeks later, respectively. Here I report that differences in weight between standard- and 'enriched'-housed rats also occur in females, and that differences exist until at least 20 months.

Spangenberg et al. (2005) and Augustsson et al. (2002) observed differences in body weight between standard- and enriched-housed rats despite finding no differences in food intake between the two groups. In contrast, Fiala et al. (1977) found that individually housed rats weighed more because they ate more than their enriched-housed littermates. I did not measure food intake between rats from the two groups, so I cannot know whether standard-housed rats gained more weight because they ate more or because they were less active, or from a combination of both factors. Both groups had *ad libitum* access to the same rat chow, but rats in the semi-naturalistic condition were also given small amounts of non-sweetened cereal, nuts, oats, seeds or toast. Of the eight types of treats given, four had higher and four had lower caloric content than the rat chow. In general, food intake is affected by affective state in humans and rats (Canetti et al., 2002; Dallman, 2010), so if standard-housed rats were indeed experiencing poorer welfare than semi-naturalistic-housed rats as suggested in Chapter 4, then this may have affected how much they ate. However, rats in a negative affective state tend to increase consumption only of high-calorie foods but not regular rat chow (Dallman et al., 2005; Ortolani et al., 2011). Indeed, stressed or anxious rats tend to decrease their intake of standard rat chow (Bazhan and Zelena, 2013; Krahn et al., 1990). Therefore, in light of the results from Chapter 4, we would expect standard-housed rats to eat less than semi-

naturalistic-housed, suggesting that heavier body weights in standard-housed rats were unlikely to have been caused by emotionally driven differences in food intake.

Few studies have reported long-term weight data on female Sprague-Dawley rats. At 21 months of age, my standard-housed rats weighed 710 ± 25 g (Fig. 5.1). In contrast, one study reported that standard-housed Sprague-Dawley females weighed approximately 550 g at 21 months (Hubert et al., 2000), and another that females weighed 485 g at 24 months (Nohynek et al., 1993). Several factors could explain why my rats were heavier. Both of the previous studies used rats from Charles River France and mine were from Charles River Canada; there may be differences between the French and Canadian stocks. Also, the rat chow given to my rats was higher in fat (5.0% vs. 3.1% for Hubert et al., 2000) and it contained higher physiological fuel value (3.43 kcal/g vs. 3.34 kcal/g). In addition, rats' thermoneutral zone – the range of temperatures at which metabolic rate is at its minimum – is in the range of 28-32 °C; below 28 °C, rats' metabolic rate is higher, resulting in higher energy loss (Poole and Stephenson, 1977). My animals' housing room was warmer at 23.3 ± 0.8 °C (mean ± SD) compared to Nohynek et al.'s (1993) 20 ± 3 °C and Hubert et al.'s (2000) 22 °C; in addition, my rats were pair-housed while theirs were housed individually. The combination of higher room temperature and the presence of another rat within the cage likely contributed to higher within-cage temperatures and resulted in lower metabolic rates, leading to heavier animals.

Heavier animals are more likely to develop cancer (e.g., Rogers et al., 1999; Williams, 2013), but I found no differences in body weight between rats who developed a tumour versus those who did not. Interestingly, mean body weight of rats who developed a tumour was numerically (but not statistically) lower than that of healthy rats. Research has also shown a link between risk of developing cancer and stress (i.e. activation of the HPA axis and serum glucocorticoid concentration), irrespective of weight (Cao et al., 2010; Hermes et al., 2009). As reviewed earlier, stressed rats tend to decrease their intake of regular rat chow. I speculate that, irrespective of

housing condition, individual rats who were more stressed were more likely to develop a tumour (Pyter and Prendergast, 2013); stress also caused these individuals to eat less, which manifested as lower body weight. Larger sample sizes as well as measurements of food intake and affective state would need to be taken to investigate this idea.

Seventy percent of my standard-housed rats and 58% of my semi-naturalistic-housed rats developed a tumour by the age of 21 months. An older study reported that 57% of female Sprague-Dawley rats had developed tumours by the end of their natural life-span, which was on average 28 months long (Davis et al., 1956). These rats were housed ten to a cage, but no more housing information was given. A more recent study also using female Sprague-Dawley rats from Charles River reported that 74% of their animals (whether housed individually or in groups of five) had developed tumours by 15 months of age (Hermes et al., 2009); this is considerably higher than the 15-25% rate observed in my rats at that age. The rats of Hermes et al. (2009) were housed at lower room temperature (22 °C) and longer photoperiod (14 h light: 10 h dark) than my rats, and these factors could account for some of the difference observed. Tumours grow faster in lower ambient temperatures (Kokolus et al., 2013; Song et al., 2009) and longer photoperiods (Mhatre et al., 1984; Shah et al., 1984).

Like Hermes et al. (2009), I found no differences in the rate at which standard- versus 'enriched'-housed female Sprague-Dawley rats developed tumours. These authors, however, found that tumours in their socially isolated rats were much larger and more likely to be malignant compared to their enriched-housed rats. My study was not designed to assess tumour growth in rats, and measuring tumour size and malignancy were not part of routine animal monitoring. Histopathology was performed on two tumours removed by the university's clinical veterinarian (one from a rat in each housing treatment), and both were found to be benign mammary tumours.

In general, lower incidence, burden and malignancy of tumours in enriched housing is linked to a better immune system, including higher natural killer cell activity and buffered immune system reactivity to stress (Benaroya-Milshtein et al., 2004; Hoffman-Goetz et al., 1992; Kingston and Hoffman-Goetz, 1996). Cao et al. (2010) found that mice housed in large groups in structurally enriched enclosures had smaller tumours and longer latencies to develop a tumour than mice housed in smaller groups in standard cages. The authors further found that lower tumour burden in the enriched-housed mice was associated with stronger immune systems (including greater natural killer cell activity) and differences in serum corticosterone compared to the standard-housed animals. The authors speculated that the enriched environment, which included more dynamic social interactions, frequent exposure to novel objects and increased physical activity, led to mild but frequent activation of the HPA axis. Such mild activation could be seen as an example of beneficial activation of the HPA axis ("eustress") with better buffering against larger, negative stressors (Benaroya-Milshtein et al., 2004; Cao et al., 2010; Larsson et al., 2002).

Similarly, Hermes et al. (2009) found that isolated rats (who developed larger tumours that were also more likely to be malignant) had very low baseline levels of corticosterone (hypocortisolemia), while being much more reactive to external stressors (e.g., exhibiting a much larger corticosterone response to a predator odour, larger response to physical restraint followed by a slower rate of recovery, and higher anxiety in an exploration test). The authors also speculated that this pattern of hypocortisolemia interrupted by long-lasting, high levels of corticosterone following exposure to acute stressors (compared to mildly elevated levels of corticosterone and buffered reactivity to acute stressors) was involved with better tumour prognosis.

5.5. Conclusion

Few studies have investigated the effects of rearing environment – especially long-term – on rat health. Here I have shown that standard-housed rats were heavier than semi-naturalistic-housed rats at 16, 20 and 21 months of age, but that rate of tumour development was similar between the two housing treatments. Overweight and obesity are associated with a multitude of health complications, so feeding diets matched to nutritional requirements and providing rats with the opportunity to exercise are recommended.

6. General Conclusions and Discussion

6.1. Thesis findings

My aim in conducting this work was to better understand the subjective experience of a rat who spends her lifetime in a standard versus a semi-naturalistic laboratory cage. Although this was not originally my intention, ultimately my approach was to investigate this query from the point of view of each of the three key concepts of animal welfare: natural living; affective states; and biological functioning (Fraser et al., 1997).

Chapter 2 reviewed scientific methods of assessing emotions in animals, and how these have been used to inform current knowledge of rats' ability to experience positive and negative emotions. Research using these methods provides compelling evidence that rats indeed experience a range of positive and negative emotions, and that the 'likes' and 'wants' of rats should be taken into account when deciding how to house and care for them.

In Chapter 3, I investigated the importance for rats of performing three 'natural' behaviours that they are unable to perform in a standard laboratory cage; namely, burrowing, climbing and standing upright. The results confirmed that rats readily and consistently engaged in these behaviours, and that burrowing and upright standing may be particularly important to rats. Results from this chapter suggest that standard laboratory caging for rats interferes with the performance of several important natural behaviours.

In Chapter 4, I used one of the methods of assessing emotions reviewed in Chapter 2 – anticipatory behaviour – to assess the affective consequences for rats living in a standard versus a semi-naturalistic laboratory cage. Results suggest that standard-housed rats experienced poorer welfare compared to rats from the semi-naturalistic environment. This chapter also provides the

first scientific description and analysis of what anticipatory behaviour looks like in rats, and how it differs between rats reared in standard versus semi-naturalistic cages.

Finally, Chapter 5 assessed long-term differences in biological functioning – specifically, body weight and incidence of tumours – between rats from the two housing conditions. I found that standard-housed rats were much heavier than rats from the semi-naturalistic condition, but that incidence of tumours was similar between the two environments. This chapter provides one of the only accounts of long-term differences in rat body weight and incidence of naturally occurring tumours in differentially housed rats.

Collectively, these results indicate that, relative to the semi-naturalistic housing assessed in this thesis, standard laboratory housing for rats leads to negative consequences in the three spheres of animal welfare by 1) preventing the performance of important natural behaviours; 2) causing negative affective states; and 3) leading to overweight animals predisposed to developing other health issues.

6.2. Significance of the results within a broader context

Rats used in science live in cages that were primarily designed for easy handling and cleaning, and the design has changed little since its original development in the 1920s (see subsection 1.2.1; Galef and Durlach, 1993). Although there is much research on rat behaviour, few studies have investigated rats' natural behaviours and behavioural priorities (Olsson et al., 2003). For example, several studies have assessed the features of rats' burrows (e.g., measurements, configuration, and sequential development) and the factors affecting these features (e.g., sex, age, and domestication) but there have been no studies investigating the importance of burrowing in rats (Boice, 1977; Price, 1977; Stryjek et al., 2012). The results presented in Chapter 3 indicate that burrowing may be especially important to these animals. This finding has implications not only for

laboratory rats, but also rats kept as pets, as in both environments rats are seldom given the opportunity to burrow. In addition to enriching rats' behavioural repertoire, I speculate that burrowing may be an activity that brings pleasure to rats. Good welfare is not simply the absence of prolonged negative experiences, but also the presence of positive ones (Boissy et al., 2007). Providing rats with burrowing substrate may be a species-relevant approach to providing opportunities for positive affect.

Rats' inability to stand fully upright in a standard laboratory cage is not a concern that I have heard raised in general, or within the laboratory animal community. In contrast, the ability of farm animals to stand upright and extend limbs without touching the walls of their enclosure is an important concern (Benard and de Cock Buning, 2013; Boogaard et al., 2011; Vanhonacker et al., 2008), prompting recent changes in legislation that will allow sows and chickens to perform this basic behaviour (National Agricultural Law Center, 2015). This legislation was enacted in large part due to citizen concerns around this issue; methods for housing laboratory animals are largely shielded from public view, so it is possible that the public does not realize that this is also an issue for laboratory rats.

Regulations around cage size and cage height are based more on tradition than scientific evidence (Galef and Durlach, 1993; Gaskill and Pritchett-Corning, 2015; Scharmann, 1991). According to Galef and Durlach (1993), in the early 1990s the Canadian Council on Animal Care (CCAC) considered recommending that large rats (i.e. adult males) should be kept in cages that were 23 cm high, rather than the 18 cm still recommended today. In response, Galef and Durlach (1993) tested large male rats' preferences for cages that were 16.8 cm versus 23 cm high, and found no differences in the amount of time rats spent in each cage. It is not clear why the CCAC had chosen a height of 23 cm, but as described in Chapter 3, this height is also too low to allow adult rats to stand fully upright. The few cm of extra height may not have been functionally significant to the rats,

explaining the lack of preference. Another study reported that the percent of daily active time rats spent standing upright was relatively low (3-14%; Büttner, 1993), and regulatory bodies in the UK have used this result to argue that standing upright must therefore not be important to rats (Dr. Penny Hawkins, head of the Research Animals Department at the RSPCA, personal communication). The results of Chapter 3 appear to be the first to show that while total daily time spent standing upright is low, the frequency of this behaviour is very high (in 3-month old rats, nearly 180 times/day, or equivalent to once every 4 min assuming rats are awake 12 h/day). These results can thus help inform new recommendations for cage height. There are substantial costs associated with replacing existing caging, but a more cost-effective solution could be to invest in 'raised lids', similar to the upper portion that clips onto the bottom pan of a hamster cage, and are already available for purchase from several caging manufacturers.

The inability to stand upright may have broader consequences beyond the inability to perform this species-specific form of kinesis (Fraser, 1988). Chapter 3 has shown that standard-housed rats performed lateral stretches much more frequently than rats housed in the semi-naturalistic environment, suggesting that they may perform more lateral stretches in part to compensate for the inability to stretch in the upright position. However, I suspect that the frequent lateral stretching in standard-housed rats is also performed as a corrective response to stiffness caused by general lack of physical activity and movement (Bertolucci, 2011; Fraser, 1989). Physical discomfort caused by a behaviourally restrictive environment is not within the realm of issues discussed around the topic of rat welfare. People tend to raise concerns about negative affective states, such as boredom (van Rooijen, 1991; Wemelsfelder, 1990) or compromised health, such as overweight and metabolically morbid (Martin et al., 2010), but not physical discomfort. Results from Chapter 3 thus point towards a yet unexplored consequence of restrictive housing for laboratory rats.

Chapter 4 explored the affective consequences of long-term standard versus semi-naturalistic housing. A similar study was performed by van der Harst and colleagues (2003a), except that their standard-housed subjects were male, housed in groups of three without a shelter, and for only two weeks. My study adds to the small literature on the negative impact of standard laboratory housing on rat affective states, and to the even smaller literature on these effects in females. Research into the affective experiences of rats housed in standard cages may be particularly important, as knowledge of animals' emotional experiences helps to inform and motivate concerns for welfare (Broida et al., 1993; Knight et al., 2009; see also subsection 6.5 below).

Differences in anticipatory behaviour are sometimes used to draw inferences about welfare in animals; for example, that animals in more impoverished conditions will show increased sensitivity to rewards and thus increased anticipatory activity. However, there is little scientific knowledge around the significance of *what* animals do during this period of increased activity. What animals do may impart further clues about their welfare, and help to develop the theory around using anticipatory behaviour as a tool for assessing welfare in animals. In Chapter 4, I have provided the first detailed account of the frequency and duration of individual behavioural elements displayed by rats during the anticipatory behaviour period. A less detailed account was previously provided for standard-housed rats (van der Harst et al., 2003b), but mine is the first for 'enriched'-housed rats. Chapter 4 has shown that not only are standard-housed rats more active (displaying more frequent transitions between individual behavioural elements), but that the behaviours displayed are also different from that displayed by semi-naturalistic-housed rats. This information can provide the basis for future investigations into using the form, and not just the quantity, of anticipatory behaviour as a tool for assessing animal welfare.

The relationships between the environment, body weight, and disease such as cancer, are a major area of scientific inquiry. My studies were not designed to investigate these relationships, but

given that rat models are an important part of these investigations (Jeong et al., 2015; Lim et al., 2013), and that very few investigators have conducted long-term studies on these relationships in rats, Chapter 5 documented the long-term effects of living a sedentary lifestyle in a standard environment versus one where the animals are more active in a semi-naturalistic environment. Not only can these results be used in future meta-analyses on these relationships and the potential implications for humans, but they also contribute to the literature on the biological consequences of standard housing on rat welfare.

6.3. Implications for rat welfare

Examining the welfare consequences of standard housing from the perspective of natural living, affective states and biological functioning helps to illustrate the degree to which behaviour, emotion and health are interconnected. An animal who becomes physically ill does not do so in a vacuum; and behavioural and psychological deficits have direct impact on physical well-being.

The inability to engage in species-specific behaviours may have consequences on affective states (for example, causing frustration, boredom, anxiety, or depression) and biological functioning (potentially causing joint and muscle stiffness, overweight, and slow metabolism). Affective states in turn influence behaviour (depression leads to the cessation of naturally motivated behaviours; anxiety, boredom or frustration may lead to the performance of abnormal behaviours) and health (stress and anxiety lead to anorexia or overeating of 'unhealthy' foods; stress leads to higher risk of cancer and many other diseases). Poor health also affects behaviour (overweight reduces agility and therefore ability to perform certain behaviours; infection and inflammation lead to lethargy) and affective states (physical ailments that cause pain cause negative judgment biases; pain as a result of physical injury is itself a negative emotional experience). The interconnectedness between

behaviour, emotion and health suggests a trickle-down effect of any one consequence of inadequate housing, and underscores the importance of looking at these responses together.

Problems associated with standard housing do not begin when the animal enters a particular study. Rats used in research are usually ordered from major animal suppliers, such as Charles River. Rats at Charles River – including breeding pairs and dams with their offspring – are housed is standard cages without enrichment, although some nesting material is provided to nursing dams (Gaskill and Pritchett-Corning, 2015; Dr. Brianna Gaskill, personal communication). The issues with standard housing identified in this thesis (inability to perform important natural behaviours, negative affective states and overweight) are likely also affecting the dams who give birth to future research subjects. The negative effects of maternal stress on rat offspring are well established (Talge et al., 2007; Van Den Bergh et al., 2005; Weinstock, 2005, 1997). For example, activation of the HPA axis in the pregnant dam impairs functioning of HPA axis in the offspring into adulthood, and creates anxious offspring with heightened responses to novelty, including freezing, defecation and lower exploration. I suggest that the intergenerational effects of conventional housing may be creating individuals with neurobiologically and behaviourally abnormal profiles (Weinstock, 1997). The creation of animals thus predisposed from birth to experience poor welfare is itself an important welfare issue.

6.4. Implications for the quality of science

Scientists using animals in research place great importance on using 'healthy' subjects. Rodents ordered from commercial breeders are guaranteed to be pathogen-free (e.g., Clifford, 2014), and this status is often included in the Material & Methods section in publications. Large-scale animal facilities usually keep in every room a cage with 'sentinel' animals who are regularly tested for the presence of certain pathogens to ensure early detection and treatment. Animals who

exhibit signs of morbidity are usually excluded from experimental analysis. The reasoning behind these procedures is that sick animals are not 'normal' and may skew the results; this is true. My question is: why limit these concerns to a limited number of 'abnormalities' in biological functioning?

Even if one were solely concerned with biological functioning, then pathogens or other overt signs of morbidity should not be the only sources of concern. Martin and colleagues (2010) reviewed evidence that standard-housed, control animals are "metabolically morbid". The authors described that the sedentary lifestyle and lack of stimulation associated with rodent standard housing lead to animals who are overweight, insulin resistant, and hypertensive, with physiological profiles consistent with increased disease susceptibility. When these animals are used in basic and translational biomedical research, including preclinical drug testing, the beneficial effects of some drugs may result from their effects on processes associated with these abnormalities rather than an effect of the drug on the actual disease process. These authors further reviewed evidence that better diet and exercise in these rodents lead to better plasma profiles, lower blood pressure and resting heart rate, and a cardiovascular system better able to recover from stress.

As reviewed in subsection 1.2.2., a standard, barren environment also leads to different behavioural and neurological outcomes compared to a more complex environment. These differences have profound effects on the data obtained from animals. For example, Lewejohann and colleagues (2009) found that when a widely used transgenic mouse model of Alzheimer's disease was housed in a semi-naturalistic environment instead of the normally used standard cage, mice still developed the typical β-amyloid plaques characteristic of the disease, but their behavioural profiles were indistinguishable from that of healthy controls. Hockley and colleagues (2002) reared transgenic mice models of Huntington's disease in standard or highly enriched environments, and found that the decline in performance on a rotating rod test as well as changes in the brain in mice

from the enriched environment occurred at a much slower rate. These authors argued that mice from the enriched environment were more representative of the human disease progression, and that standard-housed mice were likely not a good model of the disease.

The rationale behind the use of barren environments is that it allows for environmental standardization, and this is deemed important because it allows researchers to control for extraneous variables, allowing for their results to be reproducible. However, increasing evidence suggests that standardization through the use of barren environments may actually lead to idiosyncratic results particular to individual laboratories. Crabbe and colleagues (1999) tested several strains of mice on six behavioural tests simultaneously across three laboratories. All protocols, instruments and many environmental variables had been meticulously equated. Nonetheless, strains greatly differed in most behaviours between the three laboratories; for example, anxiety scores in the widely used elevated plus maze were higher in one laboratory than the another.

More recently, Richter and colleagues (2009) presented a proof of principle based on data obtained from three laboratories on behavioural differences between several mouse strains. Their findings suggest that standardization is actually a cause of poor reproducibility. In these barren environments, the smallest variations in otherwise meaningless variables lead to large changes, because in an environment where 'nothing happens', any change becomes salient to the animals.

Together, these studies suggest that not only do standard laboratory environments lead to 'abnormal' animals who are likely not representative of a typical human, but they also leave the animals vulnerable to idiosyncratic responses, yielding data with little external validity. It is suggested that environmental enrichment may actually improve scientific outcomes by reducing the number of abnormal animals and abnormal responses into experiments (Garner, 2005)

6.5. Limitations

The major limitation of the studies described in this thesis is the small sample sizes. Unfortunately, six cages in each housing treatment were the most I could physically fit into the laboratory. The already small sample sizes were further reduced by the loss of animals (leading to the loss of entire cages) due to illness. Some studies were designed to assess within-individual differences (e.g., effect of drug treatment on anticipatory behaviour in Chapter 4) specifically to increase statistical power. Despite the small sample sizes, in some cases differences between the two housing conditions were large enough that I was able to detect differences (e.g., frequency of lateral stretching in Chapter 3; behavioural frequency during anticipation of a treat in Chapter 4). In other cases, it is possible that differences were not detected due to low power (e.g., incidence of tumours between the two groups in Chapter 5).

The second major limitation of this work is that I only assessed one particular type of 'standard' housing versus one particular type of 'enriched' housing. The specific details of each housing condition matter. My aim was to compare a common standard system with something substantially different, and it is possible that had some features of either system been different, certain results would have also been different. Future work comparing other commonly used standard cages (e.g., single-housing, different type of bedding, different type or no shelter) versus other types of 'enriched' housing could yield further insights into what ultimately rats find important, and what contributes most to good or poor welfare (see subsection 6.6 below).

A third limitation has to do with the fact that I could not handle my semi-naturalistic-housed rats. I knew that once these rats were in their cages with soil, I would not be able to handle them easily because they would prefer to retreat into their burrows rather than to be picked up. To address this problem, I had come up with a plan to let these rats out into a large playpen daily for 30-60 min. The idea was that these rats would become accustomed to spending time in the playpen,

and that I could then easily pick them up from there. Presumably, the playpen would have been attractive enough to warrant continued visits even after the rats learned that sometimes they would be picked up by a human while visiting the pen. With time, they would become accustomed to being picked up regularly, just as the standard-housed rats got accustomed to being picked up twice a week during cage cleaning. I did implement the playpen in rats' early days in the laboratory, but this plan was quickly thwarted because of rats' frequent (and clever) escapes from the playpen into the room. When the playpen plan failed, I proceeded to come up with ways of testing the rats without having to handle them before testing. Unfortunately, this approach constrained the types of tests I was able to do with these animals.

Because of the difficulties associated with handling the semi-naturalistic-housed rats, I did not mark them for individual identification until it was absolutely necessary for the within-individual tests of drug effect on anticipatory behaviour. Had the rats been marked earlier, I could have obtained data on individual differences in the propensity to burrow, climb and stand upright in these rats. Had the rats been easier to handle, I also would have weighed them earlier and more regularly.

I also want to acknowledge the limitations I was faced with when planning experiments that involved the use of ketamine, which is a controlled substance. The use of a controlled substance required me to obtain a special exemption from the Canadian government, and under this exemption I was limited to sourcing the drug from a limited number of companies who were allowed to supply drugs to animal researchers. None of these suppliers carried the oral form of ketamine, so I was left with giving my rats injectable ketamine. As I found out, the injectable form of ketamine is very bitter and rats categorically refused to consume it unless, as I found through weeks of trying different cocktails, it was mixed with honey peanut butter. Others have given rats injectable ketamine orally through gavage (Shimoyama et al., 1999, 1997) or by withholding food

and water from rats for 23/h day, then providing 1 h/day access to food and water laced with the drug (Silvestre et al., 2002). For welfare reasons, I did not want to gavage my rats, nor deprive them of food and water for extended periods of time. Being limited to giving ketamine in the form of peanut butter constrained the types of experimental approaches I was able to take with these rats.

6.6. Future research

To me, some of the most interesting results are those from Chapter 3 on the importance of burrowing and standing upright for laboratory rats, in part because these were unexpected. An analysis of rats' propensity to perform these behaviours was a logical place to start given the general lack of research in this area. However, I believe that my analysis has only begun to 'scratch the surface' of what there is to learn about the importance of these behaviours for rats. Regarding burrowing, I propose using more sophisticated methods of assessing its importance. A controlled study, similar to that performed by Sherwin and colleagues (2004) where rats have to pay an increasing price to access a burrowing substrate (e.g., by pressing a weighted barrier; Manser et al., 1996; Patterson-Kane et al., 2008), could help assess the strength of motivation to burrow. Moreover, controlled investigations into rats' propensity to burrow in an area where previously built burrows have been destroyed versus left intact could inform on the importance of burrowing versus having a burrow.

Furthermore, I would like to test my theory that burrowing leads to positive affect. Testing for positive affective states is in its infancy, but one approach could be to assess judgment biases immediately after letting rats into an arena where they did or did not have access to a burrowing substrate (Brydges et al., 2011; Rygula et al., 2015). Young animals are also more likely to play when in a positive affective state (Held and Špinka, 2011), so the likelihood to play with a cage-mate

shortly after having access to an enclosure with versus without burrowing substrate, or the amount of play in cages where animals have or do not have burrowing substrate, could be investigated.

If burrowing is indeed a behaviour motivated by prevention motivation (see subsection 3.4), then propensity to burrow should vary between individuals, should be stable across time in each individual, and should correlate with the performance of other prevention-motivated behaviours (e.g., maintaining darkness or containing a manageable threat; Franks et al., 2014, 2012). I think there is scope to investigate this idea by assessing individual rats' propensity to burrow and correlating it with propensity to engage in other prevention-motivated behaviours.

I also think there is a need to test practical ways of providing a burrowing substrate to rats. The set-up that I used was messy and labour intensive, and I do not recommend it for large-scale use. However, I think there is scope to develop creative ways for letting rats dig and maintain tunnels without necessarily filling the cage with soil. For example, a network of plastic tunnels could be filled with wood wool or wood shavings rats would need to excavate to reach an inner dark chamber. To facilitate animal monitoring, one or two sides of the dark chamber could be made of red transparent material to allow observers to see the animals while providing a darkened environment for the rats. A different approach would be to investigate the suitability of providing rats with polar fleece blankets, which they burrow into, fluff up and re-arrange on a regular basis (observations in Joyce Sato-Reinhold's and my laboratory).

Similar studies to those assessing rats' motivation to burrow could be performed to assess rats' motivation to stand upright and to be able to stretch in the upright position; for example, assessing the price rats are willing to pay to access cages with increased height, or testing judgment biases in rats allowed to perform this behaviour.

There is also scope to determine which aspects of the semi-naturalistic cages led to better welfare. The cages used in my thesis provided rats with the opportunity to burrow and stand

upright, increased space that allowed running and climbing, a larger social group, a varied diet, and the provision of this diet in a way that required rats to work in order to gain access. An adequate area to retreat from the light and regulate ambient temperature also likely contributed to increased well-being. Which of these features or combination of features were most important has yet to be determined.

I am also particularly interested in further assessing the affective consequences of standard cages, as well as rats' ability to experience a range of emotions. The negative behavioural, neurological, physiological and health effects of standard housing are well established (see subsection 1.2.2), and yet there appears to be little motivation in the laboratory community for change. Indeed, I suggest that rats and mice are not afforded the same quality of care as other laboratory mammals. Support for animal experimentation is greater when experiments are performed on rats and mice than on other mammals (Knight et al., 2003; Phillips et al., 2012), and even identical experiments are judged as more acceptable if they are to be performed on rats, mice, or non-mammalian species than if they are to be performed on dogs, cats, or monkeys (Driscoll, 1992). When used, rats and mice are more likely than other mammals to be subjected to invasive procedures. In Canada, experiments involving vertebrates are rated according to four 'categories of invasiveness' ranging from B to E. Studies rated as category of invasiveness E cause "severe pain near, at, or above the pain tolerance threshold of unanaesthetized conscious animals" (CCAC, 1991). In 2013, 0.89% of rats and mice were used in category E experiments, compared to 0.007% of non-human primates, dogs and cats (CCAC, 2015). In New Zealand, the most invasive procedures are rated as Grade E – "Very High Impact" and include major surgery without anaesthesia, exposure to extremely noxious stimuli from which escape is impossible, and experiments with death as an endpoint (MAF, 2010). In 2010, 18.7% of rats and mice were subjected to Grade E manipulations;

the only other species in this category were guinea pigs and unspecified 'pest' species (NAEAC, 2011).

Rodents are also less likely than other mammals to receive analgesics after painful surgical procedures (Coulter et al., 2009; Stokes et al., 2009). Structured literature reviews that assessed the trends in the administration of analgesics to laboratory rats and mice revealed that in 1990-1992, only 3% of studies published in peer-reviewed journals reported analgesic administration to these rodents after a surgical procedure (Richardson and Flecknell, 2005). The proportion of studies that reported administration of analgesics to rats and mice increased to 10% by 2000-2001, and 20% by 2005-2006 (Stokes et al., 2009). In contrast, the reported administration of analgesics to other laboratory mammals (non-human primates, dogs, pigs, rabbits and sheep) following a surgical procedure was 50% in 2000-2001 and 63% in 2005-2006 (Coulter et al., 2009).

There is evidence to suggest that such differential treatment of rodents versus other mammals is largely driven by people's beliefs in the ability of different species to suffer and experience a range of emotions (Hills, 1995). For example, university students and scientists who conduct research on animals are less likely to attribute the capacity to experience at least moderate levels of pain, emotions, and suffering to rats, mice and non-mammalian species than to other mammals such as dolphins, chimpanzees, dogs, and cats (Herzog and Galvin, 1997; Knight et al., 2009; Phillips et al., 2012). Furthermore, research has shown that scientists and animal welfarists alike believe that animals they perceive as having less ability to experience pain, emotions and suffering deserve less moral consideration (Herzog and Galvin, 1997; Knight et al., 2009).

Thus one benefit of studies investigating the affective states of rats is that the resulting evidence may help dispel perceptions that these animals are somehow less able to experience 'pleasure and pain'. Moreover, demonstrating the affective consequences of standard housing could also help motivate the implementation of higher housing standards. For example, I would like to do

a drug self-administration study in which rats from standard versus 'enriched' housing are given the choice between regular water and water containing an antidepressant or an anxiolytic (Colpaert et al., 2001, 1980; Sherwin and Olsson, 2004). The properties of the drug should only be reinforcing to animals experiencing the state that the drug is meant to treat, so only animals experiencing that state, but not healthy controls, should have a preference for the drugged water (see section 2.3.2.1). For example, anxious animals would feel better after drinking water containing an anxiolytic, but healthy controls would not be affected. Therefore, anxious animals would show a preference for the drugged water while healthy controls would not (provided that the drugged water is not more palatable than regular water).

6.7. Final conclusion

Whether from a moral or a scientific point of view, good animal welfare matters. Rats reared in the standard cages were different from rats reared in the semi-naturalistic environment on a behavioural, affective, and physical level, and these differences were reflective of differences in welfare between the two groups.

On the basis of the results from my research, and the results of other studies reviewed in this thesis, I recommend raising current standards for housing laboratory rats, beginning by focusing on rats used for breeding. Specifically, I recommend:

1. providing a burrowing substrate;
2. increasing cage height to allow full upright standing;
3. providing opportunities for exercise (e.g., increased space and structural complexity)
4. providing diets matched to nutritional requirements

I have chosen to study animal welfare because I believe that it matters in its own right. I believe that any being with 'wants' and 'likes' and the motivation to achieve these deserves a

chance at a 'good life'. The results in this thesis, together with those from the other studies reviewed in this thesis, indicate that there is scope to do better in terms of providing laboratory rats with a good life. I suggest that the way rats are housed is an important factor contributing to their overall welfare, and that considerable improvements in welfare can be achieved by raising current housing standards.

But good animal welfare matters beyond its intrinsic value. To the animal care technician, good welfare should matter because 'happy' animals are much more interesting to work with. The animal care technicians working with my rats were simply amazed to see the rats in the semi-naturalistic housing act like rats. They had no idea rats could dig and climb and be anything other than sedentary. They confessed to spending extra time in my room just to watch the rats in action. Enjoying their job and the animals they cared for surely influenced their own quality of life, as well as the quality of care given to the animals. Compassion fatigue is common among animal care technicians (Figley, 2006), but may be lessened when there are fewer feelings of guilt thanks to the knowledge that the animals have a decent life.

To the biomedical research scientist, the welfare consequences of current standard laboratory housing should matter because these consequences directly affect the quality of the results. Considerable strides could be achieved in human health if the animals used to test new drugs or biological mechanisms were valid, replicable and reliable. To the funding agencies, good welfare should matter because yearly, in the United States alone, it is estimated that US$28 billion may be wasted on preclinical research that is irreproducible (Freedman et al., 2015). To the average citizen, good welfare should matter because their earnings fund the majority of this research, whether through paying taxes or charitable donations, and their own access to new therapies is dependent on the advances in biomedicine.

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